Experiment (8): Digestion of DNA with Restriction Enzymes

Aim:

- Restriction of genomic DNA.

Introduction:

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, which is known as **RESTRICTION SITE** or target sequence. RE naturally found in a wide variety of prokaryotes. In live bacteria, restriction enzymes function to defend the cell against invading viral bacteriophages by cleaving its DNA at specific sites and so prevent replication. Over 300 restriction enzyme have been isolated and the nomenclature depends on the organism from which they are derived e.g. *Eco*RI: is isolated from **E. coli** strain **R**Y13, **I** (Roman numeral) indicates it was the first enzyme of that type isolated from E. coli RY13. **Table.1** present few examples of restriction enzymes, their origin and restriction site.

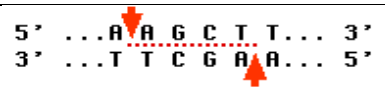
PAUSE AND THINK ➔ Bacterium is immune to its own restriction enzymes, even if it has the target sequences ordinarily targeted by them. Why?

How Restriction Enzyme cut the DNA?

A fragment of DNA produced by a pair of adjacent cuts is called a RESTRICTION FRAGMENT. Restriction enzymes can generate two different types of cuts (**Figure.1**) depending on whether they cut both strands at the center (Blunt end) of the recognition sequence or each strand closer to one end of the recognition sequence (Sticky end). The latter (sticky ends) are more cohesive compared to blunt ends, which have no nucleotide overhangs. Both are useful in molecular genetics and can be used for join DNA fragments.

Figure.1. Generation of blunt and sticky ends fragments my different RE.

Table.1. Examples of RE and their restriction site.

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	

*Arrows denote phosphodiester bonds cleaved by each restriction endonuclease.

Mechanism of Action:

Restriction Endonuclease scan the length of the DNA, binds to the DNA molecule when it recognizes a specific sequence and makes one cut in each of the sugar phosphate backbones of the double helix by hydrolyzing the phosphodiester bond. Specifically, the bond between the 3' O atom and the P atom is broken.

Uses in Biotechnology:

The ability of restriction enzymes to cut DNA at specific sequences has led to the widespread use of these tools in many molecular genetics techniques. Restriction enzymes can be used to map DNA fragments or genomes. Mapping means determining the order of the restriction enzyme sites in the genome. Perhaps the most important use of restriction enzymes has been in the generation of recombinant DNA molecules, which are DNAs that consist of genes or DNA fragments from two different organisms (gene cloning). In addition, Restriction enzymes also have applications in several methods for identifying individuals or strains of a particular species. 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. It relies on the specificity of restriction endonucleases, which are highly sequence-specific and cut the double-stranded DNA only at their recognition sites.

Principle:

Cleavage by ER is accomplished by the incubating of genomic DNA or DNA fragments obtained following amplification using PCR with the ER under appropriate experimental conditions of temperature, pH and ionic strength. The RE restricts the DNA at sites where the specific sequence recognized by the RE are presents, resulting in the production of different size fragments. These fragments can be separated on agarose gel electrophoresis.

In this experiment restriction of genomic DNA will be done using *Mst*II, which cut the DNA at '5-CCTNAGG-3'

Materials:

DNA solution (1 µg/µl), *Mst*II (1.5 U/ µl), 10X restriction buffer, NaCl solution, nuclease free water, 0.5 M EDTA.

Protocol:

1. Label a clean micro-centrifuge 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 *Mst*II (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.

Results:

Analyse the results using agarose gel electrophoresis.

References:

1. https://www.thebalance.com/what-are-restriction-enzymes-375674
2. http://medicine.jrank.org/pages/2779/Restriction-Enzymes-Use-Restriction-Enzymes-in-Biotechnology.html
3. https://www.thermofisher.com/sa/en/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/molecular-cloning/restriction-enzymes/restriction-enzymes-genome-mapping.html