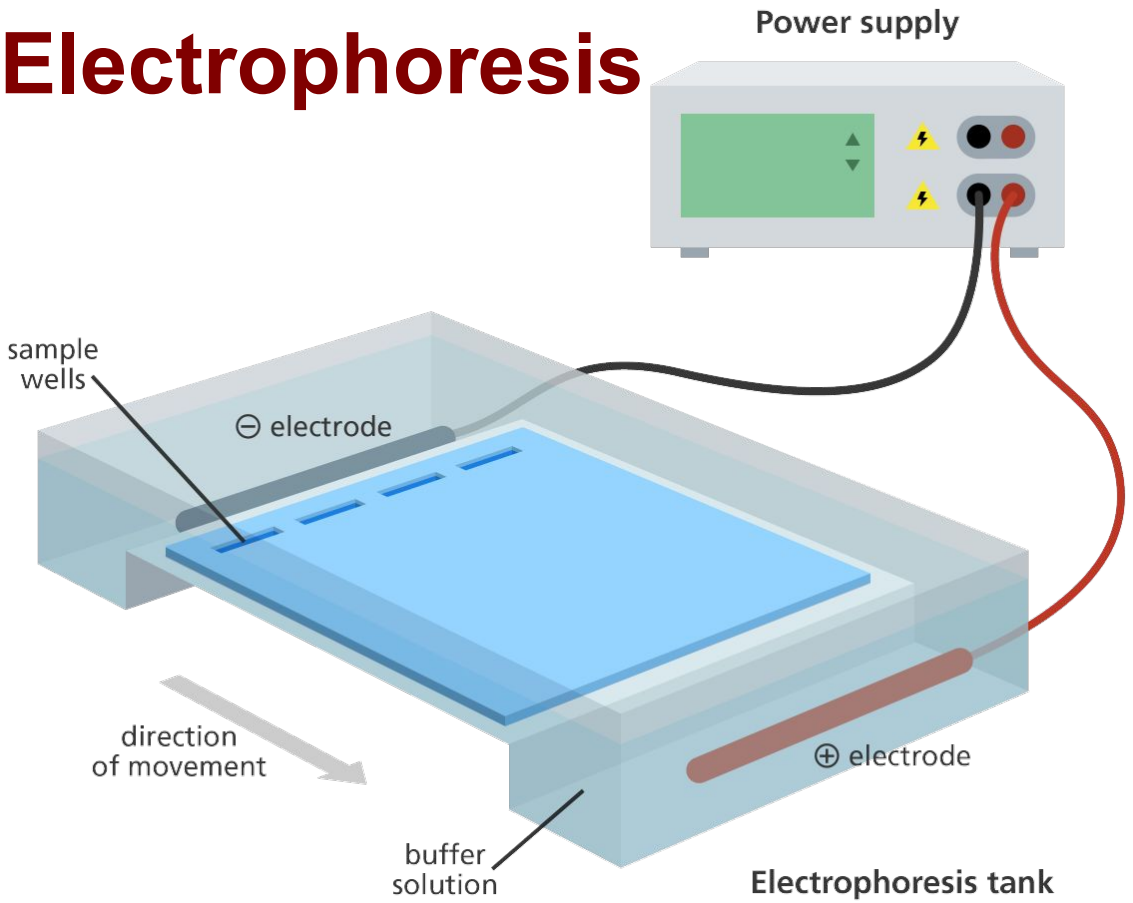
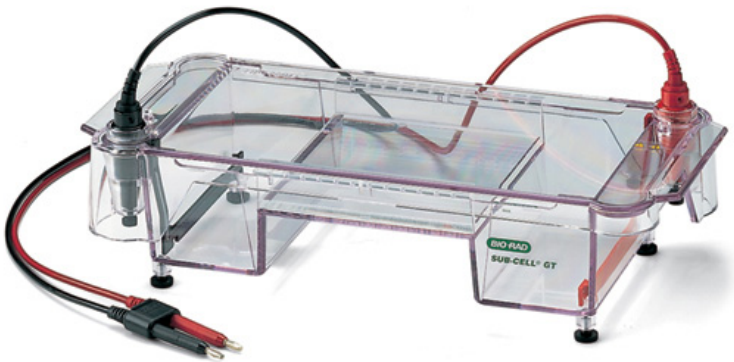
September, 2023

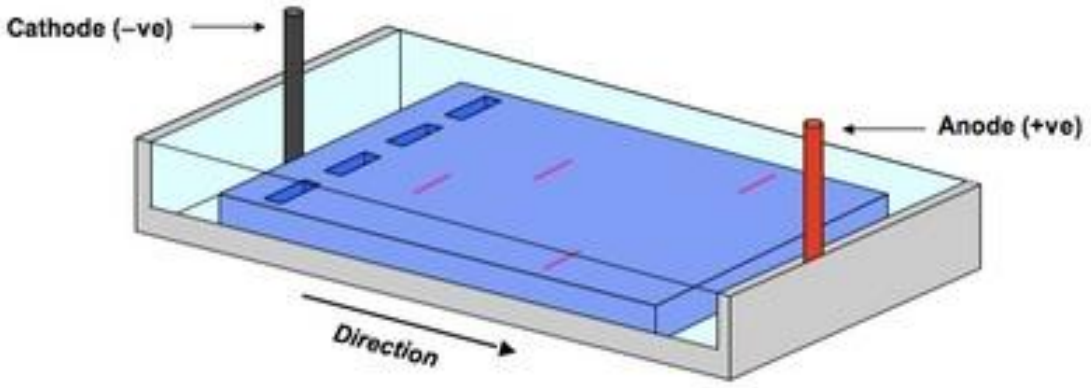
Laboratory Methods for the Analysis of DNA

Gel electrophoresis, PCR and DNA sequencing

DNA Gel Electrophoresis







DNA Gel Electrophoresis

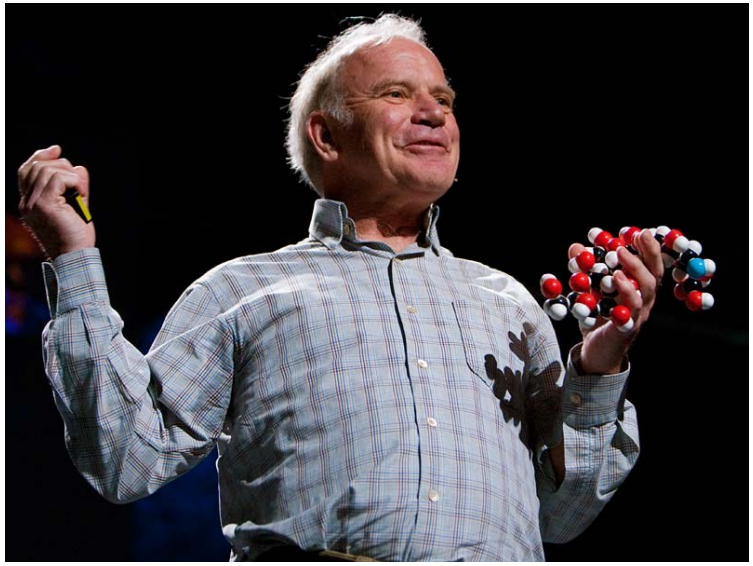
- Agarose gel is used to analyze a mixture of DNA.
- DNA is negatively charged and hence migrates towards the positive terminal in the applied electric field gradient.
- Different DNA molecules separate according to mass.
- Smaller molecules migrate faster.
- DNA is visualized in the gel by staining with ethidium bromide, which fluoresces under UV light.
- UV light at 302 nm or 365 nm wavelengths are used.

Polymerase Chain Reaction (PCR) and Its Applications

What is PCR?

PCR is an exponentially progressing synthesis of the defined target DNA sequences in vitro.

It was invented in 1983 by Dr. Kary Mullis



Nobel Prize in 1993

Polymerase Chain Reaction (PCR)

Why “Polymerase” ?

It is called “polymerase” because the only enzyme used in this reaction is DNA polymerase (Specifically Taq Polymerase).

Why “Chain” ?

It is called “chain” because the products of the first reaction become substrates of the following one, and so on.

Polymerase Chain Reaction (PCR)

The "Reaction" Components

1) Target DNA - contains the sequence to be amplified.

2) Pair of Primers - oligonucleotides that define the sequence to be amplified.

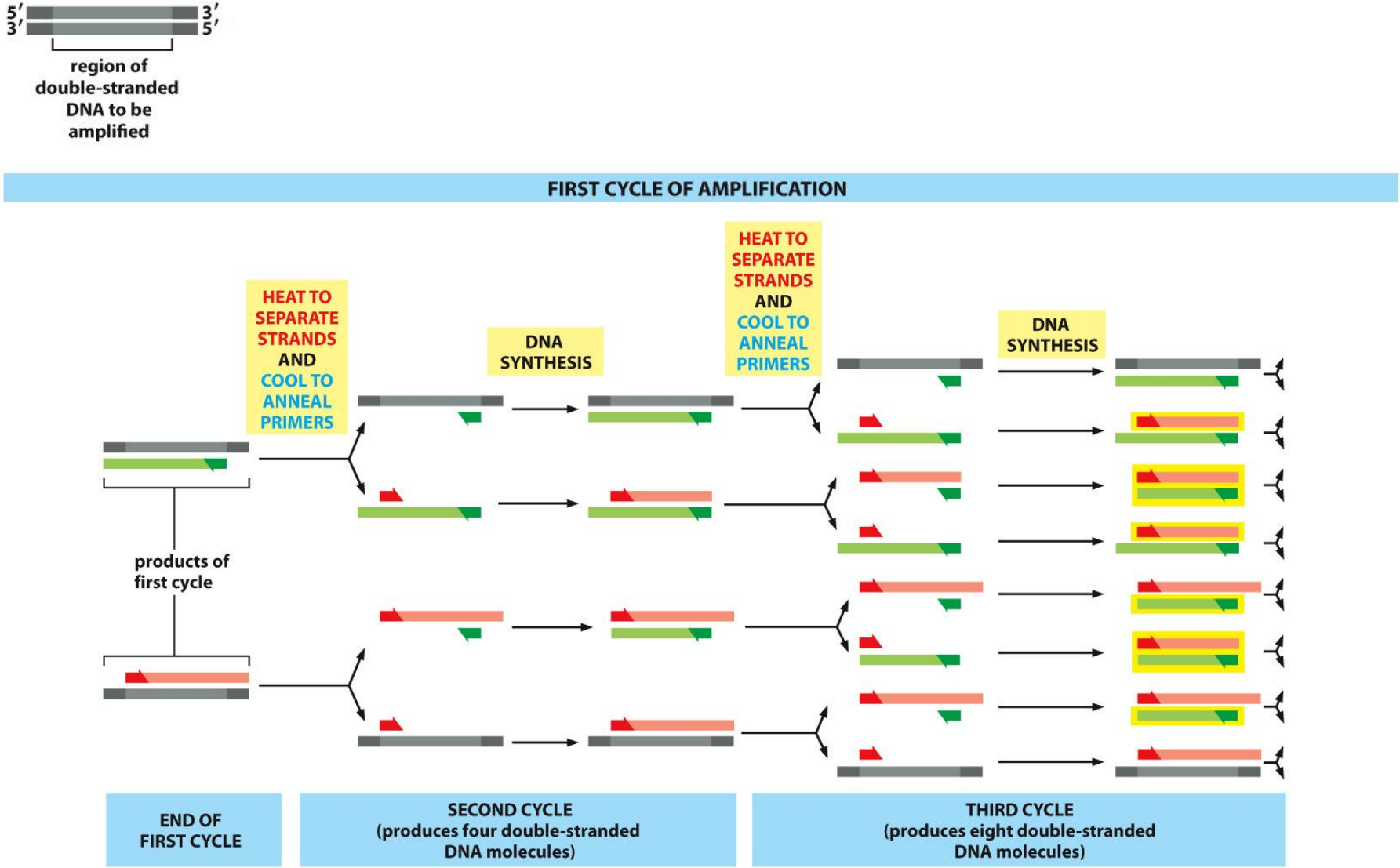
3) dNTPs - deoxynucleotidetriphosphates: DNA building blocks.

4) Thermostable DNA Polymerase – enzyme (Taq Polymerase) that catalyzes the reaction

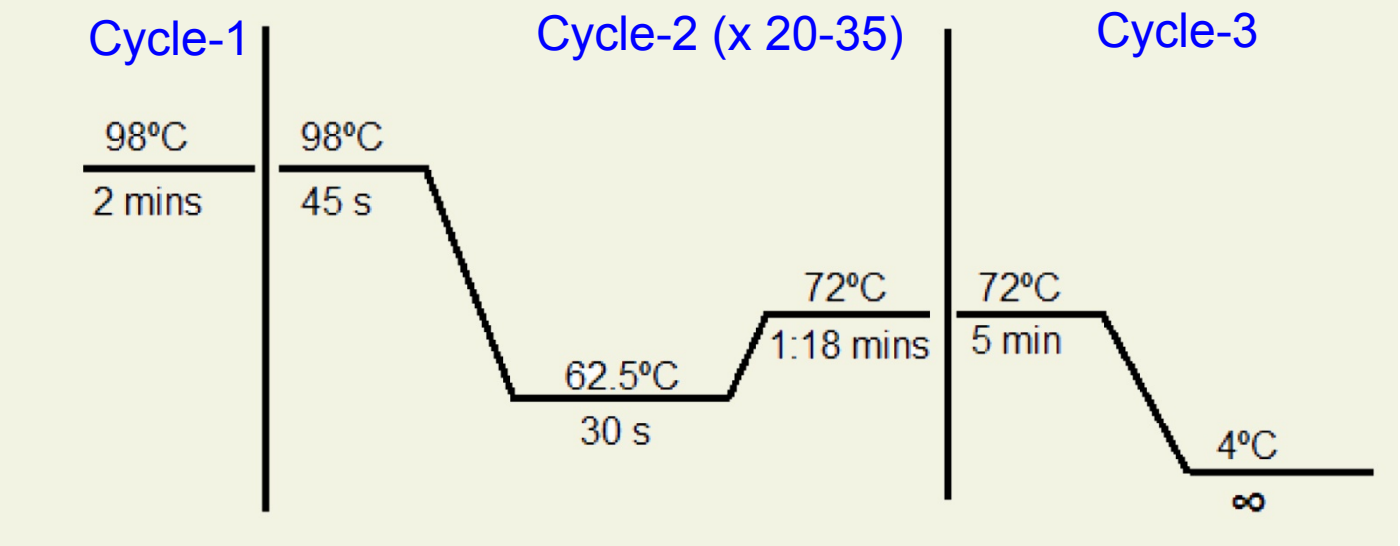
5) Mg^{++} ions - cofactor of the enzyme

6) Buffer solution - maintains pH and ionic strength of the reaction solution suitable for the activity of the enzyme

PCR Reaction Steps



Reaction Cycle and the Machine



30 cycles = 2^{30} i.e. 1,073,741,824 copies

PCR tube

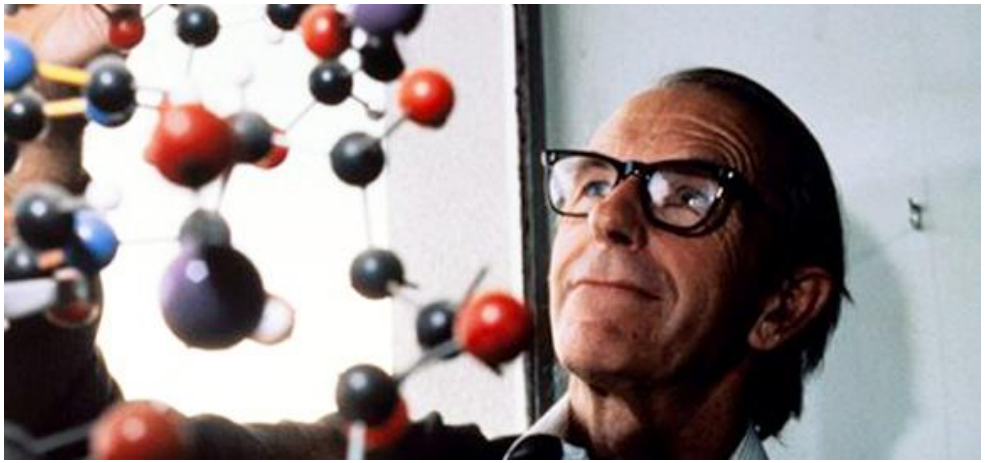
Thermocycler

DNA analysis in forensic science

- DNA analysis has become an important tool in forensic science in the past decade.
- The innocence project (http://www.innocenceproject.org) in the USA has used DNA testing to free 343 wrongfully convicted people and finding of 147 real perpetrators.

DNA Sequencing and Its Applications

DNA Sequencing



Frederick Sanger
Nobel Prize in 1980

Sequencing: **The process by which you determine the exact order of the nucleotides in a given region of DNA**

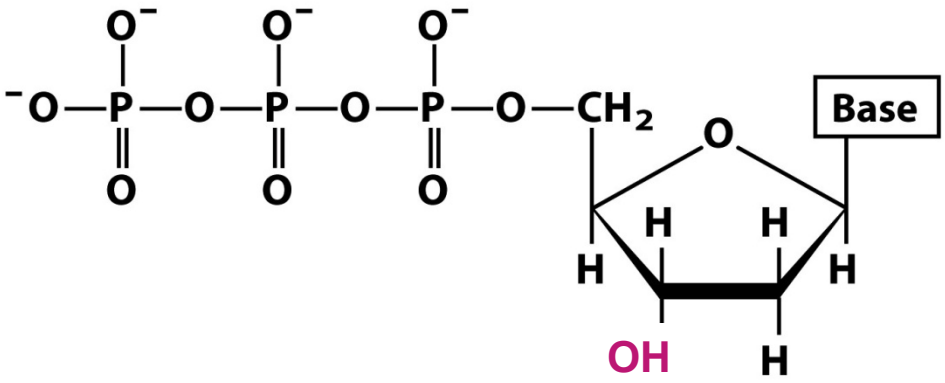
Why? **To decode the genetic information embedded in DNA**

How? **Through complementary chain synthesis and early termination**

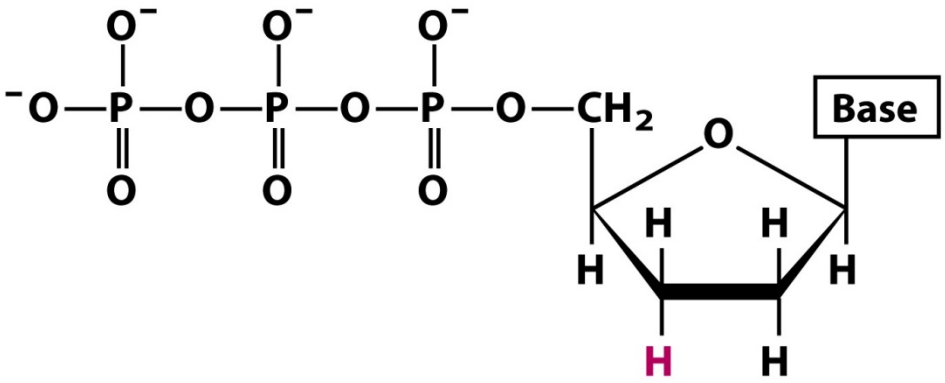
The synthesized chains are visualized by methods using:

- **Radioactive labels**
- **Nonradioactive labels**

Dideoxynucleotides (ddNTPs)



dNTP



ddNTP analog

The 3' hydroxyl has been changed to a hydrogen in ddNTP's, which terminates a DNA chain because a phosphodiester bond cannot form at this 3' location

Requirements for Sanger-Coulson Sequencing

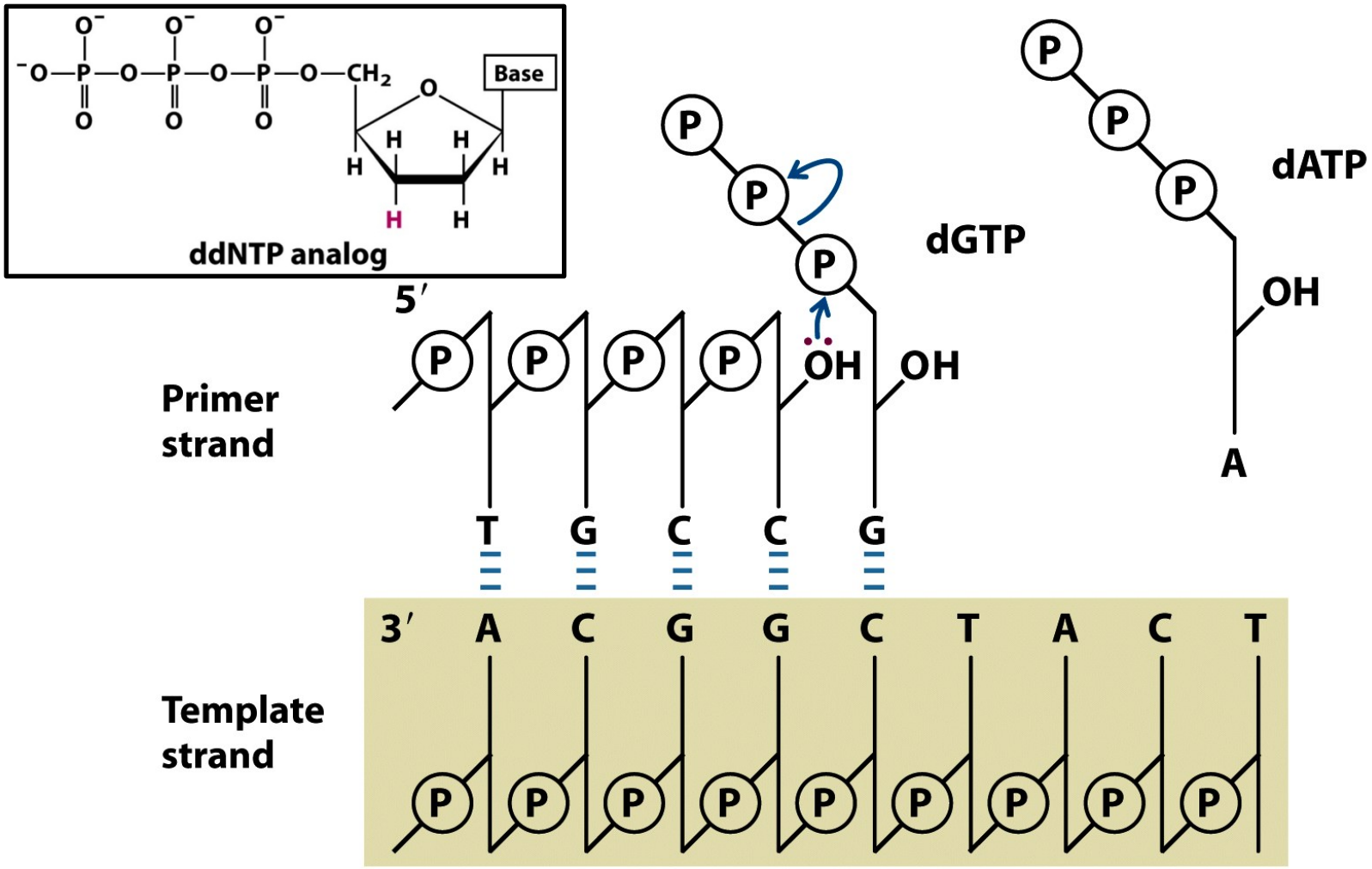
DNA to be sequenced must be in single stranded form

The region to be sequenced must be flanked by a stretch of 3' known sequence

Reagents needed are:

- **A primer complementary to the known region to direct chain synthesis.**
- **DNA polymerase.**
- **4 deoxynucleotide triphosphates (dNTPs).**
- **4 dideoxynucleotide triphosphates (ddNTPs) (one for each set)**

Mechanism of DNA polymerization



Since the 3' –OH is changed to a –H in ddNTPs, it is unable to form a phosphodiester bond by nucleophilic attack on the phosphate, and it will cause a termination in the DNA chain

Sanger's Dideoxy Sequencing Reaction

Template	3' – A T A T C T G T A C C T A G G T G A G T C A G T A C C
Primer	-5'
Differentially elongated fragments	5' – T A T A G A C
	5' – T A T A G A C ddA
	5' – T A T A G A C A T G G ddA
	5' – T A T A G A C A T G G A T C C ddA
	5' – T A T A G A C A T G G A T C C A C T C ddA
	5' – T A T A G A C A T G G A T C C A C T C A G T C ddA

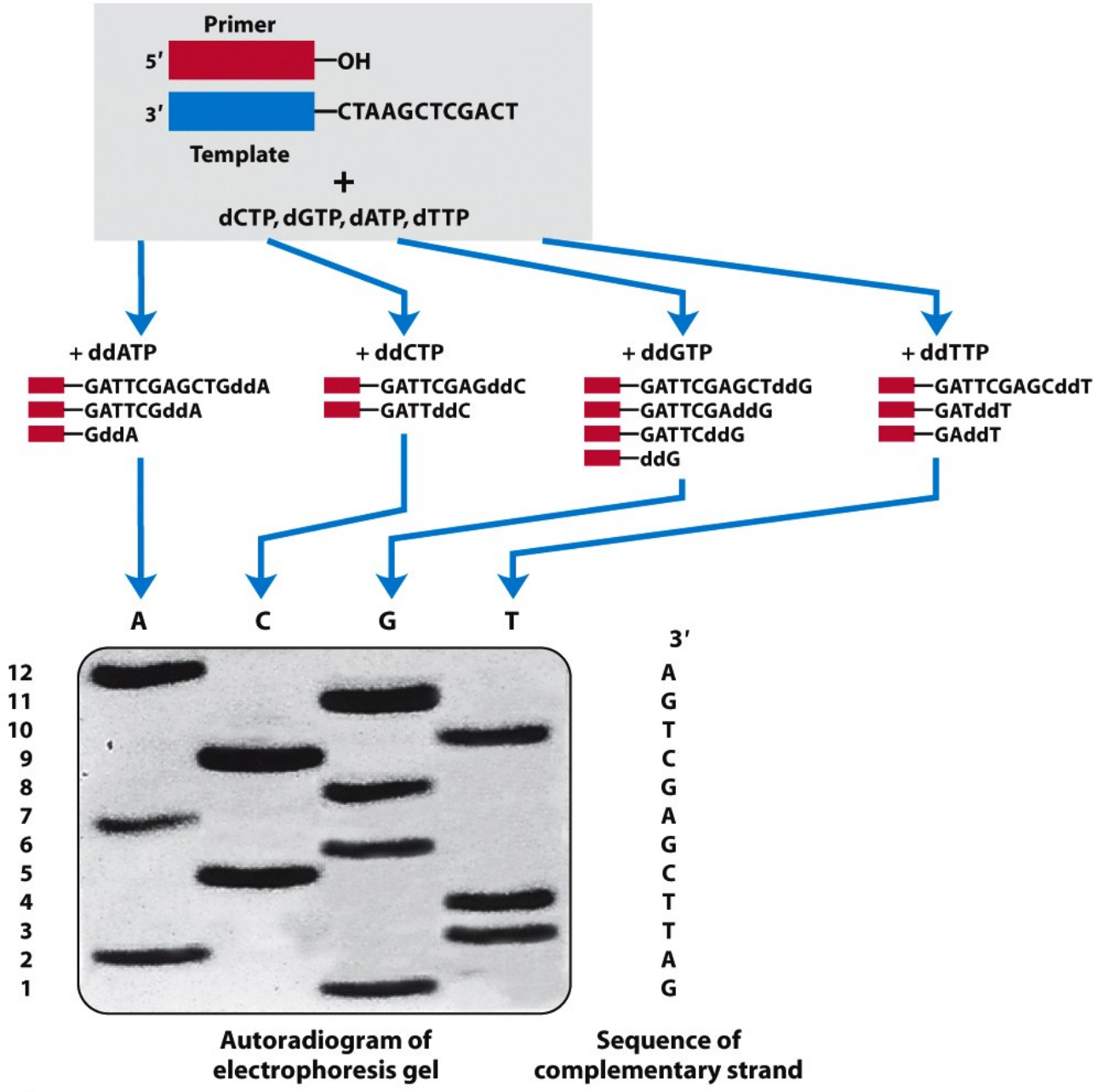
To each reaction along with the four regular dNTPs only one of the four dideoxynucleotides (ddATP in the above reaction) is added

The ddNTP is added to be approximately 100-fold lower in concentration than the corresponding dNTP

A series of differentially elongated DNA fragments (hence of different length) will be produced

Can be visualized by labelling the primers with radioactive phosphate (^{32}P or ^{33}P)

Sanger's Dideoxy Sequencing Reaction



Automated sequencing

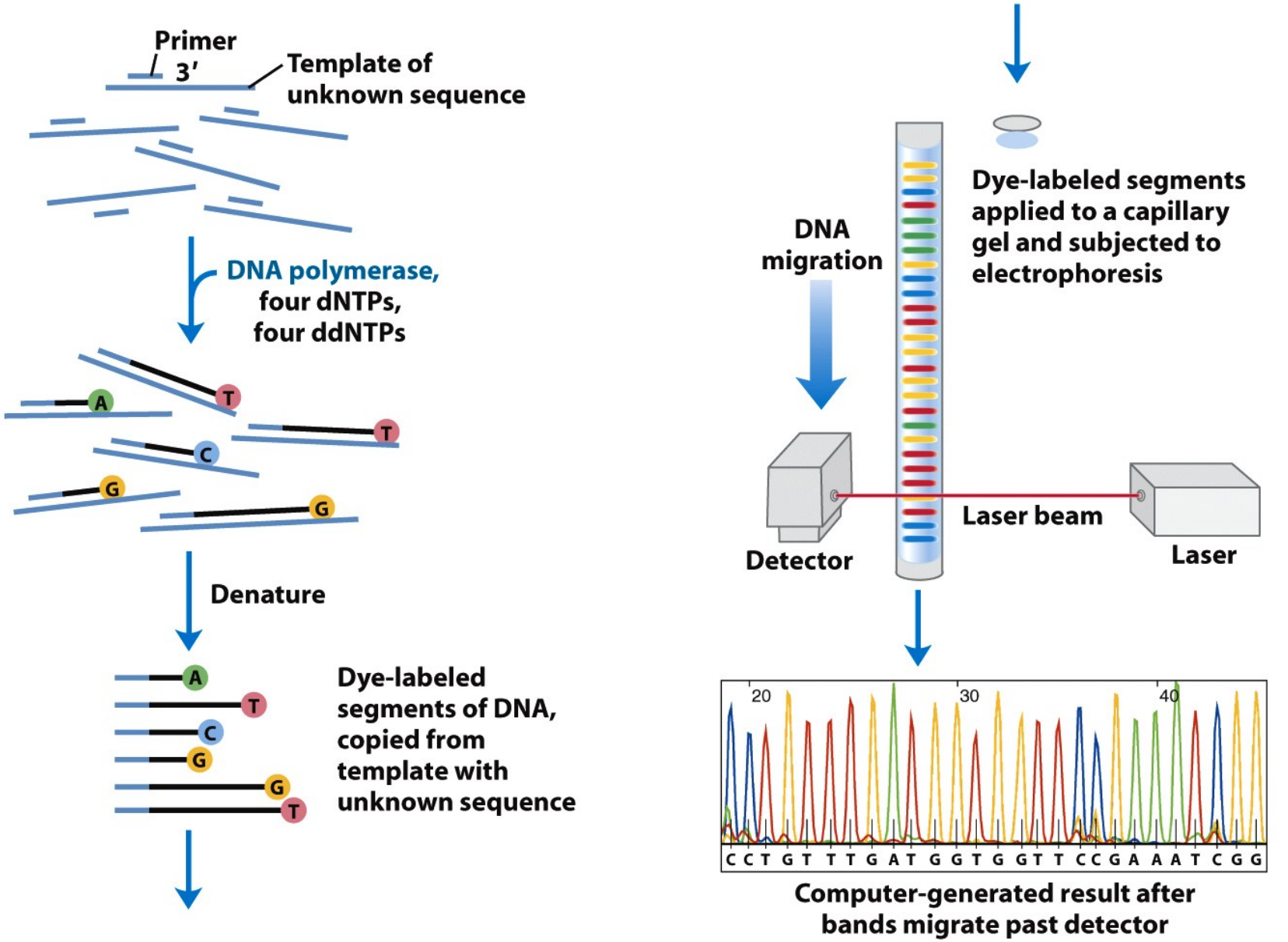


Figure 8-34
Lehninger Principles of Biochemistry, Fifth Edition
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Automated sequencing

Each dideoxynucleotide used are linked to a fluorescent molecule that gives all the fragments terminating in that nucleotide a particular color.

All four labelled ddNTPs are added to a single tube.

The resulting colored DNA fragments are then separated by capillary gel electrophoresis

The color associated with each peak is detected using a laser beam.

The DNA sequence is read by determining the sequence of colors in the peaks as they pass the detector.

This information is fed directly to a computer, which determines the sequence.

Extra resources

Videos:

Gel electrophoresis
https://www.youtube.com/watch?v=vq759wKCCUQ

Polymerase Chain Reaction (PCR)
https://www.dnalc.org/view/15475-The-cycles-of-the-polymerase-cha
-reaction-PCR-3D-animation.html