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KINDS OF DNA PROFILING ANALYSIS USED IN CRIMINAL INVESTIGATION AND ITS EFFECTS

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Abstract

Human remains may be traced via DNA profiling, as can unresolved crimes from the ancient history. It may be able to determine a corpse using parental Dna if standard approaches (including such physical appearances and dermatoglyphic prints) fail. If the Dna samples are different, the possibility of a relationship is ruled out. Dental data is more resistant to post deterioration and severe environmental factors than other parts of the body. Teeth are also transportable and a significant store of DNA, making them an important genetic resource. It has long been shown but even in a mass burial, matching premortem dental data with skeletal remains may identify individuals. Affluent cultures can rely heavily on dental records to track down individual victims. Human rights breaches associated to mass killing are more frequent in the less wealthy areas, where hospital records are less likely to be available. You might have to rely on DNA testing to verify this person.

Key Words:-DNA Profiling, Measurement, Analysis, Evidence, Techniques, Scientific, Collection of Data, Antemortem.

Introduction

Each person's DNA fingerprinting profile is unique. When a sufficient number of DNA patterns in two unrelated individuals are analysed, the chances of full resemblance range from one in 30 billion to 300 billion, or roughly half the world's population. Several instances, including paternity disputes, have been settled in India using DNA fingerprinting. DNA profiles were used to identify Dhanu and Sivarasan, the killers of the late Prime Minister Mr Rajiv Gandhi. 132 The Supreme Court upheld the Delhi High Court's decision that ND Tiwari, a former Union Minister and former Governor of Andhra Pradesh, undergo a paternity test, but

stated that the results would not be made public until they were required. In some circumstances, DNA fingerprinting is useful.

Hybridisation processing is simply to bring in contact the labelled probes with the DNA fragments on the Nylon Membrane (Southern Blotting). The complementary base pairing takes place and the fragment become radioactive. The Nylon Membrane, after removing the excess of probe, is brought in contact with an X-ray film, the same is affected at the radioactive sites. The hybridised RFLS give visible bands on the X-ray plate on development. Comparison of the bands from the questioned DNA sample with the DNA profile from the suspected sources reveals the identity or non-identity of the given questioned source.

DNA profiling has acquired tremendous importance in criminal and civil investigations. In spite of the fact that the tools and techniques are comparatively new and they are revolutionary in their concepts, they have been accepted, by and large, by all who are concerned with the identifications including courts in India. In fact the courts insist upon the DNA evidence where it has not been produced and yet it is required.Over the years the following analytical techniques have emerged:

I. **RFLP Analysis**
II. **PCR Analysis**
III. **Y-Chromosome Analysis**
IV. **Amp FLPS**
V. **Mitochondrial DNA**

I. **The RFLPS**,

1. which form the basis of identification:

(ii) Are stable throughout the lifetime of a person, like the ridge patterns of a fingerprint.

(iii) Have true genetic character.

(iv) Are inherited.

2. There is now adequate data on the frequencies of various RFLPS based upon the statistical studies, with adequate number, on various groups of people.
3. A nomenclature for RFLPS based on molecular weights has come up for all practical purposes. In fact the separation as well as identification of RFLPS is based mainly on weight.

4. Tools and techniques are available in Forensic Science Laboratories and other institutions carrying out DNA profiling. The work can be checked and cross-checked at more than one place.
5. Detailed test procedures have been laid down. Appropriate controls and standards are specified. The quantum of proof: number, quality and positional variation limits of bands are also specified.
6. Quality assurance is being practiced in laboratories where DNA profiling is done for court cases.
7. Sensitivities of the test procedures have increased tremendously. The amount of DNA required was 1 to 10 microgram. This quantity of DNA needed now stands reduced to nanogrm and picograms. The replication of the DNA fragments through PCR technique has made it possible. It has ultimately overcome the deficiency of sample's sufficiency.

The stability of DNA molecule and its fragments (RFLPs) has been established on proper footing in recent times. In fact it has been found that they are more stable than the protein and antigen genetic markers, which have been used, so far, in blood and bloodstain evaluations.

DNA profiling, through RFLP analysis, involves the following steps:

1. Isolation of high molecular weight DNA from biological material.
2. Fragmentation of DNA molecule through Restriction Enzymes (RE).
3. Separation of the Restriction Fragment Lengths (RFLs) through electrophoresis.
4. Transference of RFLS to Nylon Membrane (Southern Blotting).
5. Preparation of radioactive probes.

i. Hybridisation of the RFLS through radioactive probes.
ii. Visualisation of RFLS bands on X-ray film through autoradiography and its development to give visible patterns of the Restriction Fragment Length Polymorphs (RFLPs).

RFLP Analysis was the first process used to identify human beings. The technique was laborious, time consuming and costly. It also needed more quantity of samples which was not available in some cases. It has been more or less abandoned now.

These advantages have made the RFLPS techniques redundant.

The polymerase chain reaction (PCR) process involves the following steps:

1. DNA is incubated at a suitable high temperature (& 95°C). The double strand of DNA changes into single strand. The strand acts as template for replication.

2. The temperature is lowered. Oligonucleotides, re-application primers are added. They get attached (bound) to their complementary sequences on the template DNA strands in specific way.
3. Nucleotide primers added stepwise, extend the primers strands and new DNA molecules are formed in the presence of DNA polymerase.
4. The thermostable polymerase TAQ is used for the purpose. It is heat stable. The DNA molecules have double strands. The molecules so formed acts as templates for subsequent replication cycles and the formation of new molecules increases exponentially.
5. In automated reaction chain, the steps and the number of cycles are controlled by thermocycler. The extension steps are now carried out with greater speed at higher temperatures (up to 75° C) by the new generation of thermostable polymerase.

An enzyme is used to make many copies of a small section of the DNA. This section cut into pieces by another enzyme, and separated by electrophoresis. The fragments are then visuallised with a silver stain, with the pattern of light and dark bands seen being characteristic for an individual

II. PCR Analysis

Polymerase chain reaction was discovered in the 1980s itself. It was found that desired segments of the DNA molecule could be multiplied almost infinitely. Thus small amount of the DNA sample could be increased manifolds and analysed conveniently.

The STR is a small section of the DNA. This section is cut by an enzyme, and separated by electrophoresis. The fragments are then visuallised with a silver stain, with the patterns of light and dark bands seen being characteristic for an individual.

In this process the desired strand is denatured and treated with primers and enzymes and then hybridised to get copies of the strands. The process is repeated time and again to get the desired quantity of the STR. The desired fragments increase exponentially. The operations are now performed through instruments and replications are automatic. The process is so efficient that adequate quantity of the fragments could be obtained in about two hours.

The DNA profiling is now done through PCR based STRS (4 base) repeat sequences analysis of the high polymorphic region. 3 and 5 base repeats are also utilised. They are treated

with sequence specific primers and amplified. The fragments are then separated through electrophoresis. There are two electrophoretic techniques: capillary and gel phoresis. The former is more popular.

The STRS used are shared by other individuals also. Therefore, a number of STRS are located and compared simultaneously. The combined probabilities of the STRS give individuality of the DNA profile. USA uses 13 STRS for identity in its CODIS database; while UK uses 11 STRS in its NDNAD for individuality. The calculated probability for wrong match is given as 1 in 10^{18}. There are systems where more STRS are also used.

The PCR technique has revolutionised the DNA analysis. It can be used in both VNTR and STR techniques. It reduced the processing time and the quantity of the clue material required for the process. Even the degraded DNA or mixed samples, such as in gang rape, can be analysed. More recently quantitative PCR estimation, through quantity based varying fluorescence intensities, has improved the PCR result interpretation.

PCR is virtually a cloning process in which DNA or its fragments can be replicated any number of time - even a million or more times. The advent of PCR has proved a boon for personal identification in forensic science. Equipment is now commercially available for automatic polymerase chain reactions.

PCR offers the modern approach for DNA analysis. It has assumed tremendous importance in recent times and it is being used by most of the institutions carrying out DNA profiling work. The approach offers a number of advantages:

1. Very small amounts of evidentiary clues, considered insufficient previously, can be analysed for DNA. For example, a single hair, saliva on cigarette stubs or fine spray drop of blood can be used to provide DNA profile of the individual. The possibility of DNA profiling with smaller amounts is a distinct advantage over RFLP technique.
2. The PCR amplification can be carried out in multiplex mode. In other words a number of PCR amplifications can be carried out simultaneously.
3. Degraded DNA is amenable to PCR amplification. This extends the application of DNA analysis to many cases in which the traditional method using VNTR would fail.

4. PCR increases the amount of relevant DNA fragments. The increased amount increases the speed and ensures better quality results of analysis.
5. The minute quantities required for PCR permit division of the original clue material for independent testing by the defence, or at more than one place.
6. Related tests can be performed with the increased amount made possible by PCR.

Controlling Factors

The general representative factors, which control the quality, quantity and the speed of the replication of DNA or parts thereof, are:

1 The quality and quantity of the following materials:

- DNA material
- Oligonucleotide Primers
- Polymerase

1. Ingredients of the reaction mix for the range 1 - 5000 copies are:

1. Primers	0.05 - 0.5 mM
2. TAQ	1 - 4 units/100 ml.
3. Buffer	5 - 8.4 pH
4.Protein BEA/Gelatin	0 - 50 ml/ml
5. KCL	50 mM
6. Mg	1 - 6 mg
7. d NTP	50 - 200 uM.

2. The Reaction Condition

- The periodicity of the thermocycler.
- The frequency of the addition of the reaction mix.
- The number of cycles.

In principle each cycle double the amount. Thus, the number of copies increases rapidly.

PCR Replication

No. of cycles	No. of Copies
10	1024
20	10^6
30	10^8 (i.e., up to maximum)

1. The presence, absence, addition or extraction of promoters or inhibitors.
2. When the PCR product reaches about 10^{10}, the replication reaches the limit.

   The main aim of the process is to achieve specificity of the reproduction of the DNA or of the target sequence(s). The following steps achieve this:

1. Very high purity of the starting material is required because extremely small samples of DNA can be used as evidence, therefore, greater attention to contamination issues is necessary when identifying, collecting, and preserving DNA evidence.
2. In a case of forced oral sex a single hair was found sticking in the mouth of the victim. Mitochondrial DNA analysis provided very strong evidence to get the culprit convicted.

   The following precautions are therefore, essential:

- Wear gloves. Change them often.
- Use disposable instruments or clean them thoroughly before and after handling each sample.
- Avoid touching the area where you believe DNA may exist.
- Avoid talking, sneezing, and coughing over evidence.
- Avoid touching your face, nose, and mouth when collecting and packaging evidence.
- Air-dry evidence thoroughly before packaging.
- Put evidence into new paper bags or envelopes, not into plastic bags.
- Do not use staples.
- Strict control of the reaction conditions.
- Complete control of the quality and quantity of the oligonucleotide primers.
- Length of the target sequence: shorter the target sequence, better the specificity.
- The primers length is dependent upon the STRS.
- Annealing temperature 60 - 65°C.

- The number of cycles depends upon the number of copies of the material to be replicated. Less than fifty copies will hardly produce detectable material in 30 cycles.
- The minimal amount of the clue material which should be available for amplification is 0.1 ng amplifiable DNA. This amount contains about 40 total templates. This is the smallest amount recommended for case work.

Identification Techniques

In PCR amplification three types of techniques have emerged for the identification of individuals.

1. DNA Sequencing.
2. Sequence Variations.
3. Length Variations.

1. DNA Sequencing:

DNA sequencing has become extremely important, in fact the most important tool for:

i) Individualisation of human beings,
ii) Understanding hereditary diseases and other problems,
iii) Clinical treatment and diagnosis of refractory diseases, and
iv) Most importantly, for the evaluation of the genetic code.

Mind boggling progress has been made in all aspects of DNA technology in recent times and the progress is continuing at an astounding rate. The major developments are coming in:

i) Initial processing of DNA evidence.
ii) Electrophoretic materials (gels) and experimental condition.
iii) Automation in separation of RFLS, determination of their molecular weight, use of fluorescent labels for their detection and evaluation.

Today automatic processes are doing DNA sequencing. Thousands of nucleotides can be sequenced in a day. The human error is also reduced to the minimum. However, considerable emphasis is being given on reproducible and reliable results. It is ensured by repeating the analysis, especially in criminal cases where the lives or the liberties of the persons are concerned. Multiple sampling is also being adopted in important cases.

2. Sequence Variations:

Sequence variations are studied through Sequence Specific Oligonucleotides (SSo) which are of 15 to 30 Oligonucleotides corresponding to the target allele. The labelled probe is mixed with the PCR products on a dot-blot format. It will hybridise with the corresponding allele, not with others. A number of probes of different types are used in the same way. In one system the PCR products is fixed on a membrane in a dot-blot form. In another way the probes are fixed on the membrane in the form of blot and labelled PCR products are brought in contact. Corresponding allele get attached. The attached positions are identified through the labels in both the techniques. The former is known as dot-blot technique and the latter as reverse dot-blot technique. The latter technique has proved its worth and is being used extensively.

In a rape case there were two suspects. The samples of the two suspects, of the victim (blood, epithelial cells and swab sperms) and blank were collected and analysed by reverse dot-blot technique. The test patterns were different for the two suspects and the victim identified the first suspect and eliminated the second. The sperm DNA from the vagina corresponded only with DNA of the first suspect.

Other methods of DNA profiling through PCR amplification are:

- Direct sequence - A promising method.
- Allele specific amplification - more useful in medicines.
- Oligonucleotide - Ligation Assay.
- Variations at restriction site - a useful technique.
- Denaturing gradients Gel Electrophoresis.

These methods are not extensively used in forensic work yet. The details of the methods are beyond the scope of the book.

3. Length Variation:

The restriction fragment length polymorphism RFLP analysis of loci containing variable numbers of tandem repeats (VNTR) has been extensively used in identifying persons. Corresponding technique in PCR is PCR-VNTR and PCR_STR techniques. In the former the VNTR used are sufficiently large (15-70 bp.) In the latter, the STR are short, usually of 4 bases pairs and also of 3 or 5 base pairs. They allow separation of allele product by simple gel or capillary electrophoresis.

The PCR-VNTR technique uses automated DNA sequence coupled to fluorescence detection system. The fluorescent labels are introduced into the PCR primers. More than one fluorescent label can be used simultaneously to detect.

Problems in PCR:-

The main problems in PCR amplification are:

2. Amplification inhibition

- It is due to problems with the starting DNA material. It is overcome by:
- Reducing templates.
- Increasing polymerase.
- Changing reaction condition.

3. Mis-incorporation

The oligonucleotides get attached at wrong places. It has been estimated that this happens 1 or 2 times in 10^4 incorporations. The mis-incorporations are consequently negligible, unless, the starting material is too small.

4. Reliability of PCR product. Replication process is not perfect because:

- Polymerase is not perfect. Mis-incorporations do occur.
- Sequence insertion or deletion may take place due to alignment errors n the template.
- Tandem repeat sequences can cause misalignment on the template. Thus PCR products of different sizes are formed.
- When the sample is small the number of template is small. Here in some cases some alleles amplify preferentially. The other alleles are masked and not seen.

5. **Other problems**

There some other problems which need attention to get the correct results are:

1. The sample has to be pure, contamination free.
2. The sample should be authentic.
3. DNA sample should not be degraded.
4. The Analyst should be aware of existence of monozygotes (there are about 12 million of them in the world!), Chimeras, synthetic DNA, etc.
5. New workers may be over zealous and give results on insufficient data.

6. Some pseudo scientists follow confirmation syndrome. They give wrong findings at times.
7. The system may fail at time. Periodic check (calibration) of the system is essential.

III. Y-Chromosome Analysis

The research has been extended to male specific chromosomes. Primes have comeup which target Y-chromosomes and can thus separate Y-STRs. They are inherited paternally and thus can link an individual to his paternal male relatives.Y-chromosome analysis is also used to identify the sex of the biological samples. It is based upon the fact that the male alone have Y-chromosomes in addition to X-chromosomes. The females have X-chromosomes only. Primers have been discovered which target only polymorphic region of Y-chromosomes which are in males. A number of X and Y chromosome specific probes have been identified. They are used for discrimination.

The DNA analysis thus permits direct identification of the male source of the clue material. The female source the clue material has is inferred as there is no directly identifying allele in the female DNA.

VI. Amp FLPS

In Amplified Fragment Length Polymorphism (Amp FLPS) technique Variable Number Tandem Repeat polymorphism is utilized to identify different alleles. Separated on a polyacrimide gel, the alleles are visualised by silver spray. The technique has not been used extensively.

V. Mitochondrial DNA

Mitochondria are body gene contained in eukaryotic cells, responsible for cellular respiration and for the generation of Adenosine triphosphate (ATP) - an important body constituent for the transfer of energy in living cells. Mitochondria genes give mitochondrial DNA (mt DNA). It does not follow the Mendelian inheritance law. The mitochondrial genes are inherited from mother only. The father is not involved in their inheritance. Hence, mitochondrial genes cannot be used to determine the fatherhood of a person or child. They, however, link mother and other maternal relatives positively.

The basic composition of mitochondrial DNA and its shape vary from nucleic DNA. The functions also vary.

Human mitochondria contain 2 to 10 units of double stranded circular DNA molecule. It has 16.5 kilobase length and molecular weight is of the order of 10^7.The mitochondrial genome is highly variable. A segment, known as D-loop, provides high discrimination among non-related individuals on analysis with restricted enzymes. It has also been found that maternally related individuals show abundant correspondence in D-loop sequences. The D-loop segment is amendable to polymerase chain reaction and, thus, minute quantities of the mtdna can provide mitochondrial DNA profiling for determining maternal interrelationship or identity.

Mt DNA profiling is coming up as an important tool to establish the identity or non-identity of an individual from hair (shaft), teeth and bones even when they are old. Mt DNA has thus widened the identification field tremendously.

The most common forms of evidentiary clues available for testing and the quantity required thereof are given in the table below:

A. Caution in collection of things use in DNA analysis

The advancement in the DNA fingerprinting technology has made the forensic examiner very potential to individualise various body fluids, tissues and to determine the paternity. As a result, it is demanding that the quality of the analytical data be more closely monitored. There are cases on record in the United States, where mistakes seem to have been prompted by human errors. The Technical Working Group on DNA Analysis Methods (TWGDAM) hosted by the FBI Laboratory at the FBI Academy provided guidelines for a "Quality Assurance Programme for DNA Restriction Fragment Length Polymorphism Analysis". The quality assurance guidelines were supplemented by the publication of the "Guidelines for a Proficiency Testing program for DNA Restriction Fragment Length Polymorphism Analysis". Protocols for standardisation and proficiency testing are in place in the United States as well as in Europe. Other countries also have similar approaches for the purpose of quality assurance in DNA typing. It is also needed to interpret DNA typing with a thorough understanding of the populations involved.

B. DNA Process

Conduct of investigation in respect of DNA profiling, necessitates the investigator to ensure detail description, compliance with legal formalities maintenance of relevancy, authenticity, integrity and continuity, in the course of collection of samples/evidentiary clues, as follows:

1. Blood - Blood in the form of washed blood, blood clot, dry and wet blood stains, blood powder, liquid blood, blood crust, wet blood and blood spatter, on all absorbent as well as non-absorbent surfaces contains cells. In case of liquid blood:

- a vacuum container with anti-coagulant should be filled with 2.5 ml of the blood, the two should be properly mixed by shaking, another similar container should be prepared for reserve sample, the specimen should be packed in airtight manner until it arrives at the DNA laboratory
- transportation of the sample should be made in vacuum flask containing ice, without any delay
- thorough drying of wet blood and bloodstains is necessary before packing.

2. Semen - Collection of semen specimen needs:

- complete drying of the stain, prior to packing, as prevention for putrefaction of the semen
- preservation of liquid semen by addition of preservative (toluene or formaline) and mentioning of names of the preservatives, or the preservation at temperature of less than five degree centigrade
- collection of semen stain from the person of female victim and her vaginal swabs, with the assistance of female doctor.

3. Spots of semen in scene of crime - At the scene of crime, traces of semen is found on:

- person of the victim
- sexual organs, hands, fingers/crevices of finger nails, buttocks, pubic hairs, thighs of the victim, vagina or anus (in sodomy), or mouth (in oral sex)
- garments/undergarments of victim(s)
- person of offenders, who has not taken any wash or bath
- undergarments, trousers and handkerchief of the criminal, sofa set, bedding, other upholstery, floor or ground, walls, wastepaper basket, chairs and dustbins
- seats, floors, doors and upholstery of a vehicle, in which crime has been committed.
- Liquid semen should be collected in a refrigerated thermos bottle with a clean dropper, or collection of the liquid semen on a folded clean bandage cloth.

4. Collection of specimen semen - The investigator should seek the assistance of a doctor, in respect of collection of the semen in a sterile tube and is to be processed in the manner of the liquid semen.
5. Skin and flesh - Evidentiary clues in the form of skin and flesh are found in instances of:

- severed bodies
- dragging of victim
- torn skin on rope used for hanging of victim
- scratched or bitten off skin and flesh by victim
- some flesh and skin attached to skeletal remains
- foeticide.

6. Hair - In the course of collection of hair, the investigator should:

- look for pubic hair or hair on head of the victim combed with hair of offender, which may be entangled on the hairs of the victim, by placing a paper towel below the private parts or head of the victim, and folding the towel before packing
- pick up hairs from other spots of crime with the use of pair of forceps, in a paper fold and then packing and sealing of the evidence
- take precautions for preservation of the roots of the hair
- undertake drying of hair smeared with body fluids or attached with flesh or skin or is wet
- preserve hair in a refrigerator, containing body materials, until its despatch to a DNA laboratory
- make the collection in clean containers
- put hairs from different sources each in separate containers
- despatch the hairs collected to the DNA laboratory without loss of time.

7. Teeth and its pulp - First attempt for molar, premolar and then other teeth, which can be packed and sealed in a clean envelope, as no preservation is required for them.
8. Bones - Femure, humerus or ribs as bones are sent for DNA profiling.
9. Saliva - Resort to the process of collection of evidence of blood or semen.

Dr. Wilson J. Wall's testimony was rejected for the following reasons: -

- You are an independent consultant.
- Asked to review DrLalji Singh's testimony.

- He held conferences with a defense attorney in London and India.
- He was present in Court when DrLalji Singh was questioned by the accused's lawyer.

C. Problem Regarding DNA evidences in investigation

Forensic DNA evidence presented in criminal cases is anticipated to also be scrutinised more closely by defence lawyers after the OJ Simpson case. As a result, the defence in Simpson's case effectively put the crime laboratory on trial. Even though DNA profiling's core scientific principles aren't in doubt, there ought to be some room for debate over whether the lab processes and the experts who witness are adequate.

The scientific community believes that DNA profiling has a high probability of producing findings, but there are still issues with the lengthy process of Genetic profiling. The DNA-probe may be mismanaged at any point in the seven-step method just described, which can lead to a life term or even the death penalty in some situations. As a result, the legitimacy of laboratory processes and also the competency of the experts that testify should remain subject to dispute.

It's not uncommon for technological advances to fail because of human mistake in the lab, just like any latest tech. There have been some major flaws in DNA labs that could have led in wrongful acquittals or convictions, according to initial quality-control surveys. To identify a sample, it must be loaded in both lanes. Another problem that emerges is that, in the event of a failed first test, there is usually insufficient DNA to do a two tests. Experts may find this tough to cope with. When molecular biology enters the court, the stakes are hugely increased. As a result, it's essential to understand the risks of DNA profiling by briefly outlining the mistakes that might go awry.

In population genetics, there really are two types of errors that may occur: technical ones which can be corrected, and genetic variation ones that can be caused by incorrect computations and erroneous selection method on those calculations.

Conclusion

DNA analysis for forensic reasons is done out all over the globe. As a consequence, rising nations, such as Malaysia, should establish and assemble a national DNA database that includes a "crime scene DNA profile index," a "convicted criminal DNA profile index," as well as a database of DNA profiles of unexplained corpses and body parts. This project will compel

necessary changes to criminal legislation to aid law enforcement officials in identifying persons accused of committing significant and violent crimes, as well as empowering the collection of DNA samples for a DNA profile database. To present, information for nine STRs has been published for three ethnic malays (Malay, Chinese, and Indians) and efforts are currently underway to profile subpopulations of Malays and to begin utilising the newly validated STR profiling kit in other Malaysian groups. Criminal.

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