

Start tophat by submitting to lonestar

Start tophat-cufflinks by submitting to lonestar

Step 0. Make sure all the required modules are loaded.

```
module load bowtie
module load tophat
module load cufflinks/2.0.2
```

Step 1. Copy over the directory \$BI/ngs_course/tophat_cufflinks_enrichment to \$SCRATCH and cd into that directory.

```
cp -r $BI/ngs_course/tophat_cufflinks_enrichment/ $SCRATCH
cd $SCRATCH/tophat_cufflinks_enrichment
ls
```

Step 2. Create a file called **tophat.commands** with tophat commands. You can use your favorite editor (like nano or emacs) to open and edit a file.

```
nano tophat.commands
```

Use **ctrl+o** to write out the data then **ctrl+x** to quit. Say yes when asked whether you want to save the modifications.

```
tophat -p 8 -G reference/genes.gtf -o C1_R1_thout reference/genome data/GSM794483_C1_R1_1.fq data/GSM794483_C1_R1_2.fq
tophat -p 8 -G reference/genes.gtf -o C1_R2_thout reference/genome data/GSM794484_C1_R2_1.fq data/GSM794484_C1_R2_2.fq
tophat -p 8 -G reference/genes.gtf -o C1_R3_thout reference/genome data/GSM794485_C1_R3_1.fq data/GSM794485_C1_R3_2.fq
tophat -p 8 -G reference/genes.gtf -o C2_R1_thout reference/genome data/GSM794486_C2_R1_1.fq data/GSM794486_C2_R1_2.fq
tophat -p 8 -G reference/genes.gtf -o C2_R2_thout reference/genome data/GSM794487_C2_R2_1.fq data/GSM794487_C2_R2_2.fq
tophat -p 8 -G reference/genes.gtf -o C2_R3_thout reference/genome data/GSM794488_C2_R3_1.fq data/GSM794488_C2_R3_2.fq
```

Step 3. Create a file called **cufflinks.commands** with cufflinks commands. You can use your favorite editor (like nano or emacs) to open and edit a file.

```
cufflinks -p 8 -o C1_R1_clout C1_R1_thout/accepted_hits.bam
cufflinks -p 8 -o C1_R2_clout C1_R2_thout/accepted_hits.bam
cufflinks -p 8 -o C1_R3_clout C1_R3_thout/accepted_hits.bam
cufflinks -p 8 -o C2_R1_clout C2_R1_thout/accepted_hits.bam
cufflinks -p 8 -o C2_R2_clout C2_R2_thout/accepted_hits.bam
cufflinks -p 8 -o C2_R3_clout C2_R3_thout/accepted_hits.bam
```

Step 4. Alternatively, lets put tophat and cufflinks in one command file and make sure they run sequentially. Lets call this file **tc.commands**



Be careful in **nano** – it has an unfortunate tendency to add line breaks, and there must be exactly one command per line in a commands file.

```

tophat -p 8 -G reference/genes.gtf -o C1_R1_thout reference/genome data/GSM794483_C1_R1_1.fq data/GSM794483_C1_R1_2.fq && cufflinks -p 8 -o C1_R1_clout C1_R1_thout/accepted_hits.bam
tophat -p 8 -G reference/genes.gtf -o C1_R2_thout reference/genome data/GSM794484_C1_R2_1.fq data/GSM794484_C1_R2_2.fq && cufflinks -p 8 -o C1_R2_clout C1_R2_thout/accepted_hits.bam
tophat -p 8 -G reference/genes.gtf -o C1_R3_thout reference/genome data/GSM794485_C1_R3_1.fq data/GSM794485_C1_R3_2.fq && cufflinks -p 8 -o C1_R3_clout C1_R3_thout/accepted_hits.bam
tophat -p 8 -G reference/genes.gtf -o C2_R1_thout reference/genome data/GSM794486_C2_R1_1.fq data/GSM794486_C2_R1_2.fq && cufflinks -p 8 -o C2_R1_clout C2_R1_thout/accepted_hits.bam
tophat -p 8 -G reference/genes.gtf -o C2_R2_thout reference/genome data/GSM794487_C2_R2_1.fq data/GSM794487_C2_R2_2.fq && cufflinks -p 8 -o C2_R2_clout C2_R2_thout/accepted_hits.bam
tophat -p 8 -G reference/genes.gtf -o C2_R3_thout reference/genome data/GSM794488_C2_R3_1.fq data/GSM794488_C2_R3_2.fq && cufflinks -p 8 -o C2_R3_clout C2_R3_thout/accepted_hits.bam

```

Step 5. We have a commands file. We need a launcher file to submit this to the lonestar queue. Use `launcher_creator.py` to create a launcher file.

```
launcher_creator.py -n tophat_cufflinks -q normal -t 12:00:00 -j tc.commands -l tc_launcher.sge
```

Step 6. Submit this job to the queue.

```
qsub tc_launcher.sge
```

Use qstat to find the jobid for this job

```
qstat
```

Step 7. Create a file called **cuffmerge.commands** to run cuffmerge on results from step 6.

```

nano cuffmerge.commands
cuffmerge -g reference/genes.gtf -s reference/genome.fa -p 8 assembly_list.txt

```

Step 8. Use `launcher_creator` to create a launcher for this cuffmerge job and submit it to the queue *such that it runs after the previous job completes*.

```

launcher_creator.py -n cuffmerge -q normal -t 6:00:00 -j cuffmerge.commands -l cuffmerge_launcher.sge
qstat
qsub -hold_jid <jobid> cuffmerge_launcher.sge

```

Step 9. Create a file called **cuffdiff.commands** to run cuffdiff on results from step 7.

```

nano cuffdiff.commands
cuffdiff -o diff_out -b reference/genome.fa -p 8 -L C1,C2 -u merged_asm/merged.gtf C1_R1_thout/accepted_hits.bam,C1_R2_thout/accepted_hits.bam,C1_R3_thout/accepted_hits.bam C2_R1_thout/accepted_hits.bam,C2_R2_thout/accepted_hits.bam,C2_R3_thout/accepted_hits.bam

```

Step 10. Use `launcher_creator` to create a launcher for this cuffdiff job and submit it to the queue *such that it runs after the previous job completes*.

```

launcher_creator.py -n cuffdiff -q normal -t 6:00:00 -j cuffdiff.commands -l cuffdiff_launcher.sge
qsub -hold_jid <jobid> cuffdiff_launcher.sge

```