Digestion of DNA with Restriction Enzymes

What are restriction enzymes ?

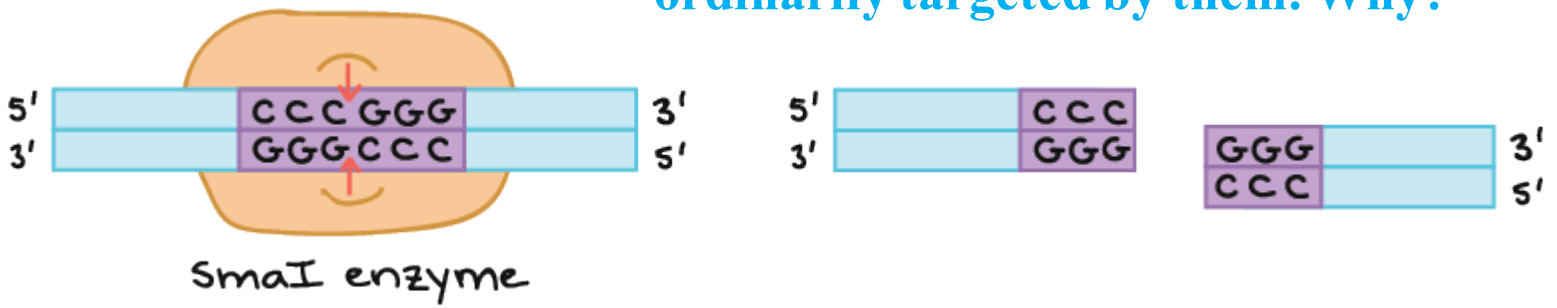
- **Restriction enzymes (RE)** are enzymes that have the ability to **recognizes a specific**, short nucleotide sequence and cleave the sugar phosphate backbones in double stranded DNA at that specific site.

- **The specific site called:** RESTRICTION SITE .

- They are biological scissors.

- RE naturally found in a wide variety of prokaryotes.

➔ **Bacterium is immune to its own restriction enzymes, even if it has the target sequences ordinarily targeted by them. Why?**



RE nomenclature:

- **EcoRI:**

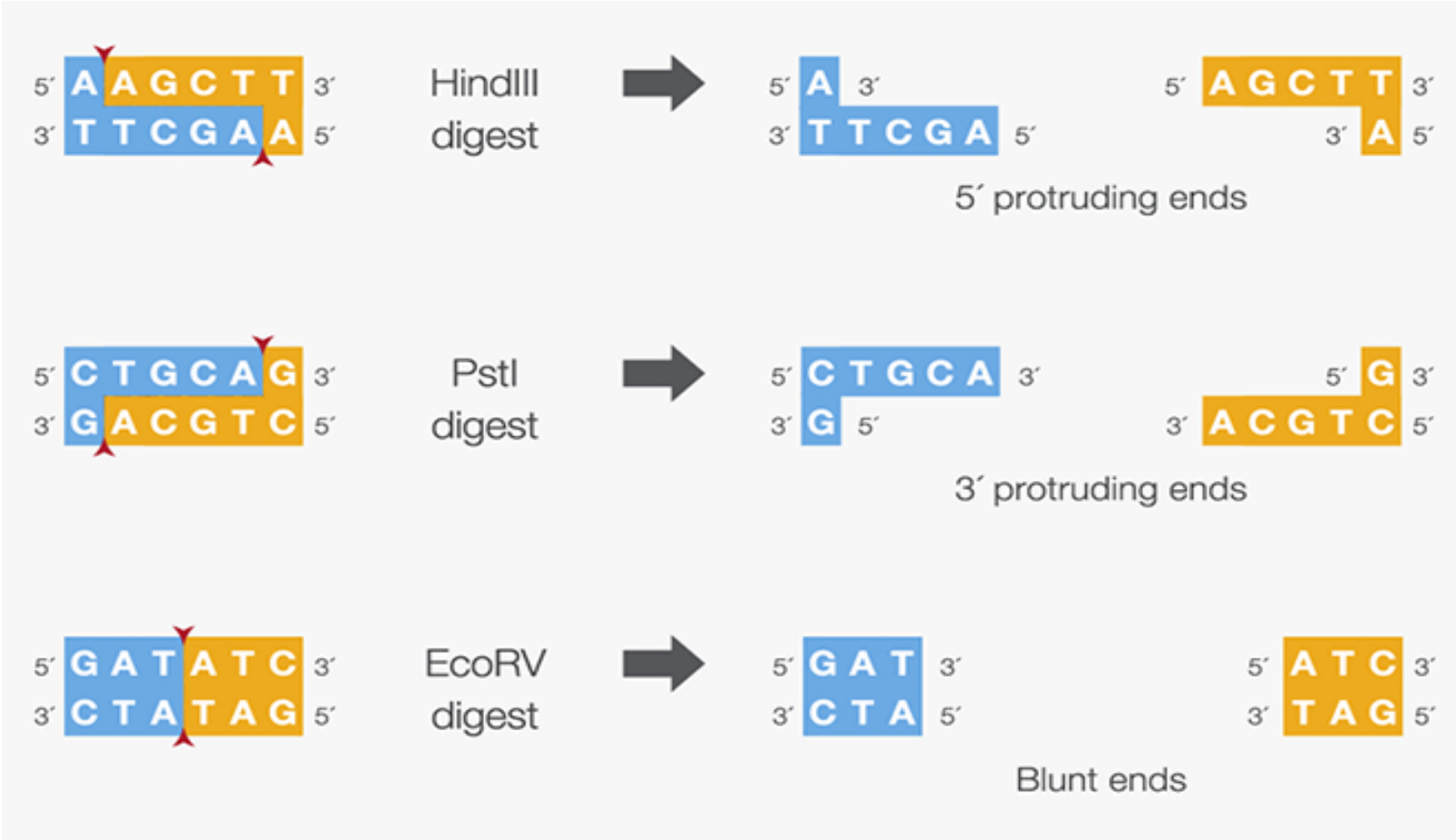
➔ is isolated from **E**.**co**li strain **R**Y13.

➔ **I** indicates it was the first enzyme of that type isolated from E. coli RY13.

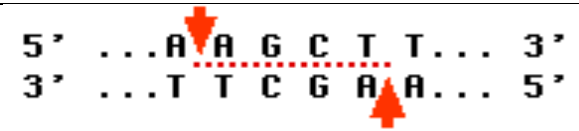
How Restriction Enzyme cut the DNA ?

Sticky end

Blunt end

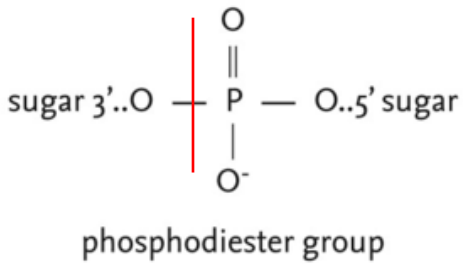


Examples of RE:

RE name	Origin	Restriction site
***Eco*RI**	Escherichia coli	
***Bam*HI**	Bacillus amyloliquefaciens H	
***Hind*III**	Haemophilus influenza RD	
***Hae*III**	Haemophilus aegyptius	
***Alu*I**	Arthrobacter luteus	

Mechanism of Action:

➔ Restriction Endonuclease scan the length of the DNA.



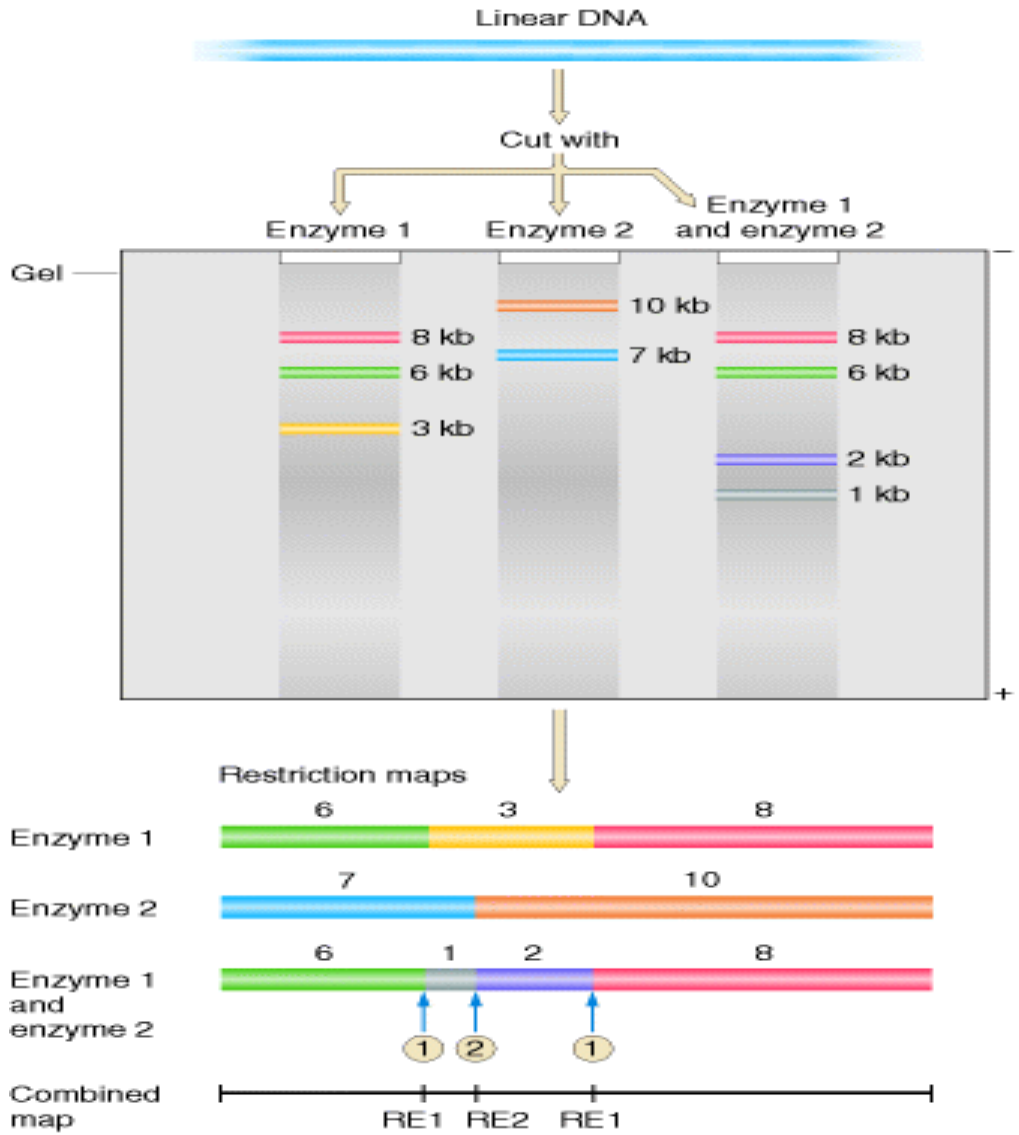
➔ Binds to the DNA molecule when it recognizes a specific sequence.

➔ Makes one cut in each of the sugar phosphate backbones of the double helix – by hydrolysing the phosphodiester bond (Specifically between the 3' O atom and the P atom is broken).

(Scan ➔Recognize ➔Cut)

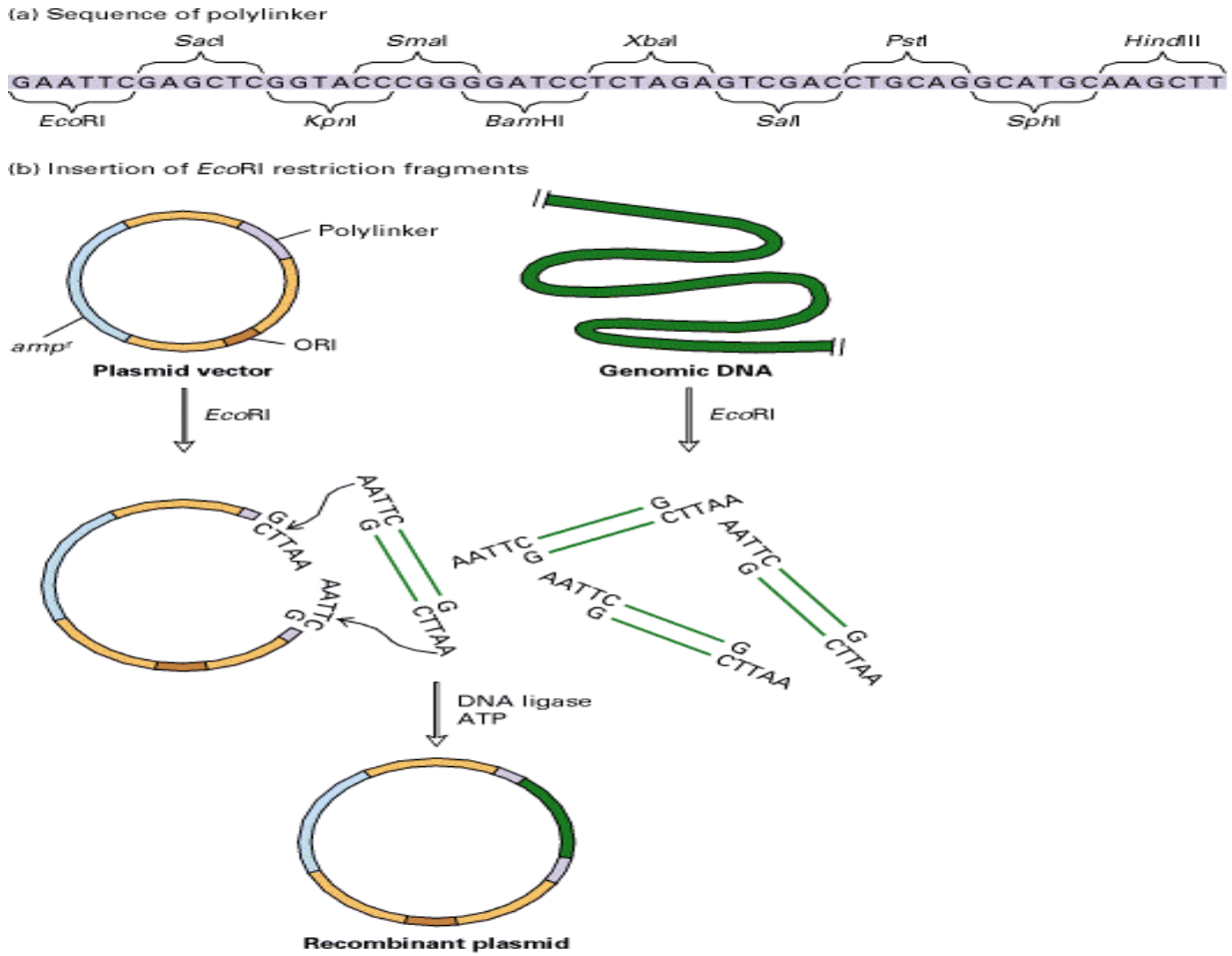
Uses in Biotechnology:

1. Generation of restriction map.



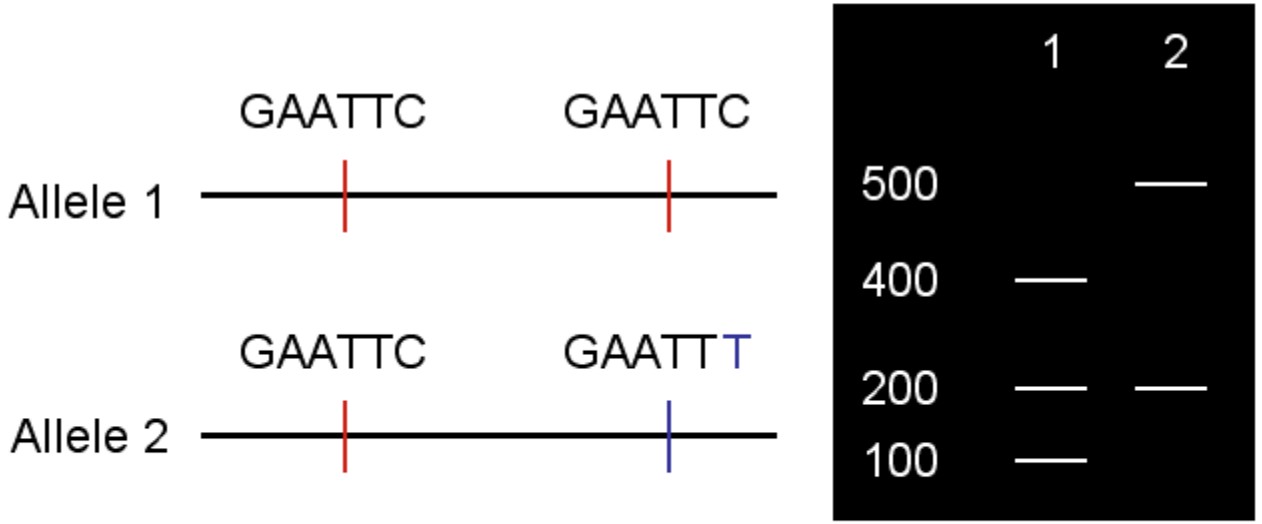
Uses in Biotechnology:

2. Recombinant DNA technology (gene cloning).



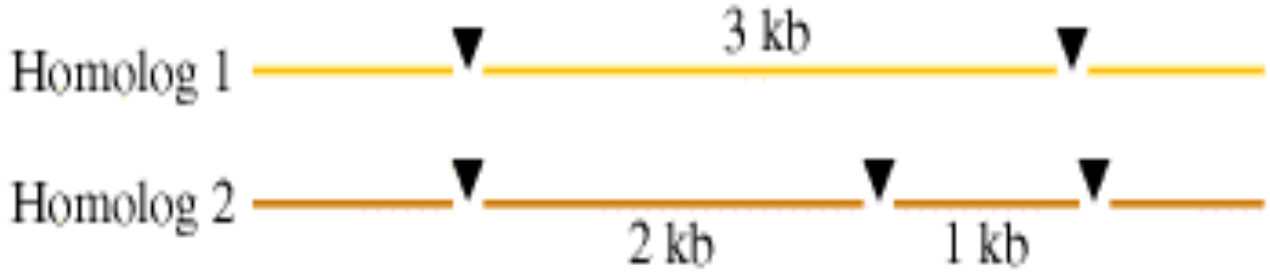
Uses in Biotechnology:

3. Restriction Fragment Length Polymorphism (RFLP).

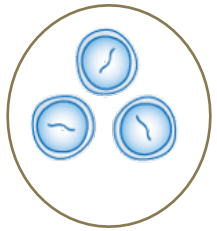


Restriction Fragment Length Polymorphism (RFLP):

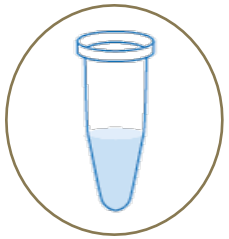
- Is a tool to study variations among individuals (humans and other species).
- This technique able to differentiate minor nucleotide sequence variations in **homologous** fragments of DNA



RFLP Workflow:



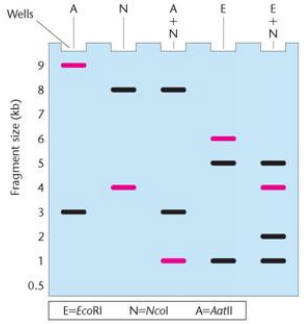
DNA Extraction



PCR for the region that you want to do the study on

Incubation of the DNA with RE

Agarose gel to visualized your results

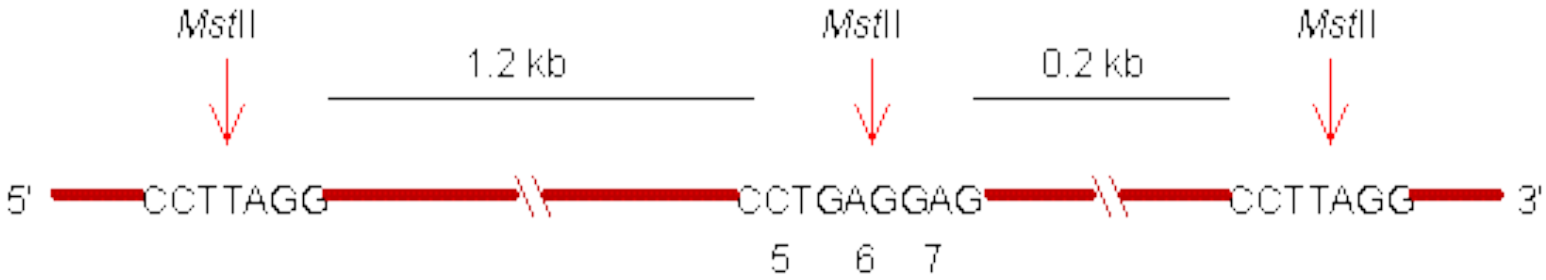


RFLP - Example:

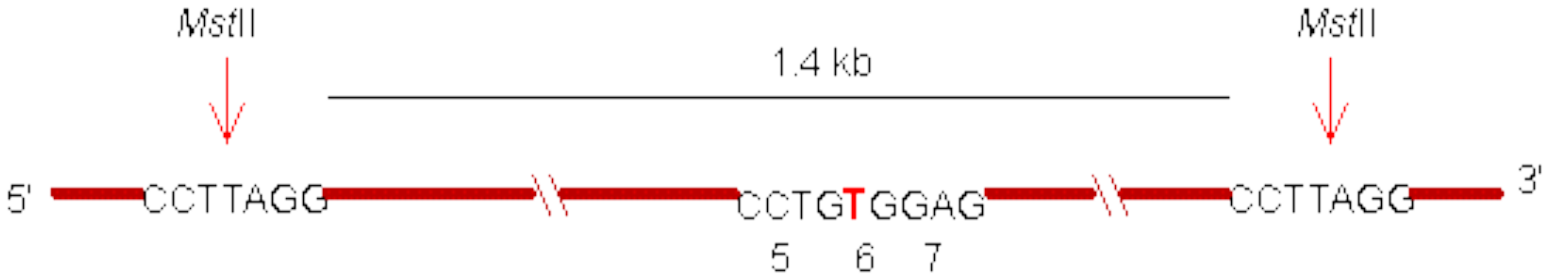
- **Genetic disease analysis as application.**

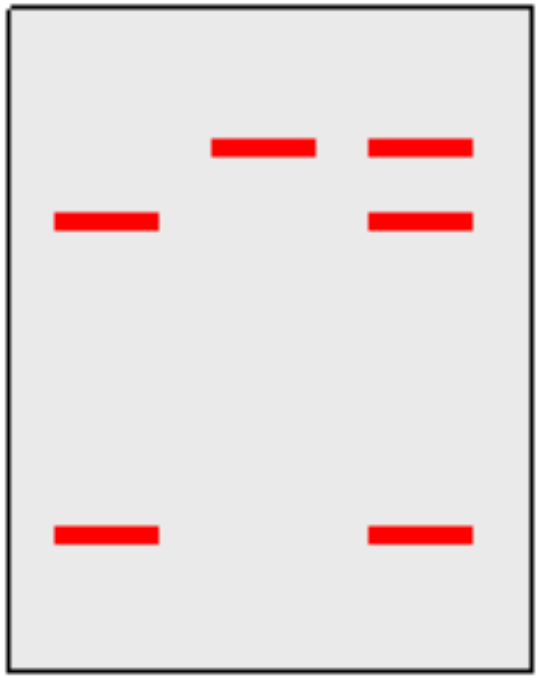
***Mst*II restriction stite:**
'5-CCTNAGG-3'

Normal cell



Sickle cell





Practical Part

Aim:

- Restriction of genomic DNA.

Principle:

- Genomic DNA or DNA fragments obtained following PCR incubated with ER under appropriate experimental conditions.
- Resulted restriction fragments can be separated on agarose gel electrophoresis by size.
- In this experiment restriction of genomic DNA will be done using *Mst*II, which cut the DNA at '5-CCTTAGG-3'

Method:

1. Label a clean microcentrifuge tube, and add the following:

Component	Volume (µl)
DNA solution (0.5 µg/µl)	1
10X restriction buffer	2
NaCl solution	1
Water	16

2. Add MstII (3 U for each one µg DNA) and incubate the reaction mixture for 20 min at 37 °C in an incubator.
3. Stop the reaction by adding 0.5 µl of 0.5 M EDTA.
4. Prepare it for agarose gel electrophoresis by adding 5 µl of gel loading buffer

Home Work:

- Refresh your knowledge about DNA polymerase by:

1. Draw the reaction of phosphodiester bond formation by the DNA polymerase.
2. Explain your drawing by your words, and make sure to mention which groups are involved in the bond formation.