

Exercise 1

RNA-seq data analysis on the Galaxy server (<http://centromere:8080/root>):

1. Upload to your history
 - a. [SharedData/Data Libraries/GTF 4 Cufflink](#) (the reference Annotation GTF files for Cufflink)
 - b. [SharedData/Data Libraries/workshop_sample_data/RNA-seq](#)
2. Map with Tophat
3. Find splice junctions using Cufflinks
4. Merge the transcripts using Cuffmerge
5. Perform DE analysis using Cuffdiff

Analyze the input/output files and the program parameters.

Homework

Analyze the input/ output files in this analysis in your Galaxy session.

1. Cufflinks
 - a. -If you have not yet done so, run Cufflinks with the options:
 - i. **Use Reference Annotation:** Use Reference Annotation, setting **Reference Annotation:** dm3_genes.gtf
 - How many transcripts have been found for cond1 and how many for cond2 ?
 - ii. **Use Reference Annotation:** No
 - How many transcripts have been found for cond1 and how many for cond2 ?

-What is the difference between Cufflinks results when **Use Reference Annotation** option is on and when it is off (between i. and ii.)?

2. Inspect the results of Cuffmerge. What is the number of significantly differentially expressed genes?