

Test for Diff. Expression with Cuffdiff Element

Cuffdiff takes a transcript file as input, along with two or more fragment alignments (e.g. in SAM format) for two or more samples. It produces a number of output files that contain test results for changes in expression at the level of transcripts, primary transcripts, and genes. It also tracks changes in the relative abundance of transcripts sharing a common transcription start site, and in the relative abundances of the primary transcripts of each gene. Tracking the former allows one to see changes in splicing, and the latter lets one see changes in relative promoter use within a gene.

Parameters in GUI

Parameter	Description	Default value
Output directory	Directory to save MACS output files.	
Time series analysis	If set to True, instructs Cuffdiff to analyze the provided samples as a time series, rather than testing for differences between all pairs of samples. Samples should be provided in increasing time order.	False
Upper quartile norm	If set to True, normalizes by the upper quartile of the number of fragments mapping to individual loci instead of the total number of sequenced fragments. This can improve robustness of differential expression calls for less abundant genes and transcripts.	False
Hits norm	Instructs how to count all fragments. Total specifies to count all fragments, including those not compatible with any reference transcript, towards the number of mapped fragments used in the FPKM denominator. Compatible specifies to use only compatible fragments. Selecting Compatible is generally recommended in Cuffdiff to reduce certain types of bias caused by differential amounts of ribosomal reads which can create the impression of falsely differentially expressed genes..	Compatible
Frag bias correct	Providing the sequences your reads were mapped to instructs Cuffdiff to run bias detection and correction algorithm which can significantly improve accuracy of transcript abundance estimates..	
Multi read correct	Do an initial estimation procedure to more accurately weight reads mapping to multiple locations in the genome.	False
Library type	Specifies RNA-Seq protocol.	Standard Illumina
Mask file	Ignore all reads that could have come from transcripts in this file. It is recommended to include 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..	
Min alignment count	The minimum number of alignments in a locus for needed to conduct significance testing on changes in that locus observed between samples. If no testing is performed, changes in the locus are deemed not significant, and the locus' observed changes don't contribute to correction for multiple testing..	10
FDR	The allowed false discovery rate used in testing.	0.05
Max MLE iterations	Sets the number of iterations allowed during maximum likelihood estimation of abundances.	5000
Emit count tables	Include information about the fragment counts, fragment count variances, and fitted variance model into the report.	False
Cuffdiff tool path	The path to the Cuffdiff external tool in UGENE.	default
Temporary directory	The directory for temporary files.	default

Parameters in Workflow File

Type: cuffdiff

Parameter	Parameter in the GUI	Type
out-dir	Output directory	string
time-series-analysis	Time series analysis	boolean
upper-quartile-norm	Upper quartile norm	boolean
hits-norm	Hits norm	numeric
frag-bias-correct	Frag bias correct	string

multi-read-correct	Multi read correct	<i>boolean</i>
library-type	Library type	<i>numeric</i>
mask-file	Mask file	<i>numeric</i>
min-alignment-count	Min alignment count	<i>string</i>
fdr	FDR	<i>numeric</i>
max-mle-iterations	Max MLE iterations	<i>numeric</i>
emit-count-tables	Emit count tables	<i>boolean</i>
path	Cuffdiff tool path	<i>string</i>
temp-dir	Temporary directory	<i>string</i>

Input/Output Ports

The element has 2 *input port*:

Name in GUI: Annotations

Name in Workflow File: in-annotations

Slots:

Slot In GUI	Slot in Workflow File	Type
Set of annotations	in-annotations	<i>ann_table</i>

Name in GUI: Assembly

Name in Workflow File: in-assembly

Slots:

Slot In GUI	Slot in Workflow File	Type
Assembly data	assembly	<i>assembly</i>
Source url	url	<i>string</i>