M.Sc. (Microbiology) 2nd Semester, Paper 2nd ,Unit 4th

RFLP (Restriction fragment length polymorphism)

And

RAPD (Rapid amplified polymorphic DNA) are genetic markers

Genetic Marker

- “Genetic markers are known DNA sequences which can be used for studying any kind of polymorphism”
- A genetic marker is a known location on a chromosome used for identification of individuals among and between species.
- The Genetic markers are used, for studying disease gene ,for studying any kind of alterations (polymorphism) ,identification of individuals, identification of species or organisms

- “The genetic markers are often known as DNA marker is a known DNA sequence located in the genome, used in the molecular genetics for identification of alteration or DNA sequence from the unknown DNA sample.”

- The genetic marker is a known DNA sequence or gene located on the chromosome which can be applied in the identification of individual species or organism or we can use it in the identification of other genes or DNA sequences.

By marking a specific sequence of DNA, we can use it in different types of genetic studies.
Let's take an example:

SPECIES A

ATGGCGGCCCG ATGGCGGCCCG ATGGCGGCCCG ATGGCGGCCCG

SPECIES B

ATGGCGGCCCG ATGGCGGCCCG ATGGCGGCCCG ATGGCGGCCCG
ATGGCGGCCCG ATTTGT

SPECIES C

ATGGCGGCCCG ATGGCGGCCCG ATGGCGGCCCG ATGGCGGCCCG
ATGGCGGCCCG ATGGCGGCCCG ATGGCGGCCCG AAATGCGCGTG

Can you identify something? 11 base pair sequence is repeatedly observed at several intervals in a sequence and it is present in all three species.
Suppose this sequence is abundantly present in all organisms on earth (assume it). Let me give some name to this marker by using its characteristics.

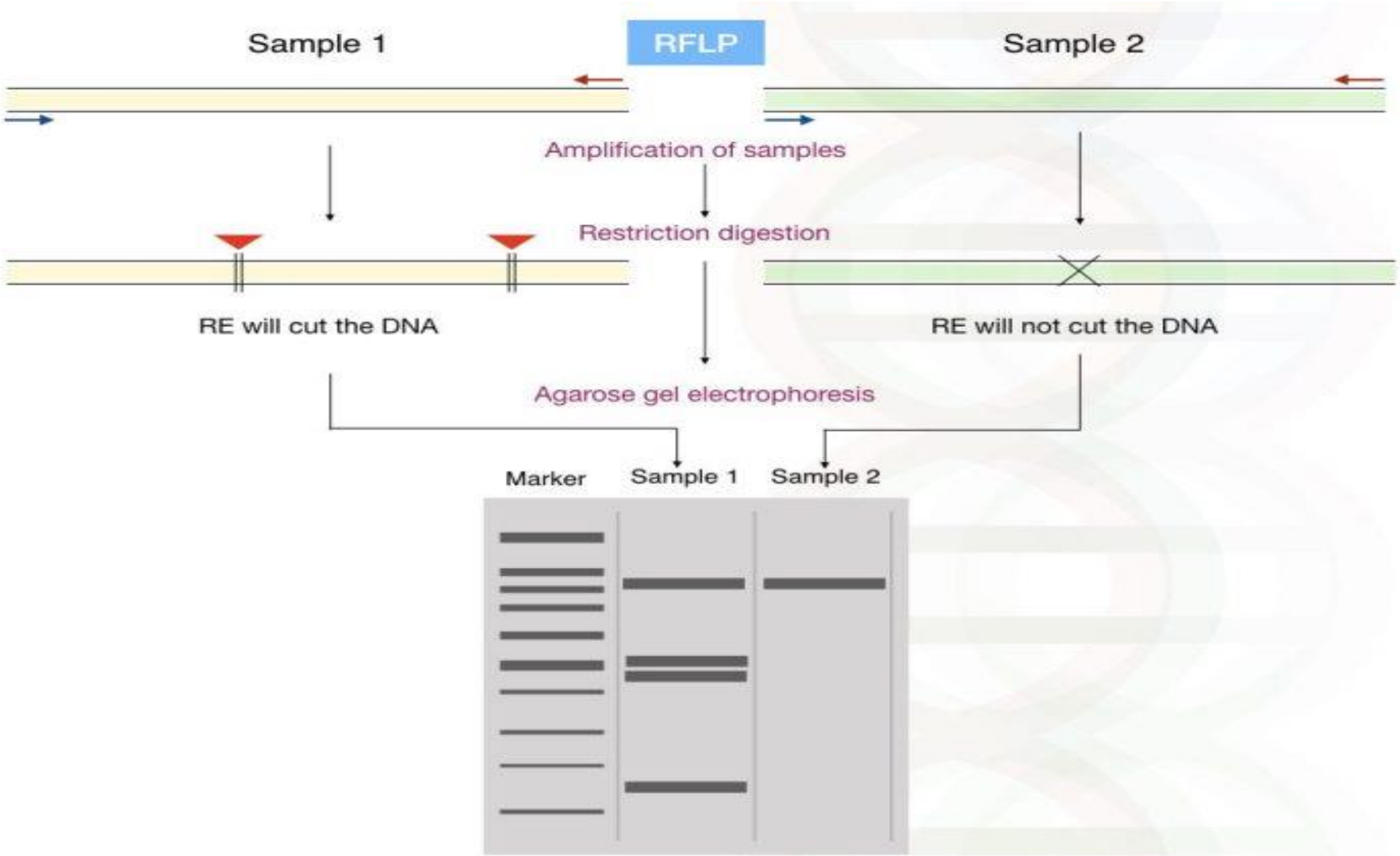
It is observed one after another, tandemly .
It is repeatedly observed after regular interval of sequences hence it is a repeat sequence.
Though the sequence is the same, sequence numbers are different in all three species i.e , 4 repeats in species A, 5 repeats in species B and 7 repeats in species C. So the number of the repeats are variable.
Now collect all three characters in a single set: it is a sequence, arranged tandemly , repeated one after another and variable so we can name it as variable numbers of tandem repeats.
That is how different markers are developed.

RFLP: Restriction fragment length polymorphism

With the help of the Restriction digestion by REase, alterations in the same/ homologous DNA sequences can be detected by analysing fragments of different length, digested with a restriction enzyme.

A single restriction endonuclease gives more specific results by cutting at one specific locus and produces fragments of different length.

different DNA fragments are created by Restriction digestion.



Firstly, let us understand the terminology

RFLP, alteration (polymorphism) in the length of different fragments (of DNA) can be analysed using restriction digestion.

Restriction digestion is performed by restriction endonuclease enzymes. It cuts DNA at its specific restriction site. Millions of restriction sites are present for individual RE in the human genome.

The length of different fragments is identified using blotting, which is now replaced by sequencing. RFLP is applicable in disease identification, genetic mapping, heterozygous detection and carrier identification.

The RFLP markers are highly locus-specific and co-dominant.

Why RFLP markers are locus-specific?
Because of the nature of the REase used in the RFLP .
The REases can cut at a specific location where their restriction site is located. One restriction digestion site generates two different fragments.

Further, it is co-dominant because both alleles in the heterozygous condition (mutant as well as normal) can be detected by RFLP .

There are two variations in the RFLP are most common.

The traditional method of the RFLP is blotting based probe hybridization method.
The PCR based RFLP method is a newer version, easy and rapid.

In the traditional method, the labelled DNA probes called an RFLP probe, used to hybridized with the digested fragments by the Southern blotting analysis.

The polymorphism can be detected by analyzing the hybridization pattern of the labelled probe using autoradiography .

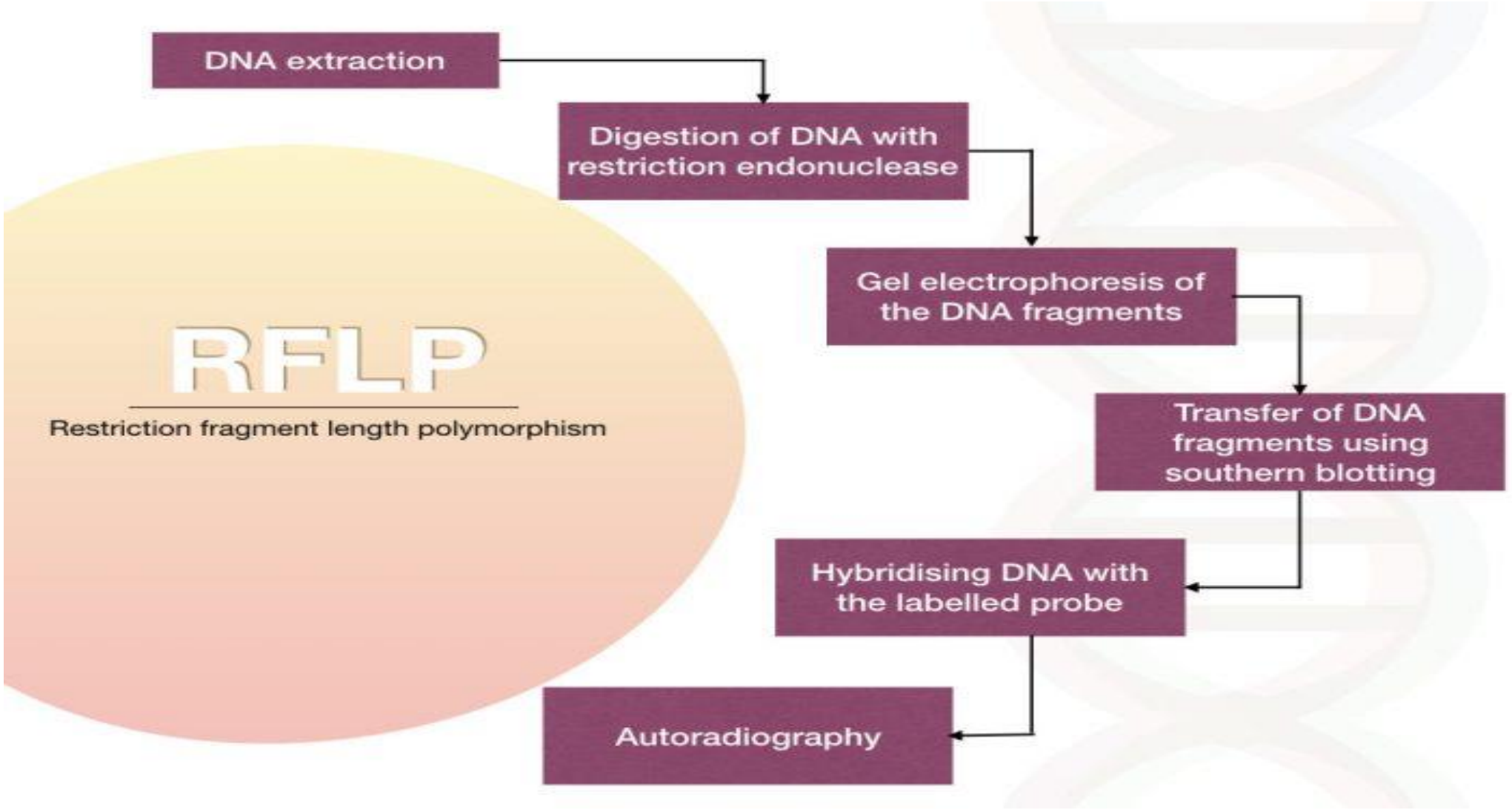
In the PCR based RFLP method, the gene of our interest is amplified using the polymerase chain reaction and proceed for the restriction digestion using the specific REase.

The results are analyzed using agarose gel electrophoresis.
No tedious labelling and hybridization techniques are required in this method.

The PCR-RFLP method is easy, simple, time-efficient and reliable.

Polymorphism in the alleles of a gene such as MTHFR and HBS can be detected using PCR-RFLP.

Graphical illustration of different steps in the restriction fragment length polymorphism: RFLP.



Applications of RFLP:

It is used in the polymorphism study, forensics, paternity verification, hereditary disease diagnosis and DNA fingerprinting and genome mapping.

RAPD: Random amplification of polymorphic DNA

By using the short, arbitrary DNA primers, the part of the genomic DNAs is randomly amplified.

Pronounced as a “rapid”, it does not require any previous sequence information, the primer of 8 to 12mer randomly amplifies the region of the genomic DNA.

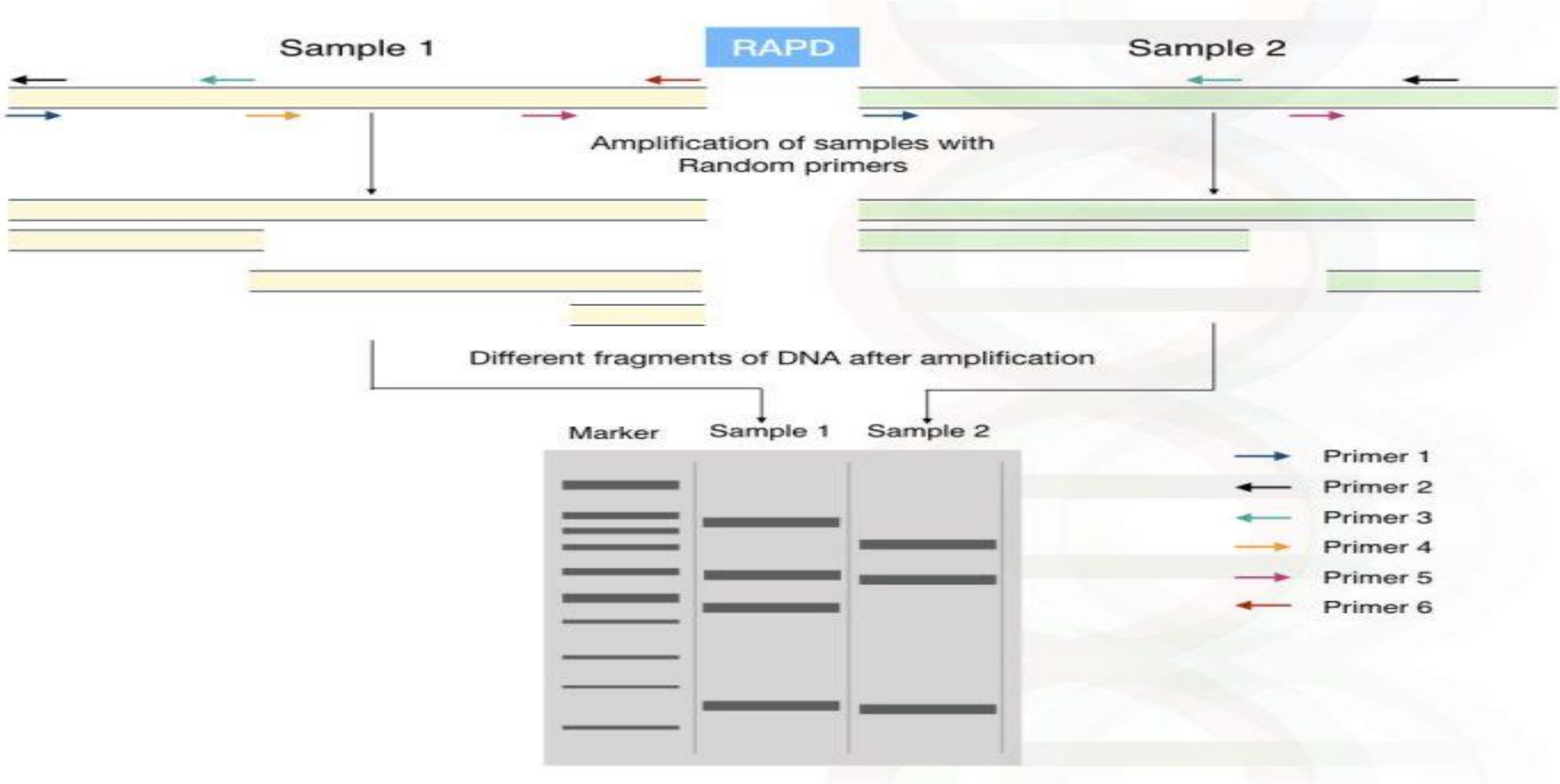
If any mutation or alteration occurred in the region of the primer binding site, the primer can not bind to that location or the amplified fragment length decreases or increases.

A different pattern of amplification is observed in different sample types.

Hence the RAPD can help in the polymorphism studies.

Detection of RAPD:

The RAPD fragments are between 0.2 to 5.0kb. It can be observed using the ethidium bromide-stained agarose gel or it can also be analyzed on polyacrylamide gel electrophoresis.



Advantages:

The RAPD markers are abundantly present into the genome and are distributed throughout the genome.
No previous sequence information is needed.
It can amplify low quantity of DNA.

Disadvantages:

The marker is not locus-specific.
The sensitivity of the RAPD is also lower.
The reproducibility is also very low.
Homozygous and heterozygous are not easily distinguished.
The RAPD results are difficult to interpret because the short primer can amplify any of the random sequences presents into the genome.
Nonetheless, RAPD markers are quite popular in gene mapping, species identification, species-specific polymorphism studies and phylogenetic analysis.
It is popular in plant genetic research.

References

Young, N.D. Plant Growth Regul (1993) 12: 229. https://doi.org/10.1007/BF00027203
Raza. S., Shoib. W. and Mubeen H. “Genetic markers: importance, uses and applications.” International Journal of Scientific and Research Publications; 2016: