Trapnell Data Set - Differential Expression (cuffdiff)

BIOL550 - Lab 3 Weekly Report (Week 3)

What I accomplished since the previous report

I produced a set of STAR BAM files that include the `XS` strand tag required by `cuffdiff`, and then ran `cuffdiff` on all six samples (3 C1 replicates and 3 C2 replicates) to complete the Trapnell differential expression step. I exported the `cuffdiff` output directory locally, summarized gene-level results from `gene_exp.diff` using `q_value <= 0.05`, and generated two sanity-check figures (volcano plot + top-DE bar chart).

Results summary

```
Lab 3: Cuffdiff gene-level DE summary
OK tests: 8289
Significant genes (q<=0.05): 265
Top up (by log2FC): Fatp, crc, scf, CTPsyn, Df31
Top down (by log2FC): Nep2, RpS19b, Amy-d, CG6847, Aplip1
```

Methods used (commands + parameters)

STAR was used to generate sorted coordinate BAMs, and `cuffdiff` was used for differential expression on the aligned reads. Then, downstream filtering and plotting were done locally from the `cuffdiff` output tables.

STAR re-alignment (to ensure `XS` tags)

STAR was re-run for all six samples with `--outSAMstrandField intronMotif` so that spliced alignments include `XS` tags.

```
STAR \
  --genomeDir /home/pzg8794/star_index/classref_trapnell_zip_bdgp6_84_v2 \
  --runThreadN 4 \
  --sjdbGTFfile "/home/pzg8794/BIOL550/Lab1/Trapnell_Data/Trapnell Data/Drosophila reference/Drosophila_melanogaster.BDGP6.84.gtf"
\
  --readFilesIn \
    "/home/pzg8794/BIOL550/Lab1/Trapnell_Data/Trapnell Data/Raw reads/GSM794483_C1_R1_1.fq.gz" \
    "/home/pzg8794/BIOL550/Lab1/Trapnell_Data/Trapnell Data/Raw reads/GSM794483_C1_R1_2.fq.gz" \
  --readFilesCommand zcat \
  --outSAMtype BAM SortedByCoordinate \
  --outSAMstrandField intronMotif \
  --limitBAMsortRAM 6000000000 \
  --outFileNamePrefix /home/pzg8794/BIOL550/Lab1/star_align_classref_v2_all_xs/GSM794483_C1_R1/
```

Differential expression (`cuffdiff`)

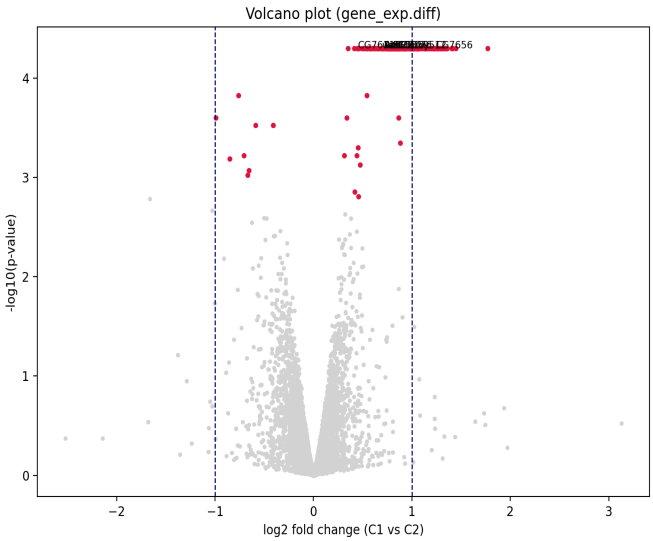
The Drosophila reference directory includes both a GTF and a GFF3; the GTF (`Drosophila_melanogaster.BDGP6.84.gtf`) was used because `cuffdiff` expects GTF2 annotation (GFF3 would require conversion before use). `Cuffdiff` was run on the XS-tagged BAMs (3 replicates per condition) with bias correction enabled via `-b`.

```
/usr/local/bin/cufflinks/cuffdiff \
  -o /home/pzg8794/BIOL550/Lab1/cuffdiff_classref_v2_xs \
  -p 4 \
  -L C1,C2 \
  -b /home/pzg8794/refs/classref_bdgp6_84_ids_v2.fa \
  "/home/pzg8794/BIOL550/Lab1/Trapnell_Data/Trapnell Data/Drosophila reference/Drosophila_melanogaster.BDGP6.84.gtf" \

/home/pzg8794/BIOL550/Lab1/star_align_classref_v2_all_xs/GSM794483_C1_R1/Aligned.sortedByCoord.out.bam,/home/pzg8794/BIOL550/Lab1/s
tar_align_classref_v2_all_xs/GSM794484_C1_R2/Aligned.sortedByCoord.out.bam,/home/pzg8794/BIOL550/Lab1/star_align_classref_v2_all_xs
/GSM794485_C1_R3/Aligned.sortedByCoord.out.bam \

/home/pzg8794/BIOL550/Lab1/star_align_classref_v2_all_xs/GSM794486_C2_R1/Aligned.sortedByCoord.out.bam,/home/pzg8794/BIOL550/Lab1/s
tar_align_classref_v2_all_xs/GSM794487_C2_R2/Aligned.sortedByCoord.out.bam,/home/pzg8794/BIOL550/Lab1/star_align_classref_v2_all_xs
/GSM794488_C2_R3/Aligned.sortedByCoord.out.bam
```

Downstream summary (local)

From the file`gene_exp.diff`, I filtered valid tests by keeping rows where `status == "OK"`, and defined significance at `q_value <= 0.05`. Then, I generated a volcano plot (log2FC vs. -log10 p-value) and a top-gene bar chart (ranked by log2FC) as sanity checks on effect-size distribution and signal presence.

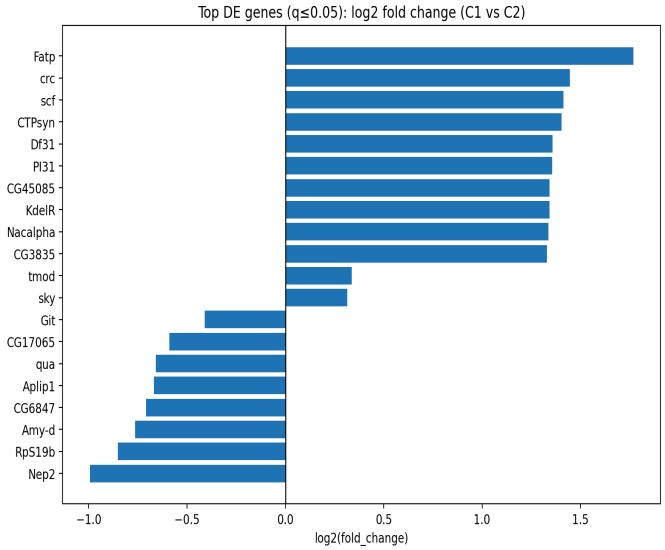
Volcano plot



Each gene is a point: x = log2 fold-change (effect size), y = -log10(p) (significance score), so "outliers" pop into corners.

Volcano plot: `gene_exp.diff`

Top 20 DE genes bar chart



Ranking genes by signed log2FC while showing the biggest movers up vs. down as bars.

Top DE genes (by log2FC)

Problems encountered

The first issue was annotation format: the reference folder includes both a GTF and a GFF3 file. The GFF3 file uses `ID=`/`Parent=` attributes, whereas the GTF uses the `gene_id`/`transcript_id` fields. After inspecting both files, I used the GTF (`Drosophila_melanogaster.BDGP6.84.gtf`) because it was the most compatible with STAR and `cuffdiff` with minimal changes. The second issue was that the initial STAR BAMs from Lab 2 did not contain `XS` strand tags on spliced reads, which caused `cuffdiff` to fail. This required going back and re-aligning all six samples with `--outSAMstrandField intronMotif` to produce XS-tagged BAMs.

Goals for the coming week

Next week I will (1) further explore and improve the visualization workflow (e.g., volcano and MA-style plots) to better interpret the `cuffdiff` outputs, (2) run the FASTQ "dump" workflow for our zebrafish project data using the SRA Toolkit (`prefetch` + `fasterq-dump`) and validate our wrapper script for downloading an arbitrary number of runs, and (3) run `FastQC` on the downloaded FASTQs and begin reviewing the QC reports to guide downstream analysis.