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# Comparative Analysis Of Dna Fingerprinting Techniques: Rflp, Pcr And Next-Generation Sequencing

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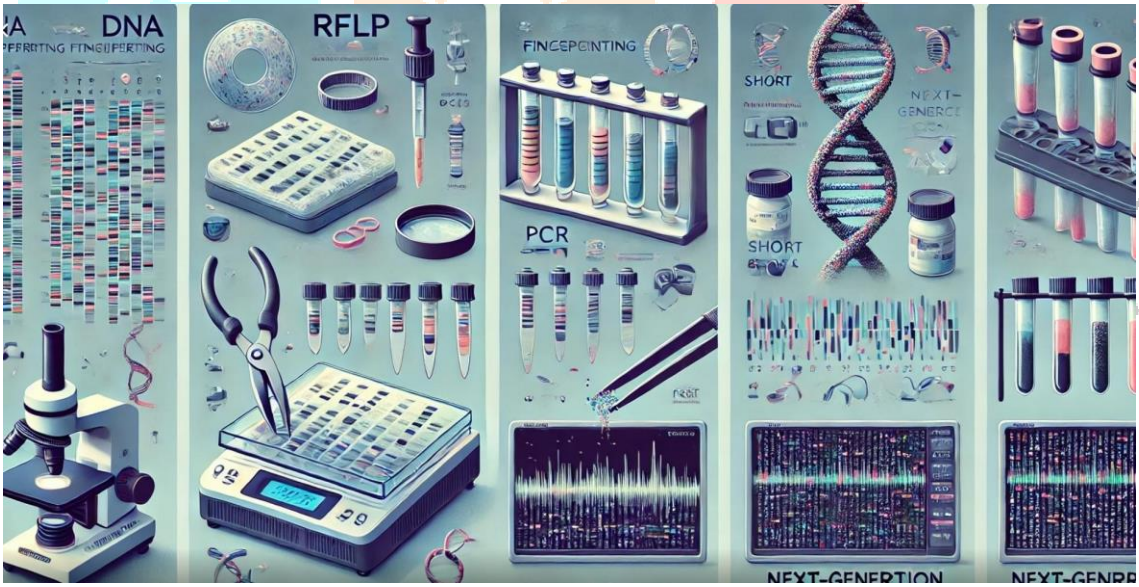


## Introduction-

DNA fingerprinting is a revolutionary molecular technique that is used to identify individuals based on their unique and variant genetic patterns. Through DNA fingerprinting we find the difference between the Satellite DNA regions in the genome. These Satellite DNA regions are stretches of repetitive DNA which are not coded for any specific protein. These are present in abundant form and use for DNA profiling of humans as they depict a very high level of polymorphism and are known to be the basis of DNA fingerprinting. This technique was discovered by Alex Jeffrey in 1984 and has revolutionized forensic science ,paternity testing, medical diagnosis and evolutionary studies since its discovery. Another name for DNA fingerprinting is DNA profiling as it identifies individuals on the basis of their unique genetic makeup present in the specific region of deoxyribonucleic acid DNA particularly the short tandem repeat STR. There are several methods that can be used for DNA brina with the help of restriction fragment length

polymorphism RFLP, polymerase chain reaction PCR based technique, and next generation sequencing. that are most widely used. Through this paper I want to present a comparative analysis of RFLP , NGS , PCR based fingerprinting by analysing their principles, methodologies, application and limitations so that we can identify which technique is best suited in different situations.

**Historical background and evolution of DNA fingerprinting**

The DNA fingerprinting that we work on today was set on the foundation by Sir Alex Jeffrey at the University of Leicester, UK who discovered repeated DNA sequences also known as mini satellites vary significantly between the individuals. This variation was visualised using a technique known as Southern blotting with radiolabeled robes. Through this he demonstrated that every individual has a unique DNA pattern similar to a biological fingerprint. This discovery came into application for the first time in solving up immigration case in 1986 proving a child's biological relationship with his family.

The molecular DNA fingerprinting method that we study today is restriction fragment length polymorphism RFLP, which is also based on Jeffrey discovery. RFLP became a standard for forensic investigation and paternity testing because of its high specificity but it requires a large amount of high quality DNA time consuming and involvement of radioactive materials that limits its application.

The polymerase chain reaction in tension was led by carrymulus in 1983 that lead to revolution in DNA analysis because it allows in the amplification of specific DNA regions even from degraded samples. This allows faster and more sensitive DNA fingerprinting technique that involves - Short Tandem Repeat (STR) Analysis, Random Amplified Polymorphic DNA (RAPD) , AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) .

This method surpasses RFLP by being more sensitive ,easy to use and fast.

In the beginning of 2000 next generation sequencing brought a major change by massive parallel sequencing of the entire genome. This, unlike RFLP and PCR techniques, provided higher resolution by detecting even single nucleotide polymorphism. It also had the ability to analyse large DNA regions which eliminated the need for target amplification and reduced error. Even though next generation sequencing is more efficient and advanced it is more expensive but the advancements are reducing the cost making it the most reliable technique of DNA fingerprint.

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

RFLP is a fingerprinting technique based on the variation in the DNA fragments length which is generated by restriction enzyme digestion. These variations arise due to differences in the DNA sequence such as mutation insertion or deletion in DNA sequences. Due to the unique genetic makeup fragment produced by digestion of DNA by restriction enzymes differ among the individual which makes RFLP a reliable method for forensic analysis, paternity test.

**Steps feel involved in the RFLP process are-**

- Isolation of DNA from biological samples and checking the quantity and quality of extracted DNA using spectrophotometry or gel electrophoresis.
- Cutting of DNA using specific restriction enzymes at palindromic sequences. These cuts produce various lengths of DNA fragments depending upon individuals genetic design.
- After this the digestive DNA fragments are separated on the basis of size by the process of agarose gel electrophoresis under an electric field.

- The DNA fragments are transferred from the agarose gel to a nylon or nitrocellulose membrane through capillary action or electroblotting is done so that DNA fragments remain intact for further analysis.
- Hybridization of the DNA fragments is done with radioactive or fluorescent labelled DNA probes which are complementary to a specific target sequence. Binding of the probe with the DNA fragment results in visualisation of the polymorphic bond.
- Auto radiography is done for visualizing the band if the fluorescent probe is used the imaging system detects the signal.

RFLP are used for the criminal investigation by DNA profiling ,establishing relationship between child and parent identity mutations associated with inherited disorder and analysing genetic variation about population.

**Advantages and limitations**-

RFLP are known to be highly specific and reliable, it can detect single base pair change, have low contamination risks and require no prior sequence knowledge but on the other side it requires a very high quality and amount of DNA, can be very time consuming and expensive and not that effective when the DNA samples are degraded.

**Polymerase Chain Reaction (PCR)-Based Fingerprinting**

The main principle of PCR based fingerprint DNA amplification is done by the repeated cycle of the denaturation annealing and elongation using short DNA primers. These primers are designed to target the specific variable region of the genome known as Short Tandem Repeats (STR), (RAPD), AFLP. These specific regions exhibit high levels of polymorphism which are useful for genetic identification.

**Technique of PCR based fingerprinting** -

- **Short Tandem Repeat STR** - these are repetitive sequences of 2 to 6 base pairs that vary in length in different individuals. The number of repeats at a given location is used for genetic identification. The STR markers are highly polymorphic and the formula of multiple STR loci the power of discrimination of different types of DNA sequence.
- **RANDOM AMPLIFIED POLYMORPHIC DNA** - This technique uses random primers for the qualification of unknown DNA regions. It does not have prior sequence information and is able to detect genetic polymorphism throughout a gene. It is fast and cost efficient that is why it is used in genetic diversity studies .
- **AMPLIFIED FRAGMENT LENGTH POLYMORPHISM**- This technique combines the principle used in RFLP and PCR by starting with the digestion of the DNA with restriction enzyme and then amplifying the resulting fragment with the help of PCR. This provides high resolution fingerprinting of a wide range of genetic polymorphism and is used for genetic mapping, phylogenetic studies.

**Method of PCR based fingerprint-**

- DNA is isolated from the biological sample such as blood saliva tissues etc but it leaves to be in sufficient quantity as well as high quality for the successful procedure of amplification.
- Primer designing is done to target polymorphic regions such as STR loci, RAPD sequence or AFLP markers.
- Dna is mixed with the primer DNA polymerase nucleotides and buffer for the amplification process. The thermocycler is programmed for multiple cycles of denaturation (94-98°C) which result in separation of DNA strands, annealing ( 50-65°C ) where primers bind to the target at sequence, and extension where DNA polymerase synthesis the complementary strands .
- The number of copies of the target region multiplies with each cycle allows amplification of even minute amounts of DNA.
- The amplified products are then analysed by the gel electrophoresis or capillary electrophoresis method that separate the amplified DNA fragment by size. STR analysis technique mostly uses capillary electrophoresis for high resolution and accurate measurements of the fragment sizes.
- Data interpretation is done by the method of STR analysis that compares the sizes of PCR products at each locale with the individual's genetic similarity or dissimilarity. In the RAPD and AFLP techniques the presence or absence of a band indicates genetic variation.

PCR based fingerprinting is used widely for criminal investigation because RAPD and AFLP are essential even when the DNA samples are degraded or present in a limited quantity.
This is also used in establishing biological relationships to identify genetic disorders such as cystic fibrosis, sickle cell anaemia by detecting mutation in specific genes. PCR is also used in marker assisted selection in plant and animal breeding programs.

**Advantages and limitations-**

PCR based fingerprinting are highly sensitive and specific to small or degraded samples. These are very cost effective ,rapid and efficient for large scale studies. They can amplify a variety of polymorphic markers such as STR, RAPD , AFLP and require no prior sequence knowledge.
But mostly PCR are prone to contamination that can lead to false results, also require high quality primer and optimal reaction conditions. The short amplification regions in some cases can reduce the power of discrimination.

**Next-Generation Sequencing (NGS)**

Next generation sequencing is a highly efficient DNA sequencing technology that allows rapid sequencing of the whole genome, transcriptomes and epigenomes with high accuracy . This next generation sequencing sequences millions of DNA fragments simultaneously making it a game changer tool in genomics , medicine and forensic science.

**The principles of NGS involve-**

- DNA fragmentation in this the genome is broken into 100 to 500 base pair small fragments.
- Adapter ligation the short DNA sequences also known as adaptors are attached to the fragments.
- Library Preparation - the fragments with the adaptor are formed into a library that is ready for sequencing.

- Amplification of the DNA fragments are now done using polymerized chain reaction PCR or bridge amplification.
- Millions of DNA fragments are sequence parallely using different platforms this process is called the massively parallel sequencing.
- Data analysis is done using advanced bioinformatics tool to reconstruct the full sequence by aligning the reads.

**Types of next generation sequencing NGS technologies-**

- SEQUENCING BY SYNTHESIS ( SBS) Illumina Technology - This technology uses fluorescent labelled nucleotides that are incorporated one at a time. The incorporated nucleotide signals are recorded. This technology provides high accuracy and his widely used for whole genome sequencing, RNA sequencing and epigenetic studies.
- PYROSEQUENCING- Roche 454 - This sequencing used to detect light signal release during the nucleotide incorporation. This sequencing was faster than illumina but they had not been useful with the long homopolymer stretches . Therefore this sequencing technology is not under use at the moment and has been discontinued.
- ION TORRENT SEQUENCING - Semiconductor based Sequencing - this sequencing is used in detecting pH changes when the nuclear tides are incorporated. It allows faster and cheaper sequencing in comparison to the other next generation sequencing methods but it is seen to have lower accuracy for homopolymer regions.
- SINGLE MOLECULE REAL TIME SEQUENCING ( SMRT) - This sequencing uses real time sequencing of single molecules and provides long read sequencing for up to 100,000 base pairs and is useful for structural variation and complex genome.
- Nano pore sequencing - In this method DNA is passed through a nanopore which changes its electric current to identify the bases. This provides real time portable sequencing useful in the field of research and pathogen detection.

Next generation sequencing has contributed in many fields including the medical genomics with whole genome sequencing that identifies the disease causing mutation and the rare genetic disorders , whole genome sequencing which mainly focuses on protein coding regions useful and cancer and inherited disease research, transcriptome analysis that studies gene expression used in Cancer Research neuro biology and drug development.

NGS contribution in forensic science contributes in analysis of degradable or mixed DNA samples that improves the criminal investigation by determining ancestry ,physical traits and event time of the death. Next generation sequencing plays a major role and identifying pathogen outbreak such as during Covid 19 , ebola. It also contributes in improving crop reading by identifying disease resistance genes that yield enhancement of the crop . Is useful in genetically modifying plants for enhancing their nutritional value.

**Advantages and limitations-**

Next generation sequences are known to provide high output sequencing of millions of DNA fragments simultaneously, it can sequence an entire genome in a specific region with accurate precision. One major advantage is that it can work well with the degraded or low quality DNA which is useful in forensic and ancient data research.

This much advancement is still rather expensive and requires large computational resources. The knowledge

of complex bioinformatics tool for data analysis is also compulsory.

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