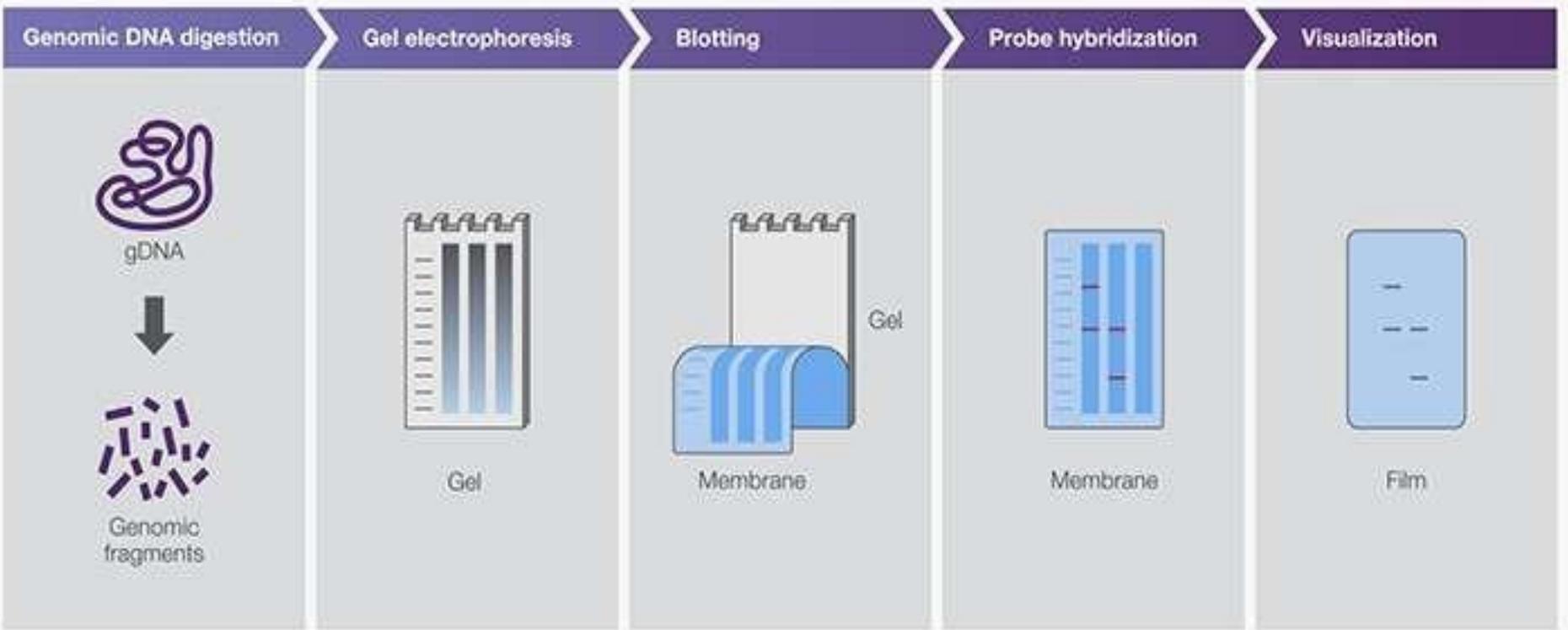




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DEPARTMENT OF BIOTECHNOLOGY

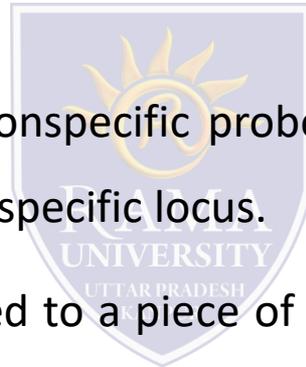
RFLP (RESTRICTION FRAGMENT LENGTH POLYMORPHISM)



Restriction Fragment
Length
Polymorphism

- Restriction fragment length polymorphism (RFLP) markers were regarded as the first shot in the genome revolution, marking the start of an entirely different era in the biological sciences.
- RFLP was the most popular approach for analysis of genetic variation during the entire 1980s.
- RFLP is based DNA fragment length differences after digesting genomic DNA with one or more restriction enzymes.
- DNA is digested by one or more restriction enzymes and separated on an agarose gel.
- The DNA in the gel transferred to nitrocellulose or nylon membranes.

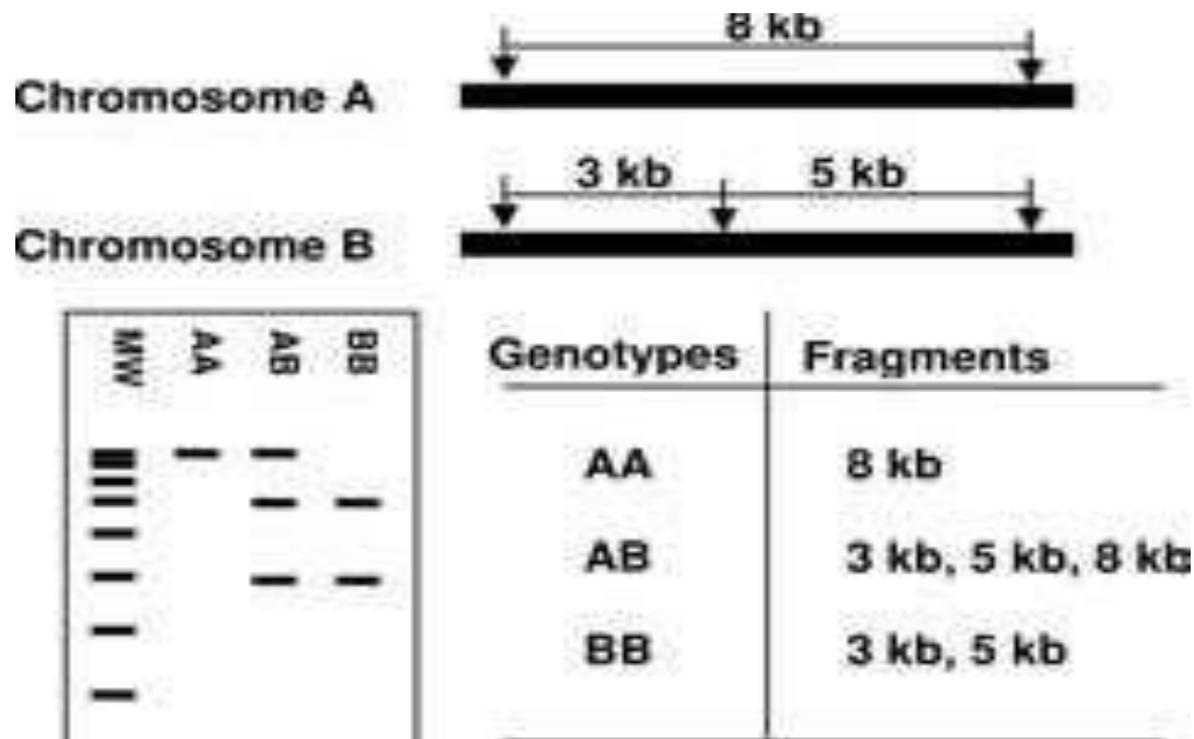
- The specific DNA locus with a potential fragment length difference is characterized by hybridization to a probe, a radioactively labeled DNA or ribonucleic acid (RNA) molecule with sequence similarities to the locus of interest.
- After hybridization, the nonspecific probes must be washed away leaving only hybridized probes to the specific locus.
- The membrane is then exposed to a piece of X-ray film for autoradiography to visualize the DNA bands.



Technology Advances - Development of RFLP

- Two specific technological advances that set the foundation for RFLP were —
 - The discovery and application of restriction enzymes and
 - The development of DNA hybridisation.





In the example, a base substitution within the 8 kb fragment leads to the gain of a new restriction site. For homozygous AA, one band of 8 kb should be generated; for homozygous BB, two bands of 3 kb and 5 kb should be generated; for heterozygous AB, three bands of 8 kb (from allele A), 3 kb and 5 kb (both from allele B) should be generated.

- *RFLP is a non-PCR based method .*
- *In this Method DNA is digested with restriction Enzymes.*
- *RFLP is the co dominant marker.*
- *RFLP is 1-10 loci detected.*

