

Restriction Fragment Length Polymorphism (RFLP)

Restriction enzymes are site-specific DNAses that cleave a DNA molecule whenever the recognition sequence, which is usually a 4-6 base palindrome, is present. Because of the enzyme's sequence specificity, digestion of a particular DNA results in a reproducible array of fragments. RFLP, or length differences in homologous fragments between different DNA, are caused by changes in the primary sequence of the DNA.

These length differences can be the result of:

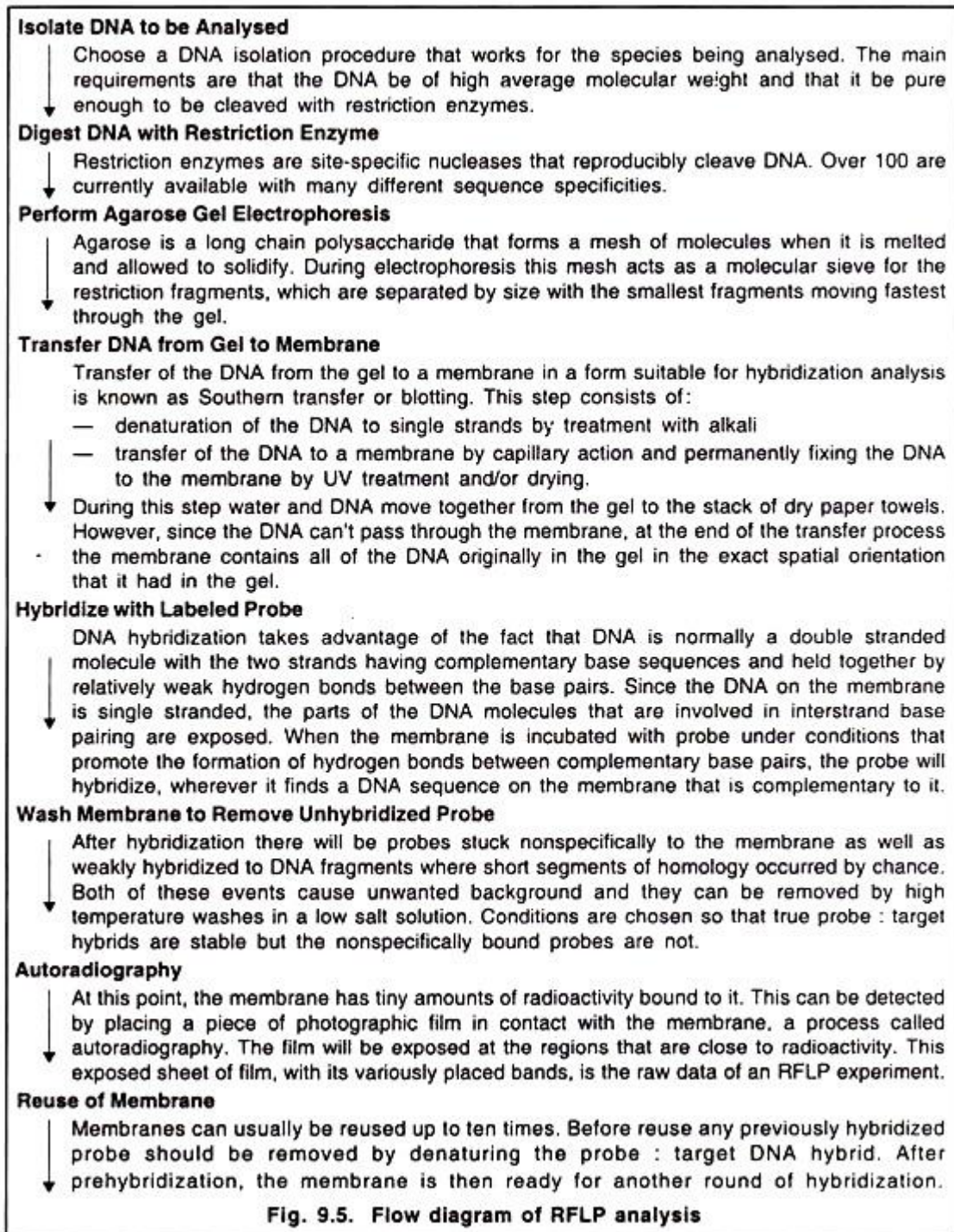
- a. A point mutation resulting in the loss or gain of restriction enzyme cut site

- b. An insertion or deletion of DNA between two restriction enzyme cut sites

- c. A deletion which overlaps a restriction enzyme site

- d. A DNA rearrangement, where one end of the rearranged segment resides between two restriction enzyme sites. When such differences occur, they can be detected by DNA hybridization and used as molecular markers in fingerprinting or genetic studies.

The flow diagram of RFLP analysis is shown in Fig. 9.5:



Advantages of RFLP:

- a. Present everywhere,
- b. Mendelian inheritance,
- c. Co-dominant expression,

- d. No pleiotropic effects,
- e. Independent of the environment,
- f. Present at each developmental stage,
- g. Long stability of cDNA probes,
- h. Different loci may be identified by one probe,
- i. Heterologous genes may be used as probes,
- j. Any number of probes can be produced,
- k. Probes are producible for coding and silent sequences,
- l. Probes show the variability of flanking sequences,
- m. Several characters can be screened in the same sample.