Sanger sequencing and phylogenetics

MMB-114

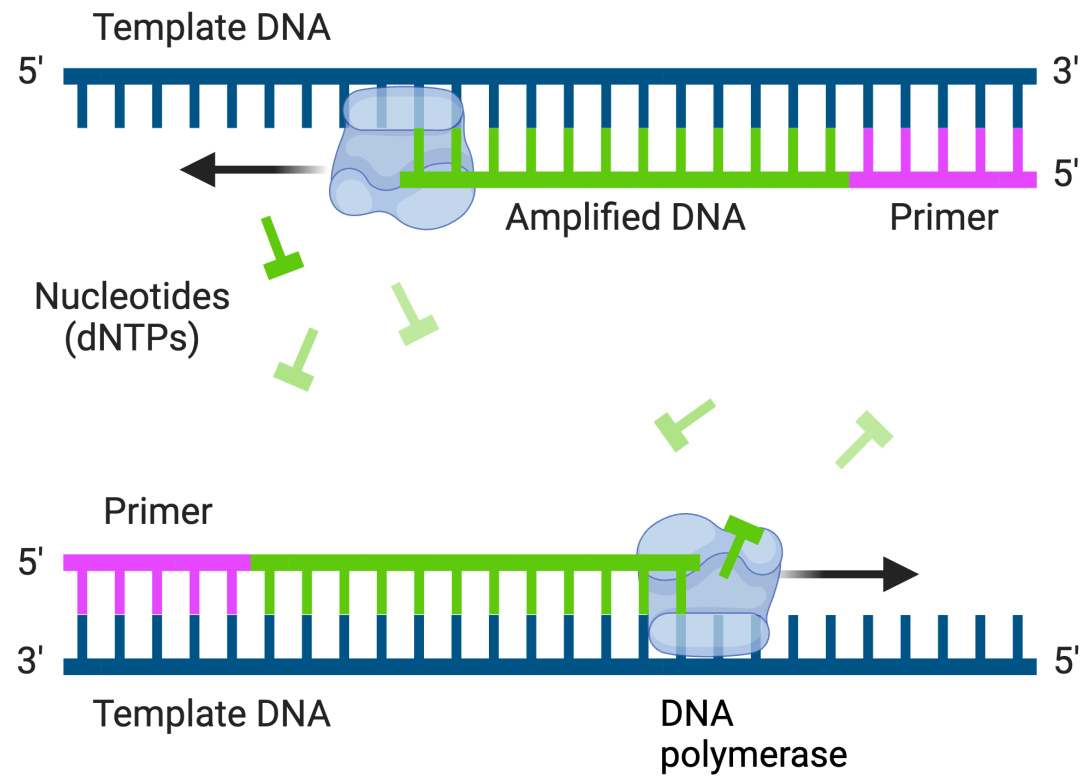## Antti Karkman

Evolution of sequencing

First generation	Second generation (next generation sequencing)	Third generation
Sanger sequencing Maxam and Gilbert Sanger chain termination	454, Solexa, Ion Torrent, Illumina	PacBio Oxford Nanopore
Infer nucleotide identity using dNTPs, then visualize with electrophoresis	High throughput from the parallelization of sequencing reactions	Sequence native DNA in real time with single-molecule resolution
500–1,000 bp fragments	~50–500 bp fragments	Tens of kb fragments, on average

Short-read sequencing | **Long-read sequencing**

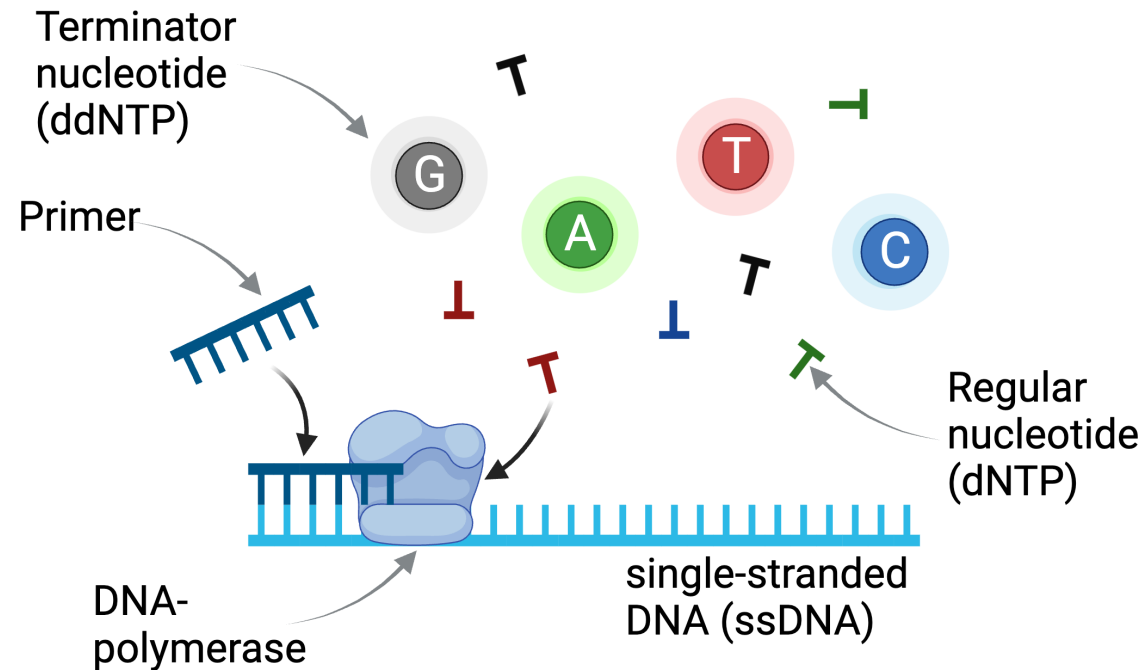https://www.pacb.com/blog/the-evolution-of-dna-sequencing-tools/
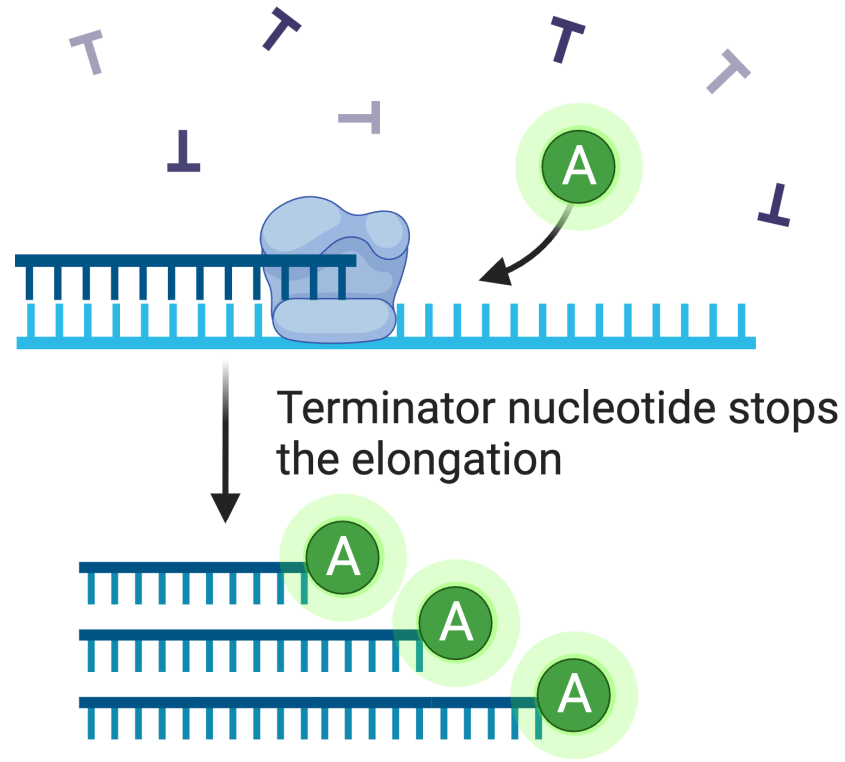
Sanger-sequencing

PCR - recap

Terminator nucleotides

- Chain termination – dideoxy nucleotides (ddNTPs)

Terminator nucleotides

Terminator nucleotide stops
the elongation

Sequencing gels

Sequencing gels
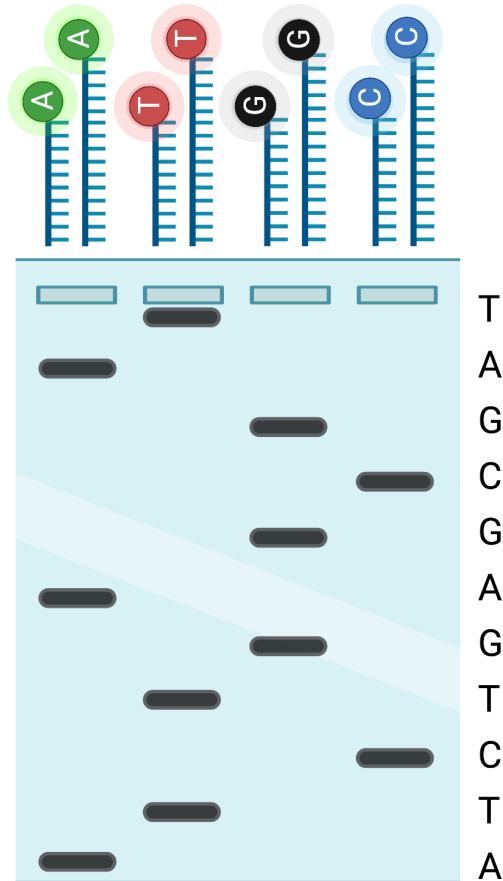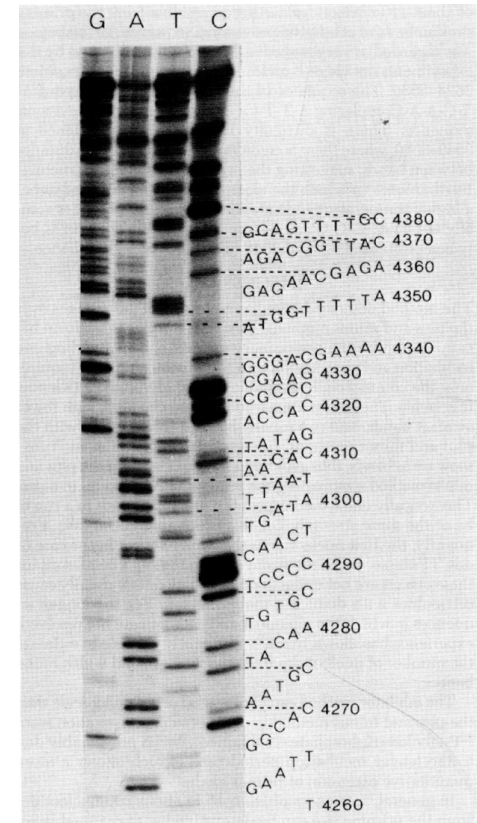
Automated Sanger-sequencing

Sequencing products

Fluorescent terminator nucleotides

Capillar electrophoresis

Detector

Laser

Nucleotide

1   10
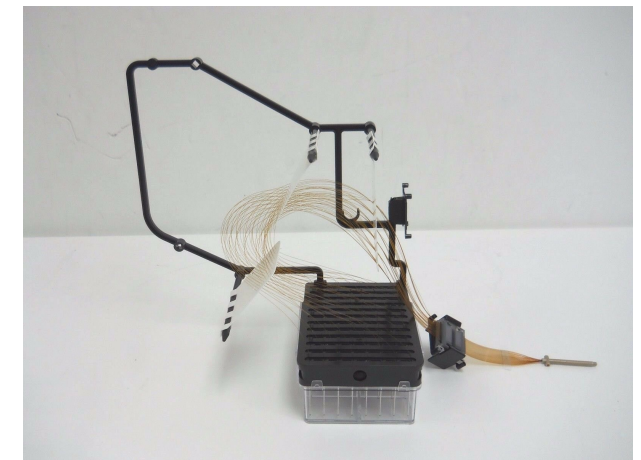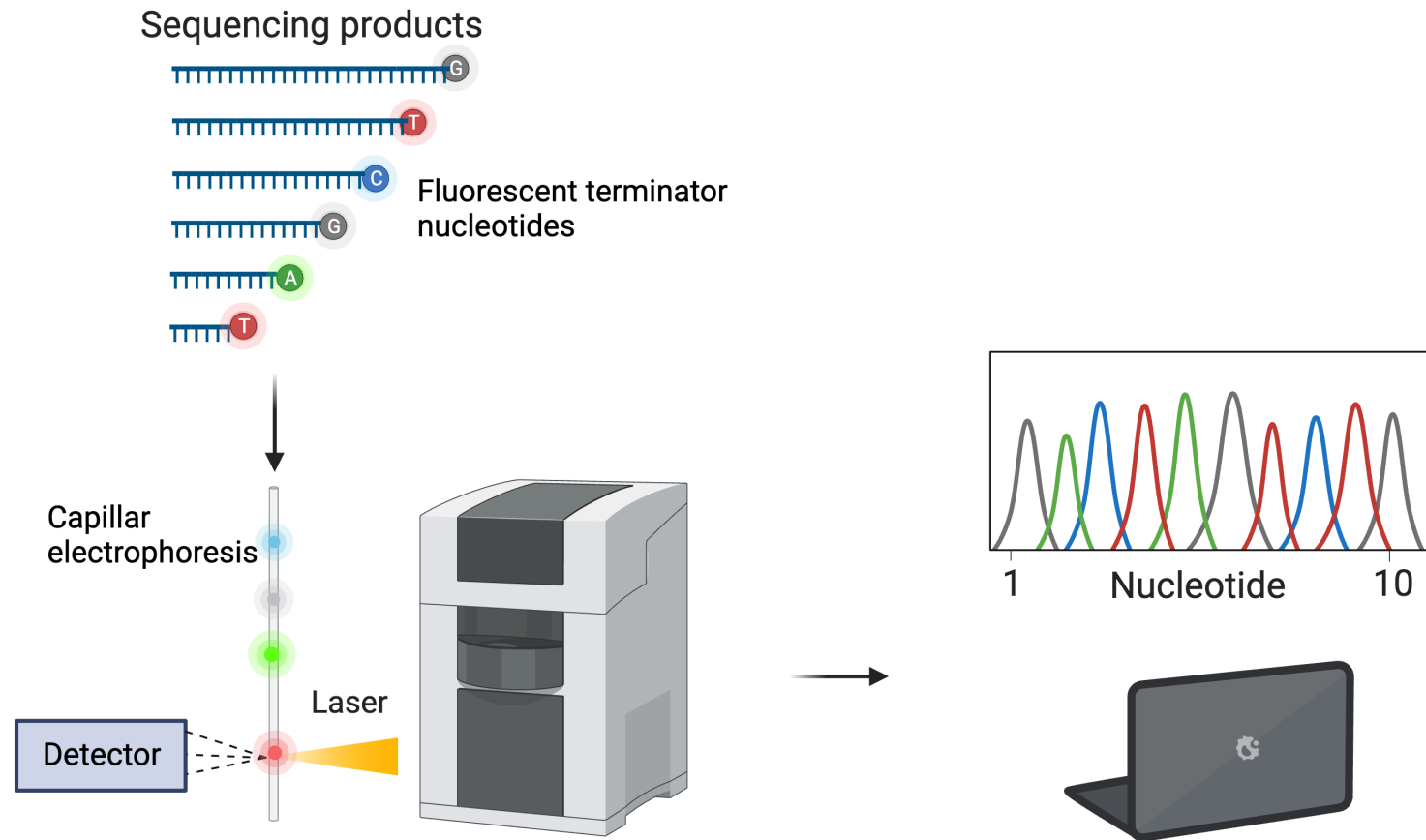
Phylogenetics

Phylogeny

- Evolutionary relationship of group of organisms

- Based on heritable traits
  - DNA sequence
  - Amino acid sequence
  - Morphology

1. Geospiza magnirostris.
2. Geospiza fortis.
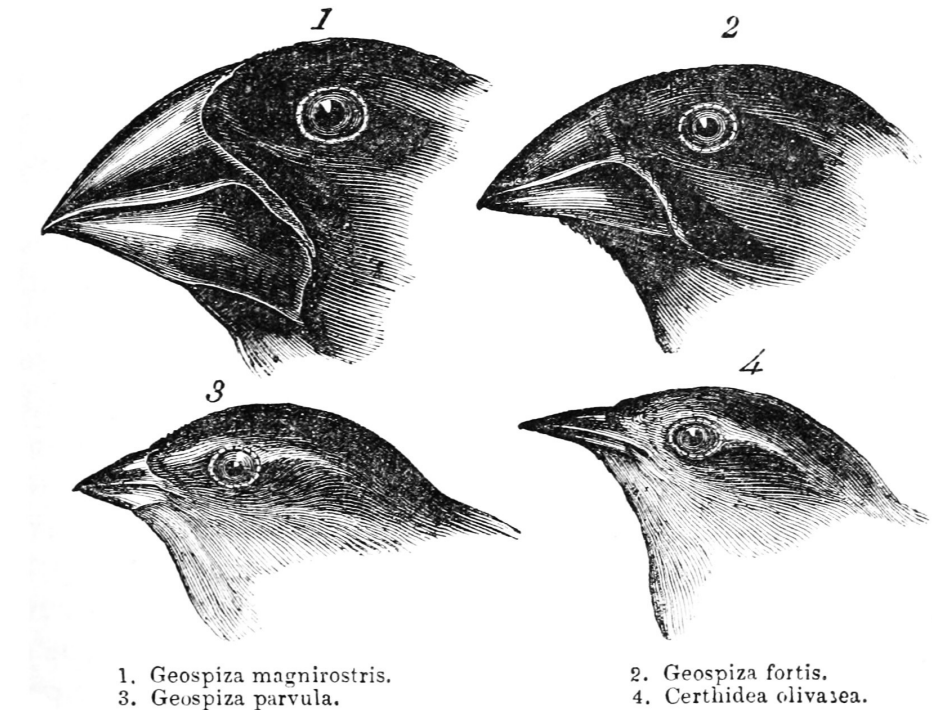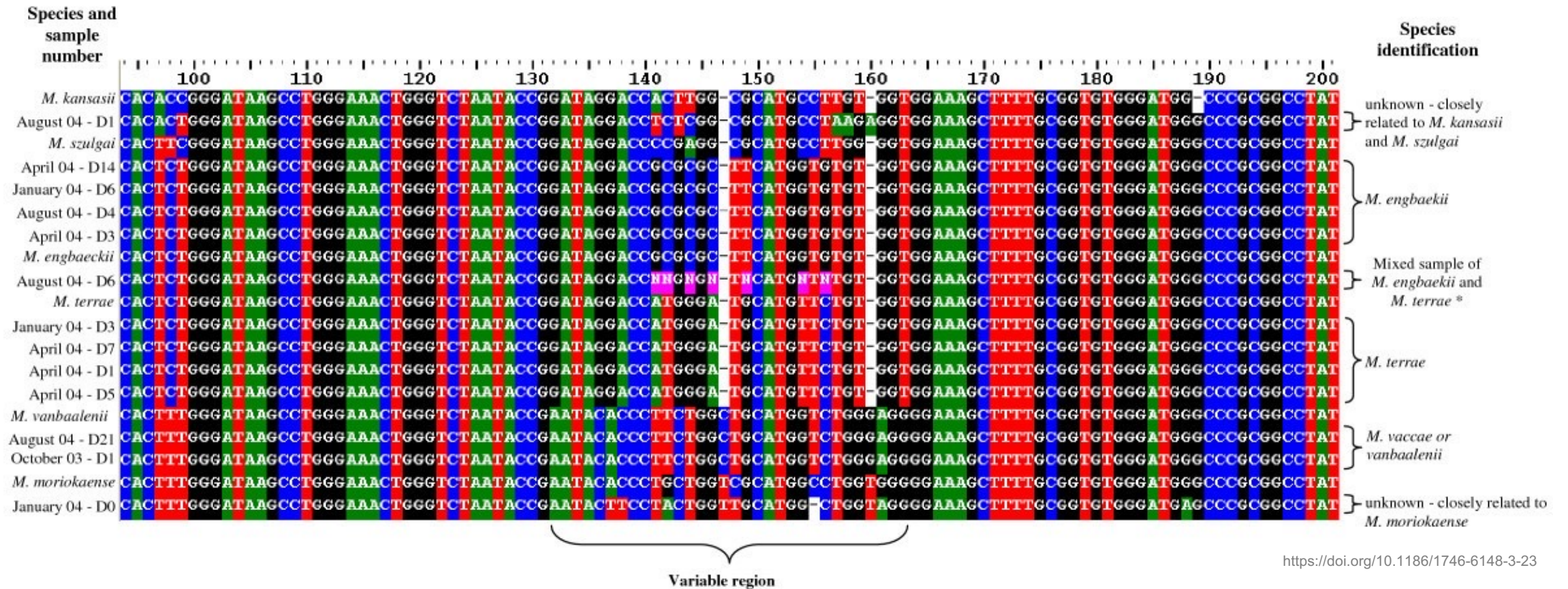3. Geospiza parvula.
4. Certhidea olivasea.

https://en.wikipedia.org/wiki/Phylogenetics#/media/File:Darwin's_finches_by_Gould.jpg

Multiple sequence alignment

Variable region

Phylogenetic trees

Bacteria

Spirochetes
Gram-positives
Proteobacteria
Cyanobacteria
Planctomyces
Bacteroides Cytophaga
Thermotoga
Aquifex

Chloroflexi

Archaea

Methanosarcina
Methanobacterium
Methanococcus
Thermococcus celer
Thermoproteus
Pyrodicticum
Haloarchaea

Eukarya

You

Entamoebae
Slime molds
Animals
Fungi
Plants
Ciliates
Flagellates
Trichomonads
Microsporidia
Diplomonads

Phylogenetic trees

Scale

Node

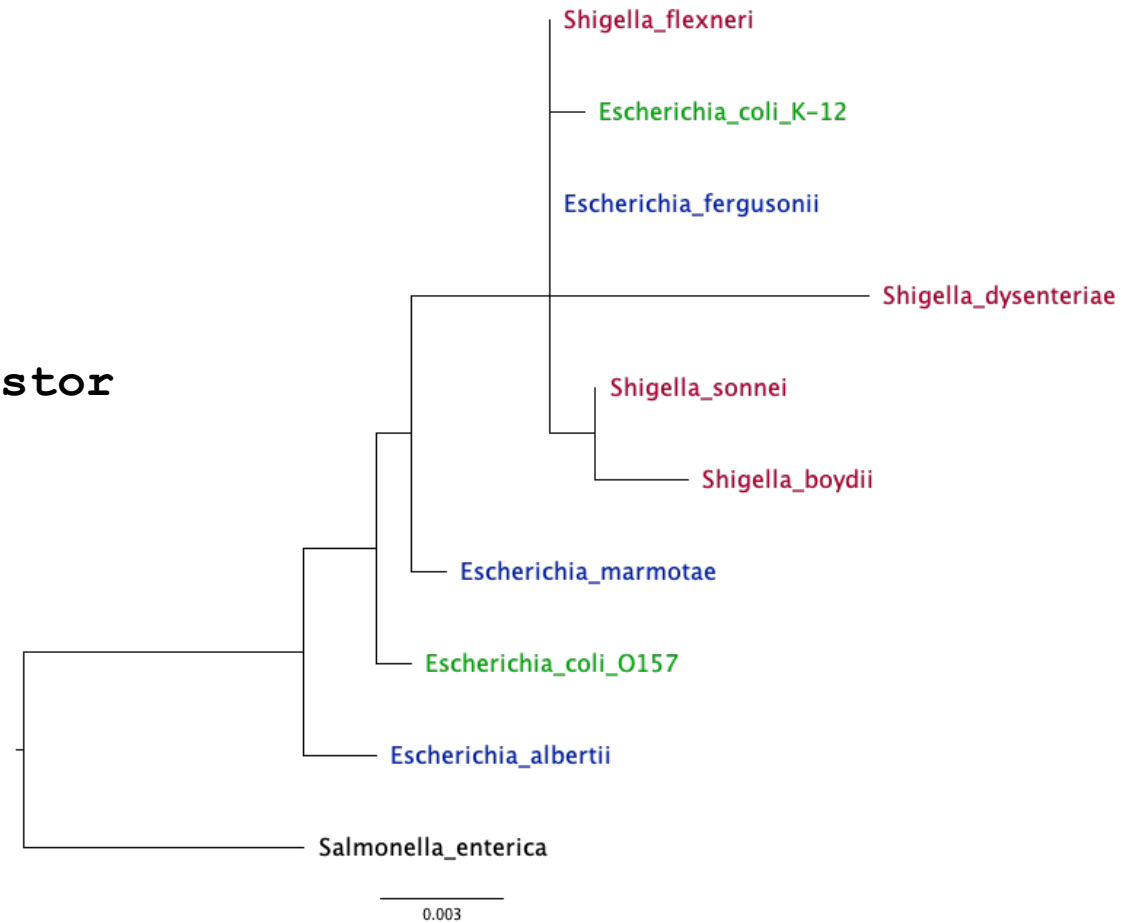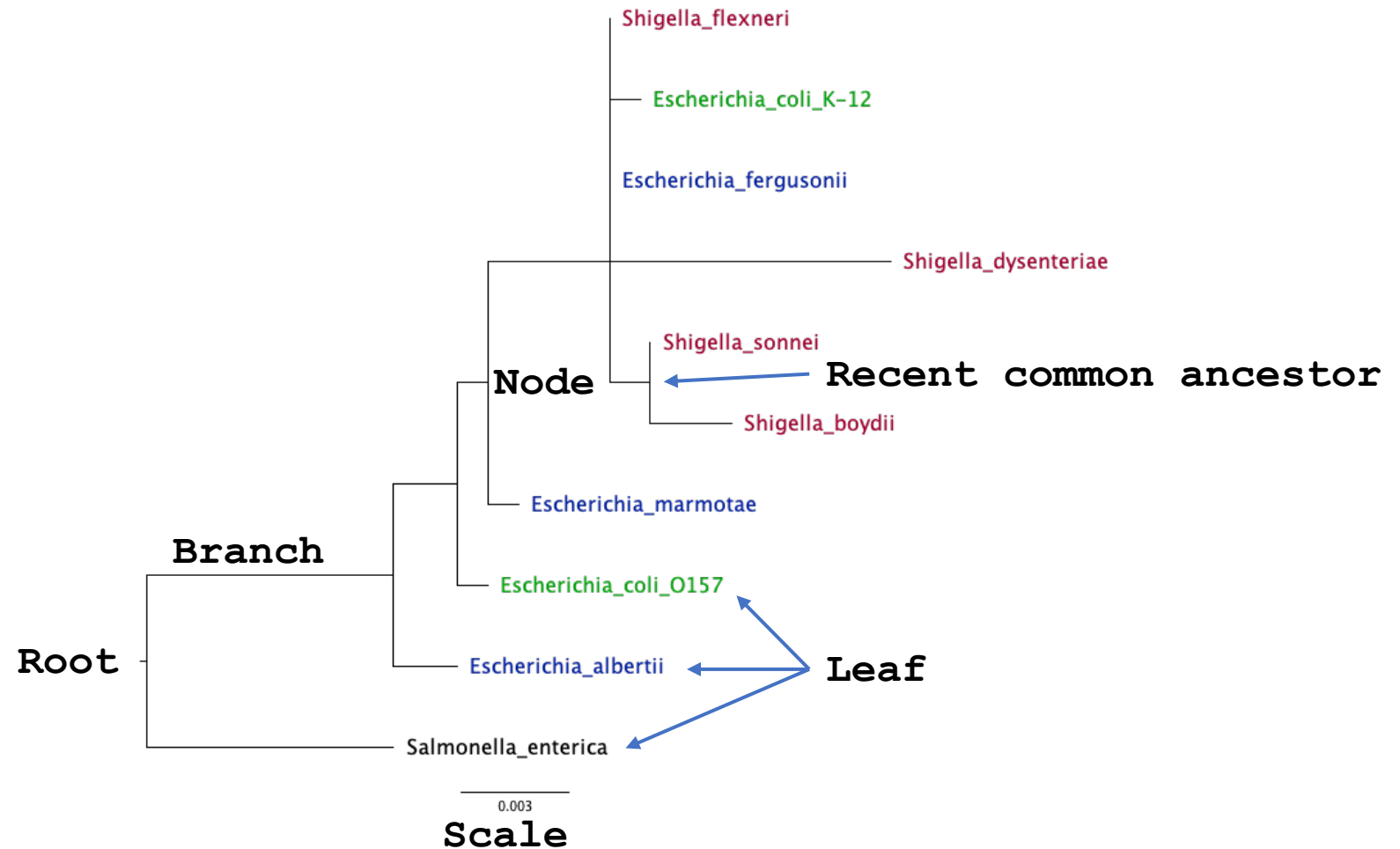**Recent common ancestor**

Root

Branch

Leaf

Phylogenetic trees

Practicals

16S rRNA gene sequence analysis and phylogenetics

Sequence data analysis

- Go to https://www.gear-genomics.com/
- Select `Teal` and upload your own sequences (`.ab1`) one at a time
  - Inspect how much of bad sequencing data needs to be removed from both ends
  - Can you find heterozygous positions from your data
- Select `Pearl` and upload both sequence files
  - Inspect how much the software clipped from both ends
  - How did it resolve possible heterozygous positions
  - Download the sequence: `Save user sequence (FA)`

Taxonomic annotation and close relatives

- Go to https://blast.ncbi.nlm.nih.gov/
  - Paste your sequence
  - Select the `rRNA/ITS databases` and `16S ribosomal`…
  - Hit `BLAST`
  - From the results select 10-20 closely related species that you want to add to your tree. You can also select outgroup sequences, if you can find some more distantly related sequences
  - Download the results
  - **Add your own sequence to the file**

Construct phylogenetic tree

- Go to https://www.arb-silva.de/
- Select `Browser`
  - Find suitable outgroup sequences. Add them to you cart.
  - When finished, download the cart and add the sequences to your own collection (fasta without gaps).
  - Put the furthest outgroup sequence first.

- Select `Search` → `Sequence Search`
  - Paste your sequence and click the `Compute tree` tab
  - Use the defaults, or change if you like
  - Name your job and click `Run tool`
  - When the job finishes, select it from the list and download the results

Modify the tree

- Go to https://itol.embl.de/
  - Upload your tree and maybe give it a name
  - Re-root the tree from your furthest outgroup, if needed
  - Change the names and modify the tree as you like.
  - When finished, export the tree as PNG (or as SVG if you want to further modify it)