

Transcript Expression Analysis - Cuffdiff

This option is only available when *Cufflinks quantification* node is selected. Detailed implementation information can be found in the Cuffdiff manual [5].

When the task is selected, the dialog will display all the categorical attributes more than one subgroups (Figure 1).

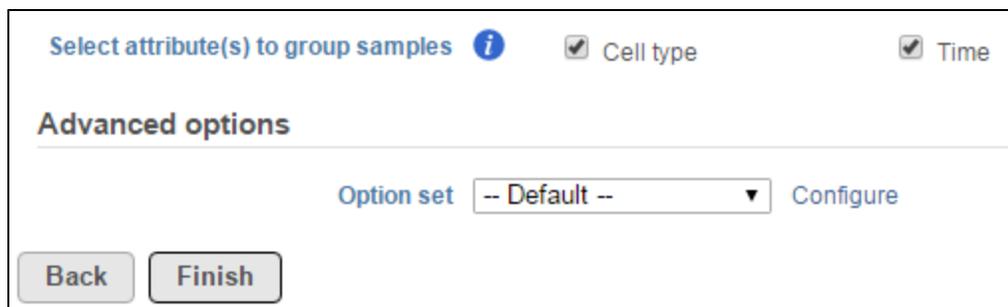


Figure 1. Cuffdiff setup dialog. "Select attributes(s) to groups samples" lists the categorical attributes which have at least two levels (e.g. "Cell type" and "Time")

When an attribute is selected, pairwise comparisons of all the levels will be performed independently.

Click on **Configure** button in the Advanced options to configure normalization method and library types (Figure 2).

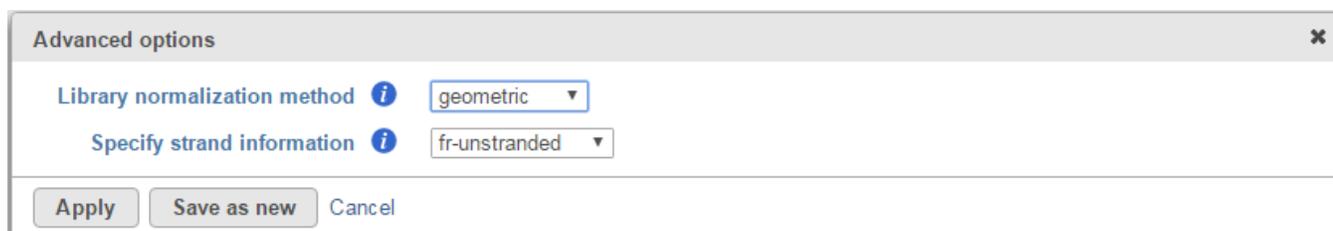


Figure 2. Advanced option of cuffdiff

There are three library normalization methods:

- Class-fpkm: library size factor is set to 1, no scaling applied to FPKM values
- Geometric: FPKM are scaled via the median of the geometric means of the fragment counts across all libraries [6]. This is the default option (and is identical to the one used by DESeq)
- Quartile: FPKMs are scaled via the ratio of the 75 quartile fragment counts to the average 75 quartile value across all libraries

The library types have three options:

- Fr-unstranded: reads from the left-most end of the fragment in transcript coordinates map to the transcript strand, and the right-most end maps to the opposite strand. E.g. standard Illumina
- Fr-firststrand: reads from the left-most end of the fragment in transcript coordinates map to the transcript strand, and the right-most end maps to the opposite strand. The right-most end of the fragment is the first sequenced or only sequenced for single-end reads. It is assumed that only the strand generated during first strand synthesis is sequenced. E.g. dUPT, NSR, NNSR
- Fr-secondstrand: reads from the left-most end of the fragment in transcript coordinates map to the transcript strand, and the right-most end maps to the opposite strand. The left-most end of the fragment is the first sequenced or only sequenced for single-end reads. It is assumed that only the strand generated during second strand synthesis is sequenced. E.g. Directional Illumina, standard SOLiD.

The report of the cuffdiff task is a table of a feature list p-values, q-value and log2 fold-change information for all the comparisons (Figure 20).

Gene ID	Test ID	P-value (5uM vs 10uM)	Q-value (5uM vs 10uM)	log2(foldChange) (5uM vs 10uM)	P-value (5uM vs 0uM)	Q-value (5uM vs 0uM)	log2(foldChange) (5uM vs 0uM)
RBFOX2	CUFF.589.1	0.59	0.97	0.24	0.81	0.97	0.13
XLOC_000042	CUFF.41.1	0.57	0.97	0.22	0.98	0.99	2.07E-3
SNORD43	NR_002439	NOTEST			NOTEST		
XRCC6	NM_001288978	NOTEST			0.47	0.97	1.70
XRCC6	NM_001288977	0.95	0.99	-0.13	0.43	0.96	1.32
FAM227A	CUFF.652.3	NOTEST			NOTEST		
FAM227A	CUFF.652.4	NOTEST			NOTEST		
GSTT2	NM_001080843.1	NOTEST			NOTEST		
NDUFA6-AS1	CUFF.1211.2	0.83	0.97	-0.10	0.38	0.95	0.38
SREBF2	CUFF.1211.1	NOTEST			NOTEST		
TPTEP1	NR_001591	NOTEST			NOTEST		
XRCC6	NM_001288976	0.78	0.97	-0.18	0.92	0.98	0.07
SEZ6L	NM_001184773	NOTEST			NOTEST		
LRP5L	CUFF.301.1	NOTEST			NOTEST		

Figure 3. Figure 20: Cuffdiff task report. Each row is a feature, p-value, q-value and log2 fold change columns are display for each comparison

In the p-value column, besides an actual p-value, which means the test was performed successfully, there is also the following flags which indicate the test was not successful:

- NOTEST: not enough alignments for testing
- LOWDATA: too complex or shallowly sequences
- HIGHDATA: too many fragments in locus
- FAIL: when an ill-conditioned covariance matrix or other numerical exception prevents testing

The table can be downloaded as a text file when clicking the **Download** button on the lower-right corner of the table.

References

1. Benjamini, Y., Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing, JRSS, B, 57, 289-300.
2. Storey JD. (2003) The positive false discovery rate: A Bayesian interpretation and the q-value. Annals of Statistics, 31: 2013-2035.
3. Auer, 2011, A two-stage Poisson model for testing RNA-Seq
4. Burnham, Anderson, 2010, Model selection and multimodel inference
5. Law C, Voom: precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biology, 2014 15:R29.
6. <http://cole-trapnell-lab.github.io/cufflinks/cuffdiff/index.html#cuffdiff-output-files>
7. Anders S, Huber W: Differential expression analysis for sequence count data. Genome Biology, 2010

Additional Assistance

If you need additional assistance, please visit [our support page](#) to submit a help ticket or find phone numbers for regional support.



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