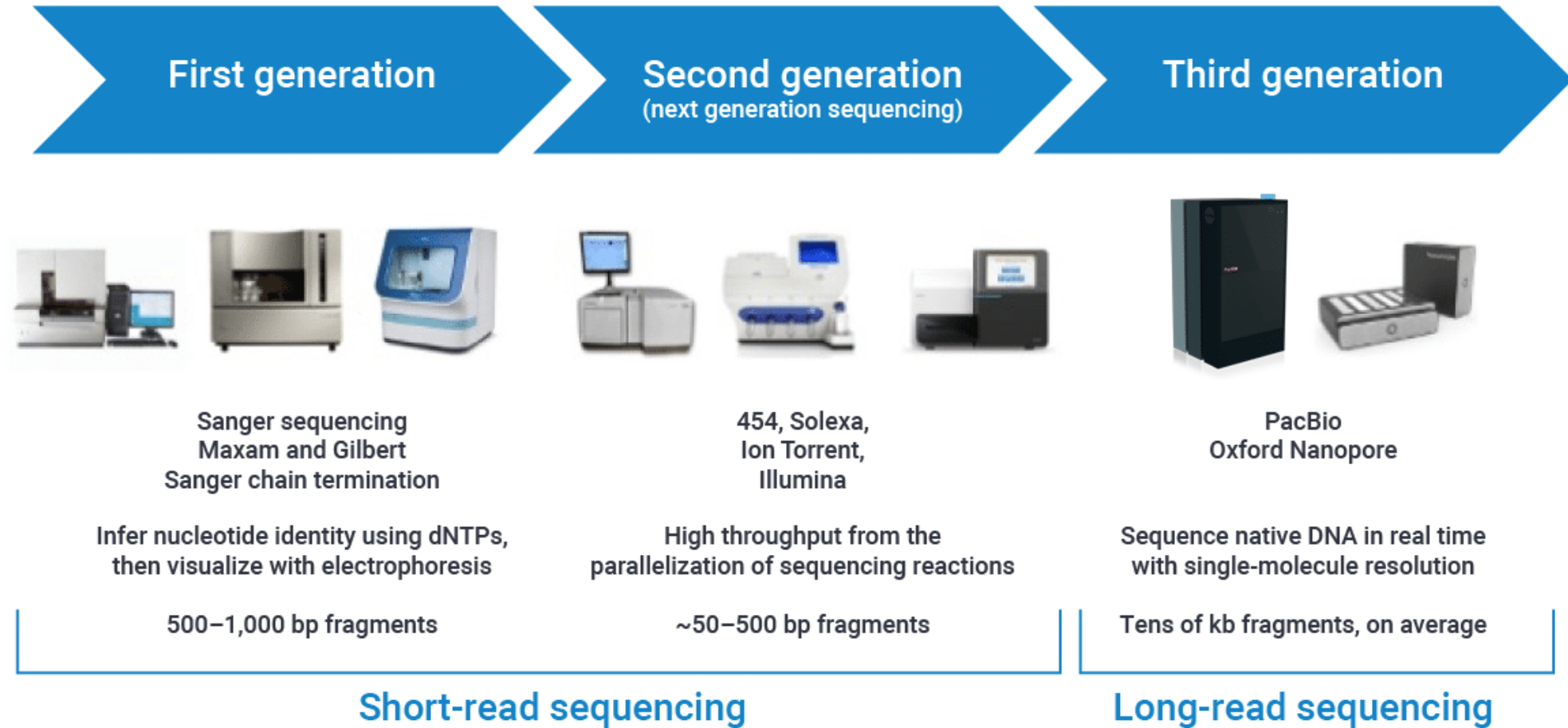


# **Sanger sequencing and phylogenetics**

**MMB-114**

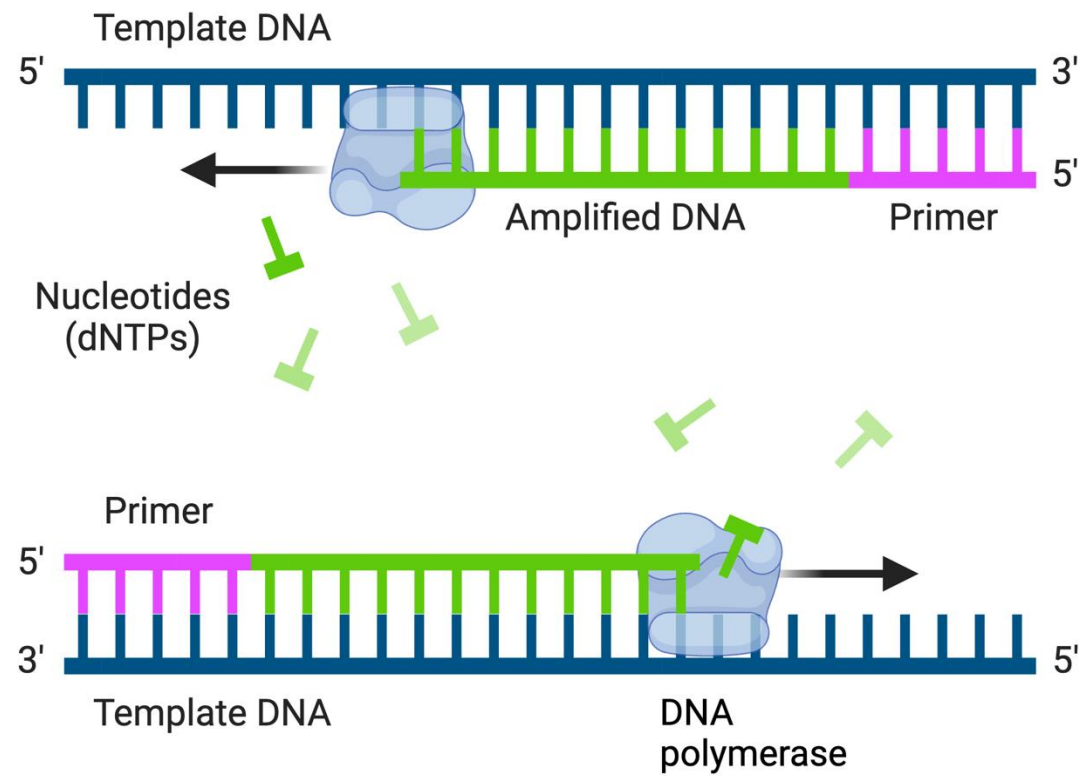
# Evolution of sequencing



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

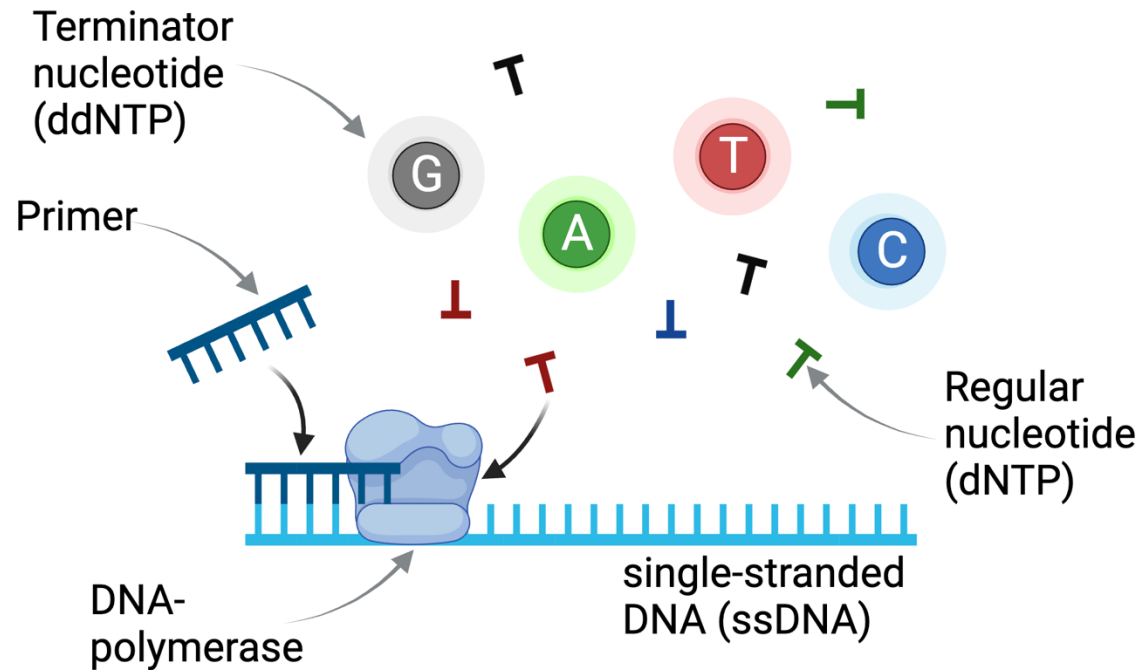
# **Sanger-sequencing**

# PCR - recap

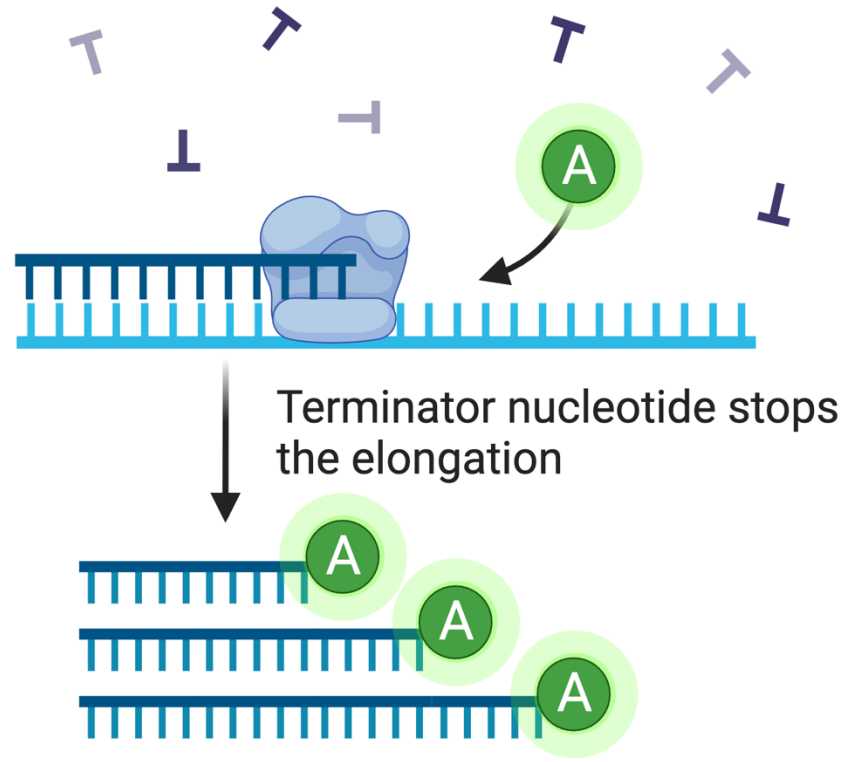


# Terminator nucleotides

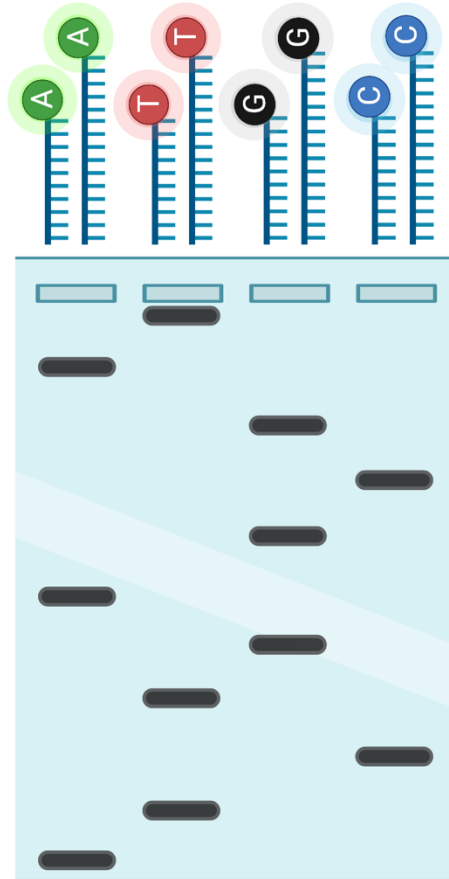
- Chain termination – dideoxy nucleotides (ddNTPs)



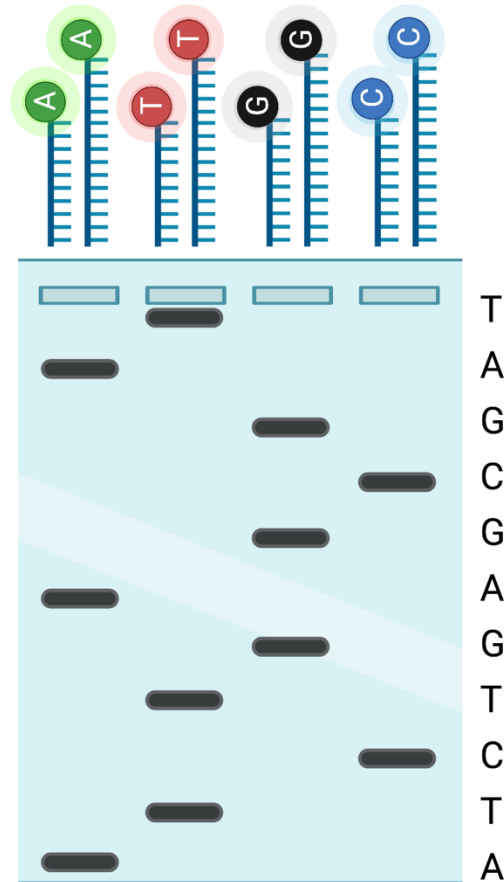
# Terminator nucleotides



# Sequencing gels



# Sequencing gels

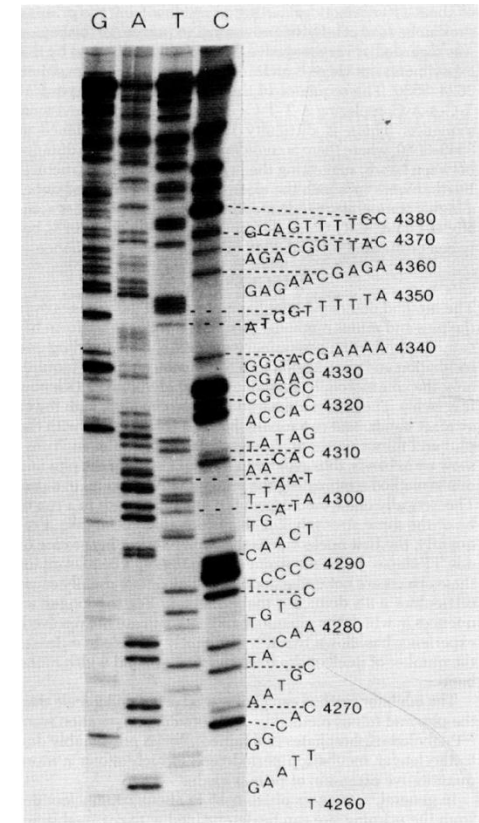


*Proc. Natl. Acad. Sci. USA*  
Vol. 74, No. 12, pp. 5463-5467, December 1977  
Biochemistry

## DNA sequencing with chain-terminating inhibitors

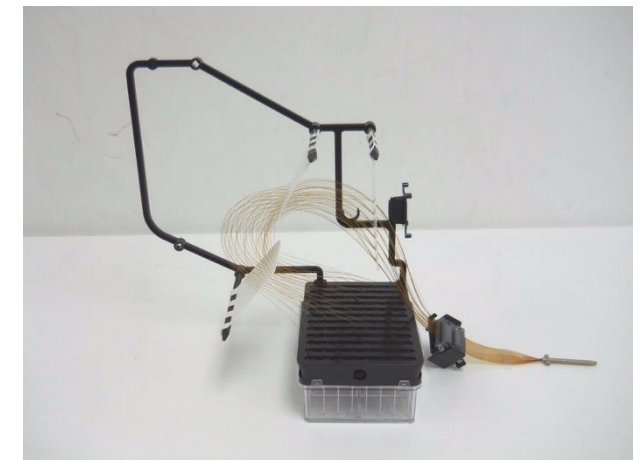
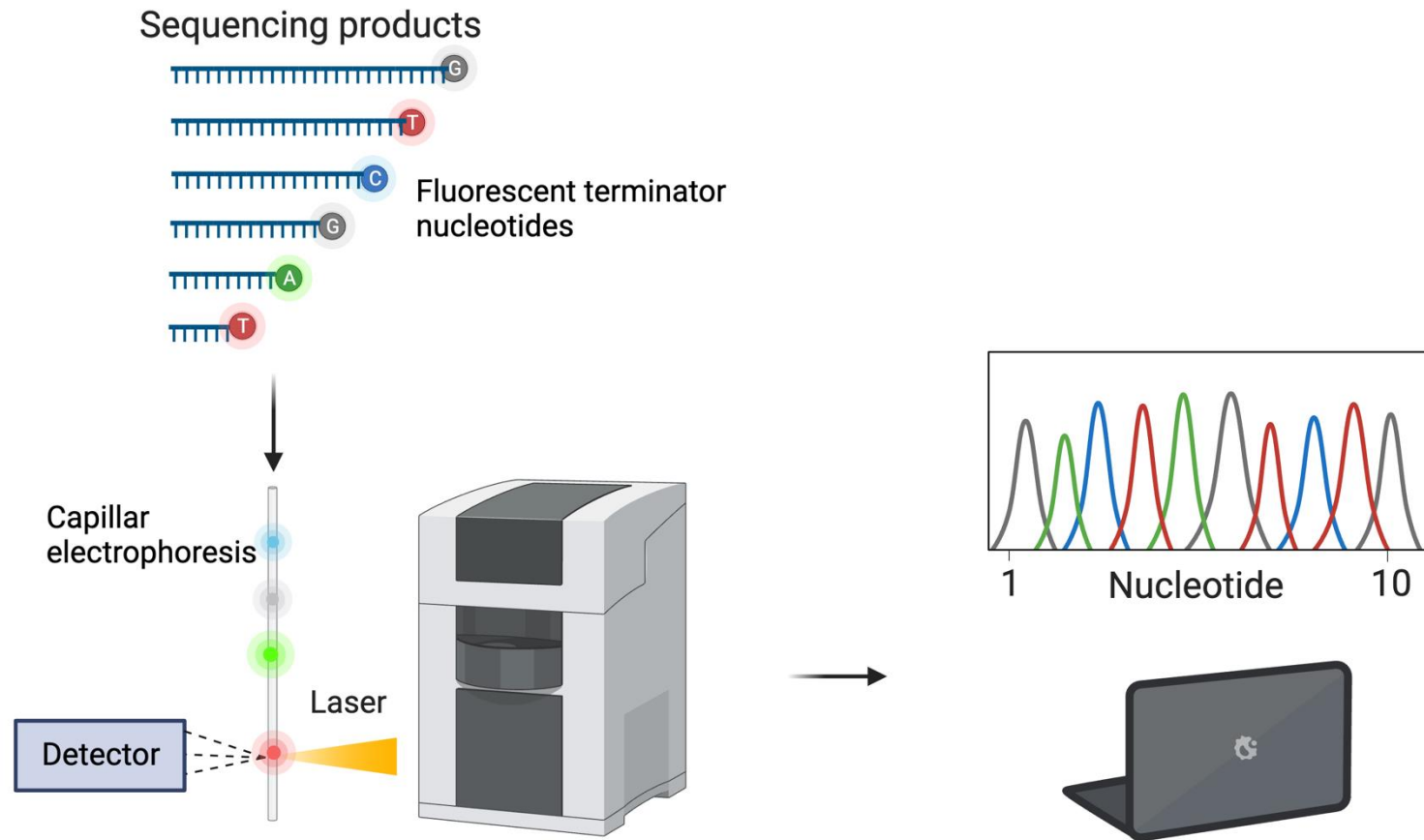
(DNA polymerase/nucleotide sequences/bacteriophage  $\phi$ X174)

F. SANGER, S. NICKLEN, AND A. R. COULSON





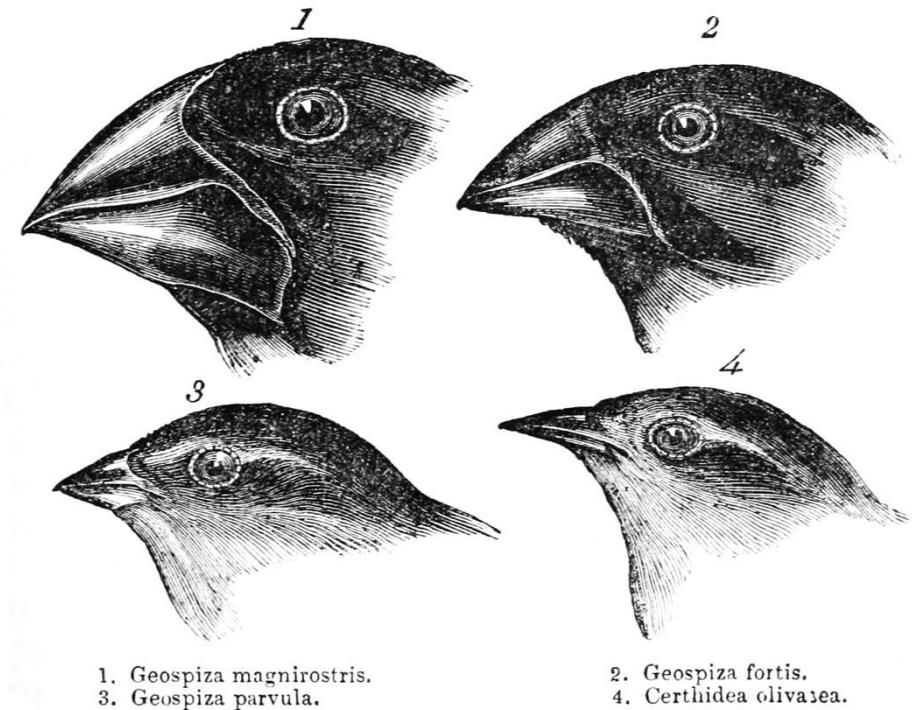
# Automated Sanger-sequencing



# Phylogenetics

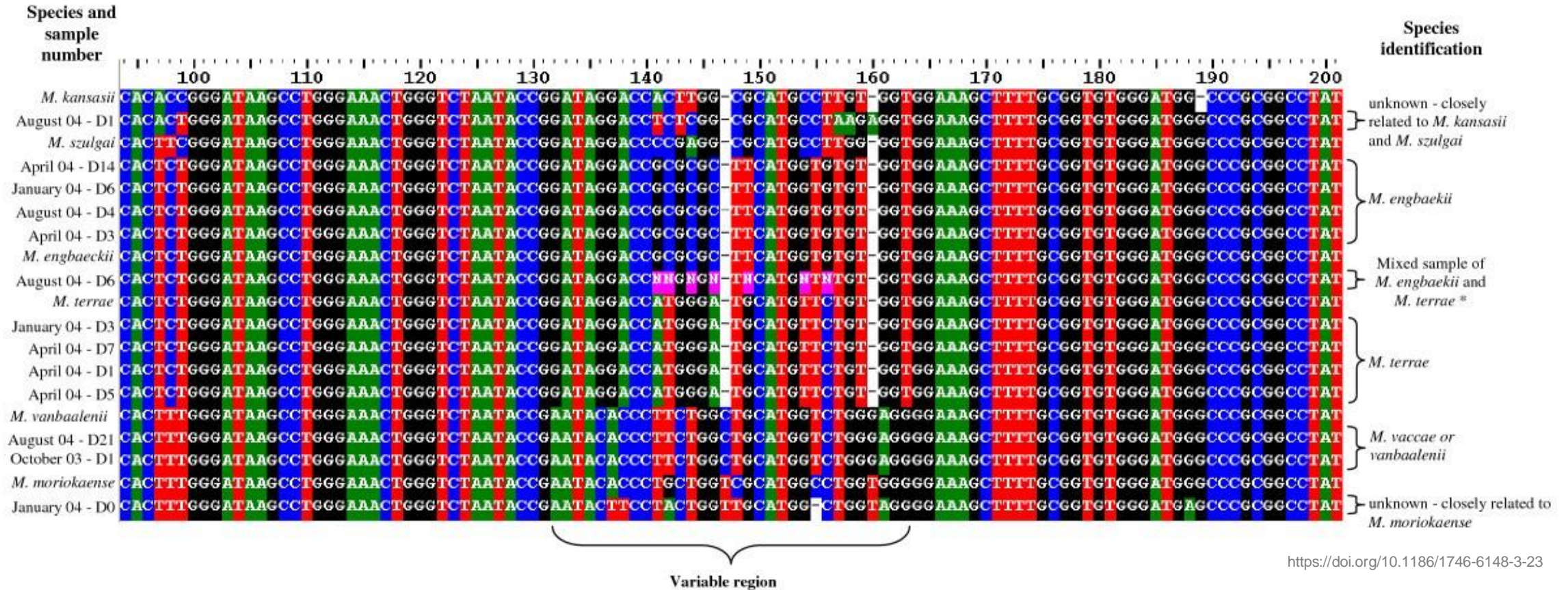
# Phylogeny

- Evolutionary relationship of group of organisms
- Based on heritable traits
  - DNA sequence
  - Amino acid sequence
  - Morphology

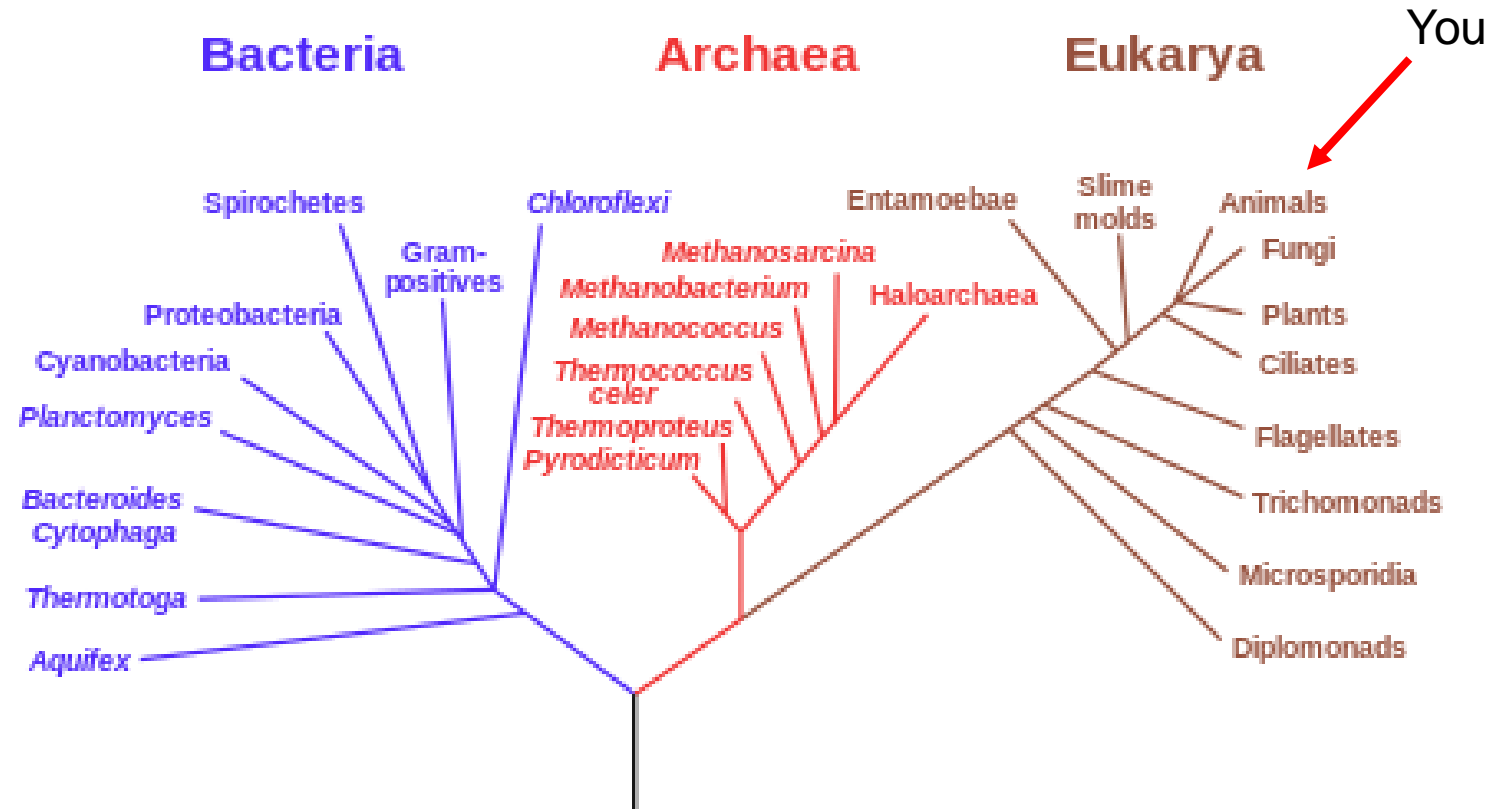


[https://en.wikipedia.org/wiki/Phylogenetics#/media/File:Darwin's\\_finches\\_by\\_Gould.jpg](https://en.wikipedia.org/wiki/Phylogenetics#/media/File:Darwin's_finches_by_Gould.jpg)

# Multiple sequence alignment



# Phylogenetic trees



# Phylogenetic trees

Scale

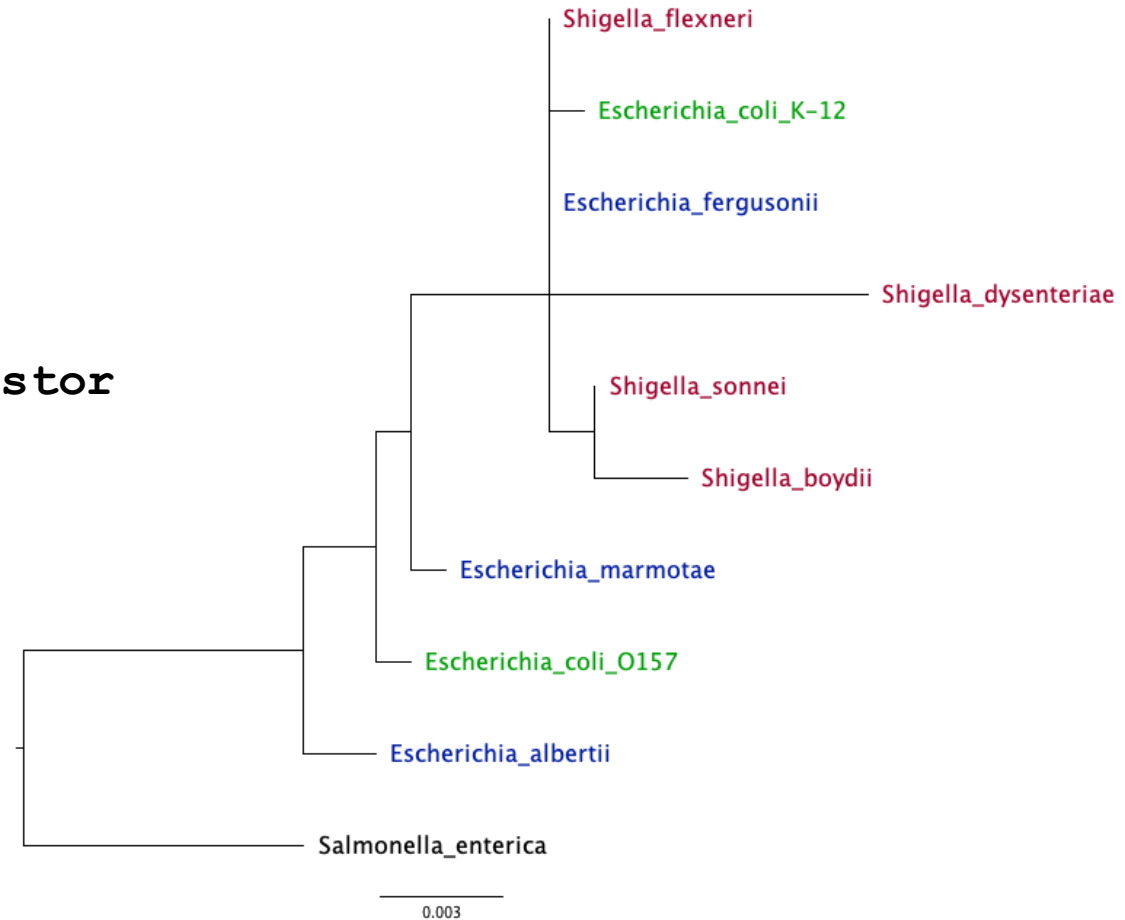
Node

Recent common ancestor

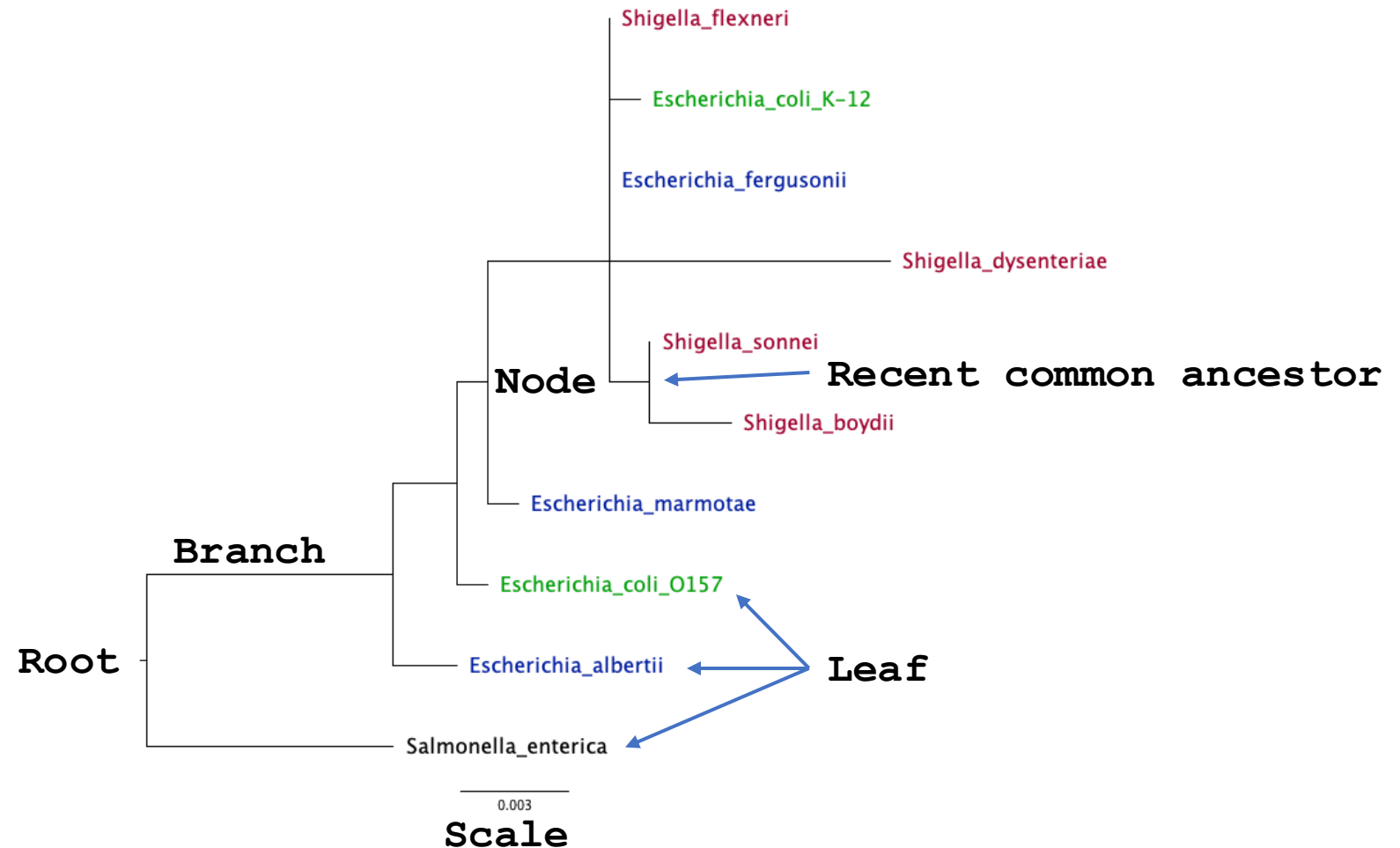
Root

Branch

Leaf



# Phylogenetic trees



# Practicals

16S rRNA gene sequence analysis and phylogenetics



# Sequence data analysis

- Go to <https://www.gear-genomics.com/>
- Select **Tea1** 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 **Pear1** 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 your cart.
  - When finished, download the cart (options: “fasta without gaps” & “[none]”)
  - Add the sequences to your own sequence file (from blast).
  - Put the furthest outgroup sequence first.
- Select **ACT**
  - Paste your sequences 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)