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PATERNITY TESTING THROUGH MULTIPLEX PCR BASED STR GENOTYPING OF GENOMIC DNA

Geetha Subramanyam[1], Harishitha Damerla[1] & Giridhar Reddy P.[2]

[1]Department of Biotechnology, Chaitanya Bharathi Institute of Technology, A.P.-500075, India
[2]Department of Chemistry, Chaitanya Bharathi Institute of Technology, A.P.-500075, India.

E-mail ID: geeven_subgee@yahoo.com

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ABSTRACT

Forensic science is the application of a broad spectrum of sciences to answer questions of interest to a legal system. Forensic scientists use a technique called DNA profiling to assist in the identification of individuals by their respective DNA profiles. DNA profiles are encrypted sets of numbers that reflect a person's DNA makeup, which can also be used as the person's identifier. The method of DNA profiling used today is based on PCR and uses short tandem repeats (STR). After DNA is extracted and amplified using PCR, genotyping is done. The different types of assays used are Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic Detection of Genomic DNA (RAPD), and Amplified Fragment Length Polymorphism detection (AFLP). The method used in the investigation is Short Tandem Repeats (STR) technology. Short tandem repeat (STR) technology is used to evaluate specific regions (loci) within nuclear DNA. Variability in STR regions can be used to distinguish one DNA profile from another. The Federal Bureau of Investigation (FBI) uses a standard set of 13 specific STR regions for CODIS. The purpose of the investigation is to solve a disputed paternity case by comparing the STR profiles of the suspect and evidence.

Keywords: Paternity testing, PCR, STR, Paternity Index, DNA profiling, allelic ladder

Number of Tables : 2 **Number of Figures :** 1 **Number of References:**21

INTRODUCTION:

Years ago, it seemed nearly impossible to catch certain criminals who were intelligent enough to get rid of indicating physical evidence like fingerprints and blood. But as technology progressed and investigators began to rely more on science, criminals were getting caught by leaving behind the very essence of their being: DNA samples. Forensic DNA analysis is the use of deoxyribonucleic acid (DNA) specimens in legal proceedings. DNA typing, since it was introduced in the mid-1980s, has revolutionized forensic science and the ability of law enforcement to match perpetrators with crime scenes. Human identity testing using DNA typing methods has been widespread since was first described in 1985 by an English geneticist named Alec Jeffreys (John M. Butler, 2005). The past 15 years have seen tremendous growth in the use of DNA evidence in crime scene investigations as well as paternity testing. The basis of DNA typing is that only one-tenth of a single percent of DNA (about 3 million bases) differs from one person to the next. Scientists can use these variable regions to generate a DNA profile of an individual, using samples from blood, bone, hair, and other body tissues and products. To identify individuals, forensic scientists scan 13 DNA regions, or loci, that vary from person to person and use the data to create a DNA profile of that individual (sometimes called a DNA fingerprint). There is an extremely small chance that another person has the same DNA profile for a particular set of 13 regions. Scientists find the markers in a DNA sample by designing small pieces of DNA (probes) that will each seek out and bind to a complementary DNA sequence in the sample. A series of probes bound to a DNA sample creates a distinctive pattern for an individual. Forensic scientists compare these DNA profiles to determine whether the suspect's sample matches the evidence sample. The gamut of DNA typing technologies used over the past 15 years for human identity testing includes single-locus probe and multi-locus probe RFLP methods and more recently PCR based assays. New and improved methods have developed over the years that tests with a high degree of discrimination can now be performed in a few hours. The best solution including a high power of discrimination and a rapid analysis speed has been achieved with short tandem repeat DNA markers. This method gained importance in the late 1990's and is used mainly in paternity testing. Short tandem repeat (STR) markers have become the workhorse of forensic DNA typing (Lygo et al., 1994). Because STR's by definition are short, three or more can be analyzed at a time. Multiple STRs can be examined in the same DNA test, or 'multiplexed'. Multiplex STRs are valuable as they can provide highly discriminating results and can successfully measure sample mixtures and biological materials containing degraded DNA molecules. In addition, the detection of multiplex STRs can be automated, which is an important benefit as demand for DNA testing increases. Since then, STR genotyping has developed greatly to serve advanced applications and use different sources, benefiting forensics in a large way.

MATERIALS AND METHODS:

The present project involves multiplex PCR based STR genotyping of genomic DNA isolated from blood samples. The various steps involved in this process are:

DNA extraction from random blood samples: DNA quantization using a UV spectrophotometer PCR amplification of multiple STR markers; Separation and detection of PCR products (STR alleles) and comparison of sample genotypes to other sample results.

DNA Extraction: Organic extraction (also called phenol-chloroform extraction), which is the oldest and widely used method of DNA extraction is used; High molecular weight DNA may be obtained most effectively by organic extraction. (John M. Butler, 2009).

Estimation of DNA: Forensic samples often contain DNA co-extracted from microbial organisms. For RFLP analysis, it is necessary to consider both the total DNA and the proportion of human DNA; quantitative information about total DNA is used to calculate how much restriction enzyme to add to each reaction mixture. (Norah Rudin & Keith Inman, 2002). Quantification was done using UV Spectrophotometer. The quality of DNA was checked using agarose gel electrophoresis.

PCR Amplification: Polymerase Chain Reaction is used to amplify the STR alleles required for genotyping. Multiplex PCR was used for the current project. The amplification is carried out in a thermocycler for 28 cycles.

STR Analysis:

Prior to starting the regular cycle of filling the capillary with polymer solution, injecting and separating DNA samples, the temperature on the capillary heating plate is brought up to 60°C to thermally equilibrate the capillary. The LASER is turned to full power. Then the samples were fed into an auto sampler ABI 310 Genetic Analyzer after sample filling details in sample sheet. The sample data was captured by CCD camera after excitation by LASER beam at capillary window.

RESULTS AND DISCUSSION:

Table 1: Quantitative analysis of DNA

S. No.	OD AT 260 nm (A˚)	OD AT 280 nm (A˚)	OD AT 260 nm/ OD AT 280 nm	[(OD AT 260 nm) * 5] = x	1/x
A1	0.108	0.084	1.285	0.54	1.85
B1	0.112	0.081	1.38	0.56	1.78
C1	0.106	0.080	1.32	0.53	1.88
D1	0.061	0.037	1.648	0.305	3.278

Blood samples were collected from 4 random people and DNA was successfully extracted from them. The results of the quantitative analysis calculated as per readings of the UV Spectrophotometer are given above.

Now the obtained DNA from the four blood samples is subjected to paternity testing by PCR amplification followed by STR analysis. The samples were run for 28 cycles to produce about 1 billion copies of the selected 16 STR loci

Table 2: Qualitative analysis of DNA

Electrophoretic well number	Sample Number	DNA source	Quality of DNA
1	A1	Whole blood from individual – A	Sharp band
3	B1	Whole blood from individual – B	Sharp band
5	C1	Whole blood from individual – C	Sharp band
7	D1	Whole blood from individual –D	Sharp band

The sample report obtained for the DNA paternity testing presents the following allelic ladder to signify the relatedness between parents and child. The complete test results show the correlation on 16 markers between the child and the tested man to draw a conclusion of whether or not the man is the biological father. The 16 STR alleles used for DNA genotyping in this project are D85S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818, FGA, Amelogenin. These loci respond to green, blue, yellow or red dyes. Electropherograms, generated by the genetic analyzer show peaks that signify the correlation of markers between the child and the suspects. This is then used to generate an allelic ladder of the STR loci peaks of the mother, child and the suspects. Considering the match probability, it can be concluded that the alleged father 1 is the biological father of the child, while alleged father 2 is not the biological father as the STR allele loci do not match. Scientifically, each marker is assigned with a Paternity Index (PI), which is a statistical measure of how powerfully a match at a particular marker indicates paternity. The PI of each marker is multiplied with each other to generate the

Combined Paternity Index (CPI), which indicates the overall probability of an individual being the biological father of the tested child relative to any random man from the entire population of the same race. The CPI is then converted into a Probability of Paternity showing the degree of relatedness between the alleged father and child.

The report shows the genetic profiles of each tested person.

If there are markers shared among the tested individuals, the probability of biological relationship is calculated to determine how likely the tested individuals share the same markers due to a blood relationship.

According to the data, the alleged father 1 is the biological father of the child. Thus the paternity dispute has been solved using multiplex PCR based STR genotyping of 16 STR allele loci.

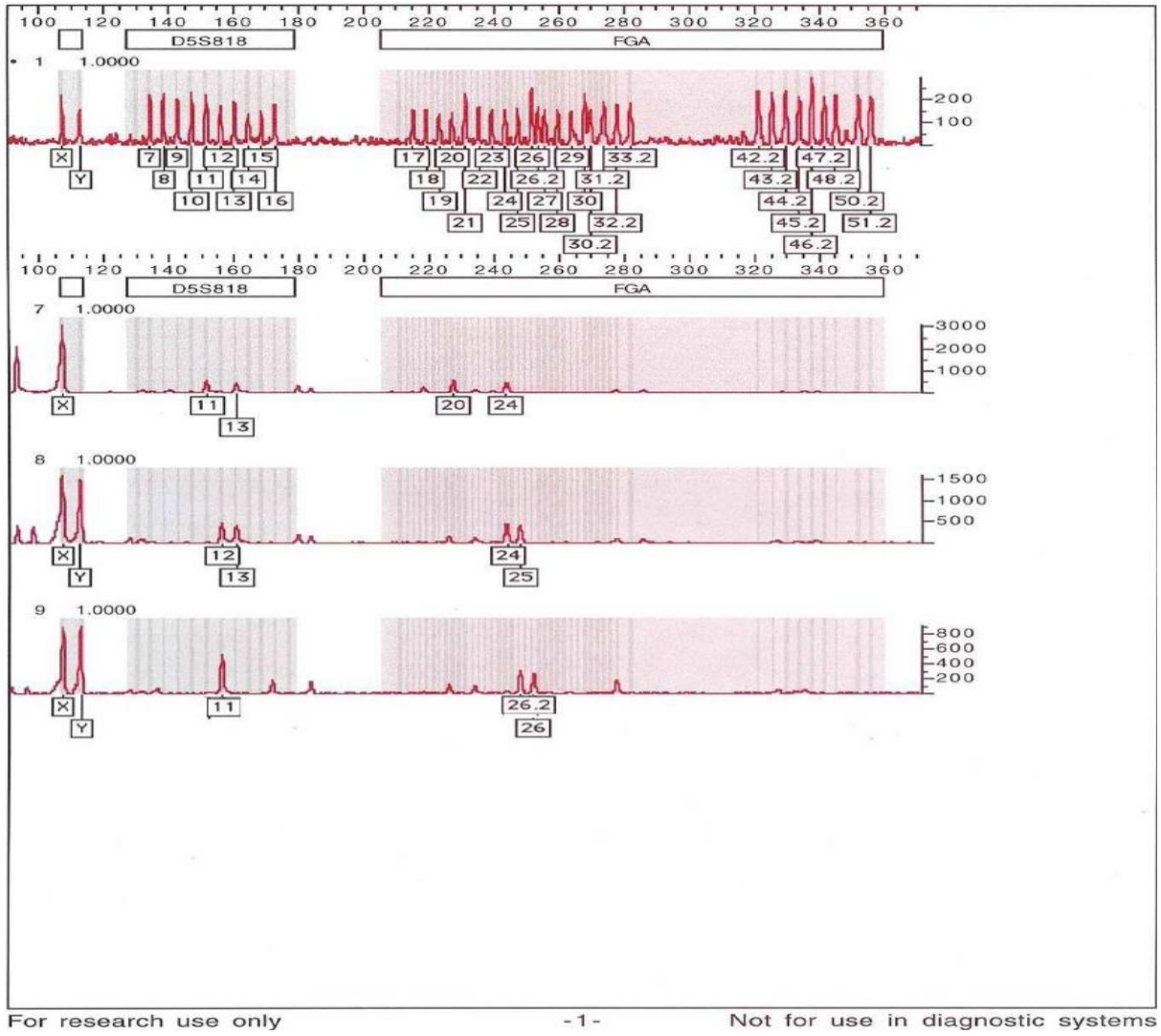

Figure 1: Electropherogram showing loci responding to red dye, of mother, child and alleged father 2

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