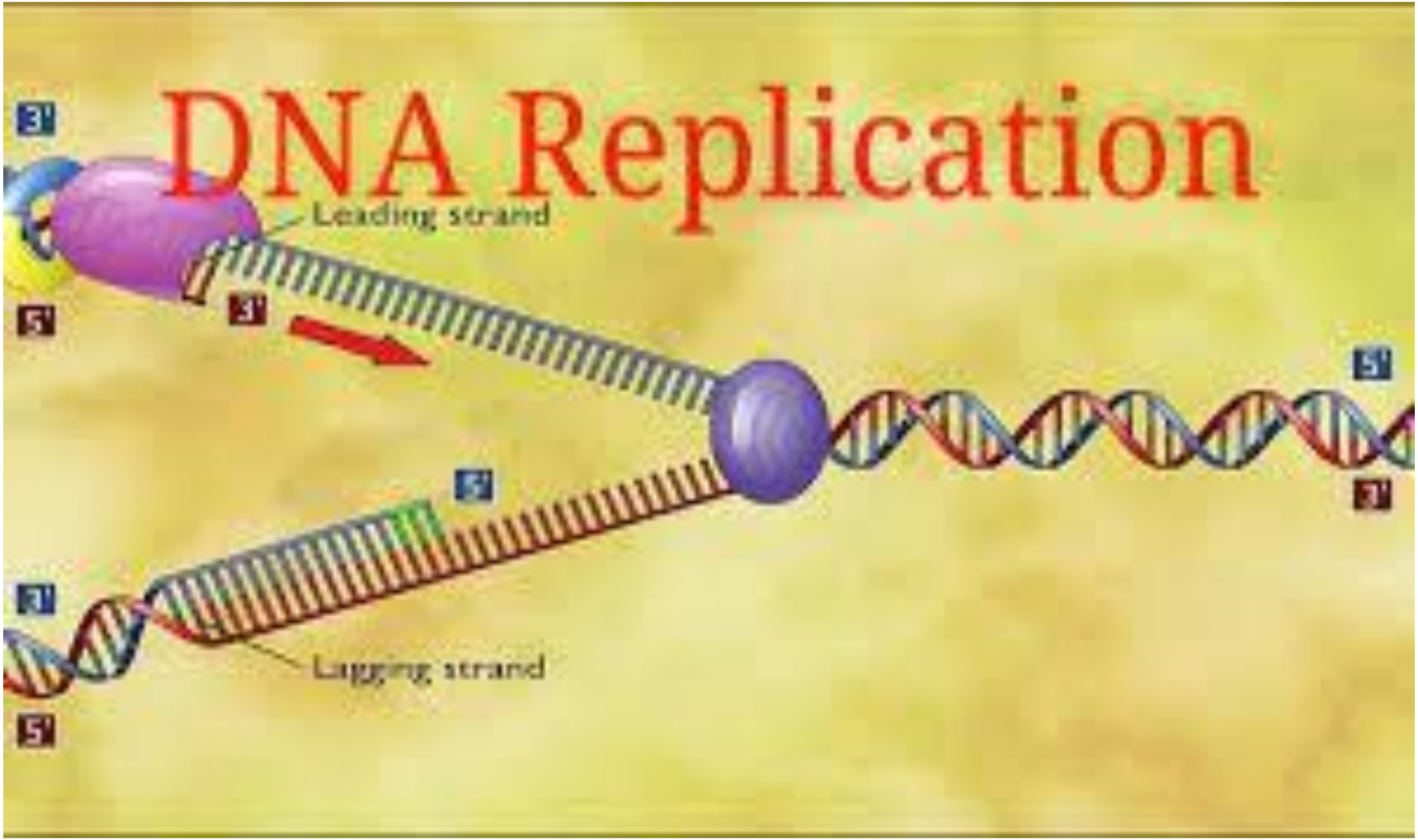
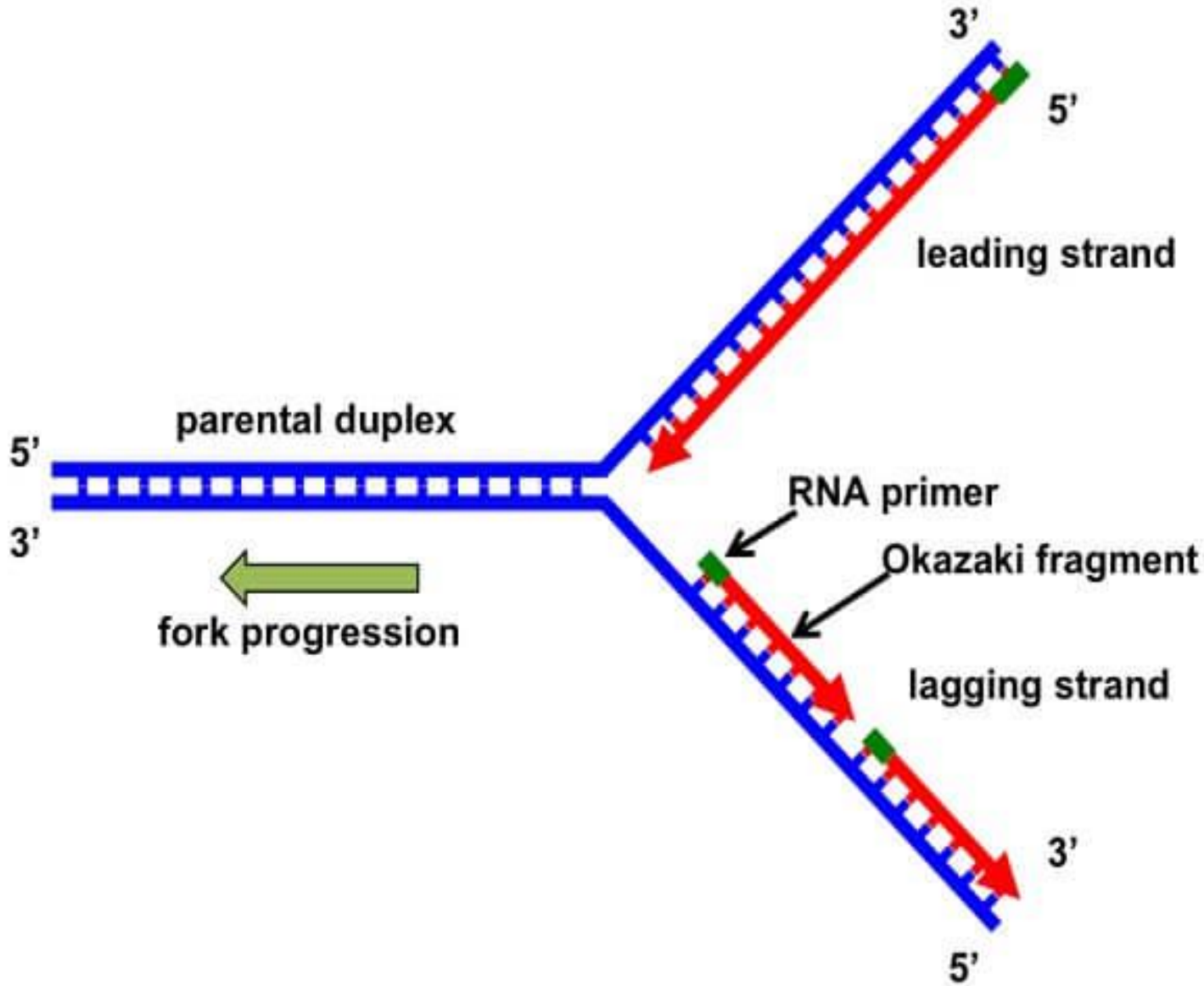
زیست شناسی مولکولی، جلسه نهم

همانند سازی DNA


DNA Replication
3'
5'
Leading strand
3'
5'
5'
3'
3'
5'
Lagging strand

Enzymes involved in Replication

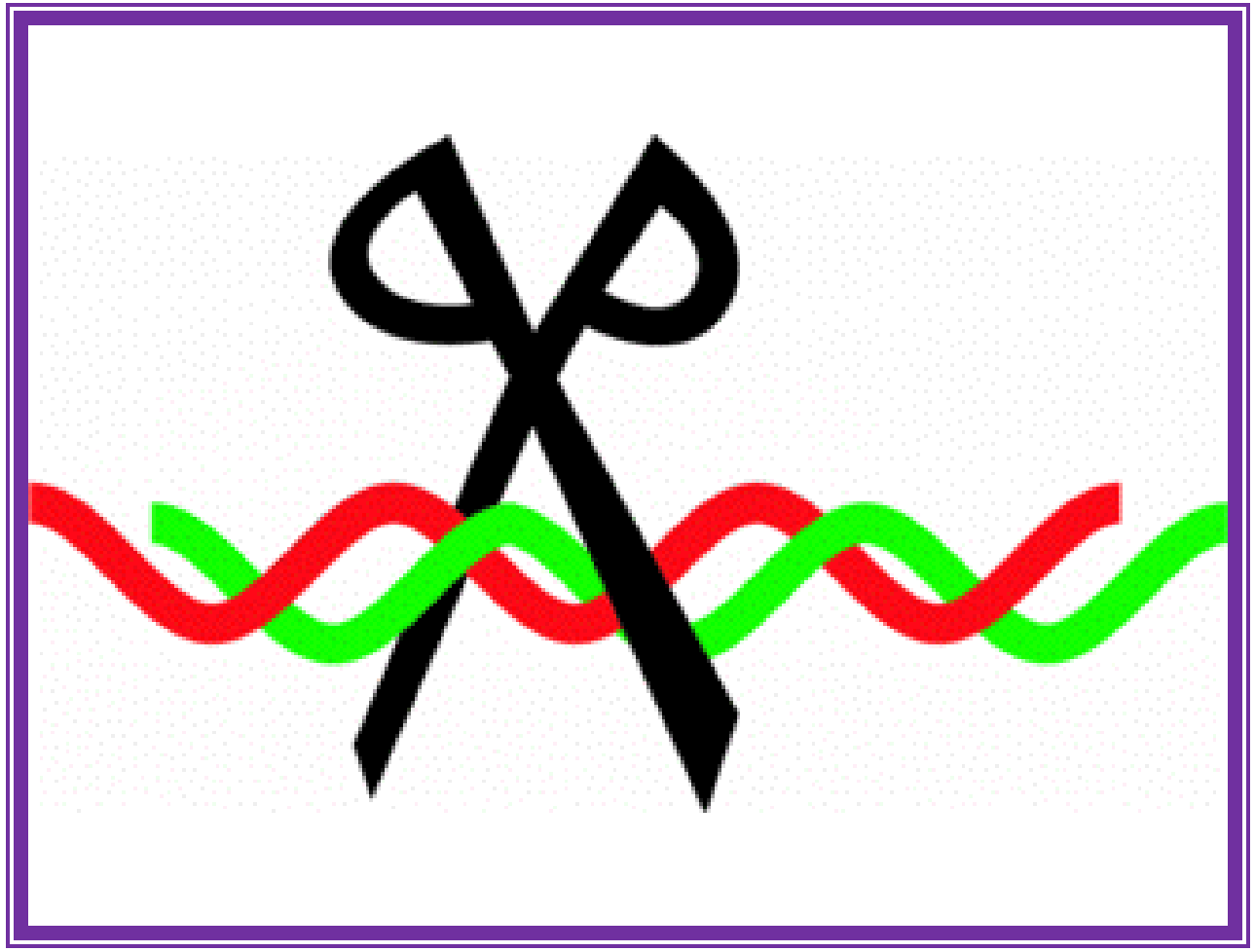
- DNA Polymerase I
  - replaces RNA primer with DNA
- DNA Polymerase III
  - adds DNA nucleotides to elongating strand
- DNA Ligase
  - connects DNA backbones
- RNA Primase
  - adds RNA primer
- Helicase
  - unzips double helix
- Single-Strand Binding Proteins
  - keeps helix separated

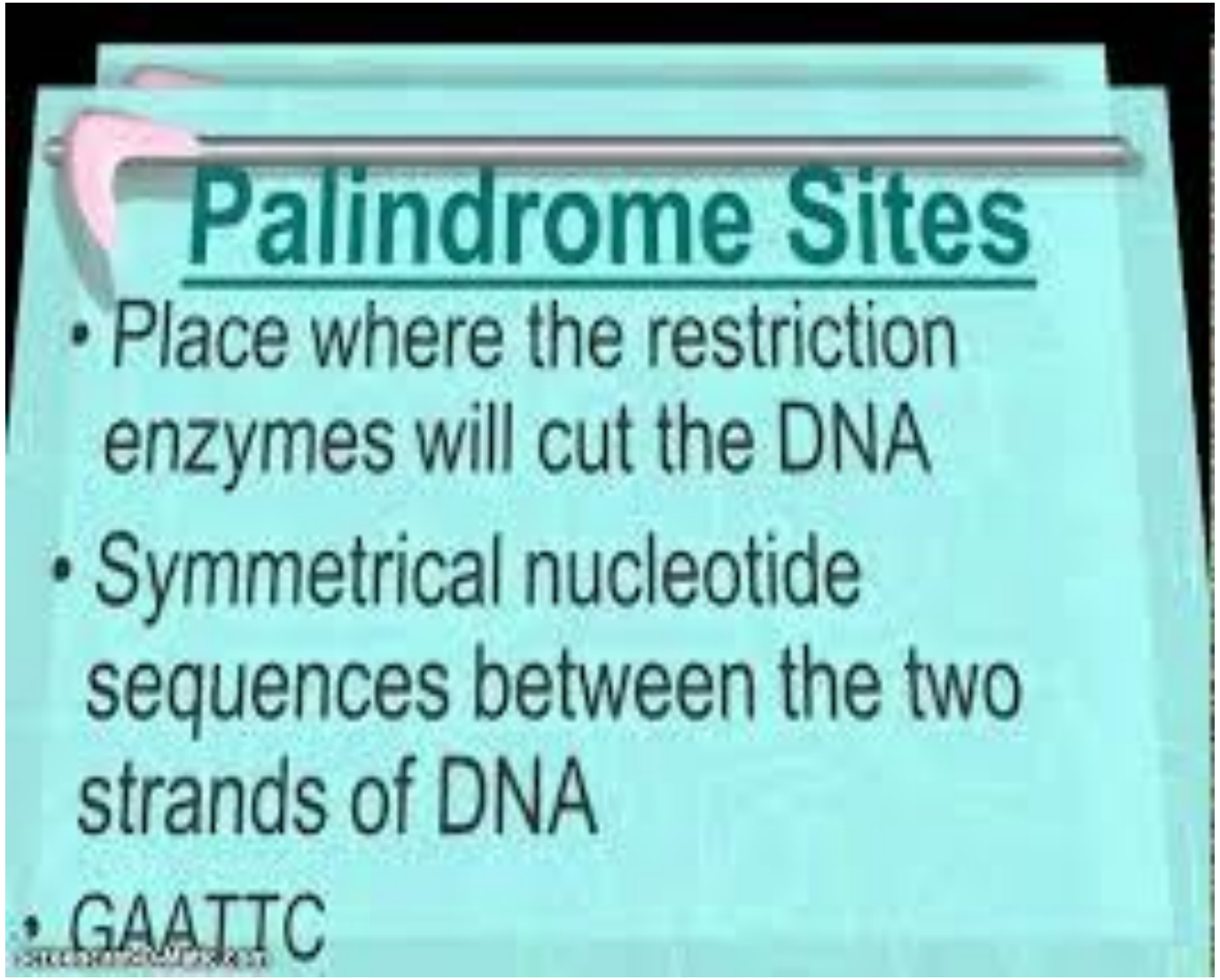

3’
5’
leading strand
parental duplex
5’
3’
RNA primer
Okazaki fragment
fork progression
lagging strand
3’
5’

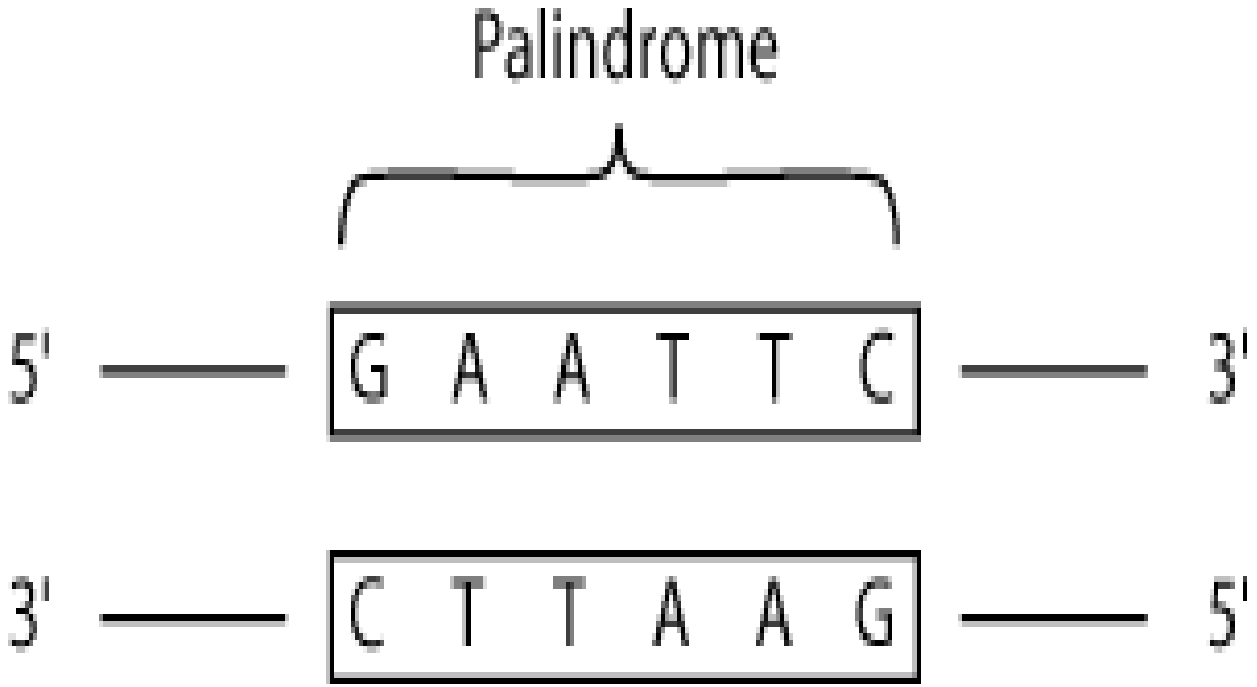
زیست شناسی مولکولی، جلسه دهم:

Palindrome و انواع آن

قیچی های مولکولی(آندونوکلئازهای محدود کننده)




Palindrome Sites
• Place where the restriction enzymes will cut the DNA
• Symmetrical nucleotide sequences between the two strands of DNA
• GAATTC

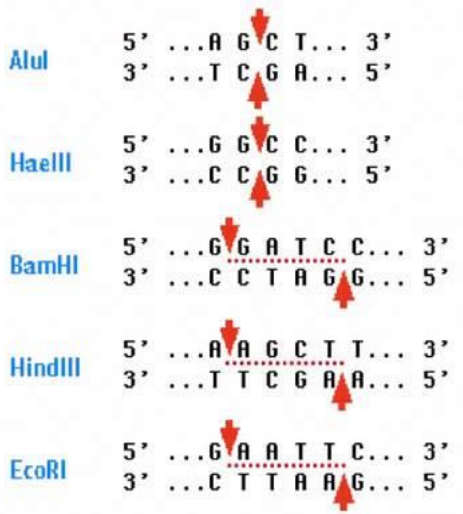


PALINDROME SEQUENCES

- The **mirror like palindrome** in which the same forward and backwards are on a single strand of DNA strand, as in GTAATG
- The **Inverted repeat palindromes** is also a sequence that reads the same forward and backwards, but the forward and backward sequences are found in complementary DNA strands (GTATAC being complementary to CATATG)
- **Inverted repeat palindromes** are more common and have greater biological importance than mirror- like palindromes.

Restriction Enzymes

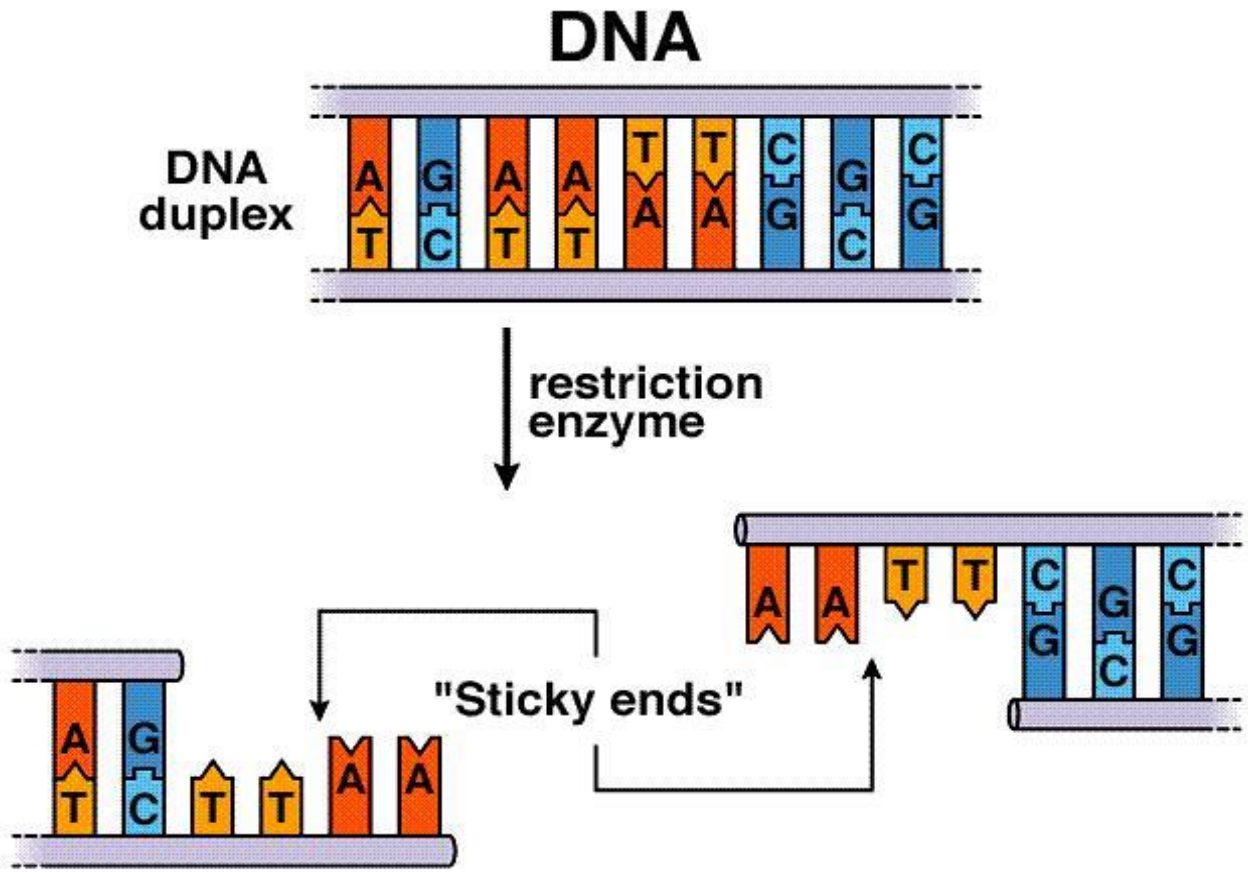
- Restriction endonucleases
  - Digest dsDNA at specific sites
  - Recognition site is palindrome
    - Word example: "Madam I'm Adam"
    - Reads the same on both strands (5' to 3')
  - Recognition sites are different sizes and unique sites
  - Cuts between same nucleotides on each strand



AluI and HaeIII produce blunt ends

BamHI HindIII and EcoRI produce "sticky" ends

http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/R/RestrictionEnzymes.html



Restriction Endonuclease Types

Type I- multi-subunit, both endonuclease and methylase activities, cleave at random up to 1000 bp from recognition sequence

Type II- most single subunit, cleave DNA within recognition sequence

Type III- multi-subunit, endonuclease and methylase about 25 bp from recognition sequence

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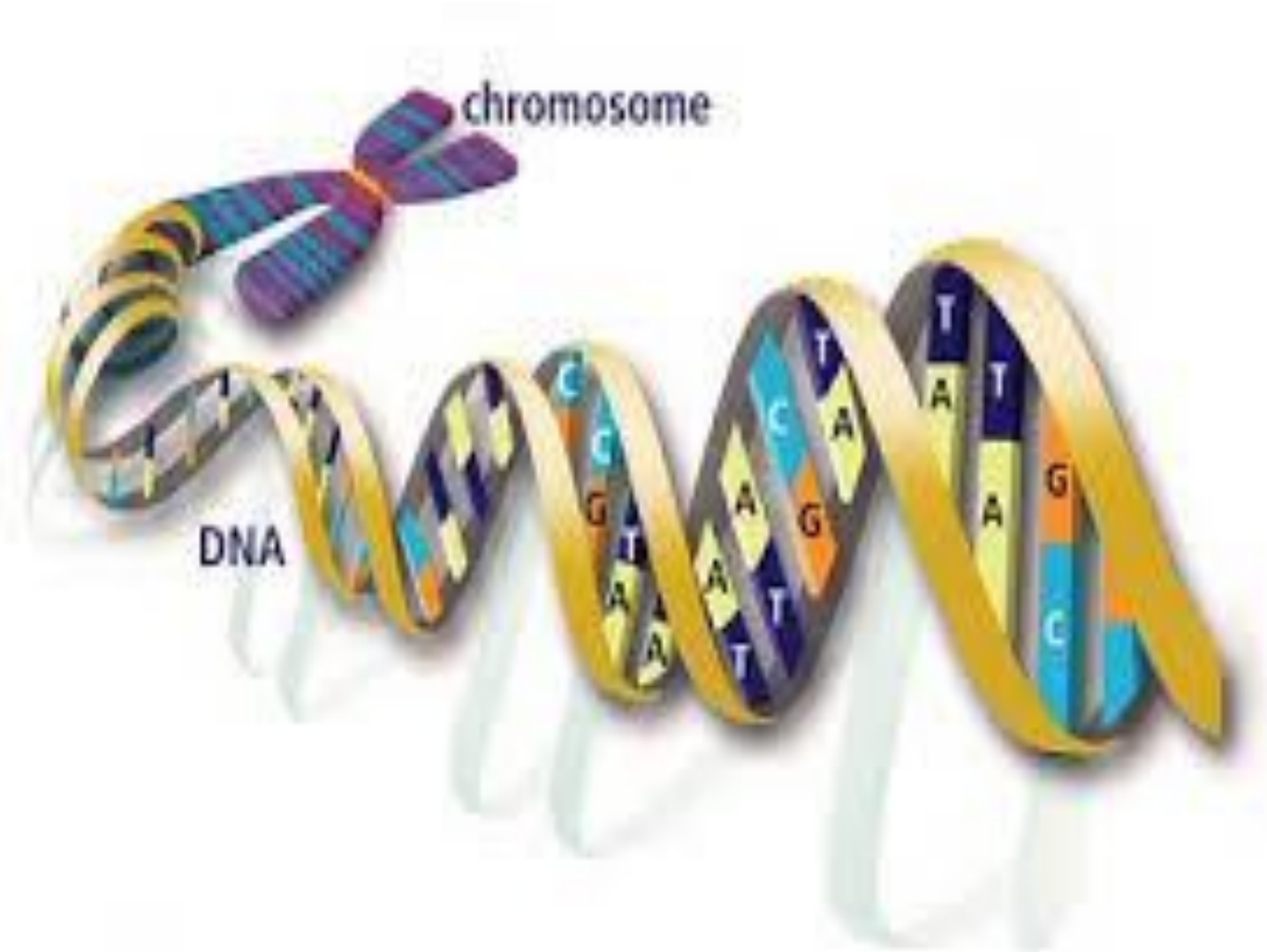
انواع Cloning

DNA Cloning

Introduction to cloning

- **There are 3 types of cloning:**
- **Gene cloning** produces copies of genes or segments of DNA.
- **Reproductive cloning** produces copies of whole animals or plants.
- **Therapeutic cloning** produces embryonic stem cells for experiments aimed at creating tissues to replace injured or diseased tissues.

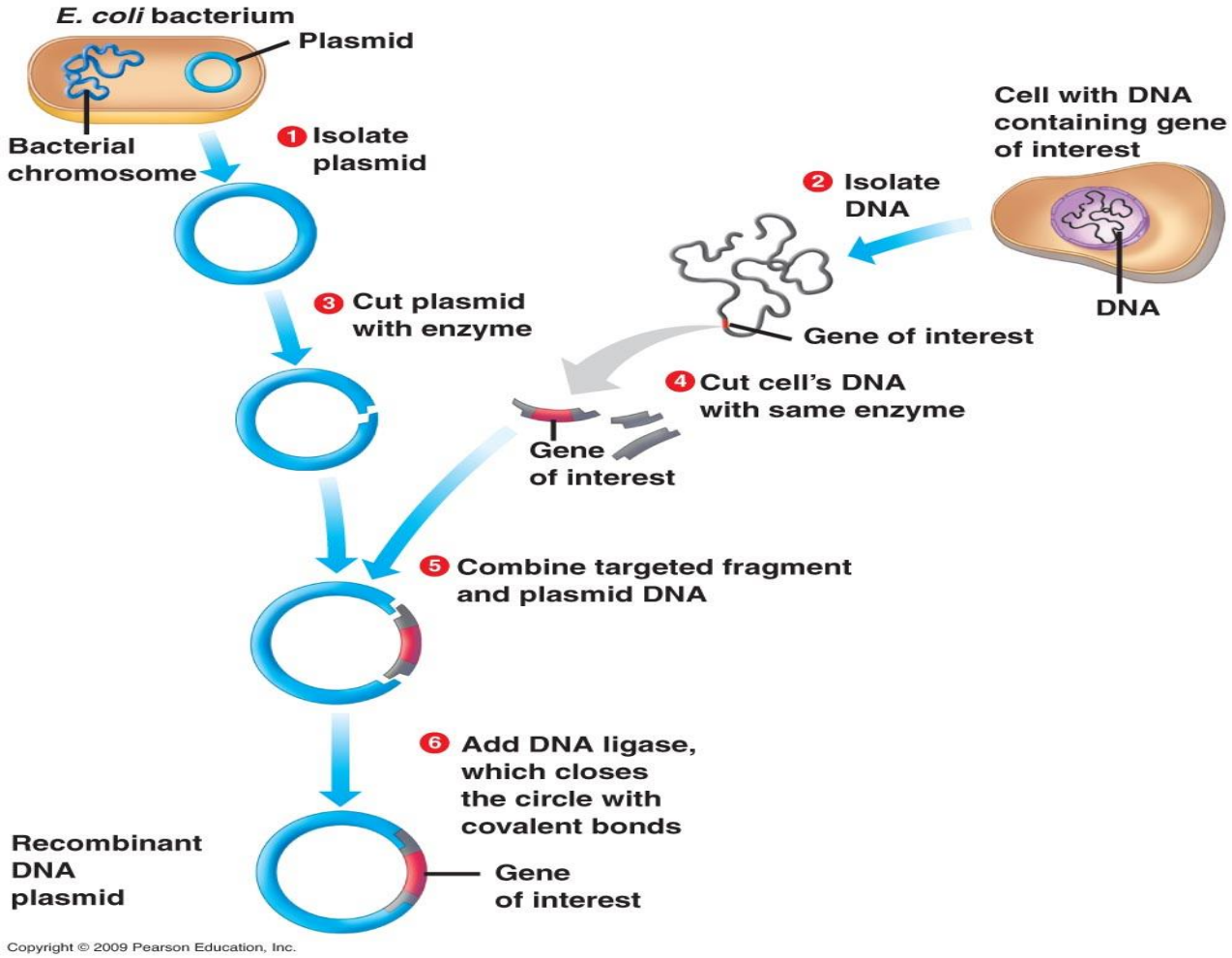

chromosome
DNA

DNA CLONING

- DNA cloning allows a copy of any specific part of a DNA (or RNA) sequence to be selected among many others and produced in an unlimited amount.
- This technique is the first stage of most of the genetic engineering experiments:
  - production of DNA libraries
  - PCR
  - DNA sequencing

DNA CLONING

- DNA cloning is a technique for reproducing DNA fragments.
- It can be achieved by two different approaches:
  - cell based
  - using polymerase chain reaction (PCR).
- a vector is required to carry the DNA fragment of interest into the host cell.



TERMS USED IN CLONING

- **DNA recombination.**
  The DNA fragment to be cloned is inserted into a vector.
- **Transformation.**
  The recombinant DNA enters into the host cell and proliferates.
- **Selective amplification.**
  A specific antibiotic is added to kill *E. coli* without any protection. The transformed *E. coli* is protected by the antibiotic-resistance gene
- **Isolation of desired DNA clones**

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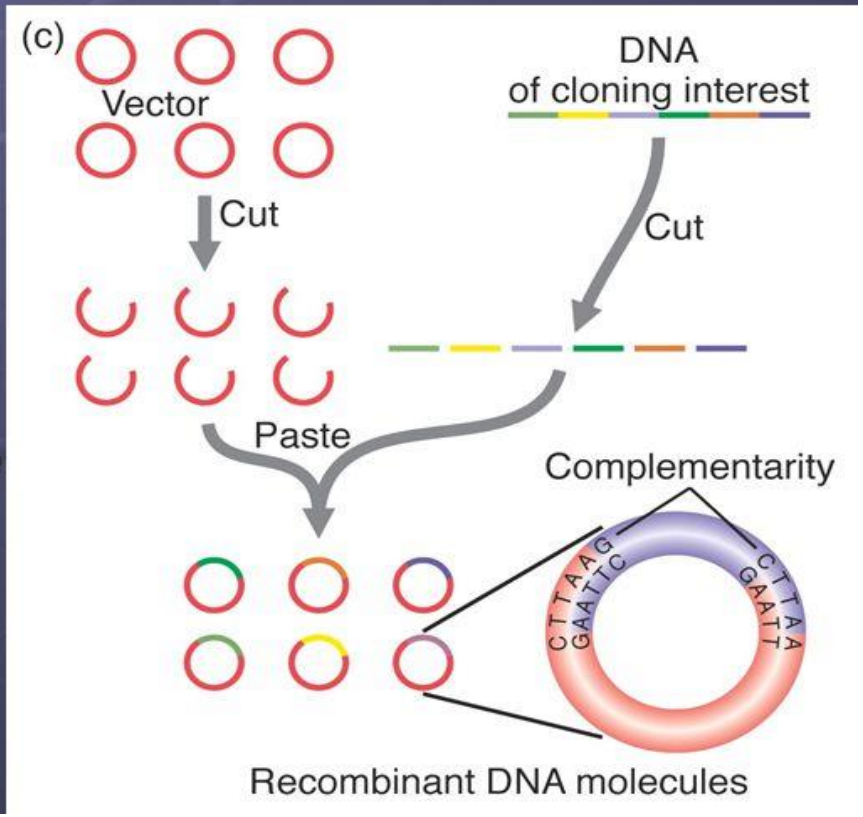
مراحل DNA Cloning

Recombinant DNA technology

- Cutting DNA and pasting it in new combinations

1) DNA purified
2) Enzymes generate fragments
3) Fragments inserted into vector
4) Vector transferred to host cell
5) As host cell replicates, recombinant molecules are passed on to progeny
6) Cloned DNA can be recovered & analyzed – can be transcribed & translated in the host cell



Summary

DNA purification methods all do the following:

- Disrupt cells and denature/digest of proteins
- Separate DNA from proteins, RNA and other cellular components
- Prepare a purified DNA solution

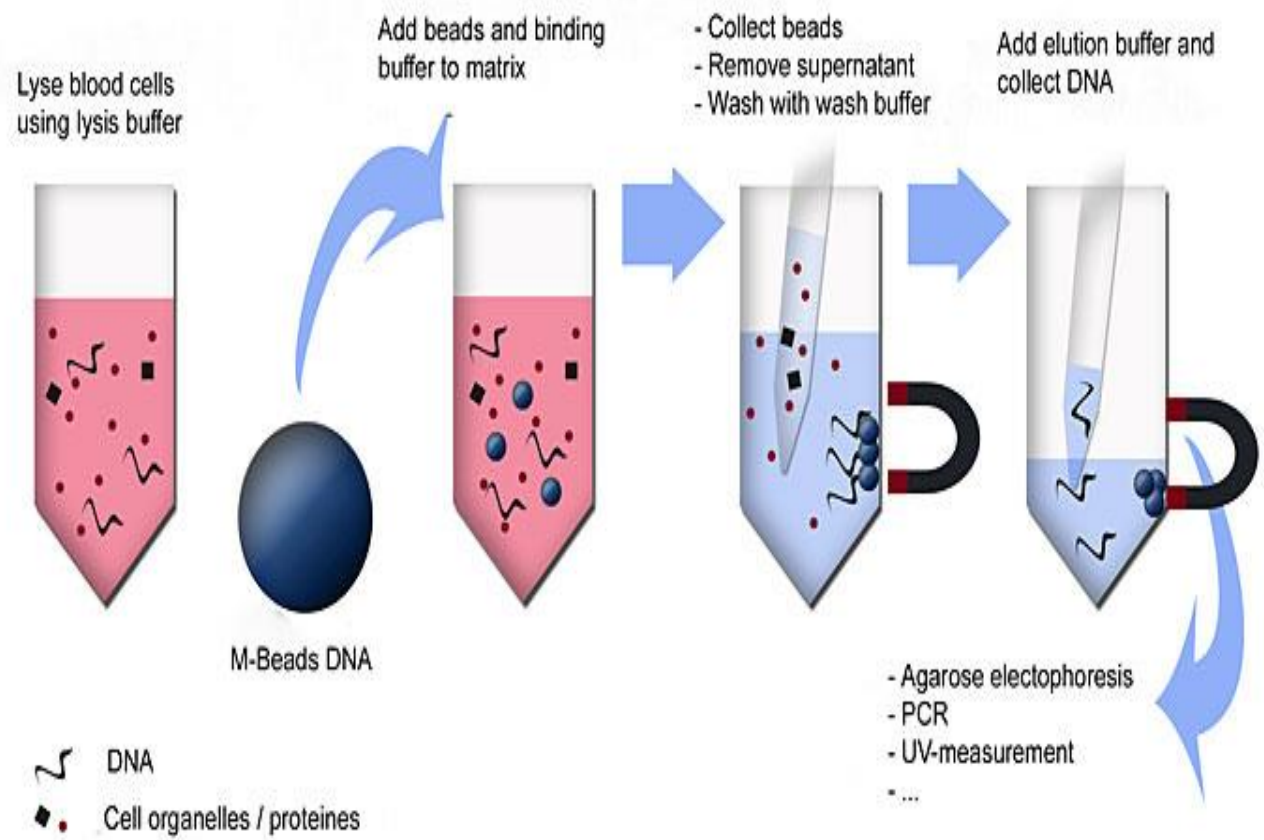
Older methods relied on laborious organic extraction and precipitation procedures.

Newer methods are faster, using selective binding of DNA to silica or magnetic beads, and are amenable to automation and miniaturization.

Nucleic Acid Purification

There are many DNA purification methods. All must:

1. Effectively disrupt cells or tissues
   (usually using detergent)
2. Denature proteins and nucleoprotein complexes
   (a protease/denaturant)
3. Inactivate endogenous nucleases
   (chelating agents)
4. Purify nucleic acid target away from other nucleic acids and protein
   (could involve RNases, proteases, selective matrix and alcohol precipitations)


Lyse blood cells
using lysis buffer
Add beads and binding
buffer to matrix
- Collect beads
- Remove supernatant
- Wash with wash buffer
Add elution buffer and
collect DNA
M-Beads DNA
- Agarose electophoresis
- PCR
- UV-measurement
- ...
DNA
Cell organelles / proteines

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PCR

What is PCR?

PCR is DNA Amplification in vitro

It has the same idea of DNA replication but in a test tube (2 DNA strands seperation, primers anealing and elongation by the DNA polymerase)

9.2 Copying DNA

The **polymerase chain reaction (PCR)** rapidly copies segments of DNA.

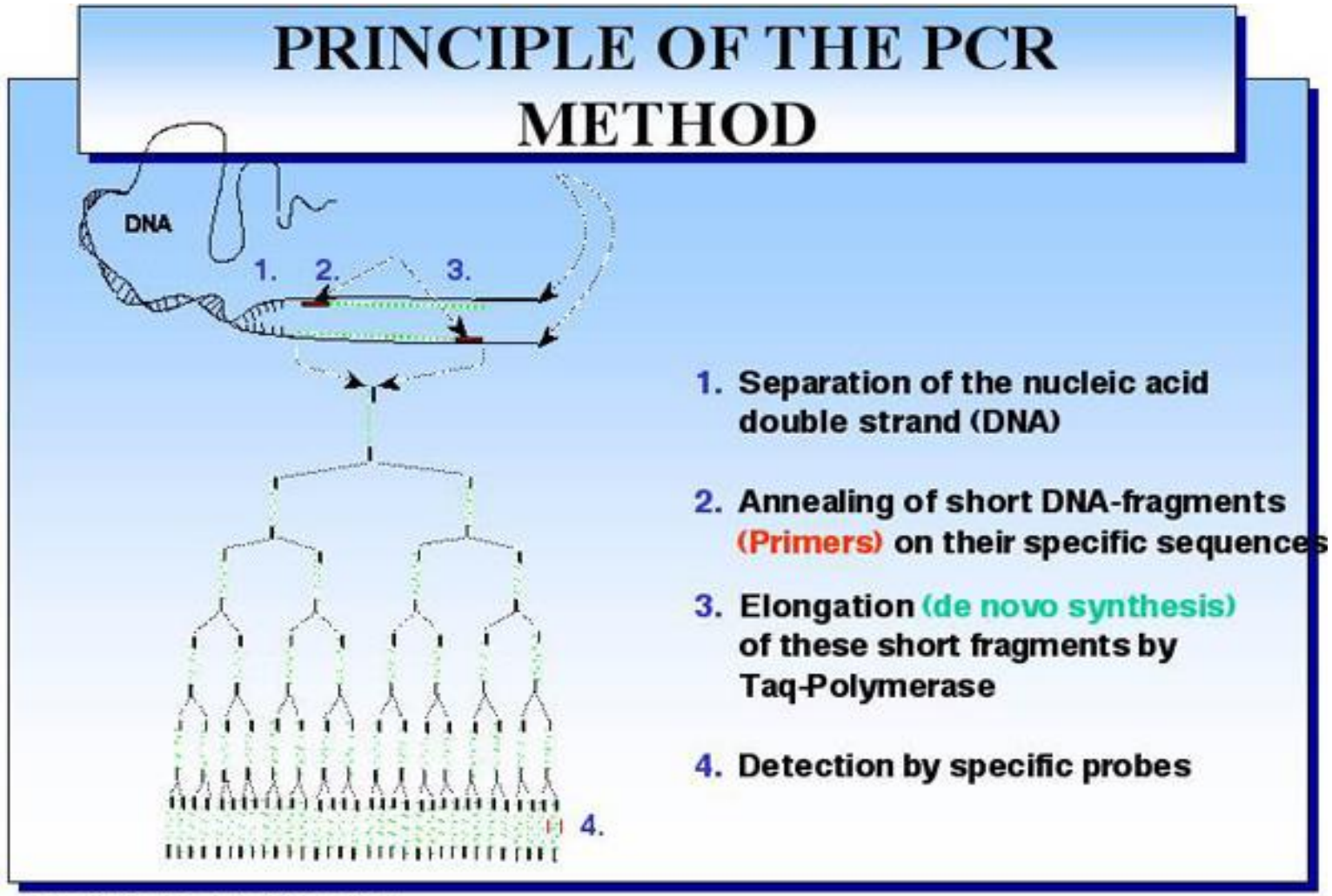
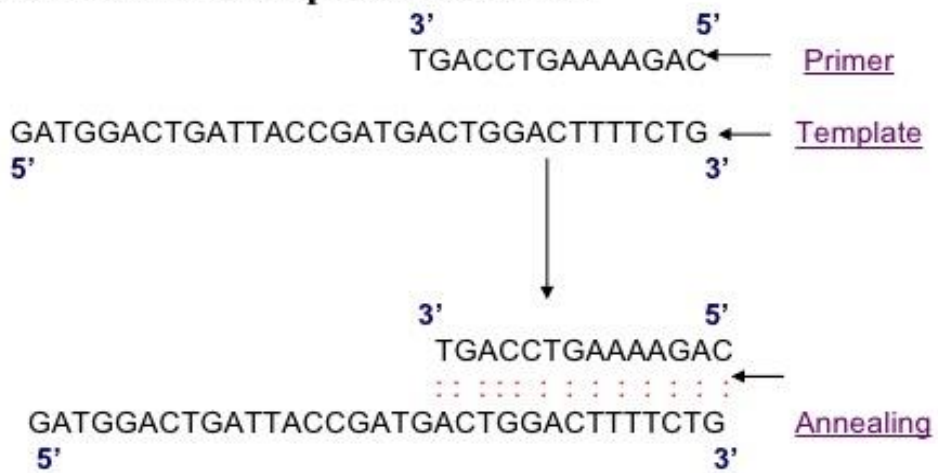
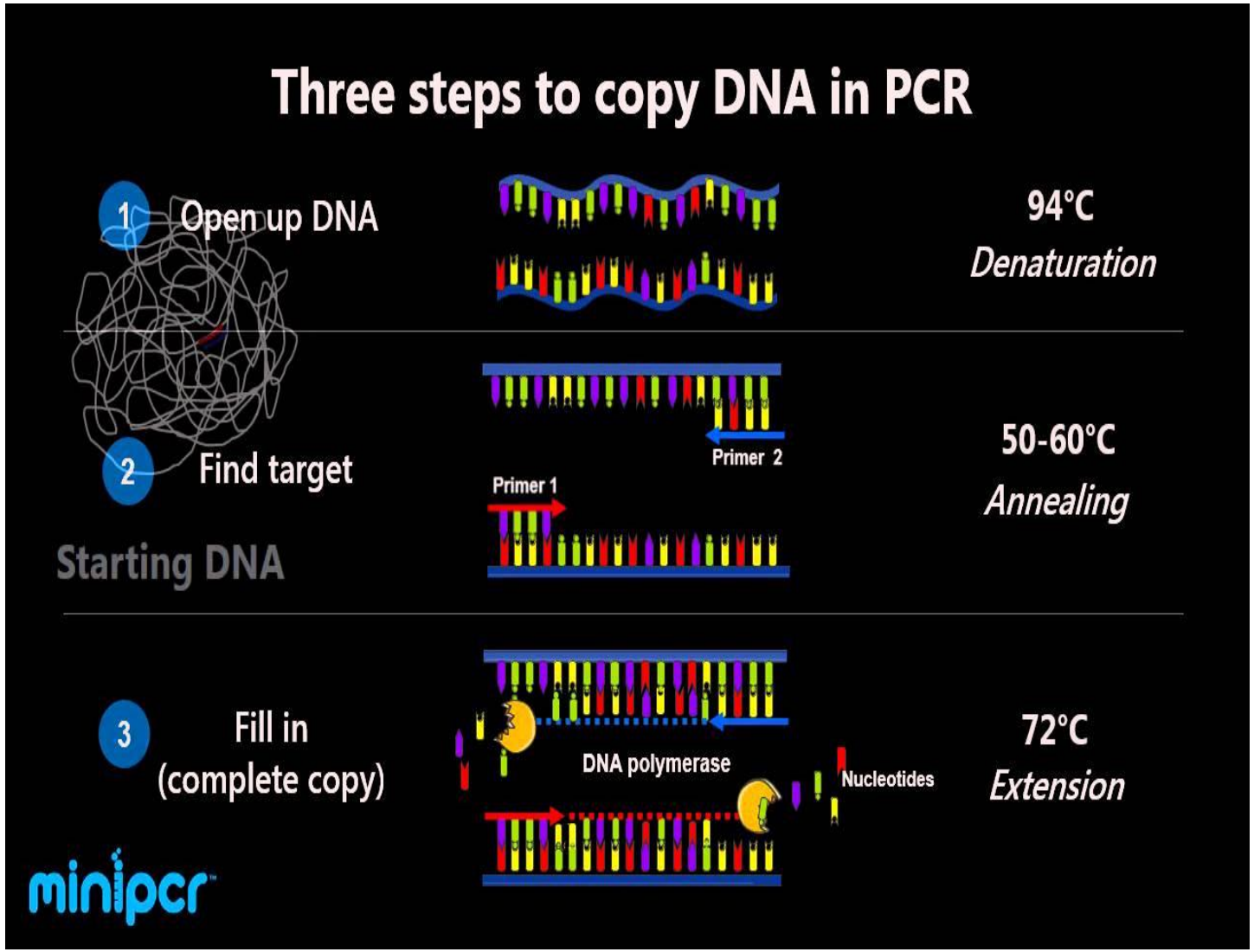


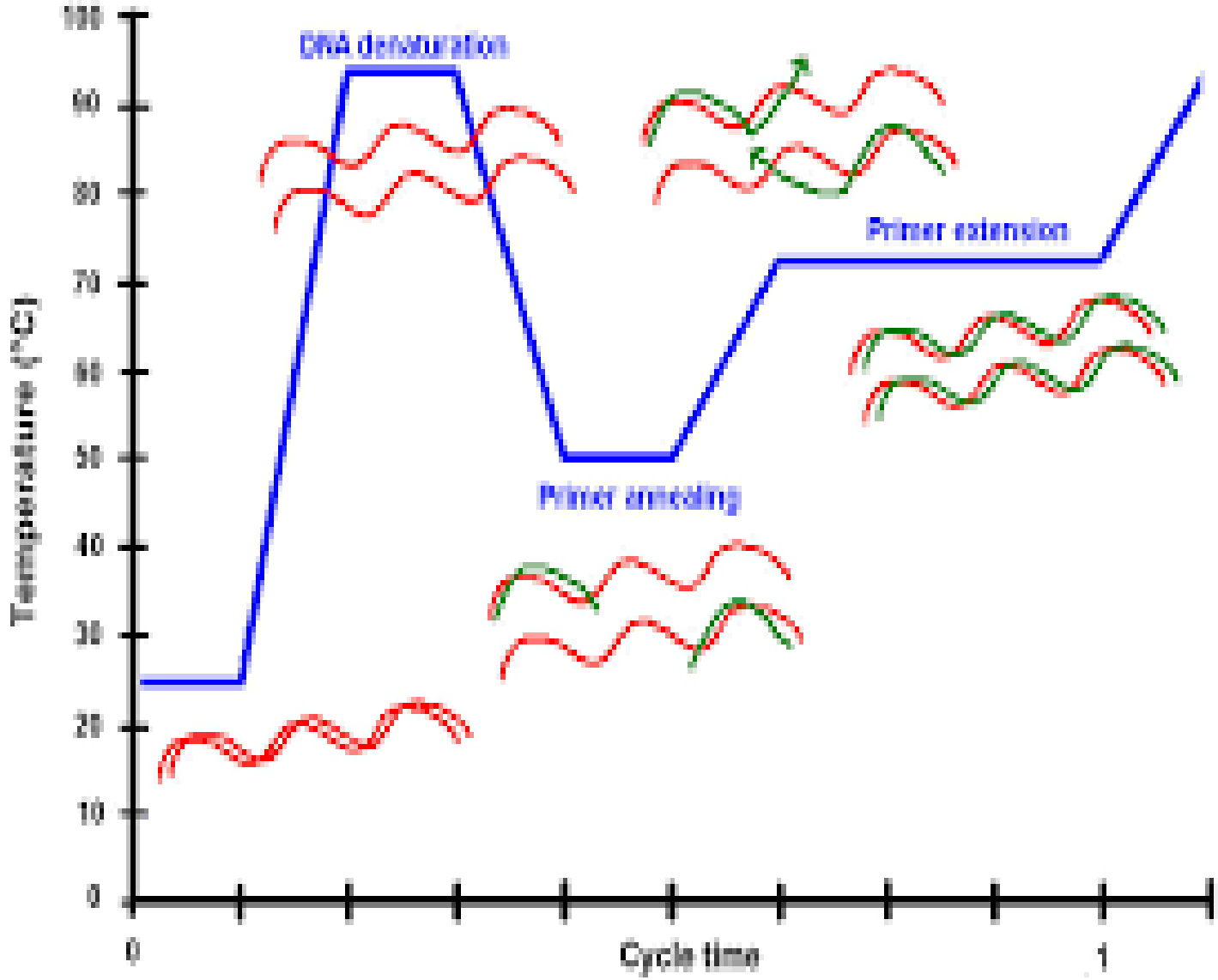
Figure 1. Principle of the PCR method.

What is a primer?

- **A primer is a short synthetic oligonucleotide which is used in many molecular techniques. These primers are designed to have a sequence which is the reverse compliment a region of template or target DNA to which we wish the primer to anneal.**



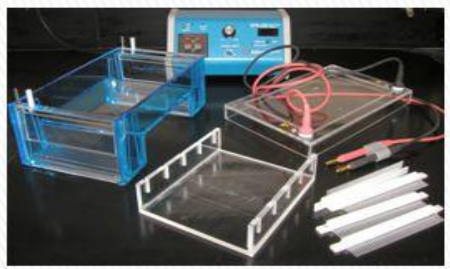

Three steps to copy DNA in PCR
1
Open up DNA
94°C
Denaturation
2
Find target
Starting DNA
Primer 2
Primer 1
50-60°C
Annealing
3
Fill in
(complete copy)
DNA polymerase
Nucleotides
72°C
Extension
minipcr


DNA denaturation
Primer annealing
Primer extension
Temperature (°C)
100
90
80
70
60
50
40
30
20
10
0
0
Cycle time
1

PCR

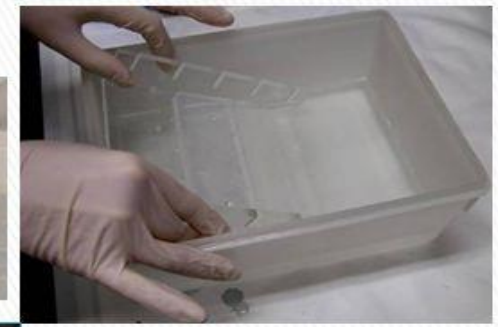
- **PCR instruments includes:**
  - Thermocycler PCR
  - Agarose gel electrophoresis
- **PCR process requires four components:**

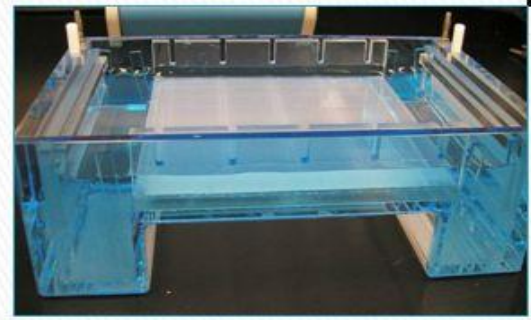
1. **Two primers:** each consisting of 15-20 bases of DNA, containing sequences complementary to the 3' end of target region of DNA that contains the polymorphism or a mutation that causes disease.
2. **Heat- stable DNA polymerase enzyme:** originally isolated from the bacterium *Thermus aquaticus* with a temperature optimum at round 70 C.
3. A large number of **free DNA nucleotides (dNTPs).**
4. Small quantity of **Genomic DNA from an individual** act as a template.

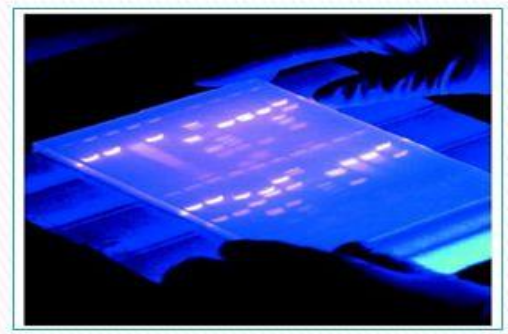


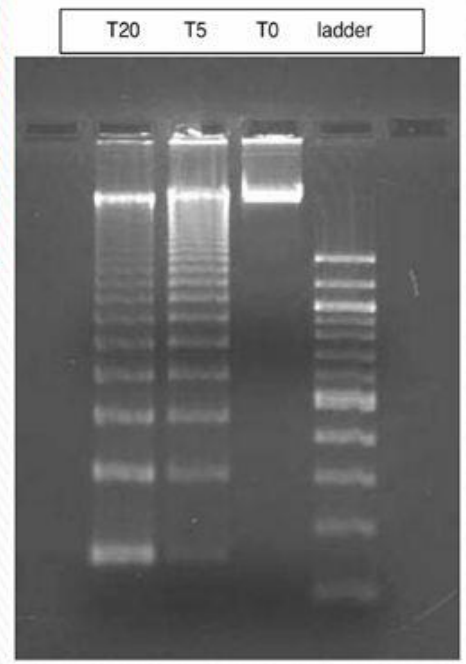
PCR product

- Finally **agarose gel electrophoresis** is employed for size separation of the PCR products. The size(s) of PCR products is determined by comparison with a DNA ladder (a molecular weight marker) which contains fragments of known size, run on a gel alongside the PCR products.

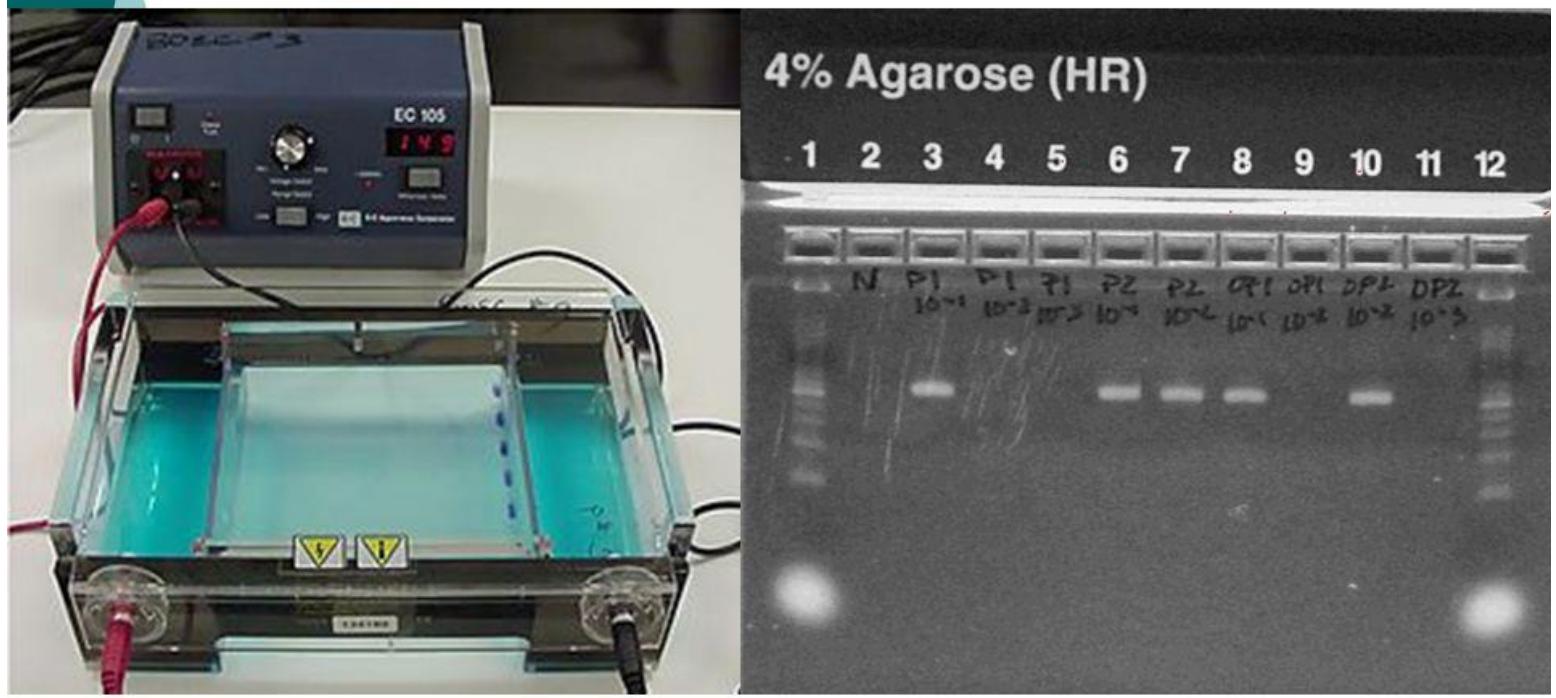






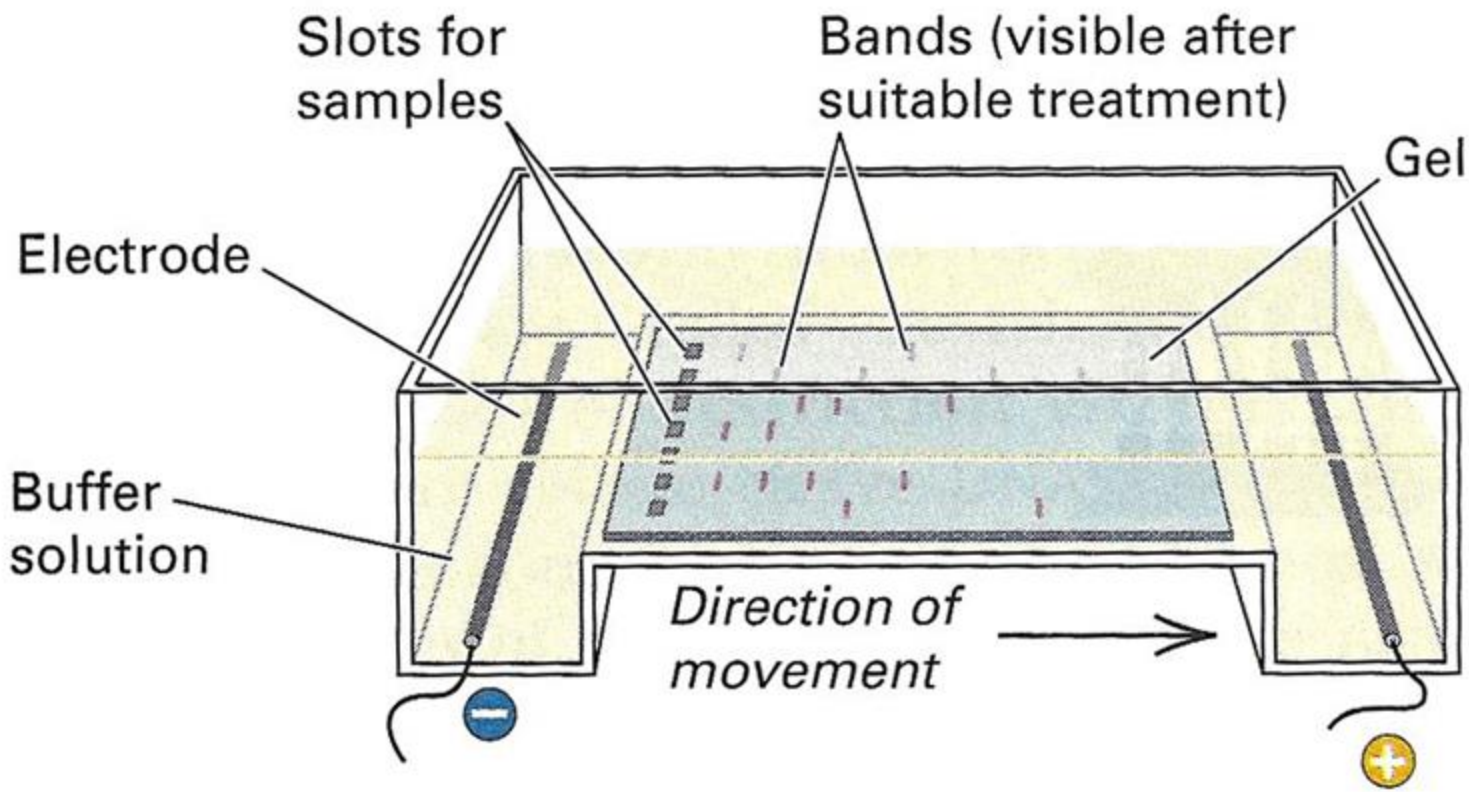


Instruments for Viewing PCR Results



•Gel Electrophoresis and Camera image of agarose gel

Agarose gel electrophoresis of DNA



Reverse Transcriptase Polymerase Chain Reaction
RT-PCR

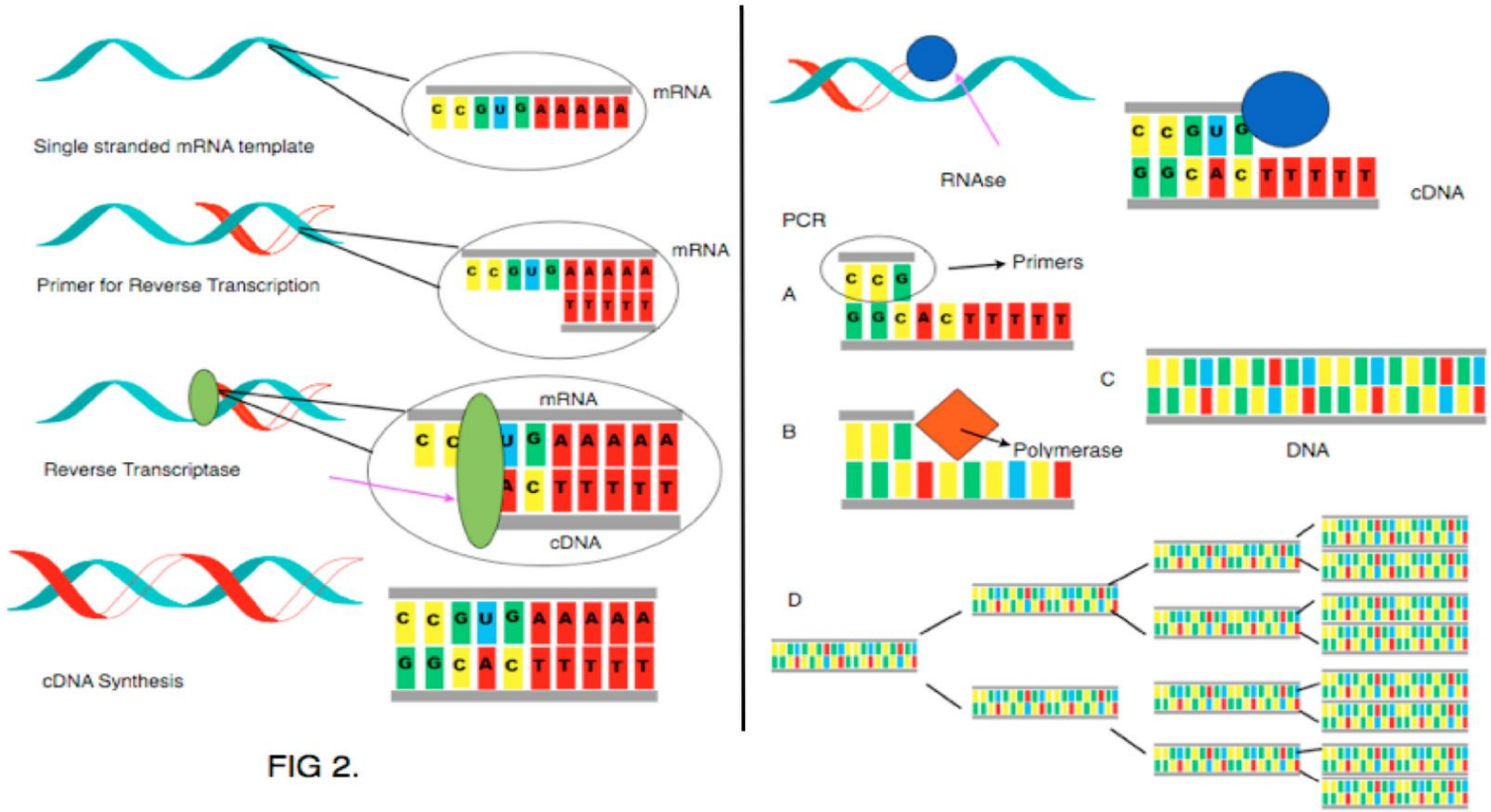


FIG 2.