

Restriction Fragment Length Polymorphism (RFLP)

Restriction Fragment Length Polymorphism (RFLP) is a technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA. If two organisms differ in the distance between sites of cleavage of a particular restriction endonuclease, the length of the fragments produced will differ when the DNA is digested with a restriction enzyme. The similarity of the patterns generated can be used to differentiate species (and even strains) from one another.

Polymorphisms are inherited differences found among the individuals in more than 1% of normal population.

Applications of RFLP:

RFLPs can be used in many different settings to accomplish different objectives.

- 1- RFLPs can be used in paternity cases or criminal cases to determine the source of a DNA sample. (i.e. it has forensic applications).
- 2- RFLPs can be used determine the disease status of an individual. (e.g. it can be used in the detection of mutations particularly known mutations)
- 3- RFLPs can be used to measure recombination rates which can lead to a genetic map with the distance between RFLP loci measured in centiMorgans.

Restriction Endonucleases

Restriction endonucleases are enzymes that cleave DNA molecules at specific nucleotide sequences depending on the particular enzyme used. Enzyme recognition sites are usually 4 to 6 base pairs in length. Generally, the shorter the recognition sequence, the greater the number of fragments generated. If molecules differ in nucleotide sequence, fragments of different sizes may be generated. The fragments can be separated by gel electrophoresis. Restriction enzymes are isolated from a wide variety of bacterial genera and are thought to be part of the cell's defenses against invading bacterial viruses. These enzymes are named by using the first letter of the genus, the first two letters of the species, and the order of discovery.

Polymerase Chain Reaction (PCR)

PCR is a technique for amplifying a specific region of DNA, defined by a set of two "primers" at which DNA synthesis is initiated by a thermostable DNA polymerase. Usually, at least a million-fold increase of a specific section of a DNA molecule can be realized and the PCR product can be detected by gel electrophoresis. The regions amplified are usually between 150-3,000 base pairs in length.

PCR/RFLP

Isolation of sufficient DNA for RFLP analysis is time-consuming and labor intensive. However, PCR can be used to amplify very small amounts of DNA, usually in 2-3 hours, to the levels required for RFLP analysis. Therefore, more samples can be analyzed in a shorter time.

Example of use of RFLP in DNA Typing:

1. Extraction

The first step in DNA typing is extraction of the DNA from the sample, be it blood, saliva, semen or some other biological sample



2. Production of Restriction Fragments

The purified DNA is then cut into fragments by RESTRICTION ENZYMES.

Take the pattern GCGC and imagine it occurs more than once in the DNA. The number of times it occurs is unique to the individual. The restriction enzyme chops the DNA in two at every place where the GCGC pattern occurs;



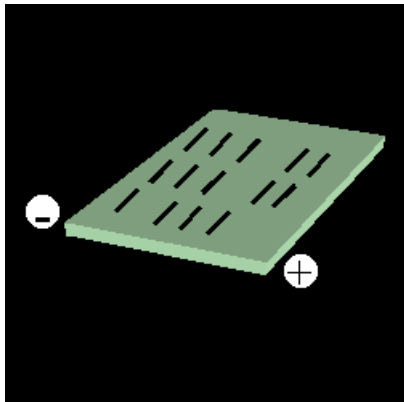
Person 1 has the repeat sequence three times while Person 2 has it twice. The restriction enzyme will cut between the first G and the first C;

Person 1 - 2 large fragments; 5' G.....CGCATGTTG.....CGCAAGAG.....CGC 3'

Person 2 - 1 large fragment; 5' G.....CGCATGAAGGCAATGAG.....CGC 3'

3. Electrophoresis

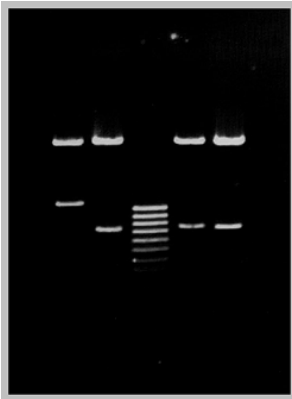
The restriction fragments have negative charge and can be separated by a technique called GEL ELECTROPHORESIS, which separates the pieces of DNA based on their size.



The samples of DNA that have been treated with restriction enzymes are placed in separate lanes on a slab of electrophoretic gel across which is placed an electric field. The fragments migrate towards the positive electrode, the smaller fragments moving faster than the larger fragments, thus separating the DNA samples into distinct bands.

4. Detection

The bands can be visualised using luminescent dyes;



This approach to DNA typing required quite large samples of biological material in order to obtain reasonable results.

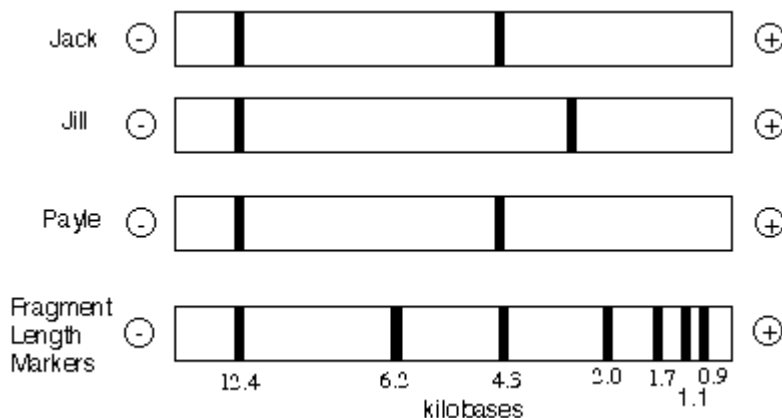
For modern forensic work RFLP typing has been superseded by methodology based on the polymerase chain reaction which requires only minute amounts of sample for a successful typing.

Example of forensic application of RFLP:

paternity Case

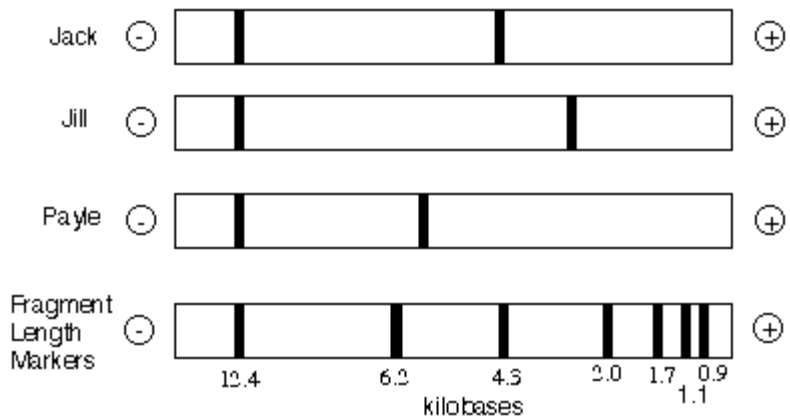
Let's use RFLP technology to determine if Jack is the father of Jill's child named Payle.

In this scenario, DNA was extracted from white blood cells from all three individuals and subjected to RFLP analysis. The results are shown below:



In this case, it appears that Jack could be the father, since Payle inherited the 12.4 kb fragment from Jill and the 4.3 fragment from Jack. However, it is possible that another man with similar RFLP pattern could be as well. To be certain, several more RFLP loci would be tested. It would be highly unlikely that two men (other than identical twins) would share multiple RFLP patterns and so paternity could be confirmed.

In a different scenario, DNA was extracted from white blood cells from all three individuals and subjected to RFLP analysis. The results are shown below:

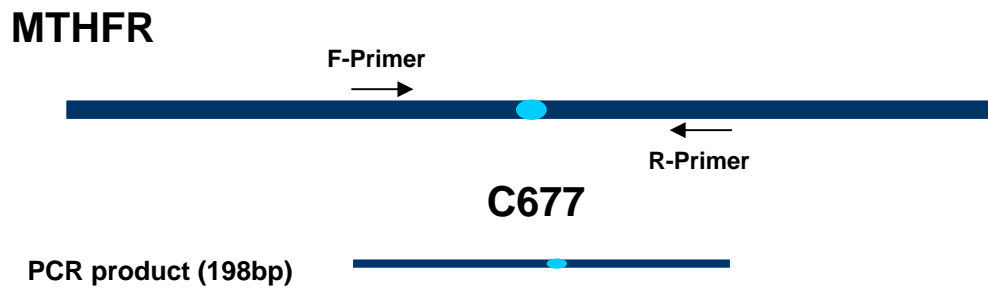


This time, it can be determined that Jack is NOT the father of Payle since Payle has a band of about 6 kb and Jack does not. Therefore, it is very probable that Payle's father is not Jack, though it is possible that Payle carries a new mutation at this locus.

Example of use of RFLP in the detection of mutation in disease state:

Mutation in the Methyl tetrahydrofolate reductase enzyme could lead to increased level of homocystein in blood and leads to increased risk of thrombosis in these individuals carrying the mutation

Method Used for MTHFR Mutation Detection



PCR product is digested with *Hinfl* restriction

