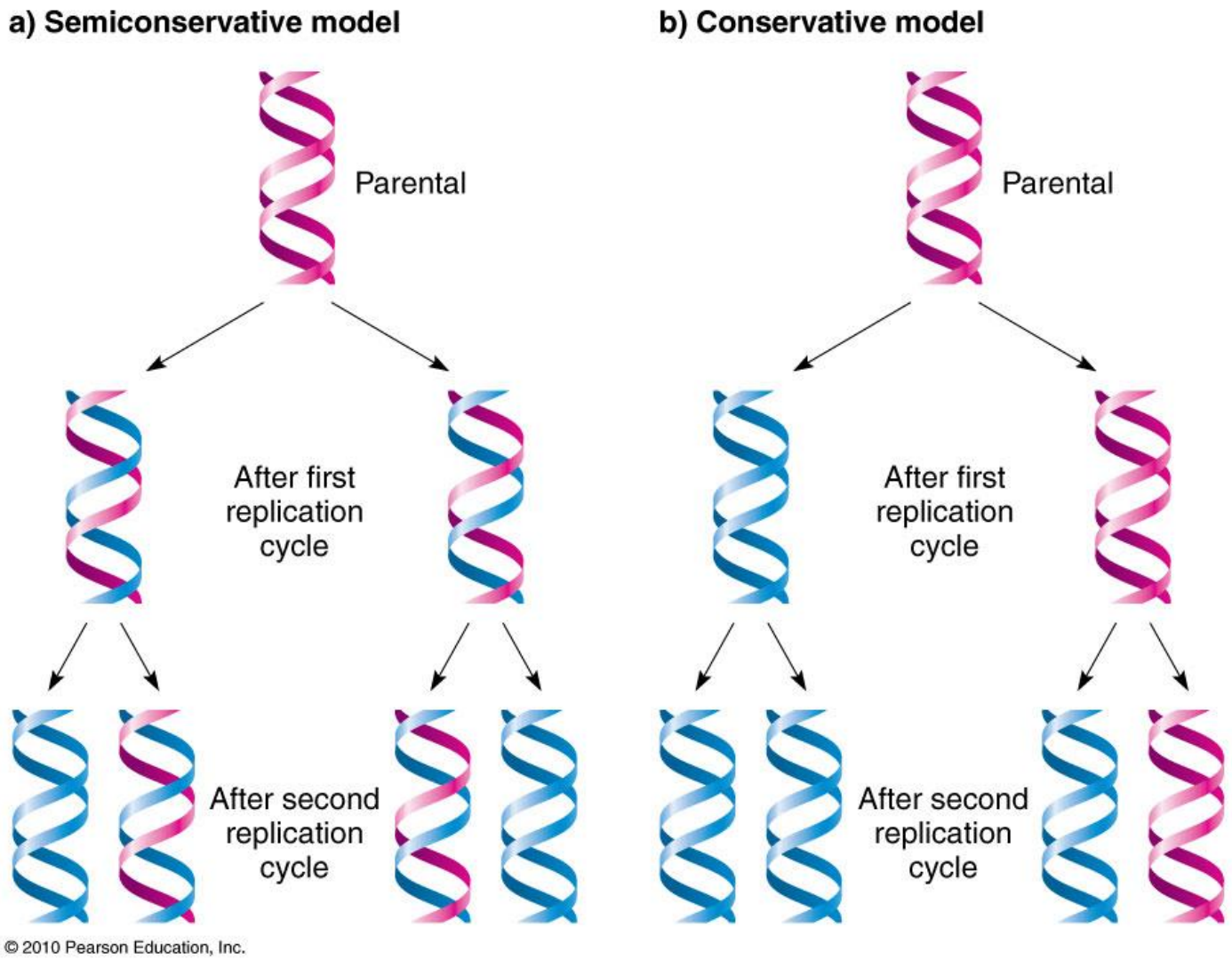
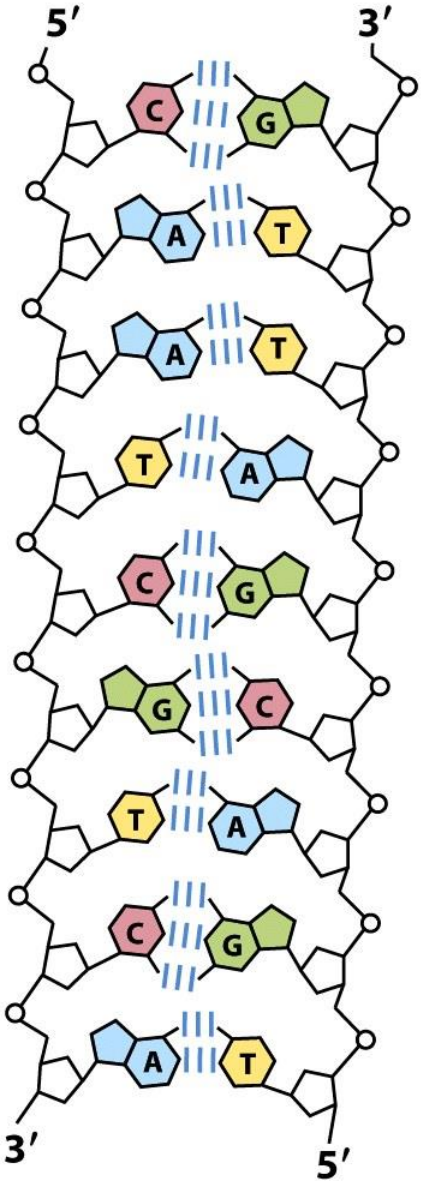
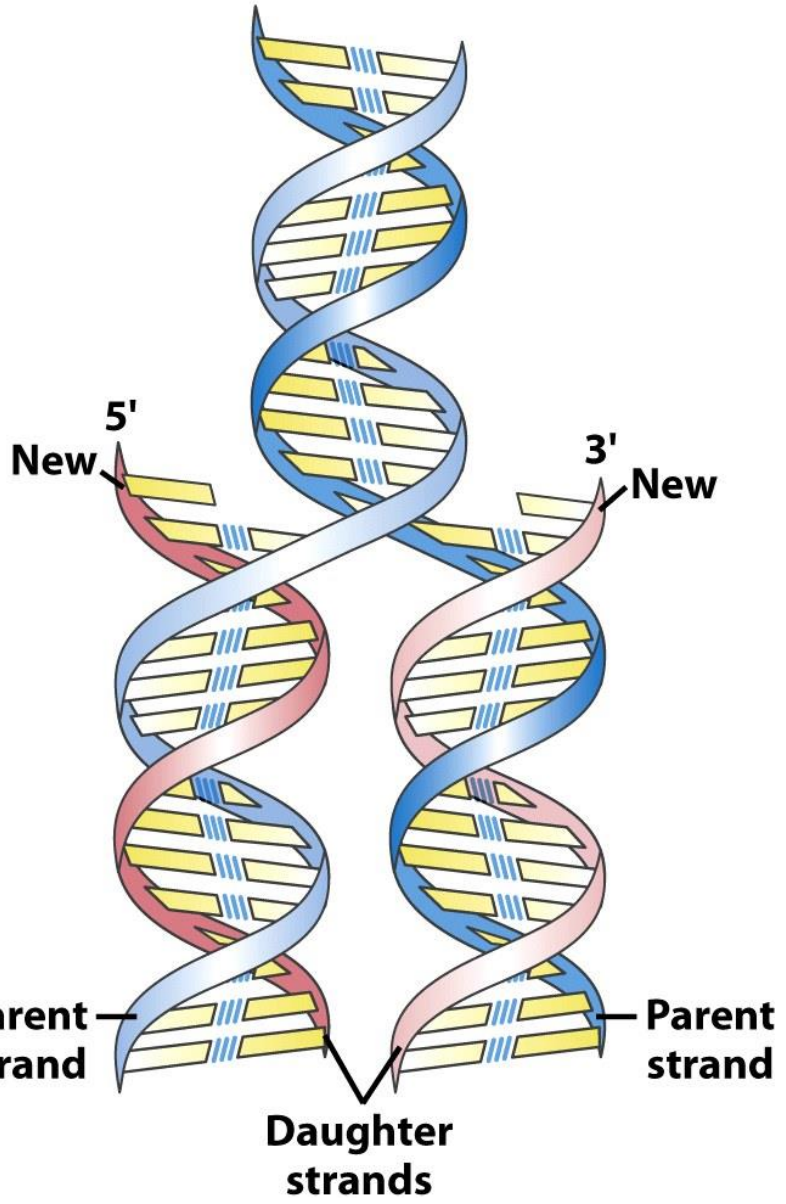
DNA Replication: An Overview

Different models for DNA replication

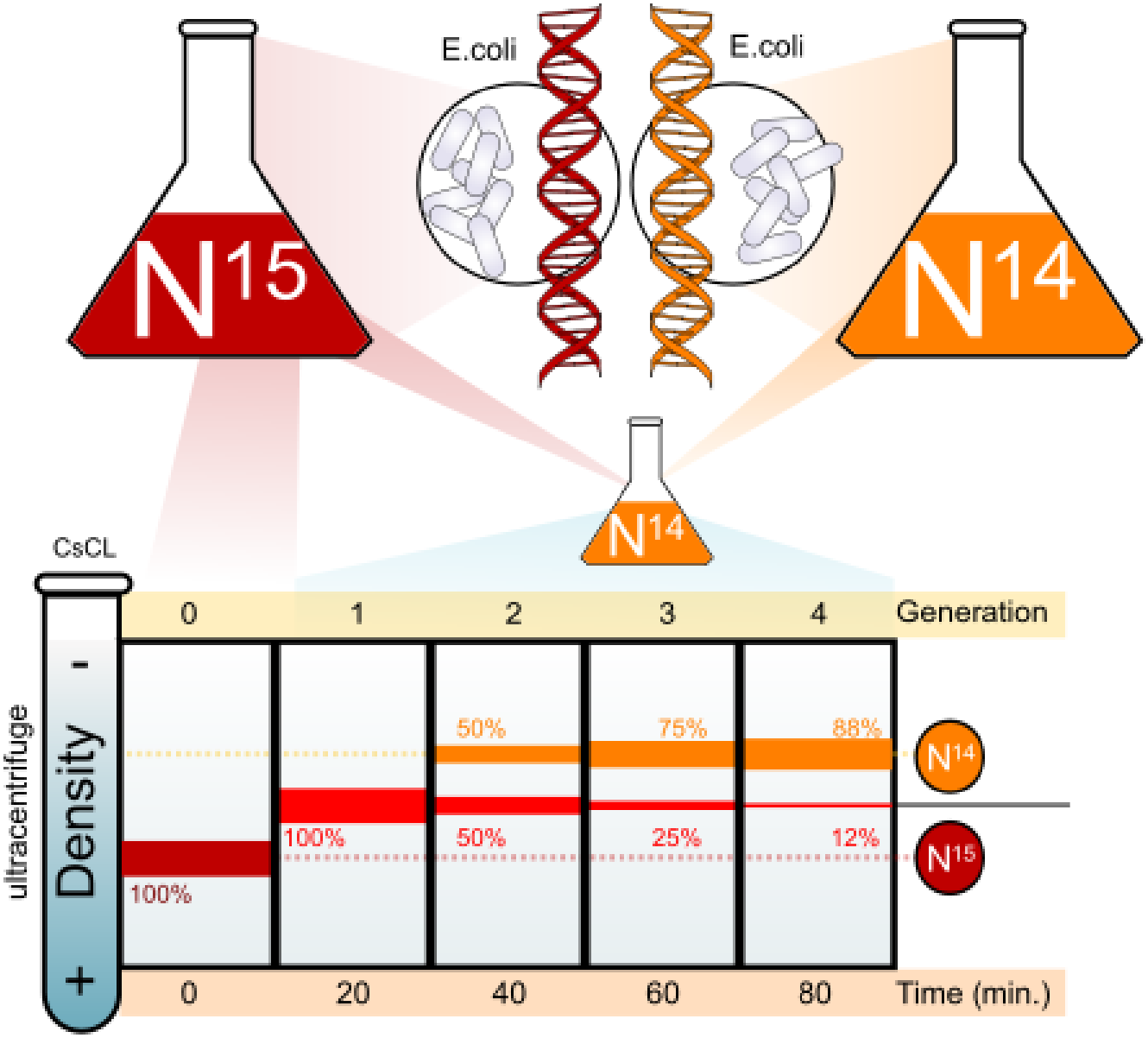


Semiconservative Replication of DNA as suggested by Watson and Crick

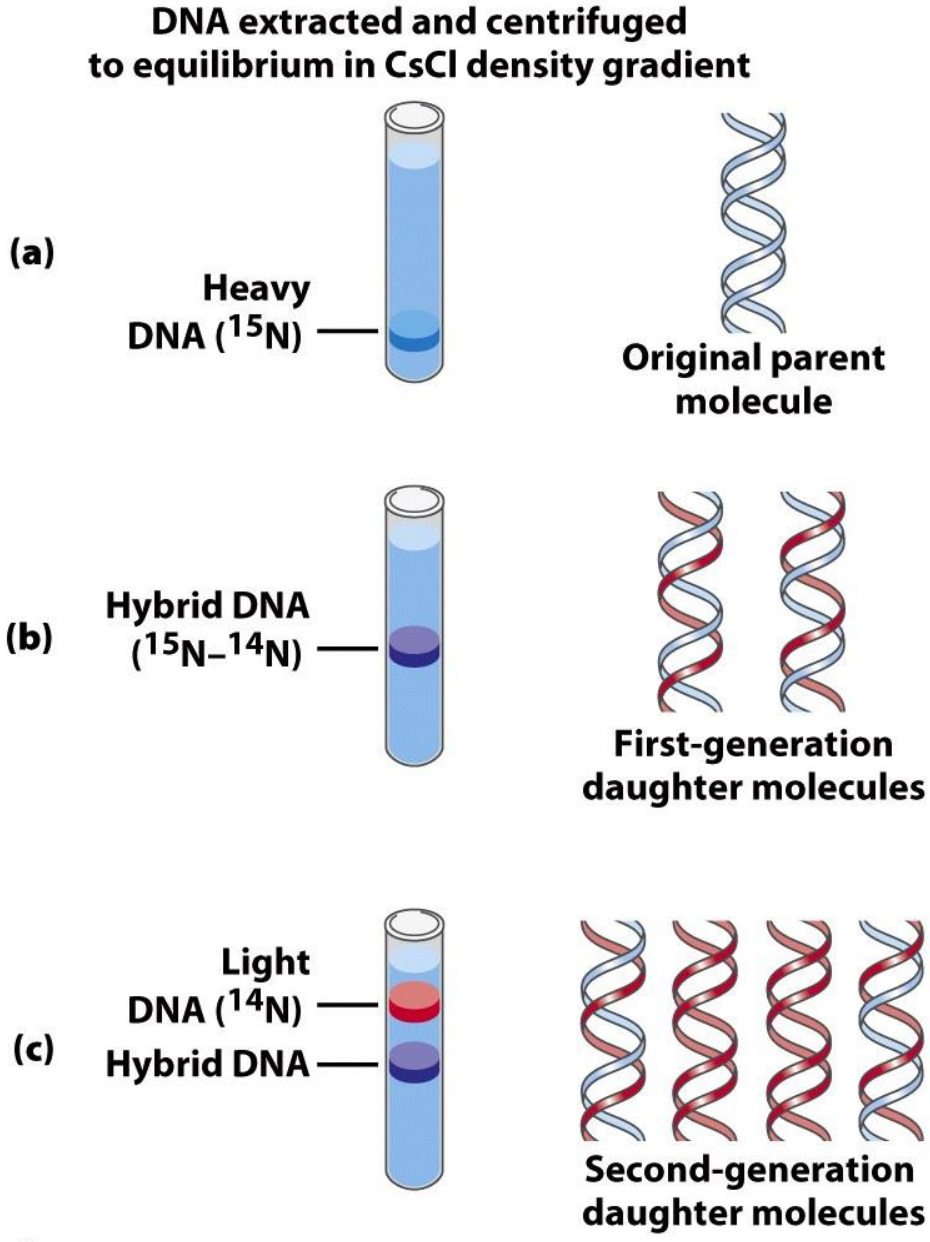




The Meselson-Stahl Experiment (1958)



The Meselson-Stahl Experiment Supports Semiconservative Replication



Meselson and Stahl grew a culture of *E.coli* for many generations in a medium that contained ^{15}N as the sole nitrogen source. ($^{15}NH4Cl$).
After many generations, all the *E.coli* cells had ^{15}N incorporated into the purine and pyrimidine bases of their DNA.

Meselson and Stahl took a sample of these bacteria and switched the rest of the bacteria to a medium that contained only ^{14}N (washed them before transferring to remove the medium containing ^{15}N).
They purified the DNA sample collected from the bacteria just before transfer to the ^{14}N containing medium.

Meselson and Stahl collected some of the bacteria after each division and extracted DNA from the bacterial cells (Collected samples of bacteria over the next few cellular generations).
Under the conditions they used, *E.coli* replicates its DNA every 20 minutes. They took samples at an interval of 20 minutes.

After extracting DNA from bacteria they checked for the density of DNA

Outline for Replication

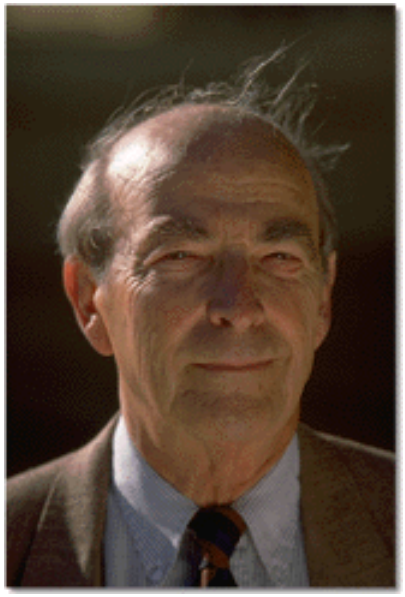
A. Initiation

B. Priming

C. Elongation

D. Proofreading and Termination

Arthur Kornberg
Nobel Prize in 1959



Worked with *E. coli*. Discovered the mechanisms of DNA synthesis in 1956.

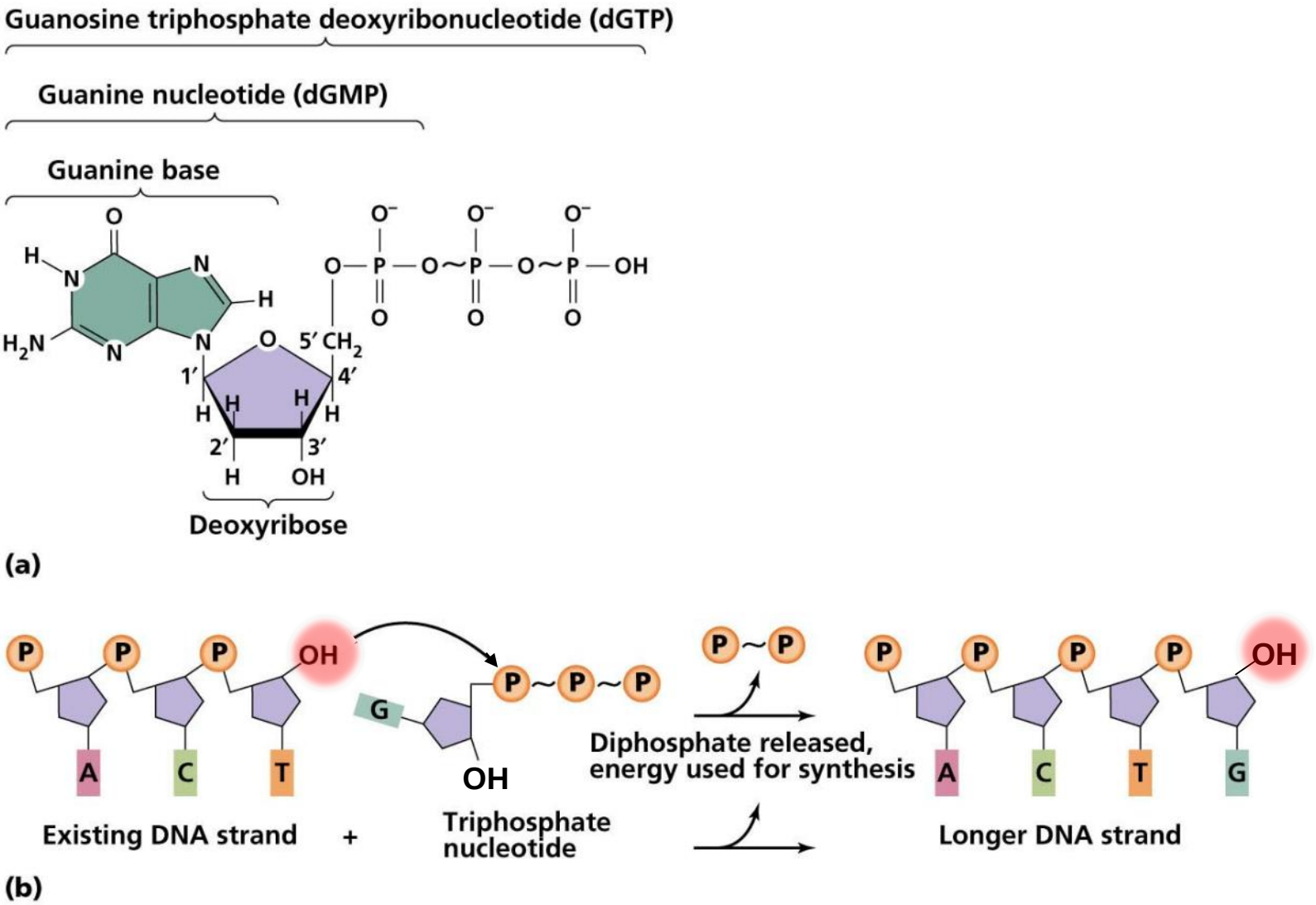
<u>Four components are required:</u>

1. dNTPs: dATP, dTTP, dGTP, dCTP

   (deoxyribonucleoside 5'-triphosphates)

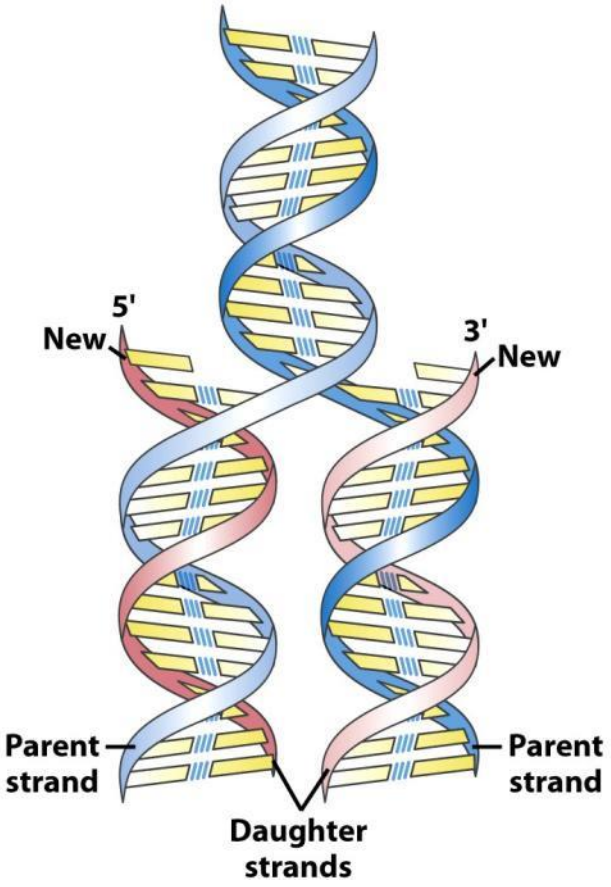
   (sugar-base + 3 phosphates)
2. DNA template
3. DNA polymerase (*Kornberg enzyme*)
4. Mg^{2+} (optimizes DNA polymerase activity)

Polymerization of nucleotide triphosphates by DNA polymerase – needs free 3'OH



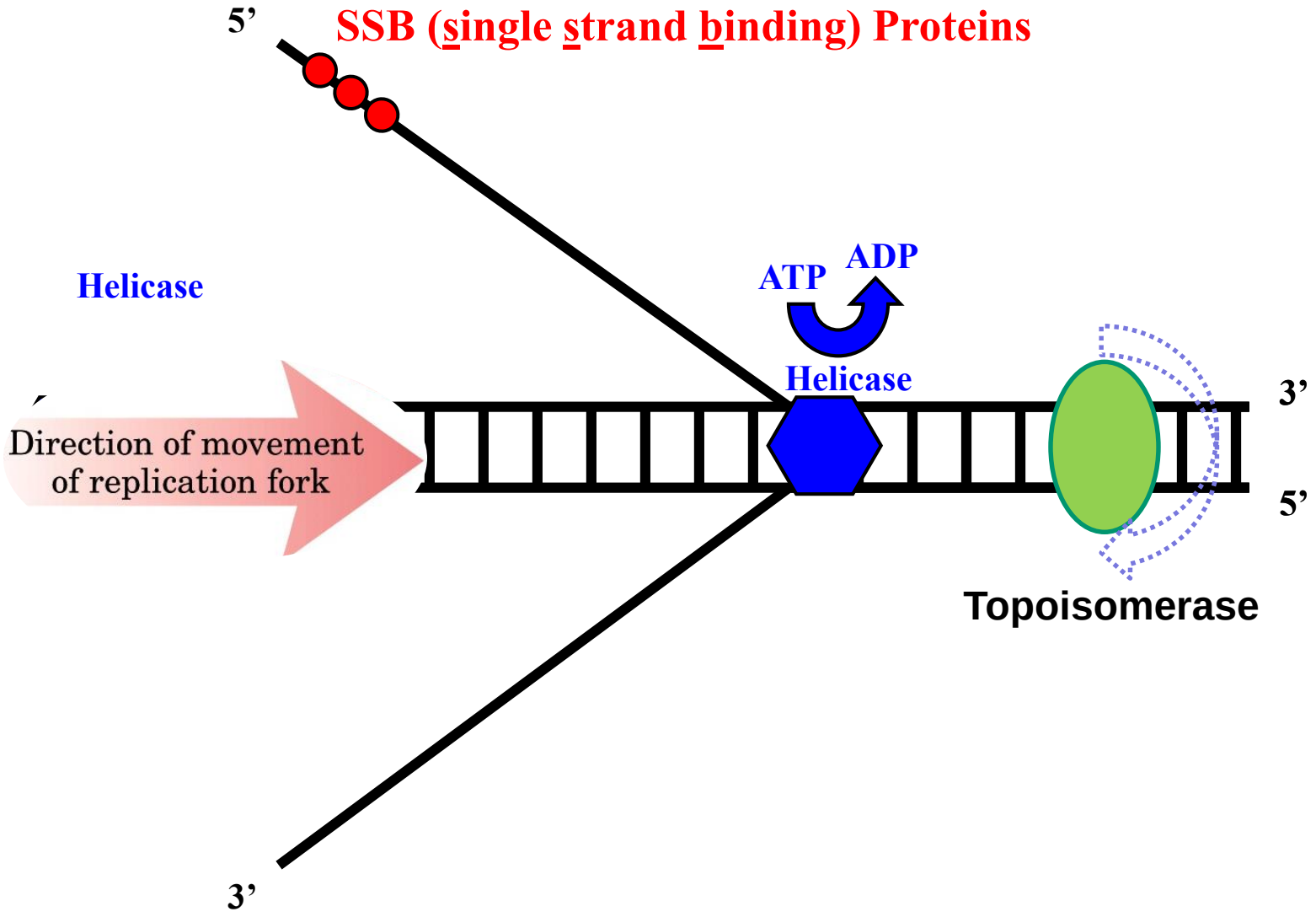
Problem -1

DNA is double helical. Hence the two strands have to be separated from each other



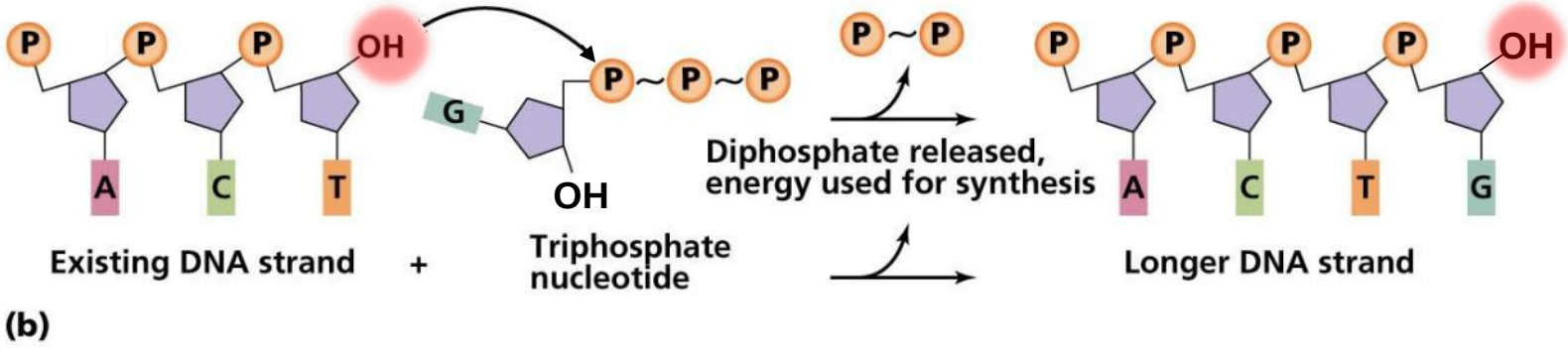
Problem - 1 : DNA unwinding

DNA is unwound by helicase, stabilized by SSB proteins;
supercoiling is relaxed by Topoisomerase



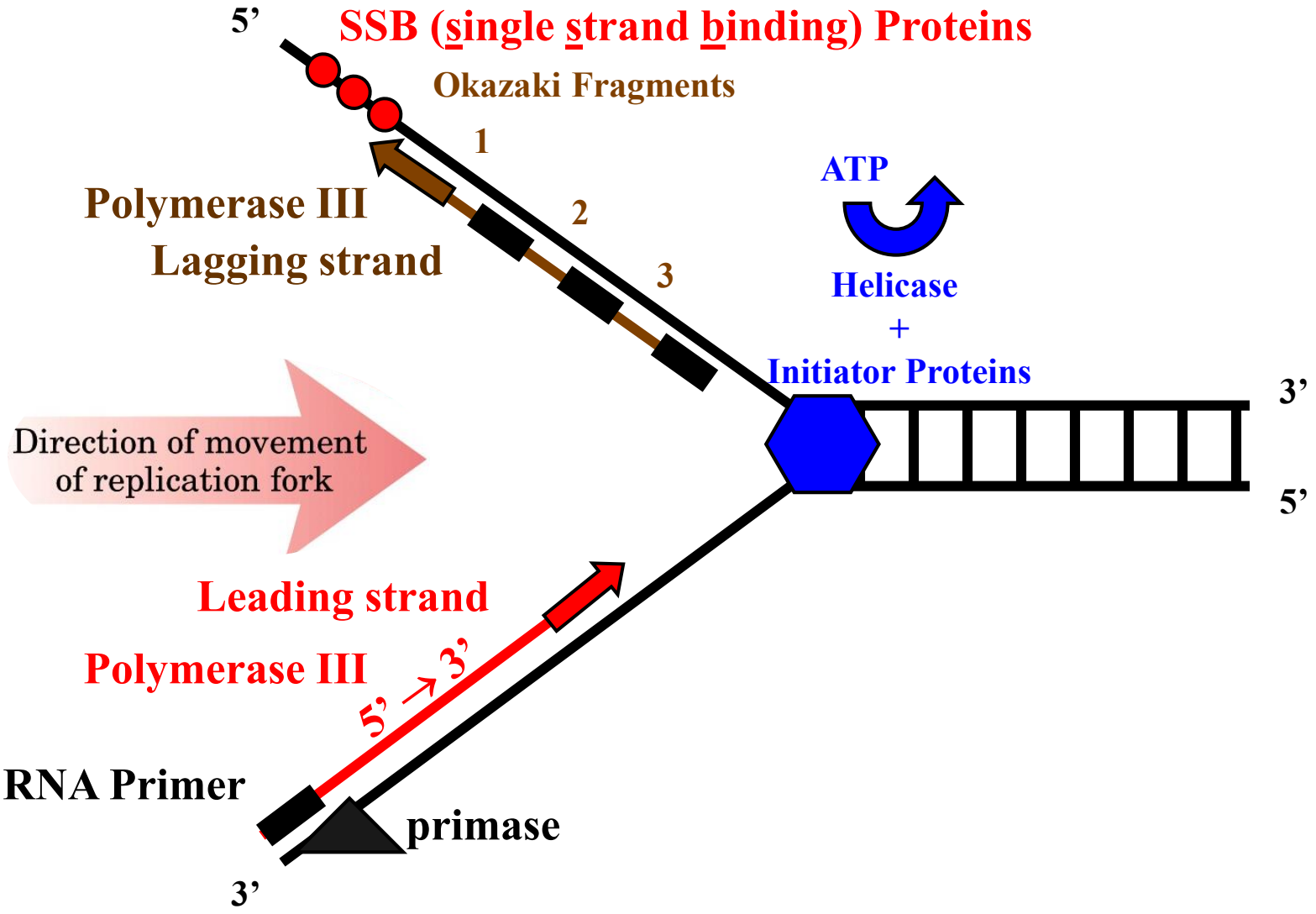
Problem - 2

DNA polymerase needs a free 3’-OH of a pre-existing nucleotide to extend the chain



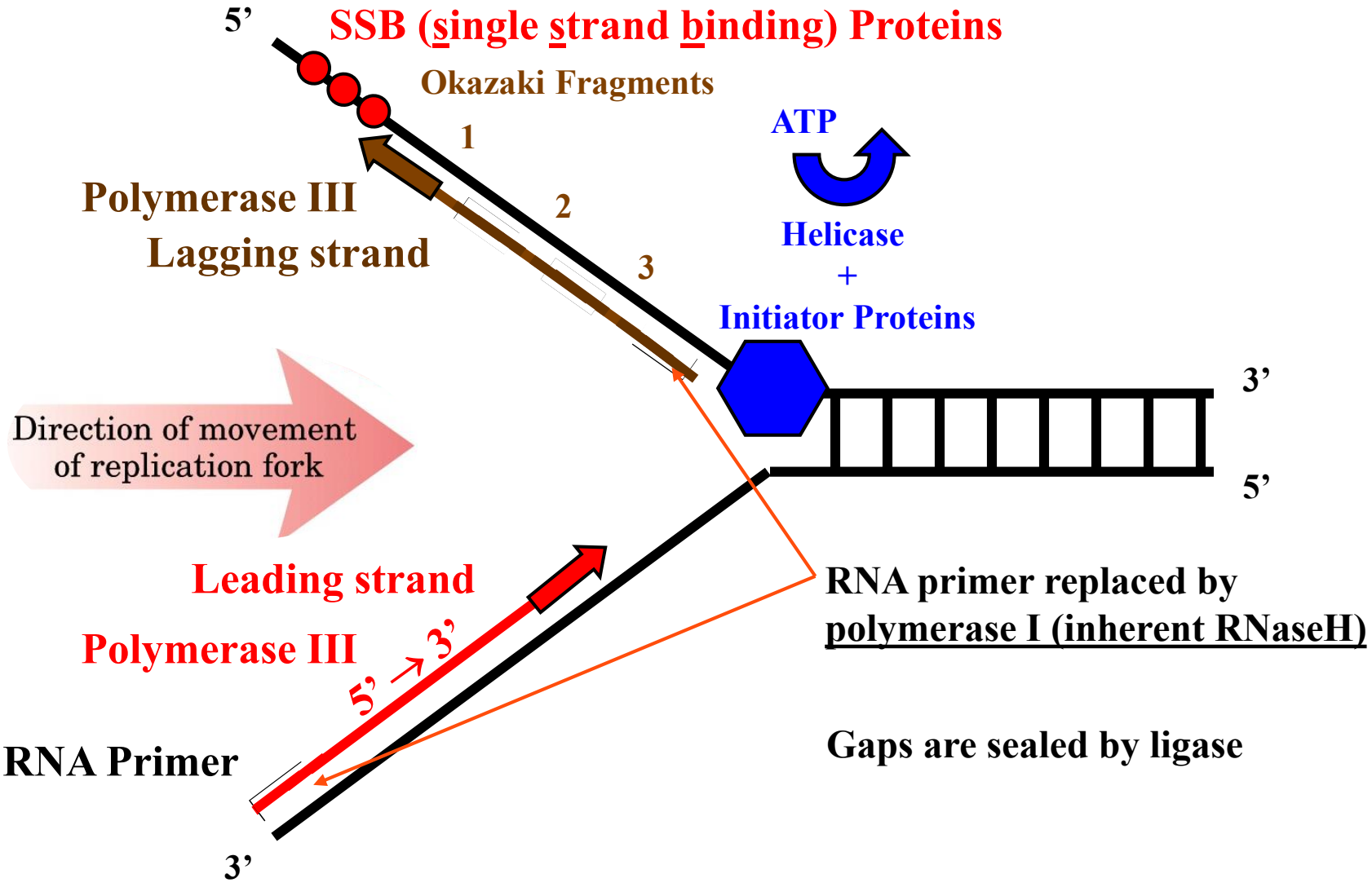
Problem – 2 : Free 3’ OH group required

Primase synthesizes small RNA primers,
DNA Polymerase III extends them



Problem -3 : RNA primers need to be removed

RNA primers are replaced with DNA by DNA Polymerase I, which has both RNaseH and DNA Polymerase activity. The gaps are sealed by Ligase

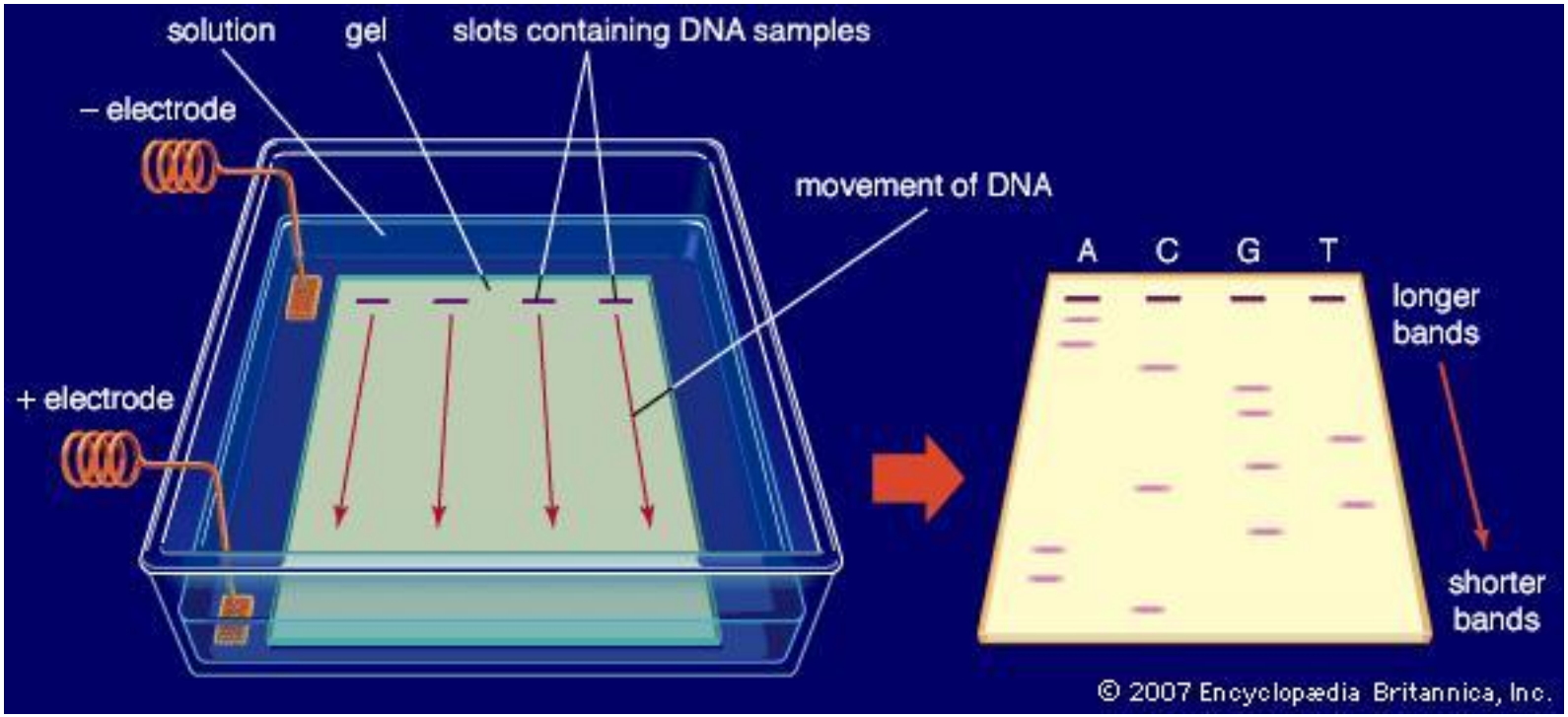


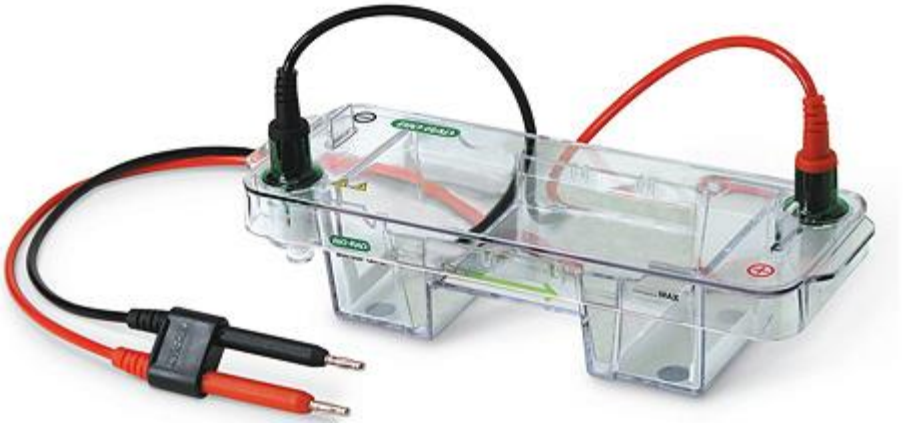
DNA Replication: Summary

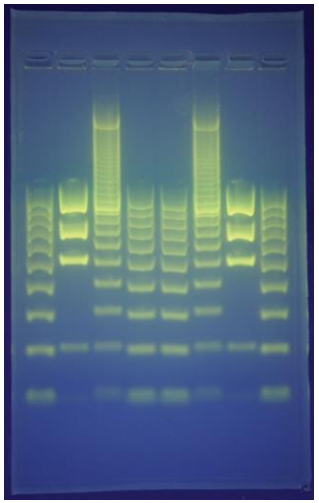
- DNA is unwound by initiator protein + helicase
- Single stranded DNA is stabilized by single stranded DNA binding proteins (SSB)
- Supercoiling is relaxed by Topoisomerase
- Small RNA primers are added by Primase (RNA polymerase)
- DNA Polymerase III extends from 3' ends of primer sequences
  - DNA Polymerase can only extend from pre-existing 3'OH group
  - Hence synthesis is always from 5'-3' direction
  - It needs DNA template, dNTPs and Mg^{2+} ions
- Replication is continuous in leading strand and discontinuous in lagging strands. Small newly synthesized DNA fragments in lagging strand are called Okazaki fragments
- RNA primers are replaced with DNA by DNA Polymerase I, which has both RNaseH and DNA Polymerase activity
- The gaps are sealed by Ligase

Laboratory methods for nucleic acid analysis

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.

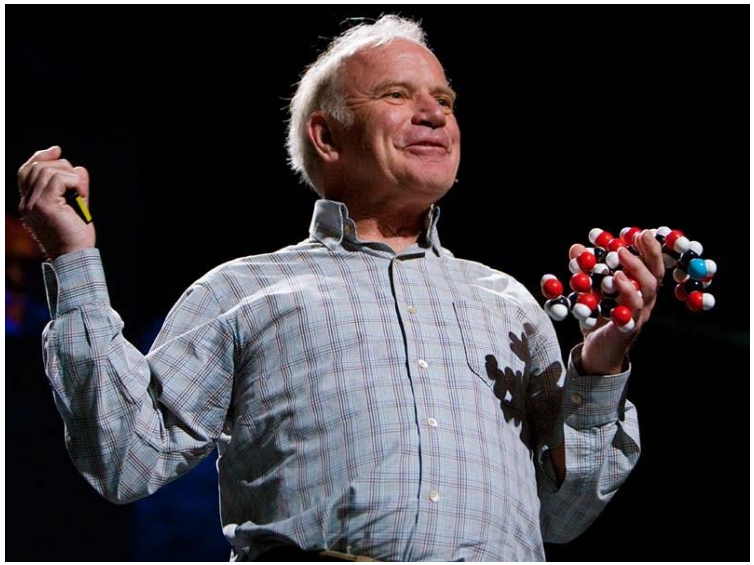
Polymerase Chain Reaction (PCR) and Its Applications

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.

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.

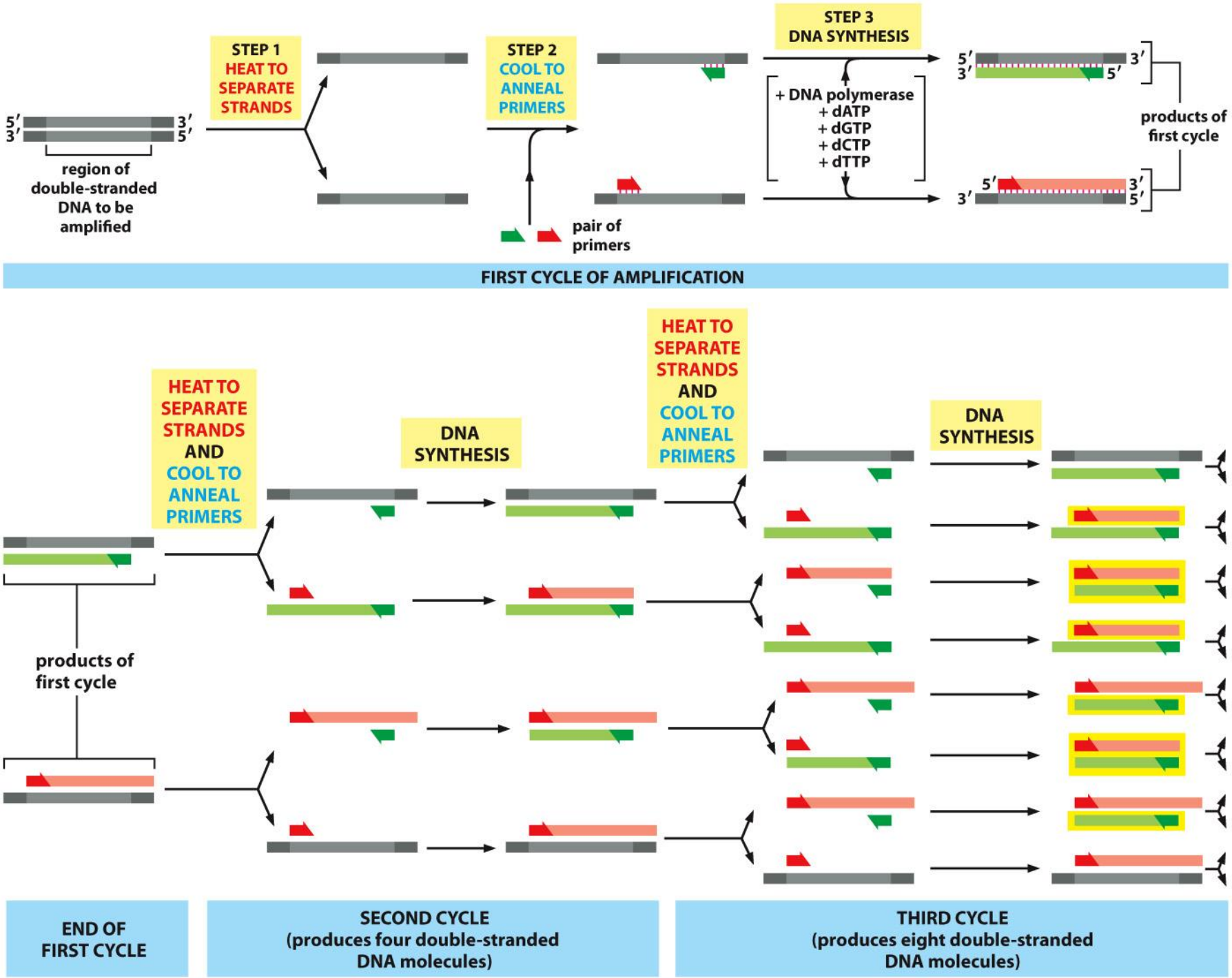
2) Pair of Primers - oligonucleotides that define the sequence to be amplified.

3) dNTPs - deoxynucleotidetriphosphates: DNA building blocks.

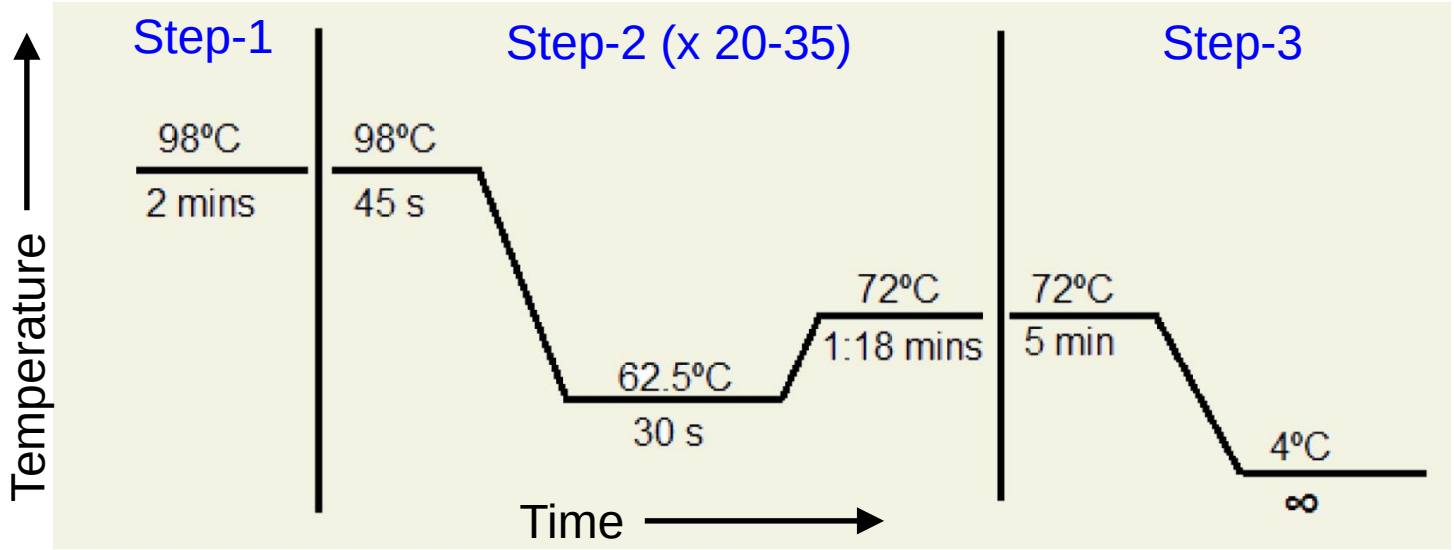
4) Thermostable DNA Polymerase - enzyme that catalyzes the reaction

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


STEP 1
HEAT TO SEPARATE STRANDS
STEP 2
COOL TO ANNEAL PRIMERS
STEP 3
DNA SYNTHESIS
+ DNA polymerase
+ dATP
+ dGTP
+ dCTP
+ dTTP
region of double-stranded DNA to be amplified
pair of primers
products of first cycle
FIRST CYCLE OF AMPLIFICATION
HEAT TO SEPARATE STRANDS AND COOL TO ANNEAL PRIMERS
DNA SYNTHESIS
HEAT TO SEPARATE STRANDS AND COOL TO ANNEAL PRIMERS
DNA SYNTHESIS
products of first cycle
END OF FIRST CYCLE
SECOND CYCLE
(produces four double-stranded DNA molecules)
THIRD CYCLE
(produces eight double-stranded DNA molecules)

Reaction Cycle and the Machine

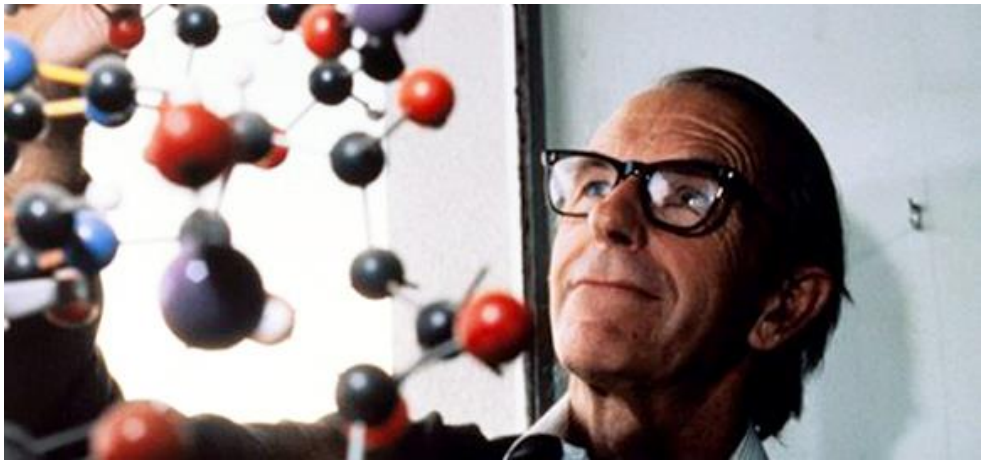


30 cycles = 2^{30} i.e. 1,073,741,824 copies

PCR tube

Thermocycler

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**

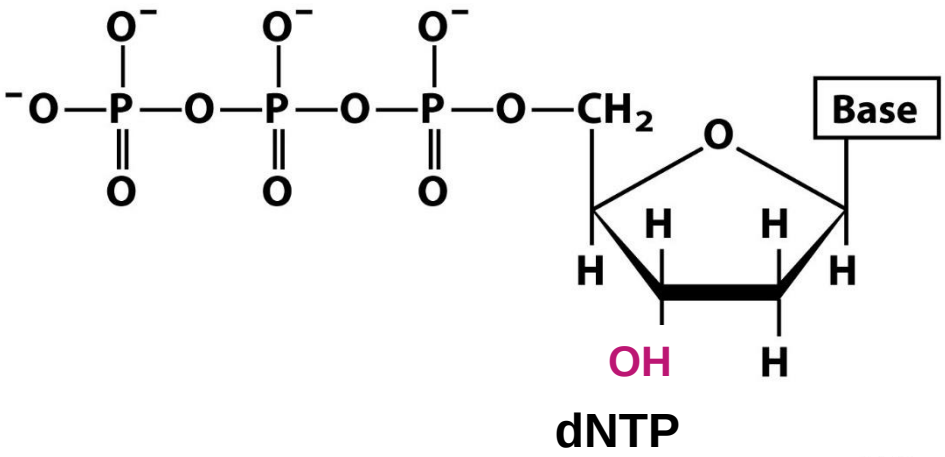
Why? **To decode the genetic information embedded in DNA**

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

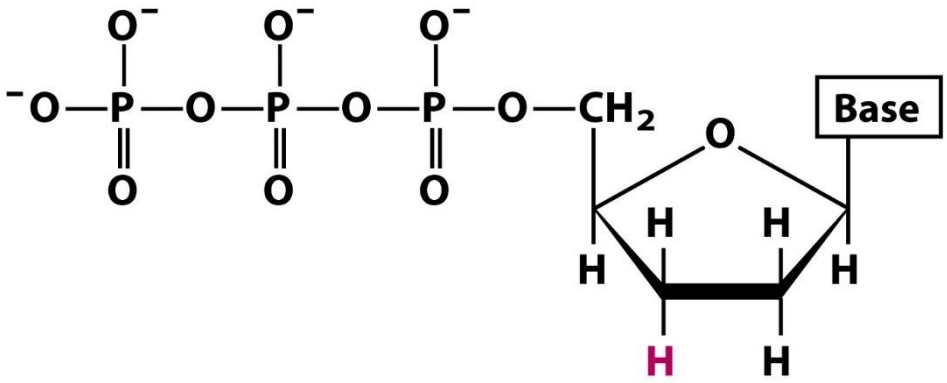
The synthesized chains are visualized by methods using:

- **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 strand form

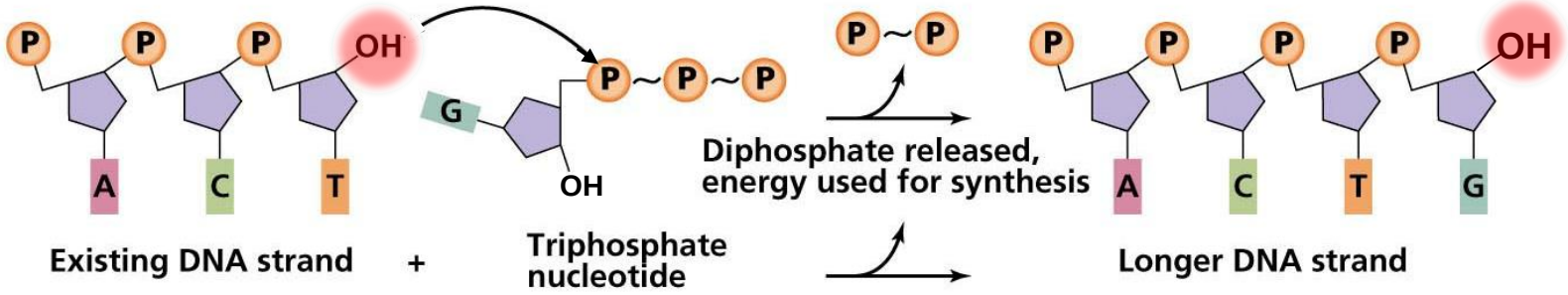
The region to be sequenced must be flanked by a stretch of 3' known sequence

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

Sequencing using denaturing gel-electrophoresis

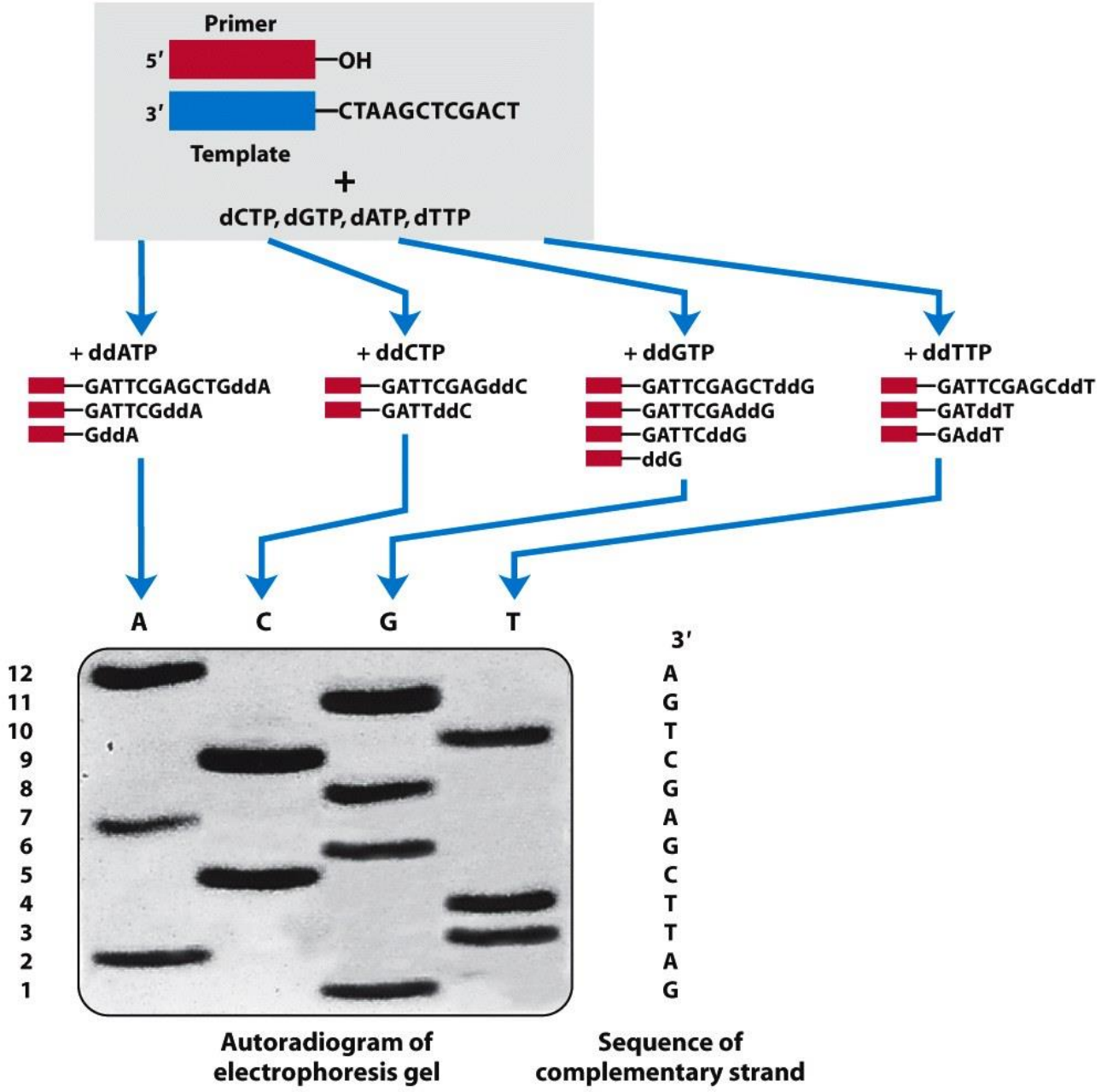
To each reaction along with the four regular dNTPs, only one of the four dideoxynucleotides (ddATP in the above reaction) are added

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)

Sequencing using denaturing gel-electrophoresis



Automated sequencing

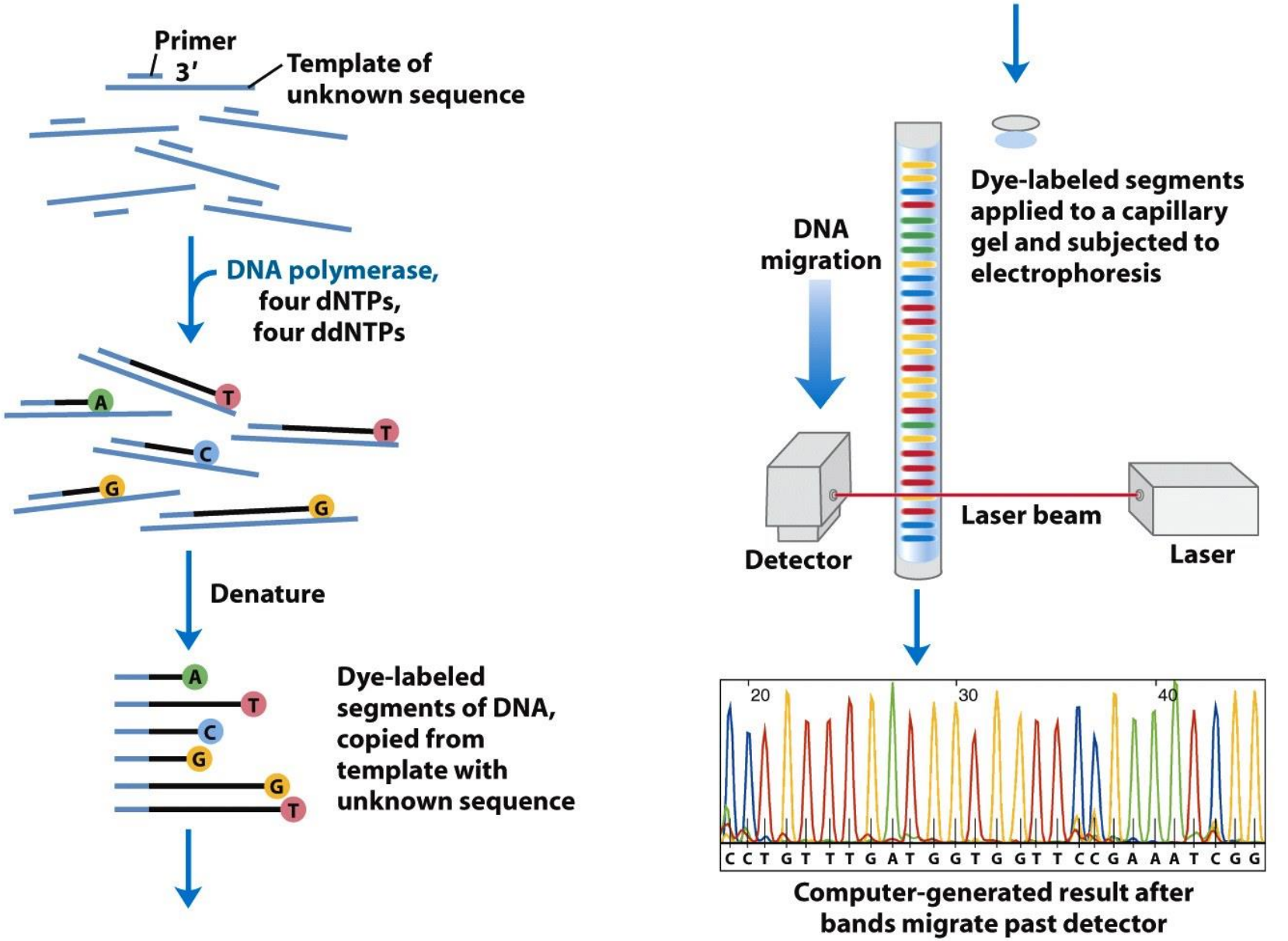


Figure 8-34
Lehninger Principles of Biochemistry, Fifth Edition

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.

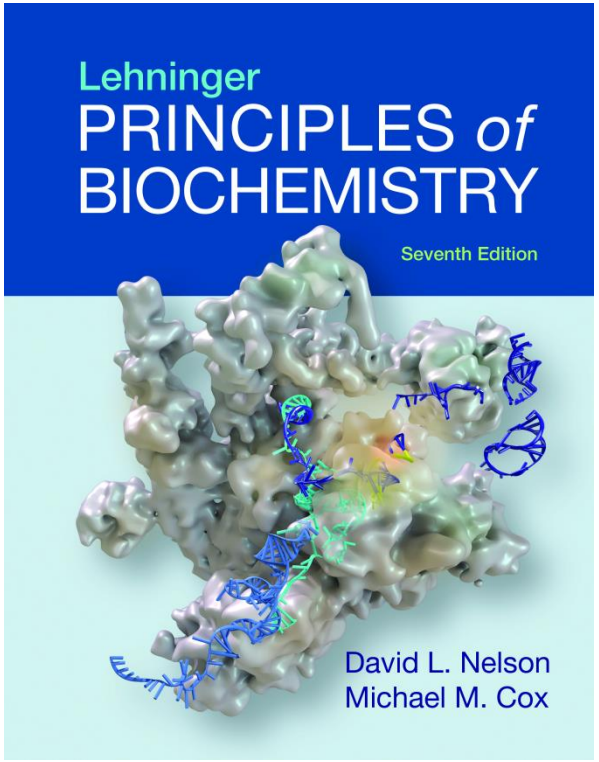
The resulting colored DNA fragments are then separated by capillary gel electrophoresis

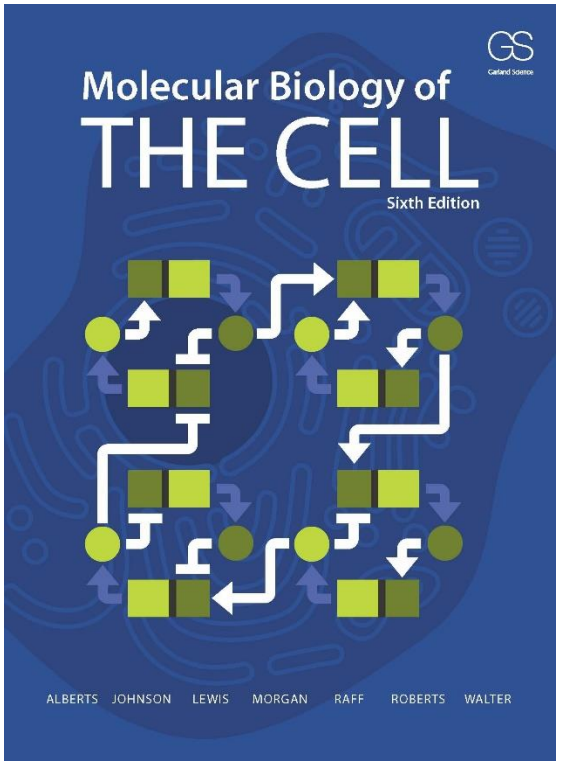
The color associated with each peak is detected using a laser beam.

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.

References


Lehninger
PRINCIPLES of
BIOCHEMISTRY
Seventh Edition
David L. Nelson
Michael M. Cox


GS
Garland Science
Molecular Biology of
THE CELL
Sixth Edition
ALBERTS JOHNSON LEWIS MORGAN RAFF ROBERTS WALTER

Extra resources

Book:

The Double Helix: A Personal Account of the Discovery of the Structure of DNA - By James D Watson

Videos:

DNA replication
https://www.youtube.com/watch?v=TNKWgcFPHqw

DNA sequencing:
https://www.youtube.com/watch?v=vK-HlMaitnE

Polymerase Chain Reaction (PCR)
http://video.mit.edu/watch/pcr-polymerase-chain-reaction-10948/
https://www.dnalc.org/view/15475-The-cycles-of-the-polymerase-chain-reaction-PCR-3D-animation.html