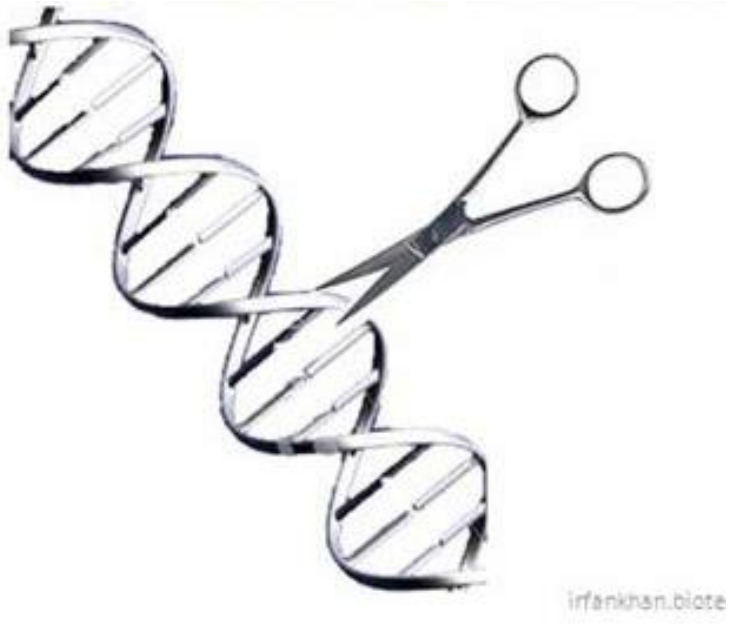
Digestion of DNA with Restriction Enzymes



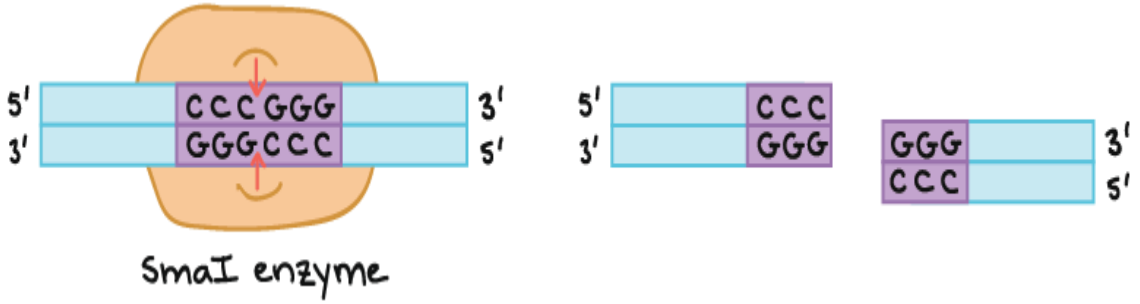
BCH361- Practical

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.**



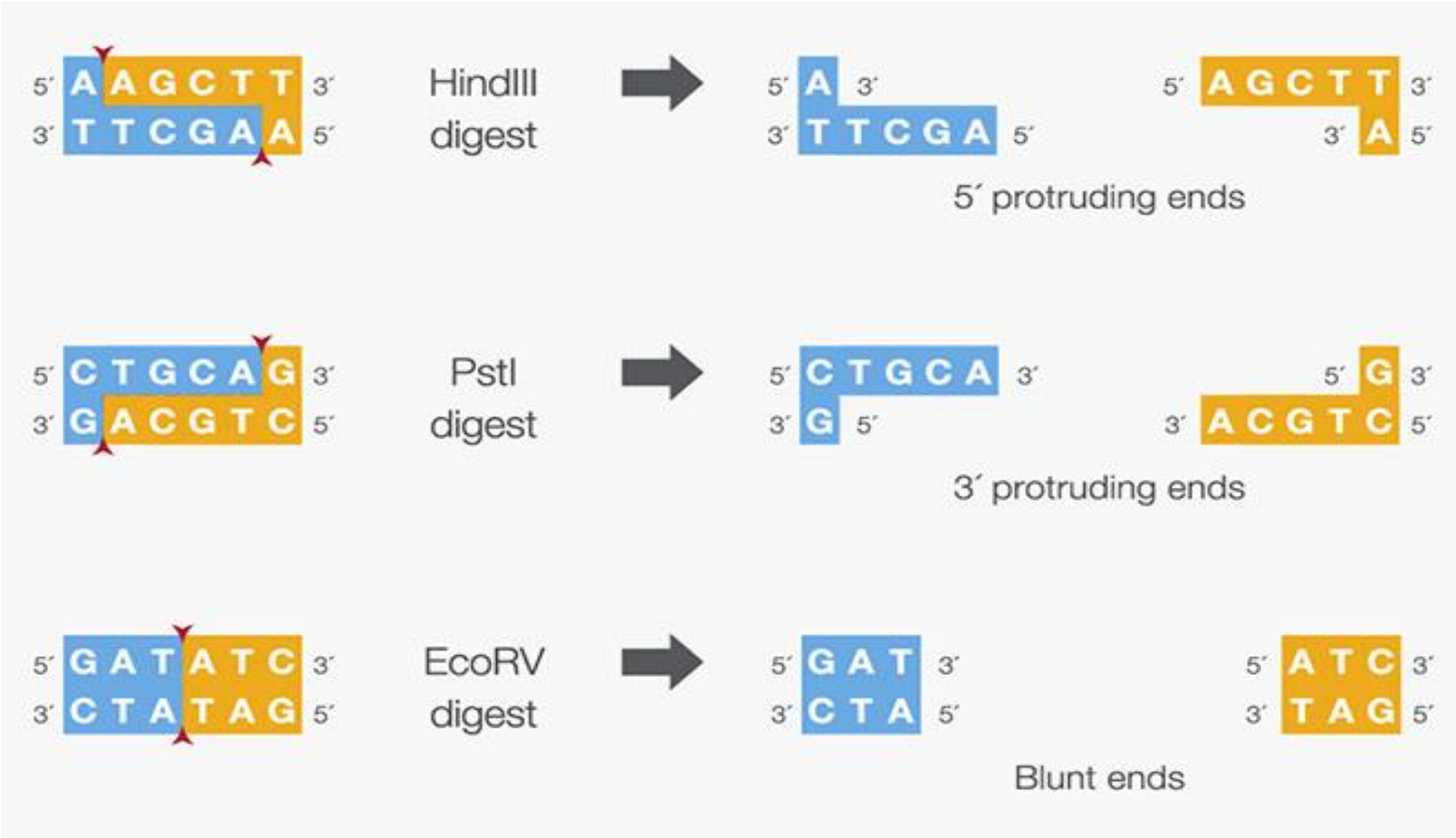
- RE naturally found in a wide variety of **prokaryotes**.
- **Bacterium is immune to its own restriction enzymes, even if it has the target sequences ordinarily targeted by them. Why?**

- **RE nomenclature: EcoRI**

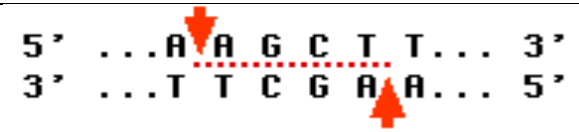
How Restriction Enzyme cut the DNA ?

Sticky end

Blunt end

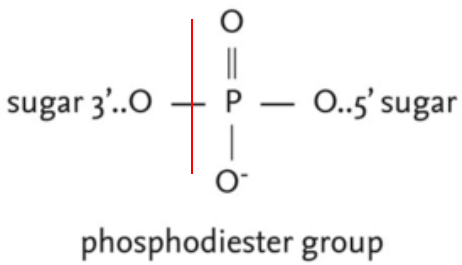


Examples of RE:

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.



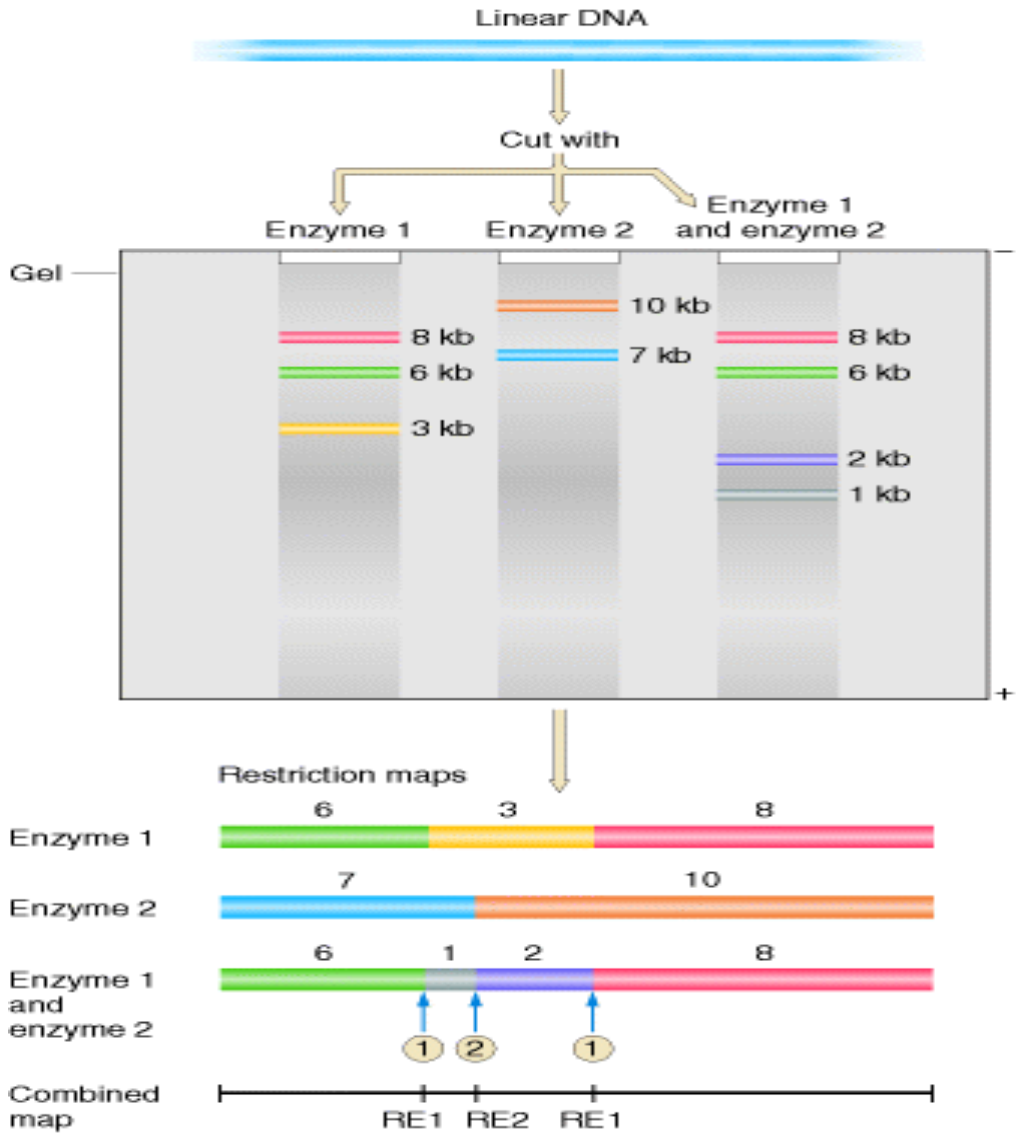
➔ Binds to the DNA molecule when it recognizes a specific sequence.

➔ 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)

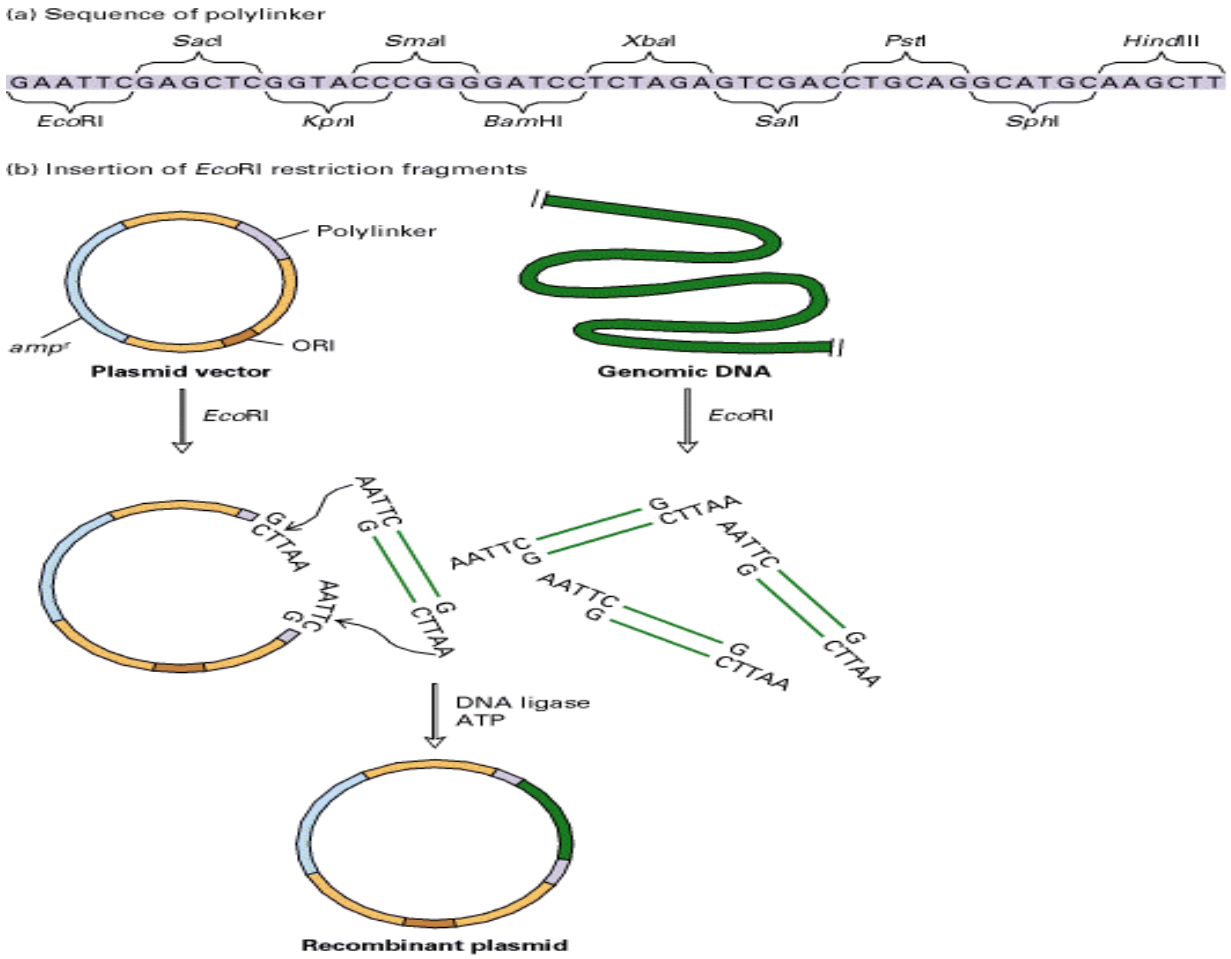
Uses in Biotechnology:

1. Generation of restriction map.



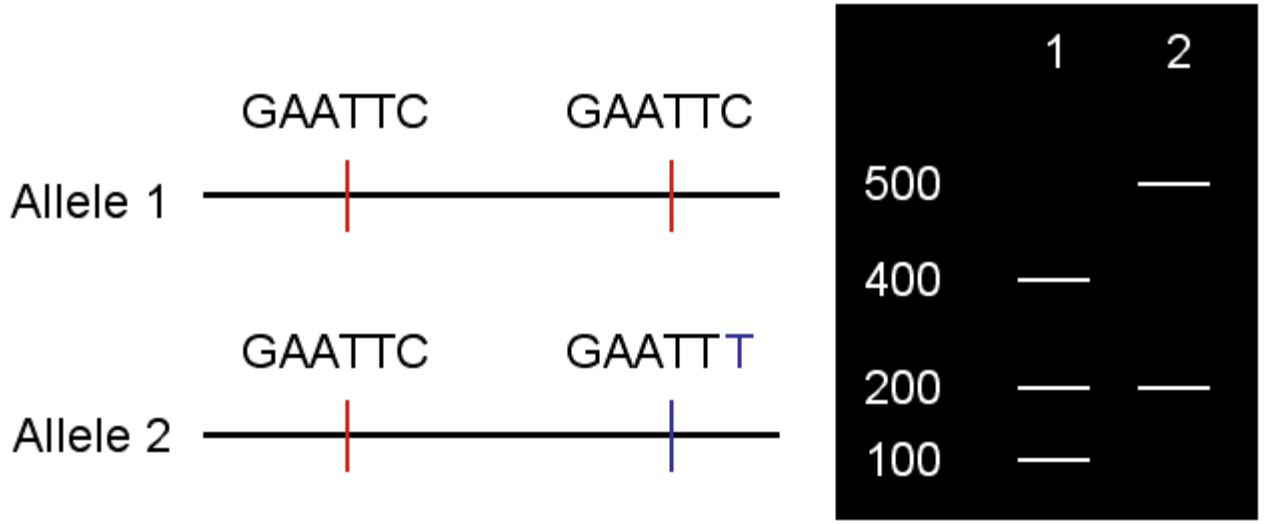
Uses in Biotechnology:

2. Recombinant DNA technology (gene cloning).



Uses in Biotechnology:

3. Restriction Fragment Length Polymorphism (RFLP).

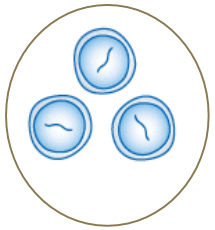


Restriction Fragment Length Polymorphism (RFLP):

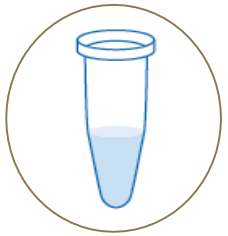
Is a tool to study variations among individuals (humans and other species).

This technique able to differentiate minor nucleotide sequence variations in **homologous** fragments of DNA

RFLP Workflow:



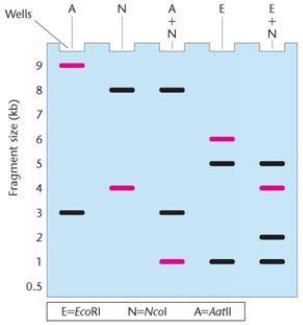
DNA Extraction



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

Incubation of the DNA with RE

Agarose gel to visualized your results

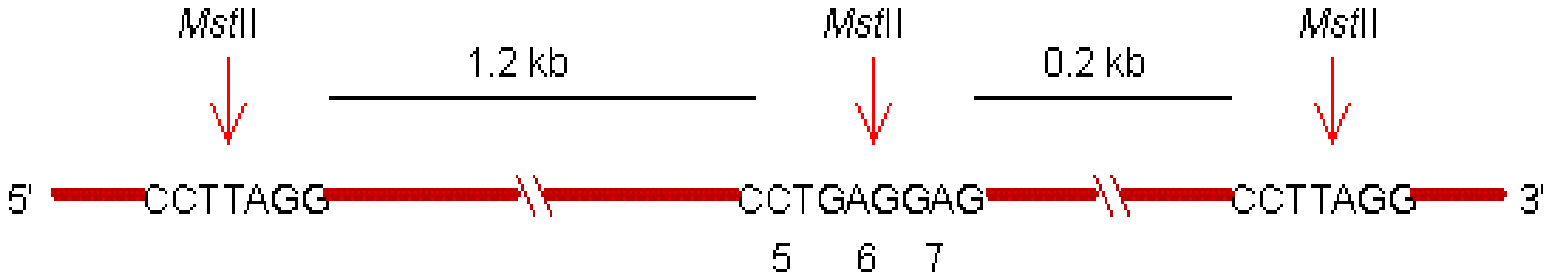


RFLP - Example:

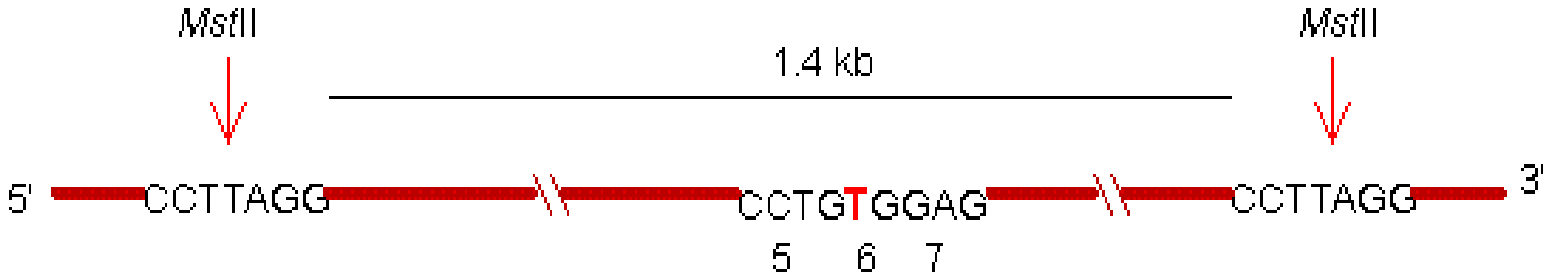
- **Genetic disease analysis as application.**

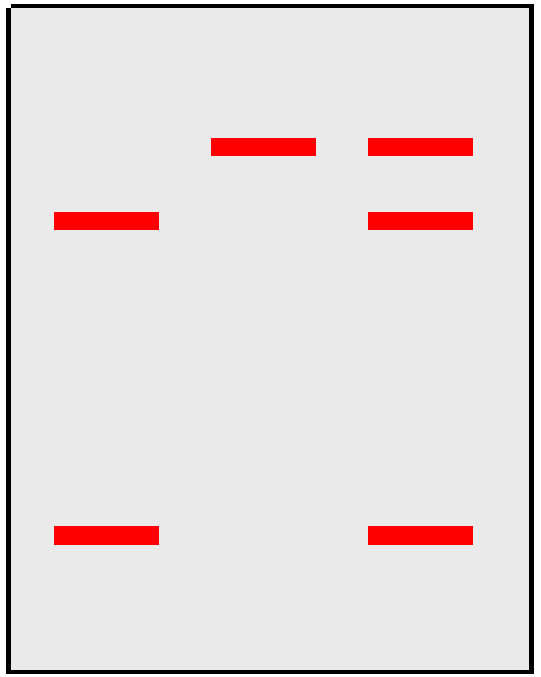
***Mst*II restriction stite:**
‘5-CCTNAGG-3’

Normal cell



Sickle cell





Practical Part

Aim:

- Restriction of genomic DNA.

Principle:

- Genomic DNA or DNA fragments obtained following PCR **incubated** with RE under appropriate experimental conditions.
- The RE **restricts** the DNA at sites at specific sequence, resulting in the production of different size fragments.
- 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 (1 µg/µl)	1
10X restriction buffer	2
NaCl solution	1
Water	14

2. Add MstII (1.5 U/µl) (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:

Draw the reaction of phosphodiester bond formation by the DNA polymerase.

Explain your drawing by your words, and make sure to mention which groups are involved in the bond formation.