



Lecture 14:

Finding the allele 4: DNA sequencing

Course 410

Molecular Evolution

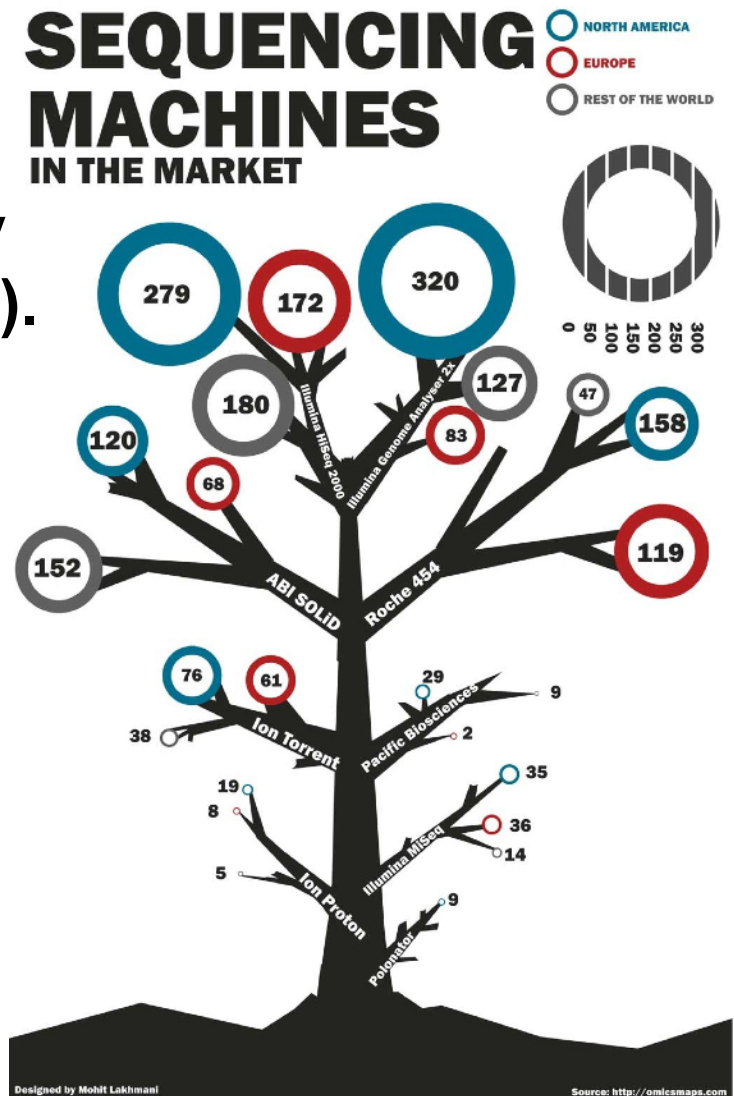
What is DNA sequencing?


It is reading the letters of the book.

It is reading the exact nucleotide sequence of the genome.



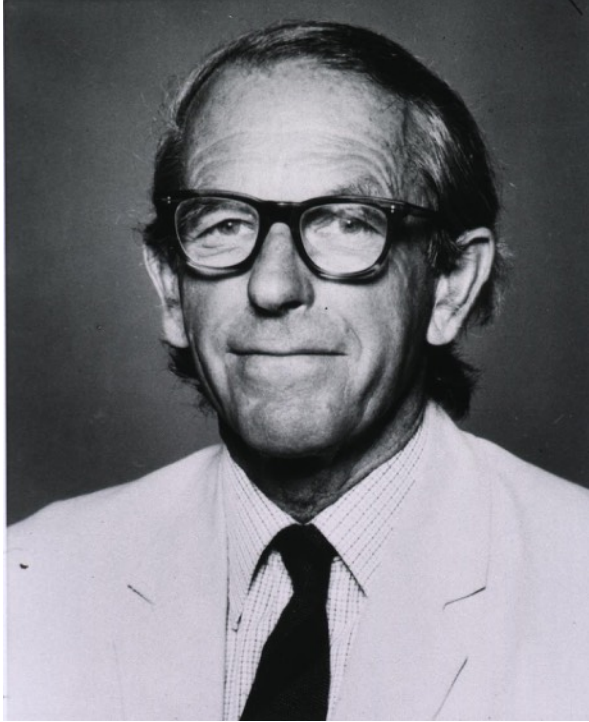
1. Maxam and Gilbert chemical degradation method (extinct).
2. Sanger sequencing (dideoxy or chain termination method).
3. Illumina sequencing.
4. SOLiD sequencing.
5. Pyrosequencing.
6. Ion Torrent method.
7. Single molecule sequencing.




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- DNA sequencing can be considered the ultimate characterization of gene(s) or fragment(s) of DNA.
 - **DNA Sequencing is used for:**
 - Mapping genomes
 - Determining gene structure and thus function
 - Detecting polymorphism (single nucleotide polymorphism SNP)
 - Analyzing genetic variation
 - Predicting the possible product(s) of DNA fragments
 - Many purposes depending on the questions one is asking



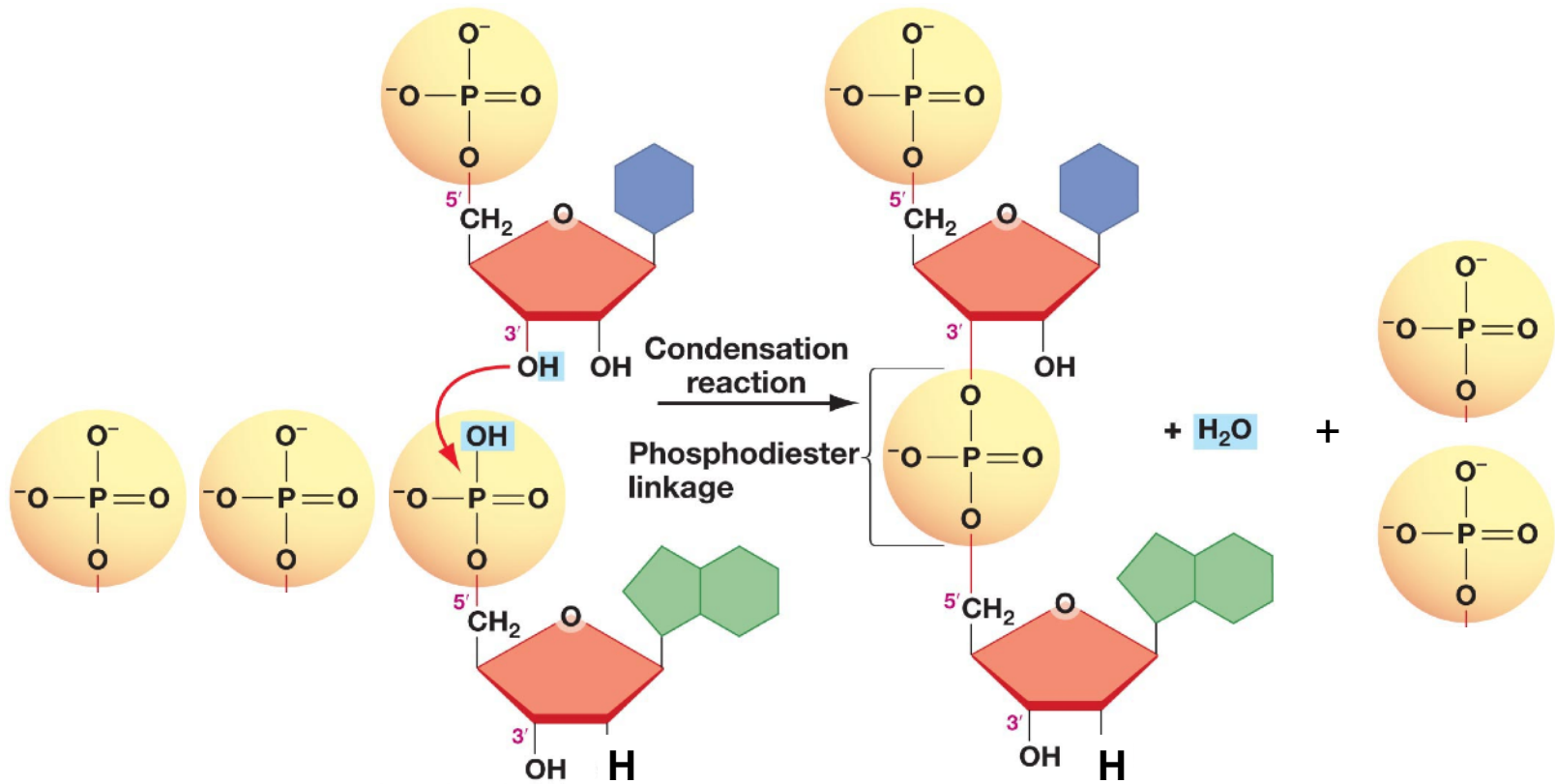
Sanger sequencing



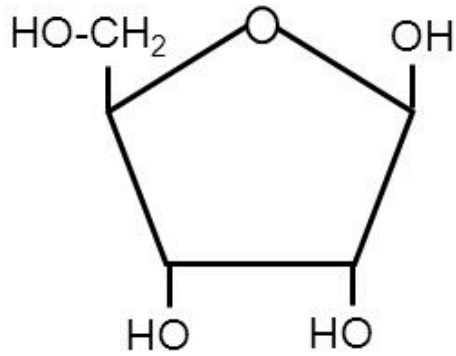
1. Fredrick Sanger has developed a sequencing method and received a Noble prize for it.
2. Sanger sequencing method is also called **Chain Termination Method** and **Dideoxy sequencing method**.

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- **Employs:**
 - specific primers
 - dNTPs
 - **ddNTPs**
 - DNA polymerase
 - DNA template

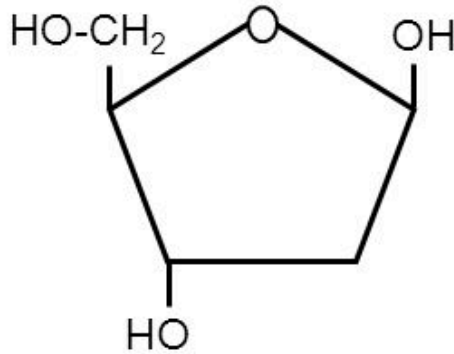
DNA synthesis requires the availability of a 3'-OH and energy



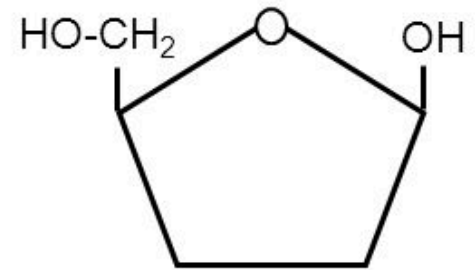
Difference in OH location in sugar and consequences



ribose

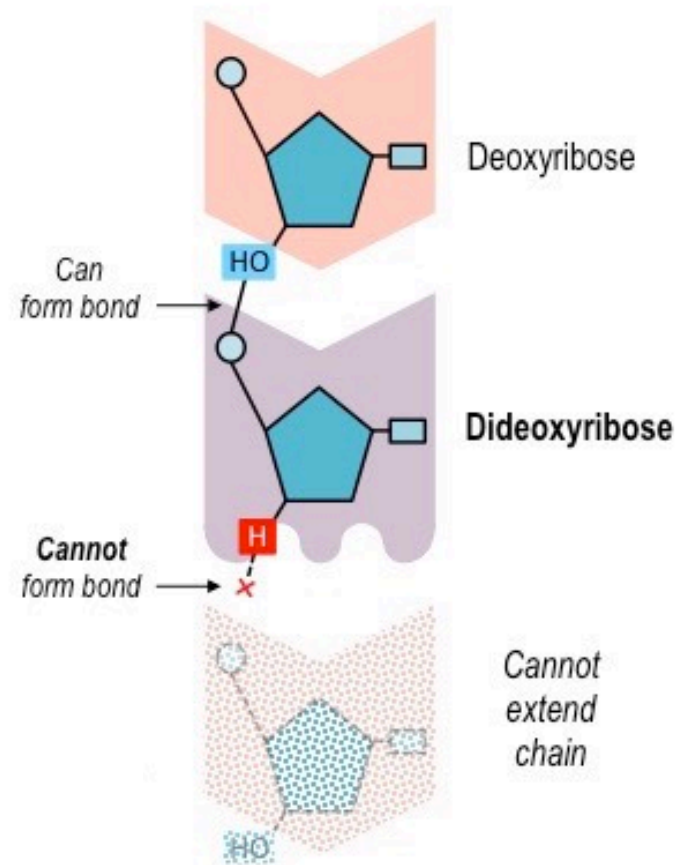
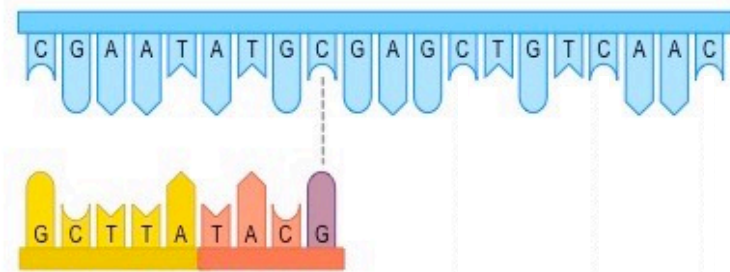


deoxyribose



dideoxyribose

The absence of OH group on the 3' carbon of the sugar blocks further addition of nucleotides

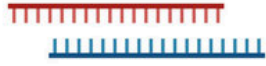


Chain termination by dideoxynucleotides

Sanger sequencing

DNA denaturation

Apply heat to convert dsDNA into ssDNA



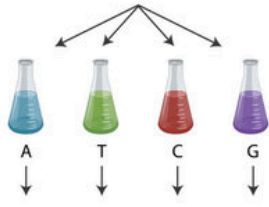
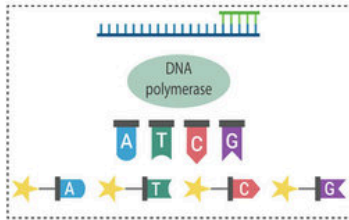
DNA amplification

Primers bind to ssDNA

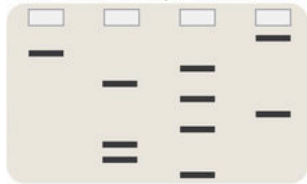


Chain reactions

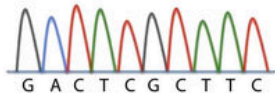
Four reactions are set, each containing:
- template DNA + primer
- DNA polymerase
- free nucleotides
- marked nucleotides



Gel electrophoresis

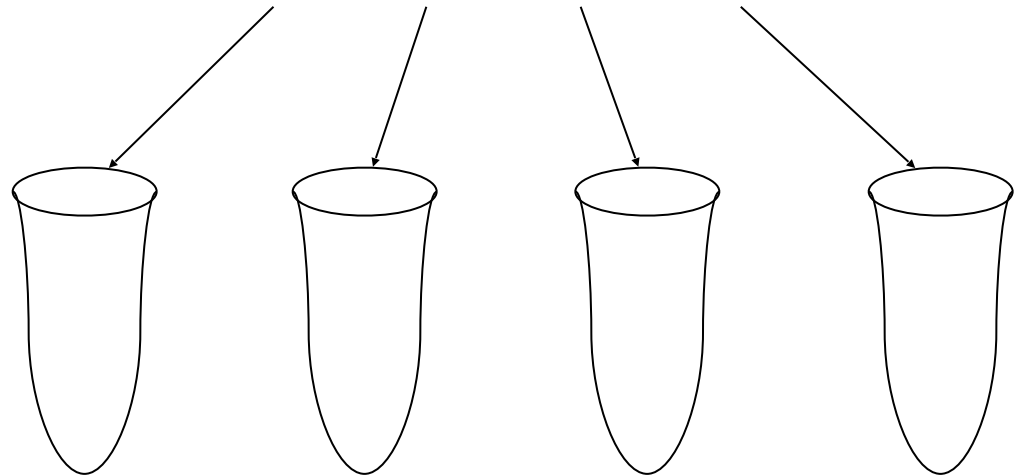


Reading of DNA sequence



DNA Template Polymerase Excess dNTPs

Primer

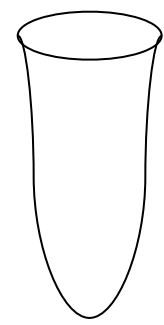
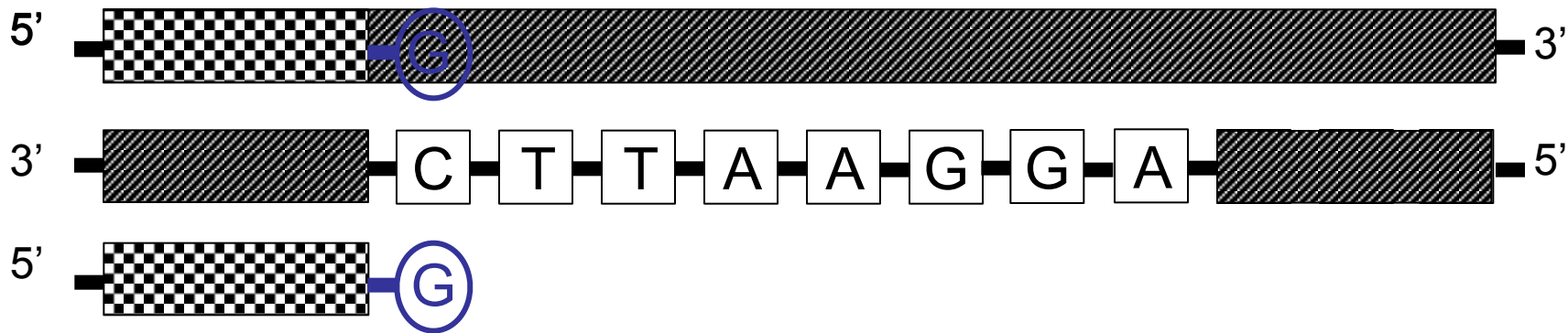


ddGTP

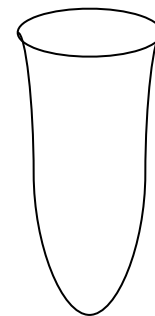
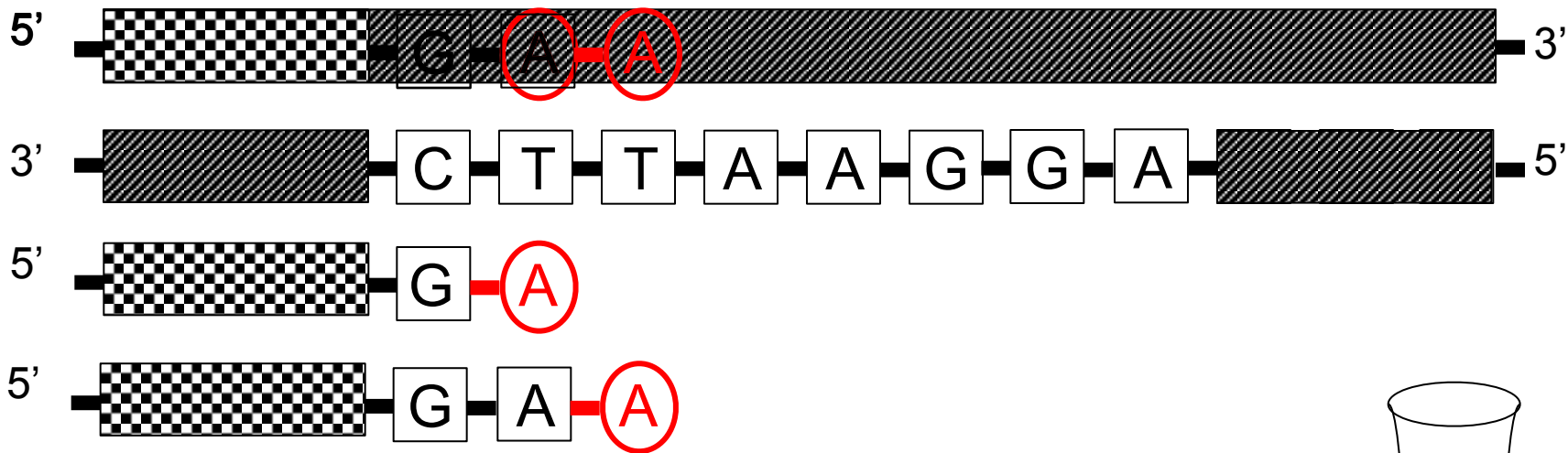
ddCTP

ddATP

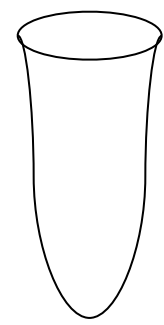
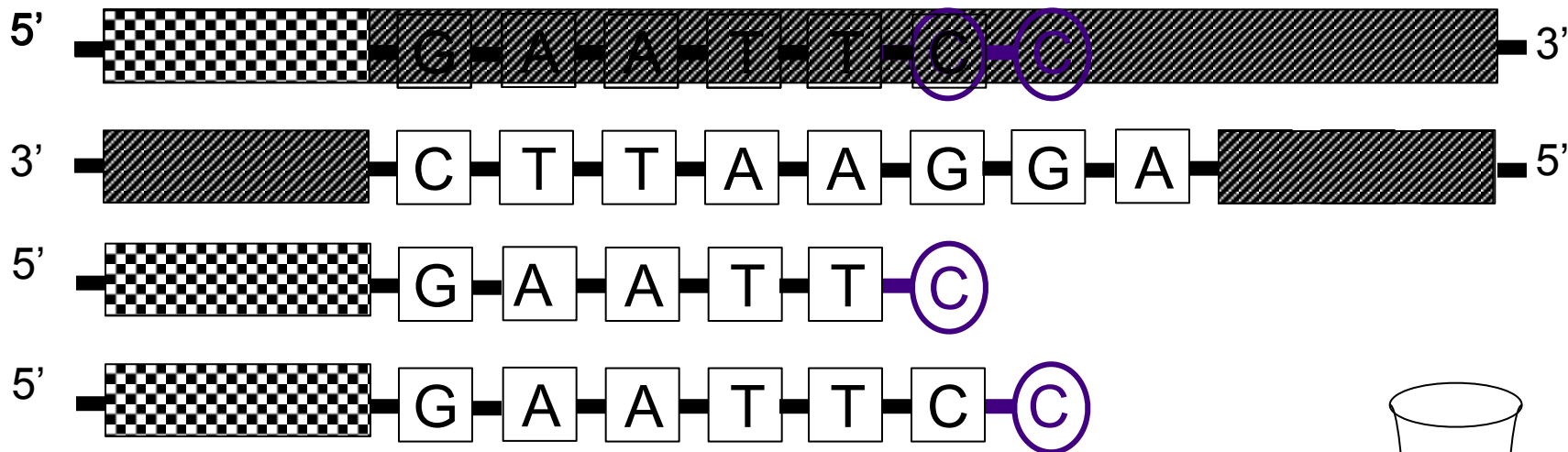
ddTTP



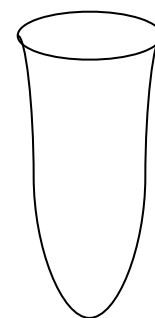
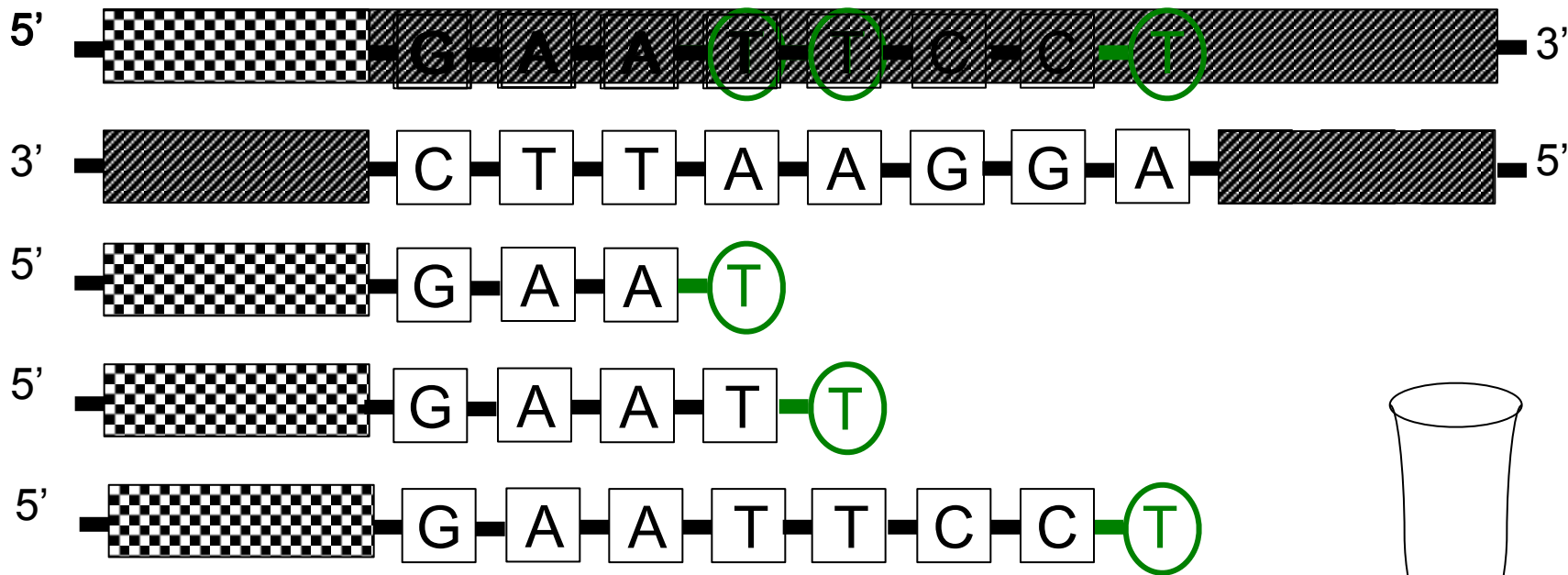
ddGTP



ddATP



ddCTP



ddTTP

Sanger sequencing - Gel

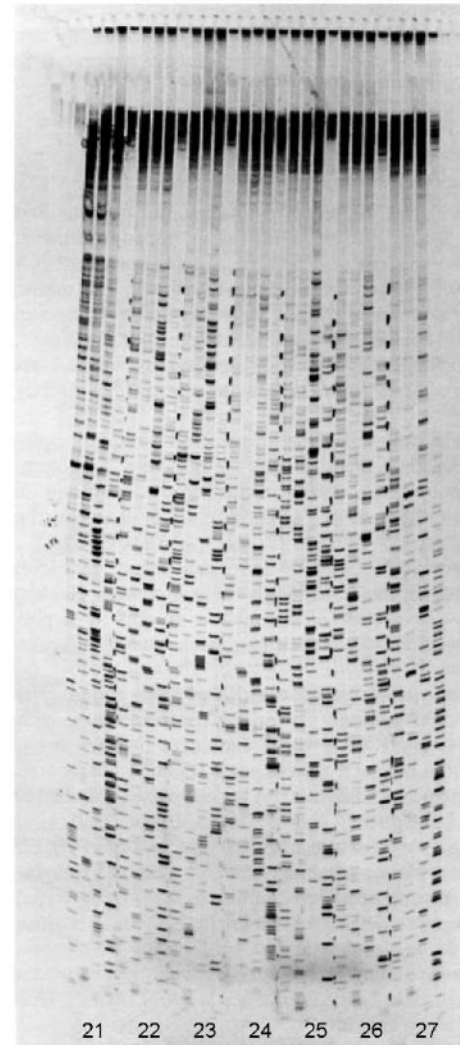
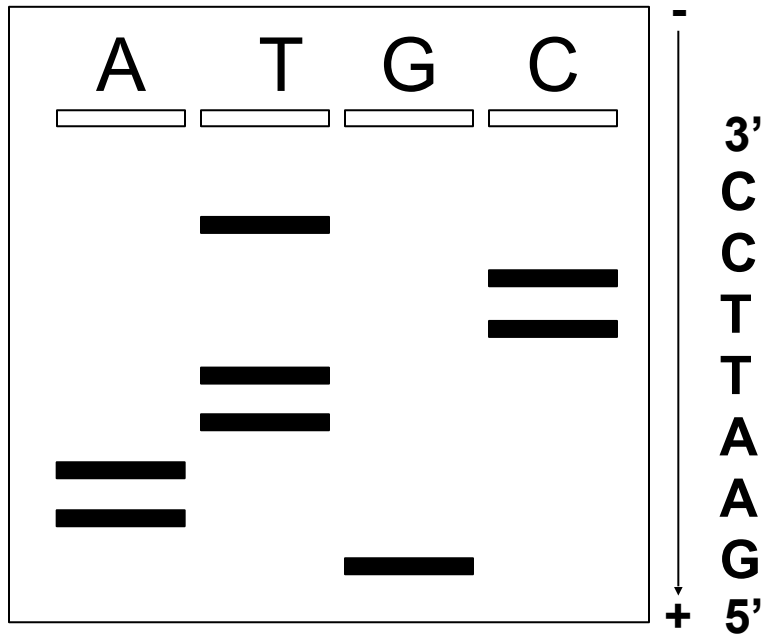

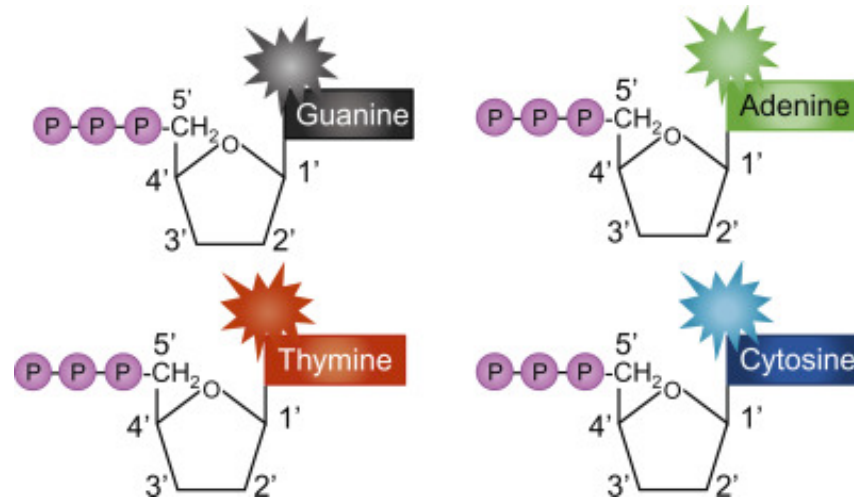


FIGURE 2.8. An autoradiogram (X-ray film) of a DNA sequencing gel. Each sequence requires Four lanes, one for each base.

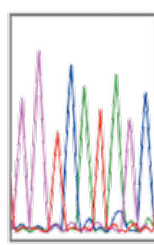
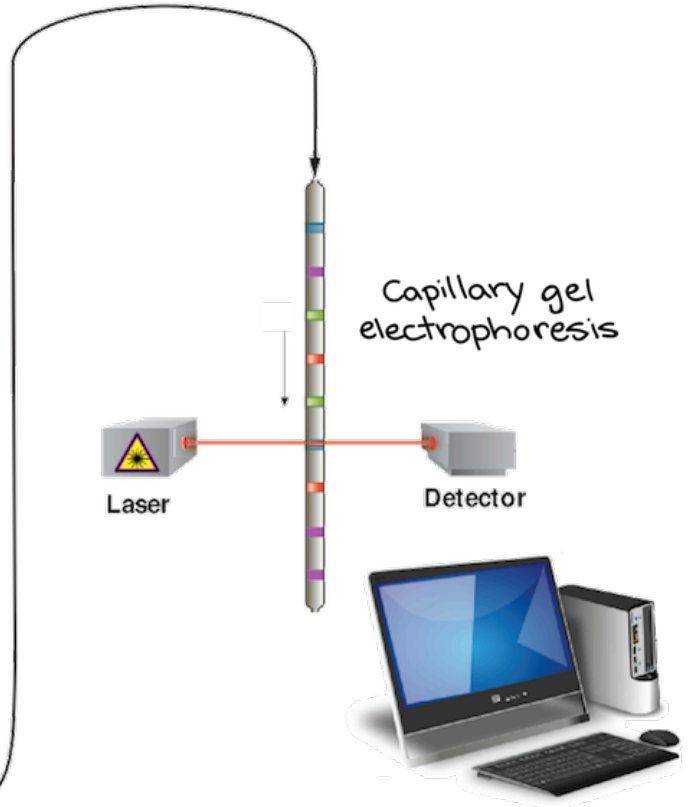
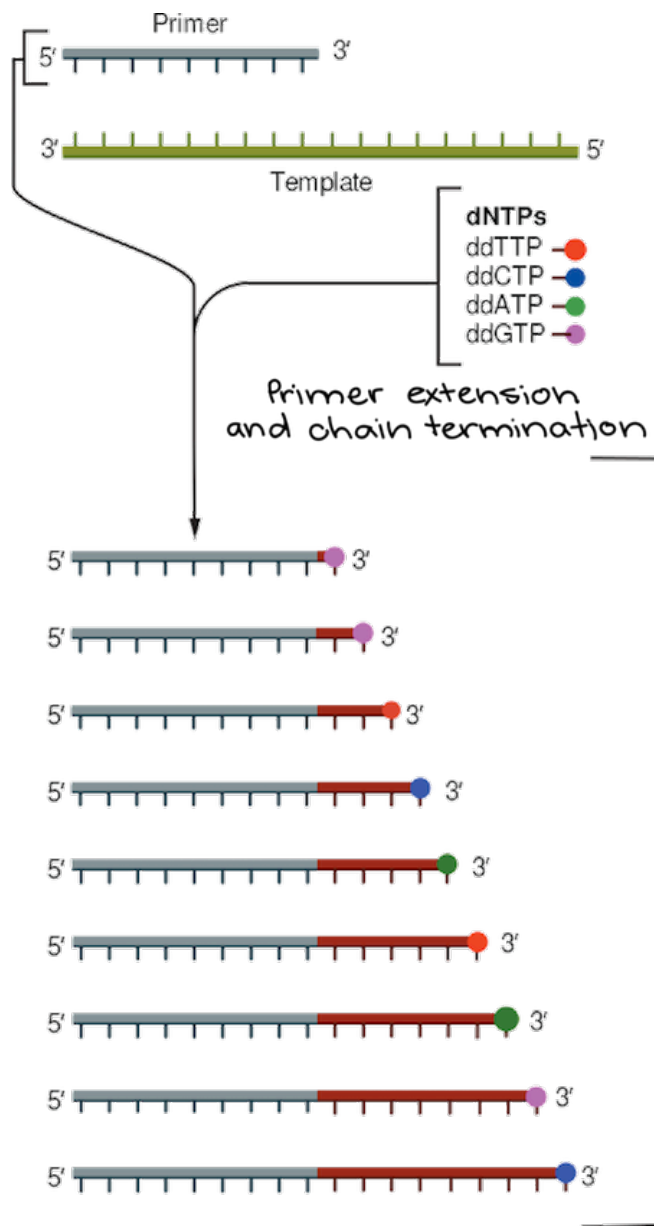
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- Analysis using high resolution polyacrylamide gel electrophoresis.
 - Fragments are detected using radioactive markers and autoradiography.

Fluorescent labeled ddNTPs



Sanger sequencing - Automated

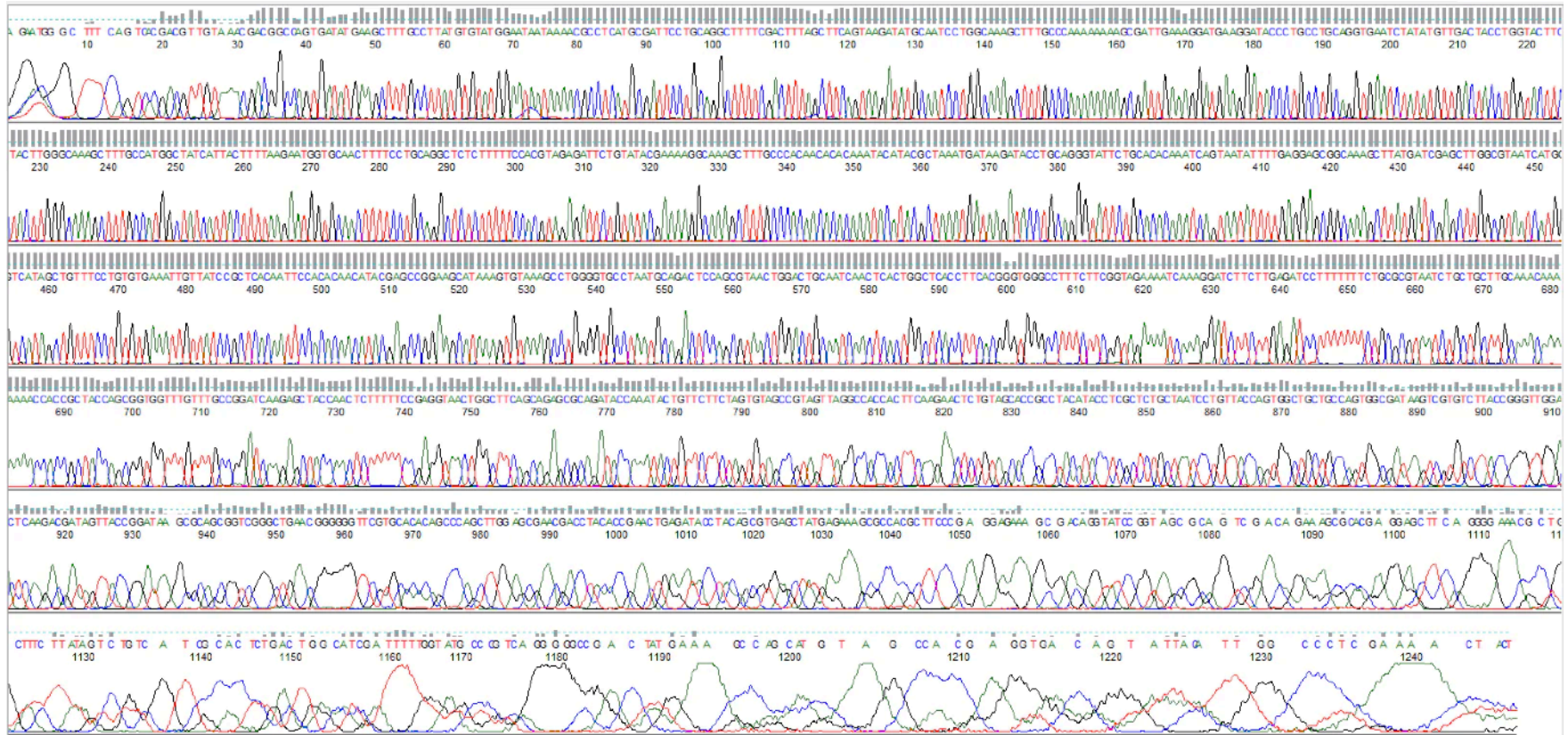
- Each dideoxy nucleotide is attached to a fluorescent marker.
- At the end of each cycle, a laser beam can detect the fluorescent marker and thus record the position of the nucleotide.



Chromatogram

GGTCATAGC ← Sequence

Chromatogram - Automated



Disclaimer

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