Introduction to Bulk RNAseq data analysis

Quantification of Gene Expression with Salmon

Exercise 1 - Create Salmon index

1. Create concatenated trancriptome/genome reference file

```
cat references/Mus_musculus.GRCm38.cdna.chr14.fa.gz \
    references/Mus_musculus.GRCm38.dna_sm.chr14.fa.gz \
    > references/gentrome.chr14.fa.gz
```

2. Create decoy sequence list from the genomic fasta

```
echo "14" > references/decoys.txt
```

3. Use `salmon index` to create the index. You will need to provide three pieces of information:
    - the **Transcript fasta file** - *references/gentrome.chr14.fa.gz*
    - the **decoys** - *references/decoys.txt*
    - the **salmon index** - a directory to write the index to, use **references/salmon_index_chr14**

Also add `-p 7` to the command to instruct salmon to use 7 threads/cores. To find the flags for the other three pieces of information use:

```
salmon index --help
```

```
Version Info: This is the most recent version of salmon.

Index
==========
Creates a salmon index.

Command Line Options:
  -v [ --version ]              print version string
  -h [ --help ]                 produce help message
  -t [ --transcripts ] arg  Transcript fasta file.
  -k [ --kmerLen ] arg (=31)    The size of k-mers that should be used
for the
                                quasi index.
  -i [ --index ] arg        salmon index.
  --gencode                     This flag will expect the input transcr
ipt
  ...
  ...
  ...
  -d [ --decoys ] arg       Treat these sequences ids from the referenc
e as
                                the decoys that may have sequence homol
ogous to
                                some known transcript. for example in c
ase of
                                the genome, provide a list of chromosom
e name
                                --- one per line
```

```
salmon index \
    -t references/gentrome.chr14.fa.gz \
    -d references/decoys.txt \
    -p 7 \
    -i references/salmon_index_chr14
```

Exercise 2 - Quantify with Salmon

1. There should already be a directory called `salmon_output` in the `Course_materials` directory. If not, create it.

```
mkdir salmon_output
```

2. Use `salmon quant` to quantify the gene expression from the raw fastq. To see all the options run `salmon quant --help-reads`. There are lot of possible parameters, we will need to provide the following:
    - **salmon index** - *references/salmon_index*
    - `-l A` - Salmon needs to use some information about the library preparation, we could explicitly give this, but it is easy enough for Salmon to **A**utomatically infer this from the data.
    - **File containing the #1 mates** - *fastq/ SRR7657883.subset_2M.sra_1.fastq.gz*
    - **File containing the #2 mates** - *fastq/ SRR7657883.subset_2M.sra_2.fastq.gz*
    - **Output quantification directory** - *salmon_output/SRR7657883*
    - `--writeMappingssalmon_output/SRR7657883.salmon.sam` - Instructs Salmon to output the read alignments in SAM format to the file *salmon_output/SRR7657883.salmon.sam*.
    - `--gcBias` - salmon can optionally correct for GC content bias, it is recommended to always use this
    - **The number of threads to use** - *7*

```
salmon quant \
    -i references/salmon_index \
    -l A \
    -1 fastq/SRR7657883.subset_2M.sra_1.fastq.gz \
    -2 fastq/SRR7657883.subset_2M.sra_2.fastq.gz \
    -o salmon_output/SRR7657883 \
    --writeMappings=salmon_output/SRR7657883/SRR7657883.salmon.sam \
    --gcBias \
    -p 7
```

Exercise 3

1. Sort and transform your aligned SAM file into a BAM file called `SRR7657883.salmon.sorted.bam`. Use the option `-@ 7` to use 7 cores, this vastly speeds up the compression.

```
samtools sort \
  -@ 7 \
  -O BAM \
  -o salmon_output/SRR7657883/SRR7657883.salmon.sorted.bam \
   salmon_output/SRR7657883/SRR7657883.salmon.sam
```

> > ⇒ *salmon_output/SRR7657883/SRR7657883.salmon.sorted.bam*

> 2. Check your bam file

```
samtools view salmon_output/SRR7657883/SRR7657883.salmon.sorted.bam | more
```