CHAPTER

EIGHT

PROJECT

8.1 Introduction

The goal is to reproduce parts of the analysis described in this paper (to read):

- https://www.nature.com/articles/s41467-020-15966-7

We want to analyze the RNA-Seq data in order to find differentially expressed genes, i.e. genes that are more (or less) expressed in one condition (persisters) compared to another (control).

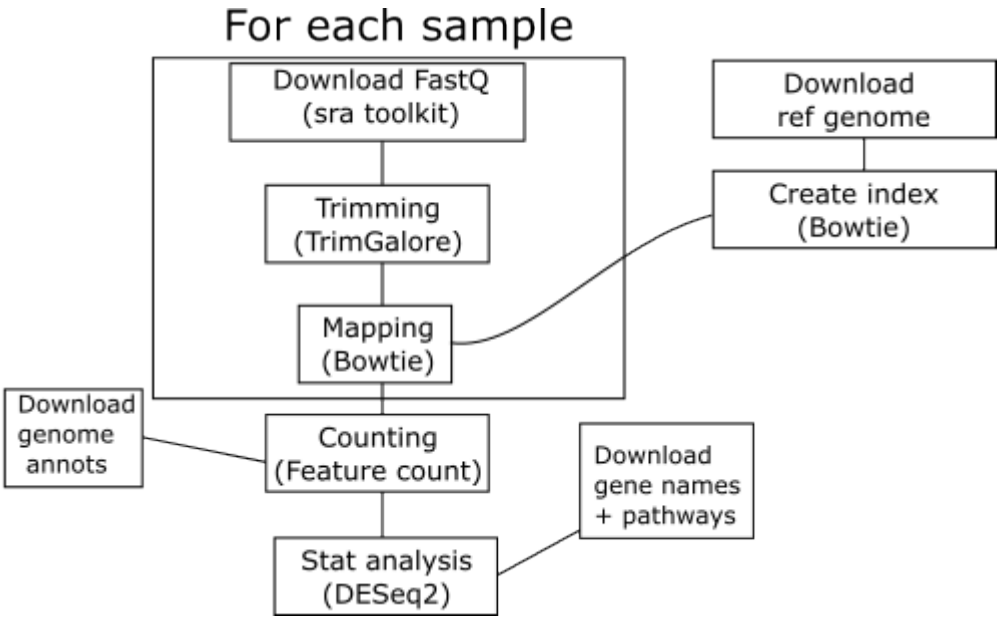
To do so you will design and implement a workflow (Snakemake or Nextflow) that must be reproducible. The work will be done in small groups (4 students).

1. This workflow must use containers that you will *build yourself* (Docker or Singularity) to run the processes;
2. The code must be readable, commented, and documented;
3. We should be able to re-execute easily the workflow;

Deliverables:

1. Container recipes (Dockerfiles, Singularity recipes)
2. Workflow code (Nextflow files + configuration or Snakemake files)
3. README.md + run.sh with all instructions for us to reproduce *easily* your analysis
4. **Report with the following parts:**
    a. Introduction (Reproducibility, biological topic of the papers to reproduce, etc.)
    b. Material and Methods (Tools used, and setup)
    c. Results (Workflow developed, results obtained after execution)
    d. Conclusion / perspectives (Interpretation of the results, and conclusion about reproducibility)
5. Oral presentation: Each group will make a presentation (**December 8th?**).
6. **Mid-project evaluation / progress report**
   [**November 3th**:]
    a. 10 minutes oral presentation
    b. **First version of the code (in the git repository) with:**
        - All the image recipes (working) and
        - First steps of the workflow (data download, genome download and indexing) (working)

8.2 Workflow to implement and execute



8.3 Downloading FASTQ files

- Tool: fastq dump
- Commands:

```
$ fasterq-dump --threads <#CPUS> --progress <SRAID>
$ gzip *.fastq
```

If you find better / quicker ways of downloading the data, feel free!

8.4 Trimming the reads

- Commands:

```
$ trim_galore -q 20 --phred33 --length 25  <FASTQ FILE>
```

8.5 Downloading reference genome

- Commands:

```
$ wget -q -O reference.fasta "https://eutils.ncbi.nlm.nih.gov/entrez/eutils/efetch.fcgi?
↪db=nuccore&id=CP000253.1&rettype=fasta"
```

8.6 Downloading reference genome annotations

- Commands:

```
$ wget -O reference.gff "https://www.ncbi.nlm.nih.gov/sviewer/viewer.cgi?db=nuccore&
↪report=gff3&id=CP000253.1"
```

8.7 Creating genome index

- Tool: Bowtie
- Commands:

```
$   bowtie-build <full genome fasta file> <index name>
```

8.8 Mapping FastQ files

- Tool: FastQC
- Commands:

```
$ bowtie -p <#cpus> -S <INDEX NAME> <(gunzip -c <GZIPED FASTQ FILE>) | \
    samtools sort -@ <#CPUS> > <NAME>.bam
$ samtools index <NAME>.bam
```

8.9 Counting reads

- Tool: subread
- Commands:

```
$ featureCounts --extraAttributes Name -t gene -g ID -F GTF -T <#CPUS> -a <GFF> -o␣
↪counts.txt <BAM FILES>
```

With options: #. **-t gene** indicates that counts should be done on gene. You may use other features such as **exon** #. **-g ID** selects the gene ID to store in the output file. This depends on your input GFF file .

8.10 Statistical analysis (differential gene expression)

- Tool: DESeq2
- Commands: You will have to find the method and write the script yourself.

8.11 Additional informations

- **Some steps are not highly described, for example:**
  - Getting the mapping between gene identifiers (in the GFF file) and gene names (needed in the final MA plot). This can be downloaded from AureoWiki.
  - Getting the list of genes involved in transcription. This can be found in KEGG, using the REST api for example.