Sanger’s method of DNA sequencing

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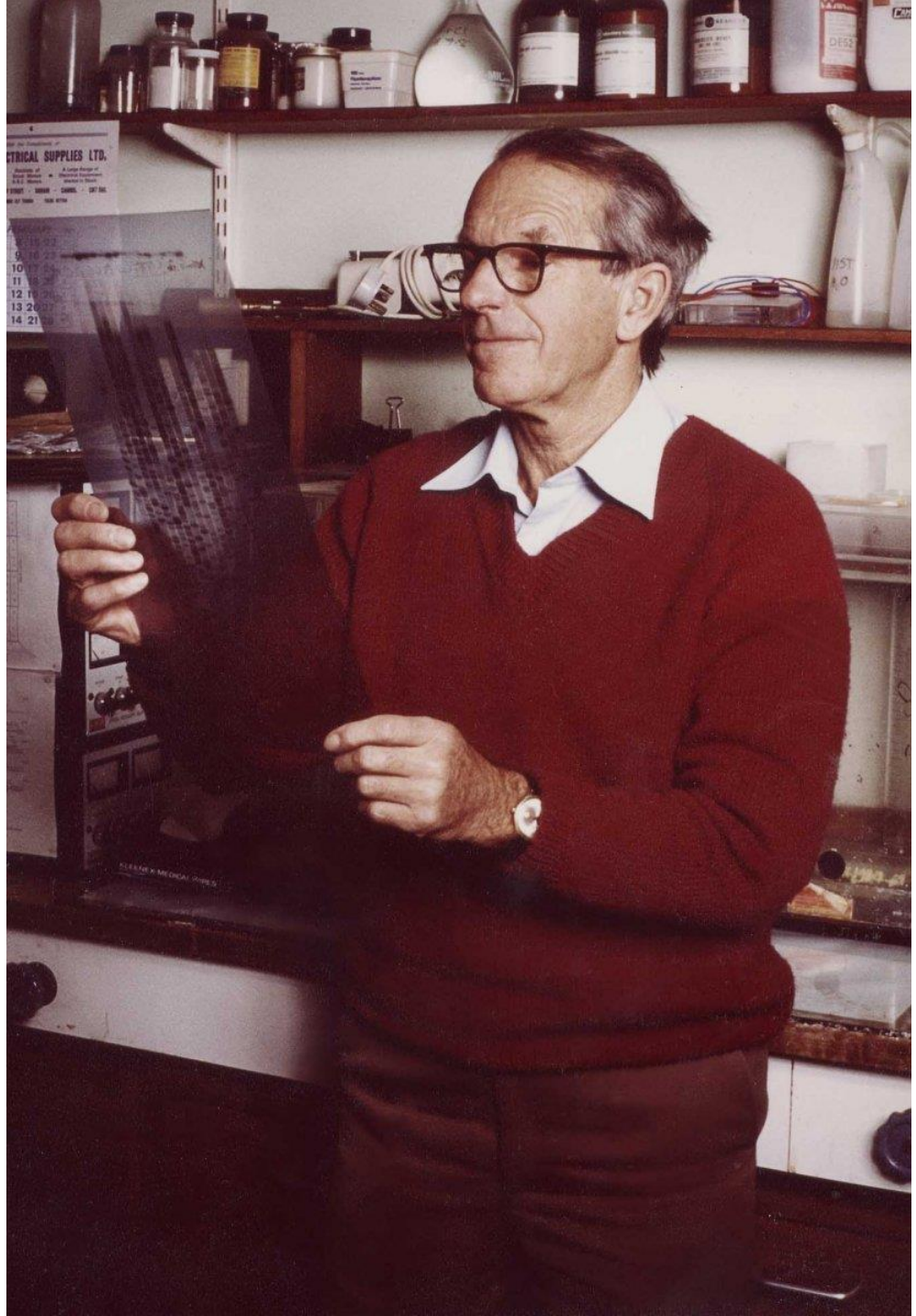


Frederick Sanger

English biochemist

- The Nobel Prize in Chemistry 1958 - for his work on the structure of proteins, especially that of insulin
- The Nobel Prize in Chemistry 1980 - for their contributions concerning the determination of base sequences in nucleic acids

This method of sequencing is also called as **Enzymatic method** or **Chain termination method**

- The key ingrediants of Sanger's DNA sequencing are

1. A DNA polymerase enzyme – helps in elongating the DNA fragment
2. A **primer**, which is a short piece of single-stranded DNA that binds to the template DNA and acts as a "starter" for the polymerase
3. The four DNA nucleotides (dATP, dTTP, dCTP, dGTP)
4. The template DNA to be sequenced
5. A unique ingredient: Dideoxy, or **chain-terminating**, versions of all four nucleotides (ddATP, ddTTP, ddCTP, ddGTP), each labeled with a different color of dye

- Dideoxy nucleotides are similar to regular, or deoxy, nucleotides, but with one key difference: they lack a hydroxyl group on the 3’ carbon of the sugar ring.
- In a regular nucleotide, the 3’ hydroxyl group acts as a “hook," allowing a new nucleotide to be added to an existing chain.
- Once a dideoxy nucleotide has been added to the chain, there is no hydroxyl available and no further nucleotides can be added.
- The chain ends with the dideoxy nucleotide, which is marked with a particular color of dye depending on the base (A, T, C or G) that it carries.
- The dye molecule on a dideoxy nucleotide is linked to the nitrogenous base.

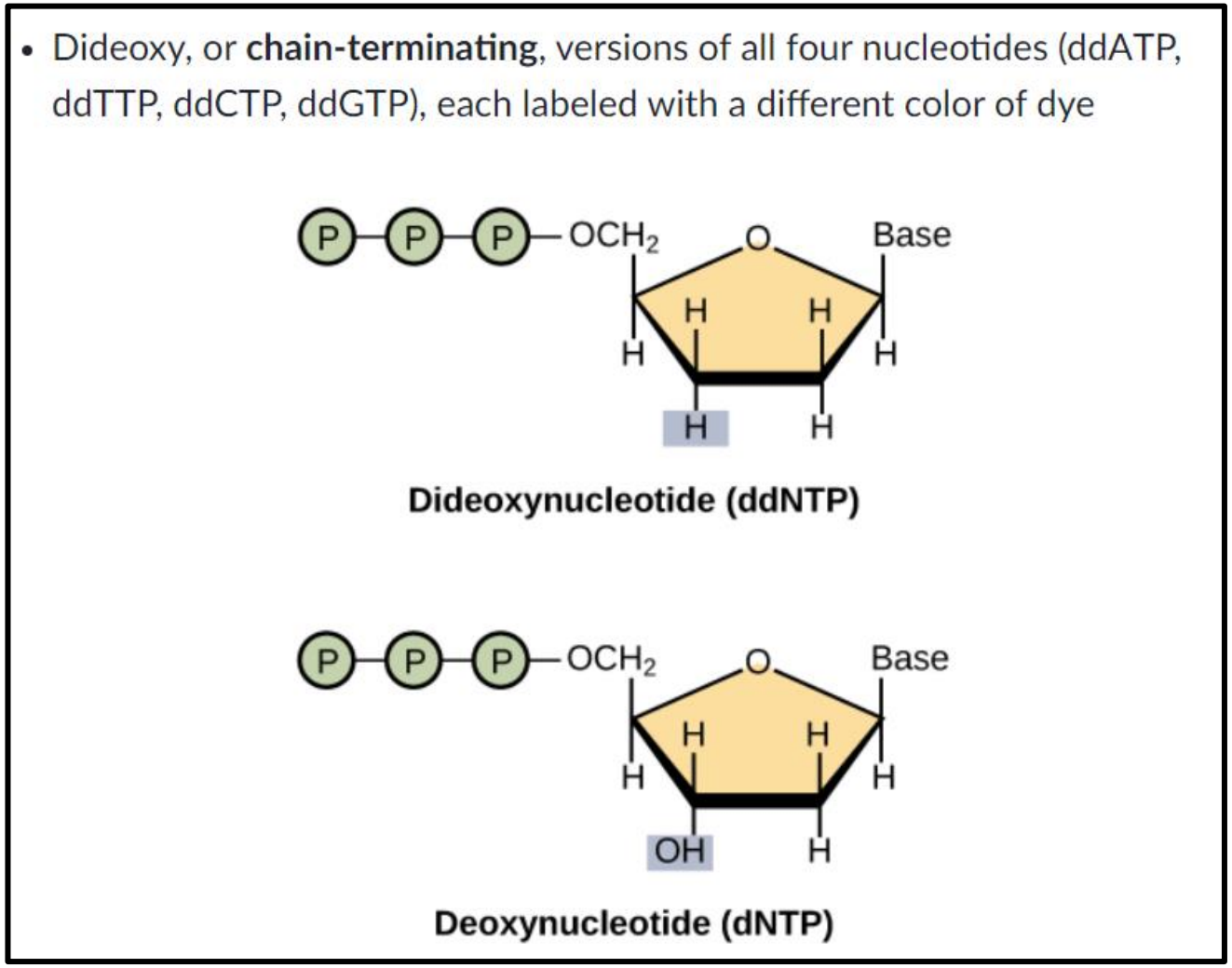
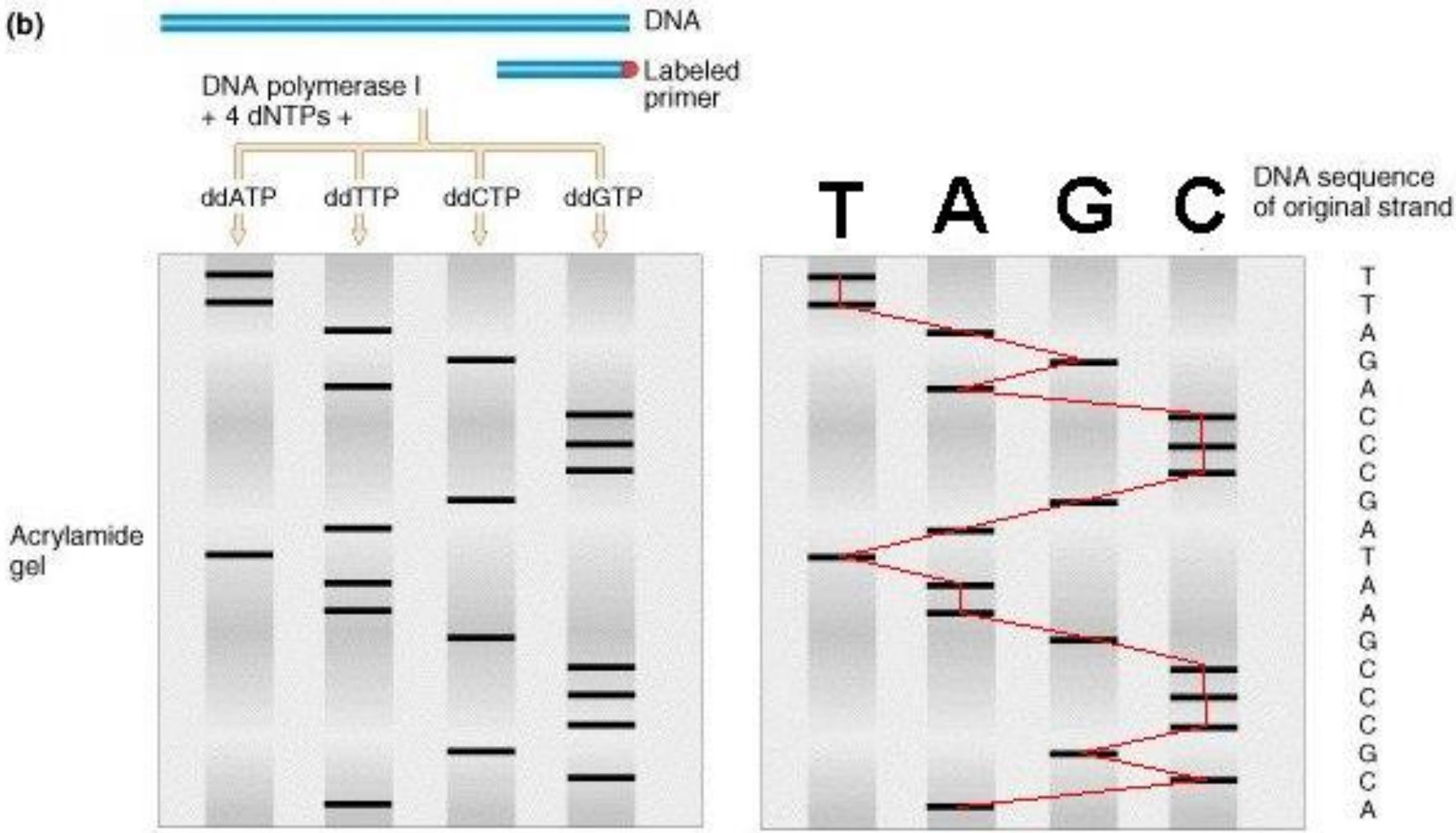


Image credit: "Whole-genome sequencing: Figure 1," by OpenStax College, Biology (CC BY 4.0).

Steps in Sanger sequencing

1. The ssDNA with a short complementary primer molecule is the beginning step of the sequencing.
2. The 3’ hydroxyl end of the primer is binds to the DNA to be sequenced.
3. The Klenow fragment of DNA polymerase I starts at the primer and synthesis a complementary copy of the particular DNA region.
4. DNA sequencing employs four different reaction mixtures, each contains all four deoxyribose nucleoside triphosphates.
5. In addition to dNTPs, each of the four mixtures contains one of the four dideoxyribonucleoside triphosphates (ddNTPs) , which lacks both 2’ and 3’ hydroxyl group.
6. As the 3’ hydroxyl group is required for the formation of phosphodiester bond, the presence of ddNTPs causes chain termination.


(b)
DNA
Labeled primer
DNA polymerase I
+ 4 dNTPs +
ddATP
ddTTP
ddCTP
ddGTP
Acrylamide gel
T A G C
DNA sequence of original strand
T T A G A C C C G A T A A G C C C G C A

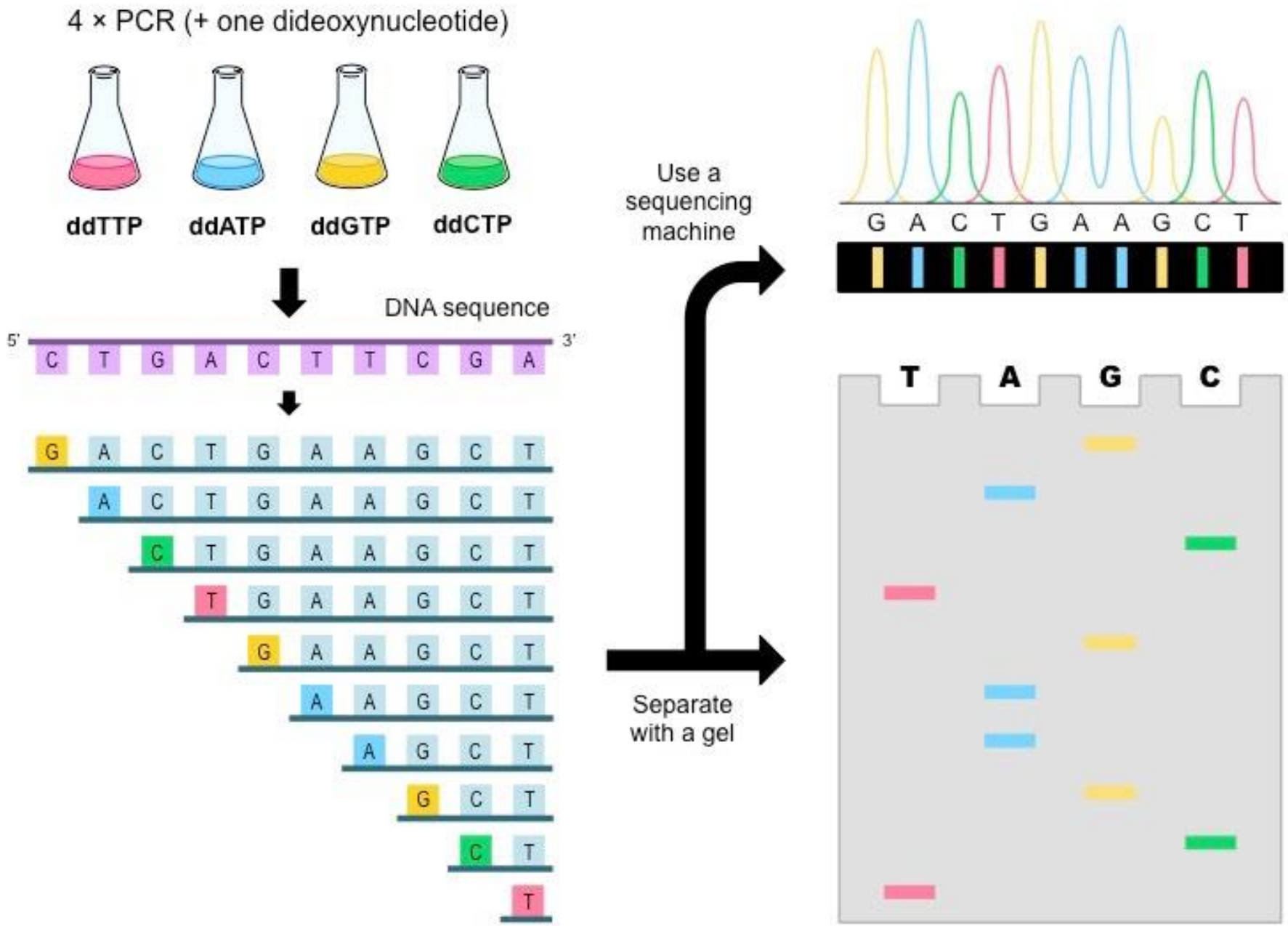
- The ratio (10: 1) is optimal for determining sequences from the primer up to =400 nucleotides.
- For determination of longer sequences either the dNTP concentration in the labeling reaction should be increased (e.g., 5-fold) or the concentration of ddNTP in the termination reaction should be decreased (e.g., dNTP/ddNTP ratio of 30:1).

Uses and limitations

1. Sanger sequencing gives high-quality sequence for relatively long stretches of DNA (up to about 900 base pairs).
2. It's typically used to sequence individual pieces of DNA, such as bacterial plasmids or DNA copied in PCR.
3. It is expensive and inefficient for larger-scale projects, such as the sequencing of an entire genome or metagenome (the “collective genome” of a microbial community)

References

- DNA Sequencing Core Facility https://biology.unt.edu/~jajohnson/Chromatogram_Interpretation
- https://agctsequencing.wordpress.com/tag/troubleshooting-sequencing/


4 × PCR (+ one dideoxynucleotide)
ddTTP
ddATP
ddGTP
ddCTP
DNA sequence
5'
3'
C T G A C T T C G A
G A C T G A A G C T
A C T G A A G C T
C T G A A G C T
T G A A G C T
G A A G C T
A A G C T
A G C T
G C T
C T
T
Use a sequencing machine
Separate with a gel
G A C T G A A G C T
T A G C