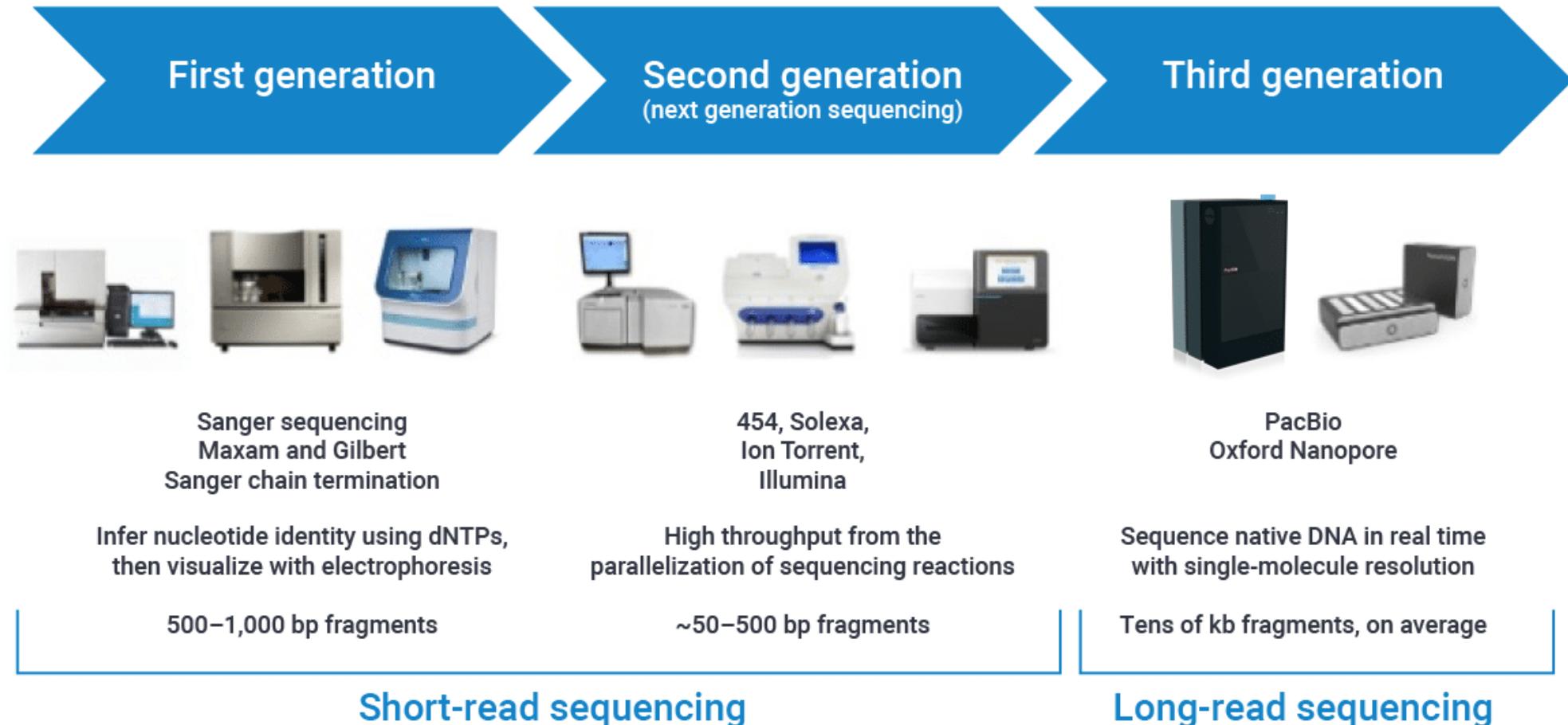


Sanger sequencing and phylogenetics

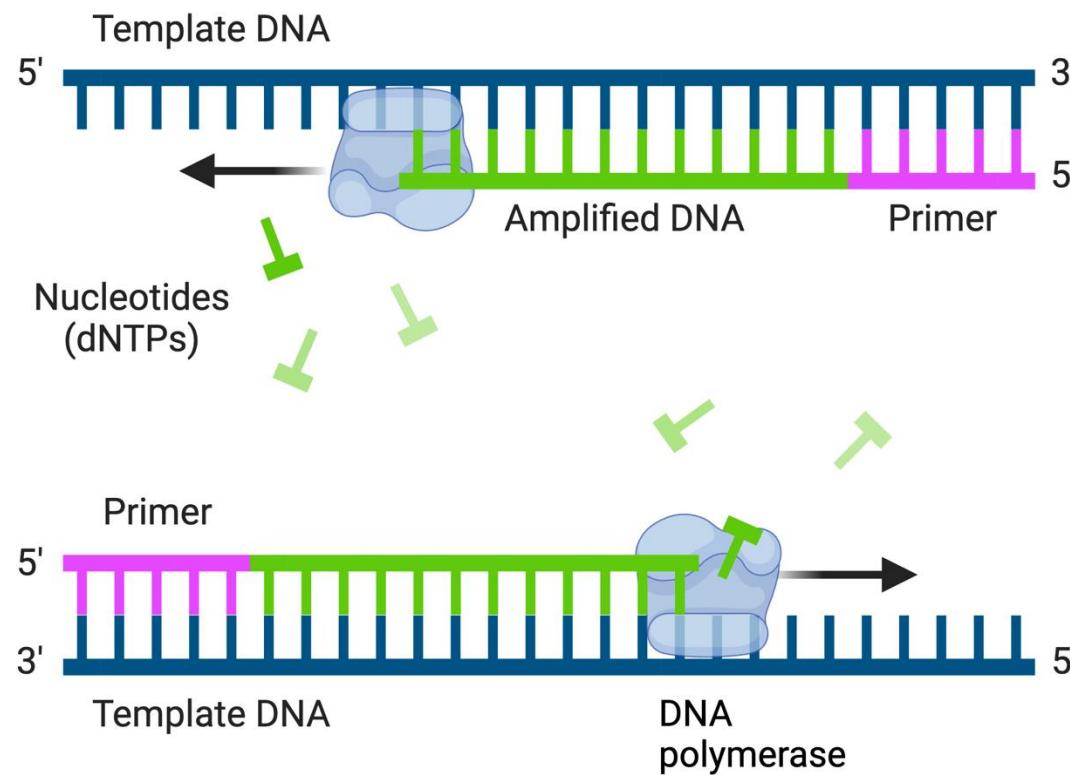
MMB-114

Evolution of sequencing



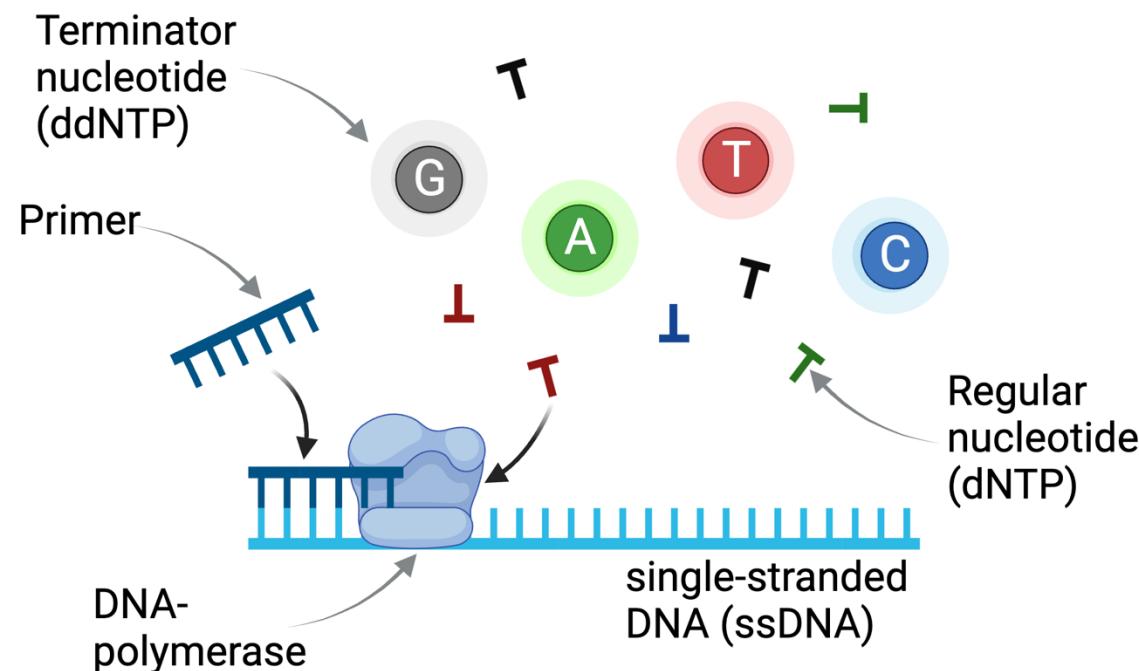
Sanger-sequencing

PCR - recap

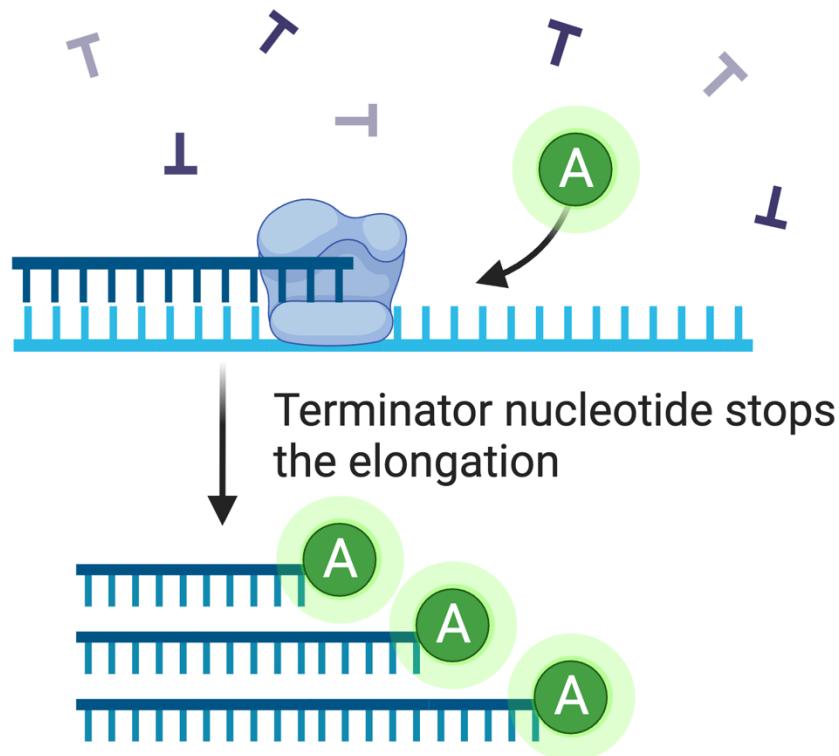


Terminator nucleotides

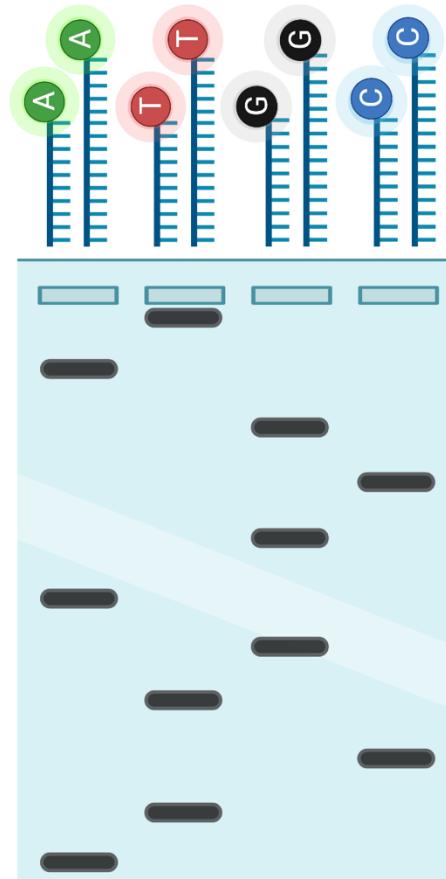
- Chain termination – dideoxy nucleotides (ddNTPs)



Terminator nucleotides



Sequencing gels



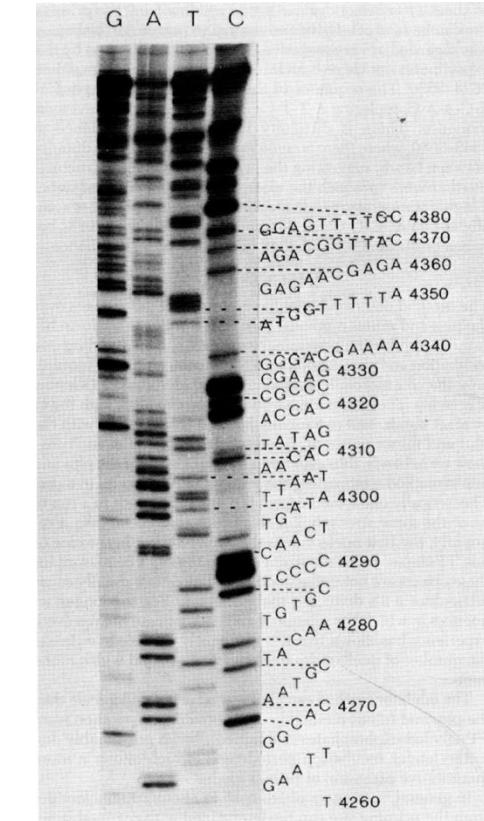
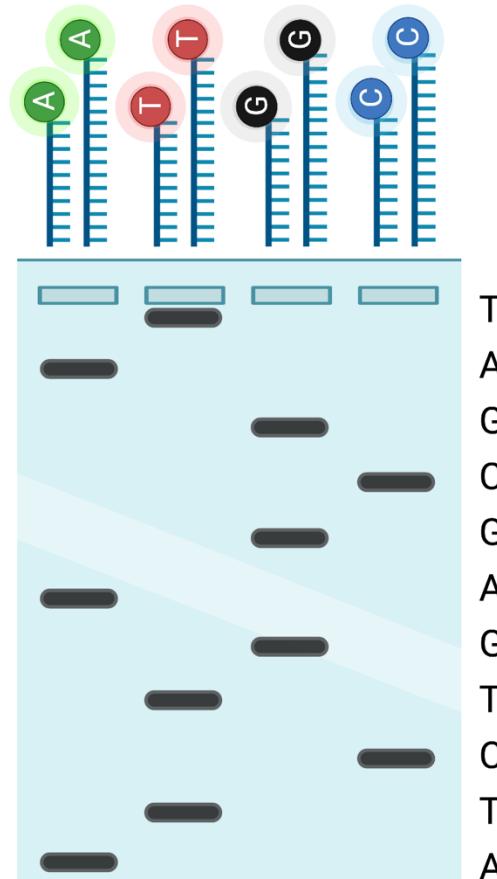
Sequencing gels

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

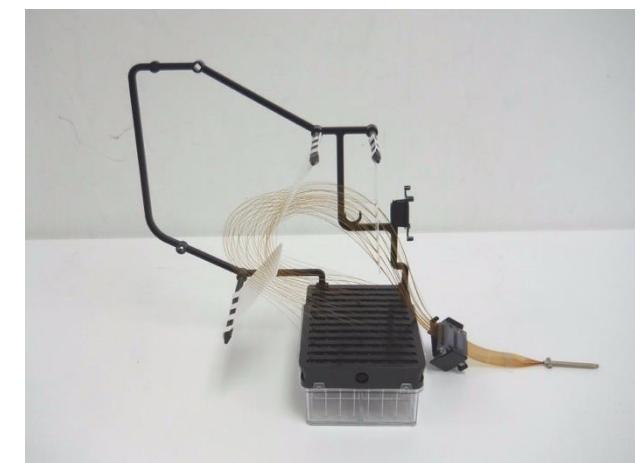
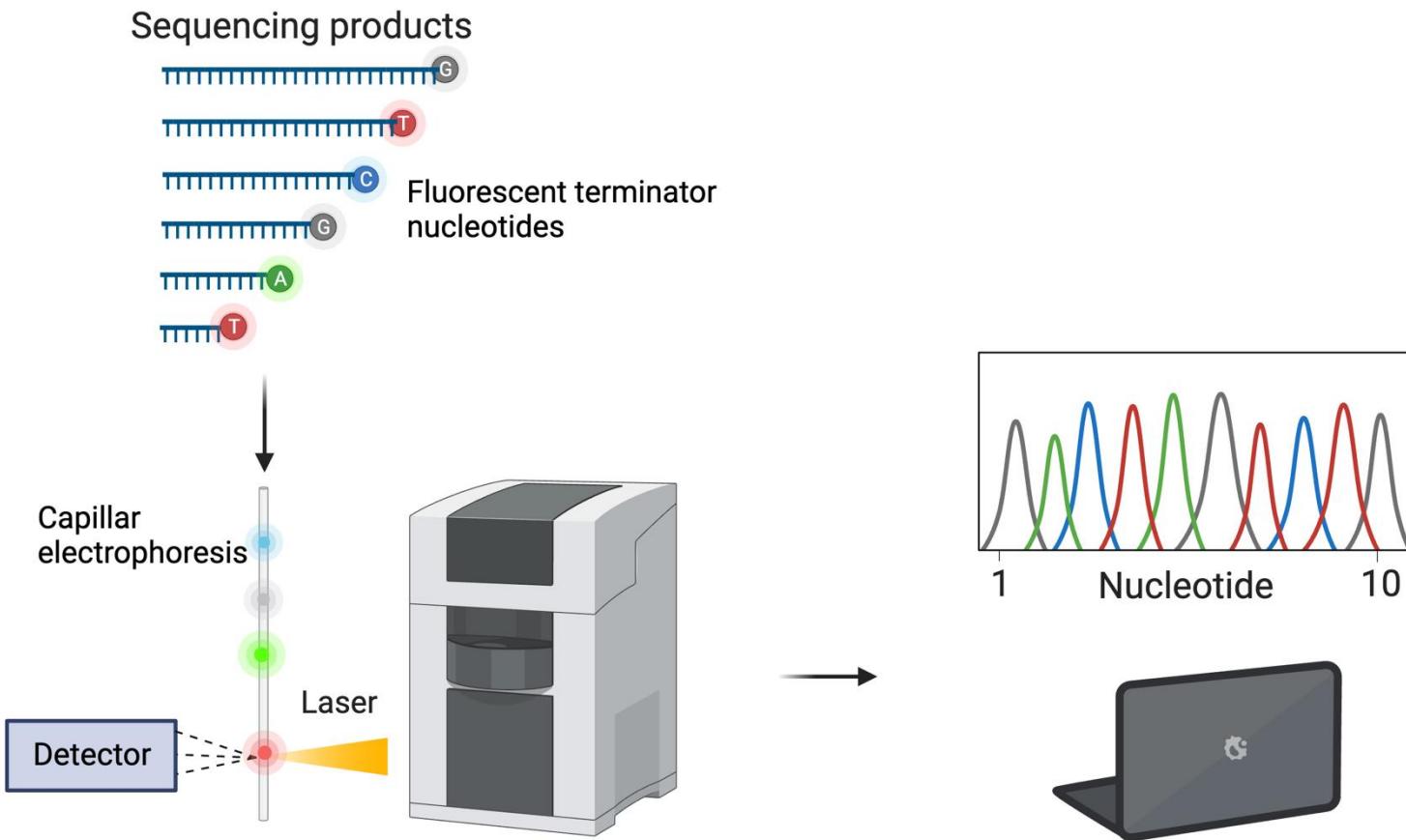
DNA sequencing with chain-terminating inhibitors

(DNA polymerase/nucleotide sequences/bacteriophage ϕ X174)

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



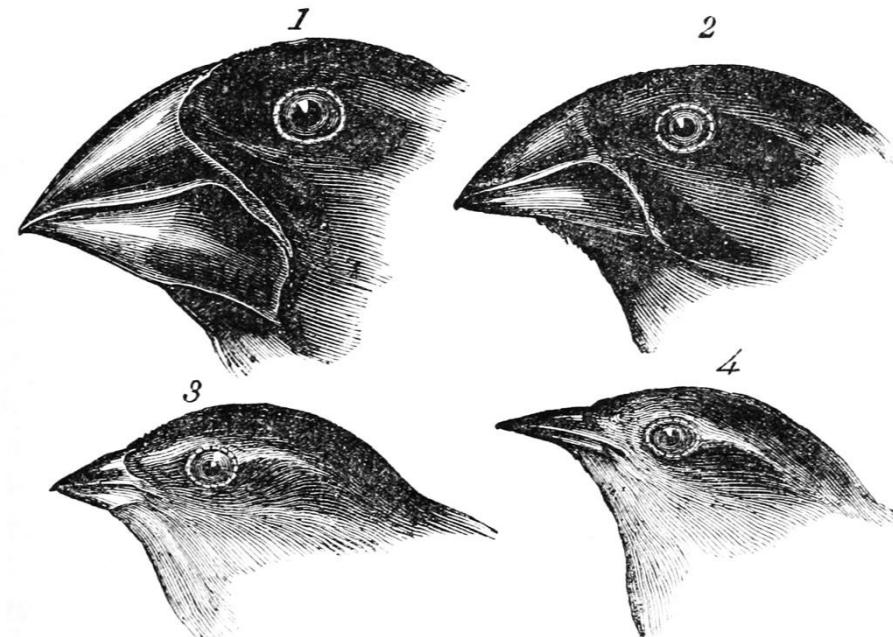
Automated Sanger-sequencing



Phylogenetics

Phylogeny

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

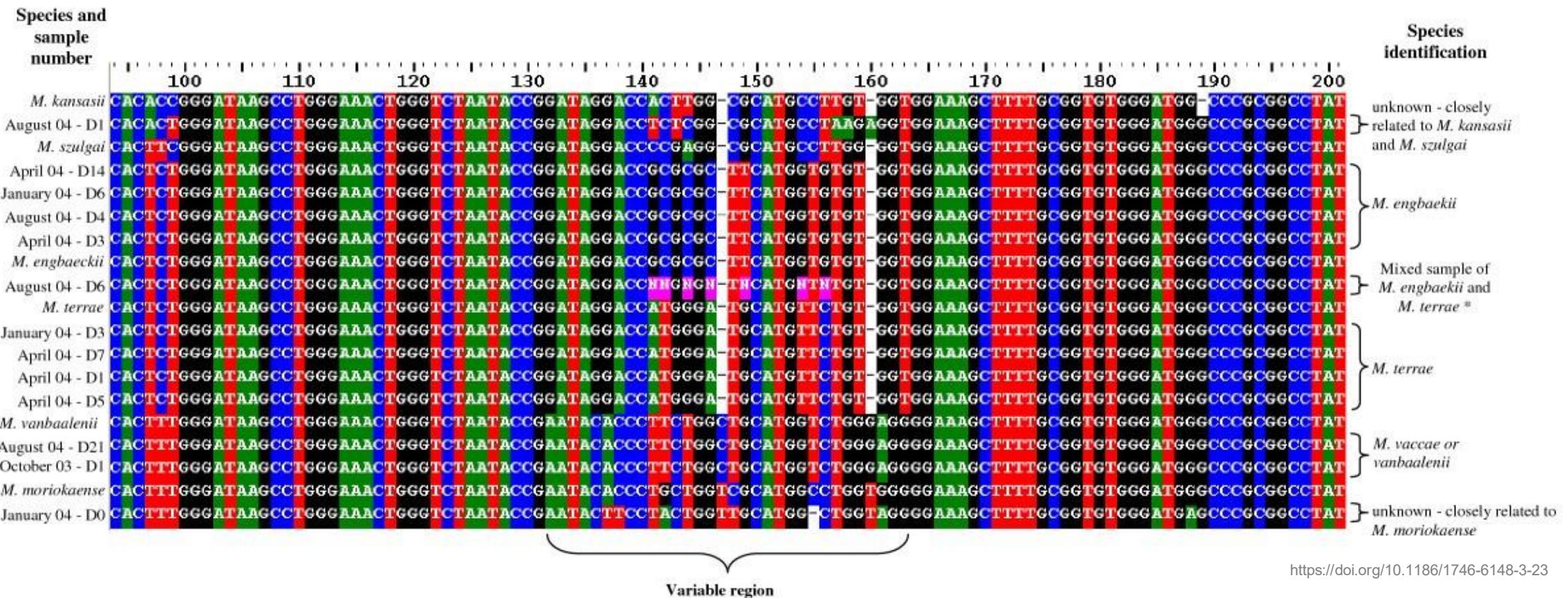


1. *Geospiza magnirostris*.
3. *Geospiza parvula*.

2. *Geospiza fortis*.
4. *Certhidea olivacea*.

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

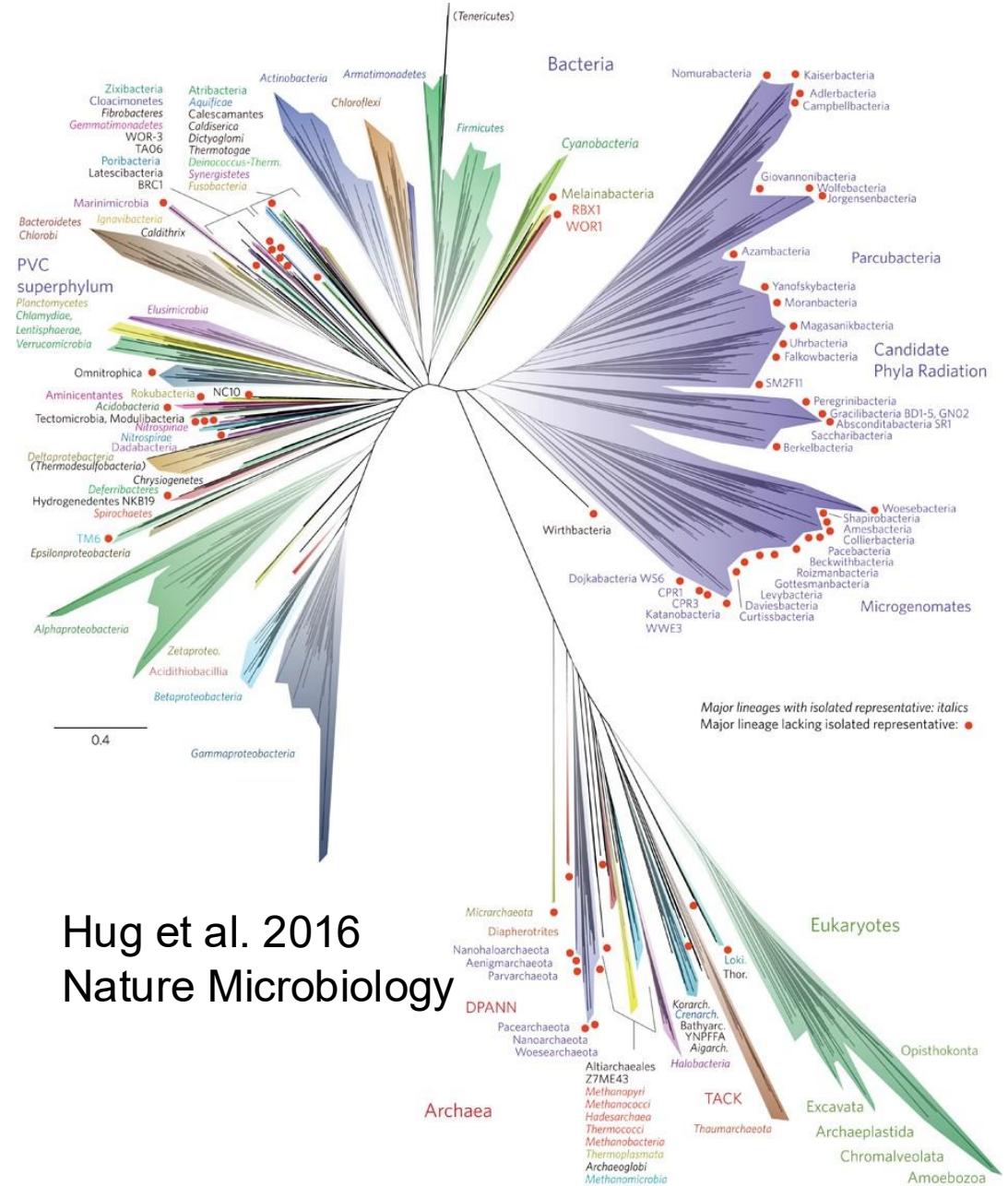
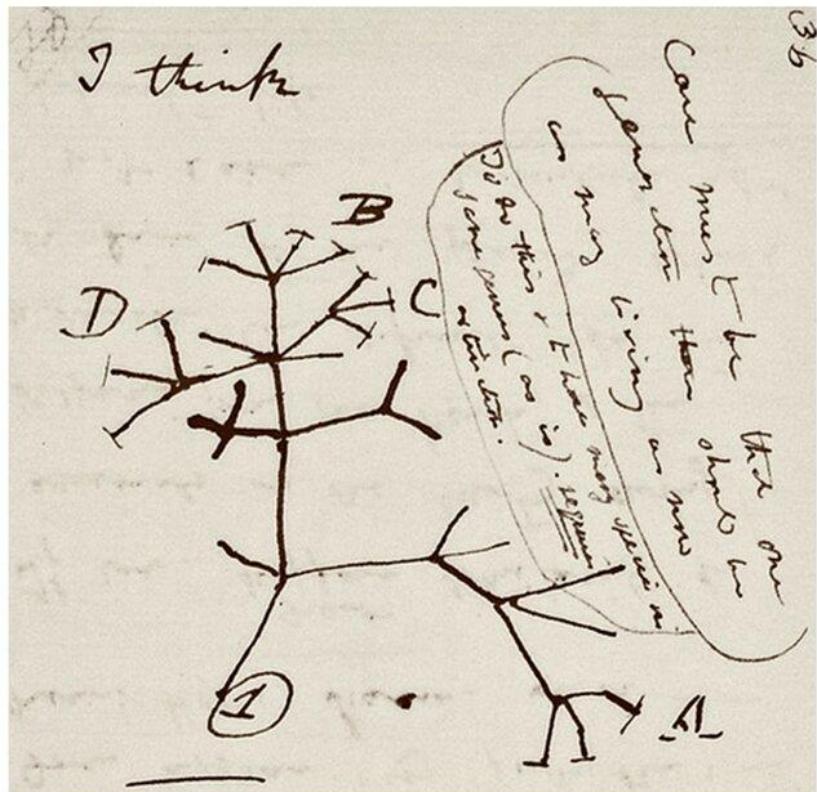
Multiple sequence alignment



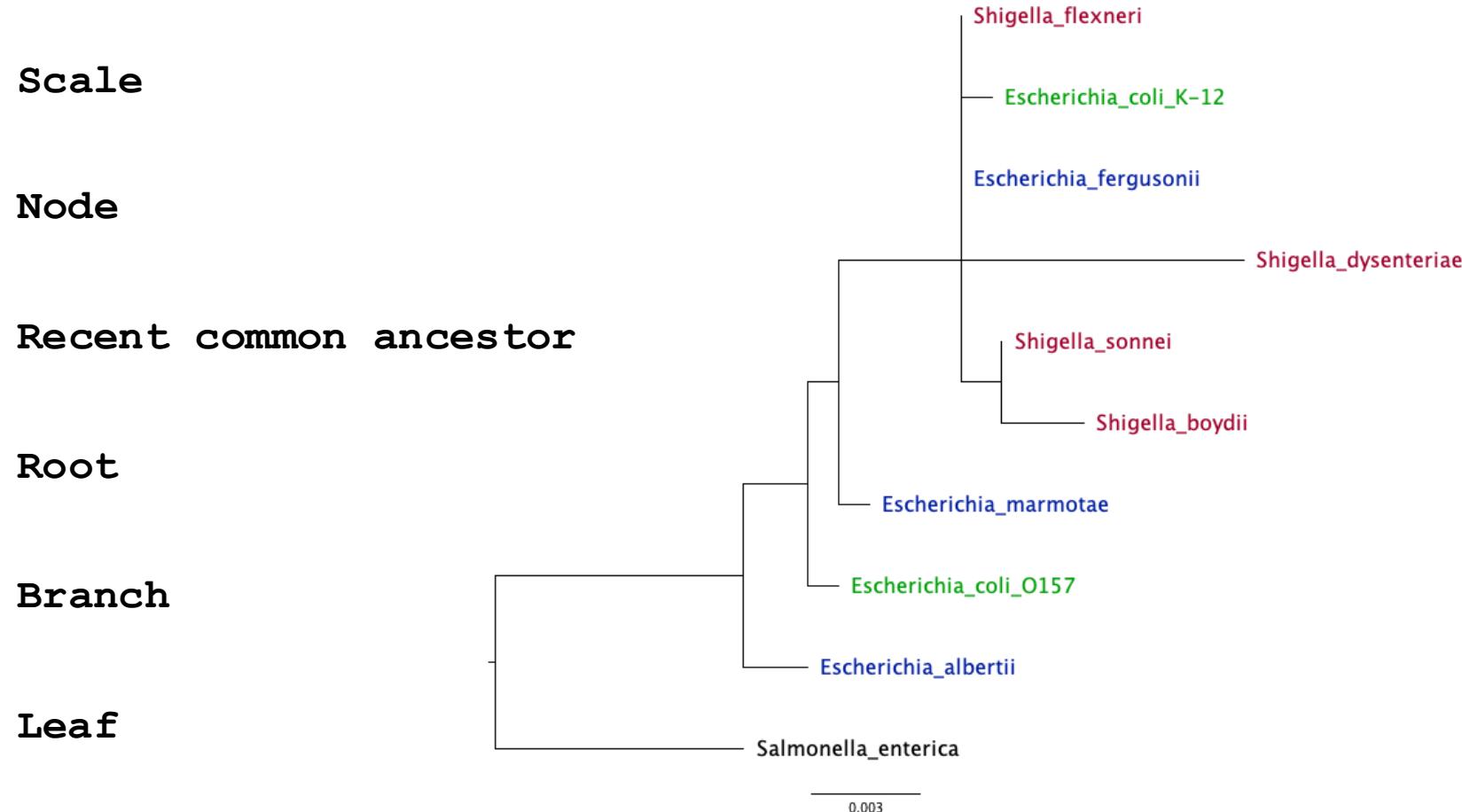
<https://doi.org/10.1186/1746-6148-3-23>

Phylogenetic trees

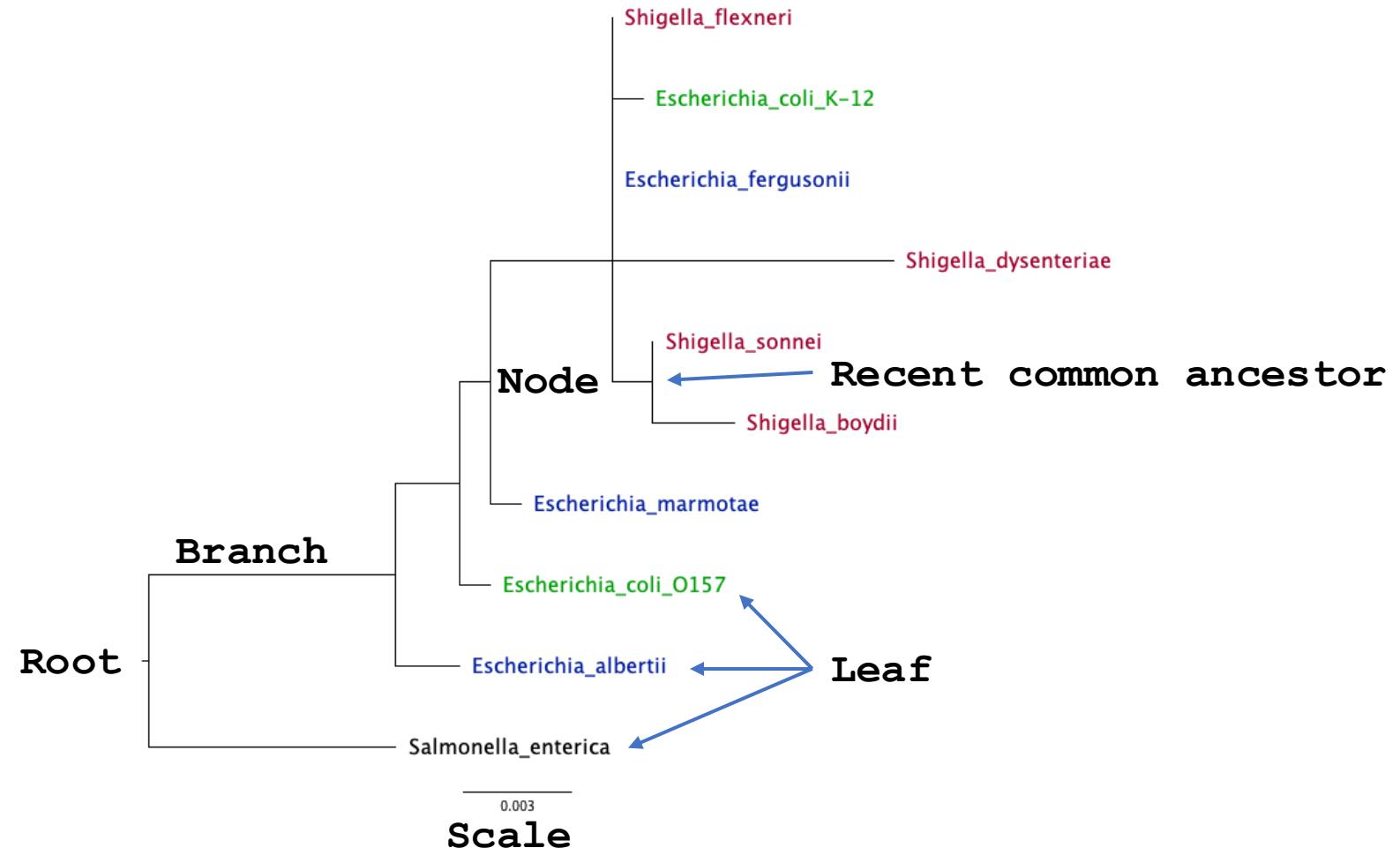
Darwin's notebook



Phylogenetic trees



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 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)