resolwe-bio

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Bioinformatics pipelines for the Resolwe dataflow package for Django framework.

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 Salmon QC (single-end)
- BBDuk Salmon QC (paired-end)
- Cutadapt STAR StringTie (Corall, single-end)
- Cutadapt STAR StringTie (Corall, 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
- Slamdunk analysis (paired-end)
- Accel Amplicon Pipeline
- shRNA quantification
- Whole exome sequencing (WES) analysis
- Trimmomatic HISAT2 HTSeq-count (single-end)
- Trimmomatic HISAT2 HTSeq-count (paired-end)
- MACS2 ROSE2
- miRNA pipeline
- Cutadapt STAR FeatureCounts (3' mRNA-Seq, single-end)
- Cutadapt STAR FeatureCounts basic QC (3' mRNA-Seq, single-end)

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:rnaseq Prepare GEO RNA-Seq
- 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:atacseq-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), Cutadapt-STAR-FeatureCounts(3'mRNA-Seq, single-end), Cutadapt-STAR-FeatureCounts-basic QC(3'mRNA-Seq, single-end)
- data:workflow:rnaseq:corall Cutadapt STAR StringTie (Corall, paired-end), Cutadapt STAR StringTie (Corall, 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:rnaseq:salmon BBDuk Salmon QC (paired-end), BBDuk Salmon QC (single-end)

- data:workflow:slamdunk Slamdunk analysis (paired-end)
- data:workflow:trimalquant shRNA quantification
- 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 rep se, *basic:integer* minins, basic:integer maxins, basic:integer trim 5, basic:integer trim 3, basic:integer trim iter, basic:integer trim nucl. **rep mode**, *basic:integer* basic:string k reports, **q** threshold, basic:integer *basic:integer* n sub, basic:boolean tn5. basic:integer shift. batagalign, *basic:string* duplicates, basic:boolean sic:string duplicates prepeak, basic:decimal qvalue, basic:decimal pvalue, basic:decimal pvalue_prepeak, *basic:integer* **cap num**, *basic:integer* mfold lower. mfold_upper, basic:integer slocal. basic:integer 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, bacall summits, basic:boolean sic: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 pairedend 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 mates.

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 2500000

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-modellike 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

data:differentialexpressionabstract-differentialexpression ()[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

data:expressionabstract-expression ()[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:fa	stq:paired rea	ds , data:genome	e:fasta genome,
	data:masterfile:amplicon			mas-
	ter_file,	data:seq:nu	cleotide	adapters,
	list:data:vari	ants:vcf		known_indels,
	list:data:vari	ants:vcf know	v n_vars , data:v	ariants:vcf db-
	snp, basic:ir	teger mbq , i	basic:integer s	tand_call_conf,
	basic:integer	min_bq,	basic:integer	min_alt_bq,
	list:data:vari	ants:vcf	known_vai	:s_db , <i>ba-</i>
	sic:decimal s	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 CollectTar-getedPcrMetrics 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 Trimmomatic.

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

(<i>data:masterfile:amplicon</i> master_file,
data:genome:fasta genome, data:reads:fastq reads,
basic:integer seed_l, basic:integer band_w, ba-
sic:decimal re_seeding, basic:boolean m, ba-
sic:integer match, basic:integer missmatch,
basic:integer gap_o, basic:integer gap_e, ba-
sic:integer clipping, basic:integer unpaired_p,
basic:boolean report_all, basic:integer re-
port_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 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

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:amplicon=report (data:picard:coverage pcr_metrics, data:coverage coverage,
data:masterfile:amplicon master_file, list:data:snpeff an-
not_vars, basic: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 annot_vars,
basic:boolean all_amplicons, basic:string ta-
ble_name) [Source: v1.1.0]
```

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		
	<i>data:annotation:gtf</i> annotation , <i>data:alignment:bam:star</i> alignment star ,	
	data:alignment:bam alignment,	
	<i>data:bed</i> input_bed_junctions) [Source:	
	v0.3.0]	

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 aligment 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/).

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 'Alignment' 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,	ba-	
sic:boolean j) [Source: v0.3.0]						

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: v1.5.0]

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

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

- 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

BAM file and index

data:alignment:bam:uploadupload-bam-indexed (basic:file src, basic:file src2, basic:string species, basic:string build) [Source: v1.5.0]

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	
	sic:integer min_length, ba-
	sic:boolean show_advanced,
	list:data:seq:nucleotide sequences, list:basic:string lit-
	eral_sequences, basic:integer kmer_length,
	<i>basic:boolean</i> check_reverse_complements ,
	basic:boolean mask_middle_base,
	basic:integer min_kmer_hits, ba-
	sic:decimal min_kmer_fraction, ba-
	sic:decimal min_coverage_fraction, ba-
	sic:integer hamming_distance, ba-
	sic:integer query_hamming_distance, basic:integer edit_distance, ba-
	basic:integer edit_distance, ba- sic:integer hamming_distance2, ba-
	sic:integer query_hamming_distance2, ba-
	sic:integer edit_distance2, basic:boolean for-
	bid_N, basic:boolean remove_if_either_bad, ba-
	sic:boolean find_best_match, basic:boolean per-
	form_error_correction, basic:string k_trim, ba-
	sic:string k_mask, basic:boolean mask_fully_covered,
	basic:integer min_k, basic:string quality_trim, ba-
	sic:integer trim_quality, basic:integer trim_poly_A,
	basic:decimal min_length_fraction,
	basic:integer max_length, ba-
	sic:integer min_average_quality, ba-
	sic:integer min_average_quality_bases,
	basic:integer min_base_quality, ba-
	sic:integer min_consecutive_bases, ba-
	sic:integer trim_pad, basic:boolean trim_by_overlap,
	basic:boolean strict_overlap, ba-
	sic:integer min_overlap, basic:integer min_insert,
	basic:boolean trim_pairs_evenly, ba-
	sic:integer force_trim_left, ba-
	sic:integer force_trim_right, ba-
	sic:integer force_trim_right2, ba-
	sic:integer force_trim_mod, basic:integer re-
	strict_left, basic:integer restrict_right, ba-
	sic:decimal min_GC, basic:decimal max_GC,
	basic:integer maxns, basic:boolean toss_junk,
	basic:boolean chastity_filter, basic:boolean bar-
	code_filter, list:data:seq:nucleotide bar-
	code_files, <i>list:basic:string</i> barcode_sequences,
	basic:integer x_min , basic:integer y_min , ba-
	sic:integer x_max , basic:integer y_max , ba-
	sic:decimal entropy, basic:integer entropy_window,
	basic:integer entropy_k, basic:boolean en-
	tropy_mask, basic:integer min_base_frequency,
	basic:boolean nogroup) [Source: v2.3.0]

BBDuk combines the most common data-quality-related trimming, filtering, and masking operations into a single highperformance 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

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 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, ba-
	sic:integer min_length, basic:ba	oolean show_advanced,
	list:data:seq:nucleotide sequen	ces, list:basic:string lit-
	eral sequences, basic:integ	
	— • •	reverse_complements,
	basic:boolean	mask_middle_base,
	basic:integer min	_kmer_hits, ba-
	sic:decimal min_kn	ner_fraction, ba-
	sic:decimal min_cove	rage_fraction, ba-
	sic:integer hammi	ng_distance, ba-
	sic:integer que	ery_hamming_distance,
		t_distance, ba-
	sic:integer hammin	g_distance2, ba-
	sic:integer query_hamn	ning_distance2, ba-
	sic:integer edit_distance2, basic	c:boolean forbid_N, ba-
	sic:boolean find_best_match, l	basic:string k_trim , ba-
	sic:string k_mask, basic:booled	<i>in</i> mask_fully_covered,
	basic:integer min_k, basic:str	ring quality_trim, ba-
	sic:integer trim_quality, basic	c:integer trim_poly_A,
	basic:decimal	min_length_fraction,
	basic:integer m	ax_length, ba-
	sic:integer min_ave	rage_quality, ba-
	sic:integer min_	_average_quality_bases,
	basic:integer min_t	base_quality, ba-
	sic:integer min_conse	ecutive_bases, ba-
	sic:integer trim_pad, basic:	integer min_overlap ,
	basic:integer min_insert, basic:	
	0	_trim_right, ba-
	0 –	rim_right2 , ba-
	sic:integer force_trim_mod	e e e e e e e e e e e e e e e e e e e
	strict_left, basic:integer	restrict_right, ba-
	sic:decimal min_GC, basi	— /
	0	c:boolean toss_junk,
	•	, basic:boolean bar-
	code_filter, list:data:seq:m	
	code_files, list:basic:string	barcode_sequences,
	basic:integer x_min , basic.	0 0-
	sic:integer x_max , basic:i	· · ·
		eger entropy_window,
	<i>basic:integer</i> entropy_k ,	basic:boolean en-
	tropy_mask, basic:integer	min_base_frequency,
	basic:boolean nogroup) [Source	e: v2.3.0]

BBDuk combines the most common data-quality-related trimming, filtering, and masking operations into a single highperformance 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:paired reads, data:genomeindex:star star_index list:data:seq:nucleotide adapters, data:annotation an- notation, ba- sic:string stranded, ba- sic:integer n_reads, ba- sic:integer seed, ba- sic:integer seed, ba- sic:decimal frac- tion, ba- sic:boolean two_pass, data:genomeindex:star rrna_refe data:genomeindex:star globin_re 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.

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 orignal 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:single reads,

data:genomeindex:star star_index list:data:seq:nucleotide adapters, data: annotation annotation, basic:string stranded, basic:integer n_reads, basic:integer seed, hasic:decimal fraction. basic:boolean two_pass, data:genomeindex:star rrna_refer data:genomeindex:star globin_ref v1.1.0]

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

Reads are preprocessed by <u>BBDuk</u> 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 <u>STAR</u> aligner. For read-count quantification, the <u>FeatureCounts</u> 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 orignal 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:genomeindex:star star_index,	
	list:data:seq:nucleotide adapters,	
	data:annotation an-	
	notation,	ba-
	sic:string stranded) [Source:	
	v1.0.1]	
This DNA sea ningling is comprised of three stone proprocessing alignment and quer	tification	

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:j	fastq:single	reads,
	data:genomeindex:star star_index,		
	list:data:seq:nucleotide adapters,		
	data:annotation annotation, 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 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)

data:workflow:rnaseq:featurecounts:qcworkflow-bbduk-star-featurecounts-qc-paired	(data:reads:fastq:paired) list:data:seq:nucleotide ; ba-
	sic:boolean show_advar list:basic:string cus-
	tom_adapter_sequences
	sic:integer kmer_length ba-
	sic:integer min_k , ba-
	<i>sic:integer</i> ham- ming_distance,
	ba- sic:integer maxns ,
	ba- sic:integer trim_quality ba-
	sic:integer min_length , data:genomeindex:star g
	ba- sic:boolean show_advar
	ba- sic:boolean un-
	stranded, ba-
	sic:boolean non- can-
	non- i- cal,
	ba- sic:boolean chimeric,
	<i>ba-</i> <i>sic:integer</i> chim-
	Seg- ment-
	Min, ba-
	sic:boolean quant- mode,
	ba- sic:boolean sin- gleend,
	ba- sic:boolean gene_counts
	ba- sic:string out-
	Fil- ter- Type,
	ba- sic:integer out-
64 Chapter 1. (Fil Co gt ents
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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 === 0

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 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

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: Normal
- BySJout: 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

a lignment. a lignment. a lign SJ over hang Min

label -alignSJoverhangMin

type basic:integer

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

required False

a lignment. a lignment. a lignSJDB over hang Min

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.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 orignal 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)

data:workflow:rnaseq:featurecounts:qcworkflow-bbduk-star-featurecounts-qc-s	ingle (data:reads:fastq:single r list:data:seq:nucleotide a ba-
	sic:boolean show_advar
	list:basic:string cus-
	tom_adapter_sequences
	ba-
	sic:integer kmer_length
	ba-
	sic:integer min_k,
	ba-
	sic:integer ham-
	ming_distance,
	ba-
	sic:integer maxns,
	ba-
	sic:integer trim_quality
	ba-
	sic:integer min_length,
	data:genomeindex:star g
	ba-
	sic:boolean show_advar
	ba-
	sic:boolean un-
	stranded,
	ba-
	sic:boolean non-
	can-
	non-
	i-
	cal,
	ba-
	sic:boolean chimeric,
	ba-
	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-
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	Mul-
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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: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 === 0

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 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

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: Normal
- BySJout: 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

a lignment. a lignment. a lign SJ over hang Min

label -alignSJoverhangMin

type basic:integer

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

required False

a lignment. a lignment. a lignSJDB over hang Min

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.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 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 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 - Salmon - QC (paired-end)

data:workflow:rnaseq:salmonworkflow-bbduk-salmon-qc-paired	(data:reads:fastq:paired reads, data:index:salmon salmon_index, data:genomeindex:star genome, data:annotation annotation, data:genomeindex:star rrna_reference, data:genomeindex:star globin_reference, ba- sic:boolean show_advanced, list:data:seq:nucleotide adapters, list:basic:string cus-
	tom_adapter_sequences,
	basic:integer kmer_length,
	basic:integer min_k,
	basic:integer ham-
	ming_distance, ba-
	sic:integer maxns , ba-
	sic:integer trim_quality,
	basic:integer min_length,
	basic:boolean seq_bias,
	basic:boolean gc_bias,
	basic:boolean vali-
	date_mappings, ba-
	sic:decimal con-
	sensus_slack, ba-
	sic:decimal min_score_fraction,
	ba-
	sic:integer range_factorization_bins,
	ba-
	sic:integer min_assigned_frag,
	basic:integer n_reads ,
	basic:integer seed, ba-
	sic:decimal fraction , ba-
	sic:boolean two_pass) [Source:
	v1.0.1]

Alignment-free RNA-seq pipeline. Salmon tool and tximport package are used in quantification step to produce genelevel abundance estimates.

rRNA and globin-sequence contamination rate in the sample is determined using STAR aligner. Quality-trimmed reads are down-sampled (using Seqtk tool) and aligned to the genome, rRNA and globin reference sequences. The rRNA and globin-sequence alignment rates indicate the percentage of the reads in the sample that are of rRNA and globin origin, respectively. Alignment of down-sampled data to a whole genome reference sequence is used to produce an alignment file suitable for Samtools and QoRTs QC analysis.

Per-sample analysis results and QC data is summarized by the MultiQC tool.

Input arguments reads

label Select sample(s)

type data:reads:fastq:paired

salmon_index

label Salmon index

type data:index:salmon

genome

label Indexed reference genome

type data:genomeindex:star

description Genome index prepared by STAR aligner indexing tool.

annotation

label Annotation

type data:annotation

rrna_reference

label Indexed rRNA reference sequence

type data:genomeindex:star

description Reference sequence index prepared by STAR aligner indexing tool.

globin_reference

label Indexed Globin reference sequence

type data:genomeindex:star

description Reference sequence index prepared by STAR aligner indexing tool.

show_advanced

label Show advanced parameters

type basic:boolean

default False

preprocessing.adapters

label Adapters

type list:data:seq:nucleotide

required 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

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.

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 === 0

default 11

preprocessing.hamming_distance

label Maximum Hamming distance for k-mers

type basic:integer

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.

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.

default 10

preprocessing.min_length

label Minimum read length

type basic:integer

description Reads shorter than minimum read length after trimming are discarded.

default 20

quantification.seq_bias

label Perform sequence-specific bias correction

type basic:boolean

default True

quantification.gc_bias

label Perform fragment GC bias correction.

type basic:boolean

default True

quantification.validate_mappings

label Validate mappings using alignment-based verification.

type basic:boolean

default True

quantification.consensus_slack

label Consensus slack

type basic:decimal

description The amount of slack allowed in the quasi-mapping consensus mechanism. Normally, a transcript must cover all hits to be considered for mapping. If this is set to a fraction, X, greater than 0 (and in [0,1)), then a transcript can fail to cover up to (100 * X)% of the hits before it is discounted as a mapping candidate. The default value of this option is 0.2 if –validateMappings is given and 0 otherwise".

required False

hidden !quantification.validate_mappings

quantification.min_score_fraction

label Minimum alignment score fraction

type basic:decimal

- **description** The fraction of the optimal possible alignment score that a mapping must achieve in order to be considered valid should be in (0,1].
- hidden !quantification.validate_mappings

default 0.65

quantification.range_factorization_bins

label Range factorization bins

type basic:integer

description Factorizes the likelihood used in quantification by adopting a new notion of equivalence classes based on the conditional probabilities with which fragments are generated from different transcripts. This is a more fine-grained factorization than the normal rich equivalence classes. The default value (0) corresponds to the standard rich equivalence classes, and larger values imply a more fine-grained factorization. If range factorization is enabled, a common value to select for this parameter is 4.

default 4

quantification.min_assigned_frag

label Minimum number of assigned fragments

type basic:integer

description The minimum number of fragments that must be assigned to the transcriptome for quantification to proceed.

default 10

downsampling.n_reads

label Number of reads

type basic:integer

default 1000000

downsampling.seed

label Seed

type basic:integer

default 11

downsampling.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.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

BBDuk - Salmon - QC (single-end)

data:workflow:rnaseq:salmonworkflow-bbduk-salmon-qc-single	(data:reads:fastq:single reads, data:index:salmon salmon_index, data:genomeindex:star genome, data:annotation annotation, data:genomeindex:star rrna_reference, data:genomeindex:star globin_reference, ba- sic:boolean show_advanced, list:data:seq:nucleotide adapters, list:basic:string cus- tom_adapter_sequences, basic:integer kmer_length, basic:integer min_k, basic:integer ham- ming_distance, ba- sic:integer maxns, ba- sic:integer min_length, basic:integer min_length, basic:boolean seq_bias, basic:boolean seq_bias, basic:boolean vali- date_mappings, ba- sic:decimal con- sensus_slack, ba- sic:decimal min_score_fraction,
	sic:integer min_assigned_frag, basic:integer n_reads, basic:integer seed, ba- sic:decimal fraction, ba- sic:boolean two_pass) [Source: v1.0.1]

Alignment-free RNA-seq pipeline. Salmon tool and tximport package are used in quantification step to produce genelevel abundance estimates.

rRNA and globin-sequence contamination rate in the sample is determined using STAR aligner. Quality-trimmed reads are down-sampled (using Seqtk tool) and aligned to the genome, rRNA and globin reference sequences. The rRNA and globin-sequence alignment rates indicate the percentage of the reads in the sample that are of rRNA and globin origin, respectively. Alignment of down-sampled data to a whole genome reference sequence is used to produce an alignment file suitable for Samtools and QoRTs QC analysis.

Per-sample analysis results and QC data is summarized by the MultiQC tool.

Input arguments reads

label Select sample(s)

type data:reads:fastq:single

salmon_index

label Salmon index

type data:index:salmon

genome

label Indexed reference genome

type data:genomeindex:star

description Genome index prepared by STAR aligner indexing tool.

annotation

label Annotation

type data:annotation

rrna_reference

label Indexed rRNA reference sequence

type data:genomeindex:star

description Reference sequence index prepared by STAR aligner indexing tool.

globin_reference

label Indexed Globin reference sequence

type data:genomeindex:star

description Reference sequence index prepared by STAR aligner indexing tool.

show_advanced

label Show advanced parameters

type basic:boolean

default False

preprocessing.adapters

label Adapters

type list:data:seq:nucleotide

required 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

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.

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 === 0

default 11

preprocessing.hamming_distance

label Maximum Hamming distance for k-mers

type basic:integer

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.

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.

default 10

preprocessing.min_length

label Minimum read length

type basic:integer

description Reads shorter than minimum read length after trimming are discarded.

default 20

quantification.seq_bias

label Perform sequence-specific bias correction

type basic:boolean

default True

quantification.gc_bias

label Perform fragment GC bias correction.

type basic:boolean

default False

quantification.validate_mappings

label Validate mappings using alignment-based verification.

type basic:boolean

default True

quantification.consensus_slack

label Consensus slack

type basic:decimal

description The amount of slack allowed in the quasi-mapping consensus mechanism. Normally, a transcript must cover all hits to be considered for mapping. If this is set to a fraction, X, greater than 0 (and in [0,1)), then a transcript can fail to cover up to (100 * X)% of the hits before it is discounted as a mapping candidate. The default value of this option is 0.2 if –validateMappings is given and 0 otherwise".

required False

hidden !quantification.validate_mappings

quantification.min_score_fraction

label Minimum alignment score fraction

type basic:decimal

- **description** The fraction of the optimal possible alignment score that a mapping must achieve in order to be considered valid should be in (0,1].
- hidden !quantification.validate_mappings

default 0.65

quantification.range_factorization_bins

label Range factorization bins

type basic:integer

description Factorizes the likelihood used in quantification by adopting a new notion of equivalence classes based on the conditional probabilities with which fragments are generated from different transcripts. This is a more fine-grained factorization than the normal rich equivalence classes. The default value (0) corresponds to the standard rich equivalence classes, and larger values imply a more fine-grained factorization. If range factorization is enabled, a common value to select for this parameter is 4.

default 4

quantification.min_assigned_frag

label Minimum number of assigned fragments

type basic:integer

description The minimum number of fragments that must be assigned to the transcriptome for quantification to proceed.

default 10

downsampling.n_reads

label Number of reads

type basic:integer

default 1000000

downsampling.seed

label Seed

type basic:integer

default 11

downsampling.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.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

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, basic:integer	q , ba-
	sic:boolean use_edit, basic:integer ed	lit_value,
	basic:decimal fraction, basic:boolean	seeds,
	<i>basic:integer</i> seed_length ,	ba-
	sic:integer seed_dist) [Source: v1.5.0]	

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

basic:integer gap_e, basic:integer clipping, basic:integer unpaired_p, basic:boolean re- port_all, basic:integer report_tr) [Source: v2.3.0]	data:alignment:bam:bwamemalignment-bwa-mem	data:reads:fastq reads, basic:integer seed_l, ba- sic:integer band_w, basic:decimal re_seeding, basic:boolean m, basic:integer match, ba- sic:integer missmatch, basic:integer gap_o, basic:integer gap_e, basic:integer clipping, basic:integer unpaired_p, basic:boolean re- port_all, basic:integer report_tr) [Source:
---	--	--

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://bio-bwa.sourceforge.net/) for more information.

Input arguments 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 from 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, ba-		
	sic:integer gap_o, basic:integer gap_e) [Source:		
	v1.4.0]		

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: v0.5.0]
```

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 analysis_type		type,		
	list:data:aligr	1ment:bam	bam, basic:string	g cell_t	type,
	basic:integer	bin_size	, data:annotatic	on:gtf	re-
	gions_gtf, data:bed regions_		bed, ba-		
	sic:integer	extension,	basic:string	sense,	ba-
	sic:boolean	skip_plot,	list:basic:string	black	_list,
	basic:integer	threads) [Se	ource: 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 Analysis type

type basic:string

default bin

choices

- Bin mode: bin
- Region mode: region
- BED mode: bed

bam

label BAM File

type list:data:alignment:bam

cell_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
sic:string
sic:integer
basic:integer
list:basic:str
```

sic:string input_region, list:data:alignment:bam bam, basic:integer stretch_input, basic:string color, basic:string sense, basic:integer extension, basic:boolean rpm, basic:string yscale, list:basic:string names, basic:string plot, basic:string title, basic:string scale, list:data:bed bed, basic:boolean multi_page) [Source: v1.3.1]

data:annotation:gtf

input_gff,

ba-

genome,

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:
	v1.1.0]		

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, basic:string mode, basic:integer m, basic:integer l, basic:boolean use_se, basic:integer trim_5, basic:integer trim_3, basic:integer trim_nucl, basic:integer trim_iter, *basic:string* r) [Source: v1.5.0]

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 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

m

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

l

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

bigwig

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, ba-		
	sic:boolean discordantly, basic:boolean rep_se,		
	basic:integer minins, basic:integer max-		
	ins, basic:integer N, basic:integer L, ba-		
	sic:integer gbar, basic:string mp, ba-		
	sic:string rdg, basic:string rfg, ba-		
	sic:string score_min, basic:integer trim_5,		
	basic:integer trim_3, basic:integer trim_iter,		
	basic:integer trim_nucl, basic:string rep_mode,		
	basic:integer k_reports) [Source: v1.6.0]		

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 pairedend 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	(<i>data:chipseq:peakscore</i> peakscore , <i>basic:decimal</i> fdr , <i>basic:decimal</i> pval , <i>basic:decimal</i> 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	(<i>data:chipseq:callpeak:macs2</i> <i>data:bed</i> bed) [Source: v2.1.0]	peaks,
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:chipseg: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, basic:string duplicates, duplicates_prepeak, basic:decimal *basic:string* qvalue. basic:decimal pvalue, basic:decimal pvalue prepeak. basic:integer **cap_num**, *basic:integer* mfold lower, *basic:integer* **mfold_upper**, *basic:integer* slocal, basic:integer llocal, basic:integer extsize, basic:integer shift, **band width**, *basic:boolean* basic:integer 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: v1.0.3]

This process runs MACS2 in batch mode. MACS2 analysis is triggered for pairs of samples as defined using treatmentbackground 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 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-modellike 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, ba-		
	sic:boolean advanced, data:bed pro-		
	moter, basic:boolean tagalign, ba-		
	sic:integer q_threshold , basic:integer n_sub ,		
	basic:boolean tn5 , basic:integer shift , ba-		
	sic:string duplicates, basic:string dupli-		
	cates_prepeak, basic:decimal qvalue, ba-		
	sic:decimal pvalue, basic:decimal pvalue_prepeak,		
	basic:integer cap_num, basic:integer mfold_lower,		
	basic:integer mfold_upper, ba-		
	sic:integer slocal, basic:integer llocal, ba-		
	sic:integer extsize, basic:integer shift, ba-		
	sic:integer band_width, basic:boolean nolambda,		
	basic:boolean fix_bimodal, basic:boolean nomodel,		
	basic:boolean nomodel_prepeak, ba-		
	sic: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: v1.0.3]		

This process runs MACS2 in batch mode. MACS2 analysis is triggered for pairs of samples as defined using treatmentbackground 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 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 1500000

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-modellike 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

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, basic:boolean br and ind ra. basic:boolean **dbsnp**, *data:variants:vcf* known sites, known indels, list:data:variants:vcf basic:integer stand_emit_conf, bastand_call_conf, basic:boolean sic:integer rf, basic:boolean advanced, basic: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: v0.4.0]

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

d (*list:data:file* src1, *list:data:file* src2, *basic:boolean* merge_lanes) [Source: v1.3.0]

Convert FASTQ files to paired-end reads.

Input arguments src1

label Mate1

type list:data:file

src2

label Mate2

type list:data:file

merge_lanes

label Merge lanes

type basic:boolean

description Merge paired-end sample data split into multiple sequencing lanes into a single pair of FASTQ files.

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

Convert files to reads (single-end)

data:reads:fastq:singlefiles-to-fastq-single	, J	src,	ba-
	sic:boolean merge	_lanes) [Source: v1.3.0]	
Convert FASTQ files to single-end reads.			
Input arguments src			

label Reads

type list:data:file

description Sequencing reads in FASTQ format

merge_lanes

label Merge lanes

type basic:boolean

description Merge sample data split into multiple sequencing lanes into a single FASTQ file.

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

Cuffdiff 2.2

data:differentialexpression:cuffdiffcuffdiff	(list:data:cufflinks:cuffquant			case,
	list:data:cufflinks:cuffquant		с	ontrol,
	list:basic:string labels, data:annotatic		a:annotation	an-
	notation, data:genome:fasta		genome,	ba-
	sic:boolean	multi_read_correct,	basic:decimal	fdr,
	basic:string	library_type,	basic:string	li-
	brary_normalization, basic:string		string	disper-
	sion_method) [Source: v2.3.0]		

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 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 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:cufflinkscufflinks
(data:alignment:bam alignment, data:annotation annotation,
data:genome:fasta genome, data:annotation:gtf mask_file, ba-
sic:string library_type, basic:string annotation_usage, ba-
sic:boolean multi_read_correct) [Source: v2.2.0]
```

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 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,	basic	integer:	threads) [S	Source:
	v1.4.0]					

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

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

build

label Build

type basic:string

Cuffnorm

data:cuffnormcuffnorm (list:data:cufflinks:cuffquant cuffquant, data:annotation annotation, basic:boolean useERCC) [Source: v2.2.0]

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

type basic:file

genes_fpkm

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	an-
	notation,	data:genome:fasta		genome,
	data:annotation:gtf	mask_file, basic:	string library_t	ype, ba-
	sic:boolean multi_r	read_correct) [Source	e: v1.4.0]	

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 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 cxb

label Abundances (.cxb)

type basic:file

source

label Gene ID database

type basic:string

species

label Species

type basic:string

build

label Build

type basic:string

Cuffquant results

```
data:cufflinks:cuffquantupload-cxb (basic:file src, basic:string source, basic:string species, ba-
sic: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, basic: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)$
```

IAI | (• / 2)

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:

v1.2.0]

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:

v1.2.0]

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 reads,
	data:seq:nucleotide mate1_5prime_file,
	data:seq:nucleotide mate1_3prime_file,
	data:seq:nucleotide mate2_5prime_file,
	<i>data:seq:nucleotide</i> mate2_3prime_file ,
	<i>list:basic:string</i> mate1_5prime_seq,
	<i>list:basic:string</i> mate1_3prime_seq ,
	<i>list:basic:string</i> mate2_5prime_seq,
	list:basic:string mate2_3prime_seq, ba-
	sic:integer times, basic:decimal er-
	ror_rate, basic:integer min_overlap,
	basic:boolean match_read_wildcards,
	basic:integer nextseq_trim, ba-
	sic:integer leading, basic:integer trailing,
	basic:integer crop, basic:integer headcrop,
	basic:integer minlen, basic:integer max_n,
	<i>basic:string</i> pair_filter) [Source: v2.3.0]
Critedant finds and more adapted assessments of	interest and the second other times of meriod

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 Select sample(s)

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.nextseq_trim

label NextSeq-specific quality trimming

type basic:integer

description NextSeq-specific quality trimming (each read). Trims also dark cycles appearing as highquality G bases. This option is mutually exclusive with the use of regular (-g) quality trimming.

required 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 reads,
	data:seq:nucleotide up_primers_file,
	data:seq:nucleotide down_primers_file,
	<i>list:basic:string</i> up_primers_seq ,
	<i>list:basic:string</i> down_primers_seq ,
	basic:integer polya_tail, ba-
	sic:integer min_overlap, ba-
	sic:integer nextseq_trim, basic:integer lead-
	ing, basic:integer trailing, ba-
	sic:integer crop, basic:integer headcrop,
	basic:integer minlen, basic:integer max_n,
	basic:boolean match_read_wildcards,
	basic:integer times, basic:decimal er-
	ror_rate) [Source: v2.1.0]

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 Select sample(s)

type data:reads:fastq:single

adapters.up_primers_file

label 5 prime adapter file

type data:seq:nucleotide

required False

adapters.down_primers_file

label 3 prime adapter file

type data:seq:nucleotide

required False

adapters.up_primers_seq

label 5 prime adapter sequence

type list:basic:string

required False

adapters.down_primers_seq

label 3 prime adapter sequence

type list:basic:string

required False

adapters.polya_tail

label Poly-A tail

type basic:integer

description Length of poly-A tail, example - AAAN -> 3, AAAAAN -> 5

required False

adapters.min_overlap

label Minimal overlap

type basic:integer

description Minimum overlap for an adapter match

default 3

modify_reads.nextseq_trim

label NextSeq-specific quality trimming

type basic:integer

description NextSeq-specific quality trimming (each read). Trims also dark cycles appearing as highquality G bases. This option is mutually exclusive with the use of regular (-g) quality trimming.

required 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. This option is mutually exclusive with the use of NextSeq-specific quality trimming.

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. This option is mutually exclusive with the use of NextSeq-specific quality trimming.

required False

modify_reads.crop

label Crop

type basic:integer

description Cut the read to a specified length by removing bases from the end

required False

modify_reads.headcrop

label Headcrop

type basic:integer

description Cut the specified number of bases from the start of the read

required False

filtering.minlen

label Min length

type basic:integer

description Drop the read if it is below a specified length

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.match_read_wildcards

label Match read wildcards

type basic:boolean

description Interpret IUPAC wildcards in reads.

required False

default False

filtering.times

label Times

type basic:integer

description Remove up to COUNT adapters from each read.

default 1

filtering.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

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

data:workflow:quant:featurecounts:singleworkflow-cutadapt-star-fc-quant-single	(data:reads:fastq:single reads, data:genomeindex:star star_i
	data:annotation an-
	nota-
	tion,
	data:genomeindex:star rrna_ 1
	data:genomeindex:star globin
	ba-
	sic:boolean show_advanced,
	ba-
	sic:integer qual-
	ity_cutoff,
	ba-
	sic:integer n_reads ,
	ba-
	sic:integer seed,
	ba-
	sic:decimal frac-
	tion,
	ba-
	sic:boolean two_pass) [Sourc
	v1.0.1]

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

Reads are preprocessed by __Cutadapt__ 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. QoRTs QC and Samtools idxstats tools are used to report alignment QC metrics.

Additional QC steps operate on downsampled reads and include an 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 Select sample(s)

type data:reads:fastq:single

star_index

label Genome

type data:genomeindex:star

description Genome index prepared by STAR aligner indexing tool.

annotation

label Annotation

type data:annotation

description Genome annotation file (GTF).

rrna_reference

label Indexed rRNA reference sequence

type data:genomeindex:star

description Reference sequence index prepared by STAR aligner indexing tool.

globin_reference

label Indexed Globin reference sequence

type data:genomeindex:star

description Reference sequence index prepared by STAR aligner indexing tool.

show_advanced

label Show advanced parameters

type basic:boolean

default False

cutadapt.quality_cutoff

label Reads quality cutoff

type basic:integer

description Trim low-quality bases from 3' end of each read before adapter removal. The use of this option will override the use of NextSeq/NovaSeq-specific trim option.

required False

downsampling.n_reads

label Number of reads

type basic:integer

default 1000000

downsampling.seed

label Seed

type basic:integer

default 11

downsampling.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.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

Cutadapt - STAR - FeatureCounts - basic QC (3' mRNA-Seq, single-end)

data:workflow:quant:featurecounts:singleworkflow-cutadapt-star-fc-quant-wo-depletion-single	(data:reads.
	data:genon
	data:annot
	no-
	ta-
	tion,
	ba-
	sic:boolear
	ba-
	sic:integer
	ity_cutoff)
	v1.0.0]
This 3' mRNA-Seq pipeline is comprised of QC, preprocessing, alignment and quantification steps.	

Reads are preprocessed by __Cutadapt__ 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. QoRTs QC and Samtools idxstats tools are used to report alignment QC metrics.

Input arguments reads

label Select sample(s)

type data:reads:fastq:single

star index

label Genome

type data:genomeindex:star

description Genome index prepared by STAR aligner indexing tool.

annotation

label Annotation

type data:annotation

description Genome annotation file (GTF).

show advanced

label Show advanced parameters

type basic:boolean

default False

cutadapt.quality_cutoff

label Reads quality cutoff

type basic:integer

description Trim low-quality bases from 3' end of each read before adapter removal. The use of this option will override the use of NextSeq/NovaSeq-specific trim option.

required False

Output results

sic:string id_attribute,

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

data:workflow:rnaseq:htseqworkflow-custom-cutadapt-star-htseq-paired	(data:reads:fasta:paired reads.
	data:genomeindex:star genome,
	data:annotation:gtf gff,
	ba-
	sic:string stranded,
	ba-
	sic:boolean ad-
	vanced, ba-
	sic:boolean non-
	cannon-
	ical, ba-
	sic:boolean chimeric,
	ba-
	sic:integer chim-
	Segment- Min, ba-
	,
	sic:boolean quant- mode, ba-
	mode , ba- sic:boolean sin-
	gleend, ba-
	sic:boolean gene_counts,
	ba-
	sic:string out-
	Filter-
	Type , <i>ba</i> -
	sic:integer out-
	FilterMul-
	timapN-
	max, ba-
	sic:integer out-
	FilterMis-
	matchN-
	max, ba-
	sic:decimal out-
	FilterMis-
	match-
	NoverL-
	max, ba-
	sic:integer align-
	SJoverhang- Min, ba-
	sic:integer align-
	SJDBover-
	hangMin, ba-
	sic:integer align-
	Intron-
	Min, ba-
	sic:integer align-
	Intron-
	Max, ba-
	sic:integer align-
	MatesGap-
	Max, ba-
	<i>sic:string</i> mode,
1.2. Process catalog	<i>ba-</i> 163
	sic:string fea-
	ture_class, ba-

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: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

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 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: 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

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

sic:string id_attribute,

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

data:workflow:rnaseq:htseqworkflow-custom-cutadapt-star-htseq-single	
	data:genomeindex:star genome,
	data:annotation:gtf gff,
	ba-
	sic:string stranded,
	ba-
	sic:boolean ad-
	vanced, ba-
	sic:boolean non-
	cannon-
	ical, ba-
	sic:boolean chimeric,
	ba- sic-integer shim
	sic:integer chim- Segment-
	Min, ba-
	sic:boolean quant-
	mode, ba-
	sic:boolean sin-
	gleend, ba-
	sic:boolean gene_counts,
	ba-
	sic:string out-
	Filter-
	Type , <i>ba</i> -
	sic:integer out-
	FilterMul-
	timapN-
	max, ba-
	sic:integer out-
	FilterMis-
	matchN-
	max, ba-
	sic:decimal out-
	FilterMis-
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	max, ba-
	sic:integer align-
	SJoverhang-
	Min, ba-
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	SJDBover-
	hangMin, ba-
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	Intron- Min ba
	Min, ba-
	sic:integer align- Intron-
	Max, ba-
	sic:integer align-
	MatesGap-
	Max, ba-
	-sic:string mode,
1.2. Process catalog	<i>ba-</i> 169
-	sic:string fea-
	ture_class, ba-
	sicestring id attribute

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

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 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: 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

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_index,
	data:index:expression ex-
	pres-
	sion_index, ba-
	sic:string stranded,
	ba-
	sic:boolean ad-
	vanced, ba-
	sic:boolean non-
	cannonical, ba-
	sic:boolean chimeric,
	ba-
	sic:integer chim-
	Segment-
	Min, ba-
	sic:boolean quant-
	mode, ba-
	sic:boolean sin-
	gleend, ba-
	sic:boolean gene_counts,
	ba-
	sic:string out-
	FilterType, ba-
	sic:integer out-
	FilterMul-
	timapN-
	max, ba-
	sic:integer out-
	FilterMis-
	matchN-
	max, ba-
	sic:decimal out-
	FilterMis-
	matchNoverL-
	max, ba-
	sic:integer align-
	SJoverhang-
	Min, ba-
	sic:integer align- SJDBover-
	hangMin, ba-
	sic:integer align-
	IntronMin, ba-
	sic:integer align-
	IntronMax, ba-
	sic:integer align-
	MatesGap-
	Max) [Source:
	v1.0.2]
	1.0.4

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: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_index,
	data:index:expression ex-
	pres-
	sion_index, ba-
	sic:string stranded,
	ba-
	sic:boolean ad-
	vanced, ba-
	sic:boolean non-
	cannonical, ba-
	sic:boolean chimeric,
	ba-
	sic:integer chim-
	Segment-
	Min, ba-
	sic:boolean quant-
	mode, ba-
	sic:boolean sin-
	gleend, ba-
	sic:boolean gene_counts,
	ba-
	sic:string out-
	FilterType, ba-
	sic:integer out-
	FilterMul-
	timapN-
	max, ba-
	sic:integer out-
	FilterMis-
	matchN-
	max, ba-
	sic:decimal out-
	FilterMis- matchNoverL-
	max , ba- sic:integer align-
	SJoverhang-
	Min, ba-
	sic:integer align-
	SJDBover-
	hangMin, ba-
	sic:integer align-
	IntronMin, ba-
	sic:integer align-
	IntronMax, ba-
	sic:integer align-
	MatesGap-
	Max) [Source:
	v1.0.2]
	· · · · ·

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 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 - StringTie (Corall, paired-end)

data:workflow:rnaseq:corallworkflow-corall-paired	(data:reads:fastq:paired	reads,
	data:genomeindex:star	star_index,
	data:annotation	annotation,
	data:genomeindex:star	rrna_reference,
	data:genomeindex:star	globin_reference,
	basic:boolean	show_advanced,
	basic:integer qua	ality_cutoff, ba-
	sic:integer n_reads , l	basic:integer seed ,
	basic:decimal	fraction, ba-
	sic:boolean two_pass)	[Source: v1.0.1]

RNA-seq pipeline optimized for the Lexogen Corall Total RNA-Seq Library Prep Kit.

UMI-sequences are extracted from the raw reads before the reads are trimmed and quality filtered using Cutadapt. Preprocessed reads are aligned by the STAR aligner and de-duplicated using UMI-tools. Gene abundance estimates are reported by the StringTie tool.

QC operates on downsampled reads and includes alignment of input reads to the rRNA/globin reference sequences. The reported alignment rate is used to asses the rRNA/globin sequence depletion rate.

The analysis results and QC reports are summarized by the MultiQC.

Input arguments reads

label Select sample(s)
type data:reads:fastq:paired

star_index

label Genome

type data:genomeindex:star

description Genome index prepared by STAR aligner indexing tool.

annotation

label Annotation

type data:annotation

description Genome annotation file (GTF).

rrna_reference

label Indexed rRNA reference sequence

type data:genomeindex:star

description Reference sequence index prepared by STAR aligner indexing tool.

globin_reference

label Indexed Globin reference sequence

type data:genomeindex:star

description Reference sequence index prepared by STAR aligner indexing tool.

show_advanced

label Show advanced parameters

type basic:boolean

default False

cutadapt.quality_cutoff

label Reads quality cutoff

type basic:integer

description Trim low-quality bases from 3' end of each read before adapter removal. Use this option when processing the data generated by older Illumina machines. The use of this option will override the NextSeq/NovaSeq-specific trimming procedure which is enabled by default and is recommended for Illumina machines that utilize 2-color chemistry to encode the four bases.

required False

downsampling.n_reads

label Number of reads

type basic:integer

default 1000000

downsampling.seed

label Seed

type basic:integer

default 11

downsampling.fraction

label Fraction

type basic:decimal

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

required False

downsampling.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

Cutadapt - STAR - StringTie (Corall, single-end)

data:workflow:rnaseq:corallworkflow-corall-single	(data:reads:fastq:single data:genomeindex:starreads, star_index, annotation,	
	data:genomeindex:star rrna_reference, data:genomeindex:star globin_reference,	
	basic:booleanshow_advanced,basic:integerquality_cutoff,basic:integern_reads,basic:integerseed,	
	<i>basic:decimal</i> fraction , <i>basic:boolean</i> two_pass) [Source: v1.0.1]	

RNA-seq pipeline optimized for the Lexogen Corall Total RNA-Seq Library Prep Kit.

UMI-sequences are extracted from the raw reads before the reads are trimmed and quality filtered using Cutadapt. Preprocessed reads are aligned by the STAR aligner and de-duplicated using UMI-tools. Gene abundance estimates are reported by the StringTie tool.

QC operates on downsampled reads and includes alignment of input reads to the rRNA/globin reference sequences. The reported alignment rate is used to asses the rRNA/globin sequence depletion rate.

The analysis results and QC reports are summarized by the MultiQC.

Input arguments reads

label Select sample(s)

type data:reads:fastq:single

star_index

label Genome

type data:genomeindex:star

description Genome index prepared by STAR aligner indexing tool.

annotation

label Annotation

type data:annotation

description Genome annotation file (GTF).

rrna_reference

label Indexed rRNA reference sequence

type data:genomeindex:star

description Reference sequence index prepared by STAR aligner indexing tool.

globin_reference

label Indexed Globin reference sequence

type data:genomeindex:star

description Reference sequence index prepared by STAR aligner indexing tool.

show_advanced

label Show advanced parameters

type basic:boolean

default False

cutadapt.quality_cutoff

label Reads quality cutoff

type basic:integer

description Trim low-quality bases from 3' end of each read before adapter removal. Use this option when processing the data generated by older Illumina machines. The use of this option will override the NextSeq/NovaSeq-specific trimming procedure which is enabled by default and is recommended for Illumina machines that utilize 2-color chemistry to encode the four bases.

required False

downsampling.n_reads

label Number of reads

type basic:integer

default 1000000

downsampling.seed

label Seed

type basic:integer

default 11

downsampling.fraction

label Fraction

type basic:decimal

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

required False

downsampling.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

DESeq2

$data: differential expression: deseq 2 {\tt differential expression-deseq 2}$	(list:data:expression case,
	list:data:expression con-
	trol, ba-
	sic:boolean beta_prior,
	basic:boolean count, ba-
	<pre>sic:integer min_count_sum,</pre>
	basic:boolean cook, ba-
	sic:decimal cooks_cutoff,
	basic:boolean indepen-
	dent, basic:decimal al-
	pha) [Source: v2.5.0]

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)

options.beta_prior

label Beta prior

type basic:boolean

description Whether or not to put a zero-mean normal prior on the non-intercept coefficients.

default False

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, basic:integer read_number, data:index:salmon index) [Source: v0.2.0]

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, ba-
	sic:string	logfc, basic:string fdr, ba-
	sic:string	logodds, basic:string fwer, ba-
	sic:string	pvalue, basic:string stat, ba-
	sic:string	source , <i>basic:string</i> species ,
	basic:string	build, basic:string fea-
	ture_type,	list:data:expression case,
	list:data:exp	pression control) [Source: v1.3.0]

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 files.

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,
	basic:string	group_by,
	data:aggregator:expressior	a expr_aggregator) [Source:
	v0.3.0]	

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, ba-
sic:string exp_type, basic:string source, basic:string species,
basic:string build, basic:string feature_type) [Source: v2.3.0]
```

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|gz) (|\.gz|\.bz2|\.tgz|\.tar\.gz|\.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

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

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 (Cuffnorm)

```
data:expressionupload-expression-cuffnorm (basic:file exp, data:cufflinks:cuffquant cxb, ba-
```

sic:string exp_type) [Source: v1.5.0]

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, basic:string source, basic:string species, basic:string build, basic:string feature_type) [Source: v1.4.0]

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
- 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 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

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: v1.2.0]

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: v1.2.0]

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: v2.1.0] 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

label Sequence file (FASTA)

type basic:file

description Sequence file (containing single or multiple sequences) in FASTA format. Supported extensions: .fasta.gz (preferred), .fa.*, .fna.* or .fasta.*

validate_regex \. (fasta|fa|fna) (|\.gz|\.bz2|\.tgz|\.tar\.gz|\.tar\.bz2|\. zip|\.rar|\.7z)\$

species

label Species

type basic:string

description Species latin name.

required False

choices

- Homo sapiens: Homo sapiens
- Mus musculus: Mus musculus
- Rattus norvegicus: Rattus norvegicus
- 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, ba-
                                                  sic:boolean merge_lanes) [Source: v2.3.0]
```

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|(.rar|(.7z))|((.bz2))
```

merge_lanes

label Merge lanes

type basic:boolean

description Merge paired-end sample data split into multiple sequencing lanes into a single pair of FASTQ files.

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

FASTQ file (single-end)

```
data:reads:fastq:singleupload-fastq-single (list:basic:file src, ba-sic:boolean merge_lanes) [Source: v2.3.0]
```

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)$
```

merge_lanes

label Merge lanes

type basic:boolean

description Merge sample data split into multiple sequencing lanes into a single FASTQ file.

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

GAF file

data:gaf:2:0upload-gaf (basic:file src, basic:string source, basic:string species) [Source: v1.2.0]

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)

data:variants:vcf:gatk:hcvc-gatk-hc (data:alignment:bam alignment, data:genome:fasta genome, data:masterfile:amplicon intervals, data:bed intervals_bed, data:variants:vcf dbsnp, basic:integer stand_call_conf, basic:integer stand_emit_conf, basic: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 intervals_bed, data:variants:vcf dbsnp, basic: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, basic:string build) [Source: v3.3.0]

Import a General Feature Format (GFF) file which is a file format used for describing genes and other features 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

- 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 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, basic:string source, basic:string species, basic:decimal pval_threshold, basic: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, basic:string build) [Source: v3.3.0]

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 \. (gtf|gff) (|\.gz|\.bz2|\.tgz|\.tar\.gz|\.tar\.bz2|\.
zip|\.rar|\.7z)$
```

source

label Gene ID database

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 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

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	(<i>list:basic:string</i> genes,	basic:string s	ource,	ba-
	sic:string species, basic:file	venn) [Source: v1	.1.2]	
Create a gene set from a Venn diagram.				
Input arguments genes				
label Genes				
<pre>type list:basic:string</pre>				
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 genes, basic:string 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, basic:file bwa_index, basic:file hisat2_index, basic:file subread_index, basic:file walt_index) [Source: v3.4.0]

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 softclip, basic:integer noncansplice,
basic:boolean cufflinks) [Source: v1.7.0]
```

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	(<i>data:alignment:bam</i> alignments,
	data:annotation:gtf gtf, basic:string mode, ba-
	sic:string stranded, basic:string feature_class,
	basic:string id_attribute, basic:string feature_type,
	basic:boolean name_ordered) [Source: v1.6.0]

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.

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 pa-per](https://academic.oup.com/bioinformatics/article/31/2/166/2366196) for more information.

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 (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

HTSeq-count (TPM)

data:expression:htseq:normalizedhtseq-count	(<i>data:alignment:bam</i> alignments,
	data:annotation:gtf gff, basic:string mode, ba-
	sic:string stranded, basic:string feature_class,
	basic:string id_attribute, basic:string feature_type,
	basic:boolean name_ordered) [Source: v1.5.0]

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,
	basic:boolean advanced,
	<i>list:basic:string</i> genes,
	basic:string source,
	basic:string species,
	basic:boolean log2, ba-
	sic:boolean z_score , ba-
	sic:string distance_metric,
	basic:string link-
	age_method, ba-
	sic:boolean order) [Source:
	v3.1.0]

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

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 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,
	basic:boolean ad-
	vanced,
	list:basic:string genes,
	basic:string source,
	basic:string species,
	basic:boolean log2,
	ba-
	sic:boolean z_score ,
	basic:string dis-
	tance_metric, ba-
	sic:string link-
	age_method, ba-
	sic:boolean or-
	der) [Source: v3.1.0]
Hierarchical clustering of samples	

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 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

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, *ba-sic: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 treatment, data:alignment:bam control, ba-sic:string pvalue*) [Source: v3.2.1]

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 con- trol, data:bed promoter, basic:boolean tagalign, basic:integer q_threshold, basic:integer n_sub, basic:boolean tn5, basic:integer shift, ba-
	sic:string duplicates, basic:string dupli- cates_prepeak, basic:decimal qvalue, ba- sic:decimal pvalue, basic:decimal pvalue_prepeak, basic:integer cap_num, basic:integer mfold_lower, basic:integer mfold_upper, ba- sic:integer slocal, basic:integer llocal, ba-
	sic:integer extsize, basic:integer shift, ba- sic:integer band_width, basic:boolean nolambda, basic:boolean fix_bimodal, basic:boolean nomodel, basic:boolean nomodel_prepeak, ba- sic:boolean down_sample, basic:boolean bed-
	graph, basic:boolean spmr, ba- sic:boolean call_summits, basic:boolean broad, basic:decimal broad_cutoff) [Source: v4.0.5]

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 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-modellike 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

narrow_peaks

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 thi 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 con-	
	trol, data:bed promoter, ba-	
	sic:boolean tagalign, ba-	
	sic:integer q_threshold , ba-	
	sic:integer n_sub, basic:boolean tn5, ba-	
	sic:integer shift, basic:string duplicates,	,
	basic:string duplicates_prepeak, ba-	
	sic:decimal qvalue, basic:decimal pvalue,	,
	basic:decimal pvalue_prepeak,	,
	basic:integer cap_num, ba-	
	sic:integer mfold_lower , ba-	
	sic:integer mfold_upper , ba-	
	sic:integer slocal, basic:integer llocal,	,
	basic:integer extsize, basic:integer shift,	,
	basic:integer band_width, ba-	
	sic:boolean nolambda, ba-	
	sic:boolean fix_bimodal, ba-	
	sic:boolean nomodel, ba-	
	sic:boolean nomodel_prepeak,	,
	basic:boolean down_sample,	
	basic:boolean bedgraph,	,
	basic:boolean spmr, ba-	
	sic:boolean call_summits,	,
	basic:boolean broad, ba-	
	sic:decimal broad_cutoff, ba-	
	sic: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 1500000

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-modellike 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

type basic:file

Mappability info

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

Upload mappability information.

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, basic: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:integer dirs_depth, basic:boolean fullnames, basic:boolean config, basic:string cl_config) [Source: v1.4.0]

Aggregate results from bioinformatics analyses across many samples into a single report. [MultiQC](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.dirs_depth

label -dirs-depth

type basic:integer

description Prepend a specified number of directories to sample names. Enter a negative number to take from start of path.

default -1

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: v1.2.0]

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, basic:string species) [Source: v2.2.0]

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 1500000

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

(list:data:reads:fastq list:data:chipseq:callpeak sic:string name) [Source: v2.0.2]	macs,	reads , ba-
	list:data:chipseq:callpeak	list:data:chipseq:callpeak macs,

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, basic: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

data:expression:shrna2quantshrna-quant (data:alignment:bam alignment, basic:integer readlengths, *basic:integer* alignscores) [Source: v1.1.0]

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:i:' 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. Default 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:genome:fasta	genome,
	data:annotation	annota-
	tion) [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

Output results

ROSE2

data:chipseq:rose2rose2(data:chipseq:callpeakinput,data:bedinput_upload,data:alignment:bamrankby,data:alignment:bamcontrol,basic:integertss,basic:integerstitch,data:bedmask)[Source: v4.3.1]

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 = 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 strandedness) [Source: v1.2.0]

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, basic:file reads2, basic:file barcodes, basic:file annotation) [Source: v1.2.0]

      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|\.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|\.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 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)

```
data:multiplexed:qseq:singleupload-multiplexed-single (basic:file reads, basic:file barcodes, basic:file annotation) [Source: v1.2.0]
```

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

data:sraimport-sra (basic:string sra_accession, basic:boolean show_advanced, basic:integer min_spot_id, basic:integer max_spot_id, basic:integer min_read_len, basic:boolean clip, basic:boolean aligned, basic:boolean unaligned) [Source: v0.2.0]

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

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

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, basic:integer max	_spot_id,
	basic:integer m	in_read_len, basic:boolean	clip, ba-
	sic:boolean align	ned, basic:boolean unaligned) [Source:
	v0.2.0]		

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 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, basic:boolean show_advanced, basic:integer min_spot_id, basic:integer max_spot_id, basic:integer min_read_len, basic:boolean clip, basic:boolean aligned, basic:boolean unaligned) [Source: v0.2.0]

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 chimSegmentMin, basic:boolean quantmode, basic:boolean singleend, basic:boolean gene_counts, basic:string outFilterType, basic:integer outFilter-MultimapNmax, basic:integer outFilterMismatchNmax, basic:decimal outFilterMismatchNoverLmax, baoutFilterScoreMin, basic:decimal sic:integer out-FilterMismatchNoverReadLmax. basic:integer align-SJoverhangMin, basic:integer alignSJDBoverhangMin, *basic:integer* alignIntronMin, basic:integer align-**IntronMax**, *basic:integer* alignMatesGapMax, basic:string alignEndsType, basic:boolean two pass mode, basic:string outSAMunmapped, basic:string out-SAMattributes, basic:string outSAMattrRGline, basic:string tool bigwig, basic:integer bin size bigwig, basic:boolean star_sort) [Source: v1.10.0]

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

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: Normal
- BySJout: 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

- Standard: Standard
- All: All
- NH HI NM MD: NH HI NM MD
- None: None

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

star_sort

label Sorting with STAR

type basic:boolean

description Set to false for sorting with samtools or to true for sorting with STAR which may be time and memory intensive.

default False

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:fa	asta gen	ome,
	data:seq:nucle	otide genome2, data: annotation	an-
	notation,	basic:string exon_n	ame,
	basic:integer	sjdbOverhang,	ba-
	sic:integer	genomeSAindexNbases,	ba-
	sic:integer	genomeChrBinNbits,	ba-
	sic:integer ger	omeSAsparseD) [Source: v1.6.0)]

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 gencode, basic:boolean keep_duplicates, basic:boolean perfect_hash, basic:string source, basic:string species, basic:string build, basic:integer kmerlen) [Source: v1.1.0]

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 transcript.

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 duplicate transcripts. If this flag is passed, then duplicate transcripts that appear in the input will be retained and quantified separately.

default False

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,	ba-
	sic:string	g buil	d) [Source: v(.6.0]	

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 Slamdunk analysis (paired-end)

(data:reads:fastq:paired reads,
data:seq:nucleotide ref_seq, data:bed re-
gions, basic:boolean show_advanced,
basic:string source, ba-
sic:boolean filter_multimappers,
basic:integer max_alignments, ba-
sic:integer read_length) [Source: v1.1.0]

Slamdunk-based pipeline for the analysis of the SLAM-Seq data.

Thiol-linked alkylation for the metabolic sequencing of RNA enables the detection of RNA transcription, processing and decay dynamics in the context of total RNA.

Input arguments reads

label Reads

type data:reads:fastq:paired

description Paired-end sequencing reads in FASTQ format.

ref_seq

label Reference sequence (FASTA)

type data:seq:nucleotide

regions

```
label Regions of interest (BED)
type data:bed
```

show_advanced

label Show advanced parameters

type basic:boolean

default False

options.source

label Gene ID database source

type basic:string

default ENSEMBL

choices

- ENSEMBL: ENSEMBL
- UCSC: UCSC

options.filter_multimappers

label Filter multimappers

type basic:boolean

description If true, filter and reasign multimappers based on the provided BED file with regions of interest.

default True

options.max_alignments

label Maximum number of multimapper alignments

type basic:integer

description The maximum number of alignments that will be reported for a multi-mapping read (i.e. reads with multiple alignments of equal best scores).

default 1

options.read_length

label Maximum read length

type basic:integer

description Maximum length of reads in the input FASTQ file.

default 150

Output results

Spike-ins quality control

data:spikeinsspikein-qc (list:data:expression samples, basic:string mix) [Source: v1.1.0]

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

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

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

required False

report

label HTML report with results

type basic:file:html

required False

hidden True

report_zip

label ZIP file contining HTML report with results

type basic:file

required False

Subread

data:alignment:bam:subreadalignment-subread (data:genome:fasta genome, data:reads:fastq reads, basic:integer indel, basic:integer consensus, bamis_matched_bp, sic:integer basic:integer cpu number, bamulti_mapping, sic:boolean basic:string reads_orientation, basic:integer consensus_subreads) [Source: v2.2.0]

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 required 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,	ba-
	sic:integer	n_reads,	basic:integer	seed,
	basic:decimal		fraction,	ba-
	sic:boolean two_pass) [Source: v1.1.0]			

[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,	ba-
	sic:integer 1	n_reads,	basic:integer	seed,
	basic:decimal		fraction,	ba-
	sic:boolean two_pass) [Source: v1.1.0]			

[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 orignal 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

fastqc_archive

label Download FastQC archive

type list:basic:file

Test basic fields

data:test:fieldstest-basic-fields (basic:boolean boolean, basic:date date, basic:datetime datetime. basic:decimal decimal. *basic:integer* integer, basic:string string, babasic:text text, sic:url:download url download, basic:url:view url view. basic:string string2, basic:string string3, basic:string string4, basic:string string5, basic:string string6, basic:string string7, *basic: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.string4

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
                                                                                               ba-
                                    sic:string width_label, basic: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
```

 $default \ {\tt FD}$

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	(<i>basic:boolean</i> broad , <i>basic:integer</i> broad_width , <i>basic:integer</i> parameter1 , <i>basic:integer</i> parameter2 , <i>basic:integer</i> 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	
<pre>type basic:integer</pre>	
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_widt	h2
label Width of peaks2	
type basic:integer	
default 5	
Output results output	

label Result

type basic:string

Test select controler

data:test:resulttest-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

type basic:string

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

Output results output

label Result

type basic:string

Trimmomatic (paired-end)

data:reads:fastq:paired:trimmomatictrimmomatic-paired	(data:reads:fastq:paired reads,
	data:seq:nucleotide adapters, ba-
	sic:integer seed_mismatches, ba-
	sic:integer simple_clip_threshold, ba-
	sic:integer palindrome_clip_threshold,
	basic:integer min_adapter_length,
	basic:boolean keep_both_reads,
	basic:integer window_size, ba-
	sic:integer required_quality,
	<i>basic:integer</i> target_length ,
	basic:decimal strictness, ba-
	sic:integer leading, basic:integer trail-
	ing, basic:integer crop, ba-
	sic:integer headcrop, ba-
	sic:integer minlen, basic:integer aver-
	age_quality) [Source: v2.2.0]

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.palindrome_clip_threshold && !illuminaclip.min_adapter_length

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. 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 1	reads,
data:seq:nucleotide adapters,	ba-
<i>sic:integer</i> seed_mismatches ,	ba-
sic:integer simple_clip_three	shold,
<i>basic:integer</i> window_size,	ba-
sic:integer required_qu	ıality,
basic:integer target_le	ength,
basic:decimal strictness,	ba-
sic:integer leading, basic:integer	trail-
ing, basic:integer crop,	ba-
sic:integer headcrop,	ba-
sic:integer minlen , basic:integer	aver-
age_quality) [Source: v2.2.0]	

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)

data:workflow:rnaseq:htseqworkflow-rnaseq-paired	(data:reads:fastq:pairedreads,data:genome:fastagenome,data:annotation:gtfannotation,
	data:seq:nucleotide adapters, ba- sic:integer seed_mismatches, ba- sic:integer palindrome_clip_threshold, basic:integer simple_clip_threshold, ba- sic:integer minlen, basic:integer trail- ing, basic:string stranded, ba- sic:string id_attribute) [Source: 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.

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

description Specifies the maximum mismatch count which will still allow a full match to be performed.

default 2

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.

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, ba-
	sic:integer minlen, basic:integer trail-
	ing, basic:string stranded, ba-
	sic:string id_attribute) [Source: 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.

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

description Specifies the maximum mismatch count which will still allow a full match to be performed.

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.

default gene_id

Output results

Upload Picard CollectTargetedPcrMetrics

data:picard:coverage:uploadupload-picard-pcrmetrics	(basic:file	target_pcr_metrics, ba-
	sic:file	target_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		src, build) [Source	<i>basic:string</i> e: v2.1.1]	species,	ba-
Upload variants in VCF format.					
Input arguments src					
label Variants (VCF)					
type basic:file					
description Variants in VCF format.					
required True					
<pre>validate_regex \.(vcf)(\.gz \.k rar \.7z)\$</pre>	oz2 ∖.tgz	: \.tar\.g	z \.tar\.bz2 `	\.zip \.	
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, list:data:alignment:bam parental_strains, *list:data:alignment:bam* mutant strains. badbsic:boolean br_and_ind_ra, basic:boolean 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, basic:integer stand call conf, basic:integer ploidy, basic:string glm, list:basic:string intervals, *basic:boolean* **rf**) [Source: v1.2.2]

"CheMut varint calling using multiple BAM input files. Note: Usage of Genome Analysis Toolkit requires a licence."

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 x

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 \mathbf{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 mu-
tant_strain, basic:integer read_depth) [Source:
v1.4.0]
```

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 variants 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 genome, data:reads:fastq reads, basic:boolean rm_dup, basic:integer mismatch, basic:integer number) [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

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

```
data:workflow:wgbsworkflow-wgbs (data:reads:fastq reads, data:genome:fasta genome, ba-
sic:boolean rm_dup, basic:integer mismatch, ba-
sic:integer number, basic:boolean cpgs, basic:boolean sym-
metric_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, reads are aligned by __WALT_ aligner. [WALT (Wildcard ALignment Tool)](https://github.com/smithlabcode/walt) is fast and accurate read mapping for bisulfite sequencing. Then, methylation level at each genomic cytosine is calculated using __methcounts__. Finally, hypo-methylated regions are identified 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 symmetric (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	(<i>data:reads:fastq:paired</i> reads, <i>data:genome:fasta</i> genome,
	<i>list:data:variants:vcf</i> known_sites , <i>data:bed</i> inter-
	vals, data:variants:vcf hc_dbsnp, basic:string vali-
	dation_stringency, data:seq:nucleotide adapters, ba-
	sic:integer seed_mismatches, basic:integer sim-
	ple_clip_threshold, basic:integer min_adapter_length, ba-
	sic:integer palindrome_clip_threshold, basic:integer lead-
	ing, basic:integer trailing, basic:integer minlen, ba-
	sic:integer seed_l, basic:integer band_w, basic:boolean m,
	basic:decimal re_seeding, basic:integer match, basic:integer mis-
	match, basic:integer gap_o, basic:integer gap_e, ba-
	sic:integer clipping, basic:integer unpaired_p, basic:integer re-
	port_tr, data:bedpe bedpe, basic:boolean skip, ba-
	sic:boolean md_skip, basic:boolean md_remove_duplicates,
	<i>basic:string</i> md_assume_sort_order , <i>basic:string</i> read_group ,
	basic:integer stand_call_conf, basic:integer mbq) [Source: v2.1.0]

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

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 parameter 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.bamclipper.bedpe

label BEDPE file used for clipping using Bamclipper

type data:bedpe

description BEDPE file used for clipping using Bamclipper tool.

required False

advanced.bamclipper.skip

label Skip Bamclipper step

type basic:boolean

description Use this option to skip Bamclipper step.

default False

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* master_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-edger (list:data:expression case,
list:data:expression control,
basic:integer filter) [Source:
v1.2.0]
```

Empirical Analysis of Digital Gene Expression Data in R (edgeR). Differential expression analysis of RNAseq 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

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	(<i>data:alignment:bam</i> aligned_reads, <i>basic:string</i> as- say_type, <i>data:index:salmon</i> cdna_index, <i>ba-</i> <i>sic:integer</i> n_reads, <i>data:annotation</i> annotation,
	basic:string feature_class, basic:string feature_type,
	basic:string id_attribute, basic:string nor-
	malization_type, data:mappability:bcm map-
	pability, basic:boolean show_advanced, ba-
	sic:boolean count_features, basic:boolean al-
	low_multi_overlap, basic:integer min_overlap,
	basic:decimal frac_overlap, ba-
	sic:decimal frac_overlap_feature, ba-
	sic:boolean largest_overlap, ba-
	sic:integer read_extension_5, ba-
	sic:integer read_extension_3, ba-
	sic:integer read_to_pos, ba-
	sic:boolean count_multi_mapping_reads, ba-
	sic:boolean fraction, basic:integer min_mqs,
	basic:boolean split_only, ba-
	sic:boolean non_split_only, basic:boolean pri-
	mary, basic:boolean ignore_dup, ba-
	sic:boolean junc_counts, data:genome genome,
	basic:boolean is_paired_end, ba-
	sic:boolean require_both_ends_mapped,
	basic:boolean check_frag_length, ba-
	sic:integer min_frag_length, ba-
	sic:integer max_frag_length, ba-
	sic:boolean do_not_count_chimeric_fragments,
	basic:boolean do_not_sort, ba-
	sic:boolean by_read_group, ba-
	sic:boolean count_long_reads, basic:boolean re-
	port_reads, basic:integer max_mop, ba-
	sic:boolean verbose) [Source: v2.6.0]

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 500000

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.frac_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 && !advanced.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 pairedend 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 alignment, ba-
sic:boolean cpgs, basic:boolean symmetric_cpgs) [Source: v1.0.1]
```

The methounts 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 symmetric (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

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

shRNA quantification

data:workflow:trimalquantworkflow-trim-align-quant	(<i>data:reads:fastq:single</i> reads,		
	<i>list:basic:string</i> up_primers_seq ,		
	<i>list:basic:string</i> down_primers_seq ,		
	<i>basic:decimal</i> error_rate_5end ,		
	<i>basic:decimal</i> error_rate_3end ,		
	data:genome:fasta genome, ba-		
	sic:string mode , basic:integer N ,		
	basic:integer L, basic:integer gbar,		
	basic:string mp, basic:string rdg, ba-		
	sic:string rfg , basic:string score_min ,		
	basic:integer readlengths, ba-		
	sic:integer alignscores) [Source:		
	v0.0.3]		

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 ($\langle int1 \rangle$) and extend ($\langle int2 \rangle$) penalties. A reference gap of length N gets a penalty of $\langle int1 \rangle + N * \langle int2 \rangle$. 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

snpEff

data:snpeff:uploadupload-snpeff	annotation, _genes) [Source:	<i>basic:file</i> v1.1.1]	summary,	ba-
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				
<pre>type basic:file:html</pre>				
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

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 Resolwe'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 discoideum', 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.

- prepare_genome () Prepare genome FASTA.
- **prepare_paired_reads** (*matel=['fw reads.fastq.gz']*, *mate2=['rw reads.fastq.gz']*) Prepare NGS reads FASTQ.
- prepare_reads (*fn=['reads.fastq.gz']*) Prepare NGS reads FASTQ.

setUp()

Initialize test files path.

class resolwe_bio.utils.test.KBBioProcessTestCase (methodName='runTest')
 Class for bioinformatics process tests that use knowledge base.

It is based on *BioProcessTestCase* and Django's LiveServerTestCase. 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 lenght 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

- Use pigz for output file compression in bbduk-single and bbduk-paired processes
- Use resolwebio/rnaseq:4.9.0 Docker image in processes bbduk-single, bbduk-paired, trimmomatic-single, alignment-bowtie, trimmomatic-paired, alignment-bowtie2, alignment-hisat2, alignment-subread, cuffmerge, pca. differentialexpression-edger, cufflinks, cuffnorm, cuffdiff. cuffquant, expression-aggregator, htseq-count, htseq-count-raw, index-fasta-nucl, rsem, upload-bam, upload-bam-indexed, upload-bam-secondary, upload-expression, upload-expression-cuffnorm, upload-expression-star, upload-genome, upload-gaf, upload-obo, upload-fasta-nucl, regtools-junctions-annotate, cutadapt-custom-single, cutadapt-custom-paired, bam-split, aff-to-atf. spikein-qc, differentialexpression-shrna, feature_counts, salmon-index, salmon-quant, library-strandedness, qorts-qc, alignment-star, alignment-star-index, cutadapt-3prime-single, cutadapt-single, cutadapt-paired, differentialexpression-deseq2, stringtie, cutadapt-corall-single, cutadapt-corall-paired, umi-tools-dedup, shrna-quant.
- resolwebio/common:1.3.1 • Use Docker image in processes amplicon-table, mergeexpressions, upload-bedpe, upload-bam-scseq-indexed, upload-diffexp, upload-etc, upload-sc-10x, upload-multiplexed-single, upload-multiplexed-paired, archive-samples, samtools-idxstats, seqtk-sample-paired, basespace-file-import, seqtk-sample-single, clustering-hierarchical-samples, clustering-hierarchical-genes, import-sra, import-sra-single, import-sra-paired.

Fixed

• Fix Mapping search for source_id/target_id

1.5.2 25.1.0 - 2020-01-14

Added

Changed

- Extend the MultiQC report so that the Sample summary table is created for the compatible Data objects
- Bump CPU and memory requirements for the alignment-bowtie2 process
- Move upload test files of differential expression to its own folder

Fixed

- Fix typo in scheduling_class variable in several Python processes
- Handle cases of improper tags passed to read_group argument of the bqsr process
- When processing differential expression files, a validation is performed for numeric columns

1.5.3 25.0.0 - 2019-12-17

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
- Support Slamdunk/Alleyoop processes in MultiQC
- Enable sorting of files in alignment-star process using Samtools

• 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

1.5.4 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 (resolwebio/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

1.5.5 23.1.1 - 2019-10-11

Changed

• Renamed workflow-trim-align-quant workflow to make the name more informative

1.5.6 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

- Test files improved for workflow-wes, bamclipper, markduplicates and bqsr
- Fix typo in differential expression shrna process docstring

Fixed

- 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

1.5.7 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

1.5.8 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 differential expression-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.

1.5.9 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/ rnaseq: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

1.5.10 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 resolwebio/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 qorts-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

Added

• 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.

1.5.11 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-single and workflow-cutadapt-star-featurecounts-pai
- 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 ouput 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

Added

- Support Python 3.7
- Add Tabix (1.7-2) to resolwebio/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 bqsr (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

1.5.12 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 qorts-qc process
- Bump memory requirements for multiqc process

1.5.13 19.0.0 2019-05-07

- Use Genialis fork of MultiQC 1.8.0b in resolwebio/common:1.1.2
- Support Samtools idxstats and QoRTs QC reports in multiqc 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

Added

- 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

1.5.14 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-gc process

Added

- Add shRNAde (v1.0) R package to the resolwebio/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

1.5.15 17.0.0 2019-03-19

Added

- Add gorts-gc (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

- **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

1.5.16 16.3.0 2019-02-19

Changed

- Bump STAR aligner version to 2.7.0c in resolwebio/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:bam:bowtie2inputs in macs2-callpeak process

1.5.17 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.

1.5.18 16.1.0 2019-01-17

Changed

- 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 resolwebio/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

1.5.19 16.0.0 2018-12-19

- 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, cuffquant, cutadapt-single, cutadapt-custom-single, cutadapt-custom-paired, cutadapt-paired, differentialexpression-deseq2, differentialexpression-edger, expression-aggregator, feature_counts, goenrichment, htseq-count, htseq-count-raw, index-fasta-nucl, library-strandedness. pca. regtools-junctions-annotate, rsem, salmon-index, trimmomatic-single, trimmomatic-paired, upload-expression, upload-expression-cuffnorm, upload-expression-star, upload-fasta-nucl, upload-fastq-single, 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

Added

- 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

1.5.20 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 resolwebio/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 resolwebio/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

1.5.21 14.0.2 2018-10-23

Fixed

• Fix htseq-count-raw process to correctly map features with associated feature symbols.

1.5.22 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

1.5.23 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

Added

- 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

1.5.24 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, xgtf2gff.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 <code>bamtobigwig.sh</code> 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.

- 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

1.5.25 12.0.0 - 2018-08-13

- 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

Added

- Add two-pass mode to alignment-star process
- Add regtools (0.5.0) to resolwebio/rnaseq Docker image
- Add KAPA experiment descriptor schema
- Add resdk Python 3 package to resolwebio/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 preprocess-ing 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 resolwebio/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

1.5.26 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 C3f fragment Complement C3c alpha' chain fragment 2" in Ensembl

- 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.

1.5.27 10.0.1 - 2018-07-06

Fixed

• Fix bamtobigwig.sh to timeout the bamCoverage calculation after defined time

1.5.28 10.0.0 - 2018-06-19

- 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 resolwebio/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

Changed

- 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 resolwebio/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

1.5.29 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
- $\bullet \ Remove \ \texttt{differential} expression-\texttt{limma} \ process$
- Use resolwebio/rnaseq:3.1.0 docker image and expand error messages in:
 - cuffdiff

- differentialexpression-deseq2
- differentialexpression-edger
- Update workflow-bbduk-star-htseq
- Update quantseq descriptor schema
- Assert species and build in htseq-count-normalized process
- Set amplicon report template in resolwebio/latex Docker image to landscape mode

Added

- 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.

1.5.30 8.1.0 - 2018-04-13

- 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 resolwebio/rnaseq 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

1.5.31 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 bgzipcompressed 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-pcrmetrics 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 resolwebio/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

1.5.32 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

1.5.33 7.0.0 - 2018-03-13

- **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 gsize 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 cooksCutoff parameter to FALSE in deseq. R tool
- Rename 'Expressions (BCM)' to 'Dicty expressions'

- 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 resolwebio/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

- 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

1.5.34 6.2.2 - 2018-02-21

Fixed

• Fix cuffnorm process to correctly use sample names as labels in output files and expand cuffnorm tests

1.5.35 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

1.5.36 6.2.0 - 2018-01-17

Added

- Add R, tabix, and CheMut R library to resolwebio/dnaseq Docker image
- Add SRA Toolkit to resolwebio/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 resolwebio/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

1.5.37 6.1.0 - 2017-12-12

- 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

1.5.38 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
- Add cufflinks, RNASeqT R library, pyarrow and sklearn Python packages to resolwebio/ <code>rnaseq</code> Docker image
- Add wigToBigWig tool to resolwebio/chipseq Docker image

- 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

- 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

1.5.39 5.0.1 - 2017-11-14

Fixed

• Update Features and Mappings ElasticSearch indices building to be compatible with Resolwe 4.0

1.5.40 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

- 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

1.5.41 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 resolwebio/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

1.5.42 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

1.5.43 4.0.0 - 2017-09-14

Added

• New base and legacy Docker images for processes, which support non-root execution as implemented by Resolwe

- BACKWARD INCOMPATIBLE: Modify all processes to explicitly use the new Docker images
- BACKWARD INCOMPATIBLE: Remove clustering-hierarchical-genes-etc process
- Require Resolwe 3.x

1.5.44 3.2.0 2017-09-13

Added

- Add index-fasta-nucl and rsem process
- Add custom Cutadapt STAR RSEM workflow

1.5.45 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

1.5.46 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

- 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

- 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

1.5.47 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

1.5.48 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

1.5.49 1.4.0 2017-07-04

- · 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 QC
- 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

- 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

- 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

1.5.50 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

- · 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

- 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

1.5.51 1.2.1 2016-07-27

Changed

• Update resolwe requirement

1.5.52 1.2.0 2016-07-27

- 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

1.5.53 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

- 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

1.5.54 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.

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:

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.:

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

То run that are skipped Docker due failures the the tests on to and errors, set 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 dist/ \star

CHAPTER 2

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