Restriction fragment length polymorphisms(RFLP)

Restriction fragment length polymorphisms

- The Variation in the restriction DNA fragment length between individuals of a species called RFLP
- *Restriction fragment length polymorphisms (RFLPs)* were the first type of molecular markers used in linkage studies.
- RFLPs arise because mutations can create or destroy the sites recognized by specific restriction enzymes, leading to variations between individuals in the length of restriction fragments produced from identical regions of the genome

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Restriction fragment length polymorphism (RFLP)

- **Restriction fragment length polymorphism** (**RFLP**) is a technique that exploits variations in homologous DNA sequences, known as polymorphisms, in order to distinguish individuals, populations, or species or to pinpoint the locations of genes within a sequence.

- The term may refer to a polymorphism itself, as detected through the differing locations of restriction enzyme sites, or to a related laboratory technique by which such differences can be illustrated.

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- In **RFLP analysis**, a DNA sample is digested into fragments by one or more restriction enzymes, and the resulting *restriction fragments* are then separated by gel electrophoresis according to their size.

- RFLP analysis was an important early tool in genome mapping localization of genes for genetic disorders, determination of risk for disease, and paternity testing.

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- Restriction fragment length polymorphism (RFLP) is a technique invented in **1984 by the English scientist Alec Jeffreys** during research into hereditary diseases.

- It is used for the analysis of unique patterns in DNA fragments in order to genetically differentiate between organisms – these patterns are called Variable Number of Tandem Repeats (VNTRs).

- Genetic polymorphism is defined as the inherited genetic differences among individuals in over 1% of normal population. The RFLP technique exploits these differences in DNA sequences to recognize and study both intraspecies and interspecies variation.

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- A restriction fragment length polymorphism is said to occur when the length of a detected fragment varies between individuals, indicating non-identical sequence homologies

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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.

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How does it Work?

- **DNA Extraction**
- To begin with, DNA is extracted from blood, saliva or other samples and purified.

- **DNA Fragmentation**
- The purified DNA is digested using restriction endonucleases. The recognition sites of these enzymes are generally 4 to 6 base pairs in length. The shorter the sequence recognized, the greater the number of fragments generated from digestion.

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- For example, if there is a short sequence of GAGC that occurs repeatedly in a sample of DNA.

- The restriction endonuclease that recognizes the GAGC sequence cuts the DNA at every repetition of the GAGC pattern.

- If one sample repeats the GAGC sequence 4 times whilst another sample repeats it 2 times, the length of the fragments generated by the enzyme for the two samples will be different.

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Restriction fragment length polymorphism

- Restriction fragment length polymorphisms (RFLPs) were developed by Botstein et al. (1980), which uses restriction enzymes that cut the DNA molecule at specific sites, called restriction sites, resulting in different fragments of variable lengths.

- After separation by electrophoresis, fragments are transferred to nitrocellulose or nylon filters through southern blotting, followed by hybridization with radioactively labelled DNA probes and visualization using photographic film. (Varshney et al., 2004).

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RFLP PROCEDURE

Genomic DNA isolation

Digestion with Restriction enzyme

Agarose gel electrophoresis of fractioned DNA

DNA transferred on membrane filter paper southern hybridization

Autoradiography

RFLP pattern with positive and

(Varshney et al., 2004

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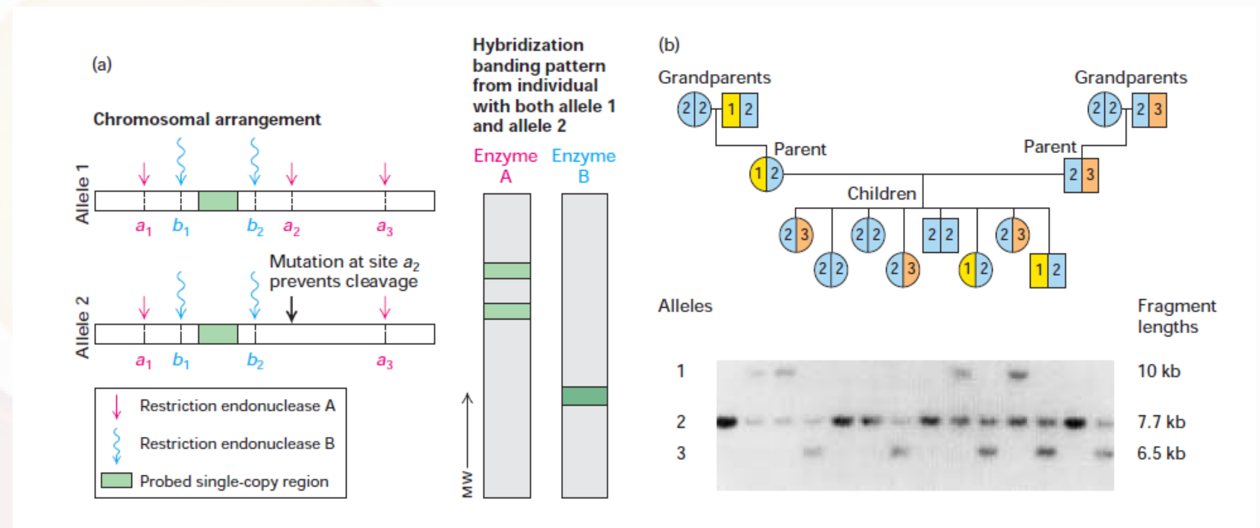
(a) In the example shown, DNA from an individual is treated with two different restriction enzymes (A and B), which cut DNA at different sequences *(a* and *b).* The resulting fragments are subjected to Southern blot analysis (Figure a) with a radioactive probe that binds to the indicated DNA region (green) to detect the fragments. Since no differences between the two homologous chromosomes occur in the sequences recognized by the B enzyme, only one fragment is recognized by the probe, as indicated by a single hybridization band. However, treatment with enzyme A produces fragments of two different lengths (two bands are seen), indicating that a mutation has caused the loss of one of the *a* sites in one of the two chromosomes.



(b) Pedigree based on RFLP analysis of the DNA from a region known to be present on chromosome 5. The DNA samples were cut with the restriction enzyme *Taq*I and analyzed by Southern blotting. In this family, this region of the genome exists in three allelic forms characterized by *Taq*I sites spaced 10, 7.7, or 6.5 kb apart. Each individual has two alleles; some contain allele 2 (7.7 kb) on both chromosomes, and others are heterozygous at this site. Circles indicate females; squares indicate males. The gel lanes are aligned below the corresponding subjects.

H. Donis-Keller et al., 1987, *Cell* **51**:319

Applications of RFLP

- RFLP has been used for several genetic analysis applications since its invention.
- Some of these key applications of RFLP are listed below:

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

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Disadvantages of RFLP

- Since its invention, RFLP has been a widely used genome analysis techniques employed in forensic science, medicine, and genetic studies.

- However, it has become almost obsolete with the advent of relatively simple and less expensive DNA profiling technologies such as the polymerase chain reaction (PCR).

- The RFLP procedure requires numerous steps and takes weeks to yield results, while techniques such as PCR can amplify target DNA sequences in a mere few hours.

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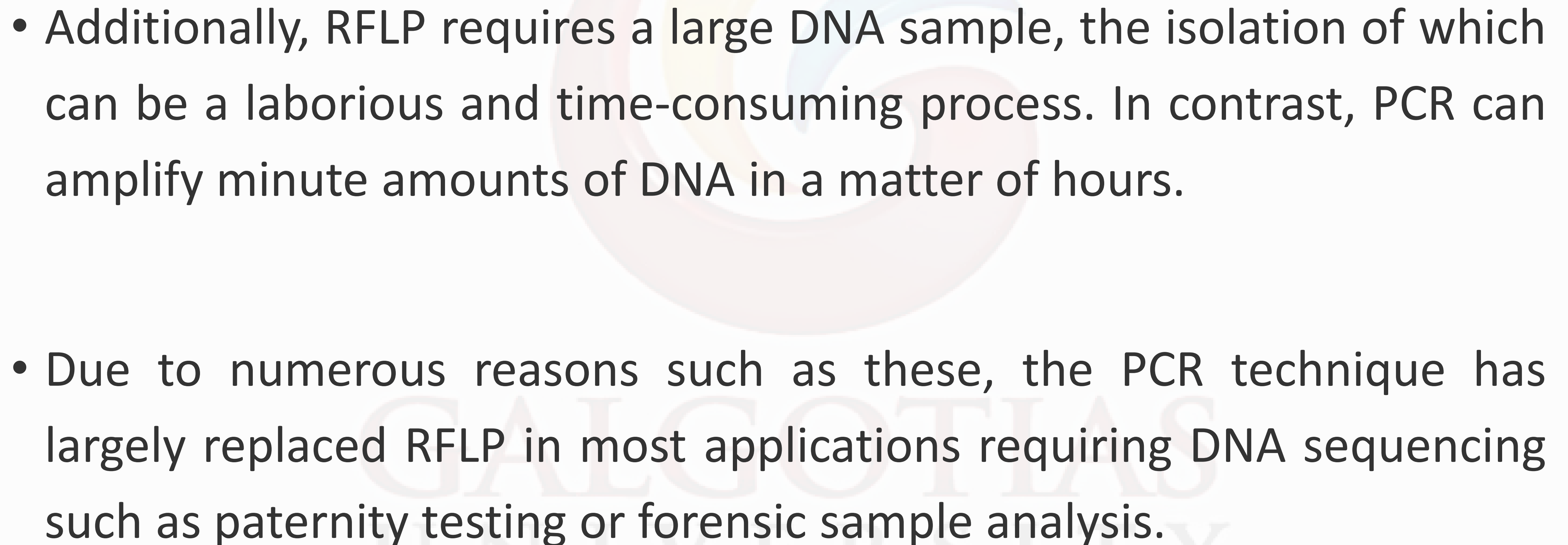
- Additionally, RFLP requires a large DNA sample, the isolation of which can be a laborious and time-consuming process. In contrast, PCR can amplify minute amounts of DNA in a matter of hours.

- Due to numerous reasons such as these, the PCR technique has largely replaced RFLP in most applications requiring DNA sequencing such as paternity testing or forensic sample analysis.



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