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Independent Study Mentorship

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**Research Assessment #8**

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**Subject:** Restriction fragment length polymorphism

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"Restriction Fragment Length Polymorphism (RFLP)." World of Forensic Science, edited by Sara Constantakis, 2nd ed., vol. 2, Gale, 2016, pp. 580-582. Science in Context, http://link.galegroup.com/apps/doc/CX3630600441/SCIC?u=j043905010&xid=ff6c2cd8. Accessed 8 Dec. 2017.

**Assessment:**

For my Original Work, I am planning on conducting different DNA fingerprinting experiments to create a guide on which process will be faster and more efficient to use depending on the circumstance. One of the main types of DNA fingerprinting is Restriction Fragment Length Polymorphism (RLFP). This process analyzes the length of the strands of the DNA molecules with repeating base pair patterns. The restriction fragment length polymorphism analysis is used to detect the repeated sequences by determining a specific pattern to the VNTR, which becomes the person's DNA fingerprint. Overall, with this research, My main goal is to learn about the RLFP and its advantages and disadvantages.

When reading the three article I have selected for this research assessment collectively, I noticed many things. The main things were how precise this procedure is. I never understood how having different methods of DNA Fingerprinting is useful if they all reach the same result. However, these articles over restriction fragment length polymorphism showed me that this procedure is very precise and requires a lot of DNA, machinery, and time. By going into the molecular level, this procedure eliminates most of the potential errors. It gives exact results which would be necessary for criminal cases to make sure the right decisions are being made. In order to do this procedure, a gel electrophoresis is needed to separate the strands of DNA. This device is one of the most important tools to help make this procedure successful and it is also necessary for the other procedures of DNA Fingerprinting as well. Another big thing I noticed was that the procedure of RFLP is beneficial for other purposes as well. It is not restricted to only DNA Fingerprinting but also “...for diagnosis of disease because it assays directly for a genotype (DNA sequence) and does not depend on expression of a gene or even phenotypic expression of the disease itself...” which is interesting since it related to my theme of connections in the being of my ISM journey.

The research I have conducted over Restriction Fragment Length Polymorphism gave me a deep look into the procedure of RFLP. I realized how crucial it was for me to recall the information I learned in my AP Biology class two years ago. With this, I was able to easily understand how to conduct my experiment due to my previous knowledge I obtained from my AP Biology class two years ago. I have gained valuable information from this article and am a lot more excited about my Original Work and what my results will bring. This research cleared up most of my confusion on how I will make this Original Work proposal work in reality. Now, I believe I understand a bit on how I will conduct my experiment which gives me more ideas on how to display my work for the community. The research overall was very beneficial to my Original Work as well as my ISM journey through Chemical Engineering. I am a lot more confident now that I know what to aim for and how to proceed with my Original Work.

**Articles:**

## **Restriction fragment length polymorphism (RFLP)**

*World of Genetics*, 2007

As [**DNA**](https://docs.google.com/document/d/1kEXQA58aEm4w7WMG5FveNqgMmcAQG6QKcA9jz4wohEM/edit#bookmark=id.gjdgxs) changes are not restricted to those that affect phenotype, restriction fragment length polymorphisms (RFLP) analysis is a powerful technique for the characterization of DNA at the molecular level. These markers are inherited in the same manner as genes that code for visible phenotypes. The **recombination** frequency between an RFLP and a detectable phenotype can be measured. Thus, genetic maps can be constructed to include both genotypic and phenotypic markers. RFLPs can thereby provide a link between genes that lie far apart. In 1980, an RFLP map was created for the human **genome**.

Restriction maps that result from different patterns of distribution of restriction sites in the DNA of individuals within a population of organisms are called restriction fragment length polymorphisms (RFLPs). Differences in individual base pairs between comparable sequences of any two individual [chromosomes](https://docs.google.com/document/d/1kEXQA58aEm4w7WMG5FveNqgMmcAQG6QKcA9jz4wohEM/edit#bookmark=id.gjdgxs) occur at a frequency of greater than 1 change per kilobase. Highly polymorphic regions are usually located between genes, where small variations in sequence do not affect [**gene expression**](https://docs.google.com/document/d/1kEXQA58aEm4w7WMG5FveNqgMmcAQG6QKcA9jz4wohEM/edit#bookmark=id.gjdgxs). The polymorphisms can be identified by the digestion of genomic DNA with a restriction enzyme (endonuclease). Differences in sequences that result in the gain or loss of a restriction site cause variations in the lengths of the fragments produced. Polymorphisms can also result from the insertion or deletion of stretches of DNA between two restriction sites.

To visualize an RFLP, Southern blotting techniques are used to identify fragments of various lengths based on the location of the sites of a particular restriction enzyme. Probes of known sequence that highlight restriction fragments that often vary in length among different individuals are used to generate clearly discernible patterns. A probe specific for a portion of a particular **chromosome** will reveal differences within that region of the genome from one individual to another.

Because restriction polymorphisms should occur near any particular target **gene**, RFLPs can be identified that show tight [linkage](https://docs.google.com/document/d/1kEXQA58aEm4w7WMG5FveNqgMmcAQG6QKcA9jz4wohEM/edit#bookmark=id.gjdgxs) with a mutant (or disease) phenotype. Comparison of restriction maps of patients suffering from a particular disease with those of unaffected individuals can reveal specific bands that are always present or absent in affected individuals.

After the [identification](https://docs.google.com/document/d/1kEXQA58aEm4w7WMG5FveNqgMmcAQG6QKcA9jz4wohEM/edit#bookmark=id.gjdgxs) of an RFLP that is tightly linked with a disease, it may be used as a molecular procedure to detect the disease, either in a prenatal screen or after birth. For instance, an RFLP has been identified that is associated with the genetic disease **sickle cell anemia**. Sickle **cell** anemia is caused by a [**mutation**](https://docs.google.com/document/d/1kEXQA58aEm4w7WMG5FveNqgMmcAQG6QKcA9jz4wohEM/edit#bookmark=id.gjdgxs) in the alpha-globin gene and results in an abnormal form of hemoglobin. Digestion of DNA with the restriction enzyme Hpa1 and Southern analysis using a probe specific for the alpha-globin gene results in the production of a 7-kb fragment in normal individuals. In contrast, patients with sickle cell anemia display a fragment of 13-kb. Carriers of the disease, those who do not have sickle-cell anemia, but who have inherited one copy of the mutant gene from a parent, can also be identified by RFLP analysis. They will produce both the 7- and 13-kb fragments. It is important to note that the change in DNA sequence that causes this RFLP is not the same change that causes sickle cell anemia itself. Instead, it is tightly linked to the alpha-globin gene. Other human genetic diseases that can be detected via RFLP analysis include Huntington's Chorea, **phenylketonuria**, and [**cystic fibrosis**](https://docs.google.com/document/d/1kEXQA58aEm4w7WMG5FveNqgMmcAQG6QKcA9jz4wohEM/edit#bookmark=id.gjdgxs).

RFLP analysis is particularly useful for diagnosis of disease because it assays directly for a genotype (DNA sequence) and does not depend on expression of a gene or even phenotypic expression of the disease itself. Thus, a disease can be identified in an individual before symptoms of the disease are apparent. Additionally, a fetus can be monitored for diseases before birth.

Additionally, RFLPs provide a beginning point for the isolation of the gene responsible for a disease. If the mutation that causes an RFLP in fact lies within the gene responsible, an RFLP at this gene must occur in all cases of the disease. Therefore, it is difficult to prove that a defect in a particular gene is in fact responsible for causing a disease. However, mapping and RFLP analysis can exclude certain genes as candidates. They may also provide a point from which researchers may proceed along the DNA to identify the causal gene itself.

The technique termed DNA fingerprinting utilizes RFLPs and other polymorphic markers to identify individuals based on their particular patterns. Through this technique, hair, blood or other bodily fluids found at a crime scene and blood obtained from a suspect can be used to compare DNA fingerprint patterns. A match between the two patterns provides strong [evidence](https://docs.google.com/document/d/1kEXQA58aEm4w7WMG5FveNqgMmcAQG6QKcA9jz4wohEM/edit#bookmark=id.gjdgxs) against the suspect. RFLP patterns are also used to establish parent-child relationships by comparison of the map of a suitable region of the chromosome between potential parents and the child.

**Source Citation**

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## **Restriction Fragment Length Polymorphism (RFLP)**

*Biology*, 2016

Restriction fragment length polymorphism (RFLP) is a technique used to analyze small differences that exist in [DNA](https://docs.google.com/document/d/108UcaMwFV_Wa7fhq6Tj00WrbDPRd50qOm_MsK0mWDo0/edit#bookmark=id.30j0zll) sequences. These comparative studies can be used to diagnose [genetic disorders](https://docs.google.com/document/d/108UcaMwFV_Wa7fhq6Tj00WrbDPRd50qOm_MsK0mWDo0/edit#bookmark=id.30j0zll), analyze evolutionary patterns, and solve forensic ambiguities and determination of paternity.

RFLP analysis requires the use of a restriction enzyme [\*](#kix.ez9tub1a474t) to make cuts in the DNA sequences being compared. Restriction [enzymes](https://docs.google.com/document/d/108UcaMwFV_Wa7fhq6Tj00WrbDPRd50qOm_MsK0mWDo0/edit#bookmark=id.30j0zll) will make cuts between specific nucleotide sequences. Each time the sequence occurs, the enzyme will make a cut. This means that if the two DNA sequences being compared both contain the specific sequence, the enzyme will make a cut that leaves two fragments for each sequence. If one of the DNA sequences lacks the restriction enzyme sequence for cutting, the enzyme will not make the cut and the fragments will be different sizes.

Once restriction enzymes have made cuts in the DNA sequences, various techniques are employed to analyze the size of the fragments. These include gel [electrophoresis](https://docs.google.com/document/d/108UcaMwFV_Wa7fhq6Tj00WrbDPRd50qOm_MsK0mWDo0/edit#bookmark=id.30j0zll) [\*](#kix.ez9tub1a474t) and hybridization [\*](#kix.ez9tub1a474t) using DNA or [RNA](https://docs.google.com/document/d/108UcaMwFV_Wa7fhq6Tj00WrbDPRd50qOm_MsK0mWDo0/edit#bookmark=id.30j0zll) probes. Gel [electrophoresis](https://docs.google.com/document/d/108UcaMwFV_Wa7fhq6Tj00WrbDPRd50qOm_MsK0mWDo0/edit#bookmark=id.30j0zll) separates the DNA fragments by size using electricity. The two DNA sequences being compared are loaded into two different agarose gel wells. As electricity is run through the DNA samples, the negatively charged DNA moves toward the positive cathode on the gel box. Small DNA fragments diffuse through the gel at a faster rate than the large fragments. These characteristics separate the fragments for analysis.

Once the fragments have been separated, hybridization techniques must be used. Hybridization utilizes radioactive probes, which will bind to the fragments of DNA and allow for analysis of the fragments. The analysis involves comparing the sizes and the distances traveled by the fragments. If the two DNA samples have the same fragments at the same distances, one can deduce that the DNA samples are the same or came from the same organism. If they are different, they are from different organisms, or one of the samples DNA sequences might contain a mutation, which might indicate the presence of a genetic disorder.

\*restriction enzyme enzymes that cut DNA (deoxyribonucleic acid) at a particular sequence

\***gel electrophoresis** technique used to separate DNA based on its size and charge

\***hybridization** technique of binding complementary DNA or RNA strands to DNA sequences

Roxanne Jamroz Argie

*See also* [**DNA**](http://docs.google.com/ic/scic/PageFinderPortletPage/PageFinderPortletWindow?javax.portlet.action=doSearch&action=1&source=RelatedDocument&query=RN+CX3629800125&p=SCIC&u=j043905010) **•** [**Electrophoresis**](http://docs.google.com/ic/scic/PageFinderPortletPage/PageFinderPortletWindow?javax.portlet.action=doSearch&action=1&source=RelatedDocument&query=RN+CX3629800138&p=SCIC&u=j043905010) **•** [**Forensic DNA Analysis**](http://docs.google.com/ic/scic/PageFinderPortletPage/PageFinderPortletWindow?javax.portlet.action=doSearch&action=1&source=RelatedDocument&query=RN+CX3629800173&p=SCIC&u=j043905010) **•** [**RNA**](http://docs.google.com/ic/scic/PageFinderPortletPage/PageFinderPortletWindow?javax.portlet.action=doSearch&action=1&source=RelatedDocument&query=RN+CX3629800377&p=SCIC&u=j043905010) **;** [**Southern and Northern Blotting**](http://docs.google.com/ic/scic/PageFinderPortletPage/PageFinderPortletWindow?javax.portlet.action=doSearch&action=1&source=RelatedDocument&query=RN+CX3629800403&p=SCIC&u=j043905010)

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## **Restriction Fragment Length Polymorphism (RFLP)**

*World of Forensic Science*, 2016

Restriction fragment length polymorphism (RFLP) is a molecular biological technique used to compare [***DNA***](https://docs.google.com/document/d/1OTbkD8eXjSLNnNZ86lhecuYB8p3ikwCoBTiAwxJ8eGs/edit#bookmark=id.gjdgxs) from two samples. Special [enzymes](https://docs.google.com/document/d/1OTbkD8eXjSLNnNZ86lhecuYB8p3ikwCoBTiAwxJ8eGs/edit#bookmark=id.gjdgxs) that cleave the DNA in specific locations are used to digest strands of DNA. Mutations within the DNA result in strands of different lengths. [***Electrophoresis***](https://docs.google.com/document/d/1OTbkD8eXjSLNnNZ86lhecuYB8p3ikwCoBTiAwxJ8eGs/edit#bookmark=id.gjdgxs) is then used to separate the strands according to their length. RFLP is used as part of DNA fingerprinting, to detect genetic diseases and to determine genetic relationships between species.

## **Nucleotides and DNA Mutations**

The DNA molecule is made up of a sequence of four smaller [molecules](https://docs.google.com/document/d/1OTbkD8eXjSLNnNZ86lhecuYB8p3ikwCoBTiAwxJ8eGs/edit#bookmark=id.gjdgxs) called [nucleotides](https://docs.google.com/document/d/1OTbkD8eXjSLNnNZ86lhecuYB8p3ikwCoBTiAwxJ8eGs/edit#bookmark=id.gjdgxs). The four nucleotides are adenine (A), guanine (G), cytosine (C), and thymine (T). The sequence of these nucleotides is extremely important, as it determines the structure of all of the biomolecules, such as proteins and enzymes, in an individual. Differences in individuals result from small variations, called mutations, in the sequence of DNA.

There are a variety of types of mutations in DNA. Insertions are regions of DNA where nucleotides have been added to a sequence. Deletions are regions where nucleotides have been removed. In vertebrates (animals with a backbone), there are regions of DNA that contain many repetitions of the same sequence. Two families of these repeats are found quite often in DNA: variable number of tandem repeats (VNTRs) and short tandem repeats (STR). Point mutations may also occur in DNA. This is simply the replacement of a single nucleotide by a different one.

A special type of protein called a restriction enzyme, or a restriction endonuclease, can recognize specific sequences of nucleotides on DNA and then cleave the DNA at these locations. For example, the restriction enzyme HaeIII recognizes the sequence GCGC and it cleaves the bond between middle cytosine and guanine. Bacteria naturally produce restriction enzymes and they use them to cleave the DNA from foreign organisms. Over 90 different restriction enzymes have been isolated from different species of bacteria. Each of these enzymes cleaves DNA between different, and specific, sequences of nucleotides.

When performing RFLP, the target DNA is usually subjected to ***polymerase chain reaction (PCR),*** which produces millions of copies of strands of DNA identical to the original. This amplified DNA is then combined with a set of restriction enzymes, which cleave the DNA in specific locations. For example, consider the strand of DNA from one individual with the sequence GCGCAAGGCGAATTCGCGC. The restriction enzymes HaeIII and EcoRI are both added to the mixture. As discussed, HaeIII cleaves between C and G on the sequence GCGC.

EcoRI recognizes the sequence GAATTC, and it cleaves the bond between the adenine and the thymine. The resulting strands from this RFLP would be GC, GCAAGGCGAA, TTCGC, and CG. Next, consider a sample of the same region of DNA from a second individual. This individual has a point [mutation](https://docs.google.com/document/d/1OTbkD8eXjSLNnNZ86lhecuYB8p3ikwCoBTiAwxJ8eGs/edit#bookmark=id.gjdgxs) so that their DNA sequence is GCGCAAGGCGAATTCGCCC. After exposure to the same restriction enzymes, the resulting strands of DNA would be GC, GCAAGGCGAA, and TTCGCCC.

After exposure to the restriction enzymes, the fragmented DNA sample is transferred to a gel and electrophoresis is performed. In [gel electrophoresis](https://docs.google.com/document/d/1OTbkD8eXjSLNnNZ86lhecuYB8p3ikwCoBTiAwxJ8eGs/edit#bookmark=id.gjdgxs), an electric current is transmitted through the gel causing the fragments of DNA to migrate through the gel according to their electrophoretic mobility. This distance is roughly proportional to the inverse of the fragment's length. As a result, shorter fragments migrate farther from the origin as they move through the gel.

After the gel is run, the DNA is labeled using a radioactive probe and the gel is exposed to x-ray film, which changes color in the presence of radioactivity. The locations of the fragments of DNA show up on the film as bands. Different samples can be loaded onto the gel in different lanes so that the banding patterns can be compared side-by-side. In the example above, if the digested DNA is loaded into two lanes on the same gel, three bands will appear in both lanes but the pattern will be different. Both lanes will have a band very far from the origin containing the small sequence GC and a band close to the origin containing the sequence GCAAGGCGAA. Both lanes will also have a third band between these two. However, the band from the first individual will be farther from the origin than the band from the second individual, because it is shorter.

In cases where the DNA under consideration contains VNTRs or STRs, restriction enzymes that do not cut within the VNTR or STR sequence are used. The resulting gel has bands closer to the origin that represent fragments with more repeats and bands farther from the origin for fragments that contain few repeats.

## **Applications**

The applications for RFLP are many. DNA fingerprinting uses the presence of STRs at 13 different locations on the [chromosomes](https://docs.google.com/document/d/1OTbkD8eXjSLNnNZ86lhecuYB8p3ikwCoBTiAwxJ8eGs/edit#bookmark=id.gjdgxs). The lengths of these STRs are detected using RFLP analysis. Several genetic diseases are detected using RFLP analysis including [cystic fibrosis](https://docs.google.com/document/d/1OTbkD8eXjSLNnNZ86lhecuYB8p3ikwCoBTiAwxJ8eGs/edit#bookmark=id.gjdgxs), Huntington's chorea, and sickle-cell anemia. In particular, sickle-cell anemia is caused by a single mutation of a single nucleotide: thymine is replaced by adenine.

This mutation occurs at a point in the DNA sequence that is recognized by the restriction enzyme MstII in a person without the disease. The RFLP from a person suffering from sickle-cell anemia will have a long band instead of two shorter ones because the cleavage by MstII will not occur. Finally, mutations in DNA between species are often investigated using RFLP analysis. Species with more different banding patterns are suspected of being less closely related than species with more similar banding patterns.

***See also*** [**DNA**](http://docs.google.com/ic/scic/PageFinderPortletPage/PageFinderPortletWindow?javax.portlet.action=doSearch&action=1&source=RelatedDocument&query=RN+CX3630600178&p=SCIC&u=j043905010) **;** [**Electrophoresis**](http://docs.google.com/ic/scic/PageFinderPortletPage/PageFinderPortletWindow?javax.portlet.action=doSearch&action=1&source=RelatedDocument&query=RN+CX3630600194&p=SCIC&u=j043905010) **;** [**Polymerase Chain Reaction (PCR)**](http://docs.google.com/ic/scic/PageFinderPortletPage/PageFinderPortletWindow?javax.portlet.action=doSearch&action=1&source=RelatedDocument&query=RN+CX3630600416&p=SCIC&u=j043905010) **;** [**Short Tandem Repeat (STR) Analysis**](http://docs.google.com/ic/scic/PageFinderPortletPage/PageFinderPortletWindow?javax.portlet.action=doSearch&action=1&source=RelatedDocument&query=RN+CX3630600474&p=SCIC&u=j043905010) **;** [**Y Chromosome Analysis**](http://docs.google.com/ic/scic/PageFinderPortletPage/PageFinderPortletWindow?javax.portlet.action=doSearch&action=1&source=RelatedDocument&query=RN+CX3630600556&p=SCIC&u=j043905010) **.**

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