resolwe-bio

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Contents

1	Cont		3	
	1.1	Writing processes		
	1.2	Process catalog	3	
	1.3	Descriptor schemas	332	
	1.4	Reference	333	
	1.5	Change Log	334	
	1.6	Contributing	373	
2 Indices and tables		379		
Python Module Index				
In	dex	3	383	

Bioinformatics pipelines for the Resolwe dataflow package for Django framework.

Contents 1

2 Contents

CHAPTER 1

Contents

1.1 Writing processes

A tutorial about writing bioinformatics pipelines (process is a step in the pipeline) is in Resolwe SDK for Python documentation.

1.1.1 **Tools**

Frequently, it is very useful to write a custom script in Python or R to perform a certain task in process' algorithm. For an example, see the tutorial in Resolwe SDK for Python documentation.

Custom scripts needed by processes included with Resolwe Bioinformatics are located in the resolwe_bio/tools directory.

Note: A Resolwe's Flow Executor searches for tools in a Django application's tools directory or directories specified in the RESOLWE_CUSTOM_TOOLS_PATHS Django setting.

1.2 Process catalog

Resolwe Bioinformatics includes over 100 processes. They are organized in categories. The type tree will help process developers with pipeline design. For process details browse process definitions.

1.2.1 Processes by category

Align

• WALT

- BWA MEM
- BWA SW
- BWA ALN
- Bowtie (Dicty)
- Bowtie2
- Subread
- HISAT2
- STAR

Chip-seq

Call peaks

- ChIP-seq (MACS2)
- MACS 2.0
- ChIP-seq (MACS2-ROSE2)
- MACS 1.4

Post process

• ROSE2

Qc report

• Pre-peakcall QC

Differential expression

- Cuffdiff 2.2
- edgeR
- DESeq2

Import

- GAF file
- VCF file
- Genome
- Expression data
- Expression data (Cuffnorm)
- Expression data (STAR)
- SAM header

- Upload Picard CollectTargetedPcrMetrics
- Metabolic pathway file
- BAM file
- · BAM file and index
- Secondary hybrid BAM file
- · Cuffquant results
- BED file
- SRA data
- SRA data (single-end)
- SRA data (paired-end)
- FASTA file
- BaseSpace file
- Differential Expression (table)
- · OBO file
- · Custom master file
- GFF3 file
- GTF file
- Mappability info
- Reads (QSEQ multiplexed, single)
- Reads (QSEQ multiplexed, paired)
- snpEff
- FASTQ file (single-end)
- FASTQ file (paired-end)
- Convert files to reads (single-end)
- Convert files to reads (paired-end)
- Gene set
- Gene set (create)
- Gene set (create from Venn diagram)
- Expression time course

Other

- · Amplicon report
- Archive and make multi-sample report for amplicon data
- PCA
- Hierarchical clustering of samples
- Hierarchical clustering of genes

- Bam split
- Prepare GEO ChIP-Seq
- Prepare GEO RNA-Seq
- MultiQC
- · Convert GFF3 to GTF
- Archive samples
- · Spike-ins quality control
- Subsample FASTQ (single-end)
- Subsample FASTQ (paired-end)
- ChIP-Seq (Peak Score)
- ChIP-Seq (Gene Score)
- Cutadapt (Diagenode CATS, single-end)
- Cutadapt (Diagenode CATS, paired-end)
- GO Enrichment analysis
- STAR genome index
- · coverageBed
- Bamliquidator
- Align (BWA) and trim adapters
- Picard CollectTargetedPcrMetrics
- Amplicon table
- Merge Expressions (ETC)
- Mappability
- Expression matrix
- Gene expression indices
- Expression aggregator
- Dictyostelium expressions
- Expression Time Course
- Indel Realignment and Base Recalibration
- Variant filtering (CheMut)
- Variant calling (CheMut)
- GATK3 (HaplotypeCaller)
- GATK4 (HaplotypeCaller)
- snpEff
- LoFreq (call)

Pipeline

- ATAC-Seq
- BBDuk STAR HTSeq-count (single-end)
- BBDuk STAR HTSeq-count (paired-end)
- WGBS
- Cutadapt STAR RSEM (Diagenode CATS, single-end)
- Cutadapt STAR RSEM (Diagenode CATS, paired-end)
- BBDuk STAR featureCounts QC (single-end)
- BBDuk STAR featureCounts QC (paired-end)
- RNA-Seq (Cuffquant)
- BBDuk STAR FeatureCounts (3' mRNA-Seq, single-end)
- BBDuk STAR FeatureCounts (3' mRNA-Seq, paired-end)
- Cutadapt STAR HTSeq-count (single-end)
- Cutadapt STAR HTSeq-count (paired-end)
- Chemical Mutagenesis
- Accel Amplicon Pipeline
- Trim, align and quantify using a library as a reference.
- Whole exome sequencing (WES) analysis
- Trimmomatic HISAT2 HTSeq-count (single-end)
- Trimmomatic HISAT2 HTSeq-count (paired-end)
- MACS2 ROSE2
- miRNA pipeline

Plot

• Bamplot

Quantify

- Cuffmerge
- Cuffnorm
- Cufflinks 2.2
- Cuffquant 2.2
- Quantify shRNA species using bowtie2
- HTSeq-count (TPM)
- HTSeq-count (CPM)
- · featureCounts
- RSEM

Splice junctions

• Annotate novel splice junctions (regtools)

Trim

- Cutadapt (single-end)
- Cutadapt (paired-end)
- Trimmomatic (single-end)
- Trimmomatic (paired-end)
- BBDuk (single-end)
- BBDuk (paired-end)

Wgbs

- methcounts
- HMR

Abstract

- Abstract bed process
- Abstract differential expression process
- Abstract alignment process
- Abstract annotation process
- Abstract expression process

Uncategorized

- Test sleep progress
- Test disabled inputs
- Test basic fields
- Test hidden inputs
- Test select controler
- Detect library strandedness
- · Salmon Index

1.2.2 Type tree

Process types are listed alphabetically. Next to each type is a list of processes of that type. Types are hierarchical, with levels of hierarchy separated by colon ":". The hierarchy defines what is accepted on inputs. For instance, Expression (Cuffnorm) process' input is data:alignment:bam. This means it also accepts all subtypes (e.g., data:alignment:bam:bwasw, data:alignment:bam:bowtiel and data:alignment:bam:tophat). We encourage the use of existing types in custom processes.

- data:aggregator:expression Expression aggregator
- data:alignment Abstract alignment process
- data:alignment:bam:bowtie1 Bowtie (Dicty)
- data:alignment:bam:bowtie2 Bowtie2
- data:alignment:bam:bwaaln-BWA ALN
- data:alignment:bam:bwamem BWA MEM
- data:alignment:bam:bwasw-BWASW
- data:alignment:bam:bwatrim Align (BWA) and trim adapters
- data:alignment:bam:hisat2-HISAT2
- data:alignment:bam:primary-Bam split
- data:alignment:bam:secondary Secondary hybrid BAM file
- data:alignment:bam:star-STAR
- data:alignment:bam:subread-Subread
- data:alignment:bam:upload BAM file, BAM file and index
- data:alignment:bam:vc Indel Realignment and Base Recalibration
- data:alignment:mr:walt-WALT
- data: annotation Abstract annotation process
- data:annotation:cuffmerge Cuffmerge
- data:annotation:gff3-GFF3 file
- data:annotation:gtf Convert GFF3 to GTF, GTF file
- data:archive:samples Archive samples
- · data:archive:samples:amplicon Archive and make multi-sample report for amplicon data
- data:bam:plot:bamliquidator Bamliquidator
- data:bam:plot:bamplot Bamplot
- data:bed Abstract bed process, BED file
- data:chipseq:batch:macs2 ChIP-seq (MACS2), ChIP-seq (MACS2-ROSE2)
- data:chipseq:callpeak:macs14 MACS 1.4
- data:chipseq:callpeak:macs2 MACS 2.0
- data:chipseq:genescore ChIP-Seq (Gene Score)
- data:chipseq:peakscore ChIP-Seq (Peak Score)
- data:chipseq:rose2 ROSE2

- data:clustering:hierarchical:gene Hierarchical clustering of genes
- · data:clustering:hierarchical:sample Hierarchical clustering of samples
- data:coverage-coverageBed
- data:cufflinks:cufflinks Cufflinks 2.2
- data:cufflinks:cuffquant Cuffquant 2.2, Cuffquant results
- data:cuffnorm Cuffnorm
- data:differentialexpression Abstract differential expression process
- data:differentialexpression:cuffdiff-Cuffdiff 2.2
- data:differentialexpression:deseq2 DESeq2
- data:differentialexpression:edger-edgeR
- data:differentialexpression:upload Differential Expression (table)
- data:etc Expression Time Course, Expression time course
- data:expression Abstract expression process, Expression data, Expression data (Cuffnorm)
- data:expression:featurecounts-featureCounts
- data:expression:htseq:cpm-HTSeq-count(CPM)
- data:expression:htseq:normalized-HTSeq-count(TPM)
- data:expression:polya Dictyostelium expressions
- data:expression:rsem-RSEM
- data:expression:shrna2quant Quantify shRNA species using bowtie2
- data:expression:star-Expression data (STAR)
- data:expressionset Expression matrix
- data:expressionset:etc-Merge Expressions (ETC)
- data:file BaseSpace file
- data:gaf:2:0 GAF file
- data:geneset Gene set, Gene set (create)
- data:geneset:venn Gene set (create from Venn diagram)
- data:genome:fasta-Genome
- data:genomeindex:star-STAR genome index
- data:goea GO Enrichment analysis
- data:index:expression Gene expression indices
- data:index:salmon Salmon Index
- data: junctions: regtools Annotate novel splice junctions (regtools)
- data:mappability:bcm Mappability, Mappability info
- data:masterfile:amplicon Custom master file
- data:metabolicpathway Metabolic pathway file
- data:multiplexed:qseq:paired-Reads (QSEQ multiplexed, paired)

- data:multiplexed:qseq:single Reads (QSEQ multiplexed, single)
- data:multiqc-MultiQC
- data:ontology:obo-OBO file
- data:other:geo:chipseq-Prepare GEO-ChIP-Seq
- data:other:geo:rnaseg-Prepare GEO-RNA-Seg
- data:pca PCA
- data:picard:coverage Picard CollectTargetedPcrMetrics
- data:picard:coverage:upload Upload Picard CollectTargetedPcrMetrics
- data:prepeakqc Pre-peakcall QC
- data:reads:fastq:paired Convert files to reads (paired-end), FASTQ file (paired-end), SRA data (paired-end)
- data:reads:fastq:paired:bbduk-BBDuk(paired-end)
- data:reads:fastq:paired:cutadapt Cutadapt (Diagenode CATS, paired-end), Cutadapt (paired-end)
- data:reads:fastq:paired:seqtk Subsample FASTQ (paired-end)
- data:reads:fastq:paired:trimmomatic Trimmomatic (paired-end)
- data:reads:fastq:single Convert files to reads (single-end), FASTQ file (single-end), SRA data (single-end)
- data:reads:fastq:single:bbduk-BBDuk(single-end)
- data:reads:fastq:single:cutadapt Cutadapt (Diagenode CATS, single-end), Cutadapt (single-end)
- data:reads:fastq:single:seqtk Subsample FASTQ (single-end)
- data:reads:fastq:single:trimmomatic Trimmomatic (single-end)
- data:report:amplicon Amplicon report
- data:sam:header SAM header
- data:seq:nucleotide FASTA file
- data:snpeff-snpEff
- data:snpeff:upload-snpEff
- data:spikeins Spike-ins quality control
- data:sra SRA data
- data:strandedness Detect library strandedness
- data:test:disabled Test disabled inputs
- data:test:fields Test basic fields
- data:test:hidden Test hidden inputs
- data:test:result Test select controler, Test sleep progress
- data:variants:vcf VCF file
- data:variants:vcf:chemut Variant calling (CheMut)

- data:variants:vcf:filtering Variant filtering (CheMut)
- data:variants:vcf:gatk:hc-GATK3 (HaplotypeCaller), GATK4 (HaplotypeCaller)
- data:variants:vcf:lofreq-LoFreq(call)
- data:varianttable:amplicon Amplicon table
- data:wgbs:hmr-HMR
- data:wgbs:methcounts methcounts
- data:workflow:amplicon Accel Amplicon Pipeline
- data:workflow:atacseg ATAC-Seq
- data:workflow:chemut Chemical Mutagenesis
- data:workflow:chipseq:macs2rose2 MACS2 ROSE2
- data:workflow:mirna miRNA pipeline
- data:workflow:quant:featurecounts:paired-BBDuk-STAR-FeatureCounts(3'mRNA-Seq, paired-end)
- data:workflow:quant:featurecounts:single-BBDuk-STAR-FeatureCounts(3'mRNA-Seq, single-end)
- data:workflow:rnaseq:cuffquant RNA-Seq (Cuffquant)
- data:workflow:rnaseq:featurecounts:qc BBDuk STAR featureCounts QC (paired-end),
 BBDuk STAR featureCounts QC (single-end)
- data:workflow:rnaseq:htseq Cutadapt STAR HTSeq-count (paired-end), Cutadapt STAR -HTSeq-count (single-end), Trimmomatic - HISAT2 - HTSeq-count (paired-end), Trimmomatic - HISAT2 -HTSeq-count (single-end)
- data:workflow:rnaseq:htseq:paired-BBDuk-STAR-HTSeq-count(paired-end)
- data:workflow:rnaseq:htseq:single-BBDuk-STAR-HTSeq-count(single-end)
- data:workflow:rnaseq:rsem Cutadapt STAR RSEM (Diagenode CATS, paired-end), Cutadapt -STAR - RSEM (Diagenode CATS, single-end)
- data:workflow:trimalquant Trim, align and quantify using a library as a reference.
- data:workflow:wes Whole exome sequencing (WES) analysis
- data:workflow:wgbs-WGBS

1.2.3 Process definitions

ATAC-Seq

data:workflow:atacseqworkflow-atac-seq (data:reads:fastq

reads. data:genome:fasta genome, data:bed promoter, basic:string mode, basic:string speed, basic:boolean use se, basic:boolean discordantly, basic:boolean barep se, minins, basic:integer sic:integer maxins. basic:integer trim_5, basic:integer trim 3, basic:integer trim_iter, basic:integer trim_nucl, basic:string rep_mode, basic:integer k_reports, basic:integer q threshold, basic:integer n sub, shift, babasic:boolean tn5, basic:integer duplisic:boolean tagalign, basic:string duplicates_prepeak, bacates, basic:string sic:decimal qvalue, basic:decimal pvalue, basic:decimal pvalue prepeak, basic:integer cap num, basic:integer mfold lower, mfold upper, basic:integer basic:integer **slocal**, basic:integer llocal. basic:integer extsize, basic:integer shift, basic:integer band_width, basic:boolean nolambda, basic:boolean fix_bimodal, basic:boolean nomodel. basic:boolean nomodel_prepeak, basic:boolean down sample, basic:boolean bedgraph, basic:boolean spmr, basic:boolean call_summits, basic:boolean broad, basic:decimal broad cutoff) [Source: v2.0.2]

This ATAC-seq pipeline closely follows the official ENCODE DCC pipeline. It is comprised of three steps; alignment, pre-peakcall QC, and calling peaks (with post-peakcall QC).

First, reads are aligned to a genome using [Bowtie2](http://bowtie-bio.sourceforge.net/index.shtml) aligner. Next, pre-peakcall QC metrics are calculated. QC report contains ENCODE 3 proposed QC metrics – [NRF](https://www.encodeproject.org/data-standards/terms/), [PBC bottlenecking coefficients, NSC, and RSC](https://genome.ucsc.edu/ENCODE/qualityMetrics.html#chipSeq). Finally, the peaks are called using [MACS2](https://github.com/taoliu/MACS/). The post-peakcall QC report includes additional QC metrics – number of peaks, fraction of reads in peaks (FRiP), number of reads in peaks, and if promoter regions BED file is provided, number of reads in promoter regions, fraction of reads in promoter regions, number of peaks in promoter regions, and fraction of reads in promoter regions.

Input arguments reads

```
label Select sample(s)
    type data:reads:fastq
genome
    label Genome
    type data:genome:fasta
promoter
    label Promoter regions BED file
    type data:bed
```

description BED file containing promoter regions (TSS+-1000bp for example). Needed to get the number of peaks and reads mapped to promoter regions.

```
required False
```

alignment.mode

```
label Alignment mode
```

```
type basic:string
```

description End to end: Bowtie 2 requires that the entire read align from one end to the other, without any trimming (or "soft clipping") of characters from either end. Local: Bowtie 2 does not require that the entire read align from one end to the other. Rather, some characters may be omitted ("soft clipped") from the ends in order to achieve the greatest possible alignment score.

```
default --local
```

choices

- end to end mode: --end-to-end
- local: --local

alignment.speed

```
label Speed vs. Sensitivity
```

```
type basic:string
```

default --sensitive

choices

- Very fast: --very-fast
- Fast: --fast
- Sensitive: --sensitive
- Very sensitive: --very-sensitive

alignment.PE_options.use_se

label Map as single-ended (for paired-end reads only)

```
type basic:boolean
```

description If this option is selected paired-end reads will be mapped as single-ended and other paired-end options are ignored.

```
default False
```

alignment.PE_options.discordantly

label Report discordantly matched read

```
type basic:boolean
```

description If both mates have unique alignments, but the alignments do not match paired-end expectations (orientation and relative distance) then alignment will be reported. Useful for detecting structural variations.

```
default True
```

alignment.PE_options.rep_se

label Report single ended

```
type basic:boolean
     description If paired alignment can not be found Bowtie2 tries to find alignments for the individual
     default True
alignment.PE options.minins
     label Minimal distance
     type basic:integer
     description The minimum fragment length for valid paired-end alignments. 0 imposes no minimum.
     default 0
alignment.PE_options.maxins
     label Maximal distance
     type basic:integer
     description The maximum fragment length for valid paired-end alignments.
     default 2000
alignment.start_trimming.trim_5
     label Bases to trim from 5'
     type basic:integer
     description Number of bases to trim from from 5' (left) end of each read before alignment.
     default 0
alignment.start_trimming.trim_3
     label Bases to trim from 3'
     type basic:integer
     description Number of bases to trim from from 3' (right) end of each read before alignment
     default 0
alignment.trimming.trim_iter
     label Iterations
     type basic:integer
     description Number of iterations.
     default 0
alignment.trimming.trim_nucl
     label Bases to trim
     type basic:integer
     description Number of bases to trim from 3' end in each iteration.
     default 2
alignment.reporting.rep_mode
     label Report mode
```

```
type basic:string
     description Default mode: search for multiple alignments, report the best one; -k mode: search for one
           or more alignments, report each; -a mode: search for and report all alignments
     default def
     choices
             • Default mode: def
             • -k mode: k
             • -a mode (very slow): a
alignment.reporting.k_reports
     label Number of reports (for -k mode only)
     type basic:integer
     description Searches for at most X distinct, valid alignments for each read. The search terminates when
          it can't find more distinct valid alignments, or when it finds X, whichever happens first.
     default 5
prepeakqc_settings.q_threshold
     label Quality filtering threshold
     type basic:integer
     default 30
prepeakqc_settings.n_sub
     label Number of reads to subsample
     type basic:integer
     default 25000000
prepeakqc_settings.tn5
     label TN5 shifting
     type basic:boolean
     description Tn5 transposon shifting. Shift reads on "+" strand by 4bp and reads on "-" strand by 5bp.
     default True
prepeakqc settings.shift
     label User-defined cross-correlation peak strandshift
     type basic:integer
     description If defined, SPP tool will not try to estimate fragment length but will use the given value as
           fragment length.
     default 0
settings.tagalign
     label Use tagAlign files
     type basic:boolean
```

description Use filtered tagAlign files as case (treatment) and control (background) samples. If extsize parameter is not set, run MACS using input's estimated fragment length.

default True

settings.duplicates

label Number of duplicates

type basic:string

description It controls the MACS behavior towards duplicate tags at the exact same location – the same coordination and the same strand. The 'auto' option makes MACS calculate the maximum tags at the exact same location based on binomal distribution using 1e-5 as pvalue cutoff and the 'all' option keeps all the tags. If an integer is given, at most this number of tags will be kept at the same location. The default is to keep one tag at the same location.

required False

hidden settings.tagalign

choices

- 1: 1
- auto: auto
- all: all

settings.duplicates_prepeak

label Number of duplicates

type basic:string

description It controls the MACS behavior towards duplicate tags at the exact same location – the same coordination and the same strand. The 'auto' option makes MACS calculate the maximum tags at the exact same location based on binomal distribution using 1e-5 as pvalue cutoff and the 'all' option keeps all the tags. If an integer is given, at most this number of tags will be kept at the same location. The default is to keep one tag at the same location.

required False

hidden !settings.tagalign

default all

choices

- 1: 1
- auto: auto
- all: all

settings.qvalue

label Q-value cutoff

type basic:decimal

description The q-value (minimum FDR) cutoff to call significant regions. Q-values are calculated from p-values using Benjamini-Hochberg procedure.

required False

disabled settings.pvalue && settings.pvalue_prepeak

settings.pvalue

label P-value cutoff

type basic:decimal

description The p-value cutoff. If specified, MACS2 will use p-value instead of q-value cutoff.

required False

disabled settings.qvalue

hidden settings.tagalign

settings.pvalue_prepeak

label P-value cutoff

type basic:decimal

description The p-value cutoff. If specified, MACS2 will use p-value instead of q-value cutoff.

disabled settings.qvalue

hidden!settings.tagalign || settings.qvalue

default 0.01

settings.cap_num

label Cap number of peaks by taking top N peaks

type basic:integer

description To keep all peaks set value to 0.

disabled settings.broad

default 300000

settings.mfold_lower

label MFOLD range (lower limit)

type basic:integer

description This parameter is used to select the regions within MFOLD range of high-confidence enrichment ratio against background to build model. The regions must be lower than upper limit, and higher than the lower limit of fold enrichment. DEFAULT:10,30 means using all regions not too low (>10) and not too high (<30) to build paired-peaks model. If MACS can not find more than 100 regions to build model, it will use the –extsize parameter to continue the peak detection ONLY if –fix-bimodal is set.

required False

settings.mfold_upper

label MFOLD range (upper limit)

type basic:integer

description This parameter is used to select the regions within MFOLD range of high-confidence enrichment ratio against background to build model. The regions must be lower than upper limit, and higher than the lower limit of fold enrichment. DEFAULT:10,30 means using all regions not too low (>10) and not too high (<30) to build paired-peaks model. If MACS can not find more than 100 regions to build model, it will use the –extsize parameter to continue the peak detection ONLY if –fix-bimodal is set.

required False

settings.slocal

label Small local region

type basic:integer

description Slocal and llocal parameters control which two levels of regions will be checked around the peak regions to calculate the maximum lambda as local lambda. By default, MACS considers 1000bp for small local region (–slocal), and 10000bps for large local region (–llocal) which captures the bias from a long range effect like an open chromatin domain. You can tweak these according to your project. Remember that if the region is set too small, a sharp spike in the input data may kill the significant peak.

required False

settings.llocal

label Large local region

type basic:integer

description Slocal and llocal parameters control which two levels of regions will be checked around the peak regions to calculate the maximum lambda as local lambda. By default, MACS considers 1000bp for small local region (–slocal), and 10000bps for large local region (–llocal) which captures the bias from a long range effect like an open chromatin domain. You can tweak these according to your project. Remember that if the region is set too small, a sharp spike in the input data may kill the significant peak.

required False

settings.extsize

label extsize

type basic:integer

description While '-nomodel' is set, MACS uses this parameter to extend reads in 5'->3' direction to fix-sized fragments. For example, if the size of binding region for your transcription factor is 200 bp, and you want to bypass the model building by MACS, this parameter can be set as 200. This option is only valid when -nomodel is set or when MACS fails to build model and -fix-bimodal is on.

default 150

settings.shift

label Shift

type basic:integer

description Note, this is NOT the legacy –shiftsize option which is replaced by –extsize! You can set an arbitrary shift in bp here. Please Use discretion while setting it other than default value (0). When –nomodel is set, MACS will use this value to move cutting ends (5') then apply –extsize from 5' to 3' direction to extend them to fragments. When this value is negative, ends will be moved toward 3'->5' direction, otherwise 5'->3' direction. Recommended to keep it as default 0 for ChIP-Seq datasets, or -1 * half of EXTSIZE together with –extsize option for detecting enriched cutting loci such as certain DNAseI-Seq datasets. Note, you can't set values other than 0 if format is BAMPE for paired-end data. Default is 0.

default -75

settings.band width

label Band width

type basic:integer

description The band width which is used to scan the genome ONLY for model building. You can set this parameter as the sonication fragment size expected from wet experiment. The previous side effect on the peak detection process has been removed. So this parameter only affects the model building.

required False

settings.nolambda

label Use backgroud lambda as local lambda

type basic:boolean

description With this flag on, MACS will use the background lambda as local lambda. This means MACS will not consider the local bias at peak candidate regions.

default False

settings.fix bimodal

label Turn on the auto paired-peak model process

type basic:boolean

description Whether turn on the auto paired-peak model process. If it's set, when MACS failed to build paired model, it will use the nomodel settings, the '-extsize' parameter to extend each tags. If set, MACS will be terminated if paired-peak model is failed.

default False

settings.nomodel

label Bypass building the shifting model

type basic:boolean

description While on, MACS will bypass building the shifting model.

hidden settings.tagalign

default False

settings.nomodel prepeak

label Bypass building the shifting model

type basic:boolean

description While on, MACS will bypass building the shifting model.

hidden !settings.tagalign

default True

$settings.down_sample$

label Down-sample

type basic:boolean

description When set, random sampling method will scale down the bigger sample. By default, MACS uses linear scaling. This option will make the results unstable and irreproducible since each time, random reads would be selected, especially the numbers (pileup, pvalue, qvalue) would change. Consider to use 'randsample' script before MACS2 runs instead.

```
default False
```

settings.bedgraph

label Save fragment pileup and control lambda

type basic:boolean

description If this flag is on, MACS will store the fragment pileup, control lambda, -log10pvalue and -log10qvalue scores in bedGraph files. The bedGraph files will be stored in current directory named NAME+'_treat_pileup.bdg' for treatment data, NAME+'_control_lambda.bdg' for local lambda values from control, NAME+'_treat_pvalue.bdg' for Poisson pvalue scores (in -log10(pvalue) form), and NAME+'_treat_qvalue.bdg' for q-value scores from Benjamini-Hochberg-Yekutieli procedure.

default True

settings.spmr

label Save signal per million reads for fragment pileup profiles

type basic:boolean

disabled settings.bedgraph === false

default True

settings.call_summits

label Call summits

type basic:boolean

description MACS will now reanalyze the shape of signal profile (p or q-score depending on cutoff setting) to deconvolve subpeaks within each peak called from general procedure. It's highly recommended to detect adjacent binding events. While used, the output subpeaks of a big peak region will have the same peak boundaries, and different scores and peak summit positions.

default True

settings.broad

label Composite broad regions

type basic:boolean

description When this flag is on, MACS will try to composite broad regions in BED12 (a gene-model-like format) by putting nearby highly enriched regions into a broad region with loose cutoff. The broad region is controlled by another cutoff through –broad-cutoff. The maximum length of broad region length is 4 times of d from MACS.

disabled settings.call summits === true

default False

settings.broad_cutoff

label Broad cutoff

type basic:decimal

description Cutoff for broad region. This option is not available unless –broad is set. If -p is set, this is a p-value cutoff, otherwise, it's a q-value cutoff. DEFAULT = 0.1

required False

disabled settings.call_summits === true || settings.broad !== true

Output results

Abstract alignment process

```
data:alignmentabstract-alignment ()[Source: v1.0.0]
Input arguments
Output results bam
     label Alignment file
     type basic:file
bai
     label Alignment index BAI
     type basic:file
species
     label Species
     type basic:string
build
     label Build
     type basic:string
Abstract annotation process
data:annotationabstract-annotation ()[Source: v1.0.0]
Input arguments
Output results annot
     label Uploaded file
     type basic:file
source
     label Gene ID source
     type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
Abstract bed process
data:bedabstract-bed ()[Source: v1.0.0]
```

```
Input arguments
Output results bed
     label BED
     type basic:file
species
     label Species
     type basic:string
build
     label Build
     type basic:string
Abstract differential expression process
{\tt data:differential expression abstract-differential expression \ () \ [Source: v1.0.0] \\
Input arguments
Output results raw
     label Differential expression (gene level)
     type basic:file
de_json
     label Results table (JSON)
     type basic:json
de_file
     label Results table (file)
     type basic:file
source
     label Gene ID source
     type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
feature_type
     label Feature type
     type basic:string
```

Abstract expression process

```
{\tt data:expressionabstract-expression} \hspace{0.2cm} \hbox{()} \hspace{0.1cm} [Source: v1.0.0] \\
Input arguments
Output results exp
     label Normalized expression
     type basic:file
rc
     label Read counts
     type basic:file
     required False
exp_json
     label Expression (json)
     type basic:json
exp_type
     label Expression type
     type basic:string
source
     label Gene ID source
     type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
feature_type
     label Feature type
     type basic:string
```

Accel Amplicon Pipeline

```
data:workflow:ampliconworkflow-accel (data:reads:fastq:paired
                                                                                               reads.
                                                    data:genome:fasta
                                                                                             genome,
                                                    data:masterfile:amplicon
                                                                                                mas-
                                                    ter file.
                                                                 data:seq:nucleotide
                                                                                            adapters.
                                                    list:data:variants:vcf
                                                                                       known indels,
                                                    list:data:variants:vcf
                                                                                         known_vars,
                                                    data:variants:vcf
                                                                       dbsnp, basic:integer
                                                                                                mbq,
                                                    basic:integer
                                                                           stand_call_conf,
                                                                                                  ba-
                                                    sic:integer
                                                                min_bq, basic:integer min_alt_bq,
                                                                              known vars db,
                                                    list:data:variants:vcf
                                                    sic:decimal af threshold) [Source: v4.0.1]
```

Processing pipeline to analyse the Accel-Amplicon NGS panel data. The raw amplicon sequencing reads are quality trimmed using Trimmomatic. The quality of the raw and trimmed data is assessed using the FASTQC tool. Quality trimmed reads are aligned to a reference genome using BWA mem. Sequencing primers are removed from the aligned reads using Primerclip. Amplicon performance stats are calculated using Bedtools coveragebed and Picard CollectTargetedPcrMetrics programs. Prior to variant calling, the alignment file is preprocessed using the GATK IndelRealigner and BaseRecalibrator tools. GATK HaplotypeCaller and Lofreq tools are used to call germline variants. Called variants are annotated using the SnpEff tool. Finally, the amplicon performance metrics and identified variants data are used to generate the PDF analysis report.

```
Input arguments reads
```

```
label Input reads
     type data:reads:fastq:paired
genome
     label Genome
     type data:genome:fasta
master_file
     label Experiment Master file
     type data:masterfile:amplicon
adapters
     label Adapters
     type data:seq:nucleotide
     description Provide an Illumina sequencing adapters file (.fasta) with adapters to be removed by Trim-
         momatic.
preprocess bam.known indels
     label Known indels
     type list:data:variants:vcf
preprocess_bam.known_vars
     label Known variants
     type list:data:variants:vcf
gatk.dbsnp
```

label dbSNP

```
type data:variants:vcf
gatk.mbq
     label Min Base Quality
     type basic:integer
     description Minimum base quality required to consider a base for calling.
     default 20
gatk.stand_call_conf
     label Min call confidence threshold
     type basic:integer
     description The minimum phred-scaled confidence threshold at which variants should be called.
     default 20
lofreq.min_bq
     label Min baseQ
     type basic:integer
     description Skip any base with baseQ smaller than the default value.
     default 20
lofreq.min_alt_bq
     label Min alternate baseQ
     type basic:integer
     description Skip alternate bases with baseQ smaller than the default value.
     default 20
var_annot.known_vars_db
     label Known variants
     type list:data:variants:vcf
report.af_threshold
     label Allele frequency threshold
     type basic:decimal
     default 0.01
Output results
```

Align (BWA) and trim adapters

```
data:alignment:bam:bwatrimalign-bwa-trim (data:masterfile:amplicon
                                                                                                mas-
                                                         ter_file,
                                                                     data:genome:fasta
                                                                                             genome,
                                                         data:reads:fastq
                                                                                     reads.
                                                                                                  ba-
                                                         sic:integer
                                                                     seed_l, basic:integer
                                                                                            band w.
                                                         basic:decimal re_seeding, basic:boolean m,
                                                         basic:integer match, basic:integer missmatch,
                                                         basic:integer
                                                                        gap_o, basic:integer
                                                         basic:integer
                                                                        clipping, basic:integer
                                                         paired_p, basic:boolean
                                                                                     report_all, ba-
                                                         sic:integer report tr) [Source: v1.2.2]
Align with BWA mem and trim the sam output. The process uses the memory-optimized Primertrim tool.
Input arguments master file
     label Master file
     type data:masterfile:amplicon
     description Amplicon experiment design file that holds the information about the primers to be removed.
genome
     label Reference genome
     type data:genome:fasta
reads
     label Reads
     type data:reads:fastq
seed_l
     label Minimum seed length
     type basic:integer
     description Minimum seed length. Matches shorter than minimum seed length will be missed. The
          alignment speed is usually insensitive to this value unless it significantly deviates 20.
     default 19
band w
     label Band width
     type basic:integer
     description Gaps longer than this will not be found.
     default 100
re_seeding
     label Re-seeding factor
     type basic:decimal
     description Trigger re-seeding for a MEM longer than minSeedLen*FACTOR. This is a key heuristic pa-
          rameter for tuning the performance. Larger value yields fewer seeds, which leads to faster alignment
          speed but lower accuracy.
     default 1.5
```

28

```
m
     label Mark shorter split hits as secondary
     type basic:boolean
     description Mark shorter split hits as secondary (for Picard compatibility)
     default False
scoring.match
     label Score of a match
     type basic:integer
     default 1
scoring.missmatch
     label Mismatch penalty
     type basic:integer
     default 4
scoring.gap_o
     label Gap open penalty
     type basic:integer
     default 6
scoring.gap_e
     label Gap extension penalty
     type basic:integer
     default 1
scoring.clipping
     label Clipping penalty
     type basic:integer
     description Clipping is applied if final alignment score is smaller than (best score reaching the end of
          query) - (Clipping penalty)
     default 5
scoring.unpaired_p
     label Penalty for an unpaired read pair
     type basic:integer
     description Affinity to force pair. Score: scoreRead1+scoreRead2-Penalty
     default 9
reporting.report_all
     label Report all found alignments
     type basic:boolean
     description Output all found alignments for single-end or unpaired paired-end reads. These alignments
          will be flagged as secondary alignments.
```

```
default False
reporting.report_tr
     label Report threshold score
     type basic:integer
     description Don't output alignment with score lower than defined number. This option only affects
          output.
     default 30
Output results bam
     label Alignment file
     type basic:file
     description Position sorted alignment
bai
     label Index BAI
     type basic:file
stats
     label Statistics
     type basic:file
bigwig
     label BigWig file
     type basic:file
     required False
species
     label Species
     type basic:string
build
     label Build
     type basic:string
Amplicon report
data:report:ampliconamplicon-report (data:picard:coverage
                                                                                      pcr_metrics,
                                                 data:coverage
                                                                                         coverage,
                                                                                       master_file,
                                                 data:masterfile:amplicon
                                                 list:data:snpeff
                                                                           annot_vars,
                                                                                               ba-
                                                 sic:decimal af_threshold) [Source: v1.0.4]
Create amplicon report.
Input arguments pcr_metrics
     label Picard TargetedPcrMetrics
     type data:picard:coverage
```

```
coverage
     label Coverage
     type data:coverage
master_file
     label Amplicon master file
     type data:masterfile:amplicon
annot_vars
     label Annotated variants (snpEff)
     type list:data:snpeff
af threshold
     label Allele frequency threshold
     type basic:decimal
     default 0.01
Output results report
     label Report
     type basic:file
panel_name
     label Panel name
     type basic:string
stats
     label File with sample statistics
     type basic:file
amplicon_cov
     label Amplicon coverage file (nomergebed)
     type basic:file
variant_tables
     label Variant tabels (snpEff)
     type list:basic:file
Amplicon table
data:varianttable:ampliconamplicon-table (data:masterfile:amplicon
                                                                                      master_file,
                                                      data:coverage coverage, list:data:snpeff an-
                                                       not_vars, basic:boolean
                                                                                   all_amplicons,
                                                      basic:string table_name) [Source: v1.0.1]
Create variant table for use together with the genome browser.
Input arguments master_file
     label Master file
```

```
type data:masterfile:amplicon
coverage
     label Amplicon coverage
     type data:coverage
annot vars
     label Annotated variants
     type list:data:snpeff
all_amplicons
     label Report all amplicons
     type basic:boolean
     default False
table_name
     label Amplicon table name
     type basic:string
     default Amplicons containing variants
Output results variant table
     label Variant table
     type basic: json
Annotate novel splice junctions (regtools)
data:junctions:regtoolsregtools-junctions-annotate (data:genome:fasta
                                                                                    genome,
                                                                data:annotation:gtf annotation,
                                                                data:alignment:bam:star align-
                                                                ment star.
```

Identify novel splice junctions by using regtools to annotate against a reference. The process accepts reference genome, reference genome annotation (GTF), and input with reads information (STAR alignent or reads aligned by any other aligner or junctions in BED12 format). If STAR aligner data is given as input, the process calculates BED12 file from STAR 'SJ.out.tab' file, and annotates all junctions with 'regtools junctions annotate' command. When reads are aligned by other aligner, junctions are extracted with 'regtools junctions extract' tool and then annotated with 'junction annotate' command. Third option allows user to provide directly BED12 file with junctions, which are then annotated. Finnally, annotated novel junctions are filtered in a separate output file. More information can be found in the [regtools manual](https://regtools.readthedocs.io/en/latest/).

data:alignment:bam

data:bed

put_bed_junctions) [Source:

ment.

v0.2.1]

align-

in-

Input arguments genome

```
label Reference genome
type data:genome:fasta
annotation
```

label Reference genome annotation (GTF)

```
type data:annotation:gtf
alignment star
     label STAR alignment
     type data:alignment:bam:star
     description Splice junctions detected by STAR aligner (SJ.out.tab STAR output file). Please provide one
          input 'STAR alignment' or 'Alignment' by any aligner or directly 'Junctions in BED12 format'.
     required False
alignment
     label Alignment
     type data:alignment:bam
     description Aligned reads from which splice junctions are going to be extracted. Please provide one
          input 'STAR alignment' or 'Alignment' by any aligner or directly 'Junctions in BED12 format'.
     required False
input_bed_junctions
     label Junctions in BED12 format
     type data:bed
     description Splice junctions in BED12 format. Please provide one input 'STAR alignment' or 'Align-
          ment' by any aligner or directly 'Junctions in BED12 format'.
     required False
Output results novel_splice_junctions
     label Table of annotated novel splice junctions
     type basic:file
splice_junctions
     label Table of annotated splice junctions
     type basic:file
novel_sj_bed
     label Novel splice junctions in BED format
     type basic:file
bed
     label Splice junctions in BED format
     type basic:file
novel_sj_bigbed_igv_ucsc
     label Novel splice junctions in BigBed format
     type basic:file
     required False
bigbed igv ucsc
     label Splice junctions in BigBed format
```

```
type basic:file
    required False

novel_sj_tbi_jbrowse
    label Novel splice junctions bed tbi index for JBrowse
    type basic:file

tbi_jbrowse
    label Bed tbi index for JBrowse
    type basic:file

species
    label Species
    type basic:string

build
    label Build
    type basic:string
```

Archive and make multi-sample report for amplicon data

```
data:archive:samples:ampliconamplicon-archive-multi-report (list:data data, list:basic:string fields, basic:boolean j) [Source: v0.2.5]
```

Create an archive of output files. The ouput folder structure is organized by sample slug and data object's output-field names. Additionally, create multi-sample report for selected samples.

Input arguments data

```
label Data list
    type list:data

fields
    label Output file fields
    type list:basic:string

j
    label Junk paths
    type basic:boolean
    description Store just names of saved files (junk the path)
    default False

Output results archive
    label Archive of selected samples and a heatmap comparing them
    type basic:file
```

Archive samples

```
data:archive:samplesarchive-samples (list:data data, list:basic:string fields, basic:boolean j) [Source: v0.2.3]
```

Create an archive of output files. The ouput folder structure is organized by sample slug and data object's output-field names.

```
Input arguments data
```

```
label Data list
    type list:data

fields
    label Output file fields
    type list:basic:string

j
    label Junk paths
    type basic:boolean
    description Store just names of saved files (junk the path)
    default False

Output results archive
    label Archive
    type basic:file
```

BAM file

```
data:alignment:bam:uploadupload-bam (basic:file src, basic:string species, basic:string build) [Source: v1.4.1]
```

Import a BAM file (.bam), which is the binary format for storing sequence alignment data. This format is described on the [SAM Tools web site](http://samtools.github.io/hts-specs/).

Input arguments src

• Mus musculus: Mus musculus

- Rattus norvegicus: Rattus norvegicus
- Dictyostelium discoideum: Dictyostelium discoideum
- Odocoileus virginianus texanus: Odocoileus virginianus texanus
- Solanum tuberosum: Solanum tuberosum

build

```
label Build
```

type basic:string

Output results bam

```
label Uploaded file
```

type basic:file

bai

label Index BAI

type basic:file

stats

label Alignment statistics

type basic:file

bigwig

label BigWig file

type basic:file

required False

species

label Species

type basic:string

build

label Build

type basic:string

BAM file and index

```
data:alignment:bam:uploadupload-bam-indexed (basic:file src, basic:file src2, basic:string species, sic:string build) [Source: v1.4.1]
```

Import a BAM file (.bam) and BAM index (.bam.bai). BAM file is the binary format for storing sequence alignment data. This format is described on the [SAM Tools web site](http://samtools.github.io/hts-specs/).

Input arguments src

```
label Mapping (BAM)
```

type basic:file

description A mapping file in BAM format.

```
validate_regex \.(bam)$
src2
     label bam index (*.bam.bai file)
     type basic:file
     description An index file of a BAM mapping file (ending with bam.bai).
     validate_regex \.(bam.bai)$
species
     label Species
     type basic:string
     description Species latin name.
     choices
            • Homo sapiens: Homo sapiens
            • Mus musculus: Mus musculus
            • Rattus norvegicus: Rattus norvegicus
            • Dictyostelium discoideum: Dictyostelium discoideum
            • Odocoileus virginianus texanus: Odocoileus virginianus texanus
            • Solanum tuberosum: Solanum tuberosum
build
     label Build
     type basic:string
Output results bam
     label Uploaded file
     type basic:file
bai
     label Index BAI
     type basic:file
stats
     label Alignment statistics
     type basic:file
bigwig
     label BigWig file
     type basic:file
     required False
species
     label Species
     type basic:string
```

build

label Build

type basic:string

BBDuk (paired-end)

data:reads:fastq:paired:bbdukbbduk-paired (data:reads:fastq:paired

reads. min_length, babasic:integer sic:boolean show_advanced, list:data:seq:nucleotide sequences, list:basic:string literal_sequences, basic:integer kmer_length, sic:boolean check_reverse_complements, basic:boolean mask_middle_base, basic:integer min_kmer_hits, basic:decimal min kmer fraction, basic:decimal min_coverage_fraction, basic:integer hamming distance, query_hamming_distance, sic:integer basic:integer edit distance, sic:integer hamming distance2, baquery hamming distance2, sic:integer edit distance2, basic:integer basic:boolean forbid N. basic:boolean remove_if_either_bad, basic:boolean find_best_match, sic:boolean perform_error_correction, basic:string **k_trim**, basic:string **k_mask**, basic:boolean mask_fully_covered, sic:integer min_k, basic:string quality_trim, basic:integer trim_quality, trim_poly_A, basic:integer basic:decimal min length fraction, max_length, basic:integer basic:integer min_average_quality, basic:integer min_average_quality_bases, basic:integer min base quality, min_consecutive_bases, sic:integer basic:integer trim pad, basic:boolean trim_by_overlap, basic:boolean strict_overlap, bamin_overlap, sic:integer bamin_insert, sic:integer basic:boolean trim_pairs_evenly, basic:integer force_trim_left, baforce_trim_right, sic:integer basic:integer force_trim_right2, basic:integer force_trim_mod, basic:integer restrict_left, basic:integer restrict right. basic:decimal min GC, basic:decimal max GC, sic:integer maxns, basic:boolean toss junk, basic:boolean chastity_filter, basic:boolean barcode filter, list:data:seq:nucleotide barcode_files, list:basic:string barcode sequences, basic:integer **x min**, basic:integer y min, basic:integer x_max, basic:integer y_max, entropy, basic:integer basic:decimal tropy_window, basic:integer entropy_k,

BBDuk combines the most common data-quality-related trimming, filtering, and masking operations into a single high-performance tool. It is capable of quality-trimming and filtering, adapter-trimming, contaminant-filtering via kmer matching, sequence masking, GC-filtering, length filtering, entropy-filtering, format conversion, histogram generation, subsampling, quality-score recalibration, kmer cardinality estimation, and various other operations in a single pass. See [here](https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide/bbduk-guide/) for more information.

Input arguments reads

```
label Reads
     type data:reads:fastq:paired
min_length
     label Minimum length [minlength=10]
     type basic:integer
     description Reads shorter than the minimum length will be discarded after trimming.
     default 10
show advanced
     label Show advanced parameters
     type basic:boolean
     default False
reference.sequences
     label Sequences [ref]
     type list:data:seq:nucleotide
     description Reference sequences include adapters, contaminants, and degenerate sequences. They can
          be provided in a multi-sequence FASTA file or as a set of literal sequences below.
     required False
reference.literal_sequences
     label Literal sequences [literal]
     type list:basic:string
     description Literal sequences can be specified by inputting them one by one and pressing Enter after
          each sequence.
     required False
     default []
processing.kmer length
     label Kmer length [k=27]
     type basic:integer
     description Kmer length used for finding contaminants. Contaminants shorter than kmer length will not
          be found. Kmer length must be at least 1.
     default 27
```

label Look for reverse complements of kmers in addition to forward kmers [rcomp=t]

1.2. Process catalog

processing.check_reverse_complements

```
type basic:boolean
     default True
processing.mask_middle_base
     label Treat the middle base of a kmer as a wildcard to increase sensitivity in the presence of errors
          [maskmiddle=t]
     type basic:boolean
     default True
processing.min_kmer_hits
     label Minimum number of kmer hits [minkmerhits=1]
     type basic:integer
     description Reads need at least this many matching kmers to be considered as matching the reference.
     default 1
processing.min kmer fraction
     label Minimum kmer fraction [minkmerfraction=0.0]
     type basic:decimal
     description A read needs at least this fraction of its total kmers to hit a reference in order to be considered
          a match. If this and 'Minimum number of kmer hits' are set, the greater is used.
     default 0.0
processing.min_coverage_fraction
     label Minimum coverage fraction [mincovfraction=0.0]
     type basic:decimal
     description A read needs at least this fraction of its total bases to be covered by reference kmers to
          be considered a match. If specified, 'Minimum coverage fraction' overrides 'Minimum number of
          kmer hits' and 'Minimum kmer fraction'.
     default 0.0
processing.hamming distance
     label Maximum Hamming distance for kmers (substitutions only) [hammingdistance=0]
     type basic:integer
     default 0
processing.query hamming distance
     label Hamming distance for query kmers [qhdist=0]
     type basic:integer
     default 0
processing.edit distance
     label Maximum edit distance from reference kmers (substitutions and indels) [editdistance=0]
     type basic:integer
     default 0
```

```
processing.hamming_distance2
     label Hamming distance for short kmers when looking for shorter kmers [hammingdistance2=0]
     type basic:integer
     default 0
processing.query hamming distance2
     label Hamming distance for short query kmers when looking for shorter kmers [qhdist2=0]
     type basic:integer
     default 0
processing.edit_distance2
     label Maximum edit distance from short reference kmers (substitutions and indels) when looking for
          shorter kmers [editdistance2=0]
     type basic:integer
     default 0
processing.forbid N
     label Forbid matching of read kmers containing N [forbidn=f]
     type basic:boolean
     description By default, these will match a reference 'A' if 'Maximum Hamming distance for kmers' > 0
          or 'Maximum edit distance from reference kmers' > 0, to increase sensitivity.
     default False
processing.remove_if_either_bad
     label Remove both sequences of a paired-end read, if either of them is to be removed [removeifei-
          therbad=t]
     type basic:boolean
     default True
processing.find best match
     label If multiple matches, associate read with sequence sharing most kmers [findbestmatch=t]
     type basic:boolean
     default True
processing.perform error correction
     label Perform error correction with BBMerge prior to kmer operations [ecco=f]
     type basic:boolean
     default False
operations.k_trim
     label Trimming protocol to remove bases matching reference kmers from reads [ktrim=f]
     type basic:string
     default f
     choices
```

```
• Don't trim: f
             • Trim to the right: r
             • Trim to the left: 1
operations.k_mask
     label Symbol to replace bases matching reference kmers [kmask=f]
     type basic:string
     description Allows any non-whitespace character other than t or f. Processes short kmers on both ends.
     default f
operations.mask_fully_covered
     label Only mask bases that are fully covered by kmers [maskfullycovered=f]
     type basic:boolean
     default False
operations.min k
     label Look for shorter kmers at read tips down to this length when k-trimming or masking [mink=0]
     type basic:integer
     description -1 means disabled. Enabling this will disable treating the middle base of a kmer as a wildcard
           to increase sensitivity in the presence of errors.
     default -1
operations.quality_trim
     label Trimming protocol to remove bases with quality below the minimum average region quality from
           read ends [qtrim=f]
     type basic:string
     description Performed after looking for kmers. If enabled, set also 'Average quality below which to trim
           region'.
     default f
     choices
             • Trim neither end: f
             • Trim both ends: rl
             • Trim only right end: r
             • Trim only left end: 1
             • Use sliding window: w
operations.trim_quality
     label Average quality below which to trim region [trimq=6]
     type basic:integer
     description Set trimming protocol to enable this parameter.
     disabled operations.quality_trim == 'f'
     default 6
```

```
operations.trim_poly_A
     label Minimum length of poly-A or poly-T tails to trim on either end of reads [trimpolya=0]
     type basic:integer
     default 0
operations.min length fraction
     label Minimum length fraction [mlf=0.0]
     type basic:decimal
     description Reads shorter than this fraction of original length after trimming will be discarded.
     default 0.0
operations.max_length
     label Maximum length [maxlength]
     type basic:integer
     description Reads longer than this after trimming will be discarded.
     required False
operations.min_average_quality
     label Minimum average quality [minavgquality=0]
     type basic:integer
     description Reads with average quality (after trimming) below this will be discarded.
     default 0
operations.min_average_quality_bases
     label Number of initial bases to calculate minimum average quality from [maqb=0]
     type basic:integer
     description Used only if positive.
     default 0
operations.min_base_quality
     label Minimum base quality below which reads are discarded after trimming [minbasequality=0]
     type basic:integer
     default 0
operations.min_consecutive_bases
     label Minimum number of consecutive called bases [mcb=0]
     type basic:integer
     default 0
operations.trim_pad
     label Number of bases to trim around matching kmers [tp=0]
     type basic:integer
     default 0
```

```
operations.trim_by_overlap
     label Trim adapters based on where paired-end reads overlap [tbo=f]
     type basic:boolean
     default False
operations.strict_overlap
     label Adjust sensitivity in 'Trim adapters based on where paired-end reads overlap' mode [strictover-
          lap=t]
     type basic:boolean
     default True
operations.min_overlap
     label Minimum number of overlapping bases [minoverlap=14]
     type basic:integer
     description Require this many bases of overlap for detection.
     default 14
operations.min_insert
     label Minimum insert size [mininsert=40]
     type basic:integer
     description Require insert size of at least this for overlap. Should be reduced to 16 for small RNA
          sequencing.
     default 40
operations.trim_pairs_evenly
     label Trim both sequences of paired-end reads to the minimum length of either sequence [tpe=f]
     type basic:boolean
     default False
operations.force trim left
     label Position from which to trim bases to the left [forcetrimleft=0]
     type basic:integer
     default 0
operations.force_trim_right
     label Position from which to trim bases to the right [forcetrimright=0]
     type basic:integer
     default 0
operations.force_trim_right2
     label Number of bases to trim from the right end [forcetrimright2=0]
     type basic:integer
     default 0
operations.force trim mod
```

```
label Modulo to right-trim reads [forcetrimmod=0]
     type basic:integer
     description Trim reads to the largest multiple of modulo.
     default 0
operations.restrict left
     label Number of leftmost bases to look in for kmer matches [restrictleft=0]
     type basic:integer
     default 0
operations.restrict_right
     label Number of rightmosot bases to look in for kmer matches [restrictright=0]
     type basic:integer
     default 0
operations.min GC
     label Minimum GC content [mingc=0.0]
     type basic:decimal
     description Discard reads with lower GC content.
     default 0.0
operations.max_GC
     label Maximum GC content [maxgc=1.0]
     type basic:decimal
     description Discard reads with higher GC content.
     default 1.0
operations.maxns
     label Max Ns after trimming [maxns=-1]
     type basic:integer
     description If non-negative, reads with more Ns than this (after trimming) will be discarded.
     default -1
operations.toss junk
     label Discard reads with invalid characters as bases [tossjunk=f]
     type basic:boolean
     default False
header_parsing.chastity_filter
     label Discard reads that fail Illumina chastity filtering [chastityfilter=f]
     type basic:boolean
     description Discard reads with id containing '1:Y:' or '2:Y:'.
     default False
```

```
header_parsing.barcode_filter
     label Remove reads with unexpected barcodes if barcodes are set, or barcodes containing 'N' otherwise
          [barcodefilter=f]
     type basic:boolean
     description A barcode must be the last part of the read header.
     default False
header_parsing.barcode_files
     label Barcode sequences [barcodes]
     type list:data:seq:nucleotide
     required False
header_parsing.barcode_sequences
     label Literal barcode sequences [barcodes]
     type list:basic:string
     description Literal barcode sequences can be specified by inputting them one by one and pressing Enter
          after each sequence.
     required False
     default []
header_parsing.x_min
     label Minimum X coordinate [xmin=-1]
     type basic:integer
     description If positive, discard reads with a smaller X coordinate.
     default -1
header_parsing.y_min
     label Minimum Y coordinate [ymin=-1]
     type basic:integer
     description If positive, discard reads with a smaller Y coordinate.
     default -1
header parsing.x max
     label Maximum X coordinate [xmax=-1]
     type basic:integer
     description If positive, discard reads with a larger X coordinate.
     default -1
header_parsing.y_max
     label Maximum Y coordinate [ymax=-1]
     type basic:integer
     description If positive, discard reads with a larger Y coordinate.
     default -1
```

```
complexity.entropy
     label Minimum entropy [entropy=-1.0]
     type basic:decimal
     description Set between 0 and 1 to filter reads with entropy below that value. Higher is more stringent.
     default -1.0
complexity.entropy window
     label Length of sliding window used to calculate entropy [entropywindow=50]
     type basic:integer
     description To use the sliding window set minimum entropy in range between 0.0 and 1.0.
     default 50
complexity.entropy_k
     label Length of kmers used to calcuate entropy [entropyk=5]
     type basic:integer
     default 5
complexity.entropy_mask
     label Mask low-entropy parts of sequences with N instead of discarding [entropymask=f]
     type basic:boolean
     default False
complexity.min_base_frequency
     label Minimum base frequency [minbasefrequency=0]
     type basic:integer
     default 0
fastqc.nogroup
     label Disable grouping of bases for reads >50bp [nogroup]
     type basic:boolean
     description All reports will show data for every base in the read. Using this option will cause fastqc to
          crash and burn if you use it on really long reads.
     default False
Output results fastq
     label Remaining upstream reads
     type list:basic:file
fastq2
     label Remaining downstream reads
     type list:basic:file
statistics
     label Statistics
```

```
type list:basic:file
fastqc_url
    label Upstream quality control with FastQC
    type list:basic:file:html
fastqc_url2
    label Downstream quality control with FastQC
    type list:basic:file:html
fastqc_archive
    label Download upstream FastQC archive
    type list:basic:file
fastqc_archive2
    label Download downstream FastQC archive
    type list:basic:file
```

BBDuk (single-end)

data:reads:fastq:single:bbdukbbduk-single

(data:reads:fastq:single reads. basic:integer min_length, basic:boolean show_advanced, sequences. list:data:seq:nucleotide list:basic:string literal sequences. basic:integer kmer_length, sic:boolean check_reverse_complements, basic:boolean mask_middle_base, basic:integer min_kmer_hits, basic:decimal min kmer fraction, basic:decimal min_coverage_fraction, basic:integer hamming distance, query_hamming_distance, sic:integer basic:integer edit distance, basic:integer hamming distance2, basic:integer query hamming distance2, edit distance2, basic:integer basic:boolean forbid N. basic:boolean find_best_match, ba**k_trim**, basic:string k mask, sic:string basic:boolean mask_fully_covered, basic:integer min_k, basic:string quality_trim, basic:integer trim_quality, basic:integer trim_poly_A, bamin_length_fraction, sic:decimal basic:integer max_length, basic:integer min average quality, basic:integer min_average_quality_bases, basic:integer min base quality, sic:integer min_consecutive_bases, basic:integer trim pad, basic:integer min_overlap, basic:integer min insert, basic:integer force trim left, basic:integer force_trim_right, baforce_trim_right2, basic:integer force_trim_mod, sic:integer basic:integer restrict_left, basic:integer restrict right, basic:decimal min GC. basic:decimal max_GC, basic:integer maxns, basic:boolean toss_junk, basic:boolean chastity_filter, basic:boolean barcode filter. list:data:seq:nucleotide barcode files, list:basic:string barcode sequences, basic:integer **x min**, basic:integer basic:integer x_max, basic:integer y_max, basic:decimal entropy, basic:integer tropy_window, basic:integer entropy_k, basic:boolean entropy mask, basic:integer min base frequency, basic:boolean nogroup) [Source: v2.2.2]

BBDuk combines the most common data-quality-related trimming, filtering, and masking operations into a single high-performance tool. It is capable of quality-trimming and filtering, adapter-trimming, contaminant-filtering via kmer matching, sequence masking, GC-filtering, length filtering, entropy-filtering, format conversion, histogram generation, subsampling, quality-score recalibration, kmer cardinality estimation, and various other operations in a single pass. See [here](https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide/bbduk-guide/) for more information.

```
Input arguments reads
```

```
label Reads
     type data:reads:fastq:single
min_length
     label Minimum length [minlength=10]
     type basic:integer
     description Reads shorter than the minimum length will be discarded after trimming.
     default 10
show advanced
     label Show advanced parameters
     type basic:boolean
     default False
reference.sequences
     label Sequences [ref]
     type list:data:seq:nucleotide
     description Reference sequences include adapters, contaminants, and degenerate sequences. They can
          be provided in a multi-sequence FASTA file or as a set of literal sequences below.
     required False
reference.literal_sequences
     label Literal sequences [literal]
     type list:basic:string
     description Literal sequences can be specified by inputting them one by one and pressing Enter after
          each sequence.
     required False
     default []
processing.kmer length
     label Kmer length [k=27]
     type basic:integer
     description Kmer length used for finding contaminants. Contaminants shorter than Kmer length will not
          be found. Kmer length must be at least 1.
     default 27
processing.check_reverse_complements
```

label Look for reverse complements of kmers in addition to forward kmers [rcomp=t]

```
type basic:boolean
     default True
processing.mask_middle_base
     label Treat the middle base of a kmer as a wildcard to increase sensitivity in the presence of errors
          [maskmiddle=t]
     type basic:boolean
     default True
processing.min_kmer_hits
     label Minimum number of kmer hits [minkmerhits=1]
     type basic:integer
     description Reads need at least this many matching kmers to be considered matching the reference.
     default 1
processing.min kmer fraction
     label Minimum kmer fraction [minkmerfraction=0.0]
     type basic:decimal
     description A read needs at least this fraction of its total kmers to hit a reference in order to be considered
          a match. If this and 'Minimum number of kmer hits' are set, the greater is used.
     default 0.0
processing.min_coverage_fraction
     label Minimum coverage fraction [mincovfraction=0.0]
     type basic:decimal
     description A read needs at least this fraction of its total bases to be covered by reference kmers to
          be considered a match. If specified, 'Minimum coverage fraction' overrides 'Minimum number of
          kmer hits' and 'Minimum kmer fraction'.
     default 0.0
processing.hamming distance
     label Maximum Hamming distance for kmers (substitutions only) [hammingdistance=0]
     type basic:integer
     default 0
processing.query hamming distance
     label Hamming distance for query kmers [qhdist=0]
     type basic:integer
     default 0
processing.edit distance
     label Maximum edit distance from reference kmers (substitutions and indels) [editdistance=0]
     type basic:integer
     default 0
```

```
processing.hamming_distance2
     label Hamming distance for short kmers when looking for shorter kmers [hammingdistance2=0]
     type basic:integer
     default 0
processing.query hamming distance2
     label Hamming distance for short query kmers when looking for shorter kmers [qhdist2=0]
     type basic:integer
     default 0
processing.edit_distance2
     label Maximum edit distance from short reference kmers (substitutions and indels) when looking for
          shorter kmers [editdistance2=0]
     type basic:integer
     default 0
processing.forbid N
     label Forbid matching of read kmers containing N [forbidn=f]
     type basic:boolean
     description By default, these will match a reference 'A' if 'Maximum Hamming distance for kmers' > 0
          or 'Maximum edit distance from reference kmers' > 0, to increase sensitivity.
     default False
processing.find_best_match
     label If multiple matches, associate read with sequence sharing most kmers [findbestmatch=f]
     type basic:boolean
     default True
operations.k_trim
     label Trimming protocol to remove bases matching reference kmers from reads [ktrim=f]
     type basic:string
     default f
     choices
             • Don't trim: f
             • Trim to the right: r
             • Trim to the left: 1
operations.k_mask
     label Symbol to replace bases matching reference kmers [kmask=f]
     type basic:string
     description Allows any non-whitespace character other than t or f. Processes short kmers on both ends.
     default f
operations.mask fully covered
```

```
label Only mask bases that are fully covered by kmers [maskfullycovered=f]
     type basic:boolean
     default False
operations.min_k
     label Look for shorter kmers at read tips down to this length when k-trimming or masking [mink=0]
     type basic:integer
     description -1 means disabled. Enabling this will disable treating the middle base of a kmer as a wildcard
           to increase sensitivity in the presence of errors.
     default -1
operations.quality_trim
     label Trimming protocol to remove bases with quality below the minimum average region quality from
           read ends [qtrim=f]
     type basic:string
     description Performed after looking for kmers. If enabled, set also 'Average quality below which to trim
           region'.
     default f
     choices
             • Trim neither end: f
             • Trim both ends: rl
             • Trim only right end: r
             • Trim only left end: 1
             • Use sliding window: w
operations.trim_quality
     label Average quality below which to trim region [trimq=6]
     type basic:integer
     description Set trimming protocol to enable this parameter.
     disabled operations.quality_trim == 'f'
     default 6
operations.trim poly A
     label Minimum length of poly-A or poly-T tails to trim on either end of reads [trimpolya=0]
     type basic:integer
     default 0
operations.min_length_fraction
     label Minimum length fraction [mlf=0]
     type basic:decimal
     description Reads shorter than this fraction of original length after trimming will be discarded.
     default 0.0
```

```
operations.max_length
     label Maximum length [maxlength]
     type basic:integer
     description Reads longer than this after trimming will be discarded.
     required False
operations.min_average_quality
     label Minimum average quality [minavgquality=0]
     type basic:integer
     description Reads with average quality (after trimming) below this will be discarded.
     default 0
operations.min_average_quality_bases
     label Number of initial bases to calculate minimum average quality from [maqb=0]
     type basic:integer
     description Used only if positive.
     default 0
operations.min base quality
     label Minimum base quality below which reads are discarded after trimming [minbasequality=0]
     type basic:integer
     default 0
operations.min_consecutive_bases
     label Minimum number of consecutive called bases [mcb=0]
     type basic:integer
     default 0
operations.trim_pad
     label Number of bases to trim around matching kmers [tp=0]
     type basic:integer
     default 0
operations.min overlap
     label Minimum number of overlapping bases [minoverlap=14]
     type basic:integer
     description Require this many bases of overlap for detection.
     default 14
operations.min_insert
     label Minimum insert size [mininsert=40]
     type basic:integer
```

```
description Require insert size of at least this for overlap. Should be reduced to 16 for small RNA
          sequencing.
     default 40
operations.force_trim_left
     label Position from which to trim bases to the left [forcetrimleft=0]
     type basic:integer
     default 0
operations.force_trim_right
     label Position from which to trim bases to the right [forcetrimright=0]
     type basic:integer
     default 0
operations.force_trim_right2
     label Number of bases to trim from the right end [forcetrimright2=0]
     type basic:integer
     default 0
operations.force_trim_mod
     label Modulo to right-trim reads [forcetrimmod=0]
     type basic:integer
     description Trim reads to the largest multiple of modulo.
     default 0
operations.restrict left
     label Number of leftmost bases to look in for kmer matches [restrictleft=0]
     type basic:integer
     default 0
operations.restrict_right
     label Number of rightmosot bases to look in for kmer matches [restricright=0]
     type basic:integer
     default 0
operations.min GC
     label Minimum GC content [mingc=0.0]
     type basic:decimal
     description Discard reads with lower GC content.
     default 0.0
operations.max_GC
     label Maximum GC content [maxgc=1.0]
     type basic:decimal
```

```
description Discard reads with higher GC content.
     default 1.0
operations.maxns
     label Max Ns after trimming [maxns=-1]
     type basic:integer
     description If non-negative, reads with more Ns than this (after trimming) will be discarded.
     default -1
operations.toss_junk
     label Discard reads with invalid characters as bases [tossjunk=f]
     type basic:boolean
     default False
header_parsing.chastity_filter
     label Discard reads that fail Illumina chastity filtering [chastityfilter=f]
     type basic:boolean
     description Discard reads with id containing '1:Y:' or '2:Y:'.
     default False
header_parsing.barcode_filter
     label Remove reads with unexpected barcodes if barcodes are set, or barcodes containing 'N' otherwise
          [barcodefilter=f]
     type basic:boolean
     description A barcode must be the last part of the read header.
     default False
header_parsing.barcode_files
     label Barcode sequences [barcodes]
     type list:data:seq:nucleotide
     required False
header_parsing.barcode_sequences
     label Literal barcode sequences [barcodes]
     type list:basic:string
     description Literal barcode sequences can be specified by inputting them one by one and pressing Enter
          after each sequence.
     required False
     default []
header_parsing.x_min
     label Minimum X coordinate [xmin=-1]
     type basic:integer
     description If positive, discard reads with a smaller X coordinate.
```

```
default -1
header_parsing.y_min
     label Minimum Y coordinate [ymin=-1]
     type basic:integer
     description If positive, discard reads with a smaller Y coordinate.
     default -1
header_parsing.x_max
     label Maximum X coordinate [xmax=-1]
     type basic:integer
     description If positive, discard reads with a larger X coordinate.
     default -1
header_parsing.y_max
     label Maximum Y coordinate [ymax=-1]
     type basic:integer
     description If positive, discard reads with a larger Y coordinate.
     default -1
complexity.entropy
     label Minimum entropy [entropy=-1]
     type basic:decimal
     description Set between 0 and 1 to filter reads with entropy below that value. Higher is more stringent.
     default -1.0
complexity.entropy_window
     label Length of sliding window used to calculate entropy [entropywindow=50]
     type basic:integer
     description To use the sliding window set minimum entropy in range between 0.0 and 1.0.
     default 50
complexity.entropy_k
     label Length of kmers used to calcuate entropy [entropyk=5]
     type basic:integer
     default 5
complexity.entropy_mask
     label Mask low-entropy parts of sequences with N instead of discarding [entropymask=f]
     type basic:boolean
     default False
complexity.min_base_frequency
     label Minimum base frequency [minbasefrequency=0]
```

```
type basic:integer
     default 0
fastqc.nogroup
     label Disable grouping of bases for reads >50bp [nogroup]
     type basic:boolean
     description All reports will show data for every base in the read. Using this option will cause fastqc to
          crash and burn if you use it on really long reads.
     default False
Output results fastq
     label Remaining reads
     type list:basic:file
statistics
     label Statistics
     type list:basic:file
fastqc_url
     label Quality control with FastQC
     type list:basic:file:html
fastqc_archive
     label Download FastQC archive
     type list:basic:file
```

BBDuk - STAR - FeatureCounts (3' mRNA-Seq, paired-end)

data:workflow:quant:featurecounts:pairedworkflow-bbduk-star-fc-quant-paired (data:reads:fastq:pa

(data:reads:fastq:pa data:genomeindex:s list:data:seq:nucleo data:annotation an

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sic:string stranded

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sic:integer seed,

sic:decimal frac-

tion,

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sic:boolean two_pa data:genomeindex:s data:genomeindex:s

v1.1.0]

This 3' mRNA-Seq pipeline is comprised of QC, preprocessing, alignment and quantification steps.

Reads are preprocessed by __BBDuk__ which removes adapters, trims reads for quality from the 3'-end, and discards reads that are too short after trimming. Preprocessed reads are aligned by __STAR__ aligner. For read-count quantification, the __FeatureCounts__ tool is used.

QC steps include downsampling, QoRTs QC analysis and alignment of input reads to the rRNA/globin reference sequences. The reported alignment rate is used to asses the rRNA/globin sequence depletion rate.

```
Input arguments reads
```

```
label Paired-end reads
    type data:reads:fastq:paired
star_index
label Star index
    type data:genomeindex:star
    description Genome index prepared by STAR aligner indexing tool.
```

adapters

```
label Adapters
type list:data:seq:nucleotide
description Provide a list of sequencing adapters files (.fasta) to be removed by BBDuk.
```

annotation

```
label Annotation
```

required False

type data:annotation

stranded

label Select the type of kit used for library preparation.

```
type basic:string
```

choices

- Strand-specific forward: forward
- Strand-specific reverse: reverse

downsampling.n_reads

```
label Number of reads
type basic:integer
default 1000000
```

downsampling.advanced.seed

```
label Seed
type basic:integer
default 11
```

downsampling.advanced.fraction

label Fraction

```
type basic:decimal
```

description Use the fraction of reads in range [0.0, 1.0] from the original input file instead of the absolute number of reads. If set, this will override the "Number of reads" input parameter.

required False

downsampling.advanced.two_pass

```
label 2-pass mode
```

type basic:boolean

description Enable two-pass mode when down-sampling. Two-pass mode is twice as slow but with much reduced memory.

default False

qc.rrna_reference

label Indexed rRNA reference sequence

type data:genomeindex:star

description Reference sequence index prepared by STAR aligner indexing tool.

qc.globin_reference

label Indexed Globin reference sequence

type data:genomeindex:star

description Reference sequence index prepared by STAR aligner indexing tool.

Output results

BBDuk - STAR - FeatureCounts (3' mRNA-Seq, single-end)

data:workflow:quant:featurecounts:singleworkflow-bbduk-star-fc-quant-single (data:reads:fastq:sin

data:genomeindex:s list:data:seq:nucleo data:annotation an

nota-

tion.

sic:string stranded

sic:integer n_reads

sic:integer seed,

sic:decimal frac-

tion,

ba-

sic:boolean two_pa data:genomeindex:s data:genomeindex:s

v1.1.0]

This 3' mRNA-Seq pipeline is comprised of QC, preprocessing, alignment and quantification steps.

Reads are preprocessed by __BBDuk__ which removes adapters, trims reads for quality from the 3'-end, and discards reads that are too short after trimming. Preprocessed reads are aligned by __STAR__ aligner. For read-count quantification, the __FeatureCounts__ tool is used.

QC steps include downsampling, QoRTs QC analysis and alignment of input reads to the rRNA/globin reference sequences. The reported alignment rate is used to asses the rRNA/globin sequence depletion rate.

```
Input arguments reads
```

```
label Input single-end reads
    type data:reads:fastq:single
star_index
    label Star index
    type data:genomeindex:star
```

description Genome index prepared by STAR aligner indexing tool.

adapters

```
label Adapters
type list:data:seq:nucleotide
description Provide a list of sequencing adapters files (.fasta) to be removed by BBDuk.
required False
```

annotation

```
label Annotation
type data:annotation
```

stranded

label Select the type of kit used for library preparation.

```
type basic:string
```

choices

- Strand-specific forward: forward
- Strand-specific reverse: reverse

$downsampling.n_reads$

```
label Number of reads
type basic:integer
default 1000000
```

downsampling.advanced.seed

```
label Seed
type basic:integer
default 11
```

downsampling.advanced.fraction

```
label Fraction
type basic:decimal
```

description Use the fraction of reads in range [0.0, 1.0] from the original input file instead of the absolute number of reads. If set, this will override the "Number of reads" input parameter.

required False

downsampling.advanced.two_pass

label 2-pass mode

type basic:boolean

description Enable two-pass mode when down-sampling. Two-pass mode is twice as slow but with much reduced memory.

default False

qc.rrna_reference

label Indexed rRNA reference sequence

type data:genomeindex:star

description Reference sequence index prepared by STAR aligner indexing tool.

qc.globin_reference

label Indexed Globin reference sequence

type data:genomeindex:star

description Reference sequence index prepared by STAR aligner indexing tool.

Output results

BBDuk - STAR - HTSeq-count (paired-end)

data:workflow:rnaseq:htseq:pairedworkflow-bbduk-star-htseq-paired (data:reads:fastq:paired reads,

(data:reads:fastq:paired reads, data:genomeindex:star star_index list:data:seq:nucleotide adapters, data:annotation an-

notation,

ba-

sic:string stranded) [Source: v1.0.1]

This RNA-seq pipeline is comprised of three steps, preprocessing, alignment, and quantification.

First, reads are preprocessed by _BBDuk_ which removes adapters, trims reads for quality from the 3'-end, and discards reads that are too short after trimming. Compared to similar tools, BBDuk is regarded for its computational efficiency. Next, preprocessed reads are aligned by _STAR_ aligner. At the time of implementation, STAR is considered a state-of-the-art tool that consistently produces accurate results from diverse sets of reads, and performs well even with default settings. For more information see [this comparison of RNA-seq aligners](https://www.nature.com/articles/nmeth.4106). Finally, aligned reads are summarized to genes by _HTSeq-count_. Compared to featureCounts, HTSeq-count is not as computationally efficient. All three tools in this workflow support parallelization to accelerate the analysis.

Input arguments reads

```
label Paired-end reads
```

type data:reads:fastq:paired

star index

```
label Star index
     type data:genomeindex:star
     description Genome index prepared by STAR aligner indexing tool.
adapters
     label Adapters
     type list:data:seq:nucleotide
     description Provide a list of sequencing adapters files (.fasta) to be removed by BBDuk.
     required False
annotation
     label Annotation
     type data:annotation
stranded
     label Select the QuantSeq kit used for library preparation.
     type basic:string
     choices
            • QuantSeq FWD: yes
            • QuantSeq REV: reverse
Output results
BBDuk - STAR - HTSeq-count (single-end)
```

```
data:workflow:rnaseq:htseq:singleworkflow-bbduk-star-htseq (data:reads:fastq:single reads,
                                                                              data:genomeindex:star star_index,
                                                                              list:data:seq:nucleotide adapters,
                                                                              data:annotation an-
                                                                              notation,
                                                                                              ba-
                                                                              sic:string stranded) [Source:
                                                                              v1.0.1]
```

This RNA-seq pipeline is comprised of three steps, preprocessing, alignment, and quantification.

First, reads are preprocessed by __BBDuk__ which removes adapters, trims reads for quality from the 3'-end, and discards reads that are too short after trimming. Compared to similar tools, BBDuk is regarded for its computational efficiency. Next, preprocessed reads are aligned by __STAR__ aligner. At the time of implementation, STAR is considered a state-of-the-art tool that consistently produces accurate results from diverse sets of reads, and performs well even with default settings. For more information see [this comparison of RNA-seq aligners](https://www.nature.com/articles/nmeth.4106). Finally, aligned reads are summarized to genes by __HTSeq-support parallelization to accelerate the analysis.

Input arguments reads

```
label Input single-end reads
     type data:reads:fastq:single
star index
```

```
label Star index
     type data:genomeindex:star
     description Genome index prepared by STAR aligner indexing tool.
adapters
     label Adapters
     type list:data:seq:nucleotide
     description Provide a list of sequencing adapters files (.fasta) to be removed by BBDuk.
     required False
annotation
     label annotation
     type data:annotation
stranded
     label Select the QuantSeq kit used for library preparation.
     type basic:string
     choices
            • QuantSeq FWD: yes
            • QuantSeq REV: reverse
```

Output results

BBDuk - STAR - featureCounts - QC (paired-end)

 $\verb|data:workflow:rnaseq:featurecounts:qcworkflow-bbduk-star-featurecounts-qc-paired| (\textit{data:reads:featurecounts-qc-paired}) | (\textit{data:reads:featurecounts-$

list:data:seq

ba-

sic:boolean

list:basic:str

tom_adapte

ba-

sic:integer k ba-

sic:integer 1

ba-

sic:integer l

ming_distar

ba-

sic:integer 1

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sic:integer 1 data:genome

ba-

sic:boolean ba-

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This RNA-seq pipeline is comprised of three steps preprocessing, alignment, and quantification.

First, reads are preprocessed by __BBDuk__ which removes adapters, trims reads for quality from the 3'-end, and discards reads that are too short after trimming. Compared to similar tools, BBDuk is regarded for its computational efficiency. Next, preprocessed reads are aligned by __STAR__ aligner. At the time of implementation, STAR is considered a state-of-the-art tool that consistently produces accurate results from diverse sets of reads, and performs well even with default settings. For more information see [this comparison of RNA-seq aligners](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5792058/). Finally, aligned reads are summarized to genes by featureCounts . Gaining wide adoption among the bioinformatics community, featureCounts yields expressions in a computationally efficient manner. All three tools in this workflow support parallelization to accelerate the analysis.

rRNA contamination rate in the sample is determined using the STAR aligner. Quality-trimmed reads are downsampled (using Seqtk tool) and aligned to the rRNA reference sequences. The alignment rate indicates the percentage of the reads in the sample that are derived from the rRNA sequences.

```
Input arguments preprocessing.reads
     label Reads
     type data:reads:fastq:paired
preprocessing.adapters
     label Adapters
     type list:data:seq:nucleotide
     required False
preprocessing.show advanced
     label Show advanced parameters
     type basic:boolean
     default False
preprocessing.custom_adapter_sequences
     label Custom adapter sequences [literal]
     type list:basic:string
     description Custom adapter sequences can be specified by inputting them one by one and pressing Enter
          after each sequence.
     required False
     hidden !preprocessing.show_advanced
     default []
preprocessing.kmer length
     label K-mer length
     type basic:integer
     description K-mer length must be smaller or equal to the length of adapters.
     hidden !preprocessing.show_advanced
     default 23
preprocessing.min_k
```

label Minimum k-mer length at right end of reads used for trimming

```
type basic:integer
     disabled preprocessing.adapters.length === 0 && preprocessing.custom_adapter_sequences.length ===
     hidden !preprocessing.show_advanced
     default 11
preprocessing.hamming_distance
     label Maximum Hamming distance for k-mers
     type basic:integer
     hidden !preprocessing.show_advanced
     default 1
preprocessing.maxns
     label Max Ns after trimming [maxns=-1]
     type basic:integer
     description If non-negative, reads with more Ns than this (after trimming) will be discarded.
     hidden !preprocessing.show_advanced
     default -1
preprocessing.trim_quality
     label Quality below which to trim reads from the right end
     type basic:integer
     description Phred algorithm is used, which is more accurate than naive trimming.
     hidden !preprocessing.show_advanced
     default 10
preprocessing.min_length
     label Minimum read length
     type basic:integer
     description Reads shorter than minimum read length after trimming are discarded.
     hidden !preprocessing.show_advanced
     default 20
alignment.genome
     label Indexed reference genome
     type data:genomeindex:star
     description Genome index prepared by STAR aligner indexing tool.
alignment.show_advanced
     label Show advanced parameters
     type basic:boolean
     default False
```

alignment.unstranded

label The data is unstranded

type basic:boolean

description For unstranded RNA-seq data, Cufflinks/Cuffdiff require spliced alignments with XS strand attribute, which STAR will generate with –outSAMstrandField intronMotif option. As required, the XS strand attribute will be generated for all alignments that contain splice junctions. The spliced alignments that have undefined strand (i.e. containing only non-canonical unannotated junctions) will be suppressed. If you have stranded RNA-seq data, you do not need to use any specific STAR options. Instead, you need to run Cufflinks with the library option –library-type options. For example, c ufflinks –library-type fr-firststrand should be used for the standard dUTP protocol, including Illumina's stranded Tru-Seq. This option has to be used only for Cufflinks runs and not for STAR runs.

hidden !alignment.show_advanced

default False

alignment.noncannonical

label Remove non-cannonical junctions (Cufflinks compatibility)

type basic:boolean

description It is recommended to remove the non-canonical junctions for Cufflinks runs using –outFilterIntronMotifs RemoveNoncanonical.

hidden !alignment.show_advanced

default False

alignment.detect_chimeric.chimeric

label Detect chimeric and circular alignments

type basic:boolean

description To switch on detection of chimeric (fusion) alignments (in addition to normal mapping), –chimSegmentMin should be set to a positive value. Each chimeric alignment consists of two "segments". Each segment is non-chimeric on its own, but the segments are chimeric to each other (i.e. the segments belong to different chromosomes, or different strands, or are far from each other). Both segments may contain splice junctions, and one of the segments may contain portions of both mates. –chimSegmentMin parameter controls the minimum mapped length of the two segments that is allowed. For example, if you have 2x75 reads and used –chimSegmentMin 20, a chimeric alignment with 130b on one chromosome and 20b on the other will be output, while 135 + 15 won't be.

default False

alignment.detect_chimeric.chimSegmentMin

label -chimSegmentMin

type basic:integer

disabled detect chimeric.chimeric!= true

default 20

alignment.t_coordinates.quantmode

label Output in transcript coordinates

type basic:boolean

description With —quantMode TranscriptomeSAM option STAR will output alignments translated into transcript coordinates in the Aligned.toTranscriptome.out.bam file (in addition to alignments in genomic coordinates in Aligned.*.sam/bam files). These transcriptomic alignments can be used with various transcript quantification software that require reads to be mapped to transcriptome, such as RSEM or eXpress.

default False

alignment.t coordinates.singleend

label Allow soft-clipping and indels

type basic:boolean

description By default, the output satisfies RSEM requirements: soft-clipping or indels are not allowed. Use –quantTranscriptomeBan Singleend to allow insertions, deletions ans soft-clips in the transcriptomic alignments, which can be used by some expression quantification software (e.g. eXpress).

disabled t_coordinates.quantmode != true

default False

alignment.t_coordinates.gene_counts

label Count reads

type basic:boolean

description With –quantMode GeneCounts option STAR will count number reads per gene while mapping. A read is counted if it overlaps (1nt or more) one and only one gene. Both ends of the paired-end read are checked for overlaps. The counts coincide with those produced by htseq-count with default parameters. ReadsPerGene.out.tab file with 4 columns which correspond to different strandedness options: column 1: gene ID; column 2: counts for unstranded RNA-seq; column 3: counts for the 1st read strand aligned with RNA (htseq-count option -s yes); column 4: counts for the 2nd read strand aligned with RNA (htseq-count option -s reverse).

disabled t_coordinates.quantmode != true

default False

alignment.filtering.outFilterType

label Type of filtering

type basic:string

description Normal: standard filtering using only current alignment; BySJout: keep only those reads that contain junctions that passed filtering into SJ.out.tab

default Normal

choices

Normal: NormalBySJout: BySJout

alignment.filtering.outFilterMultimapNmax

label -outFilterMultimapNmax

type basic:integer

description Read alignments will be output only if the read maps fewer than this value, otherwise no alignments will be output (default: 10).

required False

alignment.filtering.outFilterMismatchNmax

```
label -outFilterMismatchNmax
```

type basic:integer

description Alignment will be output only if it has fewer mismatches than this value (default: 10).

required False

alignment.filtering.outFilterMismatchNoverLmax

```
label -outFilterMismatchNoverLmax
```

type basic:decimal

description Max number of mismatches per pair relative to read length: for 2x100b, max number of mismatches is 0.06*200=8 for the paired read.

required False

alignment.filtering.outFilterScoreMin

```
label -outFilterScoreMin
```

type basic:integer

description Alignment will be output only if its score is higher than or equal to this value (default: 0).

required False

alignment.alignment.alignSJoverhangMin

```
label -alignSJoverhangMin
```

type basic:integer

description Minimum overhang (i.e. block size) for spliced alignments (default: 5).

required False

alignment.alignSJDBoverhangMin

label -alignSJDBoverhangMin

type basic:integer

description Minimum overhang (i.e. block size) for annotated (sjdb) spliced alignments (default: 3).

required False

alignment.alignment.alignIntronMin

label -alignIntronMin

type basic:integer

description Minimum intron size: genomic gap is considered intron if its length >= alignIntronMin, otherwise it is considered Deletion (default: 21).

required False

alignment.alignment.alignIntronMax

label -alignIntronMax

type basic:integer

description Maximum intron size, if 0, max intron size will be determined by (2pow(winBinNbits)*winAnchorDistNbins) (default: 0).

required False alignment.alignMatesGapMax label -alignMatesGapMax type basic:integer description Maximum gap between two mates, if 0, max intron gap will be determined by (2pow(winBinNbits)*winAnchorDistNbins) (default: 0). required False alignment.alignment.alignEndsType label -alignEndsType type basic:string description Type of read ends alignment (default: Local). required False default Local choices • Local: Local • EndToEnd: EndToEnd • Extend5pOfRead1: Extend5pOfRead1 • Extend5pOfReads12: Extend5pOfReads12 alignment.output_sam_bam.outSAMunmapped label -outSAMunmapped type basic:string description Output of unmapped reads in the SAM format. required False default None choices • None: None • Within: Within alignment.output sam bam.outSAMattributes label -outSAMattributes type basic:string description a string of desired SAM attributes, in the order desired for the output SAM. required False

default Standard

choices

• None: None

• Standard: Standard

• All: All

alignment.output sam bam.outSAMattrRGline

```
label -outSAMattrRGline
type basic:string
```

description SAM/BAM read group line. The first word contains the read group identifier and must start with "ID:", e.g. –outSAMattrRGline ID:xxx CN:yy "DS:z z z"

required False

quantification.annotation

label Annotation

type data:annotation

quantification.show_advanced

label Show advanced parameters

type basic:boolean

default False

quantification.assay_type

label Assay type

type basic:string

description In strand non-specific assay a read is considered overlapping with a feature regardless of whether it is mapped to the same or the opposite strand as the feature. In strand-specific forward assay and single reads, the read has to be mapped to the same strand as the feature. For paired-end reads, the first read has to be on the same strand and the second read on the opposite strand. In strand-specific reverse assay these rules are reversed.

hidden !quantification.show_advanced

default non_specific

choices

• Strand non-specific: non_specific

• Strand-specific forward: forward

• Strand-specific reverse: reverse

• Detect automatically: auto

quantification.cdna_index

label cDNA index file

type data:index:salmon

description Transcriptome index file created using the Salmon indexing tool. cDNA (transcriptome) sequences used for index file creation must be derived from the same species as the input sequencing reads to obtain the reliable analysis results.

required False

hidden quantification.assay_type != 'auto'

quantification.n reads

```
label Number of reads in subsampled alignment file
```

```
type basic:integer
```

description Alignment (.bam) file subsample size. Increase the number of reads to make automatic detection more reliable. Decrease the number of reads to make automatic detection run faster.

hidden quantification.assay_type != 'auto'

default 5000000

$quantification. feature_class$

label Feature class

type basic:string

description Feature class (3rd column in GTF/GFF3 file) to be used. All other features will be ignored.

hidden !quantification.show_advanced

default exon

quantification.feature_type

label Feature type

type basic:string

description The type of feature the quantification program summarizes over (e.g. gene or transcript-level analysis). The value of this parameter needs to be chosen in line with 'ID attribute' below.

hidden !quantification.show advanced

default gene

choices

• gene: gene

• transcript: transcript

quantification.id_attribute

label ID attribute

type basic:string

description GTF/GFF3 attribute to be used as feature ID. Several GTF/GFF3 lines with the same feature ID are considered as parts of the same feature. The feature ID is used to identify the counts in the output table. In GTF files this is usually 'gene_id', in GFF3 files this is often 'ID', and 'transcript_id' is frequently a valid choice for both annotation formats.

hidden !quantification.show_advanced

default gene_id

choices

- gene_id: gene_id
- transcript_id: transcript_id
- ID: ID
- geneid: geneid

downsampling.n reads

label Number of reads

```
type basic:integer
    default 1000000
downsampling.advanced.seed
    label Seed
    type basic:integer
    default 11
```

downsampling.advanced.fraction

```
label Fraction
```

type basic:decimal

description Use the fraction of reads [0 - 1.0] from the original input file instead of the absolute number of reads. If set, this will override the "Number of reads" input parameter.

required False

downsampling.advanced.two_pass

```
label 2-pass mode
```

type basic:boolean

description Enable two-pass mode when down-sampling. Two-pass mode is twice as slow but with much reduced memory.

default False

qc.rrna_reference

label Indexed rRNA reference sequence

type data:genomeindex:star

description Reference sequence index prepared by STAR aligner indexing tool.

qc.globin_reference

label Indexed Globin reference sequence

type data:genomeindex:star

description Reference sequence index prepared by STAR aligner indexing tool.

Output results

BBDuk - STAR - featureCounts - QC (single-end)

 $\verb|data:workflow:rnaseq:featurecounts:qcworkflow-bbduk-star-featurecounts-qc-single| (\textit{data:reads:featurecounts-qc-single})| (\textit{data:reads:featurecounts-qc-si$

list:data:seq

ba-

sic:boolean

list:basic:str

tom_adapte

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sic:integer k

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sic:integer l

ming_distar

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sic:integer 1

data:genome

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Multimap-

1.2. Process catalog

This RNA-seq pipeline is comprised of three steps preprocessing, alignment, and quantification.

First, reads are preprocessed by __BBDuk__ which removes adapters, trims reads for quality from the 3'-end, and discards reads that are too short after trimming. Compared to similar tools, BBDuk is regarded for its computational efficiency. Next, preprocessed reads are aligned by __STAR__ aligner. At the time of implementation, STAR is considered a state-of-the-art tool that consistently produces accurate results from diverse sets of reads, and performs well even with default settings. For more information see [this comparison of RNA-seq aligners](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5792058/). Finally, aligned reads are summarized to genes by featureCounts . Gaining wide adoption among the bioinformatics community, featureCounts yields expressions in a computationally efficient manner. All three tools in this workflow support parallelization to accelerate the analysis.

rRNA contamination rate in the sample is determined using the STAR aligner. Quality-trimmed reads are downsampled (using Seqtk tool) and aligned to the rRNA reference sequences. The alignment rate indicates the percentage of the reads in the sample that are derived from the rRNA sequences.

Input arguments preprocessing.reads

```
label Reads
     type data:reads:fastq:single
preprocessing.adapters
     label Adapters
     type list:data:seq:nucleotide
     required False
preprocessing.show advanced
     label Show advanced parameters
     type basic:boolean
     default False
preprocessing.custom_adapter_sequences
     label Custom adapter sequences [literal]
     type list:basic:string
     description Custom adapter sequences can be specified by inputting them one by one and pressing Enter
          after each sequence.
     required False
     hidden !preprocessing.show_advanced
     default []
preprocessing.kmer length
     label K-mer length
     type basic:integer
     description K-mer length must be smaller or equal to the length of adapters.
     hidden !preprocessing.show_advanced
     default 23
preprocessing.min_k
```

label Minimum k-mer length at right end of reads used for trimming

```
type basic:integer
     disabled preprocessing.adapters.length === 0 && preprocessing.custom_adapter_sequences.length ===
     hidden !preprocessing.show_advanced
     default 11
preprocessing.hamming_distance
     label Maximum Hamming distance for k-mers
     type basic:integer
     hidden !preprocessing.show_advanced
     default 1
preprocessing.maxns
     label Max Ns after trimming [maxns=-1]
     type basic:integer
     description If non-negative, reads with more Ns than this (after trimming) will be discarded.
     hidden !preprocessing.show_advanced
     default -1
preprocessing.trim_quality
     label Quality below which to trim reads from the right end
     type basic:integer
     description Phred algorithm is used, which is more accurate than naive trimming.
     hidden !preprocessing.show_advanced
     default 10
preprocessing.min_length
     label Minimum read length
     type basic:integer
     description Reads shorter than minimum read length after trimming are discarded.
     hidden !preprocessing.show_advanced
     default 20
alignment.genome
     label Indexed reference genome
     type data:genomeindex:star
     description Genome index prepared by STAR aligner indexing tool.
alignment.show_advanced
     label Show advanced parameters
     type basic:boolean
     default False
```

alignment.unstranded

label The data is unstranded

type basic:boolean

description For unstranded RNA-seq data, Cufflinks/Cuffdiff require spliced alignments with XS strand attribute, which STAR will generate with –outSAMstrandField intronMotif option. As required, the XS strand attribute will be generated for all alignments that contain splice junctions. The spliced alignments that have undefined strand (i.e. containing only non-canonical unannotated junctions) will be suppressed. If you have stranded RNA-seq data, you do not need to use any specific STAR options. Instead, you need to run Cufflinks with the library option –library-type options. For example, c ufflinks –library-type fr-firststrand should be used for the standard dUTP protocol, including Illumina's stranded Tru-Seq. This option has to be used only for Cufflinks runs and not for STAR runs.

hidden !alignment.show_advanced

default False

alignment.noncannonical

label Remove non-cannonical junctions (Cufflinks compatibility)

type basic:boolean

description It is recommended to remove the non-canonical junctions for Cufflinks runs using –outFilterIntronMotifs RemoveNoncanonical.

hidden !alignment.show_advanced

default False

alignment.detect_chimeric.chimeric

label Detect chimeric and circular alignments

type basic:boolean

description To switch on detection of chimeric (fusion) alignments (in addition to normal mapping), –chimSegmentMin should be set to a positive value. Each chimeric alignment consists of two "segments". Each segment is non-chimeric on its own, but the segments are chimeric to each other (i.e. the segments belong to different chromosomes, or different strands, or are far from each other). Both segments may contain splice junctions, and one of the segments may contain portions of both mates. –chimSegmentMin parameter controls the minimum mapped length of the two segments that is allowed. For example, if you have 2x75 reads and used –chimSegmentMin 20, a chimeric alignment with 130b on one chromosome and 20b on the other will be output, while 135 + 15 won't be.

default False

alignment.detect_chimeric.chimSegmentMin

label -chimSegmentMin

type basic:integer

disabled detect chimeric.chimeric!= true

default 20

alignment.t_coordinates.quantmode

label Output in transcript coordinates

type basic:boolean

description With —quantMode TranscriptomeSAM option STAR will output alignments translated into transcript coordinates in the Aligned.toTranscriptome.out.bam file (in addition to alignments in genomic coordinates in Aligned.*.sam/bam files). These transcriptomic alignments can be used with various transcript quantification software that require reads to be mapped to transcriptome, such as RSEM or eXpress.

default False

alignment.t coordinates.singleend

label Allow soft-clipping and indels

type basic:boolean

description By default, the output satisfies RSEM requirements: soft-clipping or indels are not allowed. Use –quantTranscriptomeBan Singleend to allow insertions, deletions ans soft-clips in the transcriptomic alignments, which can be used by some expression quantification software (e.g. eXpress).

disabled t_coordinates.quantmode != true

default False

alignment.t_coordinates.gene_counts

label Count reads

type basic:boolean

description With –quantMode GeneCounts option STAR will count number reads per gene while mapping. A read is counted if it overlaps (1nt or more) one and only one gene. Both ends of the paired-end read are checked for overlaps. The counts coincide with those produced by htseq-count with default parameters. ReadsPerGene.out.tab file with 4 columns which correspond to different strandedness options: column 1: gene ID; column 2: counts for unstranded RNA-seq; column 3: counts for the 1st read strand aligned with RNA (htseq-count option -s yes); column 4: counts for the 2nd read strand aligned with RNA (htseq-count option -s reverse).

disabled t_coordinates.quantmode != true

default False

alignment.filtering.outFilterType

label Type of filtering

type basic:string

description Normal: standard filtering using only current alignment; BySJout: keep only those reads that contain junctions that passed filtering into SJ.out.tab

default Normal

choices

Normal: NormalBySJout: BySJout

alignment.filtering.outFilterMultimapNmax

label -outFilterMultimapNmax

type basic:integer

description Read alignments will be output only if the read maps fewer than this value, otherwise no alignments will be output (default: 10).

required False

a lignment. filtering. out Filter Mismatch N max

```
label -outFilterMismatchNmax
```

type basic:integer

description Alignment will be output only if it has fewer mismatches than this value (default: 10).

required False

alignment.filtering.outFilterMismatchNoverLmax

```
label -outFilterMismatchNoverLmax
```

type basic:decimal

description Max number of mismatches per pair relative to read length: for 2x100b, max number of mismatches is 0.06*200=8 for the paired read.

required False

alignment.filtering.outFilterScoreMin

label -outFilterScoreMin

type basic:integer

description Alignment will be output only if its score is higher than or equal to this value (default: 0).

required False

alignment.alignment.alignSJoverhangMin

label -alignSJoverhangMin

type basic:integer

description Minimum overhang (i.e. block size) for spliced alignments (default: 5).

required False

alignment.alignSJDBoverhangMin

label -alignSJDBoverhangMin

type basic:integer

description Minimum overhang (i.e. block size) for annotated (sjdb) spliced alignments (default: 3).

required False

alignment.alignment.alignIntronMin

label -alignIntronMin

type basic:integer

description Minimum intron size: genomic gap is considered intron if its length >= alignIntronMin, otherwise it is considered Deletion (default: 21).

required False

alignment.alignment.alignIntronMax

label -alignIntronMax

type basic:integer

description Maximum intron size, if 0, max intron size will be determined by (2pow(winBinNbits)*winAnchorDistNbins) (default: 0).

required False alignment.alignMatesGapMax label -alignMatesGapMax type basic:integer description Maximum gap between two mates, if 0, max intron gap will be determined by (2pow(winBinNbits)*winAnchorDistNbins) (default: 0). required False alignment.alignment.alignEndsType label -alignEndsType type basic:string description Type of read ends alignment (default: Local). required False default Local choices • Local: Local • EndToEnd: EndToEnd • Extend5pOfRead1: Extend5pOfRead1 • Extend5pOfReads12: Extend5pOfReads12 alignment.output_sam_bam.outSAMunmapped label -outSAMunmapped type basic:string description Output of unmapped reads in the SAM format. required False default None choices • None: None • Within: Within alignment.output sam bam.outSAMattributes label -outSAMattributes type basic:string description a string of desired SAM attributes, in the order desired for the output SAM. required False default Standard

• None: None

• Standard: Standard

choices

• All: All

alignment.output sam bam.outSAMattrRGline

```
label -outSAMattrRGline
type basic:string
```

description SAM/BAM read group line. The first word contains the read group identifier and must start with "ID:", e.g. –outSAMattrRGline ID:xxx CN:yy "DS:z z z"

required False

quantification.annotation

label Annotation

type data: annotation

quantification.show_advanced

label Show advanced parameters

type basic:boolean

default False

quantification.assay_type

label Assay type

type basic:string

description In strand non-specific assay a read is considered overlapping with a feature regardless of whether it is mapped to the same or the opposite strand as the feature. In strand-specific forward assay and single reads, the read has to be mapped to the same strand as the feature. For paired-end reads, the first read has to be on the same strand and the second read on the opposite strand. In strand-specific reverse assay these rules are reversed.

hidden !quantification.show_advanced

default non_specific

choices

• Strand non-specific: non_specific

• Strand-specific forward: forward

• Strand-specific reverse: reverse

• Detect automatically: auto

quantification.cdna_index

label cDNA index file

type data:index:salmon

description Transcriptome index file created using the Salmon indexing tool. cDNA (transcriptome) sequences used for index file creation must be derived from the same species as the input sequencing reads to obtain the reliable analysis results.

required False

hidden quantification.assay_type != 'auto'

quantification.n reads

```
label Number of reads in subsampled alignment file
```

```
type basic:integer
```

description Alignment (.bam) file subsample size. Increase the number of reads to make automatic detection more reliable. Decrease the number of reads to make automatic detection run faster.

hidden quantification.assay_type != 'auto'

default 5000000

quantification.feature_class

label Feature class

type basic:string

description Feature class (3rd column in GTF/GFF3 file) to be used. All other features will be ignored.

hidden !quantification.show_advanced

default exon

quantification.feature_type

label Feature type

type basic:string

description The type of feature the quantification program summarizes over (e.g. gene or transcript-level analysis). The value of this parameter needs to be chosen in line with 'ID attribute' below.

hidden !quantification.show advanced

default gene

choices

• gene: gene

• transcript: transcript

quantification.id_attribute

label ID attribute

type basic:string

description GTF/GFF3 attribute to be used as feature ID. Several GTF/GFF3 lines with the same feature ID will be considered as parts of the same feature. The feature ID is used to identify the counts in the output table. In GTF files this is usually 'gene_id', in GFF3 files this is often 'ID', and 'transcript_id' is frequently a valid choice for both annotation formats.

hidden !quantification.show_advanced

default gene_id

choices

- gene_id: gene_id
- transcript_id: transcript_id
- ID: ID
- geneid: geneid

downsampling.n reads

label Number of reads

```
type basic:integer
  default 1000000

downsampling.advanced.seed
  label Seed
  type basic:integer
  default 11

downsampling.advanced.fraction
  label Fraction
  type basic:decimal
  description Use the fraction of reads [0 - 1.0] from the orignal input file instead of the absolute number
```

required False

$downs ampling. advanced. two_pass$

```
label 2-pass mode
```

type basic:boolean

description Enable two-pass mode when down-sampling. Two-pass mode is twice as slow but with much reduced memory.

default False

qc.rrna_reference

label Indexed rRNA reference sequence

type data:genomeindex:star

description Reference sequence index prepared by STAR aligner indexing tool.

of reads. If set, this will override the "Number of reads" input parameter.

qc.globin_reference

label Indexed Globin reference sequence

type data:genomeindex:star

description Reference sequence index prepared by STAR aligner indexing tool.

Output results

BED file

```
data:bedupload-bed (basic:file src, basic:string species, basic:string build) [Source: v1.3.1]
```

Import a BED file (.bed) which is a tab-delimited text file that defines a feature track. It can have any file extension, but .bed is recommended. The BED file format is described on the [UCSC Genome Bioinformatics web site](http://genome.ucsc.edu/FAQ/FAQformat#format1).

Input arguments src

```
label BED file
```

type basic:file

description Upload BED file annotation track. The first three required BED fields are chrom, chromStart and chromEnd.

```
required True
     validate_regex \.(bed|narrowPeak)$
species
     label Species
     type basic:string
     description Species latin name.
     choices
            • Homo sapiens: Homo sapiens
            • Mus musculus: Mus musculus
            • Rattus norvegicus: Rattus norvegicus
            • Dictyostelium discoideum: Dictyostelium discoideum
            • Odocoileus virginianus texanus: Odocoileus virginianus texanus
            • Solanum tuberosum: Solanum tuberosum
build
     label Genome build
     type basic:string
Output results bed
     label BED file
     type basic:file
bed_jbrowse
     label Bgzip bed file for JBrowse
     type basic:file
tbi_jbrowse
     label Bed file index for Jbrowse
     type basic:file
species
     label Species
     type basic:string
build
     label Build
     type basic:string
```

BWA ALN

```
data:alignment:bam:bwaalnalignment-bwa-aln (data:genome:fasta
                                                                                            genome,
                                                           data:reads:fastq
                                                                                     reads,
                                                                                                 ba-
                                                                        q, basic:boolean
                                                           sic:integer
                                                                                            use_edit,
                                                           basic:integer
                                                                                edit value.
                                                                                                 ba-
                                                           sic:decimal fraction, basic:boolean seeds,
                                                           basic:integer
                                                                                seed_length,
                                                                                                 ba-
                                                           sic:integer seed_dist) [Source: v1.4.2]
```

Read aligner for mapping low-divergent sequences against a large reference genome. Designed for Illumina sequence reads up to 100bp.

```
Input arguments genome
     label Reference genome
     type data:genome:fasta
reads
     label Reads
     type data:reads:fastq
q
     label Quality threshold
     type basic:integer
     description Parameter for dynamic read trimming.
     default 0
use_edit
     label Use maximum edit distance (excludes fraction of missing alignments)
     type basic:boolean
     default False
edit_value
     label Maximum edit distance
     type basic:integer
     hidden !use_edit
     default 5
fraction
     label Fraction of missing alignments
     type basic:decimal
     description The fraction of missing alignments given 2% uniform base error rate. The maximum edit
          distance is automatically chosen for different read lengths.
     hidden use edit
     default 0.04
seeds
     label Use seeds
```

```
type basic:boolean
     default False
seed_length
     label Seed length
     type basic:integer
     description Take the first X subsequence as seed. If X is larger than the query sequence, seeding will be
          disabled. For long reads, this option is typically ranged from 25 to 35 for value 2 in seed maximum
          edit distance.
     hidden !seeds
     default 35
seed_dist
     label Seed maximum edit distance
     type basic:integer
     hidden !seeds
     default 2
Output results bam
     label Alignment file
     type basic:file
     description Position sorted alignment
bai
     label Index BAI
     type basic:file
unmapped
     label Unmapped reads
     type basic:file
     required False
stats
     label Statistics
     type basic:file
bigwig
     label BigWig file
     type basic:file
     required False
species
     label Species
     type basic:string
build
```

```
label Build
type basic:string
```

BWA MEM

```
data:alignment:bam:bwamemalignment-bwa-mem (data:genome:fasta
                                                                                          genome,
                                                          data:reads:fastq
                                                                                   reads,
                                                                                               ba-
                                                          sic:integer seed_l, basic:integer band_w,
                                                          basic:decimal
                                                                               re seeding,
                                                                                               ba-
                                                          sic:boolean m, basic:integer match, ba-
                                                          sic:integer missmatch, basic:integer gap_o,
                                                          basic:integer gap_e, basic:integer clip-
                                                          ping, basic:integer
                                                                                 unpaired_p, ba-
                                                          sic:boolean report_all, basic:integer re-
                                                          port_tr) [Source: v2.2.2]
```

BWA MEM is a read aligner for mapping low-divergent sequences against a large reference genome. Designed for longer sequences ranged from 70bp to 1Mbp. The algorithm works by seeding alignments with maximal exact matches (MEMs) and then extending seeds with the affine-gap Smith-Waterman algorithm (SW). See [here](http://biobwa.sourceforge.net/) for more information.

Input arguments genome

```
label Reference genome
     type data:genome:fasta
reads
     label Reads
     type data:reads:fastq
seed 1
     label Minimum seed length
     type basic:integer
     description Minimum seed length. Matches shorter than minimum seed length will be missed. The
          alignment speed is usually insensitive to this value unless it significantly deviates 20.
     default 19
band_w
     label Band width
     type basic:integer
     description Gaps longer than this will not be found.
     default 100
re_seeding
     label Re-seeding factor
     type basic:decimal
```

description Trigger re-seeding for a MEM longer than minSeedLen*FACTOR. This is a key heuristic parameter for tuning the performance. Larger value yields fewer seeds, which leads to faster alignment speed but lower accuracy.

```
default 1.5
m
     label Mark shorter split hits as secondary
     type basic:boolean
     description Mark shorter split hits as secondary (for Picard compatibility)
     default False
scoring.match
     label Score of a match
     type basic:integer
     default 1
scoring.missmatch
     label Mismatch penalty
     type basic:integer
     default 4
scoring.gap_o
     label Gap open penalty
     type basic:integer
     default 6
scoring.gap_e
     label Gap extension penalty
     type basic:integer
     default 1
scoring.clipping
     label Clipping penalty
     type basic:integer
     description Clipping is applied if final alignment score is smaller than (best score reaching the end of
          query) - (Clipping penalty)
     default 5
scoring.unpaired_p
     label Penalty for an unpaired read pair
     type basic:integer
     description Affinity to force pair. Score: scoreRead1+scoreRead2-Penalty
     default 9
reporting.report_all
     label Report all found alignments
     type basic:boolean
```

```
description Output all found alignments for single-end or unpaired paired-end reads. These alignments
          will be flagged as secondary alignments.
     default False
reporting.report_tr
     label Report threshold score
     type basic:integer
     description Don't output alignment with score lower than defined number. This option only affects
          output.
     default 30
Output results bam
     label Alignment file
     type basic:file
     description Position sorted alignment
bai
     label Index BAI
     type basic:file
unmapped
     label Unmapped reads
     type basic:file
     required False
stats
     label Statistics
     type basic:file
bigwig
     label BigWig file
     type basic:file
     required False
species
     label Species
     type basic:string
build
     label Build
     type basic:string
```

BWA SW

```
data:alignment:bam:bwaswalignment-bwa-sw (data:genome:fasta genome, data:reads:fastq reads, basic:integer match, basic:integer missmatch, basic:integer gap_o, basic:integer gap_e) [Source: v1.3.2]
```

Read aligner for mapping low-divergent sequences against a large reference genome. Designed for longer sequences ranged from 70bp to 1Mbp. The paired-end mode only works for reads Illumina short-insert libraries.

```
Input arguments genome
```

```
label Reference genome
     type data:genome:fasta
reads
     label Reads
     type data:reads:fastq
match
     label Score of a match
     type basic:integer
     default 1
missmatch
     label Mismatch penalty
     type basic:integer
     default 3
gap_o
     label Gap open penalty
     type basic:integer
     default 5
gap_e
     label Gap extension penalty
     type basic:integer
     default 2
Output results bam
     label Alignment file
     type basic:file
     description Position sorted alignment
bai
     label Index BAI
     type basic:file
```

unmapped

```
label Unmapped reads
     type basic:file
     required False
stats
     label Statistics
     type basic:file
bigwig
     label BigWig file
     type basic:file
     required False
species
     label Species
     type basic:string
build
     label Build
     type basic:string
Bam split
data:alignment:bam:primarybam-split (data:alignment:bam bam, data:sam:header header,
                                                 data:sam:header header2) [Source: v0.4.0]
Split hybrid bam file into two bam files.
Input arguments bam
     label Hybrid alignment bam
     type data:alignment:bam
header
     label Primary header sam file (optional)
     type data:sam:header
     description If no header file is provided, the headers will be extracted from the hybrid alignment bam
          file.
     required False
header2
     label Secondary header sam file (optional)
     type data:sam:header
     description If no header file is provided, the headers will be extracted from the hybrid alignment bam
          file.
     required False
Output results bam
```

```
label Uploaded file
type basic:file
bai
label Index BAI
type basic:file
bigwig
label BigWig file
type basic:file
required False
species
label Species
type basic:string
build
label Build
type basic:string
```

Bamliquidator

```
data:bam:plot:bamliquidatorbamliquidator (basic:string list:data:alignment:bam bam, basic:string cell_type, basic:integer bin_size, data:annotation:gtf regions_gtf, data:bed regions_bed, basic:integer extension, basic:string sense, basic:boolean skip_plot, list:basic:string black_list, basic:integer threads) [Source: v0.2.1]
```

Set of tools for analyzing the density of short DNA sequence read alignments in the BAM file format.

Input arguments analysis_type

```
label Cell type
     type basic:string
     default cell_type
bin_size
     label Bin size
     type basic:integer
     description Number of base pairs in each bin. The smaller the bin size the longer the runtime and the
          larger the data files. Default is 100000.
     required False
     hidden analysis_type != 'bin'
regions_gtf
     label Region gff file / Annotation file (.gffl.gtf)
     type data:annotation:gtf
     required False
     hidden analysis_type != 'region'
regions_bed
     label Region bed file / Annotation file (.bed)
     type data:bed
     required False
     hidden analysis_type != 'bed'
extension
     label Extension
     type basic:integer
     description Extends reads by number of bp
     default 200
sense
     label Mapping strand to gff file
     type basic:string
     default .
     choices
             • Forward: +
             • Reverse: -
             • Both: .
skip_plot
     label Skip plot
     type basic:boolean
```

```
required False
black_list
     label Black list
     type list:basic:string
     description One or more chromosome patterns to skip during bin liquidation. Default is to skip any
          chromosomes that contain any of the following substrings chrUn _random Zv9_ _hap.
     required False
threads
     label Threads
     type basic:integer
     description Number of threads to run concurrently during liquidation.
     default 1
Output results analysis_type
     label Analysis type
     type basic:string
     hidden True
output_dir
     label Output directory
     type basic:file
counts
     label Counts HDF5 file
     type basic:file
matrix
     label Matrix file
     type basic:file
     required False
     hidden analysis_type != 'region'
summary
     label Summary file
     type basic:file:html
     required False
     hidden analysis_type != 'bin'
```

Bamplot

```
data:bam:plot:bamplotbamplot (basic:string)
                                                     genome, data:annotation:gtf
                                                                                   input_gff, ba-
                                                    input_region, list:data:alignment:bam
                                       sic:string
                                       basic:integer
                                                       stretch_input, basic:string
                                                                                       color,
                                                                                              ba-
                                       sic:string sense, basic:integer extension, basic:boolean rpm,
                                       basic:string yscale, list:basic:string names, basic:string plot,
                                                    title, basic:string scale, list:data:bed
                                       basic:boolean multi_page) [Source: v1.3.1]
Plot a single locus from a bam.
Input arguments genome
     label Genome
     type basic:string
     choices
            • HG19: HG19
            • HG18: HG18
            • MM8: MM8
            • MM9: MM9
            • MM10: MM10
            • RN6: RN6
            • RN4: RN4
input_gff
     label Region string
     type data:annotation:gtf
     description Enter .gff file.
     required False
input_region
     label Region string
     type basic:string
     description Enter genomic region e.g. chr1:+:1-1000.
     required False
bam
     label Bam
     type list:data:alignment:bam
     description bam to plot from
     required False
stretch_input
     label Stretch-input
     type basic:integer
```

```
description Stretch the input regions to a minimum length in bp, e.g. 10000 (for 10kb).
     required False
color
     label Color
     type basic:string
     description Enter a colon separated list of colors e.g. 255,0,0:255,125,0, default samples the rainbow.
     default 255,0,0:255,125,0
sense
     label Sense
     type basic:string
     description Map to forward, reverse or'both strands. Default maps to both.
     default both
     choices
             • Forward: forward
             • Reverse: reverse
             • Both: both
extension
     label Extension
     type basic:integer
     description Extends reads by n bp. Default value is 200bp.
     default 200
rpm
     label rpm
     type basic:boolean
     description Normalizes density to reads per million (rpm) Default is False.
     required False
yscale
     label y scale
     type basic:string
     description Choose either relative or uniform y axis scaling. Default is relative scaling.
     default relative
     choices
             • relative: relative
             • uniform: uniform
names
     label Names
```

```
type list:basic:string
     description Enter a comma separated list of names for your bams.
     required False
plot
     label Single or multiple polt
     type basic:string
     description Choose either all lines on a single plot or multiple plots.
     default merge
     choices
             • single: single
             • multiple: multiple
             • merge: merge
title
     label Title
     type basic:string
     description Specify a title for the output plot(s), default will be the coordinate region.
     default output
scale
     label Scale
     type basic:string
     description Enter a comma separated list of multiplicative scaling factors for your bams. Default is none.
     required False
bed
     label Bed
     type list:data:bed
     description Add a space-delimited list of bed files to plot.
     required False
multi_page
     label Multi page
     type basic:boolean
     description If flagged will create a new pdf for each region.
     default False
Output results plot
     label region plot
     type basic:file
```

BaseSpace file

```
data:filebasespace-file-import (basic:string file_id, basic:secret access_token_secret) [Source: v1.0.3]
```

Import a file from Illumina BaseSpace.

Input arguments file_id

```
label BaseSpace file ID
type basic:string
```

access_token_secret

label BaseSpace access token

type basic:secret

description BaseSpace access token secret handle needed to download the file.

Output results file

```
label File
type basic:file
```

Bowtie (Dicty)

```
data:alignment:bam:bowtie1alignment-bowtie (data:genome:fasta
                                                                                             genome,
                                                            data:reads:fastq
                                                                                     reads.
                                                                                                  ba-
                                                            sic:string
                                                                         mode, basic:integer
                                                                                                  m,
                                                            basic:integer
                                                                          l, basic:boolean
                                                                                              use_se,
                                                            basic:integer trim 5, basic:integer trim 3,
                                                            basic:integer
                                                                                 trim nucl,
                                                                                                  ba-
                                                            sic:integer
                                                                                 trim iter,
                                                                                                  ba-
                                                            sic:string r) [Source: v1.4.1]
```

An ultrafast memory-efficient short read aligner.

Input arguments genome

```
label Reference genome
type data:genome:fasta
```

reads

label Reads

type data:reads:fastq

mode

label Alignment mode

type basic:string

description When the -n option is specified (which is the default), bowtie determines which alignments are valid according to the following policy, which is similar to Maq's default policy. 1. Alignments may have no more than N mismatches (where N is a number 0-3, set with -n) in the first L bases (where L is a number 5 or greater, set with -l) on the high-quality (left) end of the read. The first L bases are called the "seed". 2. The sum of the Phred quality values at all mismatched positions (not just in the seed) may not exceed E (set with -e). Where qualities are unavailable (e.g. if the reads are from a FASTA file), the Phred quality defaults to 40. In -v mode, alignments may have no more

m

1

than V mismatches, where V may be a number from 0 through 3 set using the -v option. Quality values are ignored. The -v option is mutually exclusive with the -n option. default -n choices • Use qualities (-n): -n • Use mismatches (-v): -v label Allowed mismatches type basic:integer **description** When used with "Use qualities (-n)" it is the maximum number of mismatches permitted in the "seed", i.e. the first L base pairs of the read (where L is set with -l/-seedlen). This may be 0, 1, 2 or 3 and the default is 2 When used with "Use mismatches (-v)" report alignments with at most <int> mismatches. default 2 label Seed length (for -n only) type basic:integer description Only for "Use qualities (-n)". Seed length (-1) is the number of bases on the high-quality end of the read to which the -n ceiling applies. The lowest permitted setting is 5 and the default is 28. bowtie is faster for larger values of -1. default 28 use se **label** Map as single-ended (for paired end reads only) type basic:boolean description If this option is selected paired-end reads will be mapped as single-ended. default False start trimming.trim 5 label Bases to trim from 5' type basic:integer description Number of bases to trim from from 5' (left) end of each read before alignment default 0 start_trimming.trim_3 label Bases to trim from 3' type basic:integer description Number of bases to trim from from 3' (right) end of each read before alignment default 0

trimming.trim_nucl

label Bases to trim

```
type basic:integer
     description Number of bases to trim from 3' end in each iteration.
     default 2
trimming.trim_iter
     label Iterations
     type basic:integer
     description Number of iterations.
     default 0
reporting.r
     label Reporting mode
     type basic:string
     description Report up to <int> valid alignments per read or pair (-k) (default: 1). Validity of alignments
           is determined by the alignment policy (combined effects of -n, -v, -l, and -e). If more than one
           valid alignment exists and the -best and -strata options are specified, then only those alignments
           belonging to the best alignment "stratum" will be reported. Bowtie is designed to be very fast for
           small -k but bowtie can become significantly slower as -k increases. If you would like to use Bowtie
           for larger values of -k, consider building an index with a denser suffix-array sample, i.e. specify a
           smaller -o/-offrate when invoking bowtie-build for the relevant index (see the Performance tuning
           section for details).
     default -a -m 1 --best --strata
     choices
             • Report unique alignments: -a -m 1 --best --strata
             • Report all alignments: -a --best
             • Report all alignments in the best stratum: -a --best --strata
Output results bam
     label Alignment file
     type basic:file
     description Position sorted alignment
bai
     label Index BAI
     type basic:file
unmapped
     label Unmapped reads
     type basic:file
     required False
stats
     label Statistics
     type basic:file
```

```
label BigWig file
    type basic:file
    required False
species
    label Species
    type basic:string
build
    label Build
    type basic:string
```

Bowtie2

```
data:alignment:bam:bowtie2alignment-bowtie2 (data:genome:fasta
                                                                                             genome,
                                                             data:reads:fastq reads, basic:string mode,
                                                             basic:string speed, basic:boolean use_se,
                                                             basic:boolean
                                                                                 discordantly.
                                                             sic:boolean rep_se, basic:integer minins,
                                                             basic:integer maxins, basic:integer N,
                                                                           L, basic:integer
                                                             basic:integer
                                                             basic:string mp, basic:string rdg, ba-
                                                             sic:string rfg, basic:string score_min, ba-
                                                             sic:integer trim_5, basic:integer trim_3,
                                                                                   trim iter,
                                                             basic:integer
                                                                                                  ba-
                                                             sic:integer
                                                                                 trim nucl,
                                                                                                  ba-
                                                             sic:string
                                                                                 rep_mode,
                                                                                                  ba-
                                                             sic:integer k_reports) [Source: v1.5.0]
```

Bowtie is an ultrafast, memory-efficient short read aligner. It aligns short DNA sequences (reads) to the human genome at a rate of over 25 million 35-bp reads per hour. Bowtie indexes the genome with a Burrows-Wheeler index to keep its memory footprint small-typically about 2.2 GB for the human genome (2.9 GB for paired-end). See [here](http://bowtie-bio.sourceforge.net/index.shtml) for more information.

Input arguments genome

```
label Reference genome
    type data:genome:fasta
reads
    label Reads
    type data:reads:fastq
mode
    label Alignment mode
    type basic:string
```

description End to end: Bowtie 2 requires that the entire read align from one end to the other, without any trimming (or "soft clipping") of characters from either end. local: Bowtie 2 does not require

that the entire read align from one end to the other. Rather, some characters may be omitted ("soft clipped") from the ends in order to achieve the greatest possible alignment score.

default --end-to-end

choices

- end to end mode: --end-to-end
- local: --local

speed

label Speed vs. Sensitivity

type basic:string

description A quick setting for aligning fast or accurately. This option is a shortcut for parameters as follows:

For –end-to-end: –very-fast -D 5 -R 1 -N 0 -L 22 -i S,0,2.50 –fast -D 10 -R 2 -N 0 -L 22 -i S,0,2.50 –sensitive -D 15 -R 2 -N 0 -L 22 -i S,1,1.15 (default) –very-sensitive -D 20 -R 3 -N 0 -L 20 -i S,1.0.50

For -local: -very-fast-local -D 5 -R 1 -N 0 -L 25 -i S,1,2.00 -fast-local -D 10 -R 2 -N 0 -L 22 -i S,1,1.75 -sensitive-local -D 15 -R 2 -N 0 -L 20 -i S,1,0.75 (default) -very-sensitive-local -D 20 -R 3 -N 0 -L 20 -i S,1,0.50

required False

choices

- Very fast: --very-fast
- Fast: --fast
- Sensitive: --sensitive
- Very sensitive: --very-sensitive

PE_options.use_se

label Map as single-ended (for paired-end reads only)

type basic:boolean

description If this option is selected paired-end reads will be mapped as single-ended and other paired-end options are ignored.

default False

PE options.discordantly

label Report discordantly matched read

type basic:boolean

description If both mates have unique alignments, but the alignments do not match paired-end expectations (orientation and relative distance) then alignment will be reported. Useful for detecting structural variations.

default True

PE_options.rep_se

label Report single ended

type basic:boolean

description If paired alignment can not be found Bowtie2 tries to find alignments for the individual mates. default True PE options.minins label Minimal distance type basic:integer description The minimum fragment length for valid paired-end alignments. 0 imposes no minimum. default 0 PE options.maxins label Maximal distance type basic:integer description The maximum fragment length for valid paired-end alignments. default 500 alignment options.N label Number of mismatches allowed in seed alignment (N) type basic:integer **description** Sets the number of mismatches to allowed in a seed alignment during multiseed alignment. Can be set to 0 or 1. Setting this higher makes alignment slower (often much slower) but increases sensitivity. Default: 0. required False alignment options.L **label** Length of seed substrings (L) type basic:integer description Sets the length of the seed substrings to align during multiseed alignment. Smaller values make alignment slower but more sensitive. Default: the -sensitive preset is used by default for end-to-end alignment and -sensitive-local for local alignment. See documentation for details. required False alignment options.gbar **label** Disallow gaps within positions (gbar) type basic:integer **description** Disallow gaps within <int> positions of the beginning or end of the read. Default: 4. required False

alignment_options.mp

label Maximal and minimal mismatch penalty (mp)

type basic:string

description Sets the maximum (MX) and minimum (MN) mismatch penalties, both integers. A number less than or equal to MX and greater than or equal to MN is subtracted from the alignment score for each position where a read character aligns to a reference character, the characters do not match, and neither is an N. If -ignore-quals is specified, the number subtracted quals MX. Otherwise, the number subtracted is MN + floor((MX-MN)(MIN(Q, 40.0)/40.0)) where Q is the Phred quality value. Default for MX, MN: 6,2.

```
required False
```

alignment_options.rdg

label Set read gap open and extend penalties (rdg)

type basic:string

description Sets the read gap open (<int1>) and extend (<int2>) penalties. A read gap of length N gets a penalty of <int1> + N * <int2>. Default: 5,3.

required False

alignment_options.rfg

label Set reference gap open and close penalties (rfg)

type basic:string

description Sets the reference gap open (<int1>) and extend (<int2>) penalties. A reference gap of length N gets a penalty of <int1> + N * <int2>. Default: 5,3.

required False

alignment_options.score_min

label Minimum alignment score needed for "valid" alignment (score_min)

type basic:string

description Sets a function governing the minimum alignment score needed for an alignment to be considered "valid" (i.e. good enough to report). This is a function of read length. For instance, specifying L,0,-0.6 sets the minimum-score function to f(x) = 0 + -0.6 * x, where x is the read length. The default in –end-to-end mode is L,-0.6,-0.6 and the default in –local mode is G,20,8.

required False

start_trimming.trim_5

label Bases to trim from 5'

type basic:integer

description Number of bases to trim from from 5' (left) end of each read before alignment

default 0

start_trimming.trim_3

label Bases to trim from 3'

type basic:integer

description Number of bases to trim from from 3' (right) end of each read before alignment

default 0

trimming.trim_iter

label Iterations

type basic:integer

description Number of iterations.

default 0

```
trimming.trim_nucl
     label Bases to trim
     type basic:integer
     description Number of bases to trim from 3' end in each iteration.
     default 2
reporting.rep_mode
     label Report mode
     type basic:string
     description Default mode: search for multiple alignments, report the best one; -k mode: search for one
           or more alignments, report each; -a mode: search for and report all alignments
     default def
     choices
             • Default mode: def
             • -k mode: k
             • -a mode (very slow): a
reporting.k_reports
     label Number of reports (for -k mode only)
     type basic:integer
     description Searches for at most X distinct, valid alignments for each read. The search terminates when
          it can't find more distinct valid alignments, or when it finds X, whichever happens first. default: 5
     default 5
Output results bam
     label Alignment file
     type basic:file
     description Position sorted alignment
bai
     label Index BAI
     type basic:file
unmapped
     label Unmapped reads
     type basic:file
     required False
stats
     label Statistics
     type basic:file
bigwig
     label BigWig file
```

```
type basic:file
     required False
species
     label Species
     type basic:string
build
     label Build
     type basic:string
ChIP-Seq (Gene Score)
data:chipseq:genescorechipseq-genescore (data:chipseq:peakscore
                                                                                peakscore,
                                                                                              ba-
                                                                                       pval, ba-
                                                     sic:decimal
                                                                  fdr, basic:decimal
                                                     sic:decimal logratio) [Source: v1.1.1]
Chip-Seq analysis - Gene Score (BCM)
Input arguments peakscore
     label PeakScore file
     type data:chipseq:peakscore
     description PeakScore file
fdr
     label FDR threshold
     type basic:decimal
     description FDR threshold value (default = 0.00005).
     default 5e-05
pval
     label Pval threshold
     type basic:decimal
     description Pval threshold value (default = 0.00005).
     default 5e-05
logratio
     label Log-ratio threshold
     type basic:decimal
     description Log-ratio threshold value (default = 2).
     default 2.0
Output results genescore
     label Gene Score
     type basic:file
```

ChIP-Seq (Peak Score)

```
data:chipseq:peakscorechipseq-peakscore (data:chipseq:callpeak:macs2 peaks, data:bed bed) [Source: v2.1.0]
```

Chip-Seq analysis - Peak Score (BCM)

Input arguments peaks

label MACS2 results

type data:chipseq:callpeak:macs2

description MACS2 results file (NarrowPeak)

bed

label BED file

type data:bed

Output results peak_score

label Peak Score

type basic:file

ChIP-seq (MACS2)

data:chipseq:batch:macs2macs2-batch (list:data:alignment:bam

alignments, basic:boolean advanced. data:bed promoter, basic:boolean tagalign, basic:integer **q_threshold**, basic:integer n sub, basic:boolean tn5, basic:integer shift, baduplicates, basic:string duplisic:string cates prepeak, basic:decimal qvalue, sic:decimal pvalue, basic:decimal pvalue_prepeak, basic:integer cap num, basic:integer mfold lower, mfold_upper, basic:integer basic:integer **slocal**, basic:integer llocal, basic:integer extsize, basic:integer shift. basic:integer band width, basic:boolean nolambda, basic:boolean fix_bimodal, basic:boolean nomodel, basic:boolean nomodel_prepeak, badown_sample, basic:boolean sic:boolean bedgraph, basic:boolean spmr, bacall_summits, basic:boolean sic:boolean broad, basic:decimal broad cutoff) [Source: v1.0.3]

This process runs MACS2 in batch mode. MACS2 analysis is triggered for pairs of samples as defined using treatment-background sample relations. If there are no sample relations defined, each sample is treated individually for the MACS analysis.

Model-based Analysis of ChIP-Seq (MACS 2.0), is used to identify transcript factor binding sites. MACS 2.0 captures the influence of genome complexity to evaluate the significance of enriched ChIP regions, and MACS improves the spatial resolution of binding sites through combining the information of both sequencing tag position and orientation. It has also an option to link nearby peaks together in order to call broad peaks. See [here](https://github.com/taoliu/MACS/) for more information.

In addition to peak-calling, this process computes ChIP-Seq and ATAC-Seq QC metrics. Process returns a QC metrics report, fragment length estimation, and a deduplicated tagAlign file. QC report contains ENCODE 3 proposed QC

metrics – [NRF](https://www.encodeproject.org/data-standards/terms/), [PBC bottlenecking coefficients, NSC, and RSC](https://genome.ucsc.edu/ENCODE/qualityMetrics.html#chipSeq).

```
Input arguments alignments
```

```
label Aligned reads
type list:data:alignment:bam
description Select multiple treatment/background samples.
```

advanced

```
label Show advanced options
type basic:boolean
description Inspect and modify parameters.
default False
```

promoter

```
label Promoter regions BED file
```

```
type data:bed
```

description BED file containing promoter regions (TSS+-1000bp for example). Needed to get the number of peaks and reads mapped to promoter regions.

```
required False hidden !advanced
```

tagalign

```
label Use tagAlign files
type basic:boolean
```

description Use filtered tagAlign files as case (treatment) and control (background) samples. If extsize parameter is not set, run MACS using input's estimated fragment length.

```
hidden !advanced
default False
```

prepeakqc_settings.q_threshold

```
label Quality filtering threshold
type basic:integer
default 30
```

prepeakqc_settings.n_sub

```
label Number of reads to subsample
type basic:integer
default 15000000
```

prepeakqc_settings.tn5

```
label TN5 shifting
type basic:boolean
```

description Tn5 transposon shifting. Shift reads on "+" strand by 4bp and reads on "-" strand by 5bp.

```
default False
```

prepeakqc_settings.shift

label User-defined cross-correlation peak strandshift

```
type basic:integer
```

description If defined, SPP tool will not try to estimate fragment length but will use the given value as fragment length.

required False

settings.duplicates

label Number of duplicates

type basic:string

description It controls the MACS behavior towards duplicate tags at the exact same location – the same coordination and the same strand. The 'auto' option makes MACS calculate the maximum tags at the exact same location based on binomal distribution using 1e-5 as pvalue cutoff and the 'all' option keeps all the tags. If an integer is given, at most this number of tags will be kept at the same location. The default is to keep one tag at the same location.

required False

hidden tagalign

choices

• 1: 1

• auto: auto

• all: all

settings.duplicates_prepeak

label Number of duplicates

type basic:string

description It controls the MACS behavior towards duplicate tags at the exact same location – the same coordination and the same strand. The 'auto' option makes MACS calculate the maximum tags at the exact same location based on binomal distribution using 1e-5 as pvalue cutoff and the 'all' option keeps all the tags. If an integer is given, at most this number of tags will be kept at the same location. The default is to keep one tag at the same location.

required False

hidden !tagalign

default all

choices

• 1: 1

• auto: auto

• all: all

settings.qvalue

label Q-value cutoff

type basic:decimal

```
description The q-value (minimum FDR) cutoff to call significant regions. Q-values are calculated from
           p-values using Benjamini-Hochberg procedure.
     required False
     disabled settings.pvalue && settings.pvalue_prepeak
settings.pvalue
     label P-value cutoff
     type basic:decimal
     description The p-value cutoff. If specified, MACS2 will use p-value instead of q-value cutoff.
     required False
     disabled settings.qvalue
     hidden tagalign
settings.pvalue_prepeak
     label P-value cutoff
     type basic:decimal
     description The p-value cutoff. If specified, MACS2 will use p-value instead of q-value cutoff.
     disabled settings.qvalue
     hidden !tagalign || settings.qvalue
     default 1e-05
settings.cap_num
     label Cap number of peaks by taking top N peaks
     type basic:integer
     description To keep all peaks set value to 0.
     disabled settings.broad
     default 500000
settings.mfold_lower
     label MFOLD range (lower limit)
     type basic:integer
     description This parameter is used to select the regions within MFOLD range of high-confidence en-
           richment ratio against background to build model. The regions must be lower than upper limit, and
           higher than the lower limit of fold enrichment. DEFAULT:10,30 means using all regions not too
           low (>10) and not too high (<30) to build paired-peaks model. If MACS can not find more than 100
           regions to build model, it will use the -extsize parameter to continue the peak detection ONLY if
           -fix-bimodal is set.
     required False
settings.mfold_upper
     label MFOLD range (upper limit)
     type basic:integer
```

description This parameter is used to select the regions within MFOLD range of high-confidence enrichment ratio against background to build model. The regions must be lower than upper limit, and higher than the lower limit of fold enrichment. DEFAULT:10,30 means using all regions not too low (>10) and not too high (<30) to build paired-peaks model. If MACS can not find more than 100 regions to build model, it will use the –extsize parameter to continue the peak detection ONLY if –fix-bimodal is set.

required False

settings.slocal

label Small local region

type basic:integer

description Slocal and llocal parameters control which two levels of regions will be checked around the peak regions to calculate the maximum lambda as local lambda. By default, MACS considers 1000bp for small local region (–slocal), and 10000bps for large local region (–llocal) which captures the bias from a long range effect like an open chromatin domain. You can tweak these according to your project. Remember that if the region is set too small, a sharp spike in the input data may kill the significant peak.

required False

settings.llocal

label Large local region

type basic:integer

description Slocal and llocal parameters control which two levels of regions will be checked around the peak regions to calculate the maximum lambda as local lambda. By default, MACS considers 1000bp for small local region (–slocal), and 10000bps for large local region (–llocal) which captures the bias from a long range effect like an open chromatin domain. You can tweak these according to your project. Remember that if the region is set too small, a sharp spike in the input data may kill the significant peak.

required False

settings.extsize

label extsize

type basic:integer

description While '-nomodel' is set, MACS uses this parameter to extend reads in 5'->3' direction to fix-sized fragments. For example, if the size of binding region for your transcription factor is 200 bp, and you want to bypass the model building by MACS, this parameter can be set as 200. This option is only valid when -nomodel is set or when MACS fails to build model and -fix-bimodal is on.

required False

settings.shift

label Shift

type basic:integer

description Note, this is NOT the legacy –shiftsize option which is replaced by –extsize! You can set an arbitrary shift in bp here. Please Use discretion while setting it other than default value (0). When –nomodel is set, MACS will use this value to move cutting ends (5') then apply –extsize from 5' to 3' direction to extend them to fragments. When this value is negative, ends will be moved toward 3'->5' direction, otherwise 5'->3' direction. Recommended to keep it as default 0 for ChIP-Seq

datasets, or -1 * half of EXTSIZE together with –extsize option for detecting enriched cutting loci such as certain DNAseI-Seq datasets. Note, you can't set values other than 0 if format is BAMPE for paired-end data. Default is 0.

required False

settings.band_width

label Band width

type basic:integer

description The band width which is used to scan the genome ONLY for model building. You can set this parameter as the sonication fragment size expected from wet experiment. The previous side effect on the peak detection process has been removed. So this parameter only affects the model building.

required False

settings.nolambda

label Use backgroud lambda as local lambda

type basic:boolean

description With this flag on, MACS will use the background lambda as local lambda. This means MACS will not consider the local bias at peak candidate regions.

default False

settings.fix_bimodal

label Turn on the auto paired-peak model process

type basic:boolean

description Whether turn on the auto paired-peak model process. If it's set, when MACS failed to build paired model, it will use the nomodel settings, the '-extsize' parameter to extend each tags. If set, MACS will be terminated if paired-peak model is failed.

default False

settings.nomodel

label Bypass building the shifting model

type basic:boolean

description While on, MACS will bypass building the shifting model.

hidden tagalign
default False

settings.nomodel_prepeak

label Bypass building the shifting model

type basic:boolean

description While on, MACS will bypass building the shifting model.

hidden !tagalign
default True

settings.down_sample

label Down-sample

```
type basic:boolean
```

description When set, random sampling method will scale down the bigger sample. By default, MACS uses linear scaling. This option will make the results unstable and irreproducible since each time, random reads would be selected, especially the numbers (pileup, pvalue, qvalue) would change. Consider to use 'randsample' script before MACS2 runs instead.

default False

settings.bedgraph

label Save fragment pileup and control lambda

type basic:boolean

description If this flag is on, MACS will store the fragment pileup, control lambda, -log10pvalue and -log10qvalue scores in bedGraph files. The bedGraph files will be stored in current directory named NAME+'_treat_pileup.bdg' for treatment data, NAME+'_control_lambda.bdg' for local lambda values from control, NAME+'_treat_pvalue.bdg' for Poisson pvalue scores (in -log10(pvalue) form), and NAME+'_treat_qvalue.bdg' for q-value scores from Benjamini-Hochberg-Yekutieli procedure.

default True

settings.spmr

label Save signal per million reads for fragment pileup profiles

type basic:boolean

disabled settings.bedgraph === false

default True

settings.call_summits

label Call summits

type basic:boolean

description MACS will now reanalyze the shape of signal profile (p or q-score depending on cutoff setting) to deconvolve subpeaks within each peak called from general procedure. It's highly recommended to detect adjacent binding events. While used, the output subpeaks of a big peak region will have the same peak boundaries, and different scores and peak summit positions.

default False

settings.broad

label Composite broad regions

type basic:boolean

description When this flag is on, MACS will try to composite broad regions in BED12 (a gene-model-like format) by putting nearby highly enriched regions into a broad region with loose cutoff. The broad region is controlled by another cutoff through –broad-cutoff. The maximum length of broad region length is 4 times of d from MACS.

disabled settings.call_summits === true

default False

settings.broad_cutoff

label Broad cutoff

type basic:decimal

description Cutoff for broad region. This option is not available unless –broad is set. If -p is set, this is a p-value cutoff, otherwise, it's a q-value cutoff. DEFAULT = 0.1

required False

disabled settings.call summits === true || settings.broad!== true

Output results

ChIP-seq (MACS2-ROSE2)

data:chipseq:batch:macs2macs2-rose2-batch (list:data:alignment:bam

alignments. sic:boolean advanced, data:bed promoter. basic:boolean tagalign, q threshold, basic:integer basic:integer n_sub, basic:boolean tn5. **shift**, basic:string duplibasic:integer cates. basic:string duplicates prepeak. basic:decimal qvalue, basic:decimal pvalue, basic:decimal pvalue_prepeak, basic:integer cap_num, bamfold lower. basic:integer mfold upper. sic:integer basic:integer slocal, basic:integer llocal. basic:integer extsize, basic:integer shift, basic:integer band_width, basic:boolean nolambda, basic:boolean fix bimodal, basic:boolean nomodel, basic:boolean nomodel prepeak, basic:boolean down sample, basic:boolean bedgraph, basic:boolean spmr, basic:boolean call summits, basic:boolean broad. basic:decimal broad_cutoff, basic:integer tss, basic:integer stitch, data:bed mask) [Source: v1.0.31

This process runs MACS2 in batch mode. MACS2 analysis is triggered for pairs of samples as defined using treatment-background sample relations. If there are no sample relations defined, each sample is treated individually for the MACS analysis.

Model-based Analysis of ChIP-Seq (MACS 2.0), is used to identify transcript factor binding sites. MACS 2.0 captures the influence of genome complexity to evaluate the significance of enriched ChIP regions, and MACS improves the spatial resolution of binding sites through combining the information of both sequencing tag position and orientation. It has also an option to link nearby peaks together in order to call broad peaks. See [here](https://github.com/taoliu/MACS/) for more information.

In addition to peak-calling, this process computes ChIP-Seq and ATAC-Seq QC metrics. Process returns a QC metrics report, fragment length estimation, and a deduplicated tagAlign file. QC report contains ENCODE 3 proposed QC metrics – [NRF](https://www.encodeproject.org/data-standards/terms/), [PBC bottlenecking coefficients, NSC, and RSC](https://genome.ucsc.edu/ENCODE/qualityMetrics.html#chipSeq).

For identification of super enhancers R2 uses the Rank Ordering of Super-Enhancers algorithm (ROSE2). This takes the peaks called by RSEG for acetylation and calculates the distances in-between to judge whether they can be considered super-enhancers. The ranked values can be plotted and by locating the inflection point in the resulting graph, super-enhancers can be assigned. It can also be used with the MACS calculated data. See [here](http://younglab.wi.mit.edu/super enhancer code.html) for more information.

```
Input arguments alignments
     label Aligned reads
     type list:data:alignment:bam
     description Select multiple treatment/background samples.
advanced
     label Show advanced options
     type basic:boolean
     description Inspect and modify parameters.
     default False
promoter
     label Promoter regions BED file
     type data:bed
     description BED file containing promoter regions (TSS+-1000bp for example). Needed to get the num-
          ber of peaks and reads mapped to promoter regions.
     required False
     hidden !advanced
tagalign
     label Use tagAlign files
     type basic:boolean
     description Use filtered tagAlign files as case (treatment) and control (background) samples. If extsize
          parameter is not set, run MACS using input's estimated fragment length.
     hidden !advanced
     default False
prepeakqc_settings.q_threshold
     label Quality filtering threshold
     type basic:integer
     default 30
prepeakqc_settings.n_sub
     label Number of reads to subsample
     type basic:integer
     default 15000000
prepeakqc_settings.tn5
     label TN5 shifting
     type basic:boolean
     description Tn5 transposon shifting. Shift reads on "+" strand by 4bp and reads on "-" strand by 5bp.
     default False
prepeakqc_settings.shift
```

label User-defined cross-correlation peak strandshift

```
type basic:integer
```

description If defined, SPP tool will not try to estimate fragment length but will use the given value as fragment length.

required False

settings.duplicates

label Number of duplicates

type basic:string

description It controls the MACS behavior towards duplicate tags at the exact same location – the same coordination and the same strand. The 'auto' option makes MACS calculate the maximum tags at the exact same location based on binomal distribution using 1e-5 as pvalue cutoff and the 'all' option keeps all the tags. If an integer is given, at most this number of tags will be kept at the same location. The default is to keep one tag at the same location.

required False

hidden tagalign

choices

- 1: 1
- auto: auto
- all: all

settings.duplicates_prepeak

label Number of duplicates

type basic:string

description It controls the MACS behavior towards duplicate tags at the exact same location – the same coordination and the same strand. The 'auto' option makes MACS calculate the maximum tags at the exact same location based on binomal distribution using 1e-5 as pvalue cutoff and the 'all' option keeps all the tags. If an integer is given, at most this number of tags will be kept at the same location. The default is to keep one tag at the same location.

required False

hidden !tagalign

default all

choices

- 1: 1
- auto: auto
- all: all

settings.qvalue

label Q-value cutoff

type basic:decimal

description The q-value (minimum FDR) cutoff to call significant regions. Q-values are calculated from p-values using Benjamini-Hochberg procedure.

```
required False
     disabled settings.pvalue && settings.pvalue_prepeak
settings.pvalue
     label P-value cutoff
     type basic:decimal
     description The p-value cutoff. If specified, MACS2 will use p-value instead of q-value cutoff.
     required False
     disabled settings.qvalue
     hidden tagalign
settings.pvalue_prepeak
     label P-value cutoff
     type basic:decimal
     description The p-value cutoff. If specified, MACS2 will use p-value instead of q-value cutoff.
     disabled settings.qvalue
     hidden !tagalign || settings.qvalue
     default 1e-05
settings.cap_num
     label Cap number of peaks by taking top N peaks
     type basic:integer
     description To keep all peaks set value to 0.
     disabled settings.broad
     default 500000
settings.mfold_lower
     label MFOLD range (lower limit)
     type basic:integer
     description This parameter is used to select the regions within MFOLD range of high-confidence en-
           richment ratio against background to build model. The regions must be lower than upper limit, and
           higher than the lower limit of fold enrichment. DEFAULT:10,30 means using all regions not too
           low (>10) and not too high (<30) to build paired-peaks model. If MACS can not find more than 100
           regions to build model, it will use the -extsize parameter to continue the peak detection ONLY if
           -fix-bimodal is set.
     required False
settings.mfold_upper
     label MFOLD range (upper limit)
     type basic:integer
     description This parameter is used to select the regions within MFOLD range of high-confidence en-
           richment ratio against background to build model. The regions must be lower than upper limit, and
```

higher than the lower limit of fold enrichment. DEFAULT:10,30 means using all regions not too low (>10) and not too high (<30) to build paired-peaks model. If MACS can not find more than 100

regions to build model, it will use the -extsize parameter to continue the peak detection ONLY if -fix-bimodal is set.

required False

settings.slocal

label Small local region

type basic:integer

description Slocal and llocal parameters control which two levels of regions will be checked around the peak regions to calculate the maximum lambda as local lambda. By default, MACS considers 1000bp for small local region (–slocal), and 10000bps for large local region (–llocal) which captures the bias from a long range effect like an open chromatin domain. You can tweak these according to your project. Remember that if the region is set too small, a sharp spike in the input data may kill the significant peak.

required False

settings.llocal

label Large local region

type basic:integer

description Slocal and llocal parameters control which two levels of regions will be checked around the peak regions to calculate the maximum lambda as local lambda. By default, MACS considers 1000bp for small local region (–slocal), and 10000bps for large local region (–llocal) which captures the bias from a long range effect like an open chromatin domain. You can tweak these according to your project. Remember that if the region is set too small, a sharp spike in the input data may kill the significant peak.

required False

settings.extsize

label extsize

type basic:integer

description While '-nomodel' is set, MACS uses this parameter to extend reads in 5'->3' direction to fix-sized fragments. For example, if the size of binding region for your transcription factor is 200 bp, and you want to bypass the model building by MACS, this parameter can be set as 200. This option is only valid when -nomodel is set or when MACS fails to build model and -fix-bimodal is on.

required False

settings.shift

label Shift

type basic:integer

description Note, this is NOT the legacy –shiftsize option which is replaced by –extsize! You can set an arbitrary shift in bp here. Please Use discretion while setting it other than default value (0). When –nomodel is set, MACS will use this value to move cutting ends (5') then apply –extsize from 5' to 3' direction to extend them to fragments. When this value is negative, ends will be moved toward 3'->5' direction, otherwise 5'->3' direction. Recommended to keep it as default 0 for ChIP-Seq datasets, or -1 * half of EXTSIZE together with –extsize option for detecting enriched cutting loci such as certain DNAseI-Seq datasets. Note, you can't set values other than 0 if format is BAMPE for paired-end data. Default is 0.

```
required False
```

settings.band_width

label Band width

type basic:integer

description The band width which is used to scan the genome ONLY for model building. You can set this parameter as the sonication fragment size expected from wet experiment. The previous side effect on the peak detection process has been removed. So this parameter only affects the model building.

required False

settings.nolambda

label Use backgroud lambda as local lambda

type basic:boolean

description With this flag on, MACS will use the background lambda as local lambda. This means MACS will not consider the local bias at peak candidate regions.

default False

settings.fix_bimodal

label Turn on the auto paired-peak model process

type basic:boolean

description Whether turn on the auto paired-peak model process. If it's set, when MACS failed to build paired model, it will use the nomodel settings, the '-extsize' parameter to extend each tags. If set, MACS will be terminated if paired-peak model is failed.

default False

settings.nomodel

label Bypass building the shifting model

type basic:boolean

description While on, MACS will bypass building the shifting model.

hidden tagalign
default False

settings.nomodel_prepeak

label Bypass building the shifting model

type basic:boolean

description While on, MACS will bypass building the shifting model.

hidden !tagalign
default True

settings.down_sample

label Down-sample

type basic:boolean

description When set, random sampling method will scale down the bigger sample. By default, MACS uses linear scaling. This option will make the results unstable and irreproducible since each time, random reads would be selected, especially the numbers (pileup, pvalue, qvalue) would change. Consider to use 'randsample' script before MACS2 runs instead.

default False

settings.bedgraph

label Save fragment pileup and control lambda

type basic:boolean

description If this flag is on, MACS will store the fragment pileup, control lambda, -log10pvalue and -log10qvalue scores in bedGraph files. The bedGraph files will be stored in current directory named NAME+'_treat_pileup.bdg' for treatment data, NAME+'_control_lambda.bdg' for local lambda values from control, NAME+'_treat_pvalue.bdg' for Poisson pvalue scores (in -log10(pvalue) form), and NAME+'_treat_qvalue.bdg' for q-value scores from Benjamini-Hochberg-Yekutieli procedure.

default True

settings.spmr

label Save signal per million reads for fragment pileup profiles

type basic:boolean

disabled settings.bedgraph === false

default True

settings.call summits

label Call summits

type basic:boolean

description MACS will now reanalyze the shape of signal profile (p or q-score depending on cutoff setting) to deconvolve subpeaks within each peak called from general procedure. It's highly recommended to detect adjacent binding events. While used, the output subpeaks of a big peak region will have the same peak boundaries, and different scores and peak summit positions.

default False

settings.broad

label Composite broad regions

type basic:boolean

description When this flag is on, MACS will try to composite broad regions in BED12 (a gene-model-like format) by putting nearby highly enriched regions into a broad region with loose cutoff. The broad region is controlled by another cutoff through –broad-cutoff. The maximum length of broad region length is 4 times of d from MACS.

disabled settings.call_summits === true

default False

settings.broad cutoff

label Broad cutoff

type basic:decimal

description Cutoff for broad region. This option is not available unless –broad is set. If -p is set, this is a p-value cutoff, otherwise, it's a q-value cutoff. DEFAULT = 0.1

```
required False
     disabled settings.call_summits === true || settings.broad !== true
rose_settings.tss
     label TSS exclusion
     type basic:integer
     description Enter a distance from TSS to exclude. 0 = \text{no TSS} exclusion
     default 0
rose_settings.stitch
     label Stitch
     type basic:integer
     description Enter a max linking distance for stitching. If not given, optimal stitching parameter will be
           determined automatically.
     required False
rose settings.mask
     label Masking BED file
     type data:bed
     description Mask a set of regions from analysis. Provide a BED of masking regions.
     required False
Output results
Chemical Mutagenesis
data:workflow:chemutworkflow-chemut (basic:string
                                                                                         analysis_type,
                                                   data:genome:fasta
                                                                                               genome,
                                                   list:data:alignment:bam
                                                                                      parental_strains,
                                                   list:data:alignment:bam
                                                                                       mutant_strains,
                                                   basic:boolean
                                                                                advanced.
                                                                                                    ba-
                                                                 br_and_ind_ra, basic:boolean
                                                   sic:boolean
                                                                                                   db-
                                                              data:variants:vcf
                                                                                          known_sites,
                                                   snp,
                                                   list:data:variants:vcf
                                                                                        known indels,
                                                   basic:integer
                                                                           stand_emit_conf,
                                                                                                   ba-
                                                   sic:integer
                                                                           stand call conf,
                                                                                                    ba-
                                                   sic:boolean
                                                                  rf, basic:boolean
                                                                                       advanced, ba-
                                                   sic:integer read_depth) [Source: v0.0.6]
Input arguments analysis_type
     label Analysis type
     type basic:string
     description Choice of the analysis type. Use "SNV" or "INDEL" options to run the GATK analysis only
           on the haploid portion of the dicty genome. Choose options SNV_CHR2 or INDEL_CHR2 to run
           the analysis only on the diploid portion of CHR2 (-ploidy 2 -L chr2:2263132-3015703).
     default snv
```

choices

```
• SNV: snv
            • INDEL: indel
            • SNV_CHR2: snv_chr2
            • INDEL_CHR2: indel_chr2
genome
     label Reference genome
     type data:genome:fasta
parental_strains
     label Parental strains
     type list:data:alignment:bam
mutant_strains
     label Mutant strains
     type list:data:alignment:bam
Vc.advanced
     label Advanced options
     type basic:boolean
     required False
     default False
Vc.br_and_ind_ra
     label Do variant base recalibration and indel realignment
     type basic:boolean
     required False
     hidden Vc.advanced === false
     default False
Vc.dbsnp
     label Use dbSNP file
     type basic:boolean
     description rsIDs from this file are used to populate the ID column of the output. Also, the DB INFO
          flag will be set when appropriate. dbSNP is not used in any way for the calculations themselves.
     required False
     hidden Vc.advanced === false
     default False
Vc.known sites
     label Known sites (dbSNP)
     type data:variants:vcf
     required False
```

```
hidden Vc.advanced === false || Vc.br_and_ind_ra === false && Vc.dbsnp === false
Vc.known indels
     label Known indels
     type list:data:variants:vcf
     required False
     hidden Vc.advanced === false || Vc.br and ind ra === false
     default []
Vc.stand_emit_conf
     label Emission confidence threshold
     type basic:integer
     description The minimum confidence threshold (phred-scaled) at which the program should emit sites
           that appear to be possibly variant.
     required False
     hidden Vc.advanced === false
     default 10
Vc.stand_call_conf
     label Calling confidence threshold
     type basic:integer
     description The minimum confidence threshold (phred-scaled) at which the program should emit variant
           sites as called. If a site's associated genotype has a confidence score lower than the calling threshold,
           the program will emit the site as filtered and will annotate it as LowQual. This threshold separates
           high confidence calls from low confidence calls.
     required False
     hidden Vc.advanced === false
     default 30
Vc.rf
     label ReasignOneMappingQuality Filter
     type basic:boolean
     description This read transformer will change a certain read mapping quality to a different value without
           affecting reads that have other mapping qualities. This is intended primarily for users of RNA-Seq
           data handling programs such as TopHat, which use MAPQ = 255 to designate uniquely aligned
           reads. According to convention, 255 normally designates "unknown" quality, and most GATK tools
           automatically ignore such reads. By reassigning a different mapping quality to those specific reads,
           users of TopHat and other tools can circumvent this problem without affecting the rest of their
           dataset.
     required False
     hidden Vc.advanced === false
     default False
Vf.advanced
```

```
label Advanced options
     type basic:boolean
     required False
     default False
Vf.read_depth
     label Read depth cutoff
     type basic:integer
     description The minimum number of replicate reads required for a variant site to be included.
     required False
     hidden Vf.advanced === false
     default 5
Output results
Convert GFF3 to GTF
data:annotation:gtfgff-to-gtf (data:annotation:gff3 annotation) [Source: v0.3.1]
Convert GFF3 file to GTF format.
Input arguments annotation
     label Annotation (GFF3)
     type data:annotation:gff3
     description Annotation in GFF3 format.
Output results annot
     label Converted GTF file
     type basic:file
annot sorted
     label Sorted GTF file
     type basic:file
annot_sorted_idx_igv
     label Igv index for sorted GTF file
     type basic:file
annot sorted track jbrowse
     label Jbrowse track for sorted GTF
     type basic:file
source
     label Gene ID database
     type basic:string
species
```

```
label Species
     type basic:string
build
     label Build
     type basic:string
Convert files to reads (paired-end)
data:reads:fastq:pairedfiles-to-fastq-paired (list:data:file
                                                                                          src1,
                                                           list:data:file src2) [Source: v1.2.1]
Convert FASTQ files to paired-end reads.
Input arguments src1
     label Mate1
     type list:data:file
src2
     label Mate2
     type list:data:file
Output results fastq
     label Reads file (mate 1)
     type list:basic:file
fastq2
     label Reads file (mate 2)
     type list:basic:file
fastqc_url
     label Quality control with FastQC (Upstream)
     type list:basic:file:html
fastqc_url2
     label Quality control with FastQC (Downstream)
     type list:basic:file:html
fastqc_archive
     label Download FastQC archive (Upstream)
     type list:basic:file
fastqc_archive2
     label Download FastQC archive (Downstream)
     type list:basic:file
```

Convert files to reads (single-end)

```
data:reads:fastq:singlefiles-to-fastq-single (list:data:file src) [Source: v1.2.1]

Convert FASTQ files to single-end reads.

Input arguments src

label Reads

type list:data:file

description Sequencing reads in FASTQ format

Output results fastq

label Reads file

type list:basic:file

fastqc_url

label Quality control with FastQC

type list:basic:file:html

fastqc_archive

label Download FastQC archive

type list:basic:file
```

Cuffdiff 2.2

```
data:differentialexpression:cuffdiffcuffdiff (list:data:cufflinks:cuffquant
                                                                                                 case.
                                                               list:data:cufflinks:cuffquant
                                                                                                  con-
                                                               trol.
                                                                        list:basic:string
                                                                                                labels,
                                                               data:annotation
                                                                                           annotation,
                                                               data:genome:fasta
                                                                                       genome.
                                                                                                   ba-
                                                               sic:boolean
                                                                                  multi read correct,
                                                                               fdr, basic:string
                                                               basic:decimal
                                                                                                    li-
                                                               brary type,
                                                                                basic:string
                                                                                                    li-
                                                               brary_normalization, basic:string dis-
                                                               persion method) [Source: v2.2.1]
```

Cuffdiff finds significant changes in transcript expression, splicing, and promoter use. You can use it to find differentially expressed genes and transcripts, as well as genes that are being differentially regulated at the transcriptional and post-transcriptional level. See [here](http://cole-trapnell-lab.github.io/cufflinks/cuffdiff/) and [here](https://software.broadinstitute.org/cancer/software/genepattern/modules/docs/Cuffdiff/7) for more information.

Input arguments case

```
label Case samples
    type list:data:cufflinks:cuffquant
control
    label Control samples
    type list:data:cufflinks:cuffquant
labels
    label Group labels
```

```
type list:basic:string
     description Define labels for each sample group.
     default ['control', 'case']
annotation
     label Annotation (GTF/GFF3)
     type data: annotation
     description A transcript annotation file produced by cufflinks, cuffcompare, or other tool.
genome
     label Run bias detection and correction algorithm
     type data:genome:fasta
     description Provide Cufflinks with a multifasta file (genome file) via this option to instruct it to run a bias
           detection and correction algorithm which can significantly improve accuracy of transcript abundance
           estimates.
     required False
multi_read_correct
     label Do initial estimation procedure to more accurately weight reads with multiple genome mappings
     type basic:boolean
     default False
fdr
     label Allowed FDR
     type basic:decimal
     description The allowed false discovery rate. The default is 0.05.
     default 0.05
library_type
     label Library type
     type basic:string
     description In cases where Cufflinks cannot determine the platform and protocol used to generate input
```

lescription In cases where Cufflinks cannot determine the platform and protocol used to generate input reads, you can supply this information manually, which will allow Cufflinks to infer source strand information with certain protocols. The available options are listed below. For paired-end data, we currently only support protocols where reads are point towards each other: fr-unstranded - Reads from the left-most end of the fragment (in transcript coordinates) map to the transcript strand, and the right-most end maps to the opposite strand; fr-firststrand - Same as above except we enforce the rule that the right-most end of the fragment (in transcript coordinates) is the first sequenced (or only sequenced for single-end reads). Equivalently, it is assumed that only the strand generated during first strand synthesis is sequenced; fr-secondstrand - Same as above except we enforce the rule that the left-most end of the fragment (in transcript coordinates) is the first sequenced (or only sequenced for single-end reads). Equivalently, it is assumed that only the strand generated during second strand synthesis is sequenced.

```
default fr-unstranded
choices
```

fr-unstranded: fr-unstranded
 fr-firststrand: fr-firststrand
 fr-secondstrand: fr-secondstrand

library_normalization

label Library normalization method

type basic:string

description You can control how library sizes (i.e. sequencing depths) are normalized in Cufflinks and Cuffdiff. Cuffdiff has several methods that require multiple libraries in order to work. Library normalization methods supported by Cufflinks work on one library at a time.

default geometric

choices

• geometric: geometric

• classic-fpkm: classic-fpkm

• quartile: quartile

dispersion_method

label Dispersion method

type basic:string

description Cuffdiff works by modeling the variance in fragment counts across replicates as a function of the mean fragment count across replicates. Strictly speaking, models a quantitity called dispersion - the variance present in a group of samples beyond what is expected from a simple Poisson model of RNA_Seq. You can control how Cuffdiff constructs its model of dispersion in locus fragment counts. Each condition that has replicates can receive its own model, or Cuffdiff can use a global model for all conditions. All of these policies are identical to those used by DESeq (Anders and Huber, Genome Biology, 2010).

default pooled

choices

• pooled: pooled

• per-condition: per-condition

• blind: blind

• poisson: poisson

Output results raw

label Differential expression (gene level)

type basic:file

de_json

label Results table (JSON)

type basic: json

de_file

label Results table (file)

type basic:file

```
transcript_diff_exp
     label Differential expression (transcript level)
     type basic:file
tss_group_diff_exp
     label Differential expression (primary transcript)
     type basic:file
cds_diff_exp
     label Differential expression (coding sequence)
     type basic:file
cuffdiff_output
     label Cuffdiff output
     type basic:file
source
     label Gene ID database
     type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
feature_type
     label Feature type
     type basic:string
```

Cufflinks 2.2

```
data:cufflinks:cufflinks (data:alignment:bam alignment, data:annotation annotation, data:genome:fasta genome, data:annotation:gtf mask_file, basic:string library_type, basic:string annotation_usage, basic:boolean multi_read_correct) [Source: v2.1.1]
```

Cufflinks assembles transcripts, estimates their abundances, and tests for differential expression and regulation in RNA-Seq samples. It accepts aligned RNA-Seq reads and assembles the alignments into a parsimonious set of transcripts. Cufflinks then estimates the relative abundances of these transcripts based on how many reads support each one, taking into account biases in library preparation protocols. See [here](http://cole-trapnell-lab.github.io/cufflinks/) for more information.

Input arguments alignment

```
label Aligned reads
type data:alignment:bam
```

annotation

label Annotation (GTF/GFF3)
type data:annotation
required False

genome

label Run bias detection and correction algorithm

type data:genome:fasta

description Provide Cufflinks with a multifasta file (genome file) via this option to instruct it to run a bias detection and correction algorithm which can significantly improve accuracy of transcript abundance estimates.

required False

mask file

label Mask file

type data:annotation:gtf

description Ignore all reads that could have come from transcripts in this GTF file. We recommend including any annotated rRNA, mitochondrial transcripts other abundant transcripts you wish to ignore in your analysis in this file. Due to variable efficiency of mRNA enrichment methods and rRNA depletion kits, masking these transcripts often improves the overall robustness of transcript abundance estimates.

required False

library_type

label Library type

type basic:string

description In cases where Cufflinks cannot determine the platform and protocol used to generate input reads, you can supply this information manually, which will allow Cufflinks to infer source strand information with certain protocols. The available options are listed below. For paired-end data, we currently only support protocols where reads are point towards each other: fr-unstranded - Reads from the left-most end of the fragment (in transcript coordinates) map to the transcript strand, and the right-most end maps to the opposite strand; fr-firststrand - Same as above except we enforce the rule that the right-most end of the fragment (in transcript coordinates) is the first sequenced (or only sequenced for single-end reads). Equivalently, it is assumed that only the strand generated during first strand synthesis is sequenced; fr-secondstrand - Same as above except we enforce the rule that the left-most end of the fragment (in transcript coordinates) is the first sequenced (or only sequenced for single-end reads). Equivalently, it is assumed that only the strand generated during second strand synthesis is sequenced.

default fr-unstranded

choices

fr-unstranded: fr-unstranded
 fr-firststrand: fr-firststrand
 fr-secondstrand: fr-secondstrand

annotation usage

label Instruct Cufflinks how to use the provided annotation (GFF/GTF) file

```
type basic:string
```

description GTF-guide - tells Cufflinks to use the supplied reference annotation (GFF) to guide RABT assembly. Reference transcripts will be tiled with faux-reads to provide additional information in assembly. Output will include all reference transcripts as well as any novel genes and isoforms that are assembled. –GTF - tells Cufflinks to use the supplied reference annotation (a GFF file) to estimate isoform expression. It will not assemble novel transcripts, and the program will ignore alignments not structurally compatible with any reference transcript.

```
default --GTF-guide
choices
```

- Use supplied reference annotation to guide RABT assembly (-GTF-guide): --GTF-guide
- Use supplied reference annotation to estimate isoform expression (–GTF): --GTF

multi_read_correct

```
label Do initial estimation procedure to more accurately weight reads with multiple genome mappings
```

```
type basic:boolean
```

description Run an initial estimation procedure that weights reads mapping to multiple locations more accurately.

```
default False
```

Output results transcripts

```
label Assembled transcript isoforms
```

```
type basic:file
```

isoforms_fpkm_tracking

```
label Isoforms FPKM tracking
```

```
type basic:file
```

genes_fpkm_tracking

```
label Genes FPKM tracking
```

```
type basic:file
```

skipped_loci

label Skipped loci

type basic:file

source

label Gene ID database

type basic:string

species

label Species

type basic:string

build

label Build

type basic:string

Cuffmerge

```
data:annotation:cuffmergecuffmerge (list:data:cufflinks:cufflinks
                                                                                         expressions,
                                                 list:data:annotation:gtf
                                                                          gtf, data:annotation
                                                                                                  gff,
                                                 data:genome:fasta
                                                                                 genome,
                                                                                                  ba-
                                                 sic:integer threads) [Source: v1.3.1]
```

Cufflinks includes a script called Cuffmerge that you can use to merge together several Cufflinks assemblies. It also handles running Cuffcompare for you, and automatically filters a number of transfrags that are probably artifiacts. The main purpose of Cuffmerge is to make it easier to make an assembly GTF file suitable for use with Cuffdiff. See [here](http://cole-trapnell-lab.github.io/cufflinks/cuffmerge/) for more information.

```
Input arguments expressions
     label Cufflinks transcripts (GTF)
     type list:data:cufflinks:cufflinks
     required False
gtf
     label Annotation files (GTF)
     type list:data:annotation:gtf
     description Annotation files you wish to merge together with Cufflinks produced annotation files (e.g.
           upload Cufflinks annotation GTF file)
     required False
gff
     label Reference annotation (GTF/GFF3)
     type data: annotation
     description An optional "reference" annotation GTF. The input assemblies are merged together with the
           reference GTF and included in the final output.
     required False
genome
     label Reference genome
     type data:genome:fasta
     description This argument should point to the genomic DNA sequences for the reference. If a directory,
           it should contain one fasta file per contig. If a multifasta file, all contigs should be present. The
           merge script will pass this option to cuffcompare, which will use the sequences to assist in classifying
           transfrags and excluding artifacts (e.g. repeats). For example, Cufflinks transcripts consisting mostly
           of lower-case bases are classified as repeats. Note that <seq_dir> must contain one fasta file per
           reference chromosome, and each file must be named after the chromosome, and have a .fa or .fasta
           extension
     required False
threads
     label Use this many processor threads
     type basic:integer
     description Use this many threads to align reads. The default is 1.
     default 1
```

```
Output results annot
    label Merged GTF file
    type basic:file
source
    label Gene ID database
    type basic:string
species
    label Species
    type basic:string
build
    label Build
```

type basic:string

Cuffnorm

```
data:cuffnormcuffnorm (list:data:cufflinks:cuffquant cuffquant, data:annotation annotation, basic:boolean useERCC) [Source: v2.1.3]
```

Cufflinks includes a program, Cuffnorm, that you can use to generate tables of expression values that are properly normalized for library size. Cuffnorm takes a GTF2/GFF3 file of transcripts as input, along with two or more SAM, BAM, or CXB files for two or more samples. See [here](http://cole-trapnell-lab.github.io/cufflinks/cuffnorm/) for more information.

Replicate relation needs to be defined for Cuffnorm to account for replicates. If the replicate relation is not defined, each sample will be treated individually.

Input arguments cuffquant

```
label Cuffquant expression file
type list:data:cufflinks:cuffquant
annotation
label Annotation (GTF/GFF3)
type data:annotation
description A transcript annotation file produced by cufflinks, cuffcompare, or other source.
useERCC
label ERCC spike-in normalization
type basic:boolean
description Use ERRCC spike-in controls for normalization.
default False
Output results genes_count
label Genes count
```

134

genes_fpkm

type basic:file

```
label Genes FPKM
     type basic:file
genes_attr
     label Genes attr table
     type basic:file
isoform_count
     label Isoform count
     type basic:file
isoform_fpkm
     label Isoform FPKM
     type basic:file
isoform_attr
     label Isoform attr table
     type basic:file
cds_count
     label CDS count
     type basic:file
cds_fpkm
     label CDS FPKM
     type basic:file
cds_attr
     label CDS attr table
     type basic:file
tss_groups_count
     label TSS groups count
     type basic:file
tss_groups_fpkm
     label TSS groups FPKM
     type basic:file
tss_attr
     label TSS attr table
     type basic:file
run_info
     label Run info
     type basic:file
raw_scatter
```

```
label FPKM exp scatter plot
     type basic:file
boxplot
     label Boxplot
     type basic:file
fpkm_exp_raw
     label FPKM exp raw
     type basic:file
replicate_correlations
     label Replicate correlatios plot
     type basic:file
fpkm_means
     label FPKM means
     type basic:file
exp_fpkm_means
     label Exp FPKM means
     type basic:file
norm\_scatter
     label FKPM exp scatter normalized plot
     type basic:file
     required False
fpkm_exp_norm
     label FPKM exp normalized
     type basic:file
     required False
spike_raw
     label Spike raw
     type basic:file
     required False
spike_norm
     label Spike normalized
     type basic:file
     required False
R_data
     label All R normalization data
     type basic:file
```

source

```
label Gene ID database
    type basic:string
species
    label Species
    type basic:string
build
    label Build
    type basic:string
```

Cuffquant 2.2

```
data:cufflinks:cuffquantcuffquant (data:alignment:bam alignment, data:annotation annotation, data:genome:fasta genome, data:annotation:gtf mask_file, basic:string library_type, basic:boolean multi_read_correct) [Source: v1.3.1]
```

Cuffquant allows you to compute the gene and transcript expression profiles and save these profiles to files that you can analyze later with Cuffdiff or Cuffnorm. See [here](http://cole-trapnell-lab.github.io/cufflinks/manual/) for more information.

Input arguments alignment

```
label Aligned reads
    type data:alignment:bam
annotation
    label Annotation (GTF/GFF3)
    type data:annotation
```

genome

label Run bias detection and correction algorithm

```
type data:genome:fasta
```

description Provide Cufflinks with a multifasta file (genome file) via this option to instruct it to run a bias detection and correction algorithm which can significantly improve accuracy of transcript abundance estimates.

required False

mask file

label Mask file

type data:annotation:gtf

description Ignore all reads that could have come from transcripts in this GTF file. We recommend including any annotated rRNA, mitochondrial transcripts other abundant transcripts you wish to ignore in your analysis in this file. Due to variable efficiency of mRNA enrichment methods and rRNA depletion kits, masking these transcripts often improves the overall robustness of transcript abundance estimates.

```
required False
```

library_type

```
label Library type
```

```
type basic:string
```

description In cases where Cufflinks cannot determine the platform and protocol used to generate input reads, you can supply this information manually, which will allow Cufflinks to infer source strand information with certain protocols. The available options are listed below. For paired-end data, we currently only support protocols where reads are point towards each other: fr-unstranded - Reads from the left-most end of the fragment (in transcript coordinates) map to the transcript strand, and the right-most end maps to the opposite strand; fr-firststrand - Same as above except we enforce the rule that the right-most end of the fragment (in transcript coordinates) is the first sequenced (or only sequenced for single-end reads). Equivalently, it is assumed that only the strand generated during first strand synthesis is sequenced; fr-secondstrand - Same as above except we enforce the rule that the left-most end of the fragment (in transcript coordinates) is the first sequenced (or only sequenced for single-end reads). Equivalently, it is assumed that only the strand generated during second strand synthesis is sequenced.

default fr-unstranded

choices

fr-unstranded: fr-unstranded
 fr-firststrand: fr-firststrand
 fr-secondstrand: fr-secondstrand

multi_read_correct

label Do initial estimation procedure to more accurately weight reads with multiple genome mappings

```
type basic:boolean
```

description Run an initial estimation procedure that weights reads mapping to multiple locations more accurately.

default False

Output results exb

```
label Abundances (.cxb)
```

type basic:file

source

label Gene ID database

type basic:string

species

label Species

type basic:string

build

138

label Build

type basic:string

Cuffquant results

```
data:cufflinks:cuffquantupload-cxb (basic:file src, basic:string source, basic:string species,
                                             basic:string build, basic:string feature_type) [Source:
                                             v1.2.1]
Upload Cuffquant results file (.cxb)
Input arguments src
     label Cuffquant file
     type basic:file
     description Upload Cuffquant results file. Supported extention: *.cxb
     required True
     validate_regex \.(cxb)$
source
     label Gene ID database
     type basic:string
     choices
            • AFFY: AFFY
            • DICTYBASE: DICTYBASE
            • ENSEMBL: ENSEMBL
            • NCBI: NCBI
            • UCSC: UCSC
species
     label Species
     type basic:string
     description Species latin name.
     choices
            • Homo sapiens: Homo sapiens
            • Mus musculus: Mus musculus
            • Rattus norvegicus: Rattus norvegicus
            • Dictyostelium discoideum: Dictyostelium discoideum
            • Odocoileus virginianus texanus: Odocoileus virginianus texanus
            • Solanum tuberosum: Solanum tuberosum
build
     label Build
     type basic:string
feature_type
     label Feature type
     type basic:string
```

```
default gene
     choices
            • gene: gene
            • transcript: transcript
            • exon: exon
Output results cxb
     label Cuffquant results
     type basic:file
source
     label Gene ID database
     type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
feature_type
     label Feature type
     type basic:string
Custom master file
data:masterfile:ampliconupload-master-file (basic:file
                                                                                src.
                                                                                            ba-
                                                        sic:string panel_name) [Source: v1.1.1]
This should be a tab delimited file (*.txt). Please check the [example](http://genial.is/amplicon-masterfile) file for
details.
Input arguments src
     label Master file
     type basic:file
     validate_regex \.txt(|\.gz|\.bz2|\.tgz|\.tar\.gz|\.tar\.bz2|\.zip|\.
          rar|\.7z)$
panel name
     label Panel name
     type basic:string
Output results master_file
     label Master file
     type basic:file
```

```
bedfile
     label BED file (merged targets)
     type basic:file
nomergebed
     label BED file (nonmerged targets)
     type basic:file
olapfreebed
     label BED file (overlap-free targets)
     type basic:file
primers
     label Primers
     type basic:file
panel_name
     label Panel name
     type basic:string
Cutadapt (Diagenode CATS, paired-end)
data:reads:fastq:paired:cutadaptcutadapt-custom-paired (data:reads:fastq:paired reads) [Source:
                                                                       v1.1.2]
Cutadapt process configured to be used with the Diagenode CATS kits.
Input arguments reads
     label NGS reads
     type data:reads:fastq:paired
Output results fastq
     label Reads file (forward)
     type list:basic:file
fastq2
     label Reads file (reverse)
     type list:basic:file
report
     label Cutadapt report
     type basic:file
fastqc_url
     label Quality control with FastQC (forward)
     type list:basic:file:html
fastqc_url2
```

```
label Quality control with FastQC (reverse)
     type list:basic:file:html
fastqc_archive
     label Download FastQC archive (forward)
     type list:basic:file
fastqc_archive2
     label Download FastQC archive (reverse)
     type list:basic:file
Cutadapt (Diagenode CATS, single-end)
data:reads:fastq:single:cutadaptcutadapt-custom-single (data:reads:fastq:single reads) [Source:
                                                                       v1.1.2]
Cutadapt process configured to be used with the Diagenode CATS kits.
Input arguments reads
     label NGS reads
     type data:reads:fastq:single
Output results fastq
     label Reads file
     type list:basic:file
report
     label Cutadapt report
     type basic:file
fastqc_url
     label Quality control with FastQC
     type list:basic:file:html
fastqc_archive
     label Download FastQC archive
     type list:basic:file
```

Cutadapt (paired-end)

```
data:reads:fastq:paired:cutadaptcutadapt-paired (data:reads:fastq:paired
                                                                    data:seq:nucleotide mate1_5prime_file,
                                                                   data:seq:nucleotide mate1_3prime_file,
                                                                   data:seq:nucleotide mate2_5prime_file,
                                                                    data:seq:nucleotide mate2_3prime_file,
                                                                    list:basic:string mate1_5prime_seq,
                                                                    list:basic:string mate1_3prime_seq,
                                                                    list:basic:string mate2_5prime_seq,
                                                                    list:basic:string mate2_3prime_seq,
                                                                   basic:integer
                                                                                        times,
                                                                                                   ba-
                                                                   sic:decimal
                                                                                    error rate.
                                                                                                   ba-
                                                                   sic:integer
                                                                                   min overlap,
                                                                   sic:boolean match_read_wildcards,
                                                                   basic:integer
                                                                                       leading,
                                                                   sic:integer
                                                                                      trailing,
                                                                                                   ba-
                                                                   sic:integer crop, basic:integer head-
                                                                                               minlen.
                                                                    crop,
                                                                            basic:integer
                                                                                       max_ n.
                                                                    basic:integer
                                                                                                   ba-
                                                                   sic:string
                                                                                   pair_filter) [Source:
```

Cutadapt finds and removes adapter sequences, primers, poly-A tails and other types of unwanted sequence from high-throughput sequencing reads. More information about Cutadapt can be found [here](http://cutadapt.readthedocs.io/en/stable/).

v2.1.2]

Input arguments reads

```
label NGS reads
```

type data:reads:fastq:paired

adapters.mate1_5prime_file

label 5 prime adapter file for Mate 1

type data:seq:nucleotide

required False

$adapters.mate1_3prime_file$

label 3 prime adapter file for Mate 1

type data:seq:nucleotide

required False

adapters.mate2_5prime_file

label 5 prime adapter file for Mate 2

type data:seq:nucleotide

required False

adapters.mate2_3prime_file

label 3 prime adapter file for Mate 2

type data:seq:nucleotide

required False

```
adapters.mate1_5prime_seq
     label 5 prime adapter sequence for Mate 1
     type list:basic:string
     required False
adapters.mate1_3prime_seq
     label 3 prime adapter sequence for Mate 1
     type list:basic:string
     required False
adapters.mate2_5prime_seq
     label 5 prime adapter sequence for Mate 2
     type list:basic:string
     required False
adapters.mate2_3prime_seq
     label 3 prime adapter sequence for Mate 2
     type list:basic:string
     required False
adapters.times
     label Times
     type basic:integer
     description Remove up to COUNT adapters from each read.
     default 1
adapters.error_rate
     label Error rate
     type basic:decimal
     description Maximum allowed error rate (no. of errors divided by the length of the matching region).
     default 0.1
adapters.min_overlap
     label Minimal overlap
     type basic:integer
     description Minimum overlap for an adapter match.
     default 3
adapters.match_read_wildcards
     label Match read wildcards
     type basic:boolean
     description Interpret IUPAC wildcards in reads.
     default False
```

```
modify_reads.leading
     label Quality on 5 prime
     type basic:integer
     description Remove low quality bases from 5 prime. Specifies the minimum quality required to keep a
          base.
     required False
modify_reads.trailing
     label Quality on 3 prime
     type basic:integer
     description Remove low quality bases from the 3 prime. Specifies the minimum quality required to keep
           a base.
     required False
modify_reads.crop
     label Crop
     type basic:integer
     description Cut the specified number of bases from the end of the reads.
     required False
modify_reads.headcrop
     label Headcrop
     type basic:integer
     description Cut the specified number of bases from the start of the reads.
     required False
filtering.minlen
     label Min length
     type basic:integer
     description Drop the read if it is below a specified.
     required False
filtering.max n
     label Max numebr of N-s
     type basic:integer
     description Discard reads having more 'N' bases than specified.
     required False
filtering.pair_filter
     label Which of the reads have to match the filtering criterion
     type basic:string
     description Which of the reads in a paired-end read have to match the filtering criterion in order for the
```

pair to be filtered.

```
default any
choices
```

- Any of the reads in a paired-end read have to match the filtering criterion: any
- Both of the reads in a paired-end read have to match the filtering criterion: both

```
Output results fastq
     label Reads file (forward)
     type list:basic:file
fastq2
     label Reads file (reverse)
     type list:basic:file
report
     label Cutadapt report
     type basic:file
fastqc_url
     label Quality control with FastQC (forward)
     type list:basic:file:html
fastqc_url2
     label Quality control with FastQC (reverse)
     type list:basic:file:html
fastqc_archive
     label Download FastQC archive (forward)
     type list:basic:file
fastqc_archive2
     label Download FastQC archive (reverse)
     type list:basic:file
```

Cutadapt (single-end)

```
data:reads:fastq:single:cutadaptcutadapt-single (data:reads:fastq:single
                                                                   data:seq:nucleotide up_primers_file,
                                                                   data:seq:nucleotide down_primers_file,
                                                                   list:basic:string
                                                                                     up_primers_seq,
                                                                   list:basic:string down_primers_seq,
                                                                   basic:integer
                                                                                           polya_tail,
                                                                   basic:integer
                                                                                      leading,
                                                                                                  ba-
                                                                   sic:integer
                                                                                     trailing,
                                                                                                  ba-
                                                                   sic:integer crop, basic:integer head-
                                                                   crop, basic:integer min overlap,
                                                                   basic:integer
                                                                                      minlen,
                                                                   sic:integer
                                                                                      max n,
                                                                                                  ba-
                                                                   sic:boolean match_read_wildcards,
                                                                   basic:integer
                                                                                       times,
                                                                                                  ba-
                                                                   sic:decimal
                                                                                  error rate) [Source:
                                                                   v1.2.21
```

Cutadapt finds and removes adapter sequences, primers, poly-A tails and other types of unwanted sequence from high-throughput sequencing reads. More information about Cutadapt can be found [here](http://cutadapt.readthedocs.io/en/stable/).

```
Input arguments reads
```

```
label NGS reads
     type data:reads:fastq:single
up_primers_file
     label 5 prime adapter file
     type data:seq:nucleotide
     required False
down_primers_file
     label 3 prime adapter file
     type data:seq:nucleotide
     required False
up_primers_seq
     label 5 prime adapter sequence
     type list:basic:string
     required False
down_primers_seq
     label 3 prime adapter sequence
     type list:basic:string
     required False
polya_tail
     label Poly-A tail
```

type basic:integer

```
description Length of poly-A tail, example - AAAN -> 3, AAAAAN -> 5
     required False
leading
     label Quality on 5 prime
     type basic:integer
     description Remove low quality bases from 5 prime. Specifies the minimum quality required to keep a
     required False
trailing
     label Quality on 3 prime
     type basic:integer
     description Remove low quality bases from the 3 prime. Specifies the minimum quality required to keep
     required False
crop
     label Crop
     type basic:integer
     description Cut the read to a specified length by removing bases from the end
     required False
headcrop
     label Headcrop
     type basic:integer
     description Cut the specified number of bases from the start of the read
     required False
min_overlap
     label Minimal overlap
     type basic:integer
     description Minimum overlap for an adapter match
     default 3
minlen
     label Min length
     type basic:integer
     description Drop the read if it is below a specified length
     required False
max_n
     label Max numebr of N-s
     type basic:integer
```

```
description Discard reads having more 'N' bases than specified.
     required False
match_read_wildcards
     label Match read wildcards
     type basic:boolean
     description Interpret IUPAC wildcards in reads.
     required False
     default False
times
     label Times
     type basic:integer
     description Remove up to COUNT adapters from each read.
     required False
     default 1
error rate
     label Error rate
     type basic:decimal
     description Maximum allowed error rate (no. of errors divided by the length of the matching region).
     required False
     default 0.1
Output results fastq
     label Reads file
     type list:basic:file
report
     label Cutadapt report
     type basic:file
fastqc_url
     label Quality control with FastQC
     type list:basic:file:html
fastqc_archive
     label Download FastQC archive
     type list:basic:file
```

150 Chapter 1. Contents

match- 151 NoverLmax, ba-

1.2. Process catalog

Cutadapt - STAR - HTSeq-count (paired-end) data:workflow:rnaseq:htseqworkflow-custom-cutadapt-star-htseq-paired (data:reads:fastq:paired reads data:genomeindex:star genon data:annotation:gtf gff, basic:string stranded, basic:boolean advanced, basic:boolean noncannonical, basic:boolean chimeric, sic:integer chim-Segment-Min, basic:boolean quantmode, basic:boolean singleend, basic:boolean gene_counts, sic:string out-Filter-Type, basic:integer out-Filter-Multimap-Nmax, basic:integer out-Filter-Mismatch-Nmax, basic:decimal out-Filter-MisThis RNA-seq pipeline is comprised of three steps, preprocessing, alignment, and quantification.

First, reads are preprocessed by __cutadapt__ which finds and removes adapter sequences, primers, poly-A tails and other types of unwanted sequence from high-throughput sequencing reads. Next, preprocessed reads are aligned by __STAR__ aligner. At the time of implementation, STAR is considered a state-of-the-art tool that consistently produces accurate results from diverse sets of reads, and performs well even with default settings. For more information see [this comparison of RNA-seq aligners](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5792058/). Finally, aligned reads are summarized to genes by __HTSeq-count__. Compared to featureCounts, HTSeq-count is not as computationally efficient. All three tools in this workflow support parallelization to accelerate the analysis.

```
Input arguments reads
```

```
label NGS reads
    type data:reads:fastq:paired
genome
    label Indexed reference genome
    type data:genomeindex:star
    description Genome index prepared by STAR aligner indexing tool
gff
    label Annotation (GFF)
    type data:annotation:gtf
stranded
    label Assay type
```

description In strand non-specific assay a read is considered overlapping with a feature regardless of whether it is mapped to the same or the opposite strand as the feature. In strand-specific forward assay and single reads, the read has to be mapped to the same strand as the feature. For paired-end reads, the first read has to be on the same strand and the second read on the opposite strand. In strand-specific reverse assay these rules are reversed.

```
default no
```

type basic:string

choices

• Strand non-specific: no

• Strand-specific forward: yes

• Strand-specific reverse: reverse

advanced

```
label Advanced
type basic:boolean
default False
```

star.noncannonical

```
label Remove non-cannonical junctions (Cufflinks compatibility)
```

```
type basic:boolean
```

description It is recommended to remove the non-canonical junctions for Cufflinks runs using –outFilterIntronMotifs RemoveNoncanonical.

default False

star.detect chimeric.chimeric

label Detect chimeric and circular alignments

type basic:boolean

description To switch on detection of chimeric (fusion) alignments (in addition to normal mapping), –chimSegmentMin should be set to a positive value. Each chimeric alignment consists of two "segments". Each segment is non-chimeric on its own, but the segments are chimeric to each other (i.e. the segments belong to different chromosomes, or different strands, or are far from each other). Both segments may contain splice junctions, and one of the segments may contain portions of both mates. –chimSegmentMin parameter controls the minimum mapped length of the two segments that is allowed. For example, if you have 2x75 reads and used –chimSegmentMin 20, a chimeric alignment with 130b on one chromosome and 20b on the other will be output, while 135 + 15 won't be.

default False

star.detect_chimeric.chimSegmentMin

label -chimSegmentMin

type basic:integer

disabled !star.detect_chimeric.chimeric

default 20

star.t coordinates.quantmode

label Output in transcript coordinates

type basic:boolean

description With –quantMode TranscriptomeSAM option STAR will output alignments translated into transcript coordinates in the Aligned.toTranscriptome.out.bam file (in addition to alignments in genomic coordinates in Aligned.*.sam/bam files). These transcriptomic alignments can be used with various transcript quantification software that require reads to be mapped to transcriptome, such as RSEM or eXpress.

default False

star.t_coordinates.singleend

label Allow soft-clipping and indels

type basic:boolean

description By default, the output satisfies RSEM requirements: soft-clipping or indels are not allowed. Use –quantTranscriptomeBan Singleend to allow insertions, deletions and soft-clips in the transcriptomic alignments, which can be used by some expression quantification software (e.g. eXpress).

disabled !star.t_coordinates.quantmode

default False

star.t_coordinates.gene_counts

label Count reads

type basic:boolean

description With –quantMode GeneCounts option STAR will count number reads per gene while mapping. A read is counted if it overlaps (1nt or more) one and only one gene. Both ends of the paired-end read are checked for overlaps. The counts coincide with those produced by htseq-count

with default parameters. ReadsPerGene.out.tab file with 4 columns which correspond to different strandedness options: column 1: gene ID; column 2: counts for unstranded RNA-seq; column 3: counts for the 1st read strand aligned with RNA (htseq-count option -s yes); column 4: counts for the 2nd read strand aligned with RNA (htseq-count option -s reverse).

```
disabled !star.t_coordinates.quantmode
```

default False

star.filtering.outFilterType

label Type of filtering

type basic:string

description Normal: standard filtering using only current alignment; BySJout: keep only those reads that contain junctions that passed filtering into SJ.out.tab

default Normal

choices

Normal: NormalBySJout: BySJout

star.filtering.outFilterMultimapNmax

label -outFilterMultimapNmax

type basic:integer

description Read alignments will be output only if the read maps fewer than this value, otherwise no alignments will be output (default: 10).

required False

star.filtering.outFilterMismatchNmax

label -outFilterMismatchNmax

type basic:integer

description Alignment will be output only if it has fewer mismatches than this value (default: 10).

required False

star. filtering. out Filter Mismatch Nover L max

label -outFilterMismatchNoverLmax

type basic:decimal

description Max number of mismatches per pair relative to read length: for 2x100b, max number of mismatches is 0.06*200=8 for the paired read.

required False

star.alignment.alignSJoverhangMin

label -alignSJoverhangMin

type basic:integer

description Minimum overhang (i.e. block size) for spliced alignments (default: 5).

required False

star.alignment.alignSJDBoverhangMin

```
label -alignSJDBoverhangMin
     type basic:integer
     description Minimum overhang (i.e. block size) for annotated (sjdb) spliced alignments (default: 3).
     required False
star.alignment.alignIntronMin
     label -alignIntronMin
     type basic:integer
     description Minimum intron size: genomic gap is considered intron if its length >= alignIntronMin,
          otherwise it is considered Deletion (default: 21).
     required False
star.alignment.alignIntronMax
     label -alignIntronMax
     type basic:integer
                                          if 0,
     description Maximum intron size,
                                                    max intron size will
                                                                              be
                                                                                   determined
          (2pow(winBinNbits)*winAnchorDistNbins) (default: 0).
     required False
star.alignment.alignMatesGapMax
     label –alignMatesGapMax
     type basic:integer
     description Maximum gap between two mates, if 0, max intron gap will be determined by
          (2pow(winBinNbits)*winAnchorDistNbins) (default: 0).
     required False
htseq.mode
     label Mode
     type basic:string
     description Mode to handle reads overlapping more than one feature. Possible values for <mode> are
          union, intersection-strict and intersection-nonempty
     default union
     choices
            • union: union
            • intersection-strict: intersection-strict
            • intersection-nonempty: intersection-nonempty
htseq.feature_class
     label Feature class
     type basic:string
     description Feature class (3rd column in GFF file) to be used. All other features will be ignored.
     default exon
```

$htseq.id_attribute$

label ID attribute

type basic:string

description GFF attribute to be used as feature ID. Several GFF lines with the same feature ID will be considered as parts of the same feature. The feature ID is used to identity the counts in the output table.

default gene_id

htseq.name_ordered

label Use name-ordered BAM file for counting reads

type basic:boolean

description Use name-sorted BAM file for reads quantification. Improves compatibility with larger BAM files, but requires more computational time.

required False

default False

Output results

Cutadapt - STAR - HTSeq-count (single-end)

data:workflow:rnaseq:htseqworkflow-custom-cutadapt-star-htseq-single (data:reads:fastq:single reads, data:genomeindex:star genon data:annotation:gtf gff, basic:string stranded, basic:boolean advanced, basic:boolean noncannonical, basic:boolean chimeric, sic:integer chim-Segment-Min, basic:boolean quantmode, basic:boolean singleend, basic:boolean gene_counts, sic:string out-Filter-Type, basic:integer out-Filter-Multimap-Nmax, basic:integer out-Filter-Mismatch-Nmax, basic:decimal out-Filter-

1.2. Process catalog

match- 157

Mis-

This RNA-seq pipeline is comprised of three steps, preprocessing, alignment, and quantification.

First, reads are preprocessed by __cutadapt__ which finds and removes adapter sequences, primers, poly-A tails and other types of unwanted sequence from high-throughput sequencing reads. Next, preprocessed reads are aligned by __STAR__ aligner. At the time of implementation, STAR is considered a state-of-the-art tool that consistently produces accurate results from diverse sets of reads, and performs well even with default settings. For more information see [this comparison of RNA-seq aligners](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5792058/). Finally, aligned reads are summarized to genes by __HTSeq-count__. Compared to featureCounts, HTSeq-count is not as computationally efficient. All three tools in this workflow support parallelization to accelerate the analysis.

```
Input arguments reads
```

```
label NGS reads
    type data:reads:fastq:single
genome
    label Indexed reference genome
        type data:genomeindex:star
        description Genome index prepared by STAR aligner indexing tool
gff
    label Annotation (GFF)
    type data:annotation:gtf
stranded
    label Assay type
```

description In strand non-specific assay a read is considered overlapping with a feature regardless of whether it is mapped to the same or the opposite strand as the feature. In strand-specific forward assay and single reads, the read has to be mapped to the same strand as the feature. For paired-end reads, the first read has to be on the same strand and the second read on the opposite strand. In strand-specific reverse assay these rules are reversed.

```
default no
```

type basic:string

choices

• Strand non-specific: no

• Strand-specific forward: yes

• Strand-specific reverse: reverse

advanced

```
label Advanced
type basic:boolean
default False
```

star.noncannonical

```
label Remove non-cannonical junctions (Cufflinks compatibility)
```

```
type basic:boolean
```

description It is recommended to remove the non-canonical junctions for Cufflinks runs using –outFilterIntronMotifs RemoveNoncanonical.

default False

star.detect chimeric.chimeric

label Detect chimeric and circular alignments

type basic:boolean

description To switch on detection of chimeric (fusion) alignments (in addition to normal mapping), –chimSegmentMin should be set to a positive value. Each chimeric alignment consists of two "segments". Each segment is non-chimeric on its own, but the segments are chimeric to each other (i.e. the segments belong to different chromosomes, or different strands, or are far from each other). Both segments may contain splice junctions, and one of the segments may contain portions of both mates. –chimSegmentMin parameter controls the minimum mapped length of the two segments that is allowed. For example, if you have 2x75 reads and used –chimSegmentMin 20, a chimeric alignment with 130b on one chromosome and 20b on the other will be output, while 135 + 15 won't be.

default False

star.detect_chimeric.chimSegmentMin

label -chimSegmentMin

type basic:integer

disabled !star.detect_chimeric.chimeric

default 20

star.t coordinates.quantmode

label Output in transcript coordinates

type basic:boolean

description With –quantMode TranscriptomeSAM option STAR will output alignments translated into transcript coordinates in the Aligned.toTranscriptome.out.bam file (in addition to alignments in genomic coordinates in Aligned.*.sam/bam files). These transcriptomic alignments can be used with various transcript quantification software that require reads to be mapped to transcriptome, such as RSEM or eXpress.

default False

star.t_coordinates.singleend

label Allow soft-clipping and indels

type basic:boolean

description By default, the output satisfies RSEM requirements: soft-clipping or indels are not allowed. Use –quantTranscriptomeBan Singleend to allow insertions, deletions ans soft-clips in the transcriptomic alignments, which can be used by some expression quantification software (e.g. eXpress).

disabled !star.t_coordinates.quantmode

default False

star.t_coordinates.gene_counts

label Count reads

type basic:boolean

description With –quantMode GeneCounts option STAR will count number reads per gene while mapping. A read is counted if it overlaps (1nt or more) one and only one gene. Both ends of the paired-end read are checked for overlaps. The counts coincide with those produced by htseq-count

with default parameters. ReadsPerGene.out.tab file with 4 columns which correspond to different strandedness options: column 1: gene ID; column 2: counts for unstranded RNA-seq; column 3: counts for the 1st read strand aligned with RNA (htseq-count option -s yes); column 4: counts for the 2nd read strand aligned with RNA (htseq-count option -s reverse).

```
disabled !star.t_coordinates.quantmode
```

default False

star.filtering.outFilterType

label Type of filtering

type basic:string

description Normal: standard filtering using only current alignment; BySJout: keep only those reads that contain junctions that passed filtering into SJ.out.tab

default Normal

choices

Normal: NormalBySJout: BySJout

star.filtering.outFilterMultimapNmax

label -outFilterMultimapNmax

type basic:integer

description Read alignments will be output only if the read maps fewer than this value, otherwise no alignments will be output (default: 10).

required False

star.filtering.outFilterMismatchNmax

label -outFilterMismatchNmax

type basic:integer

description Alignment will be output only if it has fewer mismatches than this value (default: 10).

required False

star. filtering. out Filter Mismatch Nover L max

label -outFilterMismatchNoverLmax

type basic:decimal

description Max number of mismatches per pair relative to read length: for 2x100b, max number of mismatches is 0.06*200=8 for the paired read.

required False

star.alignment.alignSJoverhangMin

label -alignSJoverhangMin

type basic:integer

description Minimum overhang (i.e. block size) for spliced alignments (default: 5).

required False

star.alignment.alignSJDBoverhangMin

```
label -alignSJDBoverhangMin
     type basic:integer
     description Minimum overhang (i.e. block size) for annotated (sjdb) spliced alignments (default: 3).
     required False
star.alignment.alignIntronMin
     label -alignIntronMin
     type basic:integer
     description Minimum intron size: genomic gap is considered intron if its length >= alignIntronMin,
          otherwise it is considered Deletion (default: 21).
     required False
star.alignment.alignIntronMax
     label -alignIntronMax
     type basic:integer
                                          if 0,
     description Maximum intron size,
                                                    max intron size will
                                                                              be
                                                                                   determined
          (2pow(winBinNbits)*winAnchorDistNbins) (default: 0).
     required False
star.alignment.alignMatesGapMax
     label –alignMatesGapMax
     type basic:integer
     description Maximum gap between two mates, if 0, max intron gap will be determined by
          (2pow(winBinNbits)*winAnchorDistNbins) (default: 0).
     required False
htseq.mode
     label Mode
     type basic:string
     description Mode to handle reads overlapping more than one feature. Possible values for <mode> are
          union, intersection-strict and intersection-nonempty
     default union
     choices
            • union: union
            • intersection-strict: intersection-strict
            • intersection-nonempty: intersection-nonempty
htseq.feature_class
     label Feature class
     type basic:string
     description Feature class (3rd column in GFF file) to be used. All other features will be ignored.
     default exon
```

$htseq.id_attribute$

label ID attribute

type basic:string

description GFF attribute to be used as feature ID. Several GFF lines with the same feature ID will be considered as parts of the same feature. The feature ID is used to identity the counts in the output table.

default gene_id

htseq.name_ordered

label Use name-ordered BAM file for counting reads

type basic:boolean

description Use name-sorted BAM file for reads quantification. Improves compatibility with larger BAM files, but requires more computational time.

required False

default False

Output results

Cutadapt - STAR - RSEM (Diagenode CATS, paired-end)

data:workflow:rnaseq:rsemworkflow-custom-cutadapt-star-rsem-paired (data:reads:fastq:paired reads,

data:genomeindex:star star_inde

data:index:expression ex-

pres-

sion_index,

ba-

sic:string stranded,

sic:boolean ad-

vanced,

ba-

sic:boolean non-

cannon-

ical, ba-

sic:boolean chimeric,

sic:integer chim-

Seg-

ment-

Min, ba-

sic:boolean quant-

mode,

ba-

sic:boolean sin-

gleend,

ba-

sic:boolean gene_counts,

ba-

sic:string out-

Filter-

Type,

ba-

sic:integer out-

Filter-

Mul-

timapN-

max, ba-

sic:integer out-

Filter-

Mis-

match-

Nmax,

ba-

sic:decimal out-

Filter-

Mis-

match-

NoverL-

max, ba-

sic:integer align-

SJover-

hang-

Min, ba-

sic:integer align-

SJD-

Bover-

hang-Min, baThis RNA-seq pipeline is configured to be used with the Diagenode CATS RNA-seq kits. It is comprised of three steps, preprocessing, alignment, and quantification.

First, reads are preprocessed by cutadapt which finds and removes adapter sequences, primers, poly-A tails and other types of unwanted sequence from high-throughput sequencing reads. Next, preprocessed reads are aligned by STAR aligner. Finally, RSEM estimates gene and isoform expression levels from the aligned reads.

Input arguments reads

```
label NGS reads
```

type data:reads:fastq:paired

star_index

label STAR genome index

type data:genomeindex:star

expression_index

label Gene expression indices

type data:index:expression

stranded

label Assay type

type basic:string

description In strand non-specific assay a read is considered overlapping with a feature regardless of whether it is mapped to the same or the opposite strand as the feature. In strand-specific forward assay and single reads, the read has to be mapped to the same strand as the feature. For paired-end reads, the first read has to be on the same strand and the second read on the opposite strand. In strand-specific reverse assay these rules are reversed.

default no

choices

• Strand non-specific: no

• Strand-specific forward: yes

• Strand-specific reverse: reverse

advanced

label Advanced

type basic:boolean

default False

star.noncannonical

label Remove non-cannonical junctions (Cufflinks compatibility)

type basic:boolean

description It is recommended to remove the non-canonical junctions for Cufflinks runs using –outFilterIntronMotifs RemoveNoncanonical.

default False

star.detect chimeric.chimeric

label Detect chimeric and circular alignments

type basic:boolean

description To switch on detection of chimeric (fusion) alignments (in addition to normal mapping), –chimSegmentMin should be set to a positive value. Each chimeric alignment consists of two "segments". Each segment is non-chimeric on its own, but the segments are chimeric to each other (i.e. the segments belong to different chromosomes, or different strands, or are far from each other). Both segments may contain splice junctions, and one of the segments may contain portions of both mates. –chimSegmentMin parameter controls the minimum mapped length of the two segments that is allowed. For example, if you have 2x75 reads and used –chimSegmentMin 20, a chimeric alignment with 130b on one chromosome and 20b on the other will be output, while 135 + 15 won't be.

default False

star.detect_chimeric.chimSegmentMin

label -chimSegmentMin

type basic:integer

disabled !star.detect_chimeric.chimeric

default 20

star.t_coordinates.quantmode

label Output in transcript coordinates

type basic:boolean

description With —quantMode TranscriptomeSAM option STAR will output alignments translated into transcript coordinates in the Aligned.toTranscriptome.out.bam file (in addition to alignments in genomic coordinates in Aligned.*.sam/bam files). These transcriptomic alignments can be used with various transcript quantification software that require reads to be mapped to transcriptome, such as RSEM or eXpress.

default True

star.t coordinates.singleend

label Allow soft-clipping and indels

type basic:boolean

description By default, the output satisfies RSEM requirements: soft-clipping or indels are not allowed. Use –quantTranscriptomeBan Singleend to allow insertions, deletions and soft-clips in the transcriptomic alignments, which can be used by some expression quantification software (e.g. eXpress).

disabled !star.t_coordinates.quantmode

default False

star.t_coordinates.gene_counts

label Count reads

type basic:boolean

description With –quantMode GeneCounts option STAR will count number reads per gene while mapping. A read is counted if it overlaps (1nt or more) one and only one gene. Both ends of the paired-end read are checked for overlaps. The counts coincide with those produced by htseq-count with default parameters. ReadsPerGene.out.tab file with 4 columns which correspond to different strandedness options: column 1: gene ID; column 2: counts for unstranded RNA-seq; column 3: counts for the 1st read strand aligned with RNA (htseq-count option -s yes); column 4: counts for the 2nd read strand aligned with RNA (htseq-count option -s reverse).

```
disabled !star.t_coordinates.quantmode
     default False
star.filtering.outFilterType
     label Type of filtering
     type basic:string
     description Normal: standard filtering using only current alignment; BySJout: keep only those reads that
          contain junctions that passed filtering into SJ.out.tab
     default Normal
     choices
             • Normal: Normal
             • BySJout: BySJout
star.filtering.outFilterMultimapNmax
     label -outFilterMultimapNmax
     type basic:integer
     description Read alignments will be output only if the read maps fewer than this value, otherwise no
          alignments will be output (default: 10).
     required False
star.filtering.outFilterMismatchNmax
     label -outFilterMismatchNmax
     type basic:integer
     description Alignment will be output only if it has fewer mismatches than this value (default: 10).
     required False
star.filtering.outFilterMismatchNoverLmax
     label -outFilterMismatchNoverLmax
     type basic:decimal
     description Max number of mismatches per pair relative to read length: for 2x100b, max number of
          mismatches is 0.06*200=8 for the paired read.
     required False
star.alignment.alignSJoverhangMin
     label -alignSJoverhangMin
     type basic:integer
     description Minimum overhang (i.e. block size) for spliced alignments (default: 5).
     required False
star.alignment.alignSJDBoverhangMin
     label -alignSJDBoverhangMin
     type basic:integer
     description Minimum overhang (i.e. block size) for annotated (sjdb) spliced alignments (default: 3).
```

required False

star.alignment.alignIntronMin

label -alignIntronMin

type basic:integer

description Minimum intron size: genomic gap is considered intron if its length >= alignIntronMin, otherwise it is considered Deletion (default: 21).

required False

star.alignment.alignIntronMax

label -alignIntronMax

type basic:integer

description Maximum intron size, if 0, max intron size will be determined by (2pow(winBinNbits)*winAnchorDistNbins) (default: 0).

required False

star.alignment.alignMatesGapMax

label -alignMatesGapMax

type basic:integer

description Maximum gap between two mates, if 0, max intron gap will be determined by (2pow(winBinNbits)*winAnchorDistNbins) (default: 0).

required False

Output results

Cutadapt - STAR - RSEM (Diagenode CATS, single-end)

data:workflow:rnaseq:rsemworkflow-custom-cutadapt-star-rsem-single (data:reads:fastq:single reads,

data:genomeindex:star star_inde

data:index:expression ex-

pres-

sion_index,

ba-

sic:string stranded,

sic:boolean ad-

vanced,

ba-

sic:boolean non-

cannon-

ical, ba-

sic:boolean chimeric,

sic:integer chim-

Seg-

ment-

Min, ba-

sic:boolean quant-

mode,

ba-

sic:boolean sin-

gleend,

ba-

sic:boolean gene_counts,

ba-

sic:string out-

Filter-

Type,

ba-

sic:integer out-

Filter-

Mul-

timapN-

max, ba-

sic:integer out-

Filter-

Mis-

match-

Nmax,

ba-

sic:decimal out-

Filter-

Mis-

match-

NoverL-

max, ba-

sic:integer align-

SJover-

hang-

Min, ba-

sic:integer align-

ChapterStp Contents

Bover-

hang-

Min, ba-

This RNA-seq pipeline is configured to be used with the Diagenode CATS RNA-seq kits. It is comprised of three steps, preprocessing, alignment, and quantification.

First, reads are preprocessed by cutadapt which finds and removes adapter sequences, primers, poly-A tails and other types of unwanted sequence from high-throughput sequencing reads. Next, preprocessed reads are aligned by STAR aligner. Finally, RSEM estimates gene and isoform expression levels from the aligned reads.

Input arguments reads

```
label NGS reads
```

type data:reads:fastq:single

star_index

label STAR genome index

type data:genomeindex:star

expression_index

label Gene expression indices

type data:index:expression

stranded

label Assay type

type basic:string

description In strand non-specific assay a read is considered overlapping with a feature regardless of whether it is mapped to the same or the opposite strand as the feature. In strand-specific forward assay and single reads, the read has to be mapped to the same strand as the feature. For paired-end reads, the first read has to be on the same strand and the second read on the opposite strand. In strand-specific reverse assay these rules are reversed.

default no

choices

• Strand non-specific: no

• Strand-specific forward: yes

• Strand-specific reverse: reverse

advanced

label Advanced

type basic:boolean

default False

star.noncannonical

label Remove non-cannonical junctions (Cufflinks compatibility)

type basic:boolean

description It is recommended to remove the non-canonical junctions for Cufflinks runs using –outFilterIntronMotifs RemoveNoncanonical.

default False

$star. detect_chimeric.chimeric\\$

label Detect chimeric and circular alignments

type basic:boolean

description To switch on detection of chimeric (fusion) alignments (in addition to normal mapping), –chimSegmentMin should be set to a positive value. Each chimeric alignment consists of two "segments". Each segment is non-chimeric on its own, but the segments are chimeric to each other (i.e. the segments belong to different chromosomes, or different strands, or are far from each other). Both segments may contain splice junctions, and one of the segments may contain portions of both mates. –chimSegmentMin parameter controls the minimum mapped length of the two segments that is allowed. For example, if you have 2x75 reads and used –chimSegmentMin 20, a chimeric alignment with 130b on one chromosome and 20b on the other will be output, while 135 + 15 won't be.

default False

star.detect chimeric.chimSegmentMin

label -chimSegmentMin

type basic:integer

disabled !star.detect_chimeric.chimeric

default 20

star.t_coordinates.quantmode

label Output in transcript coordinates

type basic:boolean

description With —quantMode TranscriptomeSAM option STAR will output alignments translated into transcript coordinates in the Aligned.toTranscriptome.out.bam file (in addition to alignments in genomic coordinates in Aligned.*.sam/bam files). These transcriptomic alignments can be used with various transcript quantification software that require reads to be mapped to transcriptome, such as RSEM or eXpress.

default True

star.t coordinates.singleend

label Allow soft-clipping and indels

type basic:boolean

description By default, the output satisfies RSEM requirements: soft-clipping or indels are not allowed. Use –quantTranscriptomeBan Singleend to allow insertions, deletions ans soft-clips in the transcriptomic alignments, which can be used by some expression quantification software (e.g. eXpress).

disabled !star.t_coordinates.quantmode

default False

star.t_coordinates.gene_counts

label Count reads

type basic:boolean

description With –quantMode GeneCounts option STAR will count number reads per gene while mapping. A read is counted if it overlaps (1nt or more) one and only one gene. Both ends of the paired-end read are checked for overlaps. The counts coincide with those produced by htseq-count with default parameters. ReadsPerGene.out.tab file with 4 columns which correspond to different strandedness options: column 1: gene ID; column 2: counts for unstranded RNA-seq; column 3: counts for the 1st read strand aligned with RNA (htseq-count option -s yes); column 4: counts for the 2nd read strand aligned with RNA (htseq-count option -s reverse).

```
disabled !star.t_coordinates.quantmode
     default False
star.filtering.outFilterType
     label Type of filtering
     type basic:string
     description Normal: standard filtering using only current alignment; BySJout: keep only those reads that
          contain junctions that passed filtering into SJ.out.tab
     default Normal
     choices
             • Normal: Normal
             • BySJout: BySJout
star.filtering.outFilterMultimapNmax
     label -outFilterMultimapNmax
     type basic:integer
     description Read alignments will be output only if the read maps fewer than this value, otherwise no
          alignments will be output (default: 10).
     required False
star.filtering.outFilterMismatchNmax
     label -outFilterMismatchNmax
     type basic:integer
     description Alignment will be output only if it has fewer mismatches than this value (default: 10).
     required False
star.filtering.outFilterMismatchNoverLmax
     label -outFilterMismatchNoverLmax
     type basic:decimal
     description Max number of mismatches per pair relative to read length: for 2x100b, max number of
          mismatches is 0.06*200=8 for the paired read.
     required False
star.alignment.alignSJoverhangMin
     label -alignSJoverhangMin
     type basic:integer
     description Minimum overhang (i.e. block size) for spliced alignments (default: 5).
     required False
star.alignment.alignSJDBoverhangMin
```

description Minimum overhang (i.e. block size) for annotated (sjdb) spliced alignments (default: 3).

label -alignSJDBoverhangMin

type basic:integer

```
required False
```

star.alignment.alignIntronMin

```
label -alignIntronMin
```

type basic:integer

description Minimum intron size: genomic gap is considered intron if its length >= alignIntronMin, otherwise it is considered Deletion (default: 21).

required False

star.alignment.alignIntronMax

```
label -alignIntronMax
```

type basic:integer

description Maximum intron size, if 0, max intron size will be determined by (2pow(winBinNbits)*winAnchorDistNbins) (default: 0).

required False

star.alignment.alignMatesGapMax

```
label -alignMatesGapMax
```

type basic:integer

description Maximum gap between two mates, if 0, max intron gap will be determined by (2pow(winBinNbits)*winAnchorDistNbins) (default: 0).

required False

Output results

DESeq2

data:differentialexpression:deseq2differentialexpression-deseq2 (list:data:expression case,

(list:data:expression case, list:data:expression con-

trol, *ba*-

sic:boolean count,

ba-

sic:integer min_count_sum,

ba-

sic:boolean cook,

ba-

sic:decimal cooks_cutoff,

ba-

sic:boolean in-

depen-

dent. ba-

sic:decimal al-

pha) [Source:

v2.2.0]

The DESeq2 package estimates variance-mean dependence in count data from high-throughput sequencing assays and tests for differential expression based on a model using the negative binomial distribution. See [here](https://www.bioconductor.org/packages/release/bioc/manuals/DESeq2/man/DESeq2.pdf) and [here](http://bioconductor.org/packages/devel/bioc/vignettes/DESeq2/inst/doc/DESeq2.html) for more information.

```
Input arguments case
     label Case
     type list:data:expression
     description Case samples (replicates)
control
     label Control
     type list:data:expression
     description Control samples (replicates)
filter.count
     label Filter genes based on expression count
     type basic:boolean
     default True
filter.min count sum
     label Minimum raw gene expression count summed over all samples
     type basic:integer
     description Filter genes in the expression matrix input. Remove genes where the expression count sum
           over all samples is below the threshold.
     hidden !filter.count
     default 10
filter.cook
     label Filter genes based on Cook's distance
     type basic:boolean
     default False
filter.cooks cutoff
     label Threshold on Cook's distance
     type basic:decimal
     description If one or more samples have Cook's distance larger than the threshold set here, the p-value for
           the row is set to NA. If left empty, the default threshold of 0.99 quantile of the F(p, m-p) distribution
           is used, where p is the number of coefficients being fitted and m is the number of samples. This test
           excludes Cook's distance of samples belonging to experimental groups with only two samples.
     required False
     hidden !filter.cook
filter.independent
     label Apply independent gene filtering
     type basic:boolean
     default False
```

filter.alpha

```
label Significance cut-off used for optimizing independent gene filtering
     type basic:decimal
     description The value should be set to adjusted p-value cut-off (FDR).
     hidden !filter.independent
     default 0.1
Output results raw
     label Differential expression
     type basic:file
de_json
     label Results table (JSON)
     type basic: json
de_file
     label Results table (file)
     type basic:file
count matrix
     label Count matrix
     type basic:file
source
     label Gene ID database
     type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
feature_type
     label Feature type
     type basic:string
```

Detect library strandedness

```
data:strandednesslibrary-strandedness (data:reads:fastq
                                                                         reads.
                                                                                      ba-
                                                                             read_number,
                                              sic:integer
                                              data:index:salmon_index) [Source: v0.1.2]
```

This process uses the Salmon transcript quantification tool to automatically infer the NGS library strandedness. For more details, please see the Salmon [documentation](https://salmon.readthedocs.io/en/latest/library_type.html)

Input arguments reads

```
label Sequencing reads
```

```
type data:reads:fastq
```

description Sequencing reads in .fastq format. Both single and paired-end libraries are supported

read_number

label Number of input reads

type basic:integer

description Number of sequencing reads that are subsampled from each of the original .fastq files before library strand detection

default 50000

salmon_index

label Transcriptome index file

type data:index:salmon

description Transcriptome index file created using the Salmon indexing tool. cDNA (transcriptome) sequences used for index file creation must be derived from the same species as the input sequencing reads to obtain the reliable analysis results

Output results strandedness

label Library strandedness type

type basic:string

description The predicted library strandedness type. The codes U and IU indicate 'strand non-specific' library for single or paired-end reads, respectively. Codes SF and ISF correspond to the 'strand-specific forward' library, for the single or paired-end reads, respectively. For 'strand-specific reverse' library, the corresponding codes are SR and ISR. For more details, please see the Salmon [documentation](https://salmon.readthedocs.io/en/latest/library_type.html)

fragment_ratio

label Compatible fragment ratio

type basic:decimal

description The ratio of fragments that support the predicted library strandedness type

log

label Log file

type basic:file

description Analysis log file.

Dictyostelium expressions

```
data:expression:polyaexpression-dicty (data:alignment:bam alignment, data:annotation:gff3 gff, data:mappability:bcm mappable) [Source: v1.3.1]
```

Dictyostelium-specific pipeline. Developed by Bioinformatics Laboratory, Faculty of Computer and Information Science, University of Ljubljana, Slovenia and Shaulsky Lab, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA.

Input arguments alignment

```
label Aligned sequence
     type data:alignment:bam
gff
     label Features (GFF3)
     type data:annotation:gff3
mappable
     label Mappability
     type data:mappability:bcm
Output results exp
     label Expression RPKUM (polyA)
     type basic:file
     description mRNA reads scaled by uniquely mappable part of exons.
rpkmpolya
     label Expression RPKM (polyA)
     type basic:file
     description mRNA reads scaled by exon length.
rc
     label Read counts (polyA)
     type basic:file
     description mRNA reads uniquely mapped to gene exons.
rpkum
     label Expression RPKUM
     type basic:file
     description Reads scaled by uniquely mappable part of exons.
rpkm
     label Expression RPKM
     type basic:file
     description Reads scaled by exon length.
rc_raw
     label Read counts (raw)
     type basic:file
     description Reads uniquely mapped to gene exons.
exp_json
     label Expression RPKUM (polyA) (json)
     type basic: json
exp_type
```

```
label Expression Type (default output)
     type basic:string
source
     label Gene ID database
     type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
feature_type
     label Feature type
     type basic:string
Differential Expression (table)
data:differentialexpression:uploadupload-diffexp (basic:file src, basic:string gene_id,
                                                                    basic:string
                                                                                        logfc,
                                                                    sic:string
                                                                               fdr, basic:string
                                                                                                  lo-
                                                                    godds,
                                                                              basic:string
                                                                                                fwer.
                                                                    basic:string
                                                                                       pvalue,
                                                                                                  ba-
                                                                    sic:string stat, basic:string source,
                                                                                      species,
                                                                    basic:string
                                                                                                  ba-
                                                                    sic:string
                                                                                       build.
                                                                                                  ba-
                                                                    sic:string
                                                                                        feature_type,
                                                                    list:data:expression
                                                                                                 case.
                                                                    list:data:expression
                                                                                                 con-
                                                                    trol) [Source: v1.2.1]
Upload Differential Expression table.
Input arguments src
     label Differential expression file
     type basic:file
     description Differential expression file. Supported file types: *.xls, *.xlsx, *.tab (tab-delimited file),
          *.diff. DE file must include columns with log2(fold change) and FDR or pval information. DE file
          must contain header row with column names. Accepts DESeq, DESeq2, edgeR and CuffDiff output
     validate_regex \.(xls|xlsx|tab|tab.gz|diff|diff.gz)$
gene_id
     label Gene ID label
     type basic:string
logfc
```

```
label LogFC label
     type basic:string
fdr
     label FDR label
     type basic:string
     required False
logodds
     label LogOdds label
     type basic:string
     required False
fwer
     label FWER label
     type basic:string
     required False
pvalue
     label Pvalue label
     type basic:string
     required False
stat
     label Statistics label
     type basic:string
     required False
source
     label Gene ID database
     type basic:string
     choices
            • AFFY: AFFY
            • DICTYBASE: DICTYBASE
            • ENSEMBL: ENSEMBL
            • NCBI: NCBI
            • UCSC: UCSC
species
     label Species
     type basic:string
     description Species latin name.
     choices
```

```
• Homo sapiens: Homo sapiens
            • Mus musculus: Mus musculus
            • Rattus norvegicus: Rattus norvegicus
            • Dictyostelium discoideum: Dictyostelium discoideum
            • Odocoileus virginianus texanus: Odocoileus virginianus texanus
            • Solanum tuberosum: Solanum tuberosum
build
     label Build
     type basic:string
     description Genome build or annotation version.
feature_type
     label Feature type
     type basic:string
     default gene
     choices
            • gene: gene
            • transcript: transcript
            • exon: exon
case
     label Case
     type list:data:expression
     description Case samples (replicates)
     required False
control
     label Control
     type list:data:expression
     description Control samples (replicates)
     required False
Output results raw
     label Differential expression
     type basic:file
de_json
     label Results table (JSON)
     type basic:json
de_file
     label Results table (file)
```

```
type basic:file
source
     label Gene ID database
     type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
feature_type
     label Feature type
     type basic:string
Expression Time Course
data:etcetc-bcm (list:data:expression expressions, basic:boolean avg) [Source: v1.1.1]
Select gene expression data and form a time course.
Input arguments expressions
     label RPKM expression profile
     type list:data:expression
     required True
avg
     label Average by time
     type basic:boolean
     default True
Output results etcfile
     label Expression time course file
     type basic:file
etc
     label Expression time course
     type basic: json
Expression aggregator
data:aggregator:expressionexpression-aggregator (list:data:expression
                                                                                           exps,
                                                                                      group_by,
                                                               basic:string
                                                               data:aggregator:expression expr_aggregator) [Source:
```

v0.2.2]

Collect expression data from samples grouped by sample descriptor field. The Expression aggregator process should not be run in Batch Mode, as this will create redundant outputs. Rather, select multiple samples below for which you wish to aggregate the expression matrix.

```
Input arguments exps
     label Expressions
     type list:data:expression
group_by
     label Sample descriptor field
     type basic:string
expr_aggregator
     label Expression aggregator
     type data:aggregator:expression
     required False
Output results exp_matrix
     label Expression matrix
     type basic:file
box_plot
     label Box plot
     type basic: json
log_box_plot
     label Log box plot
     type basic: json
source
     label Gene ID database
     type basic:string
species
     label Species
     type basic:string
exp_type
     label Expression type
     type basic:string
```

Expression data

```
data:expressionupload-expression (basic:file rc, basic:file exp, basic:string exp_name, basic:string exp_type, basic:string source, basic:string species, basic:string build, basic:string feature_type) [Source: v2.2.1]
```

Upload expression data by providing raw expression data (read counts) and/or normalized expression data together with the associated data normalization type.

```
Input arguments rc
     label Read counts (raw expression)
     type basic:file
     description Reads mapped to genomic features (raw count data). Supported extensions: .txt.gz (pre-
         ferred), .tab.* or .txt.*
     required False
     validate_regex \.(txt|tab|qz)(|\.qz|\.bz2|\.tqz|\.tar\.qz|\.tar\.bz2|\.
         zip|\.rar|\.7z)$
exp
     label Normalized expression
     type basic:file
     description Normalized expression data. Supported extensions: .tab.gz (preferred) or .tab.*
     required False
     validate_regex \.(tab|gz)(|\.gz|\.bz2|\.tgz|\.tar\.gz|\.tar\.bz2|\.zip|\.
         rar|\.7z)$
exp_name
     label Expression name
     type basic:string
exp_type
     label Normalization type
     type basic:string
     description Normalization type
     required False
source
     label Gene ID source
     type basic:string
     choices
            • AFFY: AFFY
            • DICTYBASE: DICTYBASE
            • ENSEMBL: ENSEMBL
            • NCBI: NCBI
            • UCSC: UCSC
species
     label Species
     type basic:string
```

```
choices
            • Homo sapiens: Homo sapiens
            • Mus musculus: Mus musculus
            • Rattus norvegicus: Rattus norvegicus
            • Dictyostelium discoideum: Dictyostelium discoideum
            • Odocoileus virginianus texanus: Odocoileus virginianus texanus
            • Solanum tuberosum: Solanum tuberosum
build
     label Build
     type basic:string
     description Genome build or annotation version.
feature_type
     label Feature type
     type basic:string
     default gene
     choices
            • gene: gene
            • transcript: transcript
            • exon: exon
Output results exp
     label Normalized expression
     type basic:file
     description Normalized expression
rc
     label Read counts
     type basic:file
     description Reads mapped to genomic features.
     required False
exp_json
     label Expression (json)
     type basic: json
exp_type
     label Expression type
     type basic:string
exp_set
```

description Species latin name.

```
label Expressions
     type basic:file
exp_set_json
     label Expressions (json)
     type basic:json
source
     label Gene ID source
     type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
feature_type
     label Feature type
     type basic:string
Expression data (Cuffnorm)
data:expressionupload-expression-cuffnorm (basic:file exp, data:cufflinks:cuffquant exb,
                                                       basic:string exp_type) [Source: v1.4.1]
Upload expression data by providing Cuffnorm results.
Input arguments exp
     label Normalized expression
     type basic:file
cxb
     label Cuffquant analysis
     type data:cufflinks:cuffquant
     description Cuffquant analysis.
exp_type
     label Normalization type
     type basic:string
     default Cuffnorm
Output results exp
     label Normalized expression
     type basic:file
```

```
description Normalized expression
rc
     label Read counts
     type basic:file
     description Reads mapped to genomic features.
     required False
exp_json
     label Expression (json)
     type basic: json
exp_type
     label Expression type
     type basic:string
exp_set
     label Expressions
     type basic:file
exp_set_json
     label Expressions (json)
     type basic:json
source
     label Gene ID source
     type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
feature_type
     label Feature type
     type basic:string
Expression data (STAR)
data:expression:starupload-expression-star (basic:file rc, basic:string stranded, ba-
                                                        sic:string source, basic:string species,
                                                        basic:string
                                                                      build, basic:string
                                                                                            fea-
                                                        ture_type) [Source: v1.3.1]
```

Upload expression data by providing STAR aligner results.

```
Input arguments rc
```

```
label Read counts (raw expression)
type basic:file
description Reads mapped to genomic features (raw count data). Supported extensions: .txt.gz (pre-
ferred), .tab.* or .txt.*
validate_regex \. (txt|tab|gz) (|\.gz|\.bz2|\.tgz|\.tar\.gz|\.tar\.bz2|\.
    zip|\.rar|\.7z)$
```

stranded

label Is data from a strand specific assay?

```
type basic:string
```

description For stranded=no, a read is considered overlapping with a feature regardless of whether it is mapped to the same or the opposite strand as the feature. For stranded=yes and single-end reads, the read has to be mapped to the same strand as the feature. For paired-end reads, the first read has to be on the same strand and the second read on the opposite strand. For stranded=reverse, these rules are reversed.

default yes

choices

- yes: yes
- no: no
- reverse: reverse

source

label Gene ID source

type basic:string

choices

- AFFY: AFFY
- DICTYBASE: DICTYBASE
- ENSEMBL: ENSEMBL
- NCBI: NCBI
- UCSC: UCSC

species

label Species

type basic:string

description Species latin name.

choices

- Homo sapiens: Homo sapiens
- Mus musculus: Mus musculus
- Rattus norvegicus: Rattus norvegicus

```
• Odocoileus virginianus texanus: Odocoileus virginianus texanus
            • Solanum tuberosum: Solanum tuberosum
build
     label Build
     type basic:string
     description Genome build or annotation version.
feature_type
     label Feature type
     type basic:string
     default gene
     choices
            • gene: gene
            • transcript: transcript
            • exon: exon
Output results rc
     label Read counts (raw data)
     type basic:file
     description Reads mapped to genomic features.
exp
     label Expression data
     type basic:file
exp_json
     label Expression (json)
     type basic: json
exp_type
     label Expression type
     type basic:string
exp_set
     label Expressions
     type basic:file
exp_set_json
     label Expressions (json)
     type basic:json
source
     label Gene ID source
```

• Dictyostelium discoideum: Dictyostelium discoideum

```
type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
feature_type
     label Feature type
     type basic:string
```

Expression matrix

```
data:expressionsetmergeexpressions (list:data:expression
                                                                                           exps,
                                              list:basic:string genes) [Source: v1.1.1]
```

Merge expression data to create an expression matrix where each column represents all the gene expression levels from a single experiment, and each row represents the expression of a gene across all experiments.

Input arguments exps

```
label Gene expressions
     type list:data:expression
genes
     label Filter genes
     type list:basic:string
     required False
Output results expset
     label Expression set
     type basic:file
expset_type
     label Expression set type
     type basic:string
```

Expression time course

```
data:etcupload-etc (basic:file src) [Source: v1.1.1]
Upload Expression time course.
Input arguments src
     label Expression time course file (xls or tab)
     type basic:file
```

description Expression time course

```
required True
    validate_regex \.(xls|xlsx|tab)$
Output results etcfile
    label Expression time course file
    type basic:file
etc
    label Expression time course
    type basic:json
```

FASTA file

```
data:seq:nucleotideupload-fasta-nucl (basic:file src, basic:string species, basic:string build, basic:string source) [Source: v2.0.0]
```

Import a FASTA file, which is a text-based format for representing either nucleotide sequences or peptide sequences, in which nucleotides or amino acids are represented using single-letter codes.

Input arguments src

• Dictyostelium discoideum: Dictyostelium discoideum

build

```
label Genome build
  type basic:string
  required False
source
```

label Database source

```
type basic:string
     required False
Output results fastagz
     label FASTA file (compressed)
     type basic:file
fasta
     label FASTA file
     type basic:file
fai
     label FASTA file index
     type basic:file
number
     label Number of sequences
     type basic:integer
species
     label Species
     type basic:string
     required False
source
     label Database source
     type basic:string
     required False
build
     label Build
     type basic:string
     required False
FASTQ file (paired-end)
data:reads:fastq:pairedupload-fastq-paired (list:basic:file
                                                                                              src1,
                                                          list:basic:file src2) [Source: v2.2.1]
Import paired-end reads in FASTQ format, which is a text-based format for storing both a biological sequence (usually
nucleotide sequence) and its corresponding quality scores.
Input arguments src1
     label Mate1
     type list:basic:file
     description Sequencing reads in FASTQ format. Supported extensions: .fastq.gz (preferred), .fq.* or
          .fastq.*
```

```
validate_regex (\.(fastq|fq)(|\.gz|\.bz2|\.tgz|\.tar\.gz|\.tar\.bz2|\.
          zip|\.rar|\.7z))|(\.bz2)$
src2
     label Mate2
     type list:basic:file
     description Sequencing reads in FASTQ format. Supported extensions: .fastq.gz (preferred), .fq.* or
          .fastq.*
     validate_regex (\.(fastq|fq)(|\.gz|\.bz2|\.tgz|\.tar\.gz|\.tar\.bz2|\.
          zip|\langle .rar|\langle .7z\rangle\rangle|(\langle .bz2\rangle)$
Output results fastq
     label Reads file (mate 1)
     type list:basic:file
fastq2
     label Reads file (mate 2)
     type list:basic:file
fastqc_url
     label Quality control with FastQC (Upstream)
     type list:basic:file:html
fastqc_url2
     label Quality control with FastQC (Downstream)
     type list:basic:file:html
fastqc_archive
     label Download FastQC archive (Upstream)
     type list:basic:file
fastqc archive2
     label Download FastQC archive (Downstream)
     type list:basic:file
FASTQ file (single-end)
data:reads:fastq:singleupload-fastq-single (list:basic:file src) [Source: v2.2.1]
Import single-end reads in FASTQ format, which is a text-based format for storing both a biological sequence (usually
nucleotide sequence) and its corresponding quality scores.
Input arguments src
     label Reads
     type list:basic:file
     description Sequencing reads in FASTQ format. Supported extensions: .fastq.gz (preferred), .fq.* or
          .fastq.*
```

```
validate_regex (\.(fastq|fq)(|\.gz|\.bz2|\.tgz|\.tar\.gz|\.tar\.bz2|\.
          zip|\.rar|\.7z))|(\.bz2)$
Output results fastq
     label Reads file
     type list:basic:file
fastqc_url
     label Quality control with FastQC
     type list:basic:file:html
fastqc_archive
     label Download FastQC archive
     type list:basic:file
GAF file
data:gaf:2:Oupload-gaf (basic:file src, basic:string source, basic:string species) [Source: v1.1.1]
GO annotation file (GAF v2.0) relating gene ID and associated GO terms
Input arguments src
     label GO annotation file (GAF v2.0)
     type basic:file
     description Upload GO annotation file (GAF v2.0) relating gene ID and associated GO terms
source
     label Gene ID database
     type basic:string
     choices
           • AFFY: AFFY
            • DICTYBASE: DICTYBASE
            • ENSEMBL: ENSEMBL
            • MGI: MGI
            • NCBI: NCBI
            • UCSC: UCSC
            • UniProtKB: UniProtKB
species
     label Species
     type basic:string
Output results gaf
     label GO annotation file (GAF v2.0)
     type basic:file
```

```
gaf_obj
     label GAF object
     type basic:file
source
     label Gene ID database
     type basic:string
species
     label Species
     type basic:string
GATK3 (HaplotypeCaller)
                                                                                        alignment,
data:variants:vcf:gatk:hcvc-gatk-hc (data:alignment:bam
                                                 data:genome:fasta
                                                                                           genome,
                                                 data:masterfile:amplicon
                                                                          intervals, data:bed
                                                                                                in-
                                                 tervals_bed,
                                                                   data:variants:vcf
                                                                                               db-
                                                            basic:integer
                                                                                   stand_call_conf,
                                                 snp,
                                                 basic:integer
                                                                        stand emit conf,
                                                                                               ba-
                                                 sic:integer mbq) [Source: v0.4.0]
GATK HaplotypeCaller Variant Calling
Input arguments alignment
     label Alignment file (BAM)
     type data:alignment:bam
genome
     label Genome
     type data:genome:fasta
intervals
     label Intervals (from master file)
     type data:masterfile:amplicon
     description Use this option to perform the analysis over only part of the genome. This option is not
          compatible with "intervals_bed" option.
     required False
intervals_bed
     label Intervals (from BED file)
     type data:bed
     description Use this option to perform the analysis over only part of the genome. This options is not
          compatible with "intervals" option.
     required False
dbsnp
     label dbSNP file
```

```
type data:variants:vcf
stand_call_conf
     label Min call confidence threshold
     type basic:integer
     description The minimum phred-scaled confidence threshold at which variants should be called.
     default 20
stand_emit_conf
     label Emission confidence threshold
     type basic:integer
     description The minimum confidence threshold (phred-scaled) at which the program should emit sites
          that appear to be possibly variant.
     default 20
mbq
     label Min Base Quality
     type basic:integer
     description Minimum base quality required to consider a base for calling.
     default 20
Output results vcf
     label Variants
     type basic:file
tbi
     label Tabix index
     type basic:file
species
     label Species
     type basic:string
build
     label Build
     type basic:string
GATK4 (HaplotypeCaller)
data:variants:vcf:gatk:hcvc-gatk4-hc (data:alignment:bam
                                                                                        alignment,
                                                  data:genome:fasta
                                                                                          genome,
                                                  data:masterfile:amplicon intervals, data:bed
                                                  tervals bed.
                                                                data:variants:vcf
                                                                                     dbsnp.
                                                  sic:integer stand_call_conf, basic:integer mbq,
                                                  basic:integer max_reads) [Source: v0.2.0]
```

```
GATK HaplotypeCaller Variant Calling
Input arguments alignment
     label Alignment file (BAM)
     type data:alignment:bam
genome
     label Genome
     type data:genome:fasta
intervals
     label Intervals (from master file)
     type data:masterfile:amplicon
     description Use this option to perform the analysis over only part of the genome. This option is not
          compatible with "intervals_bed" option.
     required False
intervals bed
     label Intervals (from BED file)
     type data:bed
     description Use this option to perform the analysis over only part of the genome. This options is not
          compatible with "intervals" option.
     required False
dbsnp
     label dbSNP file
     type data:variants:vcf
stand_call_conf
     label Min call confidence threshold
     type basic:integer
     description The minimum phred-scaled confidence threshold at which variants should be called.
     default 20
mbq
     label Min Base Quality
     type basic:integer
     description Minimum base quality required to consider a base for calling.
     default 20
max_reads
     label Max reads per aligment start site
     type basic:integer
     description Maximum number of reads to retain per alignment start position. Reads above this threshold
          will be downsampled. Set to 0 to disable.
```

```
default 50
Output results vcf
     label Variants
     type basic:file
tbi
     label Tabix index
     type basic:file
species
     label Species
     type basic:string
build
     label Build
     type basic:string
GFF3 file
data:annotation:gff3upload-gff3 (basic:file src, basic:string source, basic:string species, ba-
                                           sic:string build) [Source: v3.2.1]
Import a General Feature Format (GFF) file which is a file format used for describing genes and other fea-
tures of DNA, RNA and protein sequences. See [here](https://useast.ensembl.org/info/website/upload/gff3.html) and
[here](https://en.wikipedia.org/wiki/General_feature_format) for more information.
Input arguments src
     label Annotation (GFF3)
     type basic:file
     description Annotation in GFF3 format. Supported extensions are: .gff, .gff3 and .gtf
     validate\_regex \. (gff|gff3|gtf) (|\.gz|\.bz2|\.tgz|\.tar\.gz|\.tar\.bz2|\.
          zip|\.rar|\.7z)$
source
     label Gene ID database
     type basic:string
     choices
            • AFFY: AFFY
            • DICTYBASE: DICTYBASE
            • ENSEMBL: ENSEMBL
            • NCBI: NCBI
            • UCSC: UCSC
species
     label Species
```

196

```
type basic:string
     description Species latin name.
     choices
            • Homo sapiens: Homo sapiens
            • Mus musculus: Mus musculus
            • Rattus norvegicus: Rattus norvegicus
            • Dictyostelium discoideum: Dictyostelium discoideum
            • Odocoileus virginianus texanus: Odocoileus virginianus texanus
           • Solanum tuberosum: Solanum tuberosum
build
     label Build
     type basic:string
Output results annot
     label Uploaded GFF3 file
     type basic:file
annot sorted
     label Sorted GFF3 file
     type basic:file
annot_sorted_idx_igv
     label IGV index for sorted GFF3
     type basic:file
annot_sorted_track_jbrowse
     label Jbrowse track for sorted GFF3
     type basic:file
source
     label Gene ID database
     type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
```

GO Enrichment analysis

```
data:goeagoenrichment (data:ontology:obo ontology, data:gaf gaf, list:basic:string genes, ba-
                             sic:string source, basic:string species, basic:decimal pval_threshold, ba-
                             sic:integer min_genes) [Source: v3.2.1]
Identify significantly enriched Gene Ontology terms for given genes.
Input arguments ontology
     label Gene Ontology
     type data:ontology:obo
gaf
     label GO annotation file (GAF v2.0)
     type data:gaf
genes
     label List of genes
     type list:basic:string
     placeholder new gene id
source
     label Source
     type basic:string
species
     label Species
     type basic:string
     description Species latin name. This field is required if gene subset is set.
     choices
            • Homo sapiens: Homo sapiens
            • Mus musculus: Mus musculus
            • Rattus norvegicus: Rattus norvegicus
            • Dictyostelium discoideum: Dictyostelium discoideum
            • Odocoileus virginianus texanus: Odocoileus virginianus texanus
            • Solanum tuberosum: Solanum tuberosum
pval_threshold
     label P-value threshold
     type basic:decimal
     required False
     default 0.1
min_genes
     label Minimum number of genes
```

type basic:integer

```
description Minimum number of genes on a GO term.
     required False
     default 1
Output results terms
     label Enriched terms
     type basic: json
source
     label Source
     type basic:string
species
     label Species
     type basic:string
GTF file
data:annotation:gtfupload-gtf (basic:file src, basic:string source, basic:string species, ba-
                                         sic:string build) [Source: v3.2.1]
Import a Gene Transfer Format (GTF) file. It is a file format used to hold information about gene structure. It is a
tab-delimited text format based on the general feature format (GFF), but contains some additional conventions specific
to gene information. See [here](https://en.wikipedia.org/wiki/General_feature_format) for differences between GFF
and GTF files.
Input arguments src
     label Annotation (GTF)
     type basic:file
     description Annotation in GTF format.
     validate_regex \.(qtf|qff)(|\.qz|\.bz2|\.tqz|\.tar\.qz|\.tar\.bz2|\.
          zip|\.rar|\.7z)$
source
     label Gene ID database
     type basic:string
     choices
            • AFFY: AFFY
            • DICTYBASE: DICTYBASE
            • ENSEMBL: ENSEMBL
            • NCBI: NCBI
            • UCSC: UCSC
species
     label Species
     type basic:string
```

```
description Species latin name.
     choices
            • Homo sapiens: Homo sapiens
            • Mus musculus: Mus musculus
            • Rattus norvegicus: Rattus norvegicus
            • Dictyostelium discoideum: Dictyostelium discoideum
            • Odocoileus virginianus texanus: Odocoileus virginianus texanus
            • Solanum tuberosum: Solanum tuberosum
build
     label Build
     type basic:string
Output results annot
     label Uploaded GTF file
     type basic:file
annot sorted
     label Sorted GTF file
     type basic:file
annot\_sorted\_idx\_igv
     label IGV index for sorted GTF file
     type basic:file
     required False
annot_sorted_track_jbrowse
     label Jbrowse track for sorted GTF
     type basic:file
     required False
source
     label Gene ID database
     type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
```

Gene expression indices

```
data:index:expressionindex-fasta-nucl (data:seq:nucleotide
                                                                                 nucl,
                                                                                             ba-
                                                  sic:string
                                                                                    nucl_genome,
                                                                                        genome,
                                                  data:genome:fasta
                                                  data:annotation:gtf
                                                                             annotation,
                                                                                             ba-
                                                              source, basic:string
                                                  sic:string
                                                                                    species, ba-
                                                  sic:string build) [Source: v0.3.2]
Generate gene expression indices.
Input arguments nucl
     label Nucleotide sequence
     type data:seq:nucleotide
     required False
     hidden genome
nucl_genome
     label Type of nucleotide sequence
     type basic:string
     hidden !nucl
     default gs
     choices
            • Genome sequence: gs
            • Transcript sequences: ts
genome
     label Genome sequence
     type data:genome:fasta
     required False
     hidden nucl
annotation
     label Annotation
     type data:annotation:gtf
     required False
     hidden nucl && nucl_genome == 'ts'
source
     label Gene ID database
     type basic:string
     required False
     hidden !(nucl && nucl_genome == 'ts')
     choices
            • AFFY: AFFY
```

```
• DICTYBASE: DICTYBASE
            • ENSEMBL: ENSEMBL
            • NCBI: NCBI
            • UCSC: UCSC
species
     label Species
     type basic:string
     description Species latin name.
     required False
     hidden !(nucl && nucl_genome == 'ts')
     choices
            • Homo sapiens: Homo sapiens
            • Mus musculus: Mus musculus
            • Rattus norvegicus: Rattus norvegicus
            • Dictyostelium discoideum: Dictyostelium discoideum
            • Odocoileus virginianus texanus: Odocoileus virginianus texanus
            • Solanum tuberosum: Solanum tuberosum
build
     label Genome build
     type basic:string
     required False
     hidden !(nucl && nucl_genome == 'ts')
Output results rsem_index
     label RSEM index
     type basic:dir
source
     label Gene ID database
     type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
```

Gene set

```
data:genesetupload-geneset (basic:file src, basic:string source, basic:string species) [Source:
                                   v1.1.2]
Import a set of genes. Provide one gene ID per line in a .tab, .tab.gz, or .txt file format.
Input arguments src
     label Gene set
     type basic:file
     description List of genes (.tab/.txt, one Gene ID per line. Supported extensions: .tab, .tab.gz (preferred),
         tab.*
     validate_regex (\.(tab|txt)(|\.gz|\.bz2|\.tgz|\.tar\.gz|\.tar\.bz2|\.
          zip|\.rar|\.7z))|(\.bz2)$
source
     label Gene ID source
     type basic:string
     choices
            • AFFY: AFFY
            • DICTYBASE: DICTYBASE
            • ENSEMBL: ENSEMBL
            • NCBI: NCBI
            • UCSC: UCSC
species
     label Species
     type basic:string
     description Species latin name.
     choices
            • Homo sapiens: Homo sapiens
            • Mus musculus: Mus musculus
            • Rattus norvegicus: Rattus norvegicus
            • Dictyostelium discoideum: Dictyostelium discoideum
            • Odocoileus virginianus texanus: Odocoileus virginianus texanus
            • Solanum tuberosum: Solanum tuberosum
Output results geneset
     label Gene set
     type basic:file
geneset_json
     label Gene set (JSON)
     type basic: json
```

```
source
     label Gene ID source
     type basic:string
species
     label Species
     type basic:string
Gene set (create from Venn diagram)
data:geneset:venncreate-geneset-venn (list:basic:string genes, basic:string source, ba-
                                               sic:string species, basic:file venn) [Source: v1.1.2]
Create a gene set from a Venn diagram.
Input arguments genes
     label Genes
     type list:basic:string
     description List of genes.
source
     label Gene ID source
     type basic:string
     choices
            • AFFY: AFFY
            • DICTYBASE: DICTYBASE
            • ENSEMBL: ENSEMBL
            • NCBI: NCBI
            • UCSC: UCSC
species
     label Species
     type basic:string
     description Species latin name.
     choices
            • Homo sapiens: Homo sapiens
            • Mus musculus: Mus musculus
            • Rattus norvegicus: Rattus norvegicus
            • Dictyostelium discoideum: Dictyostelium discoideum
            • Odocoileus virginianus texanus: Odocoileus virginianus texanus
```

• Solanum tuberosum: Solanum tuberosum

venn

```
label Venn diagram
     type basic:file
     description JSON file. Supported extensions: .json.gz
     validate_regex (\.json)(|\.gz|\.bz2|\.tgz|\.tar\.gz|\.tar\.bz2|\.zip|\.
          rar | .7z $
Output results geneset
     label Gene set
     type basic:file
geneset_json
     label Gene set (JSON)
     type basic:json
source
     label Gene ID source
     type basic:string
species
     label Species
     type basic:string
venn
     label Venn diagram
     type basic: json
Gene set (create)
data:genesetcreate-geneset (list:basic:string
                                                                basic:string
                                                       genes.
                                                                                 source.
                                                                                           ba-
                                   sic:string species) [Source: v1.1.2]
Create a gene set from a list of genes.
Input arguments genes
     label Genes
     type list:basic:string
     description List of genes.
source
     label Gene ID source
     type basic:string
     choices
            • AFFY: AFFY
            • DICTYBASE: DICTYBASE
            • ENSEMBL: ENSEMBL
            • NCBI: NCBI
```

```
• UCSC: UCSC
species
     label Species
     type basic:string
     description Species latin name.
     choices
            • Homo sapiens: Homo sapiens
            • Mus musculus: Mus musculus
            • Rattus norvegicus: Rattus norvegicus
            • Dictyostelium discoideum: Dictyostelium discoideum
            • Odocoileus virginianus texanus: Odocoileus virginianus texanus
            • Solanum tuberosum: Solanum tuberosum
Output results geneset
     label Gene set
     type basic:file
geneset json
     label Gene set (JSON)
     type basic: json
source
     label Gene ID source
     type basic:string
species
     label Species
     type basic:string
Genome
data:genome:fastaupload-genome (basic:file src, basic:string species, basic:string build,
                                         basic:file
                                                    bowtie_index, basic:file bowtie2_index, ba-
                                         sic:file bwa_index, basic:file hisat2_index, basic:file sub-
                                         read_index, basic:file walt_index) [Source: v3.3.2]
Import genome sequence in FASTA format which includes .fasta.gz (preferred), .fa., .fna., or .fasta extensions.
Input arguments src
     label Genome sequence (FASTA)
     type basic:file
     description Genome sequence in FASTA format. Supported extensions: .fasta.gz (preferred), .fa.*, .fna.*
          or .fasta.*
     validate\_regex \. (fasta|fa|fna|fsa) (|\.gz|\.bz2|\.tgz|\.tar\.gz|\.tar\.
```

 $bz2|\.zip|\.rar|\.7z)$ \$

```
species
     label Species
     type basic:string
     description Species latin name.
     choices
            • Homo sapiens: Homo sapiens
            • Mus musculus: Mus musculus
            • Rattus norvegicus: Rattus norvegicus
            • Dictyostelium discoideum: Dictyostelium discoideum
            • Odocoileus virginianus texanus: Odocoileus virginianus texanus
            • Solanum tuberosum: Solanum tuberosum
build
     label Genome build
     type basic:string
advanced.bowtie index
     label Bowtie index files
     type basic:file
     description Bowtie index files. Supported extensions (*.tar.gz).
     required False
     validate_regex (\.tar\.gz)$
advanced.bowtie2_index
     label Bowtie2 index files
     type basic:file
     description Bowtie2 index files. Supported extensions (*.tar.gz).
     required False
     validate_regex (\.tar\.gz)$
advanced.bwa_index
     label BWA index files
     type basic:file
     description BWA index files. Supported extensions (*.tar.gz).
     required False
     validate_regex (\.tar\.gz)$
advanced.hisat2_index
     label HISAT2 index files
     type basic:file
     description HISAT2 index files. Supported extensions (*.tar.gz).
```

```
required False
     validate_regex (\.tar\.gz)$
advanced.subread_index
     label subread index files
     type basic:file
     description Subread index files. Supported extensions (*.tar.gz).
     required False
     validate_regex (\.tar\.gz)$
advanced.walt_index
     label WALT index files
     type basic:file
     description WALT index files. Supported extensions (*.tar.gz).
     required False
     validate_regex (\.tar\.gz)$
Output results fastagz
     label Genome FASTA file (compressed)
     type basic:file
fasta
     label Genome FASTA file
     type basic:file
index_bt
     label Bowtie index
     type basic:dir
index bt2
     label Bowtie2 index
     type basic:dir
index_bwa
     label BWA index
     type basic:dir
index_hisat2
     label HISAT2 index
     type basic:dir
index_subread
     label subread index
     type basic:dir
index_walt
```

```
label WALT index
     type basic:dir
fai
     label Fasta index
     type basic:file
dict
     label Fasta dict
     type basic:file
fasta_track_jbrowse
     label Jbrowse track
     type basic:file
     hidden True
species
     label Species
     type basic:string
build
     label Build
     type basic:string
```

HISAT2

```
data:alignment:bam:hisat2alignment-hisat2 (data:genome:fasta genome, data:reads:fastq reads, basic:boolean soft-clip, basic:integer noncansplice, basic:boolean cufflinks) [Source: v1.6.1]
```

HISAT2 is a fast and sensitive alignment program for mapping next-generation sequencing reads (both DNA and RNA) to a population of genomes (as well as to a single reference genome). See [here](https://ccb.jhu.edu/software/hisat2/index.shtml) for more information.

Input arguments genome

```
label Reference genome
    type data:genome:fasta
reads
    label Reads
    type data:reads:fastq
softclip
    label Disallow soft clipping
    type basic:boolean
    default False
```

spliced_alignments.noncansplice

```
label Non-canonical splice sites penalty (optional)
     type basic:integer
     description Sets the penalty for each pair of non-canonical splice sites (e.g. non-GT/AG).
     required False
spliced_alignments.cufflinks
     label Report alignments tailored specifically for Cufflinks
     type basic:boolean
     description With this option, HISAT2 looks for novel splice sites with three signals (GT/AG, GC/AG,
          AT/AC), but all user-provided splice sites are used irrespective of their signals. HISAT2 produces
          an optional field, XS:A:[+-], for every spliced alignment.
     default False
Output results bam
     label Alignment file
     type basic:file
     description Position sorted alignment
bai
     label Index BAI
     type basic:file
stats
     label Statistics
     type basic:file
splice_junctions
     label Splice junctions
     type basic:file
unmapped_f
     label Unmapped reads (mate 1)
     type basic:file
     required False
unmapped_r
     label Unmapped reads (mate 2)
     type basic:file
     required False
bigwig
     label BigWig file
     type basic:file
     required False
```

species

```
label Species
     type basic:string
build
     label Build
     type basic:string
HMR
data:wgbs:hmrhmr (data:wgbs:methcounts methcounts) [Source: v1.1.0]
Identify hypo-methylated regions.
Input arguments methcounts
     label Methylation levels
     type data:wgbs:methcounts
     description Methylation levels data calculated using methcounts.
Output results hmr
     label Hypo-methylated regions
     type basic:file
tbi_jbrowse
     label Bed file index for Jbrowse
     type basic:file
species
     label Species
     type basic:string
build
     label Build
     type basic:string
HTSeq-count (CPM)
data:expression:htseq:cpmhtseq-count-raw (data:alignment:bam
                                                                                     alignments.
                                                      data:annotation:gtf gtf, basic:string mode,
                                                      basic:string
                                                                    stranded, basic:string
                                                      ture class,
                                                                   basic:string
                                                                                    id attribute,
```

HTSeq-count is useful for preprocessing RNA-Seq alignments for differential expression calling. It counts the number of reads that map to a genomic feature (e.g. gene). For computationally efficient quantification consider using featureCounts instead of HTSeq-count.

basic:string

The expressions with raw counts, produced by HTSeq are then normalized by computing CPM. See [the official website](https://htseq.readthedocs.io/en/release_0.9.1) and [the introductory paper](https://academic.oup.com/bioinformatics/article/31/2/166/2366196) for more information.

ba-

feature_type,

sic:boolean name_ordered) [Source: v1.5.1]

For computationally efficient quantification consider using featureCounts instead of HTSeq-count.

```
Input arguments alignments
```

```
label Aligned reads
```

type data:alignment:bam

gtf

label Annotation (GTF)

type data:annotation:gtf

mode

label Mode

type basic:string

description Mode to handle reads overlapping more than one feature. Possible values for <mode> are union, intersection-strict and intersection-nonempty

default union

choices

- union: union
- intersection-strict: intersection-strict
- intersection-nonempty: intersection-nonempty

stranded

label Is data from a strand specific assay?

```
type basic:string
```

description For stranded=no, a read is considered overlapping with a feature regardless of whether it is mapped to the same or the opposite strand as the feature. For stranded=yes and single-end reads, the read has to be mapped to the same strand as the feature. For paired-end reads, the first read has to be on the same strand and the second read on the opposite strand. For stranded=reverse, these rules are reversed

default yes

choices

- yes: yes
- no: no
- reverse: reverse

feature_class

label Feature class

type basic:string

description Feature class (3rd column in GTF file) to be used. All other features will be ignored.

default exon

id_attribute

label ID attribute

type basic:string

```
description GFF attribute to be used as feature ID. Several GTF lines with the same feature ID will be
           considered as parts of the same feature. The feature ID is used to identity the counts in the output
          table.
     default gene_id
feature_type
     label Feature type
     type basic:string
     description The type of feature the quantification program summarizes over (e.g. gene or transcript-level
          analysis).
     default gene
     choices
             • gene: gene
             • transcript: transcript
name_ordered
     label Use name-ordered BAM file for counting reads
     type basic:boolean
     description Use name-sorted BAM file for reads quantification. Improves compatibility with larger BAM
           files, but requires more computational time. Setting this to false may cause the process to fail for
          large BAM files due to buffer overflow.
     default True
Output results htseq_output
     label HTseq-count output
     type basic:file
rc
     label Read count
     type basic:file
exp
     label CPM (Counts per million)
     type basic:file
exp_json
     label CPM (json)
     type basic: json
exp_set
     label Expressions
     type basic:file
exp_set_json
     label Expressions (ison)
     type basic: json
```

```
exp_type
    label Expression Type (default output)
    type basic:string
source
    label Gene ID database
    type basic:string
species
    label Species
    type basic:string
build
    label Build
    type basic:string
feature_type
    label Feature type
    type basic:string
```

HTSeq-count (TPM)

```
data:expression:htseq:normalizedhtseq-count (data:alignment:bam
                                                                                       alignments,
                                                                                       gff,
                                                           data:annotation:gtf
                                                           sic:string mode, basic:string stranded,
                                                            basic:string
                                                                              feature class,
                                                                                               ba-
                                                           sic:string
                                                                              id_attribute,
                                                                                               ba-
                                                            sic:string
                                                                             feature_type,
                                                                                               ba-
                                                           sic:boolean
                                                                            name_ordered) [Source:
                                                            v1.4.1]
```

HTSeq-count is useful for preprocessing RNA-Seq alignments for differential expression calling. It counts the number of reads that map to a genomic feature (e.g. gene).

The expressions with raw counts, produced by HTSeq are then normalized by computing FPKM and TPM.

For computationally efficient quantification consider using featureCounts instead of HTSeq-count.

Input arguments alignments

```
label Aligned reads
    type data:alignment:bam

gff
    label Annotation (GFF)
    type data:annotation:gtf

mode
    label Mode
    type basic:string
```

description Mode to handle reads overlapping more than one feature. Possible values for <mode> are union, intersection-strict and intersection-nonempty

default union

choices

- union: union
- intersection-strict: intersection-strict
- intersection-nonempty: intersection-nonempty

stranded

label Is data from a strand specific assay?

type basic:string

description For stranded=no, a read is considered overlapping with a feature regardless of whether it is mapped to the same or the opposite strand as the feature. For stranded=yes and single-end reads, the read has to be mapped to the same strand as the feature. For paired-end reads, the first read has to be on the same strand and the second read on the opposite strand. For stranded=reverse, these rules are reversed

default yes

choices

- yes: yes
- no: no
- reverse: reverse

feature_class

label Feature class

type basic:string

description Feature class (3rd column in GFF file) to be used. All other features will be ignored.

default exon

$id_attribute$

label ID attribute

type basic:string

description GFF attribute to be used as feature ID. Several GFF lines with the same feature ID will be considered as parts of the same feature. The feature ID is used to identity the counts in the output table.

default gene_id

feature_type

label Feature type

type basic:string

description The type of feature the quantification program summarizes over (e.g. gene or transcript-level analysis).

default gene

choices

```
• gene: gene
            • transcript: transcript
name_ordered
     label Use name-ordered BAM file for counting reads
     type basic:boolean
     description Use name-sorted BAM file for reads quantification. Improves compatibility with larger BAM
          files, but requires more computational time. Setting this to false may cause the process to fail for
          large BAM files due to buffer overflow.
     default True
Output results htseq_output
     label HTseq-count output
     type basic:file
rc
     label Read counts
     type basic:file
fpkm
     label FPKM
     type basic:file
exp
     label TPM (Transcripts Per Million)
     type basic:file
exp_json
     label TPM (json)
     type basic: json
exp_type
     label Expression Type (default output)
     type basic:string
exp_set
     label Expressions
     type basic:file
exp_set_json
     label Expressions (json)
     type basic: json
source
     label Gene ID database
     type basic:string
species
```

```
label Species
    type basic:string
build
    label Build
    type basic:string
feature_type
    label Feature type
    type basic:string
```

Hierarchical clustering of genes

```
data:clustering:hierarchical:geneclustering-hierarchical-genes (list:data:expression exps,
```

```
ba-
sic:boolean ad-
vanced.
list:basic:string genes,
ba-
sic:string source,
ba-
sic:string species,
sic:boolean log2,
ba-
sic:boolean z_score,
ba-
sic:string dis-
tance_metric,
ba-
sic:string link-
age\_method,
ba-
sic:boolean or-
der) [Source:
```

v3.0.1]

Hierarchical clustering of genes.

```
Input arguments exps
```

```
label Expressions

type list:data:expression

description Select at least two data objects.

advanced

label Show advanced options

type basic:boolean
```

preprocessing.genes

default False

```
label Gene subset
     type list:basic:string
     description Select at least two genes or leave this field empty.
     required False
     placeholder new gene id
preprocessing.source
     label Gene ID database of selected genes
     type basic:string
     description This field is required if gene subset is set.
     required False
     hidden !preprocessing.genes
preprocessing.species
     label Species
     type basic:string
     description Species latin name. This field is required if gene subset is set.
     required False
     hidden !preprocessing.genes
     choices
            • Homo sapiens: Homo sapiens
            • Mus musculus: Mus musculus
            • Rattus norvegicus: Rattus norvegicus
            • Dictyostelium discoideum: Dictyostelium discoideum
            • Odocoileus virginianus texanus: Odocoileus virginianus texanus
            • Solanum tuberosum: Solanum tuberosum
preprocessing.log2
     label Log-transform expressions
     type basic:boolean
     description Transform expressions with log2(x + 1) before clustering.
     default True
preprocessing.z_score
     label Z-score normalization
     type basic:boolean
     description Use Z-score normalization of gene expressions before clustering.
     default True
processing.distance_metric
     label Distance metric
```

```
type basic:string
default pearson
choices
```

• Euclidean: euclidean

• Pearson: pearson

• Spearman: spearman

processing.linkage_method

label Linkage method
type basic:string
default average
choices

• single: single

• average: average

• complete: complete

postprocessing.order

label Order samples optimally

type basic:boolean

default True

Output results cluster

label Hierarchical clustering

type basic: json

required False

Hierarchical clustering of samples

```
data:clustering:hierarchical:sampleclustering-hierarchical-samples (list:data:expression exps,
                                                                                           ba-
                                                                                           sic:boolean ad-
                                                                                           vanced.
                                                                                           list:basic:string genes,
                                                                                           ba-
                                                                                           sic:string source,
                                                                                           sic:string species,
                                                                                           sic:boolean log2,
                                                                                           sic:boolean z_score,
                                                                                           ba-
                                                                                           sic:string dis-
                                                                                           tance metric,
                                                                                           ba-
                                                                                           sic:string link-
                                                                                           age\_method,
                                                                                           ba-
                                                                                           sic:boolean or-
                                                                                           der) [Source:
                                                                                           v3.0.1]
Hierarchical clustering of samples.
Input arguments exps
     label Expressions
     type list:data:expression
     description Select at least two data objects.
advanced
     label Show advanced options
     type basic:boolean
     default False
preprocessing.genes
     label Gene subset
     type list:basic:string
     description Select at least two genes or leave this field empty.
     required False
     placeholder new gene id
preprocessing.source
     label Gene ID database of selected genes
     type basic:string
```

description This field is required if gene subset is set.

```
required False
     hidden !preprocessing.genes
preprocessing.species
     label Species
     type basic:string
     description Species latin name. This field is required if gene subset is set.
     required False
     hidden !preprocessing.genes
     choices
            • Homo sapiens: Homo sapiens
            • Mus musculus: Mus musculus
            • Rattus norvegicus: Rattus norvegicus
            • Dictyostelium discoideum: Dictyostelium discoideum
            • Odocoileus virginianus texanus: Odocoileus virginianus texanus
            • Solanum tuberosum: Solanum tuberosum
preprocessing.log2
     label Log-transform expressions
     type basic:boolean
     description Transform expressions with log 2(x + 1) before clustering.
     default True
preprocessing.z_score
     label Z-score normalization
     type basic:boolean
     description Use Z-score normalization of gene expressions before clustering.
     default True
processing.distance_metric
     label Distance metric
     type basic:string
     default pearson
     choices
            • Euclidean: euclidean
            • Pearson: pearson
            • Spearman: spearman
processing.linkage_method
     label Linkage method
```

type basic:string

```
default average
     choices
            • single: single
            • average: average
            • complete: complete
postprocessing.order
     label Order samples optimally
     type basic:boolean
     default True
Output results cluster
     label Hierarchical clustering
     type basic: json
     required False
Indel Realignment and Base Recalibration
data:alignment:bam:vcvc-realign-recalibrate (data:alignment:bam
                                                                                    alignment,
                                                         data:genome:fasta
                                                                                      genome,
                                                         list:data:variants:vcf
                                                                                  known_vars,
                                                         list:data:variants:vcf known_indels) [Source:
                                                         v1.0.2]
Preprocess BAM file and prepare for Variant Calling.
Input arguments alignment
     label Alignment file (BAM)
     type data:alignment:bam
genome
     label Genome
     type data:genome:fasta
known_vars
     label Known sites (dbSNP)
     type list:data:variants:vcf
known_indels
     label Known indels
     type list:data:variants:vcf
Output results bam
     label Alignment file
     type basic:file
bai
```

```
label Index BAI
     type basic:file
stats
     label Stats
     type basic:file
species
     label Species
     type basic:string
build
     label Build
     type basic:string
LoFreq (call)
data:variants:vcf:lofreqlofreq (data:alignment:bam alignment, data:genome:fasta genome,
                                          data:masterfile:amplicon intervals, basic:integer min_bq,
                                          basic:integer min_alt_bq) [Source: v0.4.1]
Lofreq (call) Variant Calling.
Input arguments alignment
     label Alignment file (BAM)
     type data:alignment:bam
genome
     label Genome
     type data:genome:fasta
intervals
     label Intervals
     type data:masterfile:amplicon
     description Use this option to perform the analysis over only part of the genome.
min_bq
     label Min baseQ
     type basic:integer
     description Skip any base with baseQ smaller than the default value.
     default 6
min_alt_bq
     label Min alternate baseQ
     type basic:integer
     description Skip alternate bases with baseQ smaller than the default value.
     default 6
```

```
Output results vcf
    label Variants
    type basic:file

tbi
    label Tabix index
    type basic:file

species
    label Species
    type basic:string

build
    label Build
    type basic:string
```

MACS 1.4

```
data:chipseq:callpeak:macs14macs14 (data:alignment:bam data:alignment:bam control, sic:string pvalue) [Source: v3.2.1] treatment, ba-
```

Model-based Analysis of ChIP-Seq (MACS 1.4) empirically models the length of the sequenced ChIP fragments, which tends to be shorter than sonication or library construction size estimates, and uses it to improve the spatial resolution of predicted binding sites. MACS also uses a dynamic Poisson distribution to effectively capture local biases in the genome sequence, allowing for more sensitive and robust prediction. See the [original paper](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2592715/) for more information.

Input arguments treatment

```
label BAM File

type data:alignment:bam

control

label BAM Background File

type data:alignment:bam

required False

pvalue

label P-value

type basic:string

default 1e-9

choices

• 1e-9: 1e-9

• 1e-6: 1e-6

Output results peaks_bed
```

label Peaks (BED)

```
type basic:file
summits_bed
     label Summits (BED)
     type basic:file
peaks_xls
     label Peaks (XLS)
     type basic:file
wiggle
     label Wiggle
     type basic:file
control_bigwig
     label Control (bigWig)
     type basic:file
     required False
treat_bigwig
     label Treat (bigWig)
     type basic:file
peaks_bigbed_igv_ucsc
     label Peaks (bigBed)
     type basic:file
     required False
summits_bigbed_igv_ucsc
     label Summits (bigBed)
     type basic:file
     required False
peaks_tbi_jbrowse
     label JBrowse track peaks file
     type basic:file
summits_tbi_jbrowse
     label JBrowse track summits file
     type basic:file
model
     label Model
     type basic:file
     required False
neg_peaks
```

```
label Negative peaks (XLS)

type basic:file

required False

species

label Species

type basic:string

build

label Build

type basic:string
```

MACS 2.0

data:chipseq:callpeak:macs2macs2-callpeak (data:alignment:bam

case. data:alignment:bam control, data:bed promoter, basic:boolean tagalign, basic:integer q_threshold, ban_sub, basic:boolean sic:integer tn5. **shift**, basic:string basic:integer dupli $duplicates_prepeak,$ cates, basic:string basic:decimal qvalue, basic:decimal pvalue, basic:decimal pvalue_prepeak, basic:integer cap num, bamfold lower, sic:integer basic:integer mfold upper, basic:integer slocal, basic:integer basic:integer extsize, basic:integer shift, basic:integer band_width, basic:boolean nolambda, basic:boolean fix_bimodal, basic:boolean nomodel, basic:boolean nomodel_prepeak, basic:boolean down_sample, basic:boolean bedgraph, basic:boolean spmr, basic:boolean call summits. basic:boolean broad. basic:decimal broad cutoff) [Source: v4.0.4]

Model-based Analysis of ChIP-Seq (MACS 2.0), is used to identify transcript factor binding sites. MACS 2.0 captures the influence of genome complexity to evaluate the significance of enriched ChIP regions, and MACS improves the spatial resolution of binding sites through combining the information of both sequencing tag position and orientation. It has also an option to link nearby peaks together in order to call broad peaks. See [here](https://github.com/taoliu/MACS/) for more information.

In addition to peak-calling, this process computes ChIP-Seq and ATAC-Seq QC metrics. Process returns a QC metrics report, fragment length estimation, and a deduplicated tagAlign file. QC report contains ENCODE 3 proposed QC metrics – [NRF](https://www.encodeproject.org/data-standards/terms/), [PBC bottlenecking coefficients, NSC, and RSC](https://genome.ucsc.edu/ENCODE/qualityMetrics.html#chipSeq).

Input arguments case

```
label Case (treatment)
type data:alignment:bam
```

```
control
     label Control (background)
     type data:alignment:bam
     required False
promoter
     label Promoter regions BED file
     type data:bed
     description BED file containing promoter regions (TSS+-1000bp for example). Needed to get the num-
          ber of peaks and reads mapped to promoter regions.
     required False
tagalign
     label Use tagAlign files
     type basic:boolean
     description Use filtered tagAlign files as case (treatment) and control (background) samples. If extsize
          parameter is not set, run MACS using input's estimated fragment length.
     default False
prepeakqc settings.q threshold
     label Quality filtering threshold
     type basic:integer
     default 30
prepeakqc_settings.n_sub
     label Number of reads to subsample
     type basic:integer
     default 15000000
prepeakqc_settings.tn5
     label TN5 shifting
     type basic:boolean
     description Tn5 transposon shifting. Shift reads on "+" strand by 4bp and reads on "-" strand by 5bp.
     default False
prepeakqc_settings.shift
     label User-defined cross-correlation peak strandshift
     type basic:integer
     description If defined, SPP tool will not try to estimate fragment length but will use the given value as
          fragment length.
     required False
settings.duplicates
     label Number of duplicates
```

```
type basic:string
```

description It controls the MACS behavior towards duplicate tags at the exact same location – the same coordination and the same strand. The 'auto' option makes MACS calculate the maximum tags at the exact same location based on binomal distribution using 1e-5 as pvalue cutoff and the 'all' option keeps all the tags. If an integer is given, at most this number of tags will be kept at the same location. The default is to keep one tag at the same location.

```
required Falsehidden tagalign
```

choices

- 1:1
- auto: auto
- all: all

settings.duplicates_prepeak

label Number of duplicates

```
type basic:string
```

description It controls the MACS behavior towards duplicate tags at the exact same location – the same coordination and the same strand. The 'auto' option makes MACS calculate the maximum tags at the exact same location based on binomal distribution using 1e-5 as pvalue cutoff and the 'all' option keeps all the tags. If an integer is given, at most this number of tags will be kept at the same location. The default is to keep one tag at the same location.

```
required False
hidden !tagalign
default all
```

choices

- 1: 1
- auto: auto
- all: all

settings.qvalue

```
label Q-value cutoff
```

```
type basic:decimal
```

description The q-value (minimum FDR) cutoff to call significant regions. Q-values are calculated from p-values using Benjamini-Hochberg procedure.

```
required False
```

disabled settings.pvalue && settings.pvalue_prepeak

settings.pvalue

```
label P-value cutoff
```

```
type basic:decimal
```

description The p-value cutoff. If specified, MACS2 will use p-value instead of q-value cutoff.

required False

```
disabled settings.qvalue
```

hidden tagalign

settings.pvalue_prepeak

label P-value cutoff

type basic:decimal

description The p-value cutoff. If specified, MACS2 will use p-value instead of q-value cutoff.

disabled settings.qvalue

hidden !tagalign || settings.qvalue

default 1e-05

settings.cap_num

label Cap number of peaks by taking top N peaks

type basic:integer

description To keep all peaks set value to 0.

disabled settings.broad

default 500000

settings.mfold_lower

label MFOLD range (lower limit)

type basic:integer

description This parameter is used to select the regions within MFOLD range of high-confidence enrichment ratio against background to build model. The regions must be lower than upper limit, and higher than the lower limit of fold enrichment. DEFAULT:10,30 means using all regions not too low (>10) and not too high (<30) to build paired-peaks model. If MACS can not find more than 100 regions to build model, it will use the –extsize parameter to continue the peak detection ONLY if –fix-bimodal is set.

required False

settings.mfold_upper

label MFOLD range (upper limit)

type basic:integer

description This parameter is used to select the regions within MFOLD range of high-confidence enrichment ratio against background to build model. The regions must be lower than upper limit, and higher than the lower limit of fold enrichment. DEFAULT:10,30 means using all regions not too low (>10) and not too high (<30) to build paired-peaks model. If MACS can not find more than 100 regions to build model, it will use the –extsize parameter to continue the peak detection ONLY if –fix-bimodal is set.

required False

settings.slocal

label Small local region

type basic:integer

description Slocal and llocal parameters control which two levels of regions will be checked around the peak regions to calculate the maximum lambda as local lambda. By default, MACS considers 1000bp for small local region (–slocal), and 10000bps for large local region (–llocal) which captures the bias from a long range effect like an open chromatin domain. You can tweak these according to your project. Remember that if the region is set too small, a sharp spike in the input data may kill the significant peak.

required False

settings.llocal

label Large local region

type basic:integer

description Slocal and llocal parameters control which two levels of regions will be checked around the peak regions to calculate the maximum lambda as local lambda. By default, MACS considers 1000bp for small local region (–slocal), and 10000bps for large local region (–llocal) which captures the bias from a long range effect like an open chromatin domain. You can tweak these according to your project. Remember that if the region is set too small, a sharp spike in the input data may kill the significant peak.

required False

settings.extsize

label extsize

type basic:integer

description While '-nomodel' is set, MACS uses this parameter to extend reads in 5'->3' direction to fix-sized fragments. For example, if the size of binding region for your transcription factor is 200 bp, and you want to bypass the model building by MACS, this parameter can be set as 200. This option is only valid when -nomodel is set or when MACS fails to build model and -fix-bimodal is on.

required False

settings.shift

label Shift

type basic:integer

description Note, this is NOT the legacy –shiftsize option which is replaced by –extsize! You can set an arbitrary shift in bp here. Please Use discretion while setting it other than default value (0). When –nomodel is set, MACS will use this value to move cutting ends (5') then apply –extsize from 5' to 3' direction to extend them to fragments. When this value is negative, ends will be moved toward 3'->5' direction, otherwise 5'->3' direction. Recommended to keep it as default 0 for ChIP-Seq datasets, or -1 * half of EXTSIZE together with –extsize option for detecting enriched cutting loci such as certain DNAseI-Seq datasets. Note, you can't set values other than 0 if format is BAMPE for paired-end data. Default is 0.

required False

settings.band_width

label Band width

type basic:integer

description The band width which is used to scan the genome ONLY for model building. You can set this parameter as the sonication fragment size expected from wet experiment. The previous side

effect on the peak detection process has been removed. So this parameter only affects the model building.

required False

settings.nolambda

label Use backgroud lambda as local lambda

type basic:boolean

description With this flag on, MACS will use the background lambda as local lambda. This means MACS will not consider the local bias at peak candidate regions.

default False

settings.fix_bimodal

label Turn on the auto paired-peak model process

type basic:boolean

description Whether turn on the auto paired-peak model process. If it's set, when MACS failed to build paired model, it will use the nomodel settings, the '-extsize' parameter to extend each tags. If set, MACS will be terminated if paired-peak model is failed.

default False

settings.nomodel

label Bypass building the shifting model

type basic:boolean

description While on, MACS will bypass building the shifting model.

hidden tagalign
default False

settings.nomodel_prepeak

label Bypass building the shifting model

type basic:boolean

description While on, MACS will bypass building the shifting model.

hidden !tagalign
default True

settings.down sample

label Down-sample

type basic:boolean

description When set, random sampling method will scale down the bigger sample. By default, MACS uses linear scaling. This option will make the results unstable and irreproducible since each time, random reads would be selected, especially the numbers (pileup, pvalue, qvalue) would change. Consider to use 'randsample' script before MACS2 runs instead.

default False

settings.bedgraph

label Save fragment pileup and control lambda

```
type basic:boolean
     description If this flag is on, MACS will store the fragment pileup, control lambda, -log10pvalue and
           -log10qvalue scores in bedGraph files. The bedGraph files will be stored in current directory named
           NAME+'_treat_pileup.bdg' for treatment data, NAME+'_control_lambda.bdg' for local lambda
           values from control, NAME+'_treat_pvalue.bdg' for Poisson pvalue scores (in -log10(pvalue) form),
           and NAME+' treat qvalue.bdg' for q-value scores from Benjamini-Hochberg-Yekutieli procedure.
     default True
settings.spmr
     label Save signal per million reads for fragment pileup profiles
     type basic:boolean
     disabled settings.bedgraph === false
     default True
settings.call_summits
     label Call summits
     type basic:boolean
     description MACS will now reanalyze the shape of signal profile (p or q-score depending on cutoff
           setting) to deconvolve subpeaks within each peak called from general procedure. It's highly recom-
           mended to detect adjacent binding events. While used, the output subpeaks of a big peak region will
           have the same peak boundaries, and different scores and peak summit positions.
     default False
settings.broad
     label Composite broad regions
     type basic:boolean
     description When this flag is on, MACS will try to composite broad regions in BED12 (a gene-model-
           like format) by putting nearby highly enriched regions into a broad region with loose cutoff. The
           broad region is controlled by another cutoff through -broad-cutoff. The maximum length of broad
           region length is 4 times of d from MACS.
     disabled settings.call_summits === true
     default False
settings.broad cutoff
     label Broad cutoff
     type basic:decimal
     description Cutoff for broad region. This option is not available unless -broad is set. If -p is set, this is
           a p-value cutoff, otherwise, it's a q-value cutoff. DEFAULT = 0.1
     required False
```

disabled settings.call_summits === true || settings.broad !== true

Output results called_peaks

label Called peaks

type basic:file

```
label Narrow peaks
     type basic:file
     required False
chip_qc
     label QC report
     type basic:file
     required False
case_prepeak_qc
     label Pre-peak QC report (case)
     type basic:file
case_tagalign
     label Filtered tagAlign (case)
     type basic:file
control_prepeak_qc
     label Pre-peak QC report (control)
     type basic:file
     required False
control_tagalign
     label Filtered tagAlign (control)
     type basic:file
     required False
narrow_peaks_bigbed_igv_ucsc
     label Narrow peaks (BigBed)
     type basic:file
     required False
summits
     label Peak summits
     type basic:file
     required False
summits_tbi_jbrowse
     label Peak summits tbi index for JBrowse
     type basic:file
     required False
summits\_bigbed\_igv\_ucsc
     label Summits (bigBed)
     type basic:file
```

```
required False
broad_peaks
     label Broad peaks
     type basic:file
     required False
gappedPeak
     label Broad peaks (bed12/gappedPeak)
     type basic:file
     required False
treat_pileup
     label Treatment pileup (bedGraph)
     type basic:file
     required False
treat_pileup_bigwig
     label Treatment pileup (bigWig)
     type basic:file
     required False
control_lambda
     label Control lambda (bedGraph)
     type basic:file
     required False
control_lambda_bigwig
     label Control lambda (bigwig)
     type basic:file
     required False
model
     label Model
     type basic:file
     required False
species
     label Species
     type basic:string
build
     label Build
     type basic:string
```

MACS2 - ROSE2

data:workflow:chipseq:macs2rose2workflow-macs-rose (data:alignment:bam

case, data:alignment:bam control. data:bed promoter, basic:boolean tagalign, baq_threshold, sic:integer basic:integer n_sub, basic:boolean tn5, basic:integer shift, baduplicates, basic:string sic:string duplicates prepeak, basic:decimal qvalue, basic:decimal pvalue, basic:decimal pvalue_prepeak, basic:integer cap_num, bamfold lower, sic:integer basic:integer mfold upper, slocal, basic:integer basic:integer llocal. basic:integer extsize, bashift, basic:integer band_width, sic:integer basic:boolean nolambda, basic:boolean fix_bimodal, basic:boolean nomodel, basic:boolean nomodel_prepeak, basic:boolean down_sample, basic:boolean bedgraph, spmr, basic:boolean basic:boolean call summits, basic:boolean broad, basic:decimal broad cutoff, basic:integer tss, basic:integer stitch. data:bed mask) [Source: v1.0.1

Input arguments case

```
label Case (treatment)
```

type data:alignment:bam

control

label Control (background)

type data:alignment:bam

required False

promoter

label Promoter regions BED file

type data:bed

description BED file containing promoter regions (TSS+-1000bp for example). Needed to get the number of peaks and reads mapped to promoter regions.

```
required False
tagalign
     label Use tagAlign files
     type basic:boolean
     description Use filtered tagAlign files as case (treatment) and control (background) samples. If extsize
           parameter is not set, run MACS using input's estimated fragment length.
     default False
prepeakqc_settings.q_threshold
     label Quality filtering threshold
     type basic:integer
     default 30
prepeakqc_settings.n_sub
     label Number of reads to subsample
     type basic:integer
     default 15000000
prepeakqc_settings.tn5
     label TN5 shifting
     type basic:boolean
     description Tn5 transposon shifting. Shift reads on "+" strand by 4bp and reads on "-" strand by 5bp.
     default False
prepeakqc_settings.shift
     label User-defined cross-correlation peak strandshift
     type basic:integer
     description If defined, SPP tool will not try to estimate fragment length but will use the given value as
          fragment length.
     required False
settings.duplicates
     label Number of duplicates
     type basic:string
     description It controls the MACS behavior towards duplicate tags at the exact same location – the same
           coordination and the same strand. The 'auto' option makes MACS calculate the maximum tags at
```

the exact same location based on binomal distribution using 1e-5 as pvalue cutoff and the 'all' option keeps all the tags. If an integer is given, at most this number of tags will be kept at the same location. The default is to keep one tag at the same location.

required False hidden tagalign choices

• 1:1

```
• auto: auto
```

• all: all

settings.duplicates_prepeak

```
label Number of duplicates
```

```
type basic:string
```

description It controls the MACS behavior towards duplicate tags at the exact same location – the same coordination and the same strand. The 'auto' option makes MACS calculate the maximum tags at the exact same location based on binomal distribution using 1e-5 as pvalue cutoff and the 'all' option keeps all the tags. If an integer is given, at most this number of tags will be kept at the same location. The default is to keep one tag at the same location.

```
required False
hidden !tagalign
default all
choices
```

• 1: 1

• auto: auto

• all: all

settings.qvalue

```
label Q-value cutoff
```

```
type basic:decimal
```

description The q-value (minimum FDR) cutoff to call significant regions. Q-values are calculated from p-values using Benjamini-Hochberg procedure.

```
required False
```

disabled settings.pvalue && settings.pvalue_prepeak

settings.pvalue

```
label P-value cutoff
```

```
type basic:decimal
```

description The p-value cutoff. If specified, MACS2 will use p-value instead of q-value cutoff.

required False

disabled settings.qvalue

hidden tagalign

settings.pvalue_prepeak

```
label P-value cutoff
```

type basic:decimal

description The p-value cutoff. If specified, MACS2 will use p-value instead of q-value cutoff.

disabled settings.qvalue

hidden !tagalign || settings.qvalue

default 1e-05

settings.cap_num

label Cap number of peaks by taking top N peaks

type basic:integer

description To keep all peaks set value to 0.

disabled settings.broad

default 500000

settings.mfold_lower

label MFOLD range (lower limit)

type basic:integer

description This parameter is used to select the regions within MFOLD range of high-confidence enrichment ratio against background to build model. The regions must be lower than upper limit, and higher than the lower limit of fold enrichment. DEFAULT:10,30 means using all regions not too low (>10) and not too high (<30) to build paired-peaks model. If MACS can not find more than 100 regions to build model, it will use the –extsize parameter to continue the peak detection ONLY if –fix-bimodal is set.

required False

settings.mfold_upper

label MFOLD range (upper limit)

type basic:integer

description This parameter is used to select the regions within MFOLD range of high-confidence enrichment ratio against background to build model. The regions must be lower than upper limit, and higher than the lower limit of fold enrichment. DEFAULT:10,30 means using all regions not too low (>10) and not too high (<30) to build paired-peaks model. If MACS can not find more than 100 regions to build model, it will use the –extsize parameter to continue the peak detection ONLY if –fix-bimodal is set.

required False

settings.slocal

label Small local region

type basic:integer

description Slocal and llocal parameters control which two levels of regions will be checked around the peak regions to calculate the maximum lambda as local lambda. By default, MACS considers 1000bp for small local region (–slocal), and 10000bps for large local region (–llocal) which captures the bias from a long range effect like an open chromatin domain. You can tweak these according to your project. Remember that if the region is set too small, a sharp spike in the input data may kill the significant peak.

required False

settings.llocal

label Large local region

type basic:integer

description Slocal and llocal parameters control which two levels of regions will be checked around the peak regions to calculate the maximum lambda as local lambda. By default, MACS considers 1000bp for small local region (–slocal), and 10000bps for large local region (–llocal) which captures

the bias from a long range effect like an open chromatin domain. You can tweak these according to your project. Remember that if the region is set too small, a sharp spike in the input data may kill the significant peak.

required False

settings.extsize

label extsize

type basic:integer

description While '-nomodel' is set, MACS uses this parameter to extend reads in 5'->3' direction to fix-sized fragments. For example, if the size of binding region for your transcription factor is 200 bp, and you want to bypass the model building by MACS, this parameter can be set as 200. This option is only valid when -nomodel is set or when MACS fails to build model and -fix-bimodal is on.

required False

settings.shift

label Shift

type basic:integer

description Note, this is NOT the legacy –shiftsize option which is replaced by –extsize! You can set an arbitrary shift in bp here. Please Use discretion while setting it other than default value (0). When –nomodel is set, MACS will use this value to move cutting ends (5') then apply –extsize from 5' to 3' direction to extend them to fragments. When this value is negative, ends will be moved toward 3'->5' direction, otherwise 5'->3' direction. Recommended to keep it as default 0 for ChIP-Seq datasets, or -1 * half of EXTSIZE together with –extsize option for detecting enriched cutting loci such as certain DNAseI-Seq datasets. Note, you can't set values other than 0 if format is BAMPE for paired-end data. Default is 0.

required False

settings.band_width

label Band width

type basic:integer

description The band width which is used to scan the genome ONLY for model building. You can set this parameter as the sonication fragment size expected from wet experiment. The previous side effect on the peak detection process has been removed. So this parameter only affects the model building.

required False

settings.nolambda

label Use backgroud lambda as local lambda

type basic:boolean

description With this flag on, MACS will use the background lambda as local lambda. This means MACS will not consider the local bias at peak candidate regions.

default False

settings.fix_bimodal

label Turn on the auto paired-peak model process

type basic:boolean

description Whether turn on the auto paired-peak model process. If it's set, when MACS failed to build paired model, it will use the nomodel settings, the '-extsize' parameter to extend each tags. If set, MACS will be terminated if paired-peak model is failed.

default False

settings.nomodel

label Bypass building the shifting model

type basic:boolean

description While on, MACS will bypass building the shifting model.

hidden tagalign
default False

settings.nomodel_prepeak

label Bypass building the shifting model

type basic:boolean

description While on, MACS will bypass building the shifting model.

hidden !tagalign
default True

settings.down_sample

label Down-sample

type basic:boolean

description When set, random sampling method will scale down the bigger sample. By default, MACS uses linear scaling. This option will make the results unstable and irreproducible since each time, random reads would be selected, especially the numbers (pileup, pvalue, qvalue) would change. Consider to use 'randsample' script before MACS2 runs instead.

default False

settings.bedgraph

label Save fragment pileup and control lambda

type basic:boolean

description If this flag is on, MACS will store the fragment pileup, control lambda, -log10pvalue and -log10qvalue scores in bedGraph files. The bedGraph files will be stored in current directory named NAME+'_treat_pileup.bdg' for treatment data, NAME+'_control_lambda.bdg' for local lambda values from control, NAME+'_treat_pvalue.bdg' for Poisson pvalue scores (in -log10(pvalue) form), and NAME+'_treat_qvalue.bdg' for q-value scores from Benjamini-Hochberg-Yekutieli procedure.

default True

settings.spmr

label Save signal per million reads for fragment pileup profiles

type basic:boolean

disabled settings.bedgraph === false

default True

 $settings.call_summits$

label Call summits

type basic:boolean

description MACS will now reanalyze the shape of signal profile (p or q-score depending on cutoff setting) to deconvolve subpeaks within each peak called from general procedure. It's highly recommended to detect adjacent binding events. While used, the output subpeaks of a big peak region will have the same peak boundaries, and different scores and peak summit positions.

default False

settings.broad

label Composite broad regions

type basic:boolean

description When this flag is on, MACS will try to composite broad regions in BED12 (a gene-model-like format) by putting nearby highly enriched regions into a broad region with loose cutoff. The broad region is controlled by another cutoff through –broad-cutoff. The maximum length of broad region length is 4 times of d from MACS.

disabled settings.call_summits === true

default False

settings.broad cutoff

label Broad cutoff

type basic:decimal

description Cutoff for broad region. This option is not available unless –broad is set. If -p is set, this is a p-value cutoff, otherwise, it's a q-value cutoff. DEFAULT = 0.1

required False

disabled settings.call_summits === true || settings.broad !== true

rose_settings.tss

label TSS exclusion

type basic:integer

description Enter a distance from TSS to exclude. 0 = no TSS exclusion

default 0

rose settings.stitch

label Stitch

type basic:integer

description Enter a max linking distance for stitching. If not given, optimal stitching parameter will be determined automatically.

required False

rose_settings.mask

label Masking BED file

type data:bed

description Mask a set of regions from analysis. Provide a BED of masking regions.

required False

Output results

Mappability

```
data:mappability:bcmmappability-bcm (data:genome:fasta genome, data:annotation:gff3 gff, basic:integer length) [Source: v2.0.1]
```

Compute genome mappability. Developed by Bioinformatics Laboratory, Faculty of Computer and Information Science, University of Ljubljana, Slovenia and Shaulsky's Lab, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA.

```
Input arguments genome
```

```
label Reference genome
type data:genome:fasta

gff
label General feature format
type data:annotation:gff3

length
label Read length
type basic:integer
default 50

Output results mappability
label Mappability
```

Mappability info

```
data:mappability:bcmupload-mappability (basic:file src) [Source: v1.1.1]
```

Upload mappability information.

type basic:file

Input arguments src

```
label Mappability file
type basic:file
description Mappability file: 2 column tab separated
validate_regex \.(tab)(|\.gz|\.bz2|\.tgz|\.tar\.gz|\.tar\.bz2|\.zip|\.
rar|\.7z)$
```

Output results mappability

```
label Uploaded mappability
type basic:file
```

Merge Expressions (ETC)

```
data:expressionset:etcmergeetc (list:data:etc exps, list:basic:string genes) [Source: v1.1.1]
Merge Expression Time Course (ETC) data.
Input arguments exps
     label Expression Time Course (ETC)
     type list:data:etc
genes
     label Filter genes
     type list:basic:string
     required False
Output results expset
     label Expression set
     type basic:file
expset_type
     label Expression set type
     type basic:string
Metabolic pathway file
data:metabolicpathwayupload-metabolic-pathway (basic:file src, basic:string source, ba-
                                                            sic:string species) [Source: v1.0.1]
Upload pathway json.
Input arguments src
     label Pathway file
     type basic:file
     description JSON file. Supported extensions: '.json', '.json.gz'
     validate_regex (\.json)(\.gz)?$
source
     label Gene ID database
     type basic:string
     choices
            • BIGG: BIGG
species
     label Species
     type basic:string
     choices
            • Homo Sapiens: Homo Sapiens
```

```
• Mus musculus: Mus musculus
Output results pathway
     label Pathway json
     type basic: json
source
     label Gene ID database
     type basic:string
species
     label Species
     type basic:string
MultiQC
data:multiqcmultiqc (list:data
                                       data, basic:boolean
                                                              dirs, basic:boolean
                                                                                     fullnames, ba-
                            sic:boolean config, basic:string cl_config) [Source: v1.1.2]
Aggregate results from bioinformatics analyses across many samples into a single report.
                                                                                                     [Mul-
tiQC](http://www.multiqc.info) searches a given directory for analysis logs and compiles a HTML report. It's a general
use tool, perfect for summarising the output from numerous bioinformatics tools.
Input arguments data
     label Input data
     type list:data
     description Select multiple data objects for which the MultiQC report is to be generated.
advanced.dirs
     label -dirs
     type basic:boolean
     description Prepend directory to sample names.
     default True
advanced.fullnames
     label -fullnames
     type basic:boolean
     description Do not clean the sample names (leave as full file name).
     default False
advanced.config
     label Use configuration file
     type basic:boolean
     description Use Genialis configuration file for MultiQC report.
     default True
```

advanced.cl_config

```
label -cl-config
     type basic:string
     description Enter text with command-line configuration options to override the defaults (e.g. cus-
          tom_logo_url: https://www.genialis.com).
     required False
Output results report
     label MultiQC report
     type basic:file:html
report_data
     label Report data
     type basic:dir
OBO file
data:ontology:oboupload-obo (basic:file src) [Source: v1.1.1]
Upload gene ontology in OBO format.
Input arguments src
     label Gene ontology (OBO)
     type basic:file
     description Gene ontology in OBO format.
     required True
     validate_regex \.obo(|\.gz|\.bz2|\.tgz|\.tar\.gz|\.tar\.bz2|\.zip|\.
          rar | \.7z) $
Output results obo
     label Ontology file
     type basic:file
obo_obj
     label OBO object
     type basic:file
PCA
data:pcapca (list:data:expression
                                     exps, list:basic:string
                                                              genes, basic:string
                                                                                    source, ba-
                sic:string species) [Source: v2.1.1]
Principal component analysis (PCA)
Input arguments exps
     label Expressions
     type list:data:expression
genes
```

```
label Gene subset
     type list:basic:string
     required False
source
     label Gene ID database of selected genes
     type basic:string
     description This field is required if gene subset is set.
     required False
species
     label Species
     type basic:string
     description Species latin name. This field is required if gene subset is set.
     required False
     choices
            • Homo sapiens: Homo sapiens
            • Mus musculus: Mus musculus
            • Rattus norvegicus: Rattus norvegicus
            • Dictyostelium discoideum: Dictyostelium discoideum
            • Odocoileus virginianus texanus: Odocoileus virginianus texanus
            • Solanum tuberosum: Solanum tuberosum
Output results pca
     label PCA
     type basic: json
Picard CollectTargetedPcrMetrics
data:picard:coveragepicard-pcrmetrics (data:alignment:bam
                                                                                      alignment,
                                                  data:masterfile:amplicon
                                                                                     master file,
                                                  data:genome:fasta genome) [Source: v0.2.1]
Calculate PCR-related metrics from targeted sequencing data using the Picard CollectTargetedPcrMetrics tool
Input arguments alignment
     label Alignment file (BAM)
     type data:alignment:bam
master file
     label Master file
     type data:masterfile:amplicon
genome
     label Genome
```

```
type data:genome:fasta
Output results target_pcr_metrics
    label Target PCR metrics
    type basic:file
target_coverage
    label Target coverage
    type basic:file
```

Pre-peakcall QC

```
data:prepeakqcqc-prepeak (data:alignment:bam alignment, basic:integer q_treshold, basic:integer n_sub, basic:boolean tn5, basic:integer shift) [Source: v0.2.2]
```

ChIP-Seq and ATAC-Seq QC metrics. Process returns a QC metrics report, fragment length estimation, and a deduplicated tagAlign file. Both fragment length estimation and the tagAlign file can be used as inputs in MACS 2.0. QC report contains ENCODE 3 proposed QC metrics – [NRF, PBC bottlenecking coefficients](https://www.encodeproject.org/data-standards/terms/), [NSC, and RSC](https://genome.ucsc.edu/ENCODE/qualityMetrics.html#chipSeq).

Input arguments alignment

```
label Aligned reads
     type data:alignment:bam
q_treshold
     label Quality filtering treshold
     type basic:integer
     default 30
n sub
     label Number of reads to subsample
     type basic:integer
     default 15000000
tn5
     label TN5 shifting
     type basic:boolean
     description Tn5 transposon shifting. Shift reads on "+" strand by 4bp and reads on "-" strand by 5bp.
     default False
shift
     label User-defined cross-correlation peak strandshift
     type basic:integer
     description If defined, SPP tool will not try to estimate fragment length but will use the given value as
```

fragment length.

```
required False
Output results chip_qc
     label QC report
     type basic:file
tagalign
     label Filtered tagAlign
     type basic:file
fraglen
     label Fragnment length
     type basic:integer
species
     label Species
     type basic:string
build
     label Build
     type basic:string
Prepare GEO - ChIP-Seq
data:other:geo:chipseqprepare-geo-chipseq (list:data:reads:fastq
                                                                                          reads.
                                                       list:data:chipseq:callpeak
                                                                                            ba-
                                                                                   macs,
                                                       sic:string name) [Source: v2.0.2]
Prepare ChIP-seq data for GEO upload.
Input arguments reads
     label Reads
     type list:data:reads:fastq
     description List of reads objects. Fastq files will be used.
macs
     label MACS
     type list:data:chipseq:callpeak
     description List of MACS2 or MACS14 objects. BedGraph (MACS2) or Wiggle (MACS14) files will
          be used.
name
     label Collection name
     type basic:string
Output results tarball
     label GEO folder
     type basic:file
```

table

```
label Annotation table
type basic:file
```

Prepare GEO - RNA-Seq

```
data:other:geo:rnaseqprepare-geo-rnaseq (list:data:reads:fastq reads, list:data:expression expressions, sic:string name) [Source: v0.1.1]
```

Prepare RNA-Seq data for GEO upload.

Input arguments reads

```
label Reads
```

type list:data:reads:fastq

description List of reads objects. Fastq files will be used.

expressions

```
label Expressions
```

type list:data:expression

description Cuffnorm data object. Expression table will be used.

name

label Collection name

type basic:string

Output results tarball

label GEO folder

type basic:file

table

label Annotation table

type basic:file

Quantify shRNA species using bowtie2

Based on 'bowtie2' output (.bam file) calculate number of mapped species. Input is limited to results from 'bowtie2' since 'YT:Z:' tag used to fetch aligned species is specific to this process. Result is a count matrix (successfully mapped reads) where species are in rows columns contain read specifics (count, species name, sequence, 'AS:::' tag value).

Input arguments alignment

```
label Alignment
type data:alignment:bam
required True
```

```
readlengths
     label Species lengths threshold
     type basic:integer
     description Species with read lengths below specified threshold will be removed from final output. De-
          fault is no removal.
alignscores
     label Align scores filter threshold
     type basic:integer
     description Species with align score below specified threshold will be removed from final output. Default
          is no removal.
Output results exp
     label Normalized expression
     type basic:file
rc
     label Read counts
     type basic:file
     required False
exp_json
     label Expression (json)
     type basic: json
exp_type
     label Expression type
     type basic:string
source
     label Gene ID source
     type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
feature_type
     label Feature type
     type basic:string
mapped_species
```

label Mapped species

```
type basic:file
```

RNA-Seq (Cuffquant)

data:workflow:rnaseq:cuffquantworkflow-rnaseq-cuffquant (data:reads:fastq reads,

(data:reads:fastq reads, data:genome:fasta genome, data:annotation annotation) [Source: v1.0.0]

Input arguments reads

```
label Input reads
    type data:reads:fastq
genome
    label genome
    type data:genome:fasta
annotation
    label Annotation file
    type data:annotation
```

ROSE2

Output results

```
data:chipseq:rose2rose2 (data:chipseq:callpeak input, data:bed input_upload, data:alignment:bam rankby, data:alignment:bam control, basic:integer tss, basic:integer stitch, data:bed mask) [Source: v4.2.1]
```

For identification of super enhancers R2 uses the Rank Ordering of Super-Enhancers algorithm (ROSE2). This takes the peaks called by RSEG for acetylation and calculates the distances in-between to judge whether they can be considered super-enhancers. The ranked values can be plotted and by locating the inflection point in the resulting graph, super-enhancers can be assigned. It can also be used with the MACS calculated data. See [here](http://younglab.wi.mit.edu/super_enhancer_code.html) for more information.

Input arguments input

```
label BED/narrowPeak file (MACS results)

type data:chipseq:callpeak

required False

input_upload

label BED file (Upload)

type data:bed

required False

rankby

label BAM File

type data:alignment:bam

description bamfile to rank enhancer by
```

```
control
     label Control BAM File
     type data:alignment:bam
     description bamfile to rank enhancer by
     required False
tss
     label TSS exclusion
     type basic:integer
     description Enter a distance from TSS to exclude. 0 = \text{no TSS} exclusion
     default 0
stitch
     label Stitch
     type basic:integer
     description Enter a max linking distance for stitching. If not given, optimal stitching parameter will be
          determined automatically.
     required False
mask
     label Masking BED file
     type data:bed
     description Mask a set of regions from analysis. Provide a BED of masking regions.
     required False
Output results all_enhancers
     label All enhancers table
     type basic:file
enhancers_with_super
     label Super enhancers table
     type basic:file
plot_points
     label Plot points
     type basic:file
plot_panel
     label Plot panel
     type basic:file
enhancer_gene
     label Enhancer to gene
     type basic:file
```

```
enhancer_top_gene
     label Enhancer to top gene
     type basic:file
gene_enhancer
     label Gene to Enhancer
     type basic:file
stitch_parameter
     label Stitch parameter
     type basic:file
     required False
all_output
     label All output
     type basic:file
scatter_plot
     label Super-Enhancer plot
     type basic: json
species
     label Species
     type basic:string
build
     label Build
     type basic:string
```

RSEM

```
data:expression:rsemrsem (data:alignment:bam alignments, basic:string read_type, data:index:expression expression_index, basic:string stranded-ness) [Source: v1.1.1]
```

RSEM is a software package for estimating gene and isoform expression levels from RNA-Seq data. The RSEM package supports threads for parallel computation of the EM algorithm, single-end and paired-end read data, quality scores, variable-length reads and RSPD estimation. See [here](https://deweylab.github.io/RSEM/README.html) and the [original paper](https://bmcbioinformatics.biomedcentral.com/articles/10.1186/1471-2105-12-323) for more information.

Input arguments alignments

```
label Aligned reads
    type data:alignment:bam
read_type
    label Type of reads
    type basic:string
```

```
default se
     choices
            • Single-end: se
            • Paired-end: pe
expression_index
     label Gene expression indices
     type data:index:expression
strandedness
     label Strandedness
     type basic:string
     default none
     choices
            • None: none
            • Forward: forward
            • Reverse: reverse
Output results rc
     label Read counts
     type basic:file
fpkm
     label FPKM
     type basic:file
exp
     label TPM (Transcripts Per Million)
     type basic:file
exp_json
     label TPM (json)
     type basic: json
exp_set
     label Expressions
     type basic:file
exp_set_json
     label Expressions (json)
     type basic:json
genes
     label Results grouped by gene
     type basic:file
```

```
transcripts
     label Results grouped by transcript
     type basic:file
log
     label RSEM log
     type basic:file
exp_type
     label Type of expression
     type basic:string
source
     label Transcript ID database
     type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
feature_type
     label Feature type
     type basic:string
Reads (QSEQ multiplexed, paired)
data:multiplexed:qseq:pairedupload-multiplexed-paired (basic:file
                                                                                   reads.
                                                                                            ba-
                                                                                  reads2,
                                                                                            ba-
                                                                      sic:file
                                                                      sic:file
                                                                                      barcodes.
                                                                      basic:file
                                                                                       annota-
                                                                      tion) [Source: v1.1.1]
Upload multiplexed NGS reds in QSEQ format.
Input arguments reads
     label Multiplexed upstream reads
     type basic:file
     description NGS reads in QSeq format. Supported extensions: .qseq.txt.bz2 (preferred), .qseq.* or
          .qseq.txt.*.
     required True
     validate\_regex ((\.qseq\\.qseq\.txt)(\.gz\\.bz2\\.tgz\\.tar\\.gz\\.tar\.
         bz2|\.zip|\.rar|\.7z))|(\.bz2)$
reads2
```

```
label Multiplexed downstream reads
     type basic:file
     description NGS reads in QSeq format. Supported extensions: .qseq.txt.bz2 (preferred), .qseq.* or
         .qseq.txt.*.
     required True
     validate\_regex ((\.qseq\\.qseq\.txt)(\.gz\\.tgz\\.tar\.gz\\.tar\.
         bz2|\.zip|\.rar|\.7z))|(\.bz2)$
barcodes
     label NGS barcodes
     type basic:file
     description Barcodes in QSeq format. Supported extensions: .qseq.txt.bz2 (preferred), .qseq.* or
         .qseq.txt.*.
     required True
     validate\_regex ((\.qseq\\.qseq\.txt)(\.gz\\.tgz\\.tar\.gz\\.tar\.
         bz2|\.zip|\.rar|\.7z))|(\.bz2)$
annotation
     label Barcode mapping
     type basic:file
     description A tsv file mapping barcodes to experiment name, e.g. "TCGCAGG\tHr00".
     required True
     validate_regex (\.csv|\.tsv)$
Output results qseq_reads
     label Multiplexed upstream reads
     type basic:file
qseq_reads2
     label Multiplexed downstream reads
     type basic:file
qseq_barcodes
     label NGS barcodes
     type basic:file
annotation
     label Barcode mapping
     type basic:file
matched
     label Matched
     type basic:string
notmatched
```

```
label Not matched
     type basic:string
badquality
     label Bad quality
     type basic:string
skipped
     label Skipped
     type basic:string
Reads (QSEQ multiplexed, single)
                                                                                          ba-
                                                                                  reads.
data:multiplexed:gseq:singleupload-multiplexed-single (basic:file
                                                                                     barcodes.
                                                                     sic:file
                                                                     basic:file
                                                                                      annota-
                                                                     tion) [Source: v1.1.1]
Upload multiplexed NGS reds in QSEQ format.
Input arguments reads
     label Multiplexed NGS reads
     type basic:file
     description NGS reads in QSeq format. Supported extensions: .qseq.txt.bz2 (preferred), .qseq.* or
          .qseq.txt.*.
     required True
     validate\_regex (\.(qseq)(|\.txt)(|\.gz|\.bz2|\.tgz|\.tar\.gz|\.tar\.
         bz2|\.zip|\.rar|\.7z))|(\.bz2)$
barcodes
     label NGS barcodes
     type basic:file
     description Barcodes in QSeq format. Supported extensions: .qseq.txt.bz2 (preferred), .qseq.* or
          .qseq.txt.*.
     required True
     validate\_regex (\.(qseq)(|\.txt)(|\.gz|\.bz2|\.tgz|\.tar\.gz|\.tar\.
         bz2 | .zip | .rar | .7z) | ( .bz2 ) $
annotation
     label Barcode mapping
     type basic:file
     description A tsv file mapping barcodes to experiment name, e.g. "TCGCAGG\tHr00".
     required True
     validate_regex (\.csv|\.tsv)$
Output results qseq_reads
```

```
label Multiplexed NGS reads
     type basic:file
qseq_barcodes
     label NGS barcodes
     type basic:file
annotation
     label Barcode mapping
     type basic:file
matched
     label Matched
     type basic:string
notmatched
     label Not matched
     type basic:string
badquality
     label Bad quality
     type basic:string
skipped
     label Skipped
     type basic:string
SAM header
data:sam:headerupload-header-sam (basic:file src) [Source: v1.1.1]
Upload a mapping file header in SAM format.
Input arguments src
     label Header (SAM)
     type basic:file
     description A mapping file header in SAM format.
     validate_regex \.(sam)$
Output results sam
     label Uploaded file
     type basic:file
```

SRA data

```
sra_accession,
                                                                                     show_advanced,
data:sraimport-sra (basic:string
                                                                basic:boolean
                                             min_spot_id,
                                                                                max_spot_id,
                                                                                                 ba-
                           basic:integer
                                                              basic:integer
                           sic:integer min_read_len, basic:boolean clip, basic:boolean
                                                                                            aligned,
                           basic:boolean unaligned) [Source: v0.1.1]
Import single or paired-end reads from Sequence Read Archive (SRA) via an SRA accession number. SRA stores raw
sequencing data and alignment information from high-throughput sequencing platforms.
Input arguments sra_accession
     label SRA accession
```

show_advanced

label Show advanced options

type basic:boolean

type basic:string

default False

advanced.min_spot_id

label Minimum spot ID

type basic:integer

required False

advanced.max_spot_id

label Maximum spot ID

type basic:integer

required False

advanced.min_read_len

label Minimum read length

type basic:integer

required False

advanced.clip

label Clip adapter sequences

type basic:boolean

default False

advanced.aligned

label Dump only aligned sequences

type basic:boolean

default False

advanced.unaligned

label Dump only unaligned sequences

type basic:boolean

```
default False
```

Output results

SRA data (paired-end)

```
data:reads:fastq:pairedimport-sra-paired (basic:string
                                                                           sra accession,
                                                                                               ba-
                                                       sic:boolean
                                                                          show_advanced,
                                                                                               ba-
                                                       sic:integer
                                                                           min_spot_id,
                                                                                              ba-
                                                                                              ba-
                                                       sic:integer
                                                                           max_spot_id,
                                                       sic:integer min_read_len, basic:boolean clip,
                                                       basic:boolean aligned, basic:boolean
                                                                                              un-
                                                       aligned) [Source: v0.1.1]
```

Import paired-end reads from Sequence Read Archive (SRA) via an SRA accession number. SRA stores raw sequencing data and alignment information from high-throughput sequencing platforms.

Input arguments sra_accession

```
label SRA accession
type basic:string
```

show_advanced

```
label Show advanced options
```

 $type \; \texttt{basic:boolean}$

default False

advanced.min_spot_id

```
label Minimum spot ID
```

type basic:integer

required False

advanced.max_spot_id

label Maximum spot ID

type basic:integer

required False

advanced.min_read_len

label Minimum read length

type basic:integer

required False

advanced.clip

label Clip adapter sequences

type basic:boolean

default False

advanced.aligned

label Dump only aligned sequences

```
type basic:boolean
     default False
advanced.unaligned
     label Dump only unaligned sequences
     type basic:boolean
     default False
Output results fastq
     label Reads file (mate 1)
     type list:basic:file
fastq2
     label Reads file (mate 2)
     type list:basic:file
fastqc_url
     label Quality control with FastQC (Upstream)
     type list:basic:file:html
fastqc url2
     label Quality control with FastQC (Downstream)
     type list:basic:file:html
fastqc_archive
     label Download FastQC archive (Upstream)
     type list:basic:file
fastqc_archive2
     label Download FastQC archive (Downstream)
     type list:basic:file
SRA data (single-end)
data:reads:fastq:singleimport-sra-single (basic:string
                                                                         sra_accession,
                                                                                            ba-
                                                      sic:boolean
                                                                        show advanced,
                                                                                            ba-
                                                      sic:integer
                                                                          min_spot_id,
                                                                                            ba-
                                                      sic:integer
                                                                         max_spot_id,
                                                                                            ba-
                                                      sic:integer min_read_len, basic:boolean clip,
                                                      basic:boolean aligned, basic:boolean
                                                      aligned) [Source: v0.1.1]
```

Import single-end reads from Sequence Read Archive (SRA) via an SRA accession number. SRA stores raw sequencing data and alignment information from high-throughput sequencing platforms.

Input arguments sra_accession

```
label SRA accession
type basic:string
```

```
show_advanced
     label Show advanced options
     type basic:boolean
     default False
advanced.min_spot_id
     label Minimum spot ID
     type basic:integer
     required False
advanced.max_spot_id
     label Maximum spot ID
     type basic:integer
     required False
advanced.min_read_len
     label Minimum read length
     type basic:integer
     required False
advanced.clip
     label Clip adapter sequences
     type basic:boolean
     default False
advanced.aligned
     label Dump only aligned sequences
     type basic:boolean
     default False
advanced.unaligned
     label Dump only unaligned sequences
     type basic:boolean
     default False
Output results fastq
     label Reads file
     type list:basic:file
fastqc_url
     label Quality control with FastQC
     type list:basic:file:html
fastqc_archive
     label Download FastQC archive
```

```
type list:basic:file
```

STAR

data:alignment:bam:staralignment-star (data:reads:fastq

reads. data:genomeindex:star genome, data:annotation annotation. basic:string exon_name, basic:integer sjdbOverhang, basic:boolean unstranded. basic:boolean noncannonical. basic:boolean chimeric, basic:integer chim-SegmentMin, basic:boolean quantmode, basic:boolean singleend, basic:boolean gene_counts, basic:string outFilterType, basic:integer out-FilterMultimapNmax, basic:integer outFilterMismatchNmax, basic:decimal outFilter-MismatchNoverLmax, basic:integer outFilterScoreMin, basic:decimal outFilterMismatchNoverReadLmax, basic:integer align-S.JoverhangMin, basic:integer alignSJD-BoverhangMin, basic:integer alignIntronalignIntronMax, Min. basic:integer sic:integer alignMatesGapMax, basic:string alignEndsType, basic:boolean two pass mode, basic:string outSAMunmapped, basic:string out-SAMattributes. basic:string outSAMattrRGline. tool_bigwig, basic:string sic:integer bin size bigwig) [Source: v1.8.11]

Spliced Transcripts Alignment to a Reference (STAR) software is based on an alignment algorithm that uses sequential maximum mappable seed search in uncompressed suffix arrays followed by seed clustering and stitching procedure. In addition to unbiased de novo detection of canonical junctions, STAR can discover non-canonical splices and chimeric (fusion) transcripts, and is also capable of mapping full-length RNA sequences. More information can be found in the [STAR manual](http://labshare.cshl.edu/shares/gingeraslab/www-data/dobin/STAR/STAR.posix/doc/STARmanual.pdf) and in the [original paper](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3530905/).

Input arguments reads

```
label Reads
```

type data:reads:fastq

genome

label Indexed reference genome

type data:genomeindex:star

description Genome index prepared by STAR aligner indexing tool.

annotation

label Annotation file (GTF/GFF3)

type data: annotation

description Insert known annotations into genome indices at the mapping stage.

required False

annotation_options.exon_name

label -sjdbGTFfeatureExon

type basic:string

description Feature type in GTF file to be used as exons for building transcripts

default exon

annotation options.sjdbOverhang

label Junction length (sjdbOverhang)

type basic:integer

description This parameter specifies the length of the genomic sequence around the annotated junction to be used in constructing the splice junction database. Ideally, this length should be equal to the ReadLength-1, where ReadLength is the length of the reads. For instance, for Illumina 2x100b paired-end reads, the ideal value is 100-1=99. In case of reads of varying length, the ideal value is max(ReadLength)-1. In most cases, the default value of 100 will work as well as the ideal value.

default 100

unstranded

label The data is unstranded

type basic:boolean

description For unstranded RNA-seq data, Cufflinks/Cuffdiff require spliced alignments with XS strand attribute, which STAR will generate with –outSAMstrandField intronMotif option. As required, the XS strand attribute will be generated for all alignments that contain splice junctions. The spliced alignments that have undefined strand (i.e. containing only non-canonical unannotated junctions) will be suppressed. If you have stranded RNA-seq data, you do not need to use any specific STAR options. Instead, you need to run Cufflinks with the library option –library-type options. For example, cufflinks –library-type fr-firststrand should be used for the standard dUTP protocol, including Illumina's stranded Tru-Seq. This option has to be used only for Cufflinks runs and not for STAR runs.

default False

noncannonical

264

label Remove non-cannonical junctions (Cufflinks compatibility)

type basic:boolean

description It is recommended to remove the non-canonical junctions for Cufflinks runs using –outFilterIntronMotifs RemoveNoncanonical.

default False

detect_chimeric.chimeric

label Detect chimeric and circular alignments

type basic:boolean

description To switch on detection of chimeric (fusion) alignments (in addition to normal mapping), –chimSegmentMin should be set to a positive value. Each chimeric alignment consists of two "segments". Each segment is non-chimeric on its own, but the segments are chimeric to each other (i.e. the segments belong to different chromosomes, or different strands, or are far from each other). Both segments may contain splice junctions, and one of the segments may contain portions of both mates.

-chimSegmentMin parameter controls the minimum mapped length of the two segments that is allowed. For example, if you have 2x75 reads and used -chimSegmentMin 20, a chimeric alignment with 130b on one chromosome and 20b on the other will be output, while 135 + 15 won't be.

default False

detect_chimeric.chimSegmentMin

label -chimSegmentMin
type basic:integer
disabled detect_chimeric.chimeric!= true

default 20

$t_coordinates.quantmode$

label Output in transcript coordinates

type basic:boolean

description With –quantMode TranscriptomeSAM option STAR will output alignments translated into transcript coordinates in the Aligned.toTranscriptome.out.bam file (in addition to alignments in genomic coordinates in Aligned.*.sam/bam files). These transcriptomic alignments can be used with various transcript quantification software that require reads to be mapped to transcriptome, such as RSEM or eXpress.

default False

t coordinates.singleend

label Allow soft-clipping and indels

type basic:boolean

description By default, the output satisfies RSEM requirements: soft-clipping or indels are not allowed. Use –quantTranscriptomeBan Singleend to allow insertions, deletions and soft-clips in the transcriptomic alignments, which can be used by some expression quantification software (e.g. eXpress).

disabled t_coordinates.quantmode != true

default False

t_coordinates.gene_counts

label Count reads

type basic:boolean

description With –quantMode GeneCounts option STAR will count number reads per gene while mapping. A read is counted if it overlaps (1nt or more) one and only one gene. Both ends of the paired-end read are checked for overlaps. The counts coincide with those produced by htseq-count with default parameters. ReadsPerGene.out.tab file with 4 columns which correspond to different strandedness options: column 1: gene ID; column 2: counts for unstranded RNA-seq; column 3: counts for the 1st read strand aligned with RNA (htseq-count option -s yes); column 4: counts for the 2nd read strand aligned with RNA (htseq-count option -s reverse).

disabled t_coordinates.quantmode != true

default False

filtering.outFilterType

label Type of filtering

type basic:string

description Normal: standard filtering using only current alignment; BySJout: keep only those reads that contain junctions that passed filtering into SJ.out.tab

default Normal

choices

Normal: NormalBySJout: BySJout

filtering.outFilterMultimapNmax

label -outFilterMultimapNmax

type basic:integer

description Read alignments will be output only if the read maps fewer than this value, otherwise no alignments will be output (default: 10).

required False

filtering.outFilterMismatchNmax

label -outFilterMismatchNmax

type basic:integer

description Alignment will be output only if it has fewer mismatches than this value (default: 10).

required False

filtering.outFilterMismatchNoverLmax

label -outFilterMismatchNoverLmax

type basic:decimal

description Max number of mismatches per pair relative to read length: for 2x100b, max number of mismatches is 0.06*200=8 for the paired read.

required False

filtering.outFilterScoreMin

label -outFilterScoreMin

type basic:integer

description Alignment will be output only if its score is higher than or equal to this value (default: 0).

required False

filtering.outFilterMismatchNoverReadLmax

label -outFilterMismatchNoverReadLmax

type basic:decimal

description Alignment will be output only if its ratio of mismatches to *read* length is less than or equal to this value (default: 1.0).

required False

alignment.alignSJoverhangMin

label -alignSJoverhangMin

type basic:integer

```
description Minimum overhang (i.e. block size) for spliced alignments (default: 5).
     required False
alignment.alignSJDBoverhangMin
     label -alignSJDBoverhangMin
     type basic:integer
     description Minimum overhang (i.e. block size) for annotated (sjdb) spliced alignments (default: 3).
     required False
alignment.alignIntronMin
     label -alignIntronMin
     type basic:integer
     description Minimum intron size: genomic gap is considered intron if its length >= alignIntronMin,
          otherwise it is considered Deletion (default: 21).
     required False
alignment.alignIntronMax
     label -alignIntronMax
     type basic:integer
     description Maximum intron size,
                                          if 0,
                                                   max intron size will be
                                                                                  determined by
          (2pow(winBinNbits)*winAnchorDistNbins) (default: 0).
     required False
alignment.alignMatesGapMax
     label -alignMatesGapMax
     type basic:integer
     description Maximum gap between two mates, if 0, max intron gap will be determined by
          (2pow(winBinNbits)*winAnchorDistNbins) (default: 0).
     required False
alignment.alignEndsType
     label -alignEndsType
     type basic:string
     description Type of read ends alignment (default: Local).
     required False
     default Local
     choices
            • Local: Local
            • EndToEnd: EndToEnd
            • Extend5pOfRead1: Extend5pOfRead1
            • Extend5pOfReads12: Extend5pOfReads12
```

two_pass_mapping.two_pass_mode

```
label -twopassMode
     type basic:boolean
     description Perform first-pass mapping, extract junctions, insert them into genome index, and re-map all
          reads in the second mapping pass.
     default False
output_sam_bam.outSAMunmapped
     label -outSAMunmapped
     type basic:string
     description Output of unmapped reads in the SAM format.
     required False
     default None
     choices
            • None: None
            • Within: Within
output_sam_bam.outSAMattributes
     label -outSAMattributes
     type basic:string
     description a string of desired SAM attributes, in the order desired for the output SAM.
     required False
     default Standard
     choices
            • None: None
            • Standard: Standard
            • All: All
output_sam_bam.outSAMattrRGline
     label -outSAMattrRGline
     type basic:string
     description SAM/BAM read group line. The first word contains the read group identifier and must start
          with "ID:", e.g. -outSAMattrRGline ID:xxx CN:yy "DS:z z z"
     required False
output_sam_bam.tool_bigwig
     label Tool to calculate BigWig
     type basic:string
     description Tool to calculate BigWig.
     default deeptools
     choices
            • deepTools: deeptools
```

```
• UCSC BedGraphToBigWig: bedgraphtobigwig
output_sam_bam.bin_size_bigwig
     label Bin Size for the output of BigWig
     type basic:integer
     description Size of the bins, in bases, for the output of the bigwig. Only possible if 'Tool to calculate
          BigWig' is deepTools. If BigWig is calculated by UCSC BedGraphToBigWig then bin size is 1.
     default 50
Output results bam
     label Alignment file
     type basic:file
     description Position sorted alignment
bai
     label Index BAI
     type basic:file
unmapped_f
     label Unmapped reads (mate 1)
     type basic:file
     required False
unmapped_r
     label Unmapped reads (mate 2)
     type basic:file
     required False
sj
     label Splice junctions
     type basic:file
chimeric
     label Chimeric alignments
     type basic:file
     required False
alignment_transcriptome
     label Alignment (trancriptome coordinates)
     type basic:file
     required False
gene_counts
     label Gene counts
     type basic:file
```

```
required False
stats
     label Statistics
     type basic:file
bigwig
     label BigWig file
     type basic:file
     required False
species
     label Species
     type basic:string
build
     label Build
     type basic:string
STAR genome index
data:genomeindex:staralignment-star-index (data:genome:fasta
                                                                                        genome,
                                                       data:seq:nucleotide
                                                                                       genome2,
                                                       data:annotation
                                                                             annotation,
                                                                                             ba-
                                                       sic:string
                                                                           exon name,
                                                                                             ba-
                                                       sic:integer
                                                                          sjdbOverhang,
                                                                                             ba-
                                                       sic:integer
                                                                          genomeSAindexNbases,
                                                       basic:integer
                                                                       genomeChrBinNbits, ba-
                                                       sic:integer
                                                                      genomeSAsparseD) [Source:
                                                       v1.5.4]
Generate genome indices files from the supplied reference genome sequence and GTF files.
Input arguments genome
     label Reference genome (indexed)
     type data:genome:fasta
     required False
genome2
     label Reference genome (nucleotide sequence)
     type data:seq:nucleotide
     required False
annotation
     label Annotation file (GTF/GFF3)
     type data:annotation
     required False
```

annotation_options.exon_name

```
label -sjdbGTFfeatureExon
```

type basic:string

description Feature type in GTF file to be used as exons for building transcripts.

default exon

annotation options.sjdbOverhang

label Junction length (sjdbOverhang)

type basic:integer

description This parameter specifies the length of the genomic sequence around the annotated junction to be used in constructing the splice junction database. Ideally, this length should be equal to the ReadLength-1, where ReadLength is the length of the reads. For instance, for Illumina 2x100b paired-end reads, the ideal value is 100-1=99. In case of reads of varying length, the ideal value is max(ReadLength)-1. In most cases, the default value of 100 will work as well as the ideal value.

default 100

advanced.genomeSAindexNbases

label Small genome adjustment

type basic:integer

description For small genomes, the parameter –genomeSAindexNbases needs to be scaled down, with a typical value of min(14, log2(GenomeLength)/2 - 1). For example, for 1 megaBase genome, this is equal to 9, for 100 kiloBase genome, this is equal to 7.

required False

advanced.genomeChrBinNbits

label Large number of references adjustment

type basic:integer

description If you are using a genome with a large (>5,000) number of references (chrosomes/scaffolds), you may need to reduce the –genomeChrBinNbits to reduce RAM consumption. The following scaling is recommended: –genomeChrBinNbits = min(18, log2(GenomeLength / NumberOfReferences)). For example, for 3 gigaBase genome with 100,000 chromosomes/scaffolds, this is equal to 15.

required False

advanced.genomeSAsparseD

label Sufflux array sparsity

type basic:integer

description Suffux array sparsity, i.e. distance between indices: use bigger numbers to decrease needed RAM at the cost of mapping speed reduction (integer > 0, default = 1).

required False

Output results index

label Indexed genome

type basic:dir

source

label Gene ID source

```
type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
Salmon Index
data:index:salmonsalmon-index (data:seq:nucleotide nucl, data:file decoys, basic:boolean gen-
                                          code, basic:boolean keep_duplicates, basic:boolean per-
                                          fect_hash, basic:string source, basic:string species, ba-
                                          sic:string build, basic:integer kmerlen) [Source: v1.0.0]
Generate index files for Salmon transcript quantification tool.
Input arguments nucl
     label Nucleotide sequence
     type data:seq:nucleotide
     description A CDS sequence file in .FASTA format.
decoys
     label Decoys
     type data:file
     description Treat these sequences as decoys that may have sequence homologous to some known tran-
          script.
     required False
gencode
     label Gencode
     type basic:boolean
     description This flag will expect the input transcript FASTA to be in GENCODE format, and will split
          the transcript name at the first 'l' character. These reduced names will be used in the output and
          when looking for these transcripts in a gene to transcript GTF.
     default False
keep_duplicates
     label Keep duplicates
     type basic:boolean
     description This flag will disable the default indexing behavior of discarding sequence-identical dupli-
          cate transcripts. If this flag is passed, then duplicate transcripts that appear in the input will be
          retained and quantified separately.
     default False
```

272

perfect_hash

```
label Perfect hash
     type basic:boolean
     description Build the index using a perfect hash rather than a dense hash. This will require less memory
          (especially during quantification), but will take longer to construct.
     default False
source
     label Source of attribute ID
     type basic:string
     choices
            • DICTYBASE: DICTYBASE
            • ENSEMBL: ENSEMBL
            • NCBI: NCBI
            • UCSC: UCSC
species
     label Species
     type basic:string
     description Species latin name.
     choices
            • Homo sapiens: Homo sapiens
            • Mus musculus: Mus musculus
            • Rattus norvegicus: Rattus norvegicus
            • Dictyostelium discoideum: Dictyostelium discoideum
build
     label Genome build
     type basic:string
kmerlen
     label Size of k-mers
     type basic:integer
     description The size of k-mers that should be used for the quasi index. We find that a k of 31 seems to
          work well for reads of 75bp or longer, but you might consider a smaller k if you plan to deal with
          shorter reads.
     default 31
Output results index
     label Salmon index
     type basic:dir
source
     label Source of attribute ID
```

```
type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
Secondary hybrid BAM file
data:alignment:bam:secondaryupload-bam-secondary (data:alignment:bam
                                                                                      bam, ba-
                                                                sic:file src, basic:string species,
                                                                basic:string build) [Source: v0.5.0]
Upload a secondary mapping file in BAM format.
Input arguments bam
     label Hybrid bam
     type data:alignment:bam
     description Secondary bam will be appended to the same sample where hybrid bam is.
     required False
src
     label Mapping (BAM)
     type basic:file
     description A mapping file in BAM format. The file will be indexed on upload, so additional BAI files
          are not required.
     validate_regex \.(bam)$
species
     label Species
     type basic:string
     description Species latin name.
     choices
            • Drosophila melanogaster: Drosophila melanogaster
            • Mus musculus: Mus musculus
build
     label Build
     type basic:string
Output results bam
     label Uploaded file
     type basic:file
```

```
bai
     label Index BAI
     type basic:file
stats
     label Alignment statistics
     type basic:file
bigwig
     label BigWig file
     type basic:file
     required False
species
     label Species
     type basic:string
build
     label Build
     type basic:string
```

Spike-ins quality control

```
data:spikeinspikein-qc (list:data:expression samples, basic:string mix) [Source: v0.0.3]
```

Plot spike-ins measured abundances for samples quality control. The process will output graphs showing the correlation between known concentration of ERCC spike-ins and sample's measured abundance.

Input arguments samples

```
label Expressions with spike-ins
type list:data:expression

mix

label Spike-ins mix
type basic:string
description Select spike-ins mix.
choices

• ERCC Mix 1: ercc_mix1
• ERCC Mix 2: ercc_mix2
• SIRV-Set 3: sirv_set3

Output results plots
label Plot figures
type list:basic:file

report
```

```
label HTML report with results
    type basic:file:html
    hidden True
report_zip
    label ZIP file contining HTML report with results
    type basic:file
```

Subread

```
data:alignment:bam:subreadalignment-subread (data:genome:fasta
                                                                                          genome,
                                                           data:reads:fastq reads, basic:integer in-
                                                                 basic:integer
                                                           del,
                                                                                  consensus,
                                                                                               ba-
                                                           sic:integer
                                                                           mis_matched_bp,
                                                                                               ba-
                                                           sic:integer
                                                                             cpu_number,
                                                                                               ba-
                                                           sic:boolean
                                                                            multi_mapping,
                                                                                               ba-
                                                           sic:string
                                                                          reads_orientation,
                                                                                               ba-
                                                           sic:integer consensus_subreads) [Source:
                                                           v2.1.1]
```

Subread is an accurate and efficient general-purpose read aligner which can align both genomic DNA-seq and RNA-seq reads. It can also be used to discover genomic mutations including short indels and structural variants. See [here](http://subread.sourceforge.net/) and a paper by [Liao and colleagues](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3664803/) (2013) for more information.

Input arguments genome

```
label Reference genome
     type data:genome:fasta
reads
     label Reads
     type data:reads:fastq
options.indel
     label Number of INDEL bases
     type basic:integer
     description Specify the number of INDEL bases allowed in the mapping.
     required False
     default 5
options.consensus
     label Consensus threshold
     type basic:integer
     description Specify the consensus threshold, which is the minimal number of consensus subreads re-
          quired for reporting a hit.
     required False
     default 3
```

```
options.mis_matched_bp
     label Max number of mis-matched bases
     type basic:integer
     description Specify the maximum number of mis-matched bases allowed in the alignment.
     required False
     default 3
options.cpu_number
     label Number of threads/CPUs
     type basic:integer
     description Specify the number of threads/CPUs used for mapping
     required False
     default 1
options.multi_mapping
     label Report multi-mapping reads in addition to uniquely mapped reads.
     type basic:boolean
     description Reads that were found to have more than one best mapping location are going to be reported.
     required False
PE_options.reads_orientation
     label reads orientation
     type basic:string
     description Specify the orientation of the two reads from the same pair.
     required False
     default fr
     choices
             • ff: ff
             • fr: fr
             • rf: rf
PE_options.consensus_subreads
     label Minimum number of consensus subreads
     type basic:integer
     description Specify the minimum number of consensus subreads both reads from the sam pair must have.
     required False
     default 1
Output results bam
     label Alignment file
     type basic:file
```

```
description Position sorted alignment
bai
     label Index BAI
     type basic:file
unmapped
     label Unmapped reads
     type basic:file
     required False
stats
     label Statistics
     type basic:file
bigwig
     label BigWig file
     type basic:file
     required False
species
     label Species
     type basic:string
build
     label Build
     type basic:string
Subsample FASTQ (paired-end)
data:reads:fastq:paired:seqtkseqtk-sample-paired (data:reads:fastq:paired)
                                                                                            reads.
                                                                 basic:integer
                                                                                         n_reads,
                                                                 basic:integer
                                                                                     seed.
                                                                                              ba-
                                                                 sic:decimal
                                                                                  fraction.
                                                                                              ba-
                                                                 sic:boolean
                                                                                two_pass) [Source:
                                                                 v1.0.3]
[Seqtk](https://github.com/lh3/seqtk) is a fast and lightweight tool for processing sequences in the FASTA or FASTQ
format. The Seqtk "sample" command enables subsampling of the large FASTQ file(s).
Input arguments reads
     label Reads
     type data:reads:fastq:paired
n_reads
     label Number of reads
     type basic:integer
     default 1000000
```

```
advanced.seed
     label Seed
     type basic:integer
     default 11
advanced.fraction
     label Fraction
     type basic:decimal
     description Use the fraction of reads [0 - 1.0] from the original input file instead of the absolute number
          of reads. If set, this will override the "Number of reads" input parameter.
     required False
advanced.two_pass
     label 2-pass mode
     type basic:boolean
     description Enable two-pass mode when down-sampling. Two-pass mode is twice as slow but with much
          reduced memory.
     default False
Output results fastq
     label Remaining mate 1 reads
     type list:basic:file
fastq2
     label Remaining mate 2 reads
     type list:basic:file
fastqc_url
     label Mate 1 quality control with FastQC
     type list:basic:file:html
fastqc_url2
     label Mate 2 quality control with FastQC
     type list:basic:file:html
fastqc_archive
     label Download mate 1 FastQC archive
     type list:basic:file
fastqc_archive2
     label Download mate 2 FastQC archive
     type list:basic:file
```

Subsample FASTQ (single-end)

```
data:reads:fastq:single:seqtkseqtk-sample-single (data:reads:fastq:single
                                                                                            reads,
                                                                  basic:integer
                                                                                         n_reads,
                                                                  basic:integer
                                                                                     seed.
                                                                                              ba-
                                                                 sic:decimal
                                                                                  fraction,
                                                                                              ba-
                                                                 sic:boolean
                                                                                two_pass) [Source:
                                                                  v1.0.31
```

[Seqtk](https://github.com/lh3/seqtk) is a fast and lightweight tool for processing sequences in the FASTA or FASTQ format. The Seqtk "sample" command enables subsampling of the large FASTQ file(s).

```
Input arguments reads
     label Reads
     type data:reads:fastq:single
n_reads
     label Number of reads
     type basic:integer
     default 1000000
advanced.seed
     label Seed
     type basic:integer
     default 11
advanced.fraction
     label Fraction
     type basic:decimal
     description Use the fraction of reads [0 - 1.0] from the original input file instead of the absolute number
          of reads. If set, this will override the "Number of reads" input parameter.
     required False
advanced.two_pass
     label 2-pass mode
     type basic:boolean
     description Enable two-pass mode when down-sampling. Two-pass mode is twice as slow but with much
          reduced memory.
     default False
Output results fastq
     label Remaining reads
     type list:basic:file
fastqc_url
     label Quality control with FastQC
     type list:basic:file:html
```

280

```
label Download FastQC archive
type list:basic:file
```

Test basic fields

```
boolean, basic:date
data:test:fieldstest-basic-fields (basic:boolean
                                                                                        date,
                                                                                               ba-
                                              sic:datetime
                                                              datetime, basic:decimal
                                                                                           decimal,
                                              basic:integer
                                                              integer, basic:string
                                                                                       string, ba-
                                              sic:text
                                                        text, basic:url:download
                                                                                     url download,
                                              basic:url:view
                                                                url view, basic:string
                                                                                           string2,
                                                                                      string4, ba-
                                              basic:string
                                                             string3, basic:string
                                              sic:string
                                                            string5, basic:string
                                                                                     string6,
                                                                                               ba-
                                                           string7, basic:string
                                              sic:string
                                                                                   tricky2) [Source:
                                              v1.1.1]
```

Test with all basic input fields whose values are printed by the processor and returned unmodified as output fields.

Input arguments boolean

```
label Boolean
     type basic:boolean
     default True
date
     label Date
     type basic:date
     default 2013-12-31
datetime
     label Date and time
     type basic:datetime
     default 2013-12-31 23:59:59
decimal
     label Decimal
     type basic:decimal
     default -123.456
integer
     label Integer
     type basic:integer
     default -123
string
     label String
     type basic:string
     default Foo b-a-r.gz 1.23
```

text

```
label Text
     type basic:text
     default Foo bar in 3 lines.
url_download
     label URL download
     type basic:url:download
     default {'url': 'http://www.w3.org/TR/1998/REC-html40-19980424/html40.
         pdf'}
url_view
     label URL view
     type basic:url:view
     default {'name': 'Something', 'url': 'http://www.something.com/'}
group.string2
     label String 2 required
     type basic:string
     description String 2 description.
     required True
     disabled false
     hidden false
     placeholder Enter string
group.string3
     label String 3 disabled
     type basic:string
     description String 3 description.
     disabled true
     default disabled
group.string4
     label String 4 hidden
     type basic:string
     description String 4 description.
     hidden True
     default hidden
group.string5
     label String 5 choices
     type basic:string
     description String 5 description.
```

```
hidden False
     default choice_2
     choices
            • Choice 1: choice_1
            • Choice 2: choice 2
            • Choice 3: choice_3
group.string6
     label String 6 regex only "Aa"
     type basic:string
     default AAaAaaa
     validate_regex ^[aA] *$
group.string7
     label String 7 optional choices
     type basic:string
     description String 7 description.
     required False
     hidden False
     default choice_2
     choices
            • Choice 1: choice_1
            • Choice 2: choice_2
            • Choice 3: choice_3
tricky.tricky1.tricky2
     label Tricky 2
     type basic:string
     default true
Output results output
     label Result
     type basic:url:view
out_boolean
     label Boolean
     type basic:boolean
out_date
     label Date
     type basic:date
out_datetime
```

```
label Date and time
     type basic:datetime
out_decimal
     label Decimal
     type basic:decimal
out_integer
     label Integer
     type basic:integer
out_string
     label String
     type basic:string
out_text
     label Text
     type basic:text
out_url_download
     label URL download
     type basic:url:download
out\_url\_view
     label URL view
     type basic:url:view
out_group.string2
     label String 2 required
     type basic:string
     description String 2 description.
out\_group.string3
     label String 3 disabled
     type basic:string
     description String 3 description.
out\_group.string 4
     label String 4 hidden
     type basic:string
     description String 4 description.
out_group.string5
     label String 5 choices
     type basic:string
     description String 5 description.
```

```
out_group.string6
     label String 6 regex only "Aa"
     type basic:string
out_group.string7
     label String 7 optional choices
     type basic:string
out_tricky.tricky1.tricky2
     label Tricky 2
     type basic:string
Test disabled inputs
data:test:disabledtest-disabled (basic:boolean
                                                                                     broad_width,
                                                            broad.
                                                                    basic:integer
                                           basic:string
                                                                        width_label,
                                           sic:integer if_and_condition) [Source: v1.1.1]
Test disabled input fields.
Input arguments broad
     label Broad peaks
     type basic:boolean
     default False
broad_width
     label Width of peaks
     type basic:integer
     disabled broad === false
     default 5
width_label
     label Width label
     type basic:string
     disabled broad === false
     default FD
if_and_condition
     label If width is 5 and label FDR
     type basic:integer
     disabled broad_width == 5 && width_label == 'FDR'
     default 5
Output results output
     label Result
     type basic:string
```

Test hidden inputs

```
data:test:hiddentest-hidden (basic:boolean
                                                       broad, basic:integer
                                                                               broad_width,
                                                                                              ba-
                                                                                              ba-
                                                   parameter1, basic:integer
                                                                                 parameter2,
                                      sic:integer
                                      sic:integer broad_width2) [Source: v1.1.1]
Test hidden input fields
Input arguments broad
     label Broad peaks
     type basic:boolean
     default False
broad width
     label Width of peaks
     type basic:integer
     hidden broad === false
     default 5
parameters_broad_f.parameter1
     label parameter1
     type basic:integer
     default 10
parameters_broad_f.parameter2
     label parameter2
     type basic:integer
     default 10
parameters_broad_t.broad_width2
     label Width of peaks2
     type basic:integer
     default 5
Output results output
     label Result
     type basic:string
Test select controler
data:test:result test-list (data:test:result single, list:data:test:result multiple) [Source: v1.1.1]
Test with all basic input fields whose values are printed by the processor and returned unmodified as output fields.
Input arguments single
```

label Single

type data:test:result

multiple

```
label Multiple
    type list:data:test:result
Output results output
    label Result
```

Test sleep progress

```
data:test:resulttest-sleep-progress (basic:integer t) [Source: v1.1.1]
```

Test for the progress bar by sleeping 5 times for the specified amount of time.

Input arguments t

```
label Sleep time
type basic:integer
default 5
```

type basic:string

Output results output

```
label Result
type basic:string
```

Trim, align and quantify using a library as a reference.

data:workflow:trimalquantworkflow-trim-align-quant (data:reads:fastq:single

list:basic:string up_primers_seq, list:basic:string down_primers_seq, basic:decimal error rate 5end, basic:decimal error_rate_3end, data:genome:fasta genome, mode, basic:string basic:integer N, basic:integer L, basic:integer gbar, basic:string mp, basic:string rdg, rfg, basic:string basic:string score_min, bareadlengths, sic:integer basic:integer alignscores) [Source: v0.0.1]

Input arguments reads

```
label Untrimmed reads.
type data:reads:fastq:single
```

description First stage of shRNA pipeline. Trims 5' adapters, then 3' adapters using the same error rate setting, aligns reads to a reference library and quantifies species.

trimming_options.up_primers_seq

```
label 5' adapter sequence
     type list:basic:string
     description A string of 5' adapter sequence.
     required True
trimming options.down primers seq
     label 3' adapter sequence
     type list:basic:string
     description A string of 3' adapter sequence.
     required True
trimming_options.error_rate_5end
     label Error rate for 5'
     type basic:decimal
     description Maximum allowed error rate (no. of errors divided by the length of the matching region) for
           5' trimming.
     required False
     default 0.1
trimming options.error rate 3end
     label Error rate for 3'
     type basic:decimal
     description Maximum allowed error rate (no. of errors divided by the length of the matching region) for
           3' trimming.
     required False
     default 0.1
alignment_options.genome
     label Reference library
     type data:genome:fasta
     description Choose the reference library against which to align reads.
alignment options.mode
     label Alignment mode
     type basic:string
     description End to end: Bowtie 2 requires that the entire read align from one end to the other, without
           any trimming (or "soft clipping") of characters from either end. local: Bowtie 2 does not require
           that the entire read align from one end to the other. Rather, some characters may be omitted ("soft
           clipped") from the ends in order to achieve the greatest possible alignment score.
     default --end-to-end
     choices
             • end to end mode: --end-to-end
             • local: --local
```

alignment_options.N

label Number of mismatches allowed in seed alignment (N)

type basic:integer

description Sets the number of mismatches to allowed in a seed alignment during multiseed alignment. Can be set to 0 or 1. Setting this higher makes alignment slower (often much slower) but increases sensitivity. Default: 0.

required False

alignment_options.L

label Length of seed substrings (L)

type basic:integer

description Sets the length of the seed substrings to align during multiseed alignment. Smaller values make alignment slower but more sensitive. Default: the –sensitive preset is used by default for end-to-end alignment and –sensitive-local for local alignment. See documentation for details.

required False

alignment_options.gbar

label Disallow gaps within positions (gbar)

type basic:integer

description Disallow gaps within <int> positions of the beginning or end of the read. Default: 4.

required False

alignment_options.mp

label Maximal and minimal mismatch penalty (mp)

type basic:string

description Sets the maximum (MX) and minimum (MN) mismatch penalties, both integers. A number less than or equal to MX and greater than or equal to MN is subtracted from the alignment score for each position where a read character aligns to a reference character, the characters do not match, and neither is an N. If –ignore-quals is specified, the number subtracted quals MX. Otherwise, the number subtracted is MN + floor((MX-MN)(MIN(Q, 40.0)/40.0)) where Q is the Phred quality value. Default for MX, MN: 6,2.

required False

alignment_options.rdg

label Set read gap open and extend penalties (rdg)

type basic:string

description Sets the read gap open (<int1>) and extend (<int2>) penalties. A read gap of length N gets a penalty of <int1> + N * <int2>. Default: 5,3.

required False

alignment_options.rfg

label Set reference gap open and close penalties (rfg)

type basic:string

description Sets the reference gap open (<int1>) and extend (<int2>) penalties. A reference gap of length N gets a penalty of <int1> + N * <int2>. Default: 5,3.

required False

alignment_options.score_min

label Minimum alignment score needed for "valid" alignment (score-min)

```
type basic:string
```

description Sets a function governing the minimum alignment score needed for an alignment to be considered "valid" (i.e. good enough to report). This is a function of read length. For instance, specifying L,0,-0.6 sets the minimum-score function to f(x) = 0 + -0.6 * x, where x is the read length. The default in –end-to-end mode is L,-0.6,-0.6 and the default in –local mode is G,20,8.

required False

quant_options.readlengths

label Species lengths threshold

type basic:integer

description Species with read lengths below specified threshold will be removed from final output. Default is no removal.

quant options.alignscores

label Align scores filter threshold

type basic:integer

description Species with align score below specified threshold will be removed from final output. Default is no removal.

Output results

Trimmomatic (paired-end)

data:reads:fastq:paired:trimmomatictrimmomatic-paired (data:reads:fastq:paired reads,

data:seq:nucleotide adapters, basic:integer seed_mismatches, basic:integer simple_clip_threshold, basic:integer palindrome_clip_threshold, basic:integer min_adapter_length, basic:boolean keep_both_reads, basic:integer window_size, basic:integer required quality, basic:integer target length, basic:decimal strictness, basic:integer leading, basic:integer trailing, basic:integer crop, basic:integer headcrop, basic:integer minlen. basic:integer average_quality) [Source:

v2.1.2]

Trimmomatic performs a variety of useful trimming tasks including removing adapters for Illumina paired-end and single-end data. FastQC is performed for quality control checks on trimmed raw sequence data, which are the output of Trimmomatic. See [Trimmomatic official website](http://www.usadellab.org/cms/?page=trimmomatic), the [introductory paper](https://www.ncbi.nlm.nih.gov/pubmed/24695404), and the [FastQC official website](https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) for more information.

Input arguments reads

label Reads

type data:reads:fastq:paired

illuminaclip.adapters

label Adapter sequences

type data:seq:nucleotide

description Adapter sequence in FASTA format that will be removed from the read. This field as well as 'Seed mismatches', 'Simple clip threshold' and 'Palindrome clip threshold' parameters are needed to perform Illuminacliping. 'Minimum adapter length' and 'Keep both reads' are optional parameters.

required False

illuminaclip.seed mismatches

label Seed mismatches

type basic:integer

description Specifies the maximum mismatch count which will still allow a full match to be performed. This field as well as 'Adapter sequence', 'Simple clip threshold' and 'Palindrome clip threshold' parameters are needed to perform Illuminacliping.

required False

disabled !illuminaclip.adapters

$illuminaclip. simple_clip_threshold$

label Simple clip threshold

type basic:integer

description Specifies how accurate the match between any adapter etc. sequence must be against a read. This field as well as 'Adapter sequence', 'Seed mismatches' and 'Palindrome clip threshold' parameters are needed to perform Illuminacliping.

required False

disabled !illuminaclip.adapters

illuminaclip.palindrome_clip_threshold

label Palindrome clip threshold

type basic:integer

description Specifies how accurate the match between the two 'adapter ligated' reads must be for PE palindrome read alignment. This field as well as 'Adapter sequence', 'Simple clip threshold' and 'Seed mismatches' parameters are needed to perform Illuminacliping.

required False

disabled !illuminaclip.adapters

illuminaclip.min adapter length

label Minimum adapter length

type basic:integer

description In addition to the alignment score, palindrome mode can verify that a minimum length of adapter has been detected. If unspecified, this defaults to 8 bases, for historical reasons. However, since palindrome mode has a very low false positive rate, this can be safely reduced, even down to 1, to allow shorter adapter fragments to be removed. This field is optional for preforming Illuminaclip. 'Adapter sequences', 'Seed mismatches', 'Simple clip threshold' and 'Palindrome clip threshold' are also needed in order to use this parameter.

disabled !illuminaclip.seed_mismatches && !illuminaclip.simple_clip_threshold && !illuminaclip.palindrome_clip_threshold

default 8

illuminaclip.keep_both_reads

label Keep both reads

type basic:boolean

description After read-though has been detected by palindrome mode, and the adapter sequence removed, the reverse read contains the same sequence information as the forward read, albeit in reverse complement. For this reason, the default behaviour is to entirely drop the reverse read. By specifying this parameter, the reverse read will also be retained, which may be useful e.g. if the downstream tools cannot handle a combination of paired and unpaired reads. This field is optional for preforming Illuminaclip. 'Adapter sequence', 'Seed mismatches', 'Simple clip threshold', 'Palindrome clip threshold' and also 'Minimum adapter length' are needed in order to use this parameter.

required False

disabled !illuminaclip.seed_mismatches && !illuminaclip.simple_clip_threshold && !illuminaclip.min_adapter_length

$sliding window.window_size$

label Window size

type basic:integer

description Specifies the number of bases to average across. This field as well as 'Required quality' are needed to perform a 'Sliding window' trimming (cutting once the average quality within the window falls below a threshold).

required False

slidingwindow.required_quality

label Required quality

type basic:integer

description Specifies the average quality required. This field as well as 'Window size' are needed to perform a 'Sliding window' trimming (cutting once the average quality within the window falls below a threshold).

required False

maxinfo.target_length

label Target length

type basic:integer

description This specifies the read length which is likely to allow the location of the read within the target sequence to be determined. This field as well as 'Strictness' are needed to perform 'Maxinfo' feature (an adaptive quality trimmer which balances read length and error rate to maximise the value of each read).

required False

maxinfo.strictness

label Strictness

type basic:decimal

description This value, which should be set between 0 and 1, specifies the balance between preserving as much read length as possible vs. removal of incorrect bases. A low value of this parameter (<0.2) favours longer reads, while a high value (>0.8) favours read correctness. This field as well as 'Target length' are needed to perform 'Maxinfo' feature (an adaptive quality trimmer which balances read length and error rate to maximise the value of each read).

required False

trim_bases.leading

label Leading quality

type basic:integer

description Remove low quality bases from the beginning. Specifies the minimum quality required to keep a base.

required False

trim_bases.trailing

label Trailing

type basic:integer

description Remove low quality bases from the end. Specifies the minimum quality required to keep a base.

required False

trim_bases.crop

label Crop

type basic:integer

description Cut the read to a specified length by removing bases from the end.

required False

trim_bases.headcrop

label Headcrop

type basic:integer

description Cut the specified number of bases from the start of the read.

required False

reads_filtering.minlen

label Minimum length

type basic:integer

```
description Drop the read if it is below a specified length.
     required False
reads_filtering.average_quality
     label Average quality
     type basic:integer
     description Drop the read if the average quality is below the specified level.
     required False
Output results fastq
     label Reads file (mate 1)
     type list:basic:file
fastq_unpaired
     label Reads file
     type basic:file
     required False
fastq2
     label Reads file (mate 2)
     type list:basic:file
fastq2_unpaired
     label Reads file
     type basic:file
     required False
fastqc_url
     label Quality control with FastQC (Upstream)
     type list:basic:file:html
fastqc_url2
     label Quality control with FastQC (Downstream)
     type list:basic:file:html
fastqc_archive
     label Download FastQC archive (Upstream)
     type list:basic:file
fastqc_archive2
     label Download FastQC archive (Downstream)
     type list:basic:file
```

Trimmomatic (single-end)

```
data:reads:fastq:single:trimmomatictrimmomatic-single (data:reads:fastq:single reads,
```

data:seq:nucleotide adapters, sic:integer seed_mismatches, basic:integer simple_clip_threshold, basic:integer window_size, basic:integer required_quality, basic:integer target length, basic:decimal strictness, basic:integer leading, trailing, basic:integer basic:integer crop, basic:integer headcrop, basic:integer minlen. basic:integer average_quality) [Source: v2.1.2]

Trimmomatic performs a variety of useful trimming tasks including removing adapters for Illumina paired-end and single-end data. FastQC is performed for quality control checks on trimmed raw sequence data, which are the output of Trimmomatic. See [Trimmomatic official website](http://www.usadellab.org/cms/?page=trimmomatic), the [introductory paper](https://www.ncbi.nlm.nih.gov/pubmed/24695404), and the [FastQC official website](https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) for more information.

Input arguments reads

```
label Reads
```

type data:reads:fastq:single

illuminaclip.adapters

label Adapter sequences

type data:seq:nucleotide

description Adapter sequence in FASTA format that will be removed from the read. This field as well as 'Seed mismatches' and 'Simple clip threshold' parameters are needed to perform Illuminacliping.

required False

illuminaclip.seed mismatches

label Seed mismatches

type basic:integer

description Specifies the maximum mismatch count which will still allow a full match to be performed. This field as well as 'Adapter sequences' and 'Simple clip threshold' parameter are needed to perform Illuminacliping.

required False

disabled !illuminaclip.adapters

illuminaclip.simple_clip_threshold

label Simple clip threshold

```
type basic:integer
```

description Specifies how accurate the match between any adapter etc. sequence must be against a read. This field as well as 'Adapter sequences' and 'Seed mismatches' parameter are needed to perform Illuminacliping.

required False

disabled !illuminaclip.adapters

slidingwindow.window size

label Window size

type basic:integer

description Specifies the number of bases to average across. This field as well as 'Required quality' are needed to perform a 'Sliding window' trimming (cutting once the average quality within the window falls below a threshold).

required False

slidingwindow.required_quality

label Required quality

type basic:integer

description Specifies the average quality required in window size. This field as well as 'Window size' are needed to perform a 'Sliding window' trimming (cutting once the average quality within the window falls below a threshold).

required False

maxinfo.target_length

label Target length

type basic:integer

description This specifies the read length which is likely to allow the location of the read within the target sequence to be determined. This field as well as 'Strictness' are needed to perform 'Maxinfo' feature (an adaptive quality trimmer which balances read length and error rate to maximise the value of each read).

required False

maxinfo.strictness

label Strictness

type basic:decimal

description This value, which should be set between 0 and 1, specifies the balance between preserving as much read length as possible vs. removal of incorrect bases. A low value of this parameter (<0.2) favours longer reads, while a high value (>0.8) favours read correctness. This field as well as 'Target length' are needed to perform 'Maxinfo' feature (an adaptive quality trimmer which balances read length and error rate to maximise the value of each read).

required False

trim_bases.leading

label Leading quality

type basic:integer

```
description Remove low quality bases from the beginning, if below a threshold quality.
     required False
trim_bases.trailing
     label Trailing quality
     type basic:integer
     description Remove low quality bases from the end, if below a threshold quality.
     required False
trim_bases.crop
     label Crop
     type basic:integer
     description Cut the read to a specified length by removing bases from the end.
     required False
trim_bases.headcrop
     label Headcrop
     type basic:integer
     description Cut the specified number of bases from the start of the read.
     required False
reads_filtering.minlen
     label Minimum length
     type basic:integer
     description Drop the read if it is below a specified length.
     required False
reads_filtering.average_quality
     label Average quality
     type basic:integer
     description Drop the read if the average quality is below the specified level.
     required False
Output results fastq
     label Reads file
     type list:basic:file
fastqc_url
     label Quality control with FastQC
     type list:basic:file:html
fastqc_archive
     label Download FastQC archive
     type list:basic:file
```

Trimmomatic - HISAT2 - HTSeq-count (paired-end)

```
\verb|data:workflow:rnaseq:htseqworkflow-rnaseq-paired| (\textit{data:reads:fastq:paired}|
```

reads. data:genome:fasta genome, data:annotation:gtf annotation, data:seq:nucleotide adapters, basic:integer seed mismatches, basic:integer palindrome_clip_threshold, basic:integer simple_clip_threshold, basic:integer minlen, batrailing. sic:integer basic:string stranded. baid attribute) [Source: sic:string v1.0.1]

This RNA-seq pipeline is comprised of three steps, preprocessing, alignment, and quantification.

First, reads are preprocessed by __Trimmomatic__ which performs a variety of useful trimming tasks including removing adapters for Illumina paired-end and single-end high-throughput sequencing reads. Next, preprocessed reads are aligned by __HISAT2__ aligner. HISAT2 is a fast and sensitive alignment program for mapping next-generation sequencing reads For more information see [this comparison of RNA-seq aligners](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5792058/). Finally, aligned reads are summarized to genes by __HTSeq-count__. Compared to featureCounts, HTSeq-count is not as computationally efficient. All three tools in this workflow support parallelization to accelerate the analysis.

description Specifies the maximum mismatch count which will still allow a full match to be performed.

Input arguments reads

```
label Input reads

type data:reads:fastq:paired

genome
label Genome
type data:genome:fasta

annotation
label Annotation (GTF)
type data:annotation:gtf

adapters
label Adapter sequences (FASTA)
type data:seq:nucleotide
required False
illuminaclip.seed_mismatches
label Seed mismatches
type basic:integer
```

illuminaclip.palindrome_clip_threshold

default 2

label Palindrome clip threshold

```
type basic:integer

description Specifies how accurate the match between the two 'adapter ligated' reads must be for PE
    palindrome read alignment.

default 30

illuminaclip.simple_clip_threshold
```

label Simple clip threshold

type basic:integer

description Specifies how accurate the match between any adapter etc. sequence must be against a read.

default 10

minlen

label Min length

type basic:integer

description Drop the read if it is below a specified length.

default 10

trailing

label Trailing quality

type basic:integer

description Remove low quality bases from the end. Specifies the minimum quality required to keep a base.

default 28

stranded

label Is data from a strand specific assay?

type basic:string

description In strand non-specific assay a read is considered overlapping with a feature regardless of whether it is mapped to the same or the opposite strand as the feature. In strand-specific forward assay and single reads, the read has to be mapped to the same strand as the feature. For paired-end reads, the first read has to be on the same strand and the second read on the opposite strand. In strand-specific reverse assay these rules are reversed.

default no

choices

• Strand non-specific: no

• Strand-specific forward: yes

• Strand-specific reverse: reverse

id attribute

label ID attribute

type basic:string

description GFF attribute to be used as feature ID. Several GFF lines with the same feature ID will be considered as parts of the same feature. The feature ID is used to identity the counts in the output table.

```
default gene_id
```

Output results

Trimmomatic - HISAT2 - HTSeq-count (single-end)

```
data:workflow:rnaseq:htseqworkflow-rnaseq-single (data:reads:fastq:single
                                                                                               reads.
                                                                    data:genome:fasta
                                                                                             genome,
                                                                    data:annotation:gtf
                                                                                         annotation,
                                                                    data:seq:nucleotide adapters, ba-
                                                                    sic:integer seed mismatches, ba-
                                                                    sic:integer simple_clip_threshold,
                                                                    basic:integer
                                                                                      minlen.
                                                                                                  ba-
                                                                                     trailing,
                                                                    sic:integer
                                                                                                  ba-
                                                                    sic:string
                                                                                    stranded.
                                                                                                  ba-
                                                                                id attribute) [Source:
                                                                    sic:string
                                                                    v1.0.1]
```

This RNA-seq pipeline is comprised of three steps, preprocessing, alignment, and quantification.

First, reads are preprocessed by __Trimmomatic__ which performs a variety of useful trimming tasks including removing adapters for Illumina paired-end and single-end high-throughput sequencing reads. Next, preprocessed reads are aligned by __HISAT2__ aligner. HISAT2 is a fast and sensitive alignment program for mapping next-generation sequencing reads For more information see [this comparison of RNA-seq aligners](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5792058/). Finally, aligned reads are summarized to genes by __HTSeq-count__. Compared to featureCounts, HTSeq-count is not as computationally efficient. All three tools in this workflow support parallelization to accelerate the analysis.

description Specifies the maximum mismatch count which will still allow a full match to be performed.

Input arguments reads

```
label Input reads

type data:reads:fastq:single

genome
label Genome
type data:genome:fasta

annotation
label Annotation (GTF)
type data:annotation:gtf

adapters
label Adapter sequences (FASTA)
type data:seq:nucleotide
required False

illuminaclip.seed_mismatches
label Seed mismatches
type basic:integer
```

Chapter 1. Contents

default 2

illuminaclip.simple_clip_threshold

```
label Simple clip threshold
     type basic:integer
     description Specifies how accurate the match between any adapter etc. sequence must be against a read.
     default 10
minlen
     label Minimum length
     type basic:integer
     description Drop the read if it is below a specified length.
     default 10
trailing
     label Trailing quality
     type basic:integer
     description Remove low quality bases from the end. Specifies the minimum quality required to keep a
           base.
     default 28
stranded
     label Is data from a strand specific assay?
     type basic:string
     description In strand non-specific assay a read is considered overlapping with a feature regardless of
           whether it is mapped to the same or the opposite strand as the feature. In strand-specific forward
           assay and single reads, the read has to be mapped to the same strand as the feature. For paired-end
           reads, the first read has to be on the same strand and the second read on the opposite strand. In
           strand-specific reverse assay these rules are reversed.
     default no
     choices
             • Strand non-specific: no
             • Strand-specific forward: yes
             • Strand-specific reverse: reverse
id attribute
     label ID attribute
     type basic:string
     description GFF attribute to be used as feature ID. Several GFF lines with the same feature ID will be
           considered as parts of the same feature. The feature ID is used to identity the counts in the output
           table.
```

1.2. Process catalog

Output results

default gene_id

Upload Picard CollectTargetedPcrMetrics

```
data:picard:coverage:uploadupload-picard-pcrmetrics (basic:file target_pcr_metrics,
                                                                   basic:file
                                                                   get_coverage) [Source: v1.1.1]
Upload Picard CollectTargetedPcrMetrics result files.
Input arguments target_pcr_metrics
     label Target PCR metrics
     type basic:file
target_coverage
     label Target coverage
     type basic:file
Output results target_pcr_metrics
     label Target PCR metrics
     type basic:file
target_coverage
     label Target coverage
     type basic:file
VCF file
data:variants:vcfupload-variants-vcf (basic:file
                                                             src.
                                                                   basic:string
                                                                                  species,
                                                                                           ba-
                                                sic:string build) [Source: v2.1.1]
Upload variants in VCF format.
Input arguments src
     label Variants (VCF)
     type basic:file
     description Variants in VCF format.
     required True
     validate_regex \.(vcf)(|\.gz|\.bz2|\.tgz|\.tar\.gz|\.tar\.bz2|\.zip|\.
         rar|\.7z)$
species
     label Species
     type basic:string
     description Species latin name.
     choices
            • Homo sapiens: Homo sapiens
            • Mus musculus: Mus musculus
            • Rattus norvegicus: Rattus norvegicus
```

```
• Dictyostelium discoideum: Dictyostelium discoideum
```

- Odocoileus virginianus texanus: Odocoileus virginianus texanus
- Solanum tuberosum: Solanum tuberosum

build

```
label Genome build
type basic:string
```

Output results vcf

```
label Uploaded file
type basic:file
```

tbi

label Tabix index
type basic:file

species

label Species

type basic:string

build

label Build

type basic:string

Variant calling (CheMut)

```
data:variants:vcf:chemutvc-chemut (data:genome:fasta
                                                                                          genome,
                                                                                  parental_strains,
                                              list:data:alignment:bam
                                              list:data:alignment:bam
                                                                           mutant strains.
                                                                                               ba-
                                              sic:boolean
                                                            br_and_ind_ra, basic:boolean
                                                                                              db-
                                              snp,
                                                         data:variants:vcf
                                                                                      known_sites,
                                              list:data:variants:vcf known_indels, basic:string PL,
                                              basic:string LB, basic:string PU, basic:string CN,
                                              basic:date DT, basic:integer stand_emit_conf, ba-
                                              sic:integer
                                                           stand call conf, basic:integer
                                                                                           ploidy,
                                              basic:string
                                                            glm, list:basic:string
                                                                                    intervals, ba-
```

"CheMut varint calling using multiple BAM input files. Note: Usage of Genome Analysis Toolkit requires a licence."

sic:boolean rf) [Source: v1.2.2]

Input arguments genome

```
label Reference genome
    type data:genome:fasta

parental_strains
    label Parental strains
    type list:data:alignment:bam

mutant_strains
```

```
label Mutant strains
     type list:data:alignment:bam
br_and_ind_ra
     label Do variant base recalibration and indel realignment
     type basic:boolean
     default False
dbsnp
     label Use dbSNP file
     type basic:boolean
     description rsIDs from this file are used to populate the ID column of the output. Also, the DB INFO
          flag will be set when appropriate. dbSNP is not used in any way for the calculations themselves.
     default False
known sites
     label Known sites (dbSNP)
     type data:variants:vcf
     required False
     hidden br_and_ind_ra === false && dbsnp === false
known_indels
     label Known indels
     type list:data:variants:vcf
     required False
     hidden br_and_ind_ra === false
reads_info.PL
     label Platform/technology
     type basic:string
     description Platform/technology used to produce the reads.
     default Illumina
     choices
            • Capillary: Capillary
            • Ls454: Ls454
            • Illumina: Illumina
            • SOLiD: SOLiD
            • Helicos: Helicos
            • IonTorrent: IonTorrent
            • Pacbio: Pacbio
```

reads_info.LB

```
label Library
     type basic:string
     default \times
reads_info.PU
     label Platform unit
     type basic:string
     description Platform unit (e.g. flowcell-barcode.lane for Illumina or slide for SOLiD). Unique identifier.
     default x
reads info.CN
     label Sequencing center
     type basic:string
     description Name of sequencing center producing the read.
     default x
reads info.DT
     label Date
     type basic:date
     description Date the run was produced.
     default 2017-01-01
Varc_param.stand_emit_conf
     label Emission confidence threshold
     type basic:integer
     description The minimum confidence threshold (phred-scaled) at which the program should emit sites
           that appear to be possibly variant.
     default 10
Varc_param.stand_call_conf
     label Calling confidence threshold
     type basic:integer
     description The minimum confidence threshold (phred-scaled) at which the program should emit variant
           sites as called. If a site's associated genotype has a confidence score lower than the calling threshold,
           the program will emit the site as filtered and will annotate it as LowQual. This threshold separates
           high confidence calls from low confidence calls.
     default 30
Varc_param.ploidy
     label Sample ploidy
     type basic:integer
     description Ploidy (number of chromosomes) per sample. For pooled data, set to (Number of samples
          in each pool * Sample Ploidy).
     default 2
```

Varc_param.glm

```
label Genotype likelihoods model
```

```
type basic:string
```

description Genotype likelihoods calculation model to employ – SNP is the default option, while INDEL is also available for calling indels and BOTH is available for calling both together.

default SNP

choices

- SNP: SNP
- INDEL: INDEL
- BOTH: BOTH

Varc_param.intervals

```
label Intervals
```

```
type list:basic:string
```

description Use this option to perform the analysis over only part of the genome. This argument can be specified multiple times. You can use samtools-style intervals (e.g. -L chr1 or -L chr1:100-200).

required False

Varc_param.rf

label ReasignOneMappingQuality Filter

```
type basic:boolean
```

description This read transformer will change a certain read mapping quality to a different value without affecting reads that have other mapping qualities. This is intended primarily for users of RNA-Seq data handling programs such as TopHat, which use MAPQ = 255 to designate uniquely aligned reads. According to convention, 255 normally designates "unknown" quality, and most GATK tools automatically ignore such reads. By reassigning a different mapping quality to those specific reads, users of TopHat and other tools can circumvent this problem without affecting the rest of their dataset.

default False

Output results vcf

label Called variants file

type basic:file

tbi

label Tabix index

type basic:file

species

label Species

type basic:string

build

label Build

type basic:string

Variant filtering (CheMut)

```
data:variants:vcf:filteringfiltering-chemut (data:variants:vcf variants, basic:string analysis_type, basic:string parental_strain, basic:string mutant_strain, basic:integer read_depth) [Source: v1.3.1]
```

Filtering and annotation of Variant Calling data - Chemical mutagenesis in Dictyostelium discoideum

```
Input arguments variants
```

```
label Variants file (VCF)
    type data:variants:vcf
analysis_type
    label Analysis type
    type basic:string
```

description Choice of the analysis type. Use "SNV" or "INDEL" options for the analysis of haploid VCF files prepared by using GATK UnifiedGenotyper -glm option "SNP" or "INDEL", respectively. Choose options SNV_CHR2 or INDEL_CHR2 to run the GATK analysis only on the diploid portion of CHR2 (-ploidy 2 -L chr2:2263132-3015703).

default snv

choices

- SNV: snv
- INDEL: indel
- SNV_CHR2: snv_chr2
- INDEL_CHR2: indel_chr2

parental_strain

```
label Parental Strain Prefix
type basic:string
default parental
```

mutant_strain

```
label Mutant Strain Prefix
type basic:string
default mut
```

read_depth

```
label Read Depth Cutoff
type basic:integer
default 5
```

Output results summary

```
label Summary
type basic:file
```

```
description Summarize the input parameters and results.
vcf
     label Variants
     type basic:file
     description A genome VCF file of variants that passed the filters.
tbi
     label Tabix index
     type basic:file
variants filtered
     label Variants filtered
     type basic:file
     description A data frame of variants that passed the filters.
     required False
variants_filtered_alt
     label Variants filtered (multiple alt. alleles)
     type basic:file
     description A data frame of variants that contain more than two alternative alleles. These vairants are
           likely to be false positives.
     required False
gene_list_all
     label Gene list (all)
     type basic:file
     description Genes that are mutated at least once.
     required False
gene_list_top
     label Gene list (top)
     type basic:file
     description Genes that are mutated at least twice.
     required False
mut_chr
     label Mutations (by chr)
     type basic:file
     description List mutations in individual chromosomes.
     required False
mut_strain
     label Mutations (by strain)
```

```
type basic:file
     description List mutations in individual strains.
     required False
strain_by_gene
     label Strain (by gene)
     type basic:file
     description List mutants that carry mutations in individual genes.
     required False
species
     label Species
     type basic:string
build
     label Build
     type basic:string
WALT
data:alignment:mr:waltwalt (data:genome:fasta
                                                                  data:reads:fastq
                                                                                     reads, ba-
                                                        genome,
                                    sic:boolean rm_dup, basic:integer mismatch, basic:integer num-
                                    ber) [Source: v1.0.2]
WALT (Wildcard ALignment Tool) is a read mapping program for bisulfite sequencing in DNA methylation studies.
Input arguments genome
     label Reference genome
     type data:genome:fasta
reads
     label Reads
     type data:reads:fastq
rm_dup
     label Remove duplicates
     type basic:boolean
     default True
mismatch
     label Maximum allowed mismatches
     type basic:integer
     required False
number
     label Number of reads to map in one loop
     type basic:integer
```

310

```
description Sets the number of reads to mapping in each loop. Larger number results in program taking
                          more memory. This is especially evident in paired-end mapping.
             required False
Output results mr
             label Alignment file
             type basic:file
             description Position sorted alignment
stats
             label Statistics
             type basic:file
unmapped_f
             label Unmapped reads (mate 1)
             type basic:file
             required False
unmapped_r
             label Unmapped reads (mate 2)
             type basic:file
             required False
species
             label Species
             type basic:string
build
             label Build
             type basic:string
WGBS
                                                                                                                                                                                                                                  genome.
data:workflow:wgbsworkflow-wgbs (data:reads:fastq
                                                                                                                                                           reads, data:genome:fasta
                                                                                                             basic:boolean
                                                                                                                                                        rm dup,
                                                                                                                                                                                    basic:integer
                                                                                                                                                                                                                             mismatch,
                                                                                                             basic:integer
                                                                                                                                                      number,
                                                                                                                                                                                basic:boolean
                                                                                                                                                                                                                           cpgs,
                                                                                                                                                                                                                                             ba-
                                                                                                             sic:boolean symmetric_cpgs) [Source: v1.0.2]
This WGBS pipeline is comprised of three steps - alignment, computation of methylation levels, and identification of
hypo-methylated regions (HMRs).
First,
                                                           aligned
                                                                                    by
                                                                                                     WALT
                                                                                                                                      aligner.
                                                                                                                                                                                WALT
                                                                                                                                                                                                          (Wildcard
                                                                                                                                                                                                                                          ALignment
Tool)](https://github.com/smithlabcode/walt) is fast and accurate read mapping for bisulfite sequencing. Then, methy-
lation level at each genomic cytosine is calculated using method in the 
fied using __hmr__. Both methcounts and hmr are part of [MethPipe](http://smithlabresearch.org/software/methpipe/)
package.
Input arguments reads
             label Select sample(s)
```

```
type data:reads:fastq
genome
     label Genome
     type data:genome:fasta
alignment.rm dup
     label Remove duplicates
     type basic:boolean
     default True
alignment.mismatch
     label Maximum allowed mismatches
     type basic:integer
     default 6
alignment.number
     label Number of reads to map in one loop
     type basic:integer
     description Sets the number of reads to mapping in each loop. Larger number results in program taking
          more memory. This is especially evident in paired-end mapping.
     required False
methcounts.cpgs
     label Only CpG context sites
     type basic:boolean
     description Output file will contain methylation data for CpG context sites only. Choosing this option
          will result in CpG content report only.
     disabled methcounts.symmetric_cpgs
     default False
methcounts.symmetric_cpgs
     label Merge CpG pairs
     type basic:boolean
     description Merging CpG pairs results in symmetric methylation levels. Methylation is usually symmet-
          ric (cytosines at CpG sites were methylated on both DNA strands). Choosing this option will only
          keep the CpG sites data.
     disabled methcounts.cpgs
     default True
Output results
```

Whole exome sequencing (WES) analysis

data:workflow:wesworkflow-wes

(data:reads:fastq:paired reads, data:genome:fasta genome, data:bedpe bamclipper_bedpe, list:data:variants:vcf known sites, data:bed **vals**, data:variants:vcf he dbsnp. basic:string validation stringency, data:seq:nucleotide adapters. basic:integer seed_mismatches, basic:integer simple_clip_threshold, basic:integer min_adapter_length, basic:integer palindrome_clip_threshold, basic:integer leading, basic:integer trailing, basic:integer minlen, basic:integer seed l, basic:integer band w, basic:boolean m, basic:decimal re seeding, basic:integer match, babasic:integer sic:integer mismatch, gap o, sic:integer gap_e, basic:integer clipping, basic:integer unpaired p, basic:integer report tr, basic:boolean md skip, md remove duplicates, basic:boolean sic:string md assume sort order, basic:string read group, basic:integer stand call conf, basic:integer mbq) [Source: v2.0.01

Whole exome sequencing pipeline analyzes Illumina panel data. It consists of trimming, aligning, soft clipping, (optional) marking of duplicates, recalibration of base quality scores and finally, calling of variants.

The tools used are Trimmomatic which performs trimming. Aligning is performed using BWA (mem). Soft clipping of Illumina primer sequences is done using bamclipper tool. Marking of duplicates (MarkDuplicates), recalibration of base quality scores (ApplyBQSR) and calling of variants (HaplotypeCaller) is done using GATK4 bundle of bioinformatics tools.

To successfully run this pipeline, you will need a genome (FASTA), paired-end (FASTQ) files, BEDPE file for bamclipper, known sites of variation (dbSNP) (VCF), dbSNP database of variations (can be the same as known sites of variation), intervals on which target capture was done (BED) and illumina adapter sequences (FASTA). Make sure that specified resources match the genome used in the alignment step.

Result is a file of called variants (VCF).

```
Input arguments reads
```

```
label Raw untrimmed reads

type data:reads:fastq:paired

description Raw paired-end reads.

required True
```

genome

```
label Reference genome

type data:genome:fasta

description Against which genome to align. Further processes depend on this genome (e.g. BQSR step).

required True

bamclipper_bedpe
```

```
label BEDPE file used for clipping using Bamclipper
type data:bedpe
```

description BEDPE file used for clipping using Bamclipper tool.

required True

known_sites

label Known sites of variation used in BQSR

type list:data:variants:vcf

description Known sites of variation as a VCF file.

required True

intervals

label Intervals

type data:bed

description Use intervals to narrow the analysis to defined regions. This usually help cutting down on process time.

required True

hc_dbsnp

label dbSNP for GATK4's HaplotypeCaller

type data:variants:vcf

description dbSNP database of variants for variant calling.

required True

validation_stringency

label Validation stringency for all SAM files read by this program. Setting stringency to SILENT can improve performance when processing a BAM file in which variable-length data (read, qualities, tags) do not otherwise need to be decoded. Default is STRICT. This setting is used in BaseRecalibrator and ApplyBQSR processes.

type basic:string

default STRICT

choices

• STRICT: STRICT

• SILENT: SILENT

• LENIENT: LENIENT

advanced.trimming.adapters

label Adapter sequences

type data:seq:nucleotide

description Adapter sequence in FASTA format that will be removed from the read. This field as well as 'Seed mismatches', 'Simple clip threshold' and 'Palindrome clip threshold' parameters are needed to perform Illuminacliping. 'Minimum adapter length' and 'Keep both reads' are optional parameters.

required False

advanced.trimming.seed_mismatches

label Seed mismatches

type basic:integer

description Specifies the maximum mismatch count which will still allow a full match to be performed. This field as well as 'Adapter sequence', 'Simple clip threshold' and 'Palindrome clip threshold' parameters are needed to perform Illuminacliping.

required False

disabled !advanced.trimming.adapters

advanced.trimming.simple_clip_threshold

label Simple clip threshold

type basic:integer

description Specifies how accurate the match between any adapter etc. sequence must be against a read. This field as well as 'Adapter sequences' and 'Seed mismatches' parameter are needed to perform Illuminacliping.

required False

disabled !advanced.trimming.adapters

advanced.trimming.min_adapter_length

label Minimum adapter length

type basic:integer

description In addition to the alignment score, palindrome mode can verify that a minimum length of adapter has been detected. If unspecified, this defaults to 8 bases, for historical reasons. However, since palindrome mode has a very low false positive rate, this can be safely reduced, even down to 1, to allow shorter adapter fragments to be removed. This field is optional for preforming Illuminaclip. 'Adapter sequences', 'Seed mismatches', 'Simple clip threshold' and 'Palindrome clip threshold' are also needed in order to use this parameter.

disabled !advanced.trimming.seed_mismatches && !advanced.trimming.simple_clip_threshold && !advanced.trimming.palindrome_clip_threshold

default 8

advanced.trimming.palindrome_clip_threshold

label Palindrome clip threshold

type basic:integer

description Specifies how accurate the match between the two 'adapter ligated' reads must be for PE palindrome read alignment. This field as well as 'Adapter sequence', 'Simple clip threshold' and 'Seed mismatches' parameters are needed to perform Illuminaclipping.

required False

disabled !advanced.trimming.adapters

advanced.trimming.leading

label Leading quality

type basic:integer

description Remove low quality bases from the beginning, if below a threshold quality.

required False

advanced.trimming.trailing

label Trailing quality

```
type basic:integer
     description Remove low quality bases from the end, if below a threshold quality.
     required False
advanced.trimming.minlen
     label Minimum length
     type basic:integer
     description Drop the read if it is below a specified length.
     required False
advanced.align.seed_l
     label Minimum seed length
     type basic:integer
     description Minimum seed length. Matches shorter than minimum seed length will be missed. The
          alignment speed is usually insensitive to this value unless it significantly deviates 20.
     default 19
advanced.align.band_w
     label Band width
     type basic:integer
     description Gaps longer than this will not be found.
     default 100
advanced.align.m
     label Mark shorter split hits as secondary
     type basic:boolean
     description Mark shorter split hits as secondary (for Picard compatibility)
     default False
advanced.align.re_seeding
     label Re-seeding factor
     type basic:decimal
     description Trigger re-seeding for a MEM longer than minSeedLen*FACTOR. This is a key heuristic pa-
          rameter for tuning the performance. Larger value yields fewer seeds, which leads to faster alignment
          speed but lower accuracy.
     default 1.5
advanced.align.scoring.match
     label Score of a match
     type basic:integer
     default 1
advanced.align.scoring.mismatch
     label Mismatch penalty
```

```
type basic:integer
     default 4
advanced.align.scoring.gap_o
     label Gap open penalty
     type basic:integer
     default 6
advanced.align.scoring.gap_e
     label Gap extension penalty
     type basic:integer
     default 1
advanced.align.scoring.clipping
     label Clipping penalty
     type basic:integer
     description Clipping is applied if final alignment score is smaller than (best score reaching the end of
          query) - (Clipping penalty)
     default 5
advanced.align.scoring.unpaired_p
     label Penalty for an unpaired read pair
     type basic:integer
     description Affinity to force pair. Score: scoreRead1+scoreRead2-Penalty
     default 9
advanced.align.report_tr
     label Report threshold score
     type basic:integer
     description Don't output alignment with score lower than defined number. This option only affects
          output.
     default 30
advanced.markduplicates.md skip
     label Skip GATK's MarkDuplicates step
     type basic:boolean
     default False
advanced.markduplicates.md_remove_duplicates
     label Remove found duplicates
     type basic:boolean
     default False
advanced.markduplicates.md_assume_sort_order
     label Assume sort oder
```

```
type basic:string
     default
     choices
             • as in BAM header (default):
             • unsorted: unsorted
             • queryname: queryname
             • coordinate: coordinate
             • duplicate: duplicate
             • unknown: unknown
advanced.bqsr.read_group
     label Read group (@RG)
     type basic:string
     description If BAM file has not been prepared using a @RG tag, you can add it here. This argument
          enables the user to replace all read groups in the INPUT file with a single new read group and assign
          all reads to this read group in the OUTPUT BAM file. Addition or replacement is performed using
          Picard's AddOrReplaceReadGroups tool. Input should take the form of -name=value delimited by a
          \t, e.g. "-ID=1\t-PL=Illumina\t-SM=sample_1". See AddOrReplaceReadGroups documentation for
          more information on tag names. Note that PL, LB, PU and SM are required fields. See caveats of
          rewriting read groups in the documentation linked above.
     required False
advanced.hc.stand_call_conf
     label Min call confidence threshold
     type basic:integer
     description The minimum phred-scaled confidence threshold at which variants should be called.
     default 20
advanced.hc.mbq
     label Min Base Quality
     type basic:integer
     description Minimum base quality required to consider a base for calling.
     default 20
Output results
coverageBed
data:coveragecoveragebed (data:alignment:bam
                                                         alignment, data:masterfile:amplicon
                                                                                                mas-
                                   ter_file) [Source: v4.1.1]
Bedtools coverage (coveragebed)
Input arguments alignment
     label Alignment (BAM)
```

```
type data:alignment:bam
master file
     label Master file
     type data:masterfile:amplicon
Output results cov metrics
     label Coverage metrics
     type basic:file
mean_cov
     label Mean amplicon coverage
     type basic:file
amplicon_cov
     label Amplicon coverage file (nomergebed)
     type basic:file
covplot html
     label HTML coverage plot
     type basic:file:html
edgeR
data:differentialexpression:edgerdifferentialexpression-edger (list:data:expression case,
                                                                             list:data:expression con-
                                                                              trol.
                                                                                         ba-
```

Empirical Analysis of Digital Gene Expression Data in R (edgeR). Differential expression analysis of RNA-seq expression profiles with biological replication. Implements a range of statistical methodology based on the negative binomial distributions, including empirical Bayes estimation, exact tests, generalized linear models and quasi-likelihood tests. As well as RNA-seq, it be applied to differential signal analysis of other types of genomic data that produce counts, including ChIP-seq, Bisulfite-seq, SAGE and CAGE. See [here](https://www.bioconductor.org/packages/devel/bioc/vignettes/edgeR/inst/doc/edgeRUsersGuide.pdf) for more information.

Input arguments case

```
label Case

type list:data:expression

description Case samples (replicates)

control

label Control

type list:data:expression

description Control samples (replicates)

filter
```

fil-

sic:integer ter) [Source: v1.1.1]

```
label Raw counts filtering threshold
     type basic:integer
     description Filter genes in the expression matrix input. Remove genes where the number of counts in all
          samples is below the threshold.
     default 10
Output results raw
     label Differential expression
     type basic:file
de_json
     label Results table (JSON)
     type basic:json
de_file
     label Results table (file)
     type basic:file
source
     label Gene ID database
     type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
feature_type
     label Feature type
     type basic:string
```

featureCounts

data:expression:featurecountsfeature_counts (data:alignment:bam

aligned reads, basic:string assay_type, data:index:salmon cdna_index, basic:integer n_reads, data:annotation annotation, basic:string feature class, basic:string feature_type, basic:string id attribute. basic:string normalization_type, data:mappability:bcm mappability, basic:boolean show advanced, basic:boolean count features. sic:boolean allow multi overlap, basic:integer min_overlap, basic:decimal frac overlap, basic:decimal frac overlap feature, basic:boolean largest overlap, read extension 5, basic:integer basic:integer read extension 3, basic:integer read_to_pos, basic:boolean count_multi_mapping_reads, basic:boolean fraction, basic:integer min mgs, basic:boolean split_only, basic:boolean non_split_only, basic:boolean primary, basic:boolean ignore_dup, basic:boolean junc_counts, data:genome genome, babasic:boolean is_paired_end, sic:boolean require both ends mapped, basic:boolean check_frag_length, basic:integer min frag length, basic:integer max frag length, basic:boolean do not count chimeric fragments, basic:boolean do not sort, basic:boolean by_read_group, basic:boolean count_long_reads, basic:boolean report_reads, basic:integer max_mop, basic:boolean ver**bose**) [Source: v2.4.1]

featureCounts is a highly efficient general-purpose read summarization program that counts mapped reads for genomic features such as genes, exons, promoter, gene bodies, genomic bins and chromosomal locations. It can be used to count both RNA-seq and genomic DNA-seq reads. See the [official website](http://bioinf.wehi.edu.au/featureCounts/) and the [introductory paper](https://academic.oup.com/bioinformatics/article/30/7/923/232889) for more information.

Input arguments alignment.aligned_reads

```
label Aligned reads
    type data:alignment:bam
alignment.assay_type
    label Assay type
    type basic:string
```

description Indicate if strand-specific read counting should be performed. For paired-end reads, strand of the first read is taken as the strand of the whole fragment. FLAG field is used to tell if a read is first or second read in a pair. Automated strand detection is enabled using the [Salmon](https://salmon.readthedocs.io/en/latest/library_type.html) tool's build-in functionality. To use this option, cDNA (transcriptome) index file crated using the Salmon indexing tool must be provided.

```
default non_specific
```

choices

- Strand non-specific: non_specific
- Strand-specific forward: forward
- Strand-specific reverse: reverse
- Detect automatically: auto

alignment.cdna_index

```
label cDNA index file
```

```
type data:index:salmon
```

description Transcriptome index file created using the Salmon indexing tool. cDNA (transcriptome) sequences used for index file creation must be derived from the same species as the input sequencing reads to obtain the reliable analysis results.

```
required False
```

hidden alignment.assay_type != 'auto'

alignment.n_reads

label Number of reads in subsampled alignment file

```
type basic:integer
```

description Alignment (.bam) file subsample size. Increase the number of reads to make automatic detection more reliable. Decrease the number of reads to make automatic detection run faster.

```
hidden alignment.assay_type != 'auto'
```

default 5000000

annotation.annotation

label Annotation

type data: annotation

description GTF and GFF3 annotation formats are supported.

annotation.feature_class

label Feature class

type basic:string

description Feature class (3rd column in GTF/GFF3 file) to be used. All other features will be ignored.

default exon

annotation.feature_type

label Feature type

type basic:string

description The type of feature the quantification program summarizes over (e.g. gene or transcript-level analysis). The value of this parameter needs to be chosen in line with 'ID attribute' below.

```
default gene
```

choices

• gene: gene

• transcript: transcript

annotation.id_attribute

label ID attribute

type basic:string

description GTF/GFF3 attribute to be used as feature ID. Several GTF/GFF3 lines with the same feature ID will be considered as parts of the same feature. The feature ID is used to identify the counts in the output table. In GTF files this is usually 'gene_id', in GFF3 files this is often 'ID', and 'transcript_id' is frequently a valid choice for both annotation formats.

```
default gene_id
```

choices

• gene_id: gene_id

• transcript_id: transcript_id

• ID: ID

• geneid: geneid

normalization_type

label Normalization type

type basic:string

description The default expression normalization type.

default TPM

choices

• **TPM**: **TPM**

• **CPM**: CPM

• FPKM: FPKM

• RPKUM: RPKUM

mappability

label Mappability

type data:mappability:bcm

description Genome mappability information

required False

hidden normalization_type != 'RPKUM'

show_advanced

label Show advanced options

```
type basic:boolean
     description Inspect and modify parameters
     default False
advanced.summarization_level.count_features
     label Perform read counting at feature level
```

type basic:boolean

description Count reads for exons rather than genes.

default False

advanced.overlap.allow_multi_overlap

label Assign reads to all their overlapping features or meta-features

type basic:boolean

default False

advanced.overlap.min overlap

label Minimum number of overlapping bases in a read that is required for read assignment

type basic:integer

description Number of overlapping bases is counted from both reads if paired-end. If a negative value is provided, then a gap of up to specified size will be allowed between read and the feature that the read is assigned to.

default 1

advanced.overlap.frac_overlap

label Minimum fraction of overlapping bases in a read that is required for read assignment

type basic:decimal

description Value should be within range [0, 1]. Number of overlapping bases is counted from both reads if paired end. Both this option and 'Minimum number of overlapping bases in a read that is required for read assignment' need to be satisfied for read assignment.

default 0.0

advanced.overlap_feature

label Minimum fraction of overlapping bases included in a feature that is required for overlapping with a read or a read pair

type basic:decimal

description Value should be within range [0, 1].

default 0.0

advanced.overlap.largest_overlap

label Assign reads to a feature or meta-feature that has the largest number of overlapping bases

type basic:boolean

default False

advanced.overlap.read extension 5

label Number of bases to extend reads upstream by from their 5' end

```
type basic:integer
     default 0
advanced.overlap.read_extension_3
     label Number of bases to extend reads upstream by from their 3' end
     type basic:integer
     default 0
advanced.overlap.read_to_pos
     label Reduce reads to their 5'-most or 3'-most base
     type basic:integer
     description Read counting is performed based on the single base the read is reduced to.
     required False
advanced.multi_mapping_reads.count_multi_mapping_reads
     label Count multi-mapping reads
     type basic:boolean
     description For a multi-mapping read, all its reported alignments will be counted. The 'NH' tag in BAM
           input is used to detect multi-mapping reads.
     default False
advanced.fractional counting.fraction
     label Assign fractional counts to features
     type basic:boolean
     description This option must be used together with 'Count multi-mapping reads' or 'Assign reads to all
           their overlapping features or meta-features' or both. When 'Count multi-mapping reads' is checked,
           each reported alignment from a multi-mapping read (identified via 'NH' tag) will carry a count of
           1 / x, instead of 1 (one), where x is the total number of alignments reported for the same read.
           When 'Assign reads to all their overlapping features or meta-features' is checked, each overlapping
           feature will receive a count of 1 / y, where y is the total number of features overlapping with the
           read. When both 'Count multi-mapping reads' and 'Assign reads to all their overlapping features or
           meta-features' are specified, each alignment will carry a count of 1 / (x * y).
     required False
     disabled !advanced.multi mapping reads.count multi mapping reads
                                                                                     &&
                                                                                                     !ad-
           vanced.overlap.allow multi overlap
     default False
advanced.read_filtering.min_mqs
     label Minimum mapping quality score
     type basic:integer
     description The minimum mapping quality score a read must satisfy in order to be counted. For paired-
           end reads, at least one end should satisfy this criterion.
```

default 0

advanced.read filtering.split only

```
label Count only split alignments
type basic:boolean
default False
```

advanced.read_filtering.non_split_only

label Count only non-split alignments

type basic:boolean

default False

advanced.read_filtering.primary

label Count only primary alignments

type basic:boolean

description Primary alignments are identified using bit 0x100 in BAM FLAG field.

default False

advanced.read_filtering.ignore_dup

label Ignore duplicate reads in read counting

type basic:boolean

description Duplicate reads are identified using bit Ox400 in BAM FLAG field. The whole read pair is ignored if one of the reads is a duplicate read for paired-end data.

default False

advanced.exon_exon_junctions.junc_counts

label Count number of reads supporting each exon-exon junction

type basic:boolean

description Junctions are identified from those exon-spanning reads in input (containing 'N' in CIGAR string).

default False

advanced.exon_exon_junctions.genome

label Genome

type data:genome

description Reference sequences used in read mapping that produced the provided BAM files. This optional argument can be used to improve read counting for junctions.

required False

disabled !advanced.exon_exon_junctions.junc_counts

$advanced.paired_end.is_paired_end$

label Count fragments (or templates) instead of reads

type basic:boolean

default True

advanced.paired_end.require_both_ends_mapped

label Count only read pairs that have both ends aligned

```
type basic:boolean
     default False
advanced.paired_end.check_frag_length
     label Check fragment length when assigning fragments to meta-features or features
     type basic:boolean
     description Use minimum and maximum fragment/template length to set thresholds.
     default False
advanced.paired_end.min_frag_length
     label Minimum fragment/template length
     type basic:integer
     required False
     disabled !advanced.paired_end.check_frag_length
     default 50
advanced.paired_end.max_frag_length
     label Maximum fragment/template length
     type basic:integer
     required False
     disabled !advanced.paired_end.check_frag_length
     default 600
advanced.paired_end.do_not_count_chimeric_fragments
     label Do not count chimeric fragments
     type basic:boolean
     description Do not count read pairs that have their two ends mapped to different chromosomes or mapped
          to same chromosome but on different strands.
     default False
advanced.paired_end.do_not_sort
     label Do not sort reads in BAM input
     type basic:boolean
     default False
advanced.read_groups.by_read_group
     label Assign reads by read group
     type basic:boolean
     description RG tag is required to be present in the input BAM files.
     default False
advanced.long_reads.count_long_reads
```

label Count long reads such as Nanopore and PacBio reads

```
type basic:boolean
     default False
advanced.miscellaneous.report_reads
     label Output detailed assignment results for each read or read pair
     type basic:boolean
     default False
advanced.miscellaneous.max_mop
     label Maximum number of 'M' operations allowed in a CIGAR string
     type basic:integer
     description Both 'X' and '=' are treated as 'M' and adjacent 'M' operations are merged in the CIGAR
          string.
     default 10
advanced.miscellaneous.verbose
     label Output verbose information
     type basic:boolean
     description Output verbose information for debugging, such as unmatched chromosome / contig names.
     default False
Output results rc
     label Read counts
     type basic:file
fpkm
     label FPKM
     type basic:file
tpm
     label TPM
     type basic:file
cpm
     label CPM
     type basic:file
exp
     label Default expression output
     type basic:file
exp_json
     label Default expression output (json)
     type basic: json
exp_type
```

```
label Expression normalization type (on default output)
     type basic:string
exp_set
     label Expressions
     type basic:file
exp_set_json
     label Expressions (json)
     type basic: json
feature_counts_output
     label featureCounts output
     type basic:file
counts_summary
     label Counts summary
     type basic:file
read_assignments
     label Read assignments
     type basic:file
     description Read assignment results for each read (or fragment if paired end).
     required False
strandedness\_report
     label Strandedness report file
     type basic:file
     required False
source
     label Gene ID database
     type basic:string
species
     label Species
     type basic:string
build
     label Build
     type basic:string
feature_type
     label Feature type
     type basic:string
```

methcounts

```
data:wgbs:methcountsmethcounts (data:genome:fasta
                                                               genome, data:alignment:mr
                                                                                               align-
                                           ment, basic:boolean
                                                                    cpgs,
                                                                           basic:boolean
                                                                                            symmet-
                                           ric_cpgs) [Source: v1.0.1]
The methcounts program takes the mapped reads and produces the methylation level at each genomic cytosine, with
the option to produce only levels for CpG-context cytosines.
Input arguments genome
     label Reference genome
     type data:genome:fasta
alignment
     label Mapped reads
     type data:alignment:mr
     description WGBS alignment file in Mapped Read (.mr) format.
cpgs
     label Only CpG context sites
     type basic:boolean
     description Output file will contain methylation data for CpG context sites only. Choosing this option
          will result in CpG content report only.
     disabled symmetric_cpgs
     default False
symmetric_cpgs
     label Merge CpG pairs
     type basic:boolean
     description Merging CpG pairs results in symmetric methylation levels. Methylation is usually symmet-
          ric (cytosines at CpG sites were methylated on both DNA strands). Choosing this option will only
          keep the CpG sites data.
     disabled cpgs
     default True
Output results meth
     label Methylation levels
     type basic:file
stats
     label Statistics
     type basic:file
bigwig
     label Methylation levels BigWig file
     type basic:file
```

species

330

```
label Species
     type basic:string
build
     label Build
     type basic:string
miRNA pipeline
data:workflow:mirnaworkflow-mirna (data:reads:fastq reads, data:genome:fasta genome,
                                               data:annotation annotation, basic:string id_attribute,
                                               basic:string feature_class) [Source: v0.0.5]
Input arguments reads
     label Input miRNA reads.
     type data:reads:fastq
     description Note that these reads should already be void of adapters.
genome
     label Genome
     type data:genome:fasta
annotation
     label Annotation (GTF/GFF3)
     type data:annotation
id_attribute
     label ID attribute
     type basic:string
     description GTF/GFF3 attribute to be used as feature ID. Several GTF/GFF3 lines with the same feature
          ID will be considered as parts of the same feature. The feature ID is used to identify the counts in the
          output table. In GTF files this is usually 'gene_id', in GFF3 files this is often 'ID', and 'transcript_id'
          is frequently a valid choice for both annotation formats.
     default gene_id
     choices
             • gene_id: gene_id
             • transcript_id: transcript_id
             • ID: ID
             • geneid: geneid
feature class
     label Feature class
     type basic:string
     description Feature class (3rd column in GFF file) to be used, all features of other types are ignored.
     default miRNA
```

Output results

```
snpEff
data:snpeff:uploadupload-snpeff (basic:file
                                                       annotation,
                                                                    basic:file
                                                                                summary,
                                                                                            ba-
                                          sic:file snpeff_genes) [Source: v1.1.1]
Upload snpEff result files.
Input arguments annotation
     label Annotation file
     type basic:file
summary
     label Summary
     type basic:file
snpeff_genes
     label SnpEff genes
     type basic:file
Output results annotation
     label Annotation file
     type basic:file
summary
     label Summary
     type basic:file:html
snpeff_genes
     label SnpEff genes
     type basic:file
snpEff
data:snpeffsnpeff (data:variants:vcf variants, basic:string var_source, basic:string database,
                        list:data:variants:vcf known_vars_annot) [Source: v0.2.1]
Variant annotation using snpEff package.
Input arguments variants
     label Variants (VCF)
     type data:variants:vcf
var_source
     label Input VCF source
     type basic:string
     choices
            • GATK HC: gatk_hc
```

```
• loFreq: lofreq
database
     label snpEff database
     type basic:string
     default GRCh37.75
     choices
           • GRCh37.75: GRCh37.75
known_vars_annot
     label Known variants
     type list:data:variants:vcf
Output results annotation
     label Annotation file
     type basic:file
summary
     label Summary
     type basic:file:html
snpeff genes
     label SnpEff genes
     type basic:file
```

1.3 Descriptor schemas

When working with the biological data, it is recommended (and often required) to properly annotate samples. The annotation information attached to the samples includes information about *organism*, *source*, *cell type*, *library preparation protocols* and others.

The annotation fields associated with the samples or related sample files are defined in the descriptor schemas. This tutorial describes the descriptor schemas that are attached to the sample objects, raw sequencing reads and differential expressions files.

Other available descriptor schemas can be explored at the Resolwe-bio GitHub page. Customized descriptor schemas can be created using the Resolwe SDK.

1.3.1 Sample

When a new data object that represents a biological sample (i.e. fastq files, bam files) is uploaded to the database, the unannotated sample (presample) is automatically created. When annotation is attached to the presample object, this object is automatically converted to the annotated sample. To annotate the sample, we need to define a descriptor schema that will be used for the annotation. Together with the descriptor schema, we need to provide the annotations (descriptors) that populate the annotation fields defined in the descriptor shema. The details of this process are described in the Resolwe SDK documentation.

To annotate the sample in a GEO compliant way, we prepared the sample annotation schema. An example of the customized descriptor schema is also available.

1.3.2 Reads

To annotate raw sequencing reads we have prepared two descriptor schemas: reads and reads_detailed.

1.3.3 Differential expression

Initialize test files path.

To define the default thresholds for p-value, log fold change (FC) and to describe which samples are used as cases and which as controls in the calculation of differential expression we have prepared diffexp descriptor schema.

1.4 Reference

1.4.1 Utilities

Test helper functions.

```
class resolwe_bio.utils.test.BioProcessTestCase (methodName='runTest')
     Base class for writing bioinformatics process tests.
     It is a subclass of Resolve's ProcessTestCase with some specific functions used for testing bioinformatics
     processes.
     prepare_adapters (fn='adapters.fasta')
          Prepare adapters FASTA.
     prepare_amplicon_master_file (mfile='56G_masterfile_test.txt', pname='56G panel, v2')
          Prepare amplicon master file.
     prepare_annotation (fn='sp_test.gtf',
                                               source='DICTYBASE', species='Dictyostelium
                              coideum', build='dd-05-2009')
          Prepare annotation GTF.
     prepare_annotation_gff (fn='annotation
                                                         dicty.gff.gz',
                                                                             source='DICTYBASE',
                                   species='Dictyostelium discoideum', build='dd-05-2009')
          Prepare annotation GFF3.
     prepare_bam(fn='sp_test.bam', species='Dictyostelium discoideum', build='dd-05-2009')
          Prepare alignment BAM.
     prepare_expression (f_rc='exp_1_rc.tab.gz',
                                                       f_{exp}='exp_1_{tpm.tab.gz'}
                                                                                    f_{type}='TPM',
                              name='Expression', source='DICTYBASE',
                                                                          descriptor=None,
                              ture_type='gene', species='Dictyostelium discoideum', build='dd-05-
                              2009')
          Prepare expression.
     prepare_genome()
          Prepare genome FASTA.
     prepare_paired_reads (mate1=['fw reads.fastq.gz'], mate2=['rw reads.fastq.gz'])
          Prepare NGS reads FASTQ.
     prepare_reads (fn=['reads.fastq.gz'])
          Prepare NGS reads FASTQ.
     setUp()
```

1.4. Reference 333

class resolwe bio.utils.test.KBBioProcessTestCase(methodName='runTest')

Class for bioinformatics process tests that use knowledge base.

It is based on <code>BioProcessTestCase</code> and Django's <code>LiveServerTestCase</code>. The latter launches a live Django server in a separate thread so that the tests may use it to query the knowledge base.

setUp()

Set-up test gene information knowledge base.

```
resolwe bio.utils.test.skipDockerFailure(reason)
```

Skip decorated tests due to failures when run in Docker.

Unless TESTS_SKIP_DOCKER_FAILURES Django setting is set to False. reason should describe why the test is being skipped.

```
resolwe_bio.utils.test.skipUnlessLargeFiles(*files)
```

Skip decorated tests unless large files are available.

Parameters *files (list) – variable length files list, where each element represents a large file path relative to the TEST_LARGE_FILES_DIR directory

1.5 Change Log

All notable changes to this project are documented in this file. This project adheres to Semantic Versioning.

1.5.1 Unreleased

Added

- Add alleyoop-rates process
- Add alleyoop-utr-rates process
- Add alleyoop-summary process
- Add alleyoop-snpeval process
- Add alleyoop-collapse process
- Add slam-count process
- Add workflow-slamdunk-paired workflow

- BACKWARD INCOMPATIBLE: Refactor slamdunk-all-paired process to support genome browser visualization and add additional output fields
- Append sample and genome reference information to the summary output file in the filtering-chemut process
- Bigwig output field in bamclipper, bqsr and markduplicates processes is no longer required
- Freeze docutils package version to 0.15.2 because Sphinx has problems parsing development version numbers
- Support Slamdunk/Alleyoop processes in MultiQC
- Enable sorting of files in alignment-star process using samtools

1.5.2 24.0.0 - 2019-11-15

Added

- Add resolwebio/slamdunk Docker image
- Add Tabix (1.7-2) to resolwebio/bamliquidator: 1.2.0 Docker image
- Add seqtk-rev-complement-single and seqtk-rev-complement-paired process
- Add slamdunk-all-paired process

Changed

- BACKWARD INCOMPATIBLE: Require Resolwe 20.x
- · Make BaseSpace file download more robust
- Bump rose2 to 1.1.0, bamliquidator to 1.3.8, and use resolwebio/base:ubuntu-18.04 Docker image as a base image in resolwebio/bamliquidator:1.1.0 Docker image
- Use resolwebio/bamliquidator:1.2.0 in rose2 process
- Bump CPU, memory and Docker image (resolvebio/rnaseq:4.9.0) requirements in alignment-bwa-mem, alignment-bwa-sw and alignment-bwa-aln processes
- Use multi-threading option in Samtools commands in alignment-bwa-mem, alignment-bwa-sw and alignment-bwa-aln processes
- Support merging of multi-lane sequencing data into a single (pair) of FASTQ files in the upload-fastq-single, upload-fastq-paired, files-to-fastq-single and files-to-fastq-paired processes.

1.5.3 23.1.1 - 2019-10-11

Changed

• Renamed workflow-trim-align-quant workflow to make the name more informative

1.5.4 23.1.0 - 2019-09-30

Added

- Add Macaca mulatta species choice to the sample descriptor schema
- Add workflow-cutadapt-star-fc-quant-wo-depletion-single process

Changed

- Test files improved for workflow-wes, bamclipper, markduplicates and bqsr
- Fix typo in differential expression—shrna process docstring

- Fix transcript-to-gene_id mapping for Salmon expressions in differentialexpression-deseq2 process. Transcript versions are now ignored when matching IDs using the transcript-to-gene_id mapping table.
- Fix workflow-cutadapt-star-fc-quant-single process description

1.5.5 23.0.0 - 2019-09-17

Changed

- Update order of QC reports in MultiQC configuration file. The updated configuration file is part of the resolwebio/common:1.3.1 Docker image.
- Bump Jbrowse to version 1.16.6 in resolwebio/rnaseq: 4.9.0 Docker image
- Use JBrowse generate-names.pl script to index GTF/GFF3 features upon annotation file upload
- Support Salmon reports in MultiQC and expose dirs_depth parameter
- Expose transcript-level expression file in the salmon-quant process

Added

 Add workflow-bbduk-salmon-qc-single and workflow-bbduk-salmon-qc-paired workflows

Fixed

• Give process upload-bedpe access to network

1.5.6 22.0.0 - 2019-08-20

- **BACKWARD INCOMPATIBLE:** Require Resolwe 19.x
- BACKWARD INCOMPATIBLE: Unify cutadapt-single and cutadapt-paired process inputs and refactor to use Cutadapt v2.4
- Expose BetaPrior parameter in differentialexpression-deseq2 process
- Install R from CRAN-maintained repositories in Docker images build from the resolwebio/base:ubuntu-18.04 base image
- Prepare resolwebio/common:1.3.0 Docker image:
 - Install R v3.6.1
 - Bump Resdk to v10.1.0
 - Install gawk package
 - Fix Docker image build issues
- Use resolwebio/common:1.3.0 as a base image for resolwebio/rnaseq:4.8.0
- Update StringTie to v2.0.0 in resolwebio/rnaseq:4.8.0

• Support StringTie analysis results in DESeq2 tool

Added

- Add cutadapt-3prime-single process
- Add workflow-cutadapt-star-fc-quant-single process
- Add argument skip to bamclipper which enables skipping of the said process
- Add cutadapt-corall-single and cutadapt-corall-paired processes for pre-processing of reads obtained using Corall Total RNA-seq library prep kit
- Add umi-tools-dedup process
- Add stringtie process
- Add workflow-corall-single and workflow-corall-paired workflows optimized for Corall Total RNA-seq library prep kit data

Fixed

• Fix warning message in hierarchical clustering of genes. Incorrect gene names were reported in the warning message about removed genes. Computation of hierarchical clustering was correct.

1.5.7 21.0.1 - 2019-07-26

Changed

• Bump Cutadapt to v2.4 and use resolwebio/common:1.2.0 as a base image in resolwebio/rnaseg:4.6.0

Added

- Add pigz package to resolwebio/common:1.2.0 Docker image
- Add StringTie and UMI-tools to resolwebio/rnaseq: 4.7.0 Docker image

Fixed

- Fix spikeins-qc process to correctly handle the case where all expressions are without spikeins
- Fix an error in macs2-callpeak process that prevented correct reporting of build/species mismatch between inputs
- Support UCSC annotations in feature_counts process by assigning empty string gene_ids to the "un-known" gene

1.5.8 21.0.0 - 2019-07-16

Changed

• BACKWARD INCOMPATIBLE: Require Resolwe 18.x

- Bump the number of allocated CPU cores to 20 in alignment-bwa-mem process
- Bump memory requirements in seqtk-sample-single and seqtk-sample-paired processes
- Bump Salmon to v0.14.0 in resolvebio/rnaseq: 4.5.0 Docker image
- Expose additional inputs in salmon-index process
- Use resolwebio/rnaseq:4.5.0 Docker image in processes that call Salmon tool (library-strandedness, feature_counts and gorts-qc)
- Implement dropdown menu for upload-bedpe process
- Add validation stringency parameter to bqsr process and propagate it to the workflow-wes as well
- Add LENIENT value to validation stringency parameter of the markduplicates process
- Improve performance of RPKUM normalization in featureCounts process

• Add salmon-quant process

Fixed

- Fix genome upload process to correctly handle filenames with dots
- Fix merging of expressions in archive-samples process. Previously some genes were missing in the merged expression files. The genes that were present had expression values correctly assigned. The process was optimized for performance and now supports parallelization.

1.5.9 20.0.0 2019-06-19

- BACKWARD INCOMPATIBLE: Require Resolwe 17.x
- BACKWARD INCOMPATIBLE: Use Elasticsearch version 6.x
- BACKWARD INCOMPATIBLE: Bump Django requirement to version 2.2
- BACKWARD INCOMPATIBLE: Remove obsolete RNA-seq workflows workflow-bbduk-star-featurecounts-single, workflow-bbduk-star-featurecounts-paired, workflow-cutadapt-star-featurecounts-paired
- BACKWARD INCOMPATIBLE: Remove obsolete descriptor schemas: rna-seq-bbduk-star-featurecounts, quantseq, rna-seq-cutadapt-star-featurecounts and kapa-rna-seq-bbduk-star-featurecounts
- BACKWARD INCOMPATIBLE: In upload-fasta-nucl process, store compressed and uncompressed FASTA files in fastagz and fasta outur fields, respectively
- Allow setting the Java memory usage flags for the QoRTs tool in resolwebio/common:1.1.3 Docker image
- Use resolwebio/common:1.1.3 Docker image as a base image for resolwebio/rnaseq:4.4.2
- Bump GATK4 version to 4.1.2.0 in resolwebio/dnaseq: 4.2.0
- Use MultiQC configuration file and prepend directory name to sample names by default in multiqc process

- Bump resolwebio/common to 1.1.3 in resolwebio/dnaseq: 4.2.0
- Process vc-gatk4-hc now also accepts BED files through parameter intervals_bed

- Support Python 3.7
- Add Tabix (1.7-2) to resolvebio/wgbs docker image
- Add JBrowse index output to hmr process
- Add bamclipper tool and parallel package to resolwebio/dnaseq: 4.2.0 image
- Support hg19_mm10 hybrid genome in bam-split process
- · Support mappability-based normalization (RPKUM) in featureCounts
- · Add BEDPE upload process
- Add bamclipper process
- Add markduplicates process
- Add bgsr (BaseQualityScoreRecalibrator) process
- Add whole exome sequencing (WES) pipeline

Fixed

- Fix building problems of resolwebio/dnaseq docker
- Fix handling of no-adapters input in workflows workflow-bbduk-star-featurecounts-qc-single and workflow-bbduk-star-featurecounts-qc-paired

1.5.10 19.0.1 2019-05-13

Fixed

- Use resolwebio/rnaseq: 4.4.2 Docker image that enforces the memory limit and bump memory requirements for gorts-qc process
- Bump memory requirements for multiqc process

1.5.11 19.0.0 2019-05-07

Changed

- Use Genialis fork of MultiQC 1.8.0b in resolwebio/common:1.1.2
- Support Samtools idxstats and QoRTs QC reports in multigc process
- Support samtools-idxstats QC step in workflows:
 - workflow-bbduk-star-featurecounts-qc-single
 - workflow-bbduk-star-featurecounts-qc-paired
 - workflow-bbduk-star-fc-quant-single

- workflow-bbduk-star-fc-quant-paired
- Simplify cellranger-count outputs folder structure
- Bump STAR aligner to version 2.7.0f in resolwebio/rnaseq: 4.4.1 Docker image
- Use resolwebio/rnaseq:4.4.1 in alignment-star and alignment-star-index processes
- Save filtered count-matrix output file produced by DESeq2 differential expression process

- Add samtools-idxstats process
- Improve cellranger-count and cellranger-mkref logging
- Add FastQC report to upload-sc-10x process

Fixed

- Fix archive-samples to work with data:chipseq:callpeak:macs2 data objects when downloading only peaks without QC reports
- · Fix parsing gene set files with empty lines to avoid saving gene sets with empty string elements

1.5.12 18.0.0 2019-04-16

Changed

- BACKWARD INCOMPATIBLE: Require Resolwe 16.x
- **BACKWARD INCOMPATIBLE:** Rename and improve descriptions of processes specific to CATS RNA-seq kits. Remove related cutadapt-star-htseq descriptor schema.
- BACKWARD INCOMPATIBLE: Remove workflow-accel-gatk4 pipeline. Remove amplicon-panel, amplicon-panel-advanced and amplicon-master-file descriptor schemas.
- BACKWARD INCOMPATIBLE: Remove obsolete processes and descriptor schemas: rna-seq-quantseq, bcm-workflow-rnaseq, bcm-workflow-chipseq, bcm-workflow-wgbs, dicty-align-reads, dicty-etc, affy and workflow-chip-seq
- Expose additional parameters of bowtie2 process
- Support strandedness auto detection in gorts-qc process

Added

340

- Add shRNAde (v1.0) R package to the resolvebio/rnaseq: 4.4.0 Docker image
- Add resolwebio/scseq Docker image
- Add shRNA differential expression process. This is a two-step process which trims, aligns and quantifies short hairpin RNA species. These are then used in a differential expression.
- Add sc-seq processes:
 - cellranger-mkref

- cellranger-count
- upload-sc-10x
- upload-bam-scseq-indexed

- Bump memory requirements in seqtk-sample-single and seqtk-sample-paired processes
- Fix cellranger-count html report
- Mark spliced-alignments with XS flags in workflow-rnaseq-cuffquant
- Fix whitespace handling in cuffnorm process

1.5.13 17.0.0 2019-03-19

Added

- Add gorts-qc (Quality of RNA-seq Tool-Set QC) process
- Add workflow-bbduk-star-fc-quant-single and workflow-bbduk-star-fc-quant-paired processes
- Add independent gene filtering and gene filtering based on Cook's distance in DESeq2 differential expression process

Changed

- BACKWARD INCOMPATIBLE: Move gene filtering by expression count input to filter. min_count_sum in DESeq2 differential expression process
- BACKWARD INCOMPATIBLE: Require Resolwe 15.x
- Update resolwebio/common:1.1.0 Docker image:
 - add QoRTs (1.3.0) package
 - bump MultiQC to 1.7.0
 - bump Subread package to 1.6.3
- Expose maxns input parameter in bbduk-single and bbduk-paired processes. Make this parameter available in workflows workflow-bbduk-star-featurecounts-qc-single, workflow-bbduk-star-featurecounts-qc-paired, workflow-bbduk-star-featurecounts-single and workflow-bbduk-star-featurecounts-paired.
- Save CPM-normalized expressions in feature_counts process. Control the default expression normalization type (exp_type) using the normalization_type input.
- Bump MultiQC to version 1.7.0 in multiqc process
- Use resolwebio/rnaseq:4.3.0 with Subread/featureCounts version 1.6.3 in feature_counts process

1.5.14 16.3.0 2019-02-19

Changed

- Bump STAR aligner version to 2.7.0c in resolvebio/rnaseq: 4.2.2
- Processes alignment-star and alignment-star-index now use Docker image resolwebio/rnaseq:4.2.2 which contains STAR version 2.7.0c
- Persistence of basespace-file-import process changed from RAW to TEMP

Added

 Make prepare-geo-chipseq work with both data:chipseq:callpeak:macs2 and data:chipseq:callpeak:macs14 as inputs

Fixed

• Report correct total mapped reads and mapped reads percentage in prepeak QC report for data:alignment:bowtie2 inputs in macs2-callpeak process

1.5.15 16.2.0 2019-01-28

Changed

- Enable multithreading mode in alignment-bwa-aln and alignment-bwa-sw
- Lineary lower the timeout for BigWig calculation when running on multiple cores

Fixed

- Remove pip --process-dependency-links argument in testenv settings
- Fix walt getting killed when sort runs out of memory. The sort command buffer size was limited to the
 process memory limit.

1.5.16 16.1.0 2019-01-17

Changed

Added

- Add the FASTQ file validator script to the upload-fastq-single, upload-fastq-paired, files-to-fastq-single and files-to-fastq-paired processes
- Add spikein-qc process
- Add to resolwebio/rnaseq:4.1.0 Docker image:
 - dnaio Python library
- Add to resolwebio/rnaseq: 4.2.0 Docker image:
 - ERCC table

- common Genialis fonts and css file
- spike-in QC report template
- Set MPLBACKEND environment variable to Agg in resolvebio/common:1.0.1 Docker image

- Fix the format of the output FASTQ file in the demultiplex.py script
- Fix NSC and RSC QC metric calculation for ATAC-seq and paired-end ChIP-seq samples in macs2-callpeak and qc-prepeak processes

1.5.17 16.0.0 2018-12-19

Changed

- BACKWARD INCOMPATIBLE: Require Resolwe 14.x
- BACKWARD INCOMPATIBLE: Remove obsolete processes findsimilar
- BACKWARD INCOMPATIBLE: Include ENCODE-proposed QC analysis metrics methodology in the macs2-callpeak process. Simplified MACS2 analysis inputs now allow the use of sample relations (treatment/background) concept to trigger multiple MACS2 jobs automatically using the macs2-batch or macs2-rose2-batch processes.
- BACKWARD INCOMPATIBLE: Update workflow-atac-seq inputs to match the updated macs2-callpeak process
- Use resolwebio/rnaseq:4.0.0 Docker image in alignment-star-index, bbduk-single, bbduk-paired, cuffdiff, cufflinks, cuffmerge, cuffnorm, cutadapt-custom-single, cutadapt-custom-paired, cutadapt-single, cutadapt-paired, differentialexpression-deseq2, differentialexpression-edger, expression-aggregator, feature_counts, goenrichment, htseq-count, index-fasta-nucl, library-strandedness, htseq-count-raw, regtools-junctions-annotate, rsem, salmon-index, trimmomatic-single, trimmomatic-paired, upload-expression, upload-expression-cuffnorm, upload-fastq-single, upload-expression-star, upload-fasta-nucl, upload-fastq-paired, files-to-fastq-single, files-to-fastq-paired, upload-gaf, upload-genome, upload-gff3, upload-gtf and upload-obo
- · Order statistical groups in expression aggregator output by sample descriptor field value
- Use resolwebio/biox:1.0.0 Docker image in etc-bcm, expression-dicty and mappability-bcm processes
- Use resolwebio/common: 1.0.0 Docker image in amplicon-table, mergeexpressions, upload-diffexp, upload-etc, upload-multiplexed-single and upload-multiplexed-paired processes
- Use resolwebio/base:ubuntu-18.04 Docker image in create-geneset, create-geneset-venn, mergeetc, prepare-geo-chipseq, prepare-geo-rnaseq, upload-cxb, upload-geneset, upload-header-sam, upload-mappability, upload-snpeff and upload-picard-pcrmetrics processes
- Update GATK4 to version 4.0.11.0 in resolwebio/dnaseq: 4.1.0 Docker image. Install and use JDK v8 by default to ensure compatibility with GATK4 package.

- Use resolwebio/dnaseq:4.1.0 Docker image in align-bwa-trim, coveragebed, filtering-chemut, lofreq, picard-pcrmetrics, upload-master-file, upload-variants-vcf and vc-gatk4-hc processes
- Expose reads quality filtering (q) parameter, reorganize inputs and rename the stats output file in alignment-bwa-aln process
- Use resolwebio/chipseq: 4.0.0 Docker image in chipseq-genescore, chipseq-peakscore, macs14, upload-bed and qc-prepeak processes
- Use resolwebio/bamliquidator:1.0.0 Docker image in bamliquidator and bamplot processes

- Add biosample source field to sample descriptor schema
- Add background_pairs Jinja expressions filter that accepts a list of data objects and orders them in a list of pairs (case, background) based on the background relation between corresponding samples
- Add chipseq-bwa descriptor schema. This schema specifies the default inputs for BWA ALN aligner process as defined in ENCODE ChIP-Seq experiments.
- Add support for MACS2 result files to MultiQC process
- Add macs2-batch, macs2-rose2-batch and workflow-macs-rose processes
- Add feature symbols to expressions in archive-samples process

Fixed

- Make ChIP-seq fields in sample descriptor schema visible when ChIPmentation assay type is selected
- Fix handling of whitespace in input BAM file name in script detect_strandedness.sh
- Set available memory for STAR aligner to 36GB. Limit the available memory for STAR aligner --limitBAMsortRAM parameter to 90% of the Docker requirements setting
- Set bbduk-single and bbduk-paired memory requirements to 8GB
- Fix wrong file path in archive-samples process

1.5.18 15.0.0 2018-11-20

- BACKWARD INCOMPATIBLE: Remove obsolete processes: bsmap, mcall, coverage-garvan, igv, jbrowse-bed, jbrowse-gff3, jbrowse-gtf, jbrowse-bam-coverage, jbrowse-bam-coverage-normalized, jbrowse-refseq, fastq-mcf-single, fastq-mcf-paired, hsqutils-trim, prinseq-lite-single, prinseq-lite-paired, sortmerna-single, sortmerna-paired, bam-coverage, hsqutils-dedup, vc-samtools, workflow-heat-seq and alignment-tophat2
- BACKWARD INCOMPATIBLE: Remove jbrowse-bam-coverage process step from the workflow-accel workflow. The bigwig coverage track is computed in align-bwa-trim process instead.
- BACKWARD INCOMPATIBLE: Remove resolwebio/utils Docker image. This image is replaced by the resolwebio/common image.

- BACKWARD INCOMPATIBLE: Use resolwebio/common Docker image as a base image for the resolwebio/biox, resolwebio/chipseq, resolwebio/dnaseq and resolwebio/rnaseq images
- BACKWARD INCOMPATIBLE: Remove resolvebio/legacy Docker image.
- Use sample name as the name of the data object in:
 - alignment-bwa-aln
 - alignment-bowtie2
 - qc-prepeak
 - macs2-callpeak
- Attach macs2-callpeak, macs14 and rose2 process data to the case/treatment sample
- Use resolwebio/dnaseq:4.0.0 docker image in align-bwa-trim process
- Use resolwebio/rnaseq:4.0.0 docker image in aligners: alignment-bowtie, alignment-bwa-mem, alignment-bwa-sw, alignment-bwa-aln, alignment-hisat2, alignment-star and alignment-subread.
- Set memory limits in upload-genome, trimmomatic-single and trimmomatic-paired processes
- Improve error messages in differential expression process DESeq2

- Add makedb (WALT 1.01) callable as makedb-walt, tool to create genome index for WALT aligner, to resolvebio/rnaseq docker image
- Add resolwebio/wgbs docker image including the following tools:
 - MethPipe (3.4.3)
 - WALT (1.01)
 - wigToBigWig (kent-v365)
- Add resolwebio/common Docker image. This image includes common bioinformatics utilities and can serve as a base image for other, specialized resolwebio Docker images: resolwebio/biox, resolwebio/chipseq, resolwebio/dnaseq and resolwebio/rnaseq.
- Add shift (user-defined cross-correlation peak strandshift) input to qc-prepeak process
- Add ATAC-seq workflow
- · Compute index for WALT aligner on genome upload and support uploading the index together with the genome
- Add Whole genome bisulfite sequencing workflow and related WGBS processes:
 - WALT
 - methcounts
 - HMR
- Add bedClip to resolwebio/chipseq:3.1.0 docker image
- Add resolwebio/biox Docker image. This image is based on the resolwebio/common image and includes Biox Python library for Dictyostelium RNA-Seq analysis support.
- Add resolwebio/snpeff Docker image. The image includes SnpEff (4.3K) tool.
- Add spike-in names, rRNA and globin RNA cromosome names in resolwebio/common image

- Add UCSC bedGraphtoBigWig tool for calculating BigWig in bamtobigwig.sh script. In align-bwa-trim processor set this option (that BigWig is calculated by UCSC tool instead of deep-Tools), because it is much faster for amplicon files. In other processors update the input parameters for bamtobigwig.sh: alignment-bowtie, alignment-bowtie2, alignment-bwa-mem, alignment-bwa-sw, alignment-bwa-aln, alignment-hisat2, alignment-star alignment-subread, upload-bam, upload-bam-indexed and upload-bam-secondary.
- In bamtobigwig.sh don't create BigWig when bam file was aligned on globin RNA or rRNA (this are QC steps and BigWig is not needed)

- BACKWARD INCOMPATIBLE: Use user-specificed distance metric in hierarchical clustering
- Handle integer expression values in hierarchical clustering
- Fix Amplicon table gene hyperlinks for cases where multiple genes are associated with detected variant
- Handle empty gene name in expression files in PCA
- Fix PBC QC reporting in qc-prepeak process for a case where there are no duplicates in the input bam
- Fix macs2-callpeak process so that user defined fragment lenth has priority over the qc-prepeak estimated fragment length when shifting reads for post-peakcall QC
- Fix macs2-callpeak to prevent the extension of intervals beyond chromosome boundaries in MACS2 bedgraph outputs
- Fix warning message in hierarchical clustering of genes to display gene names

1.5.19 14.0.2 2018-10-23

Fixed

• Fix htseq-count-raw process to correctly map features with associated feature symbols.

1.5.20 14.0.1 2018-10-23

Fixed

- Handle missing gene expression in hierarchical clustering of genes. If one or more genes requested in gene filter
 are missing in selected expression files a warning is issued and hierarchical clustering of genes is computed with
 the rest of the genes instead of failing.
- Fix PCA computation for single sample case

1.5.21 14.0.0 2018-10-09

- BACKWARD INCOMPATIBLE: Require Resolwe 13.x
- BACKWARD INCOMPATIBLE: Remove gsize input from macs2-callpeak process and automate genome size selection

- BACKWARD INCOMPATIBLE: Set a new default sample and reads descriptor schema. Change slug from sample2 to sample, modify group names, add cell_type field to the new sample descriptor schema, and remove the original sample, sample-detailed, and reads-detailed descriptor schemas.
- BACKWARD INCOMPATIBLE: Unify types of macs14 and macs2-callpeak processes and make rose2 work with both
- BACKWARD INCOMPATIBLE: Remove replicates input in cuffnorm process. Use sample relation information instead.
- Use resolwebio/chipseq: 3.0.0 docker image in the following processes:
 - macs14
 - macs2-callpeak
 - rose2
- Downgrade primerclip to old version (v171018) in resolwebio/dnaseq:3.3.0 docker image and move it to google drive.
- Move bam-split process to resolwebio/rnaseq: 3.7.1 docker image
- Count unique and multimmaping reads in regtools-junctions-annotate process

- Add qc-prepeak process that reports ENCODE3 accepted ChIP-seq and ATAC-seq QC metrics
- Add QC report to macs2-callpeak process
- Add combining ChIP-seq QC reports in archive-samples process
- Add detection of globin-derived reads as an additional QC step in the workflow-bbduk-star-featurecounts-qc-single and workflow-bbduk-star-featurecounts-qc-pai processes.
- Add mappings from ENSEMBL or NCBI to UCSC chromosome names and deepTools (v3.1.0) to resolwebio/dnaseq:3.3.0 docker image
- Add BigWig output field to following processors:
 - align-bwa-trim
 - upload-bam
 - upload-bam-indexed
 - upload-bam-secondary
- Add replicate_groups Jinja expressions filter that accepts a list of data objects and returns a list of labels determining replicate groups.
- Add 'Novel splice junctions in BED format' output to regtools-junctions-annotate process, so that user can visualize only novel splice juntions in genome browsers.

Fixed

- Fix handling of numerical feature_ids (NCBI source) in create_expression_set.py script
- Make chipseq-peakscore work with gzipped narrowPeak input from macs2-callpeak

• Use uncompressed FASTQ files as input to STAR aligner to prevent issues on (network) filesystems without FIFO support

1.5.22 13.0.0 2018-09-18

Changed

- BACKWARD INCOMPATIBLE: Require Resolwe 12.x
- BACKWARD INCOMPATIBLE: Remove obsolete processes: assembler-abyss, cutadapt-amplicon, feature_location, microarray-affy-qc, reads-merge, reference_compatibility, transmart-expressions, upload-hmmer-db, upload-mappability-bigwig, upload-microarray-affy.
- BACKWARD INCOMPATIBLE: Remove obsolete descriptor schema: transmart.
- BACKWARD INCOMPATIBLE: Remove tools which are not used by any process: clustering_leaf_ordering.py, go_genesets.py, VCF_ad_extract.py, volcanoplot.py, xgff.py, xgff2gff.py.
- BACKWARD INCOMPATIBLE: Management command for inserting features and mappings requires PostgreSQL version 9.5 or newer
- Update the meta data like name, description, category, etc. of most of the processes
- Speed-up management command for inserting mappings
- Change location of cufflinks to Google Drive for resolwebio/rnaseq Docker build
- Calculate alignment statistics for the uploaded alignment (.bam) file in the upload-bam, upload-bam-indexed and upload-bam-secondary processes.
- Annotation (GTF/GFF3) file input is now optional for the creation of the STAR genome index files. Annotation file can be used at the alignment stage to supplement the genome indices with the set of known features.
- Trigger process warning instead of process error in the case when bamtobigwig.sh scripts detects an empty
 .bam file.
- Set the default reads length filtering parameter to 30 bp in the rna-seq-bbduk-star-featurecounts and kapa-rna-seq-bbduk-star-featurecounts experiment descriptor schema. Expand the kit selection choice options in the latter descriptor schema.

Added

- Add MultiQC (1.6.0) and Seqtk (1.2-r94) to the resolwebio/utils:1.5.0 Docker image
- Add sample2 descriptor schema which is the successor of the original sample and reads descriptor schemas
- Add bedToBigBed and Tabix to resolwebio/rnaseq:3.7.0 docker image
- Add HS Panel choice option to the amplicon-master-file descriptor schema
- Add MultiQC process
- Add process for the Seqtk tool sample sub-command. This process allows sub-sampling of .fastq files using either a fixed number of reads or the ratio of the input file.
- Add MultiQC analysis step to the workflow-bbduk-star-featurecounts-single and workflow-bbduk-star-featurecounts-single processes.

- Add workflow-bbduk-star-featurecounts-qc-single and workflow-bbduk-star-featurecounts-qcprocesses which support MultiQC analysis, input reads down-sampling (using Seqtk) and rRNA sequence detection using STAR aligner.
- Add to resolwebio/chipseq Docker image:

```
- bedtools (2.25.0-1)
- gawk (1:4.1.3+dfsg-0.1)
- picard-tools (1.113-2)
- run_spp.R (1.2) (as spp)
- SPP (1.14)
```

- Add regtools-junctions-annotate process that annotates novel splice junctions.
- Add background relation type to fixtures

- Track source information in the upload-fasta-nucl process.
- When STAR aligner produces an empty alignment file, re-sort the alignment file to allow successful indexing of the output .bam file.
- Create a symbolic link to the alignment file in the feature_counts process, so that relative path is used in
 the quantification results. This prevent the FeatureCounts output to be listed as a separate sample in the MultiQC
 reports.
- Fix handling of expression objects in archive-samples process

1.5.23 12.0.0 - 2018-08-13

Changed

- BACKWARD INCOMPATIBLE: Require Resolwe 11.x
- BACKWARD INCOMPATIBLE: Use read count instead of sampling rate in strandedness detection
- BACKWARD INCOMPATIBLE: Remove genome input from rose2 process and automate its selection
- BACKWARD INCOMPATIBLE: Refactor cutadapt-paired process
- BACKWARD INCOMPATIBLE: Improve leaf ordering performance in gene and sample hierarchical clustering. We now use exact leaf ordering which has been recently added to scipy instead of an approximate in-house solution based on nearest neighbor algorithm. Add informative warning and error messages to simplify troubleshooting with degenerate datasets.
- Remove igvtools from resolwebio/utils Docker image
- Improve helper text and labels in processes used for sequencing data upload
- Allow using custom adapter sequences in the workflow-bbduk-star-featurecounts-single and workflow-bbduk-star-featurecounts-paired processes
- Change chromosome names from ENSEMBL / NCBI to UCSC (example: "1" to "chr1") in BigWig files. The purpose of this is to enable viewing BigWig files in UCSC genome browsers for files aligned with ENSEBML or NCBI genome. This change is done by adding script bigwig_chroms_to_ucsc.py to bamtobigwig.sh script.
- · Reduce RAM requirement in SRA import processes

- Add two-pass mode to alignment-star process
- Add regtools (0.5.0) to resolvebio/rnaseq Docker image
- Add KAPA experiment descriptor schema
- Add resdk Python 3 package to resolvebio/utils Docker image
- Add to cutadapt-single process an option to discard reads having more 'N' bases than specified.
- Add workflows for single-end workflow-cutadapt-star-featurecounts-single and paired-end reads workflow-cutadapt-star-featurecounts-paired. Both workflows consist of preprocessing with Cutadapt, alignment with STAR two pass mode and quantification with featureCounts.
- Add descriptor schema rna-seq-cutadapt-star-featurecounts

Fixed

- BACKWARD INCOMPATIBLE: Fix the stitch parameter handling in rose2
- fix upload-gtf to create JBrowse track only if GTF file is ok
- Pin sra-toolkit version to 2.9.0 in resolvebio/utils Docker image.
- Fix and improve rose2 error messages
- Fail gracefully if bam file is empty when producing bigwig files
- Fail gracefully if there are no matches when mapping chromosome names

1.5.24 11.0.0 - 2018-07-17

Changed

- BACKWARD INCOMPATIBLE: Remove management command module
- BACKWARD INCOMPATIBLE: Remove filtering of genes with low expression in PCA analysis
- BACKWARD INCOMPATIBLE: Remove obsolete RNA-seq DSS process
- Expand error messages in rose2 process
- Check for errors during download of FASTQ files and use resolwebio/utils:1.3.0 Docker image in import SRA process
- Increase Feature's full name's max length to 350 to support a long full name of "Complement C3 Complement C3 beta chain C3-beta-c Complement C3 alpha chain C3a anaphylatoxin Acylation stimulating protein Complement C3b alpha' chain Complement C3c alpha' chain fragment 1 Complement C3dg fragment Complement C3g fragment Complement C3d fragment Complement C3c alpha' chain fragment 2" in Ensembl

Added

350

- Add *exp_set* and *exp_set_json* output fields to expression processes:
 - feature_counts
 - htseq-count

- htseq-count-raw
- rsem
- upload-expression
- upload-expression-cuffnorm
- upload-expression-star
- Add 'Masking BED file' input to rose2 process which allows masking reagions from the analysis
- Add filtering.outFilterMismatchNoverReadLmax input to alignment-star process
- Add mappings from ENSEMBL or NCBI to UCSC chromosome names to resolwebio/rnaseq:3.5.0 docker image

- Fix peaks BigBed output in macs14 process
- Remove duplicated forward of alignIntronMax input field in BBDuk STAR featureCounts workflow
- Make cuffnorm process attach correct expression data objects to samples
- Fix upload-gtf in a way that GTF can be shown in JBrowse. Because JBrowse works only with GFF files, input GTF is converted to GFF from which JBrowse track is created.

1.5.25 10.0.1 - 2018-07-06

Fixed

• Fix bamtobigwig.sh to timeout the bamCoverage calculation after defined time

1.5.26 10.0.0 - 2018-06-19

Added

- Add to resolwebio/chipseq Docker image:
 - Bedops (v2.4.32)
 - Tabix (v1.8)
 - python3-pandas
 - bedGraphToBigWig (kent-v365)
 - bedToBigBed (kent-v365)
- Add to resolwebio/rnaseq: 3.2.0 Docker image:
 - genometools (1.5.9)
 - igvtools (v2.3.98)
 - jbrowse (v1.12.0)
 - Bowtie (v1.2.2)
 - Bowtie2 (v2.3.4.1)

- BWA (0.7.17-r1188)
- TopHat (v2.1.1)
- Picard Tools (v2.18.5)
- bedGraphToBigWig (kent-v365)
- Add Debian package file to resolvebio/rnaseq: 3.3.0 Docker image
- Support filtering by type on feature API endpoint
- Add BigWig output field to following processes:
 - alignment-bowtie
 - alignment-bowtie2
 - alignment-tophat2
 - alignment-bwa-mem
 - alignment-bwa-sw
 - alignment-bwa-aln
 - alignment-hisat2
 - alignment-star
- Add Jbrowse track output field to upload-genome processor.
- Use reslowebio/rnaseq Docker image and add Jbrowse track and IGV sorting and indexing to following processes:
 - upload-gff3
 - upload-gtf
 - gff-to-gtf
- Add Tabix index for Jbrowse to upload-bed processor and use reslowebio/rnaseq Docker image
- Add BigWig, BigBed and JBrowse track outputs to macs14 process
- Add Species and Build outputs to rose2 process
- Add Species, Build, BigWig, BigBed and JBrowse track outputs to macs2 process
- Add scipy (v1.1.0) Python 3 package to resolwebio/utils Docker image

- BACKWARD INCOMPATIBLE: Drop support for Python 3.4 and 3.5
- **BACKWARD INCOMPATIBLE:** Require Resolwe 10.x
- BACKWARD INCOMPATIBLE: Upgrade to Django Channels 2
- BACKWARD INCOMPATIBLE: Count fragments (or templates) instead of reads by default in featureCounts process and BBDuk STAR featureCounts pipeline. The change applies only to paired-end data.
- BACKWARD INCOMPATIBLE: Use resolwebio/rnaseq: 3.2.0 Docker image in the following processes that output reads:
 - upload-fastq-single

- upload-fastq-paired
- files-to-fastq-single
- files-to-fastq-paired
- reads-merge
- bbduk-single
- bbduk-paired
- cutadapt-single
- cutadapt-paired
- cutadapt-custom-single
- cutadapt-custom-paired
- trimmomatic-single
- trimmomatic-paired.

This change unifies the version of FastQC tool (0.11.7) used for quality control of reads in the aforementioned processes. The new Docker image comes with an updated version of Cutadapt (1.16) which affects the following processes:

- cutadapt-single
- cutadapt-paired
- cutadapt-custom-single
- cutadapt-custom-paired.

The new Docker image includes also an updated version of Trimmomatic (0.36) used in the following processes:

- upload-fastq-single
- upload-fastq-paired
- files-to-fastq-single
- files-to-fastq-paired
- trimmomatic-single
- trimmomatic-paired.
- BACKWARD INCOMPATIBLE: Change Docker image in alignment-subread from resolwebio/legacy:1.0.0 with Subread (v1.5.1) to resolwebio/rnaseq:3.2.0 with Subread (v1.6.0).—multiMapping option was added instead of —unique_reads. By default aligner report uniquely mapped reads only.
- Update wigToBigWig to kent-v365 version in resolvebio/chipseq Docker image
- Change paths in HTML amplicon report template in resolwebio/dnaseq Docker image
- Move assay type input in BBDuk STAR featureCounts pipeline descriptor schema to advanced options
- Use resolwebio/rnaseq: 3.2.0 Docker image with updated versions of tools instead of resolwebio/legacy: 1.0.0 Docker image in following processes:
 - alignment-bowtie with Bowtie (v1.2.2) instead of Bowtie (v1.1.2)
 - alignment-bowtie2 with Bowtie2 (v2.3.4.1) instead of Bowtie2 (v2.2.6)
 - alignment-tophat2 with TopHat (v2.1.1) instead of TopHat (v2.1.0)

- alignment-bwa-mem, alignment-bwa-sw` and ``alignment-bwa-aln with BWA (v0.7.17-r1188) instead of BWA (v0.7.12-r1039)
- alignment-hisat2 with HISAT2 (v2.1.0) instead of HISAT2 (v2.0.3-beta)
- upload-genome
- Use resolwebio/base:ubuntu-18.04 Docker image as a base image in resolwebio/utils Docker image
- Update Python 3 packages in resolwebio/utils Docker image:
 - numpy (v1.14.4)
 - pandas (v0.23.0)
- Replace bedgraphtobigwig with deepTools in resolwebio/rnaseq Docker image, due to faster performance
- Use resolwebio/rnaseq:3.3.0 Docker image in alignment-star-index with STAR (v2.5.4b)

- Make management commands use a private random generator instance
- Fix output covplot_html of coveragebed process
- Fix process archive-samples and amplicon-archive-multi-report to correctly handle nested file paths
- Change rose2 and chipseq-peakscore to work with .bed or .bed.gz input files
- Fix the expression-aggregator process so that it tracks the species of the input expression data
- Fix bamtobigwig.sh to use deepTools instead of bedtools with bedgraphToBigWig due to better time performance

1.5.27 9.0.0 - 2018-05-15

- BACKWARD INCOMPATIBLE: Simplify the amplicon-report process inputs by using Latex report template from the resolwebio/latex Docker image assets
- BACKWARD INCOMPATIBLE: Simplify the coveragebed process inputs by using Bokeh assets from the resolwebio/dnaseq Docker image
- BACKWARD INCOMPATIBLE: Require Resolwe 9.x
- Update wigToBigWig tool in resolwebio/chipseq Docker image
- Use resolwebio/rnaseq:3.1.0 Docker image in the following processes:
 - cufflinks
 - cuffnorm
 - cuffquant
- Remove differential expression-limma process
- Use resolwebio/rnaseq:3.1.0 docker image and expand error messages in:
 - cuffdiff

- differentialexpression-deseq2
- differentialexpression-edger
- Update workflow-bbduk-star-htseq
- Update quant seq descriptor schema
- Assert species and build in htseq-count-normalized process
- Set amplicon report template in resolvebio/latex Docker image to landscape mode

- Support Python 3.6
- Add template_amplicon_report.tex to resolwebio/latex Docker image assets
- Add SnpEff tool and bokeh assets to resolwebio/dnaseq Docker image
- Add automated library strand detection to feature_counts quantification process
- Add FastQC option nogroup to bbduk-single and bbduk-paired processes
- Add CPM normalization to htseq-count-raw process
- Add workflow-bbduk-star-htseq-paired
- Add legend to amplicon report template in resolwebio/latex Docker image

Fixed

- · Fix manual installation of packages in Docker images to handle dots and spaces in file names correctly
- Fix COSMIC url template in amplicon-table process
- Fix Create IGV session in Archive samples process
- Fix source tracking in cufflinks and cuffquant processes
- Fix amplicon master file validation script. Check and report error if duplicated amplicon names are included. Validation will now pass also for primer sequences in lowercase.
- Fix allele frequency (AF) calculation in snpeff process
- Fix bug in script for calculating FPKM. Because genes of raw counts from featureCounts were not lexicographically sorted, division of normalized counts was done with values from other, incorrect, genes. Results from featureCounts, but not HTSeq-count process, were affected.

1.5.28 8.1.0 - 2018-04-13

Changed

- Use the latest versions of the following Python packages in resolwebio/rnaseq docker image: Cutadapt 1.16, Apache Arrow 0.9.0, pysam 0.14.1, requests 2.18.4, appdirs 1.4.3, wrapt 1.10.11, PyYAML 3.12
- Bump tools version in resolwebio/rnaseq docker image:
 - Salmon to 0.9.1
 - FastQC to 0.11.7

 Generalize the no-extraction-needed use-case in resolwebio/base Docker image download_and_verify script

Added

- Add the following Python packages to resolwebio/rnaseq docker image: six 1.11.0, chardet 3.0.4, urllib3 1.22, idna 2.6, and certifi 2018.1.18
- Add edgeR R library to resolvebio/rnaseg docker image
- Add Bedtools to resolwebio/rnaseq docker image

Fixed

- Handle filenames with spaces in the following processes:
 - alignment-star-index
 - alignment-tophat2
 - cuffmerge
 - index-fasta-nucl
 - upload-fasta-nucl
- Fix COSMIC url template in (multisample) amplicon reports

1.5.29 8.0.0 - 2018-04-11

- BACKWARD INCOMPATIBLE: Refactor trimmomatic-single, trimmomatic-paired, bbduk-single, and bbduk-paired processes
- BACKWARD INCOMPATIBLE: Merge align-bwa-trim and align-bwa-trim2 process functionality. Retain only the refactored process under slug align-bwa-trim
- BACKWARD INCOMPATIBLE: In processes handling VCF files, the output VCF files are stored in bgzip-compressed form. Tabix index is not referenced to an original VCF file anymore, but stored in a separate tbi output field
- BACKWARD INCOMPATIBLE: Remove an obsolete workflow-accel-2 workflow
- BACKWARD INCOMPATIBLE: Use Elasticsearch version 5.x
- BACKWARD INCOMPATIBLE: Parallelize execution of the following processes:
 - alignment-bowtie2
 - alignment-bwa-mem
 - alignment-hisat2
 - alignment-star
 - alignment-tophat2
 - cuffdiff
 - cufflinks

- cuffquant
- Require Resolwe 8.x
- Bump STAR aligner version in resolwebio/rnaseq docker image to 2.5.4b
- Bump Primerclip version in resolwebio/dnaseq docker image
- Use resolwebio/dnaseq Docker image in picard-permetrics process
- Run vc-realign-recalibrate process using multiple cpu cores to optimize the processing time
- Use resolwebio/rnaseq Docker image in alignment-star process

Added

- Add CNVKit, LoFreq and GATK to resolvebio/dnaseq docker image
- · Add BaseSpace files download tool
- Add process to import a file from BaseSpace
- · Add process to convert files to single-end reads
- · Add process to convert files to paired-end reads
- Add vc-gatk4-hc process which implements GATK4 HaplotypeCaller variant calling tool
- Add workflow-accel-gatk4 pipeline that uses GATK4 HaplotypeCaller as an alternative to GATK3 used in workflow-accel pipeline
- Add amplicon-master-file descriptor schema
- Add workflow-bbduk-star-featurecounts pipeline
- Add rna-seq-bbduk-star-featurecounts RNA-seq descriptor schema

Fixed

- Fix iterative trimming in bowtie and bowtie2 processes
- Fix archive-samples to use sample names for headers when merging expressions
- Improve goea.py tool to handle duplicated mapping results
- Handle filenames with spaces in the following processes:
 - alignment-hisat2
 - alignment-bowtie
 - prepare-geo-chipseq
 - prepare-geo-rnaseq
 - cufflinks
 - cuffquant

1.5.30 7.0.1 - 2018-03-27

Fixed

 Use name-ordered BAM file for counting reads in HTSeq-count process by default to avoid buffer overflow with large BAM files

1.5.31 7.0.0 - 2018-03-13

Changed

- **BACKWARD INCOMPATIBLE:** Remove Ubuntu 17.04 base Docker image since it has has reached its end of life and change all images to use the new ubuntu 17.10 base image
- BACKWARD INCOMPATIBLE: Require species and build inputs in the following processes:
 - upload-genome
 - upload-gtf
 - upload-gff3
 - upload-bam
 - upload-bam-indexed
- BACKWARD INCOMPATIBLE: Track species and build information in the following processes:
 - cuffmerge
 - alignment processes
 - variant calling processes
 - JBrowse processes
- BACKWARD INCOMPATIBLE: Track species, build and feature_type in the following processes:
 - upload-expression-star
 - quantification processes
 - differential expression processes
- BACKWARD INCOMPATIBLE: Track species in gene set (Venn) and goenrichment processes
- BACKWARD INCOMPATIBLE: Rename genes_source input to source in hierarchical clustering and PCA processes
- BACKWARD INCOMPATIBLE: Remove the following obsolete processes:
 - Dictyostelium-specific ncRNA quantification
 - go-geneset
 - bayseq differential expression
 - cuffmerge-gtf-to-gff3
 - transdecoder
 - web-gtf-dictybase
 - upload-rmsk
 - snpdat

- BACKWARD INCOMPATIBLE: Unify output fields of processes of type data: annotation
- BACKWARD INCOMPATIBLE: Rename the organism field names to species in rna-seq and cutadapt-star-htseq descriptor schemas
- BACKWARD INCOMPATIBLE: Rename the genome_and_annotation field name to species in bcm-* descriptor schemas and use the full species name for the species field values
- BACKWARD INCOMPATIBLE: Refactor featureCounts process
- BACKWARD INCOMPATIBLE: Change import-sra process to work with resolwebio/utils Docker image and refactor its inputs
- Require Resolwe 7.x
- · Add environment export for Jenkins so that the manager will use a globally-unique channel name
- Set scheduling_class of gene and sample hierarchical clustering processes to interactive
- Change base Docker images of resolwebio/rnaseq and resolwebio/dnaseq to resolwebio/base:ubuntu-18.04
- Use the latest versions of the following Python packages in resolwebio/rnaseq Docker image: Cutadapt 1.15, Apache Arrow 0.8.0, pysam 0.13, and xopen 0.3.2
- Use the latest versions of the following Python packages in resolwebio/dnaseq Docker image: Bokeh 0.12.13, pandas 0.22.0, Matplotlib 2.1.2, six 1.11.0, PyYAML 3.12, Jinja2 2.10, NumPy 1.14.0, Tornado 4.5.3, and pytz 2017.3
- Use the latest version of wigToBigWig tool in resolwebio/chipseq Docker image
- Use resolwebio/rnaseq:3.0.0 Docker image in goenrichment, upload-gaf and upload-obo processes
- Use resolwebio/dnaseq:3.0.0 Docker image in filtering_chemut process
- Change cuffnorm process type to data: cuffnorm
- Set type of coverage-garvan process to data: exomecoverage
- Remove qsize input from macs14 process and automate genome size selection
- Adjust bam-split process so it can be included in workflows
- Make ID attribute labels in featureCounts more informative
- Change 'source' to 'gene ID database' in labes and descriptions
- Change archive-samples process to create different IGV session files for build and species
- Expose advanced parameters in Chemical Mutagenesis workflow
- Clarify some descriptions in the filtering_chemut process and chemut workflow
- Change expected genome build formatting for hybrid genomes in bam-split process
- Set the ${\tt cooksCutoff}$ parameter to ${\tt FALSE}$ in ${\tt deseq.R}$ tool
- Rename 'Expressions (BCM)' to 'Dicty expressions'

Added

- Mechanism to override the manager's control channel prefix from the environment
- Add Ubuntu 17.10 and Ubuntu 18.04 base Docker images
- Add resolwebio/utils Docker image

- Add BBMap, Trimmomatic, Subread, Salmon, and dexseq_prepare_annotation2 tools and DEXSeq and loadSubread R libraries to resolvebio/rnaseq Docker image
- Add abstract processes that ensure that all processes that inherit from them have the input and output fields that are defined in them:
 - abstract-alignment
 - abstract-annotation
 - abstract-expression
 - abstract-differentialexpression
 - abstract-bed
- · Add miRNA workflow
- Add prepare-geo-chipseq and prepare-geo-rnaseq processes that produce a tarball with necessary data and folder structure for GEO upload
- Add library-strandedness process which uses the Salmon tool built-in functionality to detect the library strandedness information
- Add species and genome build output fields to macs14 process
- Expose additional parameters in alignment-star, cutadapt-single and cutadapt-paired processes
- Add merge expressions to archive-samples process
- Add description of batch mode to Expression aggregator process
- Add error and warning messages to the cuffnorm process
- Add optional species input to hierarchical clustering and PCA processes
- Add Rattus norvegicus species choice to the rna-seq descriptor schema to allow running RNA-seq workflow for this species from the Recipes

Fixed

- Fix custom argument passing script for Trimmomatic in resolwebio/rnaseq Docker image
- Fix installation errors for dexseq-prepare-annotation2 in resolwebio/rnaseq Docker image
- Fix consensus_subreads input option in Subread process
- Limit variant-calling process in the chemical mutagenesis workflow and the Picard tools run inside to 16 GB of memory to prevent them from crashing because they try to use too much memory
- The chemical mutagenesis workflow was erroneously categorized as data:workflow:rnaseq:cuffquant type. This is switched to data:workflow:chemut type.
- Fix handling of NA values in Differential expression results table. NA values were incorrectly replaced with value 0 instead of 1
- Fix cuffnorm process to work with samples containing dashes in their name and dispense prefixing sample names starting with numbers with 'X' in the cuffnorm normalization outputs
- Fix cuffnorm process' outputs to correctly track species and build information
- Fix typos and sync parameter description common to featureCounts and miRNA workflow

1.5.32 6.2.2 - 2018-02-21

Fixed

• Fix cuffnorm process to correctly use sample names as labels in output files and expand cuffnorm tests

1.5.33 6.2.1 - 2018-01-28

Changed

- Update description text of cutadapt-star-htseq descriptor schema to better describe the difference between gene/transcript-type analyses
- Speed-up management command for inserting mappings

1.5.34 6.2.0 - 2018-01-17

Added

- Add R, tabix, and CheMut R library to resolvebio/dnaseq Docker image
- Add SRA Toolkit to resolvebio/rnaseq Docker image

Changed

- Require Resolwe 6.x
- Extend pathway map with species and source field
- Move template and logo for multi-sample report into resolvebio/latex Docker image
- Refactor amplicon-report process to contain all relevant inputs for amplicon-archive-multi-report
- Refactor amplicon-archive-multi-report
- Use resolwebio/dnaseq:1.2.0 Docker image in filtering_chemut process

Fixed

- Enable DEBUG setting in tests using Django's LiveServerTestCase
- Wait for ElasticSeach to index the data in KBBioProcessTestCase
- Remove unused parameters in TopHat (2.0.13) process and Chip-seq workflow

1.5.35 6.1.0 - 2017-12-12

Added

- Add amplicon-archive-multi-report process
- Add upload-metabolic-pathway process
- Add memory-optimized primerclip as a separate align-bwa-trim2 process

• Add workflow-accel-2 workflow

Changed

- Improve PCA process performance
- Use resolwebio/chipseq:1.1.0 Docker image in macs14 process
- Change formatting of EFF [*]. AA column in snpeff process
- Save unmapped reads in aligment-hisat2 process
- Turn off test profiling

Fixed

- Fix pre-sorting in upload-master-file process
- Revert align-bwa-trim process to use non-memory-optimized primerclip
- Fix file processing in cutadapt-custom-paired process

1.5.36 6.0.0 - 2017-11-28

Added

- Add AF filter to amplicon report
- Add number of samples to the output of expression aggregator
- Add ChIP-Rx, ChIPmentation and eClIP experiment types to reads descriptor schema
- Add pandas Python package to resolwebio/latex Docker image
- Add primerclip, samtools, picard-tools and bwa to resolwebio/dnaseq Docker image
- ullet Add cufflinks, RNASeqT R library, pyarrow and sklearn Python packages to resolwebio/rnaseq Docker image
- Add wigToBigWig tool to resolvebio/chipseq Docker image

Changed

- BACKWARD INCOMPATIBLE: Drop Python 2 support, require Python 3.4 or 3.5
- BACKWARD INCOMPATIBLE: Make species part of the feature primary key
- BACKWARD INCOMPATIBLE: Substitute Python 2 with Python 3 in resolwebio/rnaseq Docker image. The processes to be updated to this version of the Docker image should also have their Python scripts updated to Python 3.
- Require Resolwe 5.x
- Set maximum RAM requirement in bbduk process
- Move Assay type input parameter in RNA-Seq descriptor schema from advanced options to regular options
- Use resolwebio/rnaseq Docker image in Cutadapt processes
- Use additional adapter trimming option in cutadapt-custom-single/paired processes

- Show antibody information in reads descriptor for ChIP-Seq, ChIPmentation, ChIP-Rx, eClIP, MNase-Seq, MeDIP-Seq, RIP-Seq and ChIA-PET experiment types
- Use resolwebio/dnaseq Docker image in align-bwa-trim process
- Refactor resolwebio/chipseq Docker image
- Use Resolwe's Test Runner for running tests and add ability to only run a partial test suite based on what processes have Changed
- Configure Jenkins to only run a partial test suite when testing a pull request
- · Make tests use the live Resolwe API host instead of external server

Fixed

- Fix merging multiple expressions in DESeq process
- Fix resolwebio/rnaseq Docker image's README
- Handle multiple ALT values in amplicon report
- Fix BAM file input in rsem process

1.5.37 5.0.1 - 2017-11-14

Fixed

• Update Features and Mappings ElasticSearch indices building to be compatible with Resolwe 4.0

1.5.38 5.0.0 - 2017-10-25

Added

- Add automatic headers extractor to bam-split process
- Add HTML amplicon plot in coveragebed process
- Add raw RSEM tool output to rsem process output
- Add support for transcript-level differential expression in deseq2 process

Changed

- BACKWARD INCOMPATIBLE: Bump Django requirement to version 1.11.x
- BACKWARD INCOMPATIBLE: Make BioProcessTestCase non-transactional
- Require Resolwe 4.x
- Add the advanced options checkbox to the rna-seq descriptor schema
- Remove static amplicon plot from coveragebed and amplicon-report processes
- Update Dockerfile for resolwebio/latex with newer syntax and add some additional Python packages

1.5.39 4.2.0 - 2017-10-05

Added

- Add resolwebio/base Docker image based on Ubuntu 17.04
- Add resolwebio/dnaseq Docker image
- Add DESeq2 tool to resolvebio/rnaseq docker image
- Add input filename regex validator for upload-master-file process

Changed

- · Remove obsolete mongokey escape functionality
- Report novel splice-site junctions in HISAT2
- Use the latest stable versions of the following bioinformatics tools in resolwebio/rnaseq docker image: Cutadapt 1.14, FastQC 0.11.5, HTSeq 0.9.1, and SAMtools 1.5

1.5.40 4.1.0 - 2017-09-22

Added

- Add Mus musculus to all BCM workflows' schemas
- Add bam-split process with supporting processes upload-bam-primary, upload-bam-secondary and upload-header-sam

Changed

• Enable Chemut workflow and process tests

Fixed

• Fix chemut intervals input option

1.5.41 4.0.0 - 2017-09-14

Added

 New base and legacy Docker images for processes, which support non-root execution as implemented by Resolwe

Changed

- BACKWARD INCOMPATIBLE: Modify all processes to explicitly use the new Docker images
- BACKWARD INCOMPATIBLE: Remove clustering-hierarchical-genes-etc process
- Require Resolwe 3.x

1.5.42 3.2.0 2017-09-13

Added

- Add index-fasta-nucl and rsem process
- Add custom Cutadapt STAR RSEM workflow

1.5.43 3.1.0 2017-09-13

Added

- Add statistics of logarithmized expressions to expression-aggregator
- Add input field description to cutadapt-star-htseq descriptor schema
- Add HISAT2 and RSEM tool to resolwebio/rnaseq docker image

Changed

- Remove eXpress tool from resolwebio/rnaseq docker image
- Use system packages of RNA-seq tools in resolwebio/rnaseq docker image
- Set hisat2 process' memory resource requirement to 32GB
- Use resolwebio/rnaseq docker image in hisat2 process

1.5.44 3.0.0 2017-09-07

Added

- Add custom Cutadapt STAR HT-seq workflow
- · Add expression aggregator process
- Add resolwebio/rnaseq docker image
- Add resolwebio/latex docker image
- Add access to sample field of data objects in processes via sample filter

Changed

- BACKWARD INCOMPATIBLE: Remove threads input in STAR aligner process and replace it with the cores resources requirement
- BACKWARD INCOMPATIBLE: Allow upload of custom amplicon master files (make changes to amplicon-panel descriptor schema, upload-master-file and amplicon-report processes and workflow-accel workflow)
- BACKWARD INCOMPATIBLE: Remove threads input in cuffnorm process and replace it with the cores resources requirement
- Add sample descriptor to prepare_expression test function
- Prettify amplicon report

Fixed

- Fix upload-expression-star process to work with arbitrary file names
- Fix STAR aligner to work with arbitrary file names
- Fix cuffnorm group analysis to work correctly
- Do not crop Amplicon report title as this may result in malformed LaTeX command
- Escape LaTeX's special characters in make_report.py tool
- Fix validation error in Test sleep progress process

1.5.45 2.0.0 2017-08-25

Added

- Support bioinformatics process test case based on Resolwe's TransactionProcessTestCase
- Custom version of Resolwe's with_resolwe_host test decorator which skips the decorated tests on non-Linux systems
- · Add optimal leaf ordering and simulated annealing to gene and sample hierarchical clustering
- Add resolwebio/chipseq docker image and use it in ChIP-Seq processes
- Add Odocoileus virginianus texanus (deer) organism to sample descriptor
- Add test for import-sra process
- Add RNA-seq DSS test
- Add Cutadapt and custom Cutadapt processes

Changed

- Require Resolwe 2.0.x
- Update processes to support new input sanitization introduced in Resolwe 2.0.0
- Improve variant table name in amplicon report
- Prepend api/ to all URL patterns in the Django test project
- Set hisat2 process' memory resource requirement to 16GB and cores resource requirement to 1
- Filter LoFreq output VCF files to remove overlapping indels
- Add Non-canonical splice sites penalty, Disallow soft clipping and Report alignments tailored specifically for Cufflinks parameters to hisat2 process
- Remove threads input from cuffquant and rna-seq workfows
- Set core resource requirement in cuffquant process to 1

Fixed

- Correctly handle paired-end parameters in featureCount
- Fix NaN in explained variance in PCA. When PC1 alone explained more than 99% of variance, explained variance for PC2 was not returned

- Fix input sanitization error in dss-rna-seq process
- Fix gene source check in hierarchical clustering and PCA
- Enable network access for all import processes
- Fix RNA-seq DSS adapters bug
- Fix sample hierarchical clustering output for a single sample case

1.5.46 1.4.1 2017-07-20

Changed

· Optionally report all amplicons in Amplicon table

Fixed

• Remove remaining references to calling pip with --process-dependency-links argument

1.5.47 1.4.0 2017-07-04

Added

- · Amplicon workflow
- Amplicon descriptor schemas
- Amplicon report generator
- Add Rattus norvegicus organism choice to sample schema
- Transforming form Phred 64 to Phred 33 when uploading fastq reads
- Add primertrim process
- RNA-Seq experiment descriptor schema
- iCount sample and reads descriptor schemas
- iCount demultiplexing and sample annotation
- ICount OC
- · Add MM8, RN4 and RN6 options to rose2 process
- · Add RN4 and RN6 options to bamplot process
- Archive-samples process
- · Add bamliquidator
- · CheMut workflow
- · Dicty primary analysis descriptor schema
- IGV session to Archive-samples process
- Use Resolwe's field projection mixins for knowledge base endpoints
- amplicon-table process
- Add C. griseus organism choice to Sample descriptor schema

- Add S. tuberosum organism choice to Sample descriptor schema
- Add log2 to gene and sample hierarchical clustering
- Add new inputs to import SRA, add read type selection process
- Set memory resource requirement in jbrowse annotation gff3 and gtf processes to 16GB
- Set memory resource requirement in star alignment and index processes to 32GB
- Add C. elegans organism choice to Sample descriptor schema
- · Add D. melanogaster organism choice to Sample descriptor schema
- Set core resource requirement in Bowtie process to 1
- Set memory resource requirement in amplicon BWA trim process to 32GB
- Add new master file choices to amplicon panel descriptor schema
- Add S. tuberosum organism choice to RNA-seq workflow
- Add Cutadapt process
- · Add leaf ordering to gene and sample hierarchical clustering

Fixed

- Use new import paths in resolwe.flow
- Upload reads (paired/single) containing whitespace in the file name
- Fix reads filtering processes for cases where input read file names contain whitespace
- · Add additional filtering option to STAR aligner
- Fix bbduk-star-htseq count workflow
- Fix cuffnorm process: Use sample names as labels (boxplot, tables), remove group labels input, auto assign group labels, add outputs for Rscript output files which were only available compressed
- Derive output filenames in hisat2 from the first reads filename
- Correctly fetch KB features in goea.py
- Append JBrowse tracks to sample
- Replace the BAM MD tag in align-bwa-trim process to correct for an issue with the primerclip tool
- Fix typo in trimmomatic and bbduk processes
- Use re-import in etc and hmmer database processes

Changed

- Support Resolwe test framework
- Run tests in parallel with Tox
- Use Resolwe's new FLOW DOCKER COMMAND setting in test project
- Always run Tox's docs, linters and packaging environments with Python 3
- Add extra Tox testing environment with a check that there are no large test files in resolwe_bio/tests/ files

- Replace Travis CI with Genialis' Jenkins for running the tests
- Store compressed and uncompressed .fasta files in data:genome:fasta objects
- Change sample_geo descriptor schema to have strain option available for all organisms
- More readable rna-seq-quantseq schema, field stranded
- Remove obsolete Gene Info processes
- Change log2(fc) default from 2 to 1 in diffexp descriptor schema
- Change Efective genome size values to actual values in macs14 process
- Change variable names in bowtie processes
- · Remove iClip processes, tools, files and tests

1.5.48 1.3.0 2017-01-28

Changed

- Add option to save expression JSON to file before saving it to Storage
- Update upload-expression process
- No longer treat resolwe_bio/tools as a Python package
- Move processes' test files to the resolwe_bio/tests/files directory to generalize and simplify handling
 of tests' files
- Update differential expression (DE) processors
- Update generate_diffexpr_cuffdiff django-admin command
- Save gene_id source to output.source for DE, expression and related objects
- Refactor upload-diffexp processor
- Update sample descriptor schema
- Remove obsolete descriptor schemas
- Add stitch parameter to rose2 processor
- Add filtering to DESeq2
- Set Docker Compose's project name to resolwebio to avoid name clashes
- GO enrichment analysis: map features using gene Knowledge base
- Add option to upload .gff v2 files with upload-gtf processor
- · Replace Haystack with Resolwe Elastic Search API
- Require Resolwe 1.4.1+
- Update processes to be compatible with Resolwe 1.4.0

Added

- Process definition documentation style and text improvements
- Add resolwe_bio.kb app, Resolwe Bioinformatics Knowledge Base
- Add tests to ensure generators produce the same results

- Upload Gene sets (data:geneset)
- Add generate_geneset django-admin command
- Add generate_diffexpr_deseq django-admin command
- Add 'Generate GO gene sets' processor
- Add generic file upload processors
- Add upload processor for common image file types (.jpg/.tiff/.png/.gif)
- Add upload processor for tabular file formats (.tab/.tsv/.csv/.txt/.xls/.xlsx)
- Add Trimmomatic process
- · Add featureCounts process
- Add Subread process
- Add process for hierarchical clusteing of samples
- Add gff3 to gtf file converter
- · Add microarray data descriptor schema
- · Add process for differential expression edgeR
- BioCollectionFilter and BidDataFilter to support filtering collections and data by samples on API
- Added processes for automatically downloading single and paired end SRA files from NCBI and converting them to FASTQ
- Added process for automatically downloading SRA files from NCBI and converting them to FASTQ
- Add HEAT-Seq pipeline tools
- Add HEAT-Seq workflow
- Add create-geneset, create-geneset-venn processors
- Add source filter to feature search endpoint
- · Add bamplot process
- Add gene hiererhical clustering
- Add cuffquant workflow
- Support Django 1.10 and versionfield 0.5.0
- django-admin commands insert_features and insert_mappings for importing features and mappings to the Knowledge Base
- · Add bsmap and mcall to analyse WGBS data
- · Vaccinesurvey sample descriptor schema
- · Add RNA-Seq single and paired-end workflow

Fixed

- Set presample to False for Samples created on Sample endpoint
- Fix FastQC report paths in processors
- Fix htseq_count and featureCounts for large files
- Fix upload gtf annotation

- Fix gene_id field type for differential expression storage objects
- Order data objects in SampleViewSet
- Fix sample hiererhical clustering
- Fix name in gff to gtf process
- Fix clustering to read expressed genes as strings
- Fix protocol labels in rna-seq-quantseq descriptor schema

1.5.49 1.2.1 2016-07-27

Changed

• Update resolve requirement

1.5.50 1.2.0 2016-07-27

Changed

- Decorate all tests that currently fail on Docker with skipDockerFailure
- Require Resolwe's master git branch
- Put packaging tests in a separate Tox testing environment
- Rename DB user in test project
- Change PostgreSQL port in test project
- Add ROSE2 results parser
- Compute index for HISAT2 aligner on genome upload
- Updated Cuffquant/Cuffnorm tools
- Change ROSE2 enhancer rank plot labels
- Refactor processor syntax
- Move processes tests into processes subdirectory
- Split sample API endpoint to sample for annotated Samples and presample for unannotated Samples
- Rename test project's data and upload directories to .test_data and .test_upload
- Save fastq files to lists:basic:file field. Refactor related processors.
- Reference genome-index path when running aligners.
- Add pre-computed genome-index files when uploading reference fasta file.
- Include all necessary files for running the tests in source distribution
- Exclude tests from built/installed version of the package
- Move testing utilities from resolwe_bio.tests.processes.utils to resolwe_bio.utils. test
- Update Cuffdiff processor inputs and results table parsing
- Refactor processes to use the updated resolwe.flow.executors.run command

• Refactor STAR aligner - export expressions as separate objects

Fixed

- Make Tox configuration more robust to different developer environments
- Set required: false in processor input/output fields where necessary
- Add Sample's Data objects to Collection when Sample is added
- Fixed/renamed Cufflinks processor field names

Added

- skipDockerFailure test decorator
- Expand documentation on running tests
- Use Travis CI to run the tests
- Add Sample model and corresponding viewset and filter
- · Add docker-compose command for PostgreSQL
- API endpoint for adding Samples to Collections
- · HISAT2 aligner
- Use Git Large File Storage (LFS) for large test files
- Test for generate_samples django-admin command
- django-admin command: generate_diffexpr

1.5.51 1.1.0 2016-04-18

Changed

- · Remove obsolete utilities superseded by resolwe-runtime-utils
- Require Resolwe 1.1.0

Fixed

- Update sample descriptor schema
- Include all source files and supplementary package data in sdist

Added

- flow_collection: sample to processes
- · MACS14 processor
- Initial Tox configuration for running the tests
- Tox tests for ensuring high-quality Python packaging
- · ROSE2 processor

• django-admin command: generate_samples

1.5.52 1.0.0 2016-03-31

Changed

- Renamed assertFileExist to assertFileExists
- · Restructured processes folder hierarchy
- Removed re-require and hard-coded tools' paths

Fixed

- Different line endings are correctly handled when opening gzipped files
- Fail gracefully if the field does not exist in assertFileExists
- Enabled processor tests (GO, Expression, Variant Calling)
- Enabled processor test (Upload reads with old Illumina QC encoding)
- · Made Resolwe Bioinformatics work with Resolwe and Docker

Added

- · Import expressions from tranSMART
- Limma differential expression (tranSMART)
- VC filtering tool (Chemical mutagenesis)
- Additional analysis options to Abyss assembler
- API endpoint for Sample
- Initial documentation

1.6 Contributing

1.6.1 Installing prerequisites

Make sure you have Python 3.6 installed on your system. If you don't have it yet, follow these instructions.

Resolwe Bioinformatics requires PostgreSQL (9.4+). Many Linux distributions already include the required version of PostgreSQL (e.g. Fedora 22+, Debian 8+, Ubuntu 15.04+) and you can simply install it via distribution's package manager. Otherwise, follow these instructions.

The pip tool will install all Resolwe Bioinformatics' dependencies from PyPI. Installing some (indirect) dependencies from PyPI will require having a C compiler (e.g. GCC) as well as Python development files installed on the system.

Note: The preferred way to install the C compiler and Python development files is to use your distribution's packages, if they exist. For example, on a Fedora/RHEL-based system, that would mean installing gcc and python3-devel packages.

1.6. Contributing 373

Optional prerequisites

If you want to run or develop tests with large input or output files, then install the Git Large File Storage extension.

1.6.2 Preparing environment

Fork the main Resolwe Bioinformatics' git repository.

If you don't have Git installed on your system, follow these instructions.

Clone your fork (replace <username> with your GitHub account name) and change directory:

```
git clone https://github.com/<username>/resolwe-bio.git
cd resolwe-bio
```

Prepare Resolwe Bioinformatics for development:

```
pip install --pre -e .[docs,package,test]
```

Note: We recommend using pyvenv to create an isolated Python environment for Resolwe Bioinformatics.

1.6.3 Preparing database

Add a postgres user:

```
createuser -s -r postgres
```

1.6.4 Running tests

Manually

Change directory to the tests Django project:

```
cd tests
```

Run docker:

```
docker-compose up
```

Note: On Mac or Windows, Docker might complain about non-mounted volumes. You can edit volumes in *Docker* => *Preferences* => *File Sharing* The following volumes need to be shared:

- /private
- /tmp
- /var/folders

/private is shared by default. When you attempt to add /var/folders it might try to add /private/var/folders which will cause Docker complaining about overlapping volumes. Here's a workaround: Change /private to /var/folders and then add /private again.

To run the tests, use:

```
./manage.py test resolwe_bio --parallel 2
```

Note: If you don't specify the number of parallel test processes (i.e. you just use --parallel), Django will run one test process per each core available on the machine.

Warning: If you run Docker in a virtual machine (i.e. if you use MacOS or Windows) rather that directly on your machine, the virtual machine can become totally unresponsive if you set the number of parallel test processes too high. We recommend using at most --parallel 2 in such cases.

To run a specific test, use:

```
./manage.py test resolwe_bio.tests.<module-name>.<class-name>.<method-name>
```

For example, to run the test_macs14 test of the ChipSeqProcessorTestCase class in the test_chipseq module, use:

```
./manage.py test resolwe_bio.tests.processes.test_chipseq.ChipSeqProcessorTestCase. {\color{red} \hookrightarrow} test\_macs14
```

Using Tox

To run the tests with Tox, use:

tox

To re-create the virtual environment before running the tests, use:

```
tox -r
```

To only run the tests with a specific Python version, use:

```
tox -e py<python-version>
```

For example, to only run the tests with Python 3.5, use

```
tox -e py35
```

Note: To see the list of available Python versions, see tox.ini.

Note: To control the number of test processes Django will run in parallel, set the DJANGO_TEST_PROCESSES environment variable.

Since running tests for all processes may take a long time, there is an option to run partial tests based on what files have been changed between HEAD and a specific commit (e.g. master). The Tox environments that run partial tests have the -partial suffix, e.g.:

1.6. Contributing 375

```
tox -e py35-partial
```

To configure the commit against which the changes are compared you should set the RESOLWE_TEST_ONLY_CHANGES_TO environmental variable (it is set to master by default).

Running tests skipped on Docker

To run the tests that are skipped on Docker due to failures and errors, set the RESOLWEBIO_TESTS_SKIP_DOCKER_FAILURES environment variable to no.

For example, to run the skipped tests during a single test run, use:

```
RESOLWEBIO_TESTS_SKIP_DOCKER_FAILURES=no ./manage.py test resolwe_bio
```

To run the skipped tests for the whole terminal session, execute:

```
export RESOLWEBIO_TESTS_SKIP_DOCKER_FAILURES=no
```

and then run the tests as usual.

Running tests with large files

To run the tests with large input or output files, ensure you have the Git Large File Storage extension installed and run the tests as usual.

Adding tests with large files

If a test file is larger than 1 MiB, then put it in the resolwe_bio/tests/files/large/ directory. Git Large File Storage (LFS) extension will automatically pick it up and treat it appropriately.

To ensure contributors without Git LFS or users using the source distribution can smoothly run the tests, decorate the tests using large files with the following:

```
@skipUnlessLargeFiles(<large-file1>, <large-file2>, ...)
```

where <large-file1>, <large-file2>, ... represent the names of large files used inside a particular test.

The decorator will ensure the test is skipped unless these files are present and represent real large files (not just Git LFS pointers).

1.6.5 Building documentation

```
python setup.py build_sphinx
```

Note: To build the documentation, you must use Python 3 (Python 2 is not supported).

1.6.6 Preparing release

Follow Resolwe's documentation on preparing a release. Resolwe code is automatically released to PyPI when tagged, but this is not supported in Resolwe Bioinformatics yet. After you have completed the first part, follow the steps below to release the code on PyPI.

Clean build directory:

python setup.py clean -a

Remove previous distributions in dist directory:

rm dist/*

Remove previous egg-info directory:

rm -r *.egg-info

Create source distribution:

python setup.py sdist

Build wheel:

python setup.py bdist_wheel

Upload distribution to PyPI:

twine upload $\mathrm{dist}/*$

1.6. Contributing 377

378 Chapter 1. Contents

CHAPTER 2

Indices and tables

- genindex
- modindex
- search

Python Module Index

r

resolwe_bio.utils,333
resolwe_bio.utils.test,333

382 Python Module Index

A	BWA SW, 92
Abstract alignment process, 22	С
Abstract annotation process, 22	
Abstract bed process, 23	Chemical Mutagenesis, 125
Abstract differential expression process, 23	ChIP-Seq (Gene Score), 107
Abstract expression process, 24	ChIP-seq (MACS2), 115
Accel Amplicon Pipeline, 26	ChIP-seq (MACS2-ROSE2), 122
Align (BWA) and trim adapters, 29	ChIP-Seq (Peak Score), 108
Amplicon report, 30	Convert files to reads (paired-end), 126
Amplicon table, 31	Convert files to reads (single-end), 127
Annotate novel splice junctions (regtools), 33	Convert GFF3 to GTF, 126
Archive and make multi-sample report for amplicon data,	coverageBed, 318
33	Cuffdiff 2.2, 130
Archive samples, 34	Cufflinks 2.2, 132
ATAC-Seq, 21	Cuffmerge, 134
-	Cuffnorm, 137
В	Cuffquant 2.2, 138
BAM file, 35	Cuffquant results, 140
BAM file and index, 37	Custom master file, 141
Bam split, 93	Cutadapt (Diagenode CATS, paired-end), 142
Bamliquidator, 95	Cutadapt (Diagenode CATS, single-end), 142
Bamplot, 98	Cutadapt (paired-end), 146
BaseSpace file, 99	Cutadapt (single-end), 149
BBDuk (paired-end), 48	Cutadapt - STAR - HTSeq-count (paired-end), 156
BBDuk (single-end), 58	Cutadapt - STAR - HTSeq-count (single-end), 162
BBDuk - STAR - FeatureCounts (3' mRNA-Seq, pairedend), 60	Cutadapt - STAR - RSEM (Diagenode CATS, pairedend), 167
BBDuk - STAR - FeatureCounts (3' mRNA-Seq, single-	Cutadapt - STAR - RSEM (Diagenode CATS, single-end),
end), 62	172
BBDuk - STAR - featureCounts - QC (paired-end), 74	D
BBDuk - STAR - featureCounts - QC (single-end), 84	D
BBDuk - STAR - HTSeq-count (paired-end), 63	DESeq2, 174
BBDuk - STAR - HTSeq-count (single-end), 64	Detect library strandedness, 175
BED file, 85	Dictyostelium expressions, 177
BioProcessTestCase (class in resolwe_bio.utils.test), 333	Differential Expression (table), 180
Bowtie (Dicty), 102	
Bowtie (107), 102	E
BWA ALN, 88	edgeR, 319
BWA MEM, 90	Expression aggregator, 181
,	1 66 6 7

Expression data, 184 Expression data (Cuffnorm), 185	miRNA pipeline, 331 MultiQC, 245
Expression data (STAR), 188	
Expression matrix, 188	0
Expression Time Course, 180	OBO file, 245
Expression time course, 189	P
F	PCA, 246
FASTA file, 190	Picard CollectTargetedPcrMetrics, 247
FASTQ file (paired-end), 191	Pre-peakcall QC, 248
FASTQ file (single-end), 192	Prepare GEO - ChIP-Seq, 249
featureCounts, 328	Prepare GEO - RNA-Seq, 249
	prepare_adapters() (resolwe_bio.utils.test.BioProcessTestCase
G	method), 333
GAF file, 193	prepare_amplicon_master_file()
GATK3 (HaplotypeCaller), 194	(resolwe_bio.utils.test.BioProcessTestCase
GATK4 (HaplotypeCaller), 196	method), 333
Gene expression indices, 202	prepare_annotation() (resolwe_bio.utils.test.BioProcessTestCase
Gene set, 204	method), 333
Gene set (create from Venn diagram), 205	prepare_annotation_gff()
Gene set (create), 206	(resolwe_bio.utils.test.BioProcessTestCase
Genome, 209	method), 333
GFF3 file, 197	prepare_bam() (resolwe_bio.utils.test.BioProcessTestCase
GO Enrichment analysis, 199	method), 333
GTF file, 200	prepare_expression() (resolwe_bio.utils.test.BioProcessTestCase method), 333
Н	prepare_genome() (resolwe_bio.utils.test.BioProcessTestCase
Hierarchical clustering of genes, 219	method), 333
Hierarchical clustering of samples, 222	<pre>prepare_paired_reads() (resolwe_bio.utils.test.BioProcessTestCase</pre>
HISAT2, 211	method), 333
HMR, 211	prepare_reads() (resolwe_bio.utils.test.BioProcessTestCase
HTSeq-count (CPM), 214	method), 333
HTSeq-count (TPM), 217	
	Q
	Quantify shRNA species using bowtie2, 251
Indel Realignment and Base Recalibration, 223	
•	R
K	Reads (QSEQ multiplexed, paired), 257
KBBioProcessTestCase (class in resolwe_bio.utils.test),	Reads (QSEQ multiplexed, single), 258
333	resolwe_bio.utils (module), 333
1	resolwe_bio.utils.test (module), 333
L	RNA-Seq (Cuffquant), 251
LoFreq (call), 224	ROSE2, 253 RSEM, 255
M	
MACS 1.4, 226	S
MACS 2.0, 234	Salmon Index, 274
MACS2 - ROSE2, 242	SAM header, 258
Mappability, 242	Secondary hybrid BAM file, 275
Mappability info, 242	setUp() (resolwe_bio.utils.test.BioProcessTestCase
Merge Expressions (ETC), 243	method), 333
Metabolic pathway file, 244	setUp() (resolwe_bio.utils.test.KBBioProcessTestCase
methocunts 330	method), 334

384 Index

```
skipDockerFailure() (in module resolwe_bio.utils.test),
         334
skipUnlessLargeFiles()
                                               module
                                 (in
         resolwe_bio.utils.test), 334
snpEff, 331, 332
Spike-ins quality control, 276
SRA data, 260
SRA data (paired-end), 261
SRA data (single-end), 263
STAR, 270
STAR genome index, 272
Subread, 278
Subsample FASTQ (paired-end), 279
Subsample FASTQ (single-end), 281
Т
Test basic fields, 285
Test disabled inputs, 285
Test hidden inputs, 286
Test select controler, 287
Test sleep progress, 287
Trim, align and quantify using a library as a reference.,
Trimmomatic (paired-end), 294
Trimmomatic (single-end), 297
Trimmomatic - HISAT2 - HTSeq-count (paired-end), 300
Trimmomatic - HISAT2 - HTSeq-count (single-end), 301
U
Upload Picard CollectTargetedPcrMetrics, 302
V
Variant calling (CheMut), 306
Variant filtering (CheMut), 309
VCF file, 303
W
WALT, 310
WGBS, 311
Whole exome sequencing (WES) analysis, 317
Writing processes, 3
```

Index 385