# Introduction to Mini SIMEX

## (mini simulated exercises)

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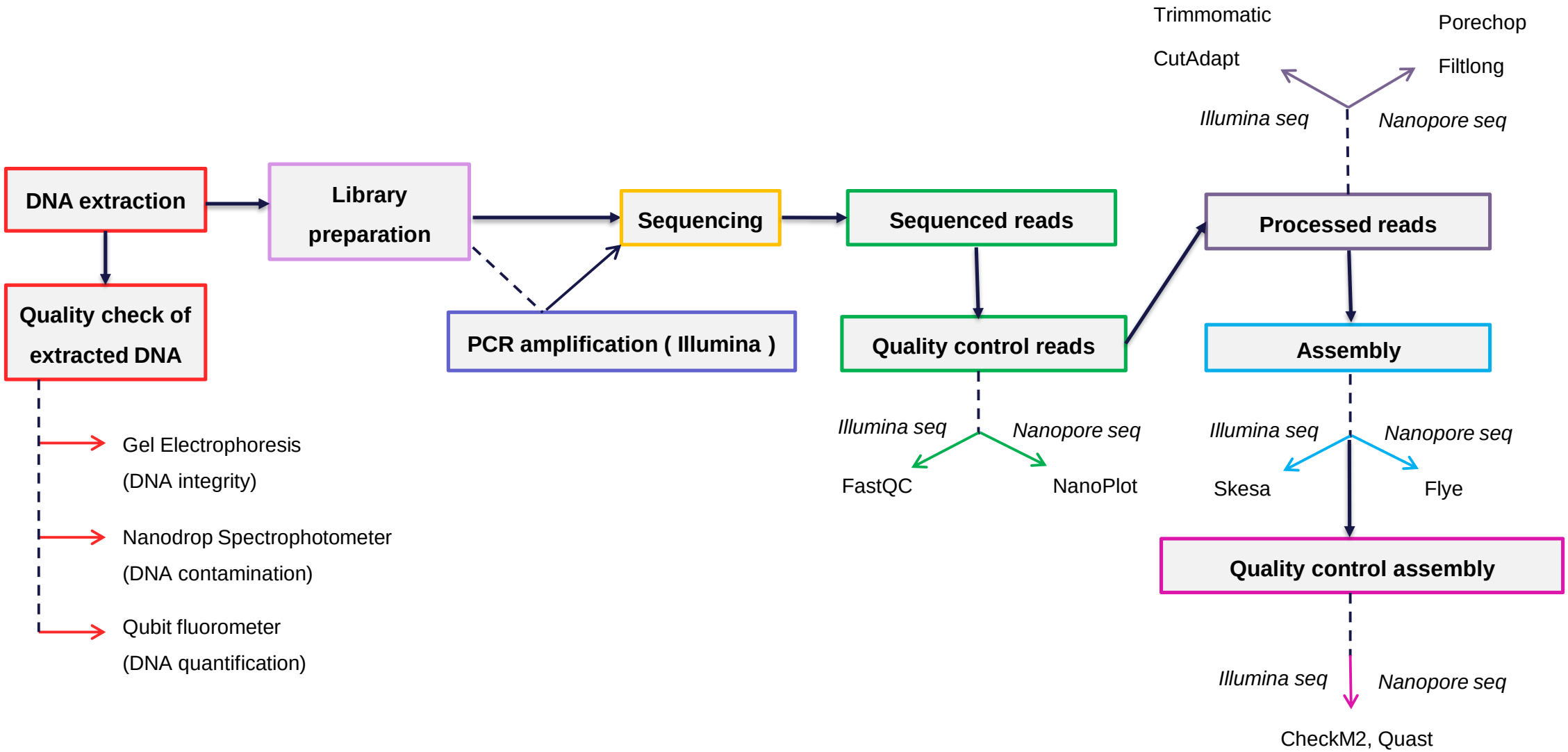
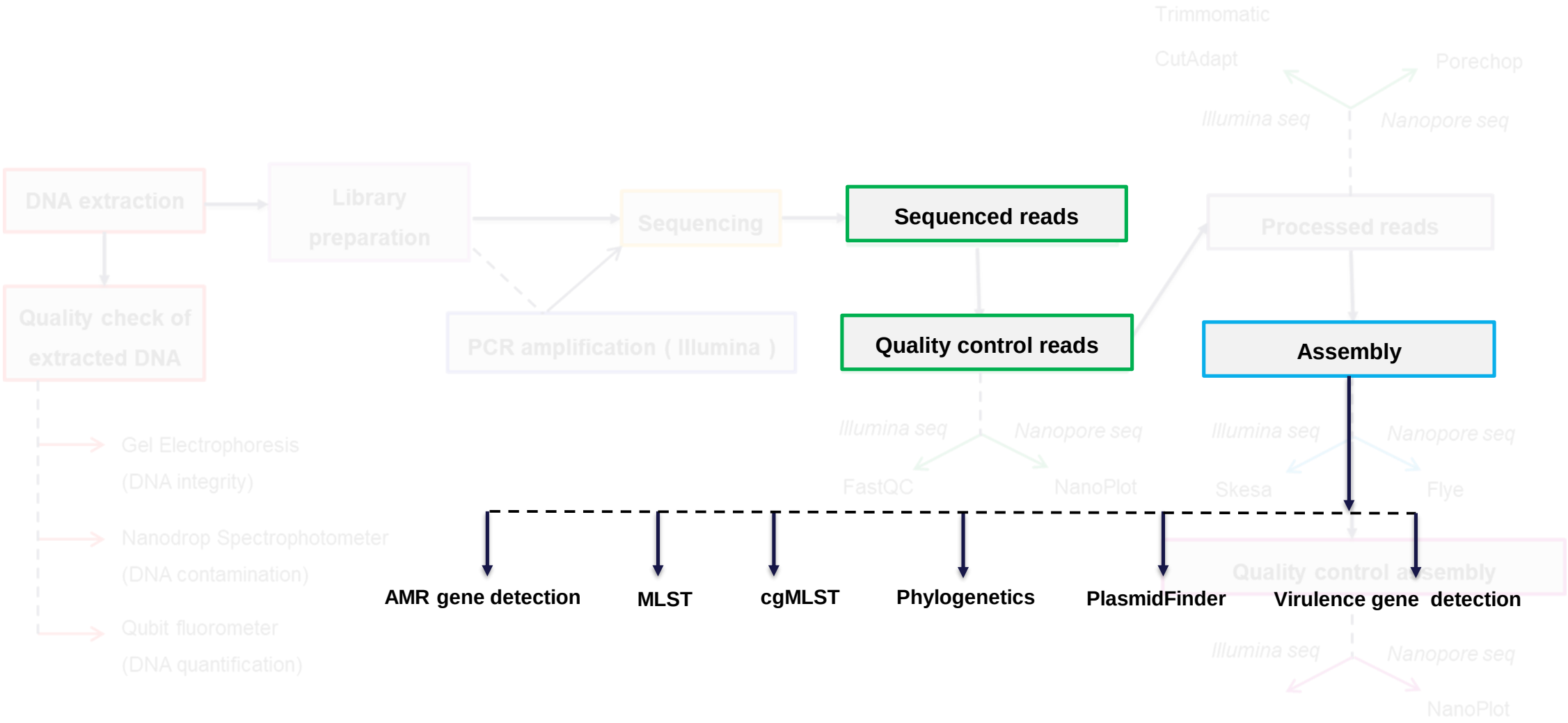
# BACTERIAL WHOLE GENOME SEQUENCING



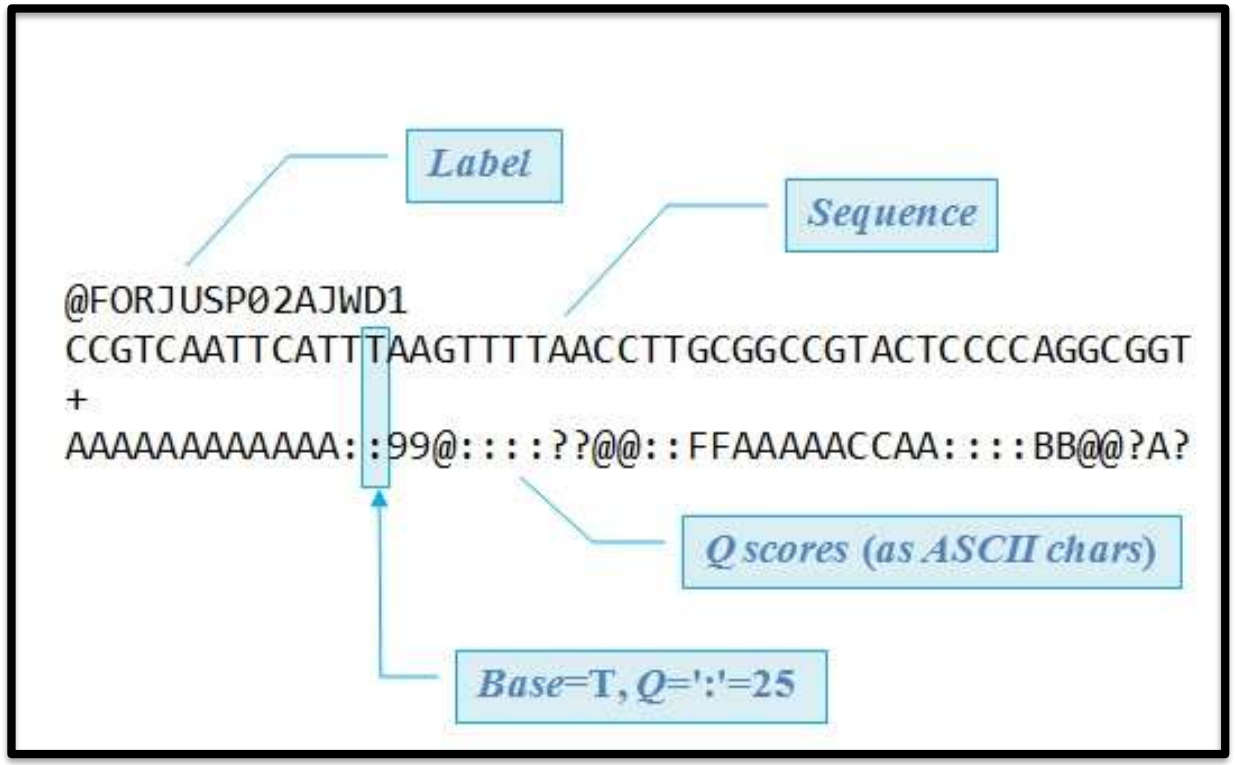
# BACTERIAL WHOLE GENOME SEQUENCING



# OUTPUT FILES

Sequenced reads

## Illumina sequencing → FASTQ file



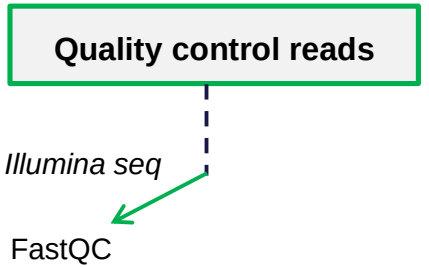

## Nanopore sequencing → FASTA file

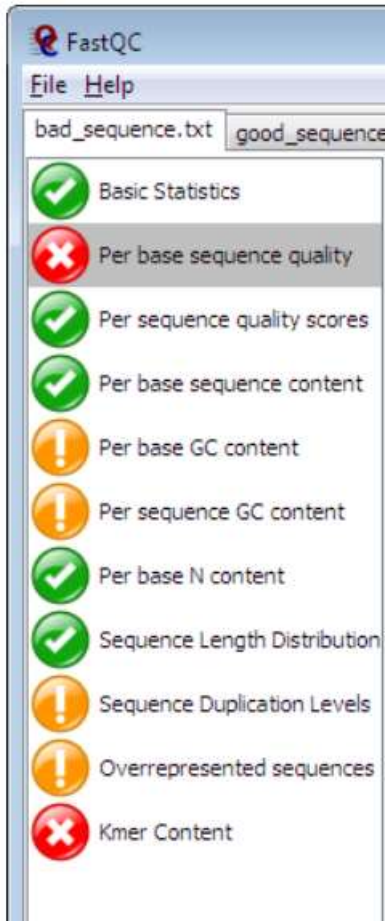
```
>NG_008679.1:5001-38170 Homo sapiens paired box 6 (PAX6)
ACCCTCTTTTCTTATCATTGACATTTAAACTCTGGGGCAGGTCCTCGCGTAGAACGCGGCTGTCAGATCT
GCCACTTCCCCTGCCGAGCGGCGGTGAGAAGTGTGGGAACCGGCGCTGCCAGGCTCACCTGCCTCCCCGC
CCTCCGCTCCCAGGTAACCGCCCGGGCTCCGGCCCCGGCCCGGCTCGGGGCCCGCGGGGCCTCTCCGCTG
CCAGCGACTGCTGTCCCCAAATCAAAGCCCGCCCCAAGTGGCCCCGGGGCTTGATTTTTGCTTTTAAAAG
GAGGCATACAAAGATGGAAGCGAGTTACTGAGGGAGGGATAGGAAGGGGGGTGGAGGAGGGACTTGTCTT
TGCCGAGTGTGCTCTTCTGCAAAAGTAGCAAAATGTTCCACTCCTAAGAGTGGACTTCCAGTCCGGCCCT
GAGCTGGGAGTAGGGGGCGGGAGTCTGCTGCTGCTGTCTGCTAAAGCCACTCGCGACCGCGAAAAATGCA
GGAGGTGGGGACGCACTTTGCATCCAGACCTCCTCTGCATCGCAGTTCACGACATCCACGCTTGGGAAAG
TCCGTACCCGCGCCTGGAGCGCTTAAAGACACCCTGCCGCGGGTCGGGCGAGGTGCAGCAGAAGTTTCCC
GCGGTTGCAAAGTGCAGATGGCTGGACCGCAACAAAGTCTAGAGATGGGGTTCGTTTCTCAGAAAGACGC
```

**Q30 → 1 in 1000 chance that a base is called incorrectly**

**=> 1/1000 = 0.001 error rate**

# QUALITY CONTROL OF READS




## Illumina - FASTQC

**FastQC aims to provide a QC report which can spot problems which originate either in the sequencer or in the starting library material.**

**PASS**

**WARNING**

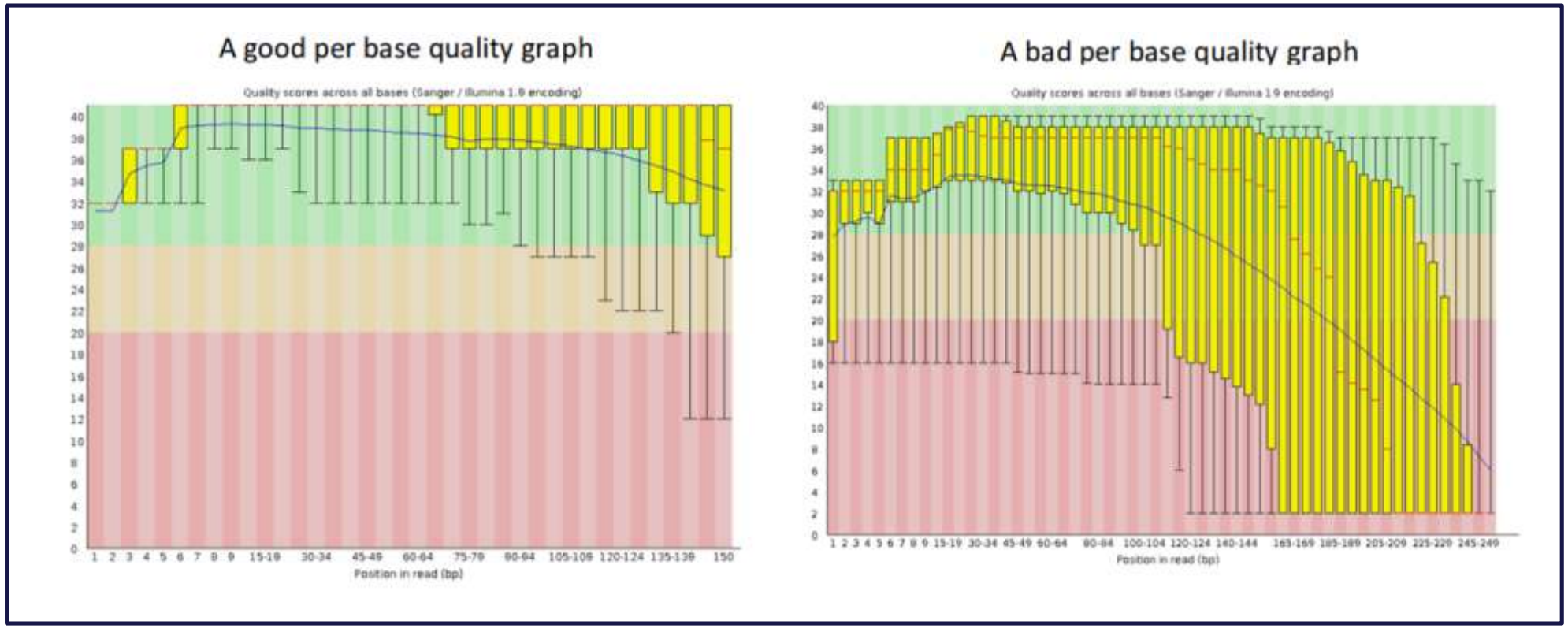
**FAIL**

### Basic Statistics

| Measure | Value |
|---|---|
| Filename | Mov10_oe_1.subset.fq |
| File type | Conventional base calls |
| Encoding | Sanger / Illumina 1.9 |
| Total Sequences | 305900 |
| Sequences flagged as poor quality | 0 |
| Sequence length | 100 |
| %GC | 47 |

# ILLUMINA SEQUENCING – QUALITY CONTROL

1)




@SEQ_ID_1
GATAAAGCAGTATCGAT
+
!''*%%%).1***+*''))**
@SEQ_ID_2
TTTGGGGTTCAAAT
+
%%%).1***CCF>>>>>>CC
@SEQ_ID_3
GATCAAAGCAGTATCGAT
+
!''*(1***+*''))**55CCF>>>>>>

# TRIMMING AND REMOVING ADAPTERS

Trimmomatic , CutAdapt

*Illumina seq*

**Processed reads**

## trimmomatic

**Command line tool → trim and crop FASTQ data**

**Single end data: 1 INPUT FILE → 1 OUTPUT FILE**

**Paired end data: 2 INPUT FILES → 4 OUTPUT FILES ; forward paired, forward unpaired, reverse paired , reverse unpaired**


Trimmomatic_Manual

| COMMAND | action |
|---|---|
| ILLUMINACLIP | Cut adapter and other illumina-specific sequences from the read |
| SLIDINGWINDOW | starts scanning at the 5' end and clips the read once the average quality within the window falls below a threshold |

## cutadapt

**Finds and removes adapter sequences, primers, poly-A tails**

**ERROR TOLERANCE**

**Allowed errors : matches, mismatches, deletions, insertions**

**Maximum error rate = 0.1 (10%) default**

**Actual error rate = number of errors in the match / length of matching part of adapter**

**Adapter occurrence is found only if actual error rate of the match does NOT exceed maximum error rate**

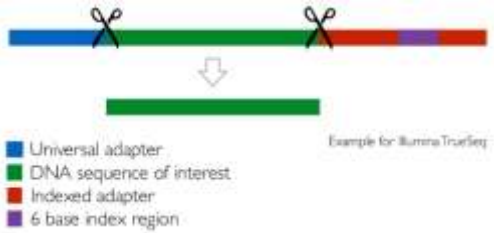
**Eg 1: adapter match of length 8 containing 1 error →**

**error rate = 1 / 8 = 0.125**

**Default maximum error rate = 0.1**

***Will this be detected or not? No!***

***- e → allows to change maximum error rate between 0 - 1***



# ONT SEQUENCING - NANOPLOT

Quality control reads

*Nanopore seq*

NanoPlot

Plotting tool for long read sequencing data and alignments

Also available as a web service → submit the summary .txt file

*INSTALLATION*: **`pip install NanoPlot`**

Upgrade to a newer version using:

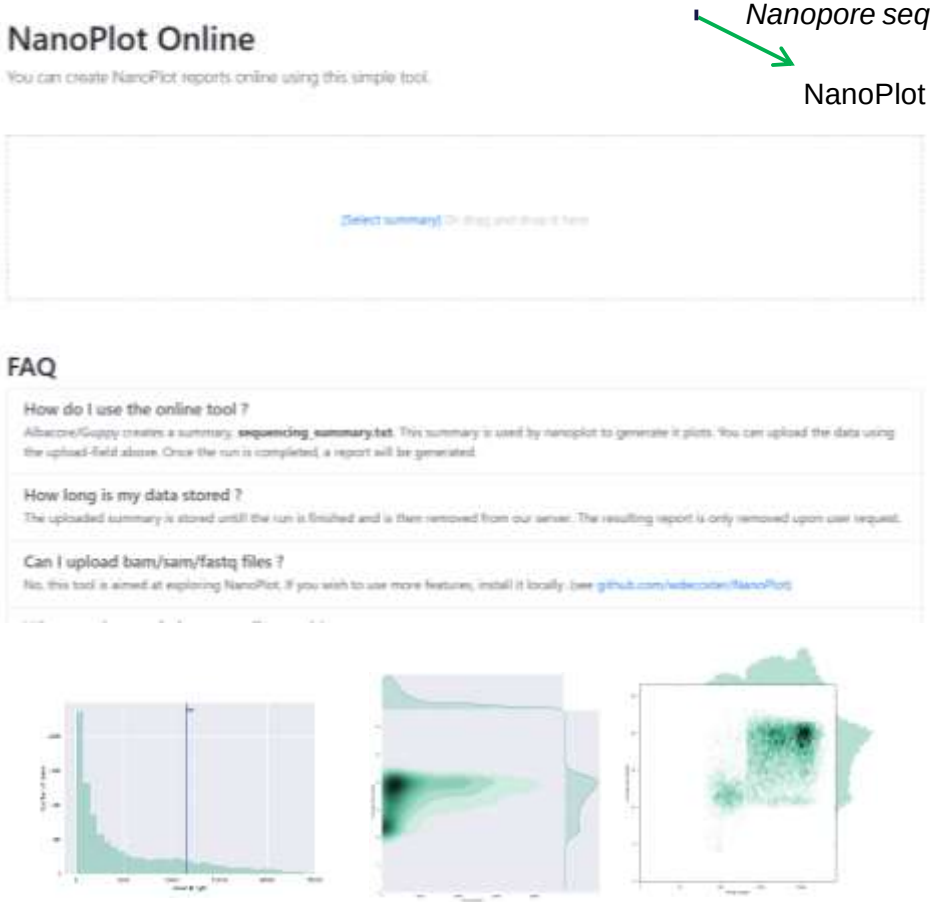
**`pip install NanoPlot --upgrade`**

(or)

**`conda badge`**

**`conda install -c bioconda nanoplot`**

NanoPlot creates → a statistical summary, a number of plots, a html summary file




```
NanoPlot --summary sequencing_summary.txt --loglength -o summary-plots-log-transformed
NanoPlot -t 2 --fastq reads1.fastq.gz reads2.fastq.gz --maxlength 40000 --plots dot --legacy hex
NanoPlot -t 12 --color yellow --bam alignment1.bam alignment2.bam alignment3.bam --downsample 10000 -o bamplots_downsampled
```

# Trimming tools – Nanopore sequencing

## PORECHOP


Porechop, Filtlong

*Nanopore seq*

**Processed reads**

**Basic adapter trimming:**

```
porechop -i input_reads.fastq.gz -o output_reads.fastq.gz
```

**Trimmed reads to stdout, if you prefer:**

```
porechop -i input_reads.fastq.gz > output_reads.fastq
```

**Demultiplex barcoded reads:**

```
porechop -i input_reads.fastq.gz -b output_dir
```

**Demultiplex barcoded reads, straight from Albacore output directory:**

```
porechop -i albacore_dir -b output_dir
```

**Also works with FASTA:**

```
porechop -i input_reads.fasta -o output_reads.fasta
```

**More verbose output:**

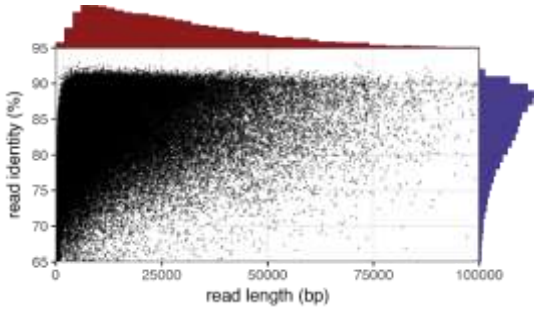
```
porechop -i input_reads.fastq.gz -o output_reads.fastq.gz --verbosity 2
```

**Got a big server?**

```
porechop -i input_reads.fastq.gz -o output_reads.fastq.gz --threads 40
```

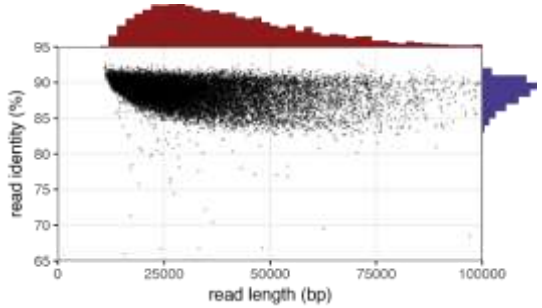
## FILTLONG


**Figure A)**




filtlong --min_length 1000 --keep_percent 90 --target_bases 500000000 input.fastq.gz | gzip > output.fastq.gz

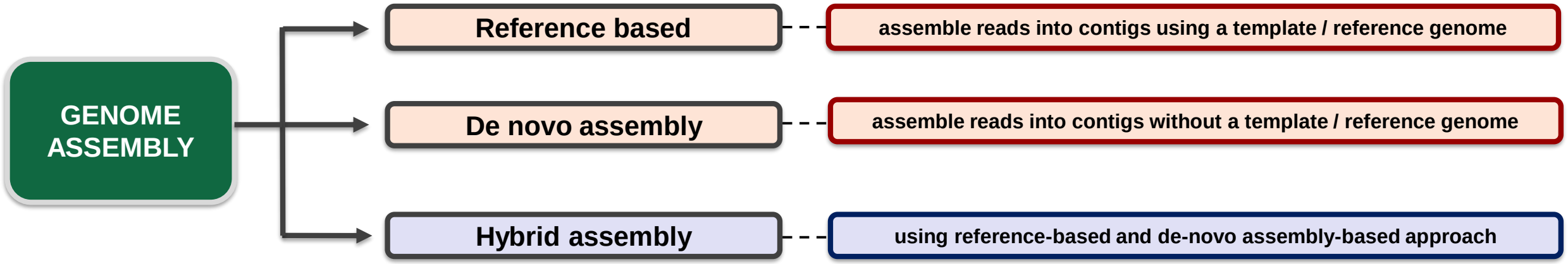
**Figure B)**



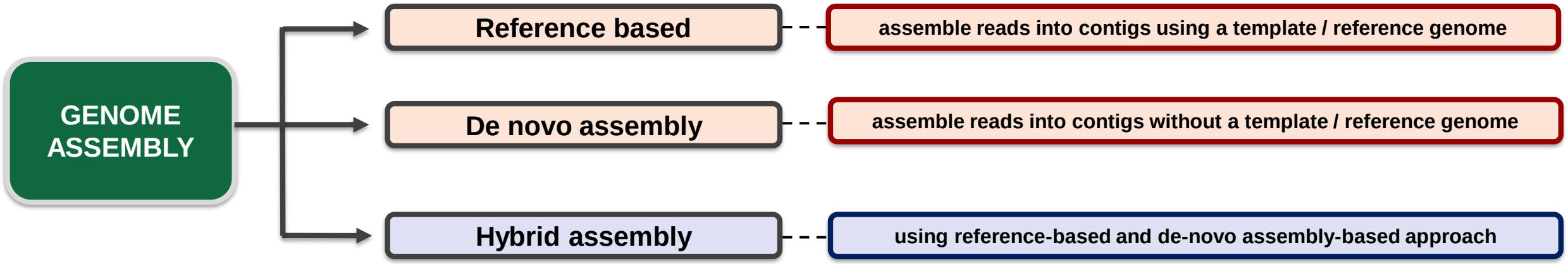

filtlong -1 illumina_1.fastq.gz -2 illumina_2.fastq.gz --min_length 1000 --keep_percent 90 --target_bases 500000000 --trim --split 500 input.fastq.gz | gzip > output.fastq.gz

# GENOME ASSEMBLY

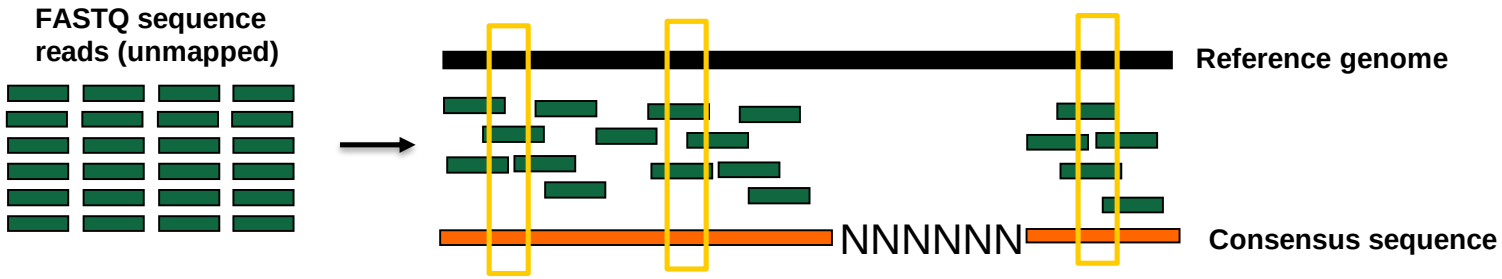
Assembly



# GENOME ASSEMBLY




## REFERENCE BASED ASSEMBLY




**TOOLS USED**

1) **Burrows-Wheeler aligner (short reads)**
   https://bio-bwa.sourceforge.net/
2) **MiniMap2 (long reads)**
   https://github.com/lh3/minimap2

# GENOME ASSEMBLY

**GENOME ASSEMBLY**

- **Reference based** — assemble reads into contigs using a template / reference genome
- **De novo assembly** — assemble reads into contigs without a template / reference genome
- **Hybrid assembly** — using reference-based and de-novo assembly-based approach

## De novo ASSEMBLY

1. Fragment DNA and sequence it
2. Identify overlaps
3. Assemble overlaps into contigs
4. Assemble contigs into scaffolds
5. scaffolds → assembly

## Assembly tools

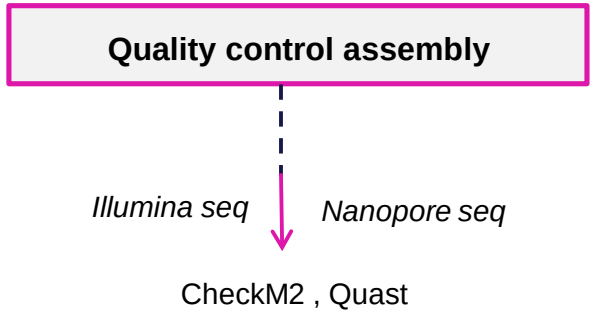
**Short read assembly**

- SPAdes
- SKESA

**Long read assembly**

- Flye (OLC-style repeat graph)
- Canu

# ASSEMBLY QUALITY ASSESSMENT




## CheckM2 - GitHub

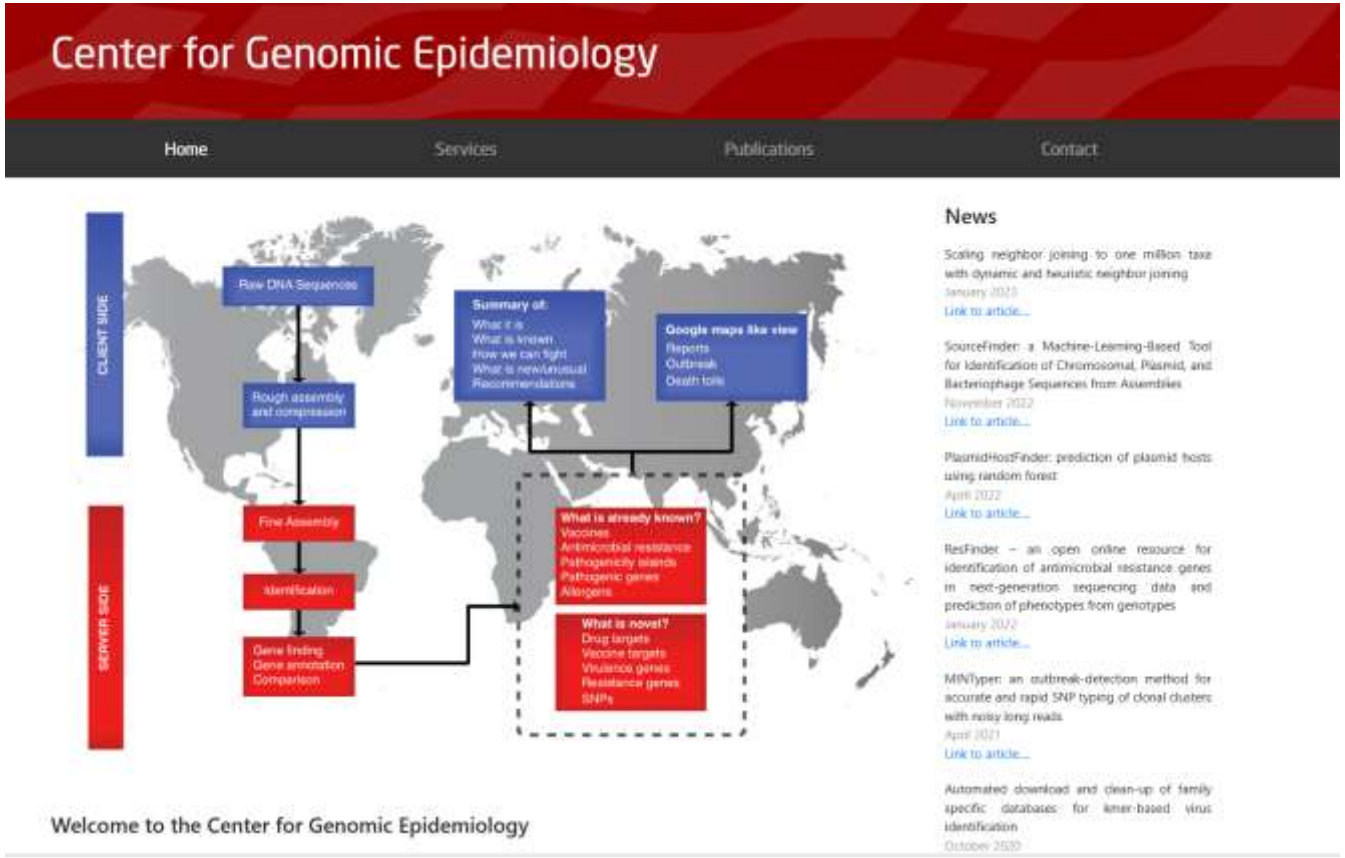
- Assess the quality of genome assembly – specifically completeness and contamination
- >90% completeness ; <5% contamination

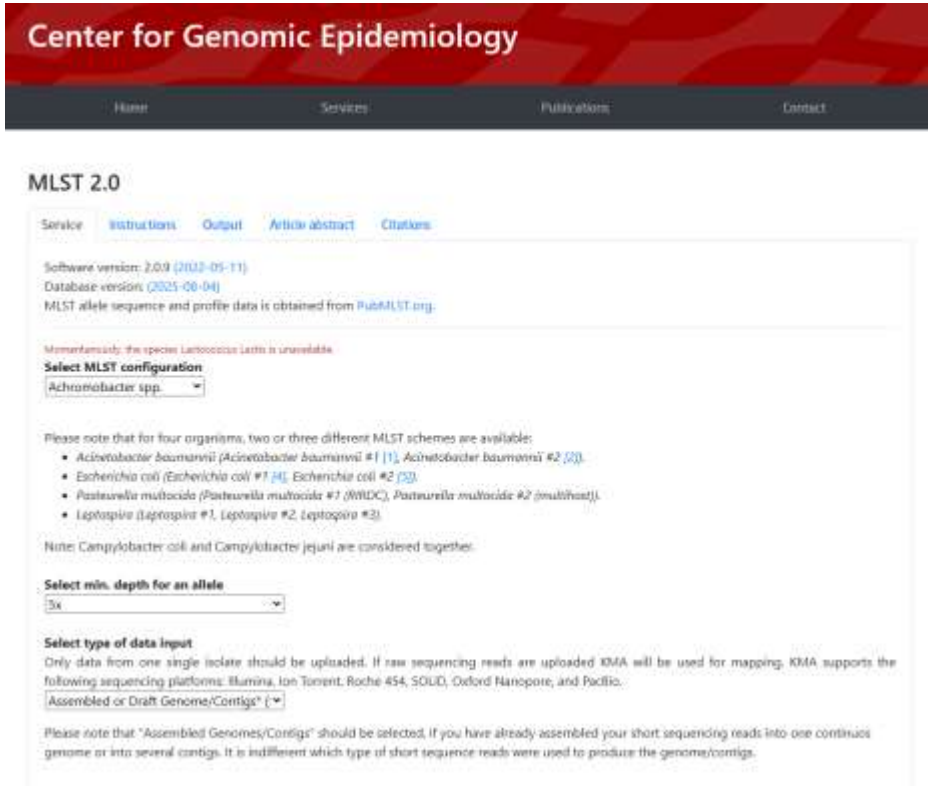
## QUAST - manual

- Provides comprehensive metrics and visual reports to check assembly quality
- Suitable for *de novo* short-read, long-read and hybrid assembly

**Let's look at how it works and the output!**

# MLST and cgMLST




https://cge.food.dtu.dk/services/MLST/

# Scenario

| Species | Date | Region | Travel | MLST | Sequence | Carba genotype (PCR) |
|---|---|---|---|---|---|---|
| E. coli | 2015 | Copenhagen | Pakistan | ST410 | Ec001 | OXA |
| E. coli | 2015 | Copenhagen | Thailand | ST410 | Ec002 | OXA |
| E. coli | 2015 | Jutland - M | India | ST410 | Ec003 | NDM |
| E. coli | 2015 | Copenhagen | Lebanon | Missing | Ec004 | OXA |
| E. coli | 2016 | Zealand | No | Missing | Ec005 | NDM, OXA |
| E. coli | 2016 | Zealand | No | ST410 | Ec006 | NDM, OXA |
| E. coli | 2017 | Copenhagen | Pakistan | ST410 | Ec007 | OXA |
| E. coli | 2018 | Jutland - N | Thailand | ST410 | Ec008 | NDM |
| E. coli | 2018 | Zealand | No | ST410 | Ec009 | NDM, OXA |
| E. coli | 2018 | Zealand | No | ST410 | Ec010 | NDM, OXA |
| E. coli | 2018 | Zealand | No | ST410 | Ec011 | NDM |
| E. coli | 2018 | Zealand | No | ST410 | Ec012 | OXA |

**Scenario**

A recent rise in cases of carbapenemase producing *E. coli* in several regional hospitals indicate one or more ongoing outbreaks, and it has been suggested that the NRL could give assistance by performing outbreak investigation by WGS. Patients involve both domestic and travel-related cases and a batch of samples has already been sequenced using Illumina sequencing platform (NextSeq). From these sequences, subtyping by MLST was performed and a selection (12 *E. coli* isolates) of the most predominant MLST (ST410) isolates has been transported to your laboratory for further analysis.

# Data Quality and SNP Calling

- Good data quality ensures reliabilitiy of your analysis
  - Poor quality sequences can rarely be used for SNP analysis

- For assembled contigs - good coverage is essential (≥30x)
- Consider the quality of your raw data (specifically phred scores)

**Preferably analyse raw reads for better resolution!**

- CSI Phylogeny SNP filtering criteria:

- SNP quality: ≥30 (Phred score, base call accuracy: 99.9%)
- SNPs with a sequence depth of <10 are removed
- A SNP is removed if it is <10 bps from the nearest SNP (Pruning)

(recombination do not reflect naturally evolved SNPs)

## CSI Phylogeny 1.4 (Call SNPs & Infer Phylogeny)

CSI Phylogeny calls SNPs, filters the SNPs, does site validation and infers a phylogeny based on the concatenated alignment of the high quality* SNPs.

The service is having some issues with files compressed. Please submit all files uncompressed.

**Select min. depth at SNP positions**
10x

**Select min. relative depth at SNP positions**
10 %

**Select minimum distance between SNPs (prune)**
10 bp

**Select min. SNP quality**
30

**Select min. read mapping quality**
25

**-score**

### Input data

**Upload reference genome (fasta format)**
Note: Reference genome must not be compressed.

Vælg fil | Der er ikke valgt nogen fil

Include reference in final phylogeny.

**Select min. depth at SNP positions**
10x

**Select min. relative depth at SNP positions**
10 %

**Select minimum distance between SNPs (prune)**
10 bp

**Select min. SNP quality**
30

**Select min. read mapping quality**
25

**Select min. Z-score**
1.96

Ignore heterozygous SNPs

**Comment (to yourself)**
This comment will appear unaltered on your output page. It has no effect on the analysis

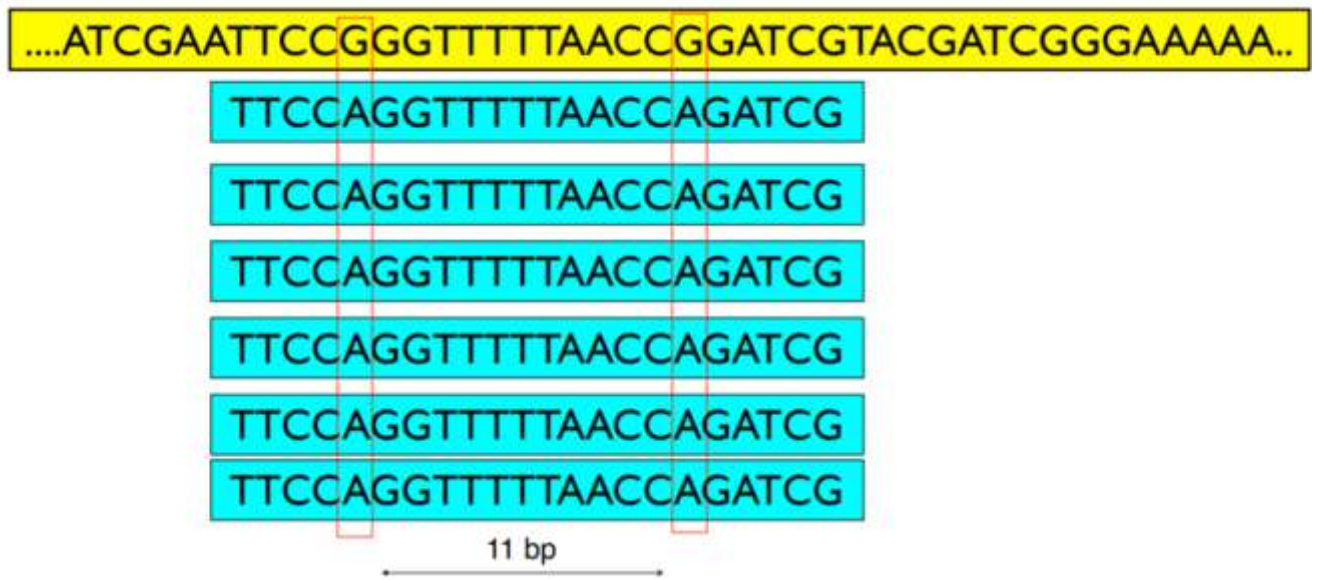
Use altered FastTree (more accurate)
Note: Read more here

**Upload read files and/or assembled genomes (fasta or**
Note: Read files must be compressed with gzip (compressed files often e
If you get an "Access forbidden. Error 403": Make sure the start of the we

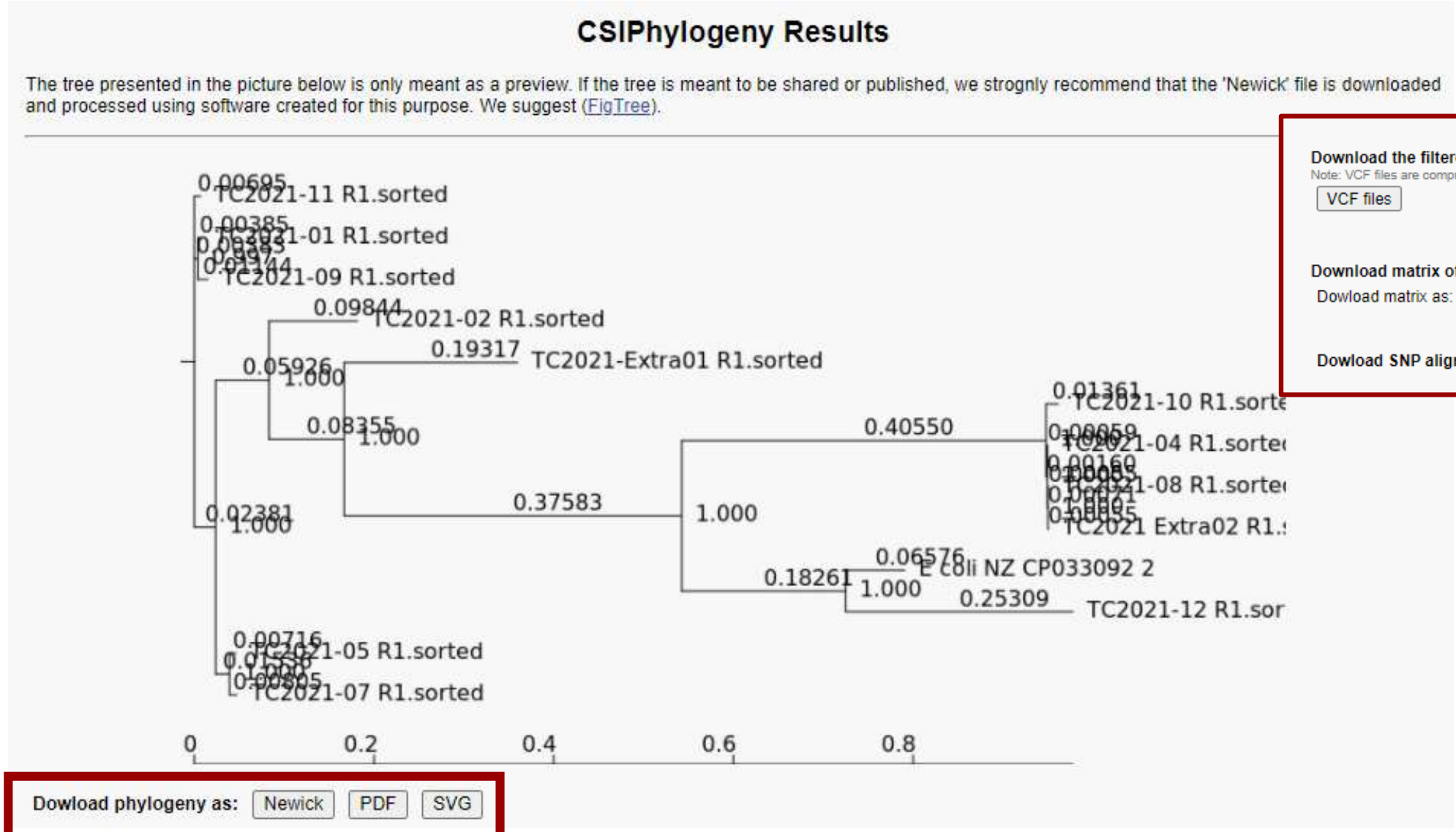
Isolate File

Name

Upload | Remove



# CSI output – web interface



# CSIPhylogeny output page

**Percentage of reference genome covered by all isolates: 71.473402371081**
3504699 positions was found in all analyzed genomes.
Size of reference genome: 4903501

Below is listed the number of positions that are shared and trusted between each isolate and the reference genome.

| File | Valid positions | Pct. of reference |
|---|---|---|
| TC2021-05_R1.ignored_snps | 3978591 | 81.137762590443 |
| TC2021-12_R1.ignored_snps | 4307863 | 87.852801498358 |
| TC2021-02_R1.ignored_snps | 4039549 | 82.3809151869246 |
| TC2021-01_R1.ignored_snps | 4048331 | 82.5600117140794 |
| TC2021-09_R1.ignored_snps | 4003614 | 81.6480714493583 |
| TC2021-08_R1.ignored_snps | 4101898 | 83.6524352702284 |
| TC2021-10_R1.ignored_snps | 4117054 | 83.9615205543957 |
| TC2021-Extra01_R1.ignored_snps | 3985371 | 81.2760311459098 |
| TC2021-07_R1.ignored_snps | 4048219 | 82.5577276317472 |
| E_coli_NZ_CP033092_2.ignored_snps | 4903501 | 100 |
| TC2021-11_R1.ignored_snps | 3986463 | 81.2983009486487 |
| TC2021-04_R1.ignored_snps | 4142652 | 84.4835557288558 |
| TC2021_Extra02_R1.ignored_snps | 4067475 | 82.9504266441467 |

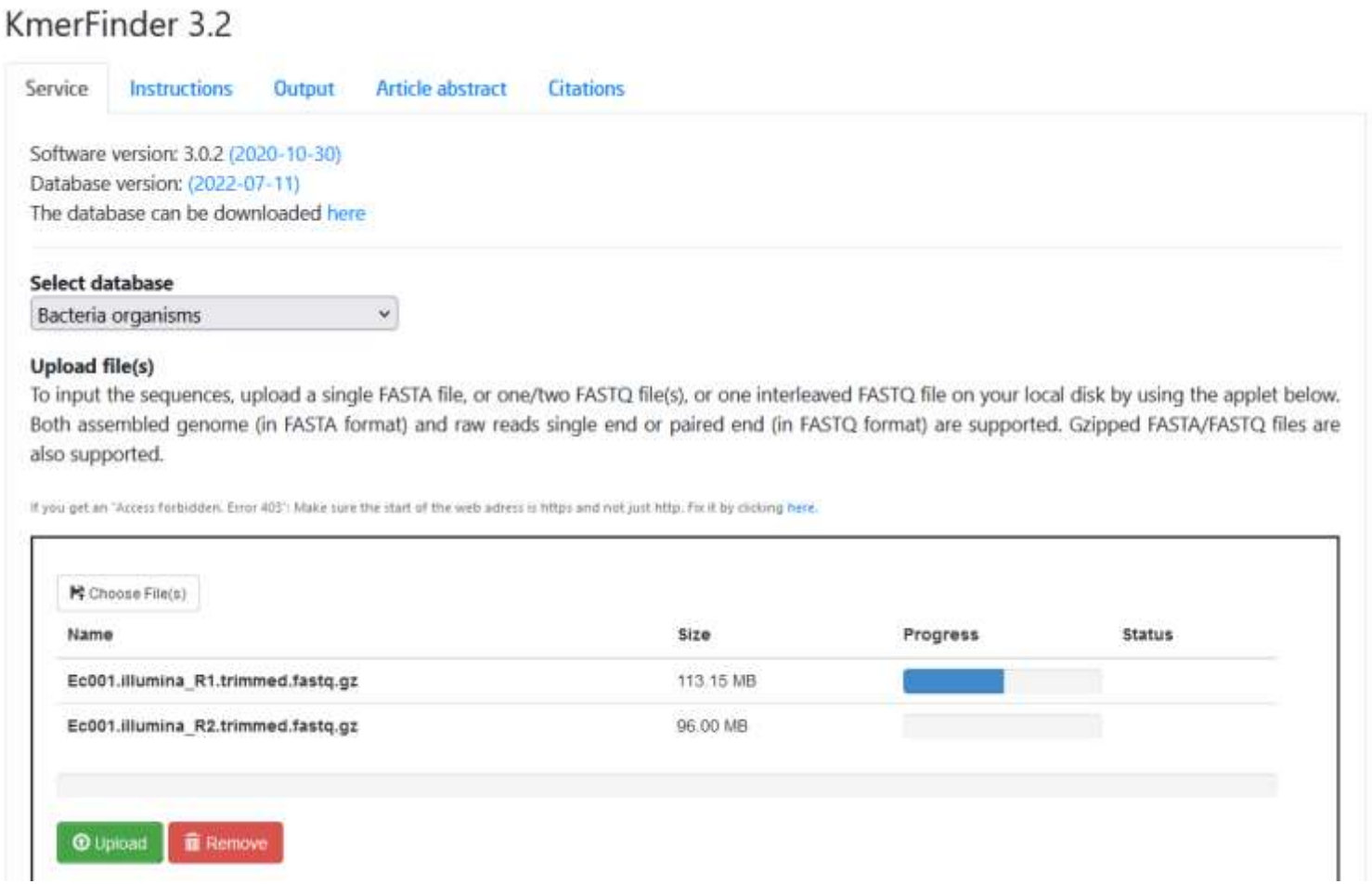
# How to choose a reference genome

- The reference should be somewhat similar to the isolates you test
  – You can use an internal reference in your collection

- Better described (annotated strain)
  – Search for something similar in kmerFinder

- The more distant your reference is from the dataset you analyse, the less bases you will build the SNP analysis on
  – -> false lower number of SNPs if you choose a bad reference

# KmerFinder –species ID and contamination



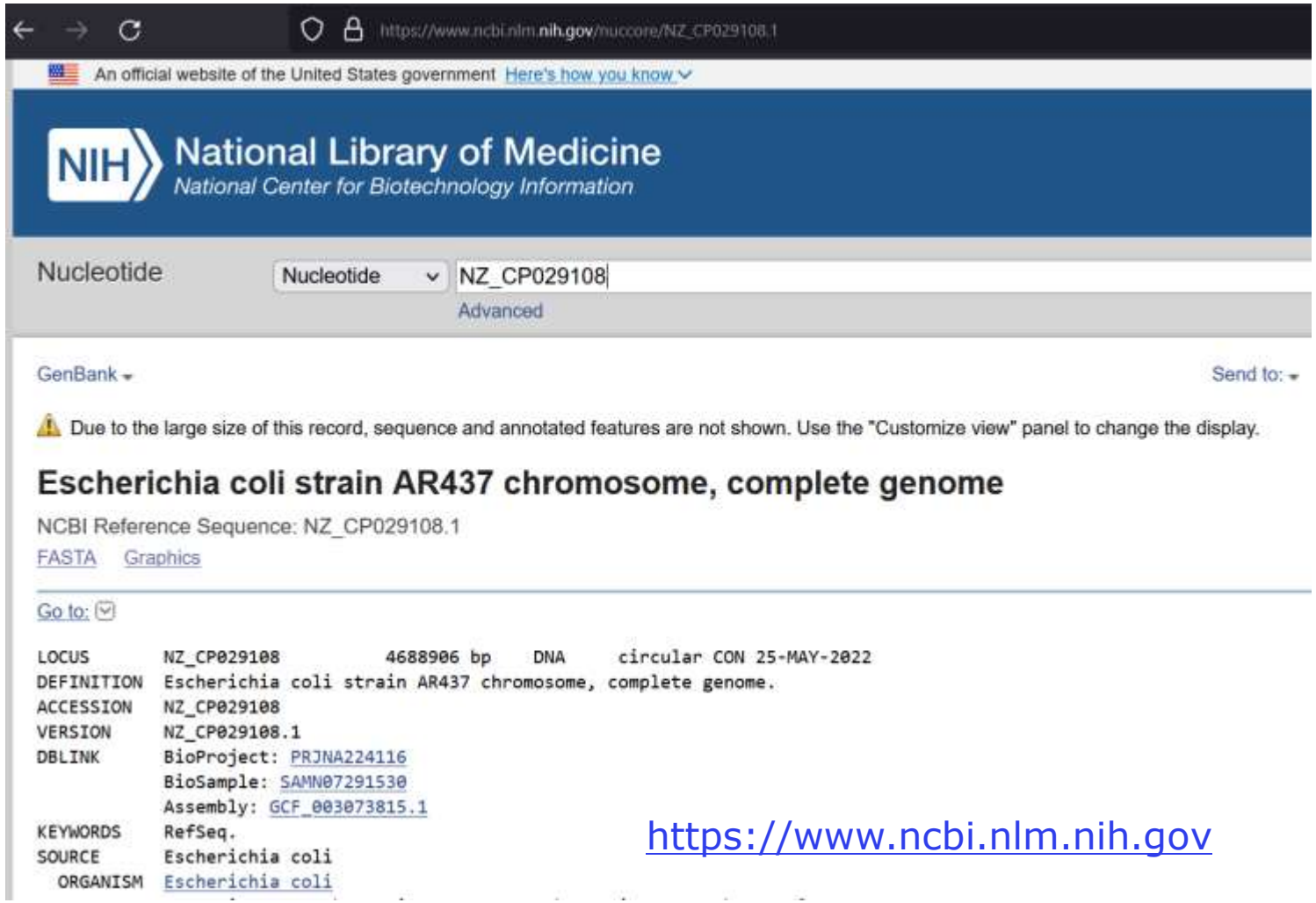
# KmerFinder – find a reference

## KmerFinder-3.2 Server - Results

### KmerFinder 3.2 results:

| Template | Num | Score | Expected | Template_length | Query_Coverage | Template_Coverage | Depth | tot_query_Coverage | tot_template |
|---|---|---|---|---|---|---|---|---|---|
| NZ_CP029108.1 Escherichia coli strain AR437 chromosome, complete genome | 14538 | 7191229 | 231 | 154903 | 82.45 | 99.04 | 46.42 | 82.45 | 99.04 |
| NZ_CP018991.1 Escherichia coli strain Ecol_AZ146 chromosome, complete genome | 18701 | 168049 | 2651 | 181206 | 1.93 | 3.19 | 0.93 | 49.86 | 51.43 |
| NZ_CP083869.1 Escherichia coli strain NDM6 chromosome, complete genome | 24430 | 68824 | 2318 | 156510 | 0.79 | 1.20 | 0.44 | 64.63 | 76.67 |
| NZ_CP080139.1 Escherichia coli strain PK8241 chromosome, complete genome | 2178 | 32981 | 2655 | 184405 | 0.38 | 1.21 | 0.18 | 65.23 | 68.71 |
| NZ_CP031653.1 Escherichia coli strain UK_Dog_Liverpool chromosome, complete genome | 9127 | 27836 | 2406 | 161066 | 0.32 | 1.00 | 0.17 | 81.94 | 95.45 |
| NC_011586.2 Acinetobacter baumannii AB0057, complete genome | 18517 | 6592 | 2266 | 152543 | 0.08 | 1.98 | 0.04 | 0.54 | 2.13 |

# Download reference




https://www.ncbi.nlm.nih.gov

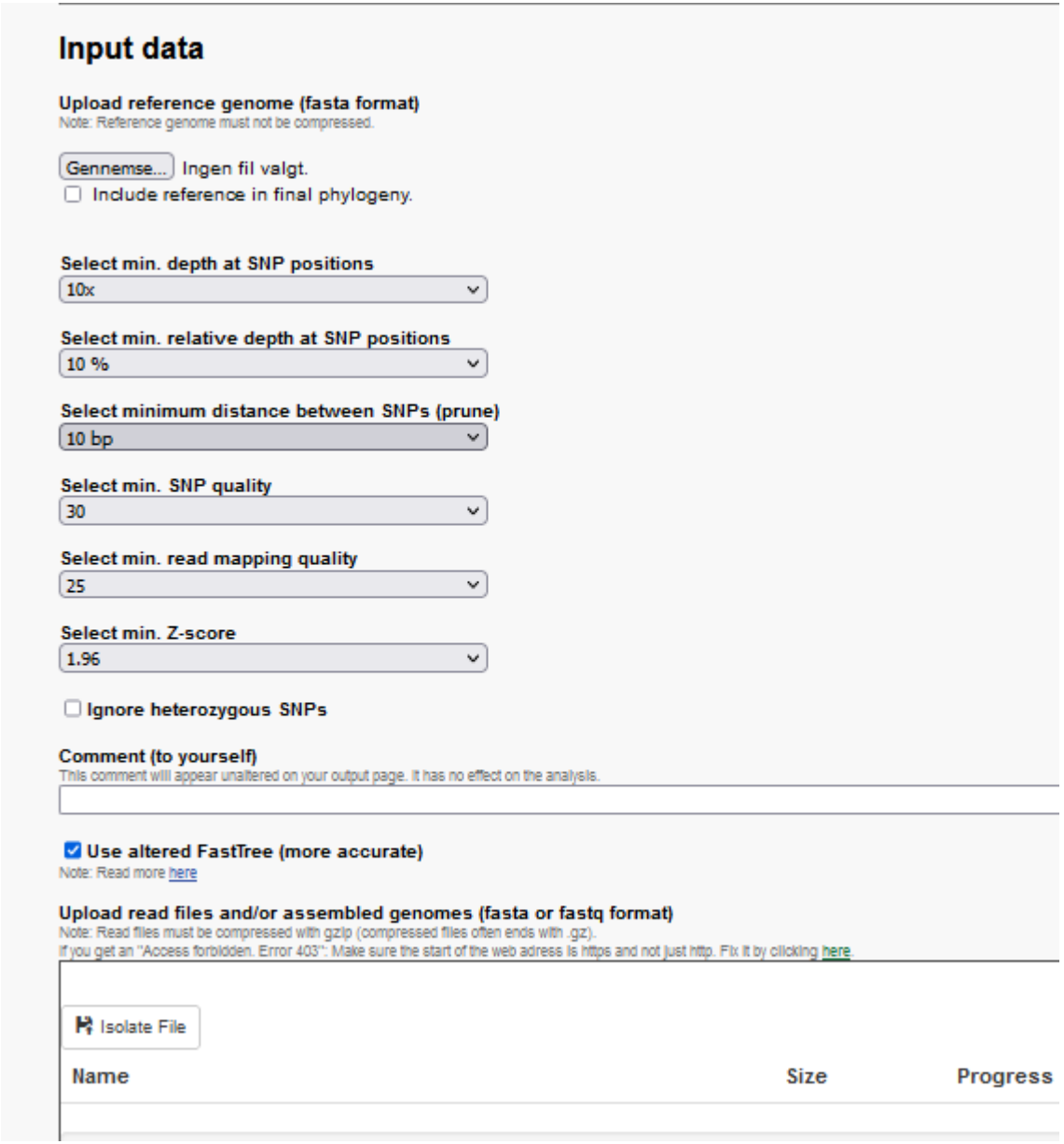
# Guidelines – how to get started

- Iterative process – what should be included?
  - Inclusion criteria
    - Time/origin/other epi-data
    - Resistance phenotype or gene
    - Sub-typing
  - Pre-analysis - Fast check for overview
    - Include all isolates
    - Consider choice of reference & settings of tool
    - Fasta files
  - Refine analysis
    - Exclude most distant isolates
    - Run analysis on fastq files

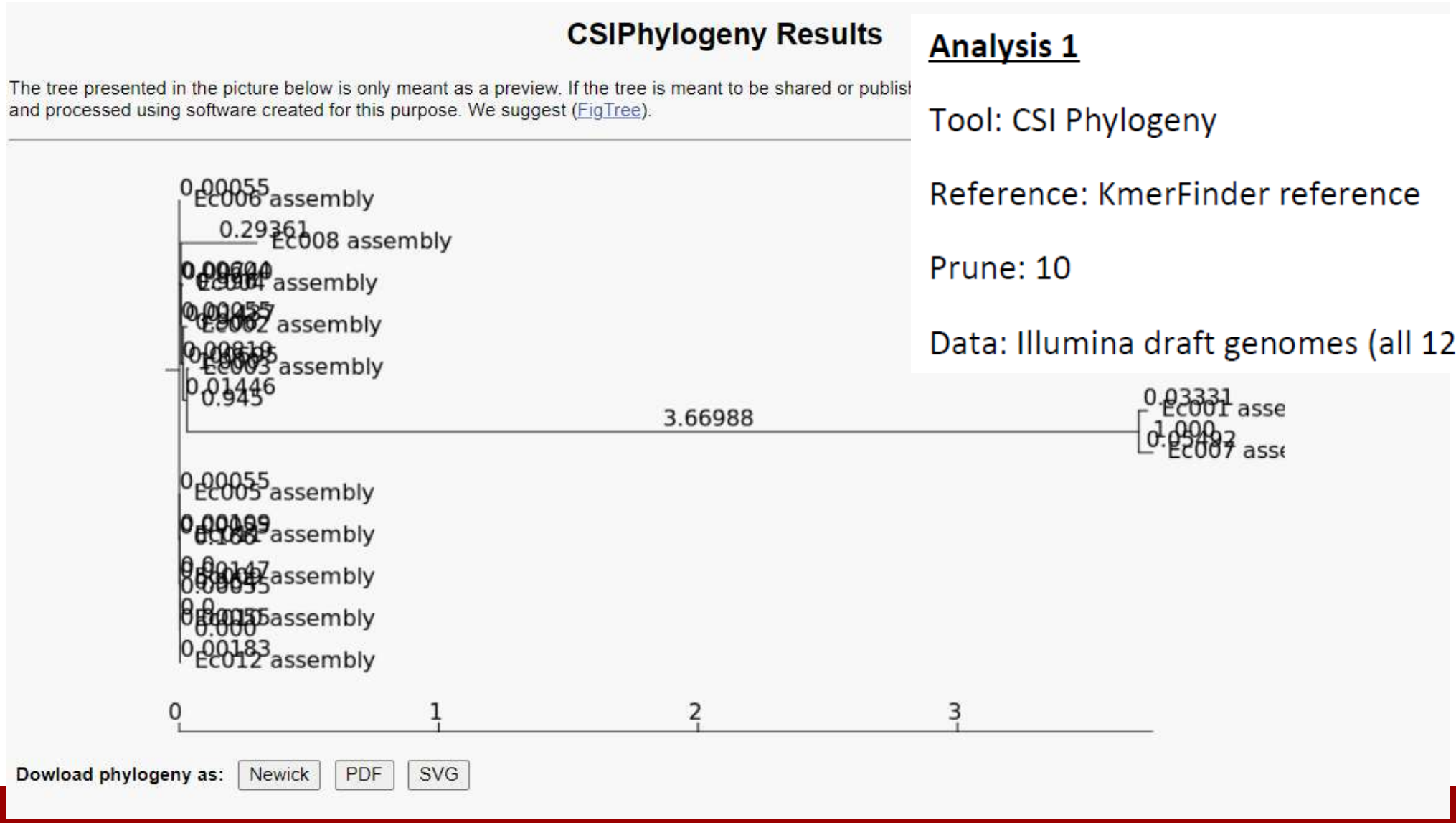
# Pre-analysis

- All strains

- Fasta files – faster analysis
- Test of pruning 10 vs 100



# Analysis 1 output: Illumina assembly




**Analysis 1**

Tool: CSI Phylogeny

Reference: KmerFinder reference

Prune: 10

Data: Illumina draft genomes (all 12 isolates)

# Analysis 1 output: Illumina assembly

**Percentage of reference genome covered by all isolates: 90.018466029857**
4383433 positions was found in all analyzed genomes.
Size of reference genome: 4869482

Below is listed the number of positions that are shared and trusted between each isolate and the reference genome.

| File | Valid positions | Pct. of reference |
|---|---|---|
| Ec002_assembly.ignored_snps | 4582041 | 94.0970928735336 |
| Ec003_assembly.ignored_snps | 4593110 | 94.324406579591 |
| Ec001_assembly.ignored_snps | 4612609 | 94.7248393155576 |
| Ec009_assembly.ignored_snps | 4544249 | 93.3209938962707 |
| Ec005_assembly.ignored_snps | 4607895 | 94.6280323040521 |
| Ec004_assembly.ignored_snps | 4607871 | 94.6275394384865 |
| Ec008_assembly.ignored_snps | 4578451 | 94.0233683993493 |
| Ec007_assembly.ignored_snps | 4622929 | 94.936771508756 |
| Ec011_assembly.ignored_snps | 4567232 | 93.792974283507 |
| Ec012_assembly.ignored_snps | 4586595 | 94.1906141146019 |
| Ec006_assembly.ignored_snps | 4610605 | 94.6836850408319 |
| Ec010_assembly.ignored_snps | 4570946 | 93.8692452297801 |

# Visualisation of tree (newick file)


**We cannot determine clustering based on the visual tree only**

# SNP matrix – pairwise comparison of SNPs

A1 prune 10

| | Ec001_assembly/1-2735 | Ec002_assembly/1-2735 | Ec003_assembly/1-2735 | Ec004_assembly/1-2735 | Ec005_assembly/1-2735 | Ec006_assembly/1-2735 | Ec007_assembly/1-2735 | Ec008_assembly/1-2735 | Ec009_assembly/1-2735 | Ec010_assembly/1-2735 | Ec011_assembly/1-2735 | Ec012_assembly/1-2735 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ec001_assembly/1-2735 | 0 | 2176 | 2122 | 2180 | 2176 | 2176 | 216 | 2280 | 2179 | 2179 | 2182 | 2184 |
| Ec002_assembly/1-2735 | 2176 | 0 | 94 | 80 | 78 | 78 | 2212 | 644 | 81 | 81 | 84 | 86 |
| Ec003_assembly/1-2735 | 2122 | 94 | 0 | 98 | 96 | 96 | 2170 | 662 | 99 | 99 | 102 | 104 |
| Ec004_assembly/1-2735 | 2180 | 80 | 98 | 0 | 38 | 38 | 2222 | 604 | 41 | 41 | 44 | 46 |
| Ec005_assembly/1-2735 | 2176 | 78 | 96 | 38 | 0 | 2 | 2218 | 598 | 5 | 5 | 8 | 10 |
| Ec006_assembly/1-2735 | 2176 | 78 | 96 | 38 | 2 | 0 | 2218 | 598 | 5 | 5 | 8 | 10 |
| Ec007_assembly/1-2735 | 216 | 2212 | 2170 | 2222 | 2218 | 2218 | 0 | 2322 | 2221 | 2221 | 2224 | 2226 |
| Ec008_assembly/1-2735 | 2280 | 644 | 662 | 604 | 598 | 598 | 2322 | 0 | 601 | 601 | 604 | 606 |
| Ec009_assembly/1-2735 | 2179 | 81 | 99 | 41 | 5 | 5 | 2221 | 601 | 0 | 0 | 3 | 5 |
| Ec010_assembly/1-2735 | 2179 | 81 | 99 | 41 | 5 | 5 | 2221 | 601 | 0 | 0 | 3 | 5 |
| Ec011_assembly/1-2735 | 2182 | 84 | 102 | 44 | 8 | 8 | 2224 | 604 | 3 | 3 | 0 | 8 |
| Ec012_assembly/1-2735 | 2184 | 86 | 104 | 46 | 10 | 10 | 2226 | 606 | 5 | 5 | 8 | 0 |

min: 0 max: 2322

# Analysis 2: Pruning 10 vs 100

**Percentage of reference genome covered by all isolates: 90.018466029857**
4383433 positions was found in all analyzed genomes.
Size of reference genome: 4869482

Below is listed the number of positions that are shared and trusted between each isolate and the reference genome.

| File | Valid positions | Pct. of reference |
|---|---|---|
| Ec008_assembly.ignored_snps | 4578451 | 94.0233683993493 |
| Ec006_assembly.ignored_snps | 4610605 | 94.6836850408319 |
| Ec007_assembly.ignored_snps | 4622929 | 94.936771508756 |
| Ec012_assembly.ignored_snps | 4586595 | 94.1906141146019 |
| Ec010_assembly.ignored_snps | 4570946 | 93.8692452297801 |
| Ec001_assembly.ignored_snps | 4612609 | 94.7248393155576 |
| Ec011_assembly.ignored_snps | 4567232 | 93.792974283507 |
| Ec004_assembly.ignored_snps | 4607871 | 94.6275394384865 |
| Ec009_assembly.ignored_snps | 4544249 | 93.3209938962707 |
| Ec005_assembly.ignored_snps | 4607895 | 94.6280323040521 |
| Ec003_assembly.ignored_snps | 4593110 | 94.324406579591 |
| Ec002_assembly.ignored_snps | 4582041 | 94.0970928735336 |

# Different pruning (100) -> different number of SNPs

Pruning 10: min: 0 max: 2322 vs. Pruning 100: min:3 max: 516

| A2 prune 100 | Ec001_assembly/1-676 | Ec002_assembly/1-676 | Ec003_assembly/1-676 | Ec004_assembly/1-676 | Ec005_assembly/1-676 | Ec006_assembly/1-676 | Ec007_assembly/1-676 | Ec008_assembly/1-676 | Ec009_assembly/1-676 | Ec010_assembly/1-676 | Ec011_assembly/1-676 | Ec012_assembly/1-676 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ec001_assembly/1-676 | 0 | 478 | 461 | 471 | 468 | 469 | 111 | 495 | 473 | 472 | 473 | 479 |
| Ec002_assembly/1-676 | 478 | 0 | 45 | 47 | 44 | 45 | 499 | 121 | 49 | 48 | 49 | 55 |
| Ec003_assembly/1-676 | 461 | 45 | 0 | 38 | 35 | 36 | 482 | 112 | 40 | 39 | 40 | 46 |
| Ec004_assembly/1-676 | 471 | 47 | 38 | 0 | 35 | 36 | 492 | 112 | 40 | 39 | 40 | 46 |
| Ec005_assembly/1-676 | 468 | 44 | 35 | 35 | 0 | 3 | 489 | 105 | 7 | 6 | 7 | 13 |
| Ec006_assembly/1-676 | 469 | 45 | 36 | 36 | 3 | 0 | 490 | 106 | 8 | 7 | 8 | 14 |
| Ec007_assembly/1-676 | 111 | 499 | 482 | 492 | 489 | 490 | 0 | 516 | 494 | 493 | 494 | 500 |
| Ec008_assembly/1-676 | 495 | 121 | 112 | 112 | 105 | 106 | 516 | 0 | 106 | 109 | 110 | 114 |
| Ec009_assembly/1-676 | 473 | 49 | 40 | 40 | 7 | 8 | 494 | 106 | 0 | 3 | 4 | 8 |
| Ec010_assembly/1-676 | 472 | 48 | 39 | 39 | 6 | 7 | 493 | 109 | 3 | 0 | 3 | 9 |
| Ec011_assembly/1-676 | 473 | 49 | 40 | 40 | 7 | 8 | 494 | 110 | 4 | 3 | 0 | 10 |
| Ec012_assembly/1-676 | 479 | 55 | 46 | 46 | 13 | 14 | 500 | 114 | 8 | 9 | 10 | 0 |
| min: 3 max: 516 | | | | | | | | | | | | |

# Analysis 3: fasta vs fastq

**Percentage of reference genome covered by all isolates: 91.005716829839 4**
4431507 positions was found in all analyzed genomes.
Size of reference genome: 4869482

| File | Valid positions | Pct. of reference |
|---|---|---|
| Ec011.illumina_R1.trimmed.ignored_snps | 4602885 | 94.5251466172377 |
| Ec006.illumina_R1.trimmed.ignored_snps | 4649224 | 95.4767673440419 |
| Ec001.illumina_R1.trimmed.ignored_snps | 4650803 | 95.5091937910439 |
| Ec009.illumina_R1.trimmed.ignored_snps | 4557370 | 93.590447608185 |
| Ec003.illumina_R1.trimmed.ignored_snps | 4652967 | 95.5536338362068 |
| Ec005.illumina_R1.trimmed.ignored_snps | 4650928 | 95.5117607991979 |
| Ec004.illumina_R1.trimmed.ignored_snps | 4623318 | 94.9447600381314 |
| Ec012.illumina_R1.trimmed.ignored_snps | 4602711 | 94.5215733418873 |
| Ec008.illumina_R1.trimmed.ignored_snps | 4620255 | 94.8818580703245 |
| Ec010.illumina_R1.trimmed.ignored_snps | 4598637 | 94.4379094121305 |
| Ec002.illumina_R1.trimmed.ignored_snps | 4639182 | 95.2705441769782 |
| Ec007.illumina_R1.trimmed.ignored_snps | 4673824 | 95.981954548759 |

# Fasta vs fastq -> different number of SNPs

Fasta: min:3 max: 516 vs. fastq: min: 0 max 413

Remove 3 most distant strains 1,7,8

A4

| | Ec001.illumina_R1.trimmed | Ec002.illumina_R1.trimmed | Ec003.illumina_R1.trimmed | Ec004.illumina_R1.trimmed | Ec005.illumina_R1.trimmed | Ec006.illumina_R1.trimmed | Ec007.illumina_R1.trimmed | Ec008.illumina_R1.trimmed | Ec009.illumina_R1.trimmed | Ec010.illumina_R1.trimmed | Ec011.illumina_R1.trimmed | Ec012.illumina_R1.trimmed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ec001.illumina_R1.trimmed | 0 | 374 | 361 | 369 | 361 | 362 | 99 | 390 | 365 | 365 | 367 | 370 |
| Ec002.illumina_R1.trimmed | 374 | 0 | 41 | 47 | 39 | 40 | 397 | 88 | 43 | 43 | 45 | 48 |
| Ec003.illumina_R1.trimmed | 361 | 41 | 0 | 36 | 28 | 29 | 384 | 77 | 32 | 32 | 34 | 37 |
| Ec004.illumina_R1.trimmed | 369 | 47 | 36 | 0 | 34 | 35 | 392 | 83 | 38 | 38 | 40 | 43 |
| Ec005.illumina_R1.trimmed | 361 | 39 | 28 | 34 | 0 | 1 | 384 | 71 | 4 | 4 | 6 | 9 |
| Ec006.illumina_R1.trimmed | 362 | 40 | 29 | 35 | 1 | 0 | 385 | 72 | 5 | 5 | 7 | 10 |
| Ec007.illumina_R1.trimmed | 99 | 397 | 384 | 392 | 384 | 385 | 0 | 413 | 388 | 388 | 390 | 393 |
| Ec008.illumina_R1.trimmed | 390 | 88 | 77 | 83 | 71 | 72 | 413 | 0 | 75 | 75 | 77 | 80 |
| Ec009.illumina_R1.trimmed | 365 | 43 | 32 | 38 | 4 | 5 | 388 | 75 | 0 | 0 | 2 | 5 |
| Ec010.illumina_R1.trimmed | 365 | 43 | 32 | 38 | 4 | 5 | 388 | 75 | 0 | 0 | 2 | 5 |
| Ec011.illumina_R1.trimmed | 367 | 45 | 34 | 40 | 6 | 7 | 390 | 77 | 2 | 2 | 0 | 7 |
| Ec012.illumina_R1.trimmed | 370 | 48 | 37 | 43 | 9 | 10 | 393 | 80 | 5 | 5 | 7 | 0 |
| min: 0 max: 413 | | | | | | | | | | | | |

# Sum-up of analysis

- Improved analysis result by:
  - Pruning 100 instead of 10
  - Using fastq instead of fasta

# Exercise

- Instructions will be sent out today!

- Following data will be provided
  - Raw sequencing data
  - Assembled data
  - Results of the SNP analysis performed (No requirement to run CSIPhylogeny but you will be given a link to the CSIPhylogeny output page)

- Survey with questions will be sent out and must be completed by Sep 12, 2026 (23:59 CET)