



Molecular Investigation of Biological Samples for Human Identification

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Received: 20th June 2015, Accepted: 16th July 2015, Published: 1st September 2015

Abstract

The remarkable advances in DNA technologies over the past two decades had an enormous impact on human identification, medical diagnosis, population genetics, and crime scene investigation. Currently, DNA can be used very specifically to discriminate between individuals, using a wide variety of techniques. Depending on the amount of sample and the level of degradation, several techniques can be applied to narrow the likelihood that a particular individual was present at a crime scene. This paper presents a discussion of the investigative procedures of biological samples for human identification. A brief overview of crime scene, various techniques of molecular biology for identification purpose, PCR and the biological samples involved has been provided. Contrary to popular perceptions, DNA profiling in its present form can't identify an individual. In RFLP analysis, the long DNA helix is cut into a set of fragments. This can be done with one of a number of specific enzymes called restriction endonucleases, which cut the DNA of all humans at specific points in the DNA base sequence. Quantitative estimation of DNA would be performed and the results stated to determine the concentration of DNA. Sequence analysis would be performed for a particular gene to determine the gender from the samples.

Keywords

Biological tools, forensic evidence, RFLP analysis, sequence analysis, DNA profiling

Introduction

A crime scene is any physical scene, anywhere that may provide potential evidence to an investigator and some perspective on the crime that may have transpired. It may include a person's body, any type of building, vehicles, places in the open air or objects found at those locations. Number of people working on the case depends on the type of case. DNA forensics is the study of forensic evidence leading to various activities or happenings. It can be used to identify individuals by the characteristics of their DNA. DNA forensics has also played an important role in present day medical diagnosis, human identification, crime scene investigation etc. DNA

forensics can also be used to exonerate individuals, who have been falsely accused of committing crimes, and to connect two different crimes scenes that may share some evidence. It can also be used to identify the father of a child achieved through rape or incest. In some cases, it can also be used to identify the rapist without the child. Biological samples such as blood, hair, semen etc play an important role in crime scene investigation.

Once the DNA (required amount) is detected, it can further be analysed by using one of the many DNS testing methods such as RFLP analysis, STR analysis, Y-chromosome typing, or mitochondrial DNA (mDNA) sequence analysis.

The testing method used depends on

- Type of sample
- Amount of sample
- The condition of the sample

Variations in DNA sequence between individuals are termed "polymorphisms". Sequences with the highest degree of polymorphism are very useful for DNA analysis in forensics cases and paternity testing.

STR technology has enabled the rapid generation of highly informative DNA data for use in human identification. Human review (in duplicate) of forensic STR data is currently required to assure that DNA profiles are correctly scored. However, this human analysis step has become a labor-intensive bottleneck impeding the rapid construction of high-quality DNA databases. The practical application of DNA technology to the identification of biological material has had a significant impact on forensic biology, because it enables much stronger conclusions of identity or non-identity to be made. The vast majority of the DNA in a cell has no known function, and this non-coding DNA varies very greatly from one individual to another. This is the DNA which is examined in most DNA profiling systems.

In RFLP analysis, the long DNA helix is cut into a set of fragments. This can be done with one of a number of specific enzymes called restriction endonucleases, which cut the DNA of all humans at specific points in the DNA base sequence. Thus a set of fragments is obtained, one of which contains the

region of interest. This fragment is identified using a short piece of synthetic DNA called a probe. The probe is designed so that its base sequence is complementary to (that is forms A-T and G-C pairs with) the base sequence of one of the two strands in the fragment of interest. It measures fragments of DNA containing short sequences that vary from person to person, called VNTRs.

The technical complexity of performing RFLP analysis coupled with the widespread use of short-lived radioisotopes for detection have prompted researchers to look for alternative methods.

The advent of the PCR has resulted in the development of a large number of molecular techniques, which offer an effective alternative to the hybridization methods of RFLP analysis.

The technical complexity of RFLP analysis coupled with widespread use of radioisotopes for detection have persuaded researchers to look for and apply alternative methods. Advent of PCR has resulted in the development of a large number of molecular techniques alternative to the RFLP analysis.

PCR based approaches have few advantages i.e they use only small amount of DNA and also avoid the use of radioactivity. They are even susceptible to automation. Lineage markers have special applications in forensic genetics. Y chromosome analysis is very helpful in cases where there is an excess of DNA from a female victim and only a low proportion from a male perpetrator. The steady growth in the size of forensic DNA databases raises issues on the criteria of inclusion and retention and doubts on the efficiency, commensurability, and infringement of privacy of such large personal data collections. In contrast to the past, not only serious but all crimes are subject to DNA analysis generating millions and millions of DNA profiles, many of which are stored and continuously searched in national DNA databases.

Extraction of DNA basically consists of four major steps:

- Preparation of a cell extract
- Purification of DNA from cell extract
- Concentration of DNA samples
- Measurement of purity and DNA concentration

A new forensic DNA approach that is being more broadly applied is mitochondrial DNA (mtDNA) sequencing. Mitochondria are structures within cells that contain DNA that is distinct from the DNA in the nucleus. Mitochondrial sequencing is used extensively in FBI labs, as well as state and private crime labs. This technique, which takes into account

small differences between people's mtDNA, is very useful for minute samples or ancient or degraded samples. This is because cells are loaded with mitochondria, so even in highly degraded samples or old hair, there is often enough mtDNA to obtain a sequence. DNA extraction and purification is a very important part of DNA forensics.

Materials and Methods

Buccal DNA Isolation:

Hypersaline solution is taken into the mouth for creating a friction effect, this isolates cheek or buccal cells which is then centrifuged gives the pellet and supernatant. DNase requires Mg ions as cofactor and when EDTA is added metal ions are used away. Due to the absence of Mg+2 DNase can't perform its function of degrading the DNA, thus the DNA is protected. Sucrose plays an important role by providing density and creating osmotic imbalance in the solution. SDS plays the role of a detergent and sodium chloride brings back the osmotic balance thereby helping in protein precipitation. Ethanol added at the end helps in identifying the DNA precipitation.

Blood DNA Isolation:

Anti coagulated blood is taken as the coagulated blood doesn't contain WBC's in their native state. EDTA is a chelating agent used to prevent the coagulation of blood. Ca ions are required as cofactors to activate the steps of blood regulation i.e. the activation of thrombinase (holoenzyme form) from apoenzyme form. Tris HCl is used to regulate pH, sucrose to provide density to the cells and MgCl₂ for the RBC lysis. Sucrose also plays another important role of providing a protective layer around the WBC. Sodium Acetate is acidic in nature and provides acidic environment to the DNA, helps in the transportation of DNA outside the cell membrane. Chloroform is used as a bleaching agent to remove hemoglobin and do decolourization of the blood as even after the removal of RBC the blood retains red colour. Iso propanol is added to enhance precipitation of the DNA, as the OH⁻ ions compete with the DNA to combine with the H⁺ ions present in the H₂O molecule. Chloroform helps to remove traces of phenol present in mixtures. Isoamyl alcohol is a antifoaming agent. It should be in very small quantity because it might take part in the reaction. ethanol will keep DNA intact and rest 30% water will solublize salt.

Hair DNA Isolation:

Human hair is one of the most common biological materials associated with legal investigations and has been used for the statistics-based population work

and DNA-based analysis in criminology. The most valuable method of DNA testing is short tandem repeat analysis of nuclear DNA. This is possible when the root portion of the hair and/or adhering tissue is present. However, telogen hairs (shed hair), often associated with a crime scene, may not contain any nuclear material. Hair samples were washed by immersing them in fresh water to remove the surface dirt and other contaminants. The lysis buffer used in this experiment contains the components Tris HCl, KCl and Tween. A buffer solution that provides an ionic environment similar to that of the cell *in vivo* to maintain the stability of our protein of interest is used. Salts (NaCl, KCl, (NH₄)₂SO₄) are added to maintain or increase the strength of the ionic medium and detergents (sodium dodecyl sulfate or SDS, deoxycholate, Triton X-100) to dissolve the cell membranes and facilitate the release of soluble proteins and other cellular components into the solution. Some lysis buffers also call for the addition of ligands and/or metal ions, chelating agents, protease and phosphatase inhibitors, glycerol, glucose or sucrose, and other reducing agents to improve the stability of the target protein and keep it in the solution.

Electrophoresis:

Electrophoresis is a method of separating substances based on the rate of movement under the influence of an electric field. Agarose is a polysaccharide purified from seaweed. An agarose gel is created by suspending dry agarose in a buffer solution, boiling until the solution becomes clear, and then pouring it into a casting tray and allowing it to cool. The result is a flexible gelatin-like slab. During electrophoresis, the gel is submerged in a chamber containing a buffer solution and a positive and negative electrode. The DNA to be analyzed is forced through the pores of the gel by the electrical current. Under an electrical field, DNA will move to the positive electrode (red) and away from the negative electrode (black). Several factors influence how fast the DNA moves, including; the strength of the electrical field, the concentration of agarose in the gel and most importantly, the size of the DNA molecules. Smaller DNA molecules move through the agarose faster than larger molecules. DNA itself is not visible within an agarose gel. The DNA will be visualized by the use of a dye that binds to DNA.

Colorimetric Estimation by DPA Method:

DPA reagent is used for measuring the concentration of DNA. 200µL of freshly prepared reagent {150mg DPA, 150µL H₂SO₄, 50µL acetaldehyde (16 mg/ml)/10ml of glacial acetic acid} was added to the DNA samples provided for estimation. The color

reaction was developed at 30°C overnight and samples were read at 590nm along with a standard curve prepared from standard DNA. The fraction of DNA from cells in the supernatant, expressed as % of total DNA is an index of cell shedding.

When DNA is treated with DPA under acidic conditions, a blue coloured complex is formed with a sharp absorbance at 595 nm. Treatment given with 2-deoxy-pentoses, which are converted into highly reactive α -hydroxy-levinaldehyde which further reacts with DPA to give a blue coloured complex. Above reaction is given by deoxyriboses of purines and not pyrimidines. Value obtained represents only DNA content.

Primer designing and Sequence:

The gene considered here for the analysis is the AMEL gene for its known importance in the determination of the sample Gender. The sequence was retrieved from NCBI database to which primer was designed using Primer 3 data base. The primer thus obtained was used for PCR amplification and sequencing. The sequencing results were then analyzed to check for the gender of the samples used.

Results and Discussion:

Extraction of DNA from the samples:

Various biological samples were used from the sample source like hair, Blood and buccal. Specific DNA extraction techniques were employed to obtain the DNA from the samples. The DNA thus obtained was used for further analysis.

Electrophoresis of the samples:

All the DNA samples extracted were run on Gel, for Electrophoresis. The separated bands were shown in the Fig 1. The presence of the band is a confirmation of the DNA obtained from the samples. All the bands were equally placed and same width as all the samples were from same individual and same genome is obtained.

Colorimetric Estimation of DNA:

The DNA extracted from all the sources was subjected for estimation by DPA method based on colorimetric. The OD values were recorded and the graph was plotted. The graph is shown in the Fig 2. As per the results of Colorimetry, it can be observed that the maximum concentration of DNA was obtained in buccal cells than all the other sources.

Gene Sequence Retrieval, Primer Designing and Sequencing:

The gene selected here is the AMEL gene which is specifically used for the sex determination of the samples. The gene sequence was retrieved from

NCBI database and was subjected for primer designing using Primer3 tool. The Primer was thus used for the PCR based amplification. The amplified product was then subjected for sequencing. The sequences thus obtained were analyzed and the sex determination was performed.

CTGATCTACAAGGGAACATTTCGTGAGACAATTCTC
TCCTTGTTGATATTTCTTTATT

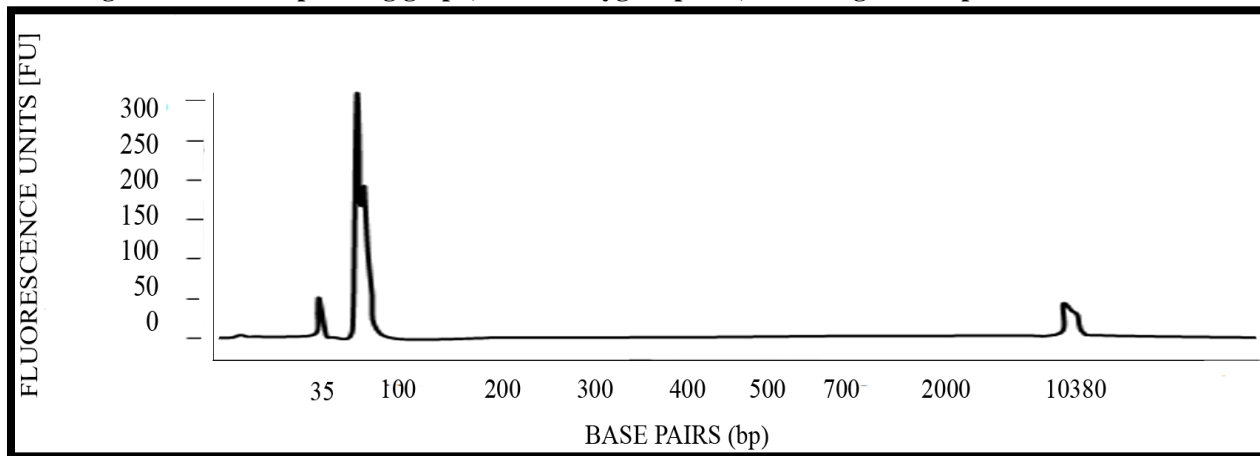
Difference between the Male and Female Sequences:

AMEL-Y CCCTTTGAAGTGGTACCAGAGCATGATAAGACCACCAGTATGTAGACATTTTGTCTTTATTCCC
AMEL-X CCCTTTGAAGTGGTACCAGAGCAT---AAGGCCACCGGTATGTAGACATTTTGTCTTTATTCCC
c1one1 CCCTTTGAAGTGGTACCAGAGCAT---AAGGCCACCGGTATGTAGACATTTTGTCTTTATTCCC

The sequence obtained for AMEL gene:

TCCTTTGATACTTAAAAGTACTTGACCACCTC

Fig shows the STR profiling graph, the homozygous peaks, indicating the sample to be of Female.



Conclusion:

The above work concludes that the maximum concentration of the DNA obtained was from the Buccal sample. All the other samples were equally quantified for the extracted DNA. However the DNA sequence remains the same whatever is the source of extraction from the same organism. The study was focused on sex determination using the AMEL gene sequencing. The sequence of AMEL was retrieved from NCBI and a specific primer was designed for the amplification using PCR. The amplicon thus obtained was sequenced and the sequence was analyzed for sex determination. The STR profile showed homozygous peaks representing the Female sample. These techniques can be applied for CARIME INVESTIGATION, Disease identification etc.

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