

ITBI2026-2027, Exercise-lecture3, 20-10-2026, M-Reczko

1.

Add required environment variables to your shell configuration:

```
echo "export IGV=/usr/share/java" >> ~/.bashrc
```

```
echo "export PATH=$PATH:/home/reczko/tools/samtools-0.1.19" >> ~/.bashrc
```

Activate the shell configuration:

```
source ~/.bashrc
```

Unpack and run the RNAseq-tutorial using

```
cd ~
```

```
tar -xzf /mnt/hdd_b/home/reczko/tools/rnaseq_workshop.taz
```

```
cd rnaseq_workshop
```

```
./runTuxedoDemo.pl
```

Answer 'y' to all steps until the IGV visualization starts.

For the data in the RNAseq-tutorial, using the IGV tool and reporting each location as "genome:<start>-<end>":

1.1 find a read with unmapped pair

1.2 find a read that has 2 mismatches

1.3 find a read-pair with pair orientation: F2R1

1.4 find a gene that agrees in 6 splice-sites with its annotation

1.5 find a location where less than 96% of the reads agree with the annotation and at least 2 other nucleotides occur.

1.6 find a gene where the peak with the highest number of reads has at least 3 times the reads of the highest peak in a different sample (with non-zero reads for that gene)

2. Quit IGV and finish the rest of the demo.

Using the cuffcompare tool (see

<http://cole-trapnell-lab.github.io/cufflinks/cuffcompare/index.html>),

calculate the statistics describing the match between the reconstructed transcriptome (in merged_asm/merged.gtf) and the reference annotation (in genes.gff3). How many novel exons and novel introns were discovered?

3.

Using tophat's align_summary.txt, what is the mapping percentage for left and right reads, averaged over all 4 samples?

4.

Using the commands in the tutorial Tuxedo_workshop_activities.pdf (load from eclass) generate a (bash) shell script to execute the tuxedo pipeline. Modify all relevant program invocations to use 4 CPUs. Help for shell scripts can be found e.g. at <http://tldp.org/HOWTO/Bash-Prog-Intro-HOWTO.html> . An efficient solution will have one script to analyze one sample and a second script calling the first script for the 4 samples.

5.

Bonus: Modify a copy of the script(s) in question 4 to use the Hisat2 mapper instead of tophat and compare the obtained results in terms of the number of significantly differentially expressed genes.

SEND SOLUTIONS (for M.Reczko exercises) ONLY TO:
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DEADLINE: 2/11/2026