Experiment (7): Restriction Fragment Length Polymorphism

Aim:

- To get familiar with the RFLP technique.

Introduction:

Restriction endonucleases have come to play a key role in all aspects of molecular biology. These enzymes recognise certain DNA sequences, usually 4–6 bp in length, and cleave them in a defined manner.

PAUSE AND THINK ➔ How restriction enzyme cut the DNA?

Individuality in humans and other species derives from their genetic polymorphism; homologous human chromosomes differ in sequence, on average, every ~1250 bp. These genetic differences create and eliminate restriction sites.
Restriction fragment length polymorphism (RFLP) is an inherited difference in the **pattern of restriction**, which is type of polymorphism that results from variation in the DNA sequence (i.e. Genetic polymorphism is defined as the inherited genetic differences among individuals in over 1% of normal population). Some of the sequence changes affect recognition sites for restriction enzymes, resulting in variation in the size of DNA fragments produced by digestion with a particular restriction enzyme. RFLPs may disrupt the function of the gene or may have no biologic consequences depending on they occurrences at known gene loci and/or in sequences that have no known function.

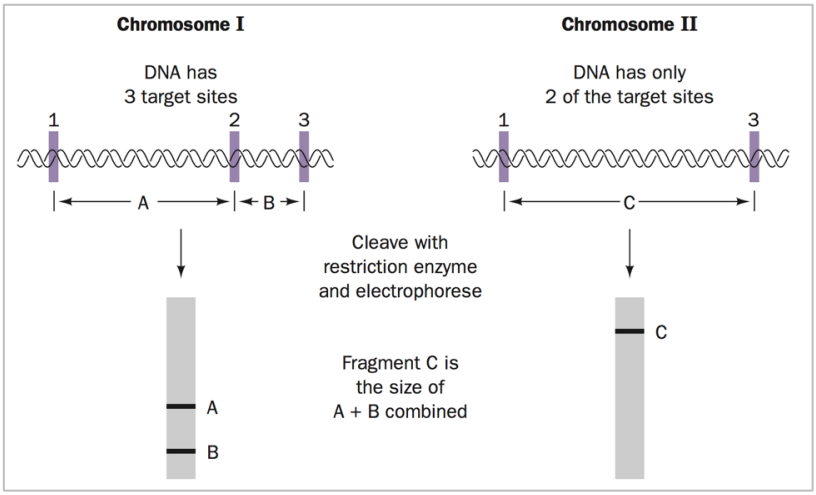

Figure 1: RFLP. A mutational change that affect a RS in a DNA segment alters the number and sizes of its restriction fragments.

RFLP Technique

Restriction fragment length polymorphism (RFLP) is a standard laboratory technique that used to analyse the DNA of genes, it was invented in 1984 by the English scientist Alec Jeffreys during research into hereditary diseases. RFLP analysis allows individuals to be identified based on unique patterns of restriction enzyme cutting in specific regions of DNA. it was an important early tool in genome mapping, localization of genes for genetic disorders, determination of risk for disease, and paternity testing.

➢ Principle:

Restriction endonucleases are enzymes that cut lengthy DNA into short pieces. Each restriction endonuclease targets different nucleotide sequences in a DNA strand and therefore cuts at different sites. The distance between the cleavage sites of a certain restriction endonuclease differs between individuals. Hence, the length of the DNA fragments produced by a restriction endonuclease will differ across both individual organisms and species.

RFLP Workflow:

1st DNA Extraction.
2nd Perform PCR for the region of interest.
3rd DNA Fragmentation by RE.
4th Gel Electrophoresis.
5th Visualization of Bands.

RFLP in molecular analysis and its Applications:

An extensive RFLP map of the human genome has been constructed. This is proving useful in the human genome sequencing project and is an important component of the effort to understand various single-gene and multigenic diseases.

➢ RFLP in different molecular analysis of disease state:

It is a useful diagnostic tools in genetic diseases where the RFLP can result from single-base changes such in sickle cell disease and in phenylketonuria. Moreover, in thalassemias the RFLP result from deletions or insertions of DNA into a restriction fragment.

➢ Applications of RFLP:

- To determine or confirm the source of a DNA sample such as in **paternity tests** or **criminal investigations.**
- In genetic mapping to determine recombination rates that show the genetic distance between the loci.
- To identify a carrier of a disease-causing mutation in a family.
- To determine the status of genetic diseases such as Cystic Fibrosis in an individual.

References:

Saraswathy, N., & Ramalingam, P. (2011). Genome mapping. Concepts and Techniques in Genomics and Proteomics, 77–93. doi:10.1533/9781908818058.77