Restriction fragment length polymorphism (RFLP)

Aim: To perform Restriction fragment length polymorphism (RFLP) of unknown DNA sample by restriction enzyme and analysis of restriction profile on agarose gel.

Introduction:

Restriction Fragment Length Polymorphism (RFLP) is a difference in homologous DNA sequences that can be detected by the presence of fragments of different lengths after digestion of the DNA samples in question with specific restriction endonucleases. RFLP, as a molecular marker, is specific to a single restriction enzyme combination. The RFLP probes are frequently used in genome mapping and in variation analysis (genotyping, forensics, paternity tests, hereditary disease diagnostics, etc.).

Principle :

The principle of RFLP markers is that any genomic DNA can be differentiated according to the presence or absence of restriction enzyme sites. Restriction enzymes recognize and cut at the particular site. Due to the accumulation of single nucleotide mutations in genomic DNA, the restriction enzyme sites on DNA change, resulting in a difference in restriction patterns of two closely related genomes. This restriction pattern can be detected using the Southern hybridization technique. Thousands of DNA markers are detected and located throughout the genome. RFLP- based genetic maps have been prepared for many organisms (**Figure 1**)

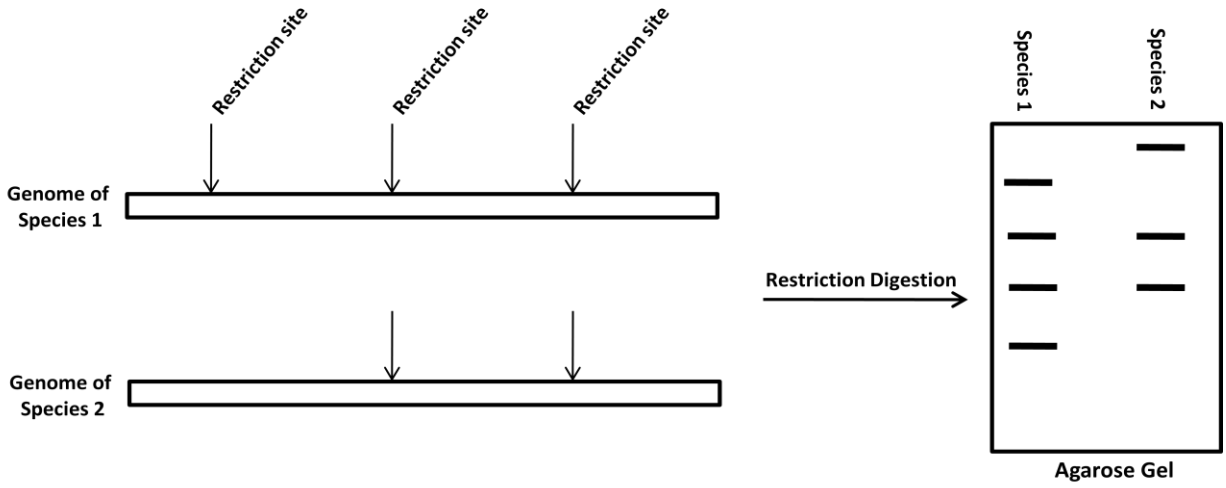

Figure1

Procedure:

In the present experiment we will not perform the southern blot, we will perform only restriction digestion. after restriction digestion we will compare restriction profile of unknown DNA sample with reference samples.

Restriction digestion of unknown DNA sample with given restriction enzymes

1. prepare the reaction mix as follow:

Component	**Vol. (Micro litre)**
Unknown DNA sample	1 2
Restriction Enzyme , *EcoRI*	1
Restriction Enzyme , *PstI*	1
10X Digestion Buffer	2
Molecular biology grade water	4
Total Volume of Reaction	**20**

2. After adding the above components, mix by tapping and give a short spin.
3. Incubate at 37 ^{O}C water bath for 3 hr .
4. Analyze the digested sample by agarose gel electrophoresis. (Load DNA ladder , reference samples and digested unknown DNA sample)
5. Compare the restriction profile of unknown sample to reference samples and identify the unknown DNA sample.

Results:

observations: