TESTING BY PCR BASED SHORT TANDEM REPEAT (STR) GENOTYPING OF GENOMIC DNA

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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

INTRODUCTION:

Besides others, DNA evidence collection plays a vital role in crime scene investigation and the detection rate of crimes. 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. 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. In forensic genetics, methods, technologies and knowledge have evolved tremendously, but Locard's exchange principle remains the same: every contact leaves a trace [1]. At a crime scene, biological material left behind by the perpetrator is collected and analyzed by crime scene investigators and forensic experts, respectively. The interpretation of a person's trace contribution finalizes in a written report for the public prosecutor and the judicial authority, respectively. Therefore, the effective collection of target DNA left by an individual marks the initial step of genetic analysis and fundamentally determines the success of DNA profiling.

In addition to the various collection techniques, several diverse swab types with different properties, such as the swab head material [5] or density and associated absorption capacity [18], [19] as well as their potential chemical treatments [19] have become commercially available over the last decades. According to the manufacturers and suppliers, the unique characteristics of each given swab promise to improve swab collection performance. In addition, the secured biological material is further exposed to e.g., the swab solutions used [20], specific surfaces from which DNA is collected, or the drying system of the swab packaging [3], [6], [24]. Moreover, these factors impact the quality of DNA, not only in the short term but also over a more extended storage interval. However, to date, only a limited number of studies have been published addressing the DNA preservation for various swabs, and none of them includes a storage period longer than one year [3], [6],

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.

Human DNA can remain unchanged for years and extracts stored at −20 °C or −80 °C can preserve important genetic information for long periods of time, which

can be submitted as evidence in court [1]. This makes such extracts particularly valuable in (older) cases in which other evidence has been destroyed and further analyses are required. Forensic genetics uses various techniques for human DNA extraction, including traditional methods such as organic extraction (phenol-chloroform) [2] and extraction with Chelex 100 Resin [3], depending on the type of biological material. Over time and with the possibility of automating multiple steps in DNA analysis, many laboratories have switched to solid phase extraction (*e.g.*, ion exchange columns, magnetic beads) in which DNA is selectively bound to a substrate such as silica particles. In this way, the DNA is retained while the proteins and other cellular components are washed away, releasing the DNA in a purified form. The extracted human DNA is typically stored at −20 °C, or even at −80 °C in order to prevent the activity of nucleases and preserve DNA for genetic profile typing [4].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:

1. DNA extraction from random blood samples
2. DNA quantitation using a UV spectrophotometer
3. PCR amplification of multiple STR markers
4. Separation and detection of PCR products (STR alleles) and comparison of sample genotypes to other sample results.

DNA Extraction:

The forensics community switched to STRs, which are a shorter type of repeat unit. The STRs used for forensics range from three to five bases long. Strung together with flanking sequences on either side, these

STRs make up overall DNA fragments that are less than 500 bases long. The length of a DNA fragment correlates with the number of repeats it contains.

500 µl of blood was drawn from 4 random people (A1,B1,C1,&D1)of different ages and sex and added into an EDTA coated Eppendorf tube. Equal quantity of Lysis Buffer I was added, and the contents were mixed thoroughly by shaking the tube. The sample is stored at -70˚C in a freezer until needed. Samples were taken out from the freezer, sealed with parafilm, and were subjected to heat shock at 65°C in a water bath for about 8-10 minutes for thawing to lyse the red cells in blood samples. The thawed samples were centrifuged at 10,000rpm for 10mins at 15°C. The lysate is discarded, and the pellet is suspended in 0.5ml of Lysis Buffer II. The mixture was mixed/homogenized properly using vortex and then 0.05ml of 20% Sodium dodecyl sulphate and 2.5µl of proteinase K were added. The resultant samples are incubated at 56°C for 2 hours or at 37˚C for 4 hours. An equal volume of Tris saturated phenol was added to an aqueous DNA sample in a micro centrifuge tube. The mixture was manually shaken and centrifuged at 10000rpm for 10 minutes at 15˚C to enact phase separation.

Estimation of DNA:

Quantification was done using UV Spectrophotometer; DNA is calculated in µg/µl. The cuvettes are filled with water or TE buffer. The spectrophotometer is set to zero at 260 nm with this blank. The DNA samples are usually diluted before measuring its absorbance with TE buffer in 1:1000 dilution as it is typically at a concentration exceeding 1 µg/µl. The solution is mixed thoroughly and taken in the cuvette. The optical density (OD) of the sample is measured at 260nm and 280nm one after the other.

PCR Amplification:

Polymerase Chain Reaction is used to amplify the STR alleles required for genotyping. Multiplex PCR was used for the current project. The extracted DNA was diluted to get 0.125ng/µl and 5 µl of this was used for PCR. This was calculated from the OD readings at 260nm obtained during quantification. First the DNA was diluted to 1ng/µl and this was again diluted to 0.125ng. The amplification is conducted 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 (10mW). The auto sampler platform is moved around in order to verify if the instrument is working well. Then the following steps are carried. To the 24.5µl master mix (24.5µl of HiDi formamide and 0.5µl of Liz that is internal size standard) 1.0µl of PCR product was added. The above mixture was spun at 2000rpm for 1min and then denatured at 95°C for 5mins. The denatured product was snap cooled on ice for 5mins. The samples were run with IX electrophoresis buffer in pop4 medium. The sample data was captured

by CCD camera after excitation by LASER beam at capillary window.

RESULTS AND DISCUSSION:

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 as follows:

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	0.114	0.090	1.297	0.59	1.69
B1	0.118	0.088	1.58	0.59	1.69
C1	0.109	0.088	1.42	0.55	1.81
D1	0.061	0.056	1.678	0.350	2.857

Table 1: Quantitative analysis of DNA

The results of gel electrophoresis are as follows:

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

Table 2: Qualitative analysis of DNA

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.

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 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.

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