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DNA Finger Printing and its Medico Legal Importance

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Abstract

The use of DNA finger printing in solving crime is proving to be as revolutionary as the introduction of fingerprint evidence in court more than a century ago. It has emerged as one of the most powerful tools available for solving many medical as well as legal complexities.

The DNA molecule is very stable and can withstand significant environmental challenge which enables forensic scientists to obtain new information from very old biological evidence or establish important data from badly degraded samples. The stability of molecule combined with the discrimination features of each individual's DNA and the accuracy of current DNA analysis techniques, makes this technology a vital component of a number of medico-legal investigations.

Key words: De-oxyribose nucleic Acid, DNA fingerprinting, DNA Probe, Polymerase Chain reaction

Introduction

DNA organised into genes, the fundamental units of genetic information. It is a linear polymer of four different monomeric units, collectively called as deoxyribouncleotides or simply nucleotides. A typical DNA molecule consists of two interwound polynucleotide chains, each containing many thousand to several million base pairs. Each nucleotide in one chain is specially linked by hydrogen bonds to a nucleotide in the other chain. Only two types of nucleotide pairings are found in DNA. Adenine with thymidine with a double bond (A=T) and guanine with cytosine using tripple bond (G _= C). Thus the sequence of nucleotides of one chain fixes the sequence of the other and the two chains are therefore said to be complementary to each other.

The sequence of these four nucleotides along a chain vary among the DNA's of unrelated organisms and indeed is the molecular basis of their genetic diversities. There are about 3x109 base pairs in each human haploid genome. If an average gene length is 3x103 base pairs, the genome could consist of 106 genes, assuming that there is no overlap. But it is thought that there

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are only 105 genes in the human body and only 10% of the DNA codes for proteins. The function of remaining 90% of DNA was unknown, so it was called as junk DNA. Every strand of DNA has pieces that contain genetic information (exons) and pieces that, apparently supply no genetic information at all (introns). Although introns may seem useless, it has been found that they contain repeated sequence of base pairs. These sequence repeat many times tandemly and are called tandem repeats. Depending upon the variable number of base pairs, these are called variable number tandem repeats (VNTR). A given person's VNTR came from the genetic information donated by his or her parents, he could have VNTRs inherited from his father or mother or a combination of both.

DNA fingerprinting

DNA fingerprinting or DNA profiling is a technique, in which virtually unique sequence of basis in the DNA strands of chromosomes are used to compare one biological sample with another to investigate genetic relationship. Using these sequences of base pairs, every person could be identified solely by the sequence of their base pairs. However because there are so many millions of base pairs the task would be very time consuming, instead, scientists are able to use a shorter method, because of repeating patterns in DNA.

These patterns do not, however, give an individual finger print, but they are able to determine whether two DNA samples are from the same person, related people or non-related people. Only the uniovular twins may have the same DNA profile.

This technique was first devised by Sir Alec Jeffreys of University of Leicester in 1984 in an attempt to identify genetic markers for disease. At first, DNA profiling was used to establish genetic relationships in paternity and immigration cases. Colin Pitchfork was the first criminal to be convicted on the basis of DNA evidence. In India, DNA profiling is mainly done at CDFD, Hydrabad (Centre for DNA Fingerprinting and Diagnosistics), an autonomous centre of the department of Biotechnology, Ministry of Science and Technology, Govt. of India.

Collection and storage of samples (biological material) needed for DNA printing

The primary requisite for any DNA based forensic analysis is proper handling of the evidence materials. Carelessness or any sort of ignorance in handling procedure during collection, transport and storage can lead to a specimen, which is unfit for analysis or yield false results, secondly the quantity of evidence material collected for forensic work is very less, so proper handling, collection and storage is very essential.

A variety of biological evidences are encountered in routine forensic cases like blood, blood stains, saliva, hairs, semen, vaginal fluid, preserved or un-preserved post-mortem body tissues. Following are the certain guidelines to be considered while the collection and storage of Forensic biological material for successful DNA based analysis.

1. Blood: As red cells have no DNA, sufficient blood must be obtained to extract DNA from much sparse leucocytes. At least 1 ml and preferably 5 ml are taken in an EDTA tube, which not only prevents clotting but inhibits enzymes in blood which may break DNA during storage. Heparin may also be used instead of EDTA but there are reports suggesting its interference with the authority of certain restriction enzymes.

The blood should be stored at – 200C, if long time storage is required, otherwise for few days, it can be kept at 40C.

There are certain guidelines issued specifically by CDFD, Hyderabad for collection and storage of blood samples. 2-5 ml of blood of the concerned person should be collected in the presence of Magistrate and should be sent in a tube containing EDTA or Heparin in a sealed thermos flask containing ice so as to reach CDFD within 72 hours after collection. The blood samples of the nearest relations of the diseased (e.g. mother, father, children) if available should also be sent along with for comparison. The person whose blood is sent must not have received any blood transfusion in last 3 months.

2. Blood stains: Blood stains should ideally be sent intact or surfaces, kept as cool as possible before and during transit to the laboratory.

Dried blood stains from the hard surfaces can be scraped off with a scalpel or if the specimen is too small to scrape off use a sterile gauge pad moistened with same solution, air dry and place in a sterile test tube. The stain should be kept in a dry polythene bag and sent. This method is used for other stains also e.g. seminal stains.

3. Seminal and vaginal fluids: Swabs from vagina, rectum, mouth etc should be air dried as quickly as possible, but not heated. They should be stored in deep freezer, unless send straight to the laboratory. Liquid semen found in the vagina or elsewhere should be recovered with a fine pipette, placed in a sterile plain tube and frozen solid.

Place two cotton tipped swabs in the vagina and leave in place for 5 minutes. Remove them, make two slides from swabs (if some other examination is also required), air dry and send the swabs for DNA finger printing in a sealed clean dry glass vial/bottle. Polythene bags should not be used in this case as it may encourage mould formation.

Mouth swabs can also be taken either to seek semen in suspected oral intercourse or to obtain buccal mucosa for DNA analysis. In the later case, as the yield is small, so at least 3-4 swabs must be rubbed hard against the inside of cheek or mouth lining scraped with an instrument and then smeared on to a swab. All such swabs should be frozen in the usual manner.

Any other material like cloth/some other object having seminal stain should also be sent along with for DNA analysis.

4. Bite marks/saliva: If an odontologist is available, he should conduct the examination of the bite mark prior to cleaning. If none is available, swab the bite mark with a sterile gauge pad

moistened with saline followed by dry cotton swab, air dry and place both in sterile tubes. This is done to collect saliva. The material then can be analyzed with STR DNA analysis.

Otherwise saliva is collected in a test tube, diluted with an equal volume of normal saline and placed in boiling water for 10 minutes (for destroying enzymes, which inactivate blood group substances), allowed to cool and transferred to a clean, sterile and well stoppered bottle (properly sealed and labeled).

5. Hairs: DNA profiting can only be performed from the hairs having nucleated root or follicle cells. The keratinized shaft contains no DNA extractable material. So it is essential to have plucked hairs, from the living or dead, as the naturally shed hairs from combs or hairs brushes may have very little or no root material. At least 10-20 hairs are required (RFLP method).

Control hairs should also be taken from the body of deceased to rule out the possibility that the hairs found on the body came from the deceased. In case of head hairs, the sample should ideally be taken from different areas.

A properly labeled (mentioning the area from where the hairs are taken) sealed, dry polythene bag should be used for sending the hairs for DNA analysis. It should also be freezed, if it has to be stored for a long time.

6. Autopsy tissue: Ideally the muscle tissue close to the bone should be dissected and about 100 gm of tissue should be sent in a clean glass bottle without any preservative on ice. As far as the organs are concerned, spleen is said to be one of the best organs for DNA recovery although liver, kidney, brain may also be used. The fresher the tissue, better it is for DNA analysis but with new techniques available (PCR), DNA can be obtained from the putrefied tissue also. Tissue samples, if possible should be collected in sufficient amounts and from more than one organ. For storage of tissues, they should be wrapped in aluminium foil, placed in clean plastic bag and stored in lysis buffer and then refrigerated. Samples collected so will be less prone to degradation but it will be useful only for DNA analysis.

7. Miscellaneous: Under the guidelines issued by CDFD, Hyderabad, teeth, bones, dry skin may also be sent for DNA analysis in a properly sealed and labeled dry polythene bag.

Plant materials should be sent after wrapping in a clean sterile material and then putting in a polythene cover. Any other material like pistol, clothes, glass, bidi, cigarette having some biological stain may also be sent for DNA analysis.

Precautions to be taken while collecting and sending the biological materials for DNA analysis
All the samples should be clearly labeled, sealed as to source, tissue type, storage conditions and date of collection.

Labels should ideally be placed inside the storage containers, if possible, outside the containers, labels should be covered with transparent adhesive tape.

A separate record should be kept, mentioning possible contamination with other DNA sources, cause and time of death, case number, storage temperature and other relevant information. Specimen of seal, requisition letter should be sent along with the specimens.

The proforma prescribed by CDFD, Hyderabad should be filled properly and sent along with the specimens.

Isolation of DNA from biological materials

Isolation of good quality DNA is a primary step in DNA finger printing. Although separate techniques are available for the separation of DNA from blood, blood stains and other tissues, but the basic principle behind all these techniques is as follows:-

Lysis of cell membrane by treatment with lysis buffer.

Incubation with proteinase K to breakdown nucleoproteins.

Removal of protein from solution by extraction with phenol/chloroform.

Discard the protein part.

Precipitation of DNA with chilled ethanol.

The DNA obtained is washed with 70% ethanol and after drying it is dissolved either in distilled water and Tris-EDTA buffer and stored at – 200C till further analysis.

Techniques for isolating DNA have undergone numerous modifications to suit the different sources of DNA, their future implications etc. For studies involving multi-locus finger printing, southern blotting, DNA digestion etc a high molecular weight DNA of good quality and quantity is essential. On the other hand for PCR, a small quantity of DNA is generally required and that too need not be of high purity. In humans, blood is the most easy and good material available for isolation of DNA.

Methods of DNA analysis

I. RFLP (Restriction Fragment Length Polymorphism) method

RFLP analysis was the first method of DNA analysis widely used. In this method, DNA is cut into VNTR fragments by restriction sequences within the molecule as opposed to exonucleases which digest restriction enzymes as their presence in the given bacteria restricted the growth of certain bacterial viruses called bacteriophages. These enzymes are named after the bacteria from which they are isolated e.g. Eco RI from Escherichia coli, R indicates the R strain of E. coli and I indicate the order of discovery. Various restriction endonucleases are used for DNA finger printing like Eco RI, Hpa I, Taq I etc etc. These enzymes recognize and cleave a specific double stranded DNA sequence i.e. 4-7 base pairs long. When DNA is digested with a given enzyme, the ends of all the fragments will have same DNA sequence e.g. Eco RI will cut the DNA when it would find the sequence “GAATTC” and Taq I will cut at “TCGA” sequence. Therefore if we

put the DNA of an individual with one of these enzymes, it would be cut into fragments of various lengths, size of these fragments being from several thousand to several million base pairs.

Southern Blotting

The fragments of DNA obtained are electrophoresed on agerose gel for size fractionalization. The DNA is poured into agarose gel and an electrical charge is applied to the gel, with positive charge at the bottom and the negative charge at its top. Because DNA has slightly negative charge, the pieces of DNA will be attracted towards the bottom of the gel, the smaller pieces however, will be able to move more quickly and thus further towards the bottom than the larger pieces. The different sized pieces of DNA will therefore be separated by size, with the smaller pieces towards the bottom and the larger pieces towards the top.

The DNA is then denatured using strong alkali, so that all of the DNA is rendered single stranded. This can also be done by heating the DNA in gel.

Now the gel with size fractionated DNA is applied to a sheet of nitrocellulose paper and then baked to permanently attach the DNA to the sheet. The southern blot is now ready to be analysed. The process mentioned above is termed as blot transfer and in case of DNA it is called southern blot as Southern was the name of the person who devised this technique.

In order to analyse a southern blot, radioactive genetic probe is used in a hybridisation reaction with the DNA in question. If an X-ray is taken of the Southern blot after a radioactive probe has been allowed to bind with denatured DNA, only areas where the radioactive probe binds will show up on the film. This allows researchers to identify in a particular person's DNA, the occurrence and frequency of the particular genetic pattern contained in the probe.

DNA probe

A molecule used to detect the presence of a specific fragment of DNA during analysis by blot transfer technique is called probe. These are short stretches of known DNA sequences that have the tendency to bind with their complementary sequences in a pool of unknown DNA. These probes may be cloned in some bacteria or chemically synthesized. These are called synthetic oligonucleotide probes and can be labelled with radioactive material like P32, S35, I125 or even with some non-radioactive fluorescent dyes. In the DNA pattern, when analysed by means of a probe, the core sequence, if occurs at only one DNA locus, it is called single locus probe. The core sequence if occurs at many different loci, is called multilocus probe.

At centre for cellular and molecular biology, Hyderabad scientists have developed another probe called Banded Krait Probe. BKm which detects qualitatively more polymorphic regions in the human genome than the Jeffrey's probe.

II. Polymerase chain reaction (PCR method)

PCR, first conceived by Kary Mullis in 1983. It is method for amplifying or copying a short sequence of DNA repeatedly, thus going from a very small amount of DNA to a very large amount. Originally, a fragment of E.coli DNA polymerase was used in PCR for chain elongation. This enzyme worked at 370C and would become inactive during denaturation step, requiring fresh enzymes to be added after each cycle. With the introduction of the thermostable taq DNA polymerase, procedure has become very simple and can be applied to reactions involving high temperature.

The basic requirement of this technique is that the sequence of DNA to be amplified or the target DNA should be known so that short stretches of DNA complimentary to the basis on the flanking regions of it can be synthesized. These are called primers which limit the length of sequence to be amplified. One cycle of PCR consists of three basic steps denaturation , annealing and extension. This cycle is repeated 20-30 times to get approximately 2n copies of target DNA (n= number of cycle). However beyond 25-30 cycles, reaction reaches the palataeu phase and ceases to be exponential.

Visualization of PCR product

On completion of PCR, the amplified product is checked by electrophoresis on agarose gel. Amplified PCR product as well as DNA marker (probe) are loaded on agarose gel. Gel is run at 1-5 volt/cm. On completion of the run, gel is visualized under UV transillumination and photograph. A single sharp band indicates a single clean amplified product.

Precautions

Since PCR is an extremely sensitive technique, detecting a specific sequence within a single DNA molecule, false positive or mistyping may occur if the majority of molecules being detected arise from a contamination rather than the sample itself.

Therefore precautions should be taken when performing PCR to reduce the risk of contamination by presence of unwanted DNA which may get amplified.

Comparison with RELP method

Advantages

PCR method is relatively simple and easily carried out in the laboratory.

Results are obtained in short time.

PCR based methods permit analysis of extremely tiny amounts of DNA.

Disadvantages

Highly susceptible to contamination.

It is less specific than RELP method.

III. Short tandem repeates (STR method)

It is the latest method used for DNA finger printing. These are very short sequences of DNA usually 2-6 bp long. STR loci occur throughout the genome at an estimated frequency of 1 STR every 300,000 to 500,000 base pairs. While most STR loci have only s6-12 alleles, there are a large number of such systems that can be exploited for identification purposes. STR often occurs in the untranslated part of known genes. While STR fragments from different STR loci differ in size, they are still very small. Thus it is possible to run the products from several loci simultaneously on one gel as long as the fragment size does not overlap. The basic steps involved in STR analysis are :-

Isolation of DNA

Replication of STR fragments by PCR

Performing gel electrophoresis

Identifying the specimens using stains, chemiluminescence or laser techniques.

STR technology has several advantages over conventional RFLP technology.

It is more rapid and can be done in 2-3 days.

It can be performed on very small quantities of DNA (from 1/10th to 1/100th the amount required for RFLP method).

It can be performed on wipings from full metal jacketed bullets that have perforated the body even if no tissue is visible.

Using 10 different STRs, the chance that two people picked at random have the same pattern is about 1 in 3 trillion. In the U.S., where 13 STRs are now routinely examined, the odds of a random match are even higher.

Dscussion

DNA fingerprinting is of great use in various medico legal works like:

1. Paternity and maternity disputes

Because a person inherits his or her VNTRs from his or her parents. VNTR pattern can be used to establish paternity and maternity. The patterns are so specific that a parental VNTR pattern can be reconstituted even if only the children's VNTR patterns are known. Parent child VNTR pattern analysis has been used to solve standard father identification cases. The coding is

made from mother, child and the putative father. The bars in the child's profile are first matched with the mother's, so the remaining bars must all come from alleged father. If there is any discrepancy, then that man cannot be the father.

2. Criminal identification

DNA isolated from the blood, hairs, skin cells or other genetic evidence left at the scene of crime can be compared through VNTR pattern with the DNA of a criminal suspect to determine his/her guilt or innocence.

3. Rape cases

DNA fingerprinting is of great importance in solving cases of rape, buccal coitus, paedophily etc. The swab taken from the vagina, mouth or the rectum respectively may have semen of the suspected person. The DNA evidence may also be present as seminal stain on clothes of the victim, bite marks (saliva) on the body parts of the victim or saliva stain may also be obtained from some glass, cup or cigarette/bidi etc which the person used before crime.

The DNA evidence obtained from such materials may be compared with the DNA obtained from the blood sample of the suspected person and thus his guilt or innocence can be proved.

4. Personal identification

DNA finger printing may be of great use in the personal identification as the chances of the two persons having same DNA finger printing is very rare other than in the cases of uniovular twins.

5. Mass disasters/accidents

Identity of the victims of the mass disasters can be established by comparing their DNA profile with the profile of their parents/children. Even if the sample cannot be obtained from the family members, the control samples may be obtained from the belongings of the person itself like tooth brush, licked envelope, stamps etc.

6. Postmortem identification of mutilated bodies/skeletal remains

The samples of DNA profiling may be obtained from muscle mass, bone, some organ etc. and thus can be compared with the DNA profile of the parents/children of the suspected/absconding person.

7. Miscellaneous

In addition to the above mentioned cases, DNA profiles may be of great use in solving cases of custody of a child born out of wedlock, legacy disputes, extortion cases (saliva samples from envelopes/face masks), soldiers ran out of brigade because of fear of war, immigration cases etc.

8. Besides the medicolegal importance of DNA profiling, it is also of great use in tumour biology/ cancer studies, studies of genetic disorders as well as population genetics, fossil studies etc.

Admissibility of the DNA profile as an evidence in the court of law

Although the scientific validity of DNA as an evidence in the court of law is not subject to serious dispute, but for the maintenance of DNA reliability, it is important that judges strictly apply and enforce the national guidelines governing chain of custody and laboratory procedures when making decisions regarding DNA profile. If any of these standards are not followed judges must find the evidence inadmissible. If compliance with the standards is established, the judge must determine and admit evidence of either a positive or negative DNA match.

Problems with DNA finger printing

Like nearly everything else in the scientific world, nothing about DNA fingerprinting is 100% assured. The term DNA fingerprint, in one sense, misnomer, it implies that, like a fingerprint, the VNTR pattern for a given person is utterly and completely unique to that person. Actually, all that a VNTR pattern can do is present a probability that the person in question is indeed the person to whom the VNTR pattern (of the child, the criminal evidence or whatever else) belongs. The probability of a DNA finger print belonging to a specific person needs to be reasonably high especially in criminal cases, where the association helps establish a suspect's guilt or innocence. Using certain rare VNTRs or combinations of VNTRs to create the VNTR pattern increases the probability that the two DNA samples do indeed match (as opposed to look alike , but not actually come from the same period) or correlate (in the case of parents and children). Errors in the hybridization and probing process must also be figured into the probability, and often the idea of error is simply not acceptable. Most people will agree that an innocent person should not be sent to jail, a guilty person allowed to walk free, or a biological mother denied her legal right to custody of her children, simply because a lab technician did not conduct an experiment accurately. When the DNA sample available is minuscule, this is an important consideration, because there is not much room for error, especially if the analysis of the DNA sample involves amplification of the sample (creating a much larger sample of generally identical DNA from what little material is available), because if the wrong DNA is amplified (i.e. skin cell from the lab technician) the consequences can be profoundly detrimental. Until recently, the standards for determining DNA fingerprinting matches, and for laboratory security and accuracy which would minimize error, were neither stringent nor universally codified, causing a great deal of public outcry.

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