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Advantage of a SupplementaryX-STRs"Investigator Argus X-12 QS" PCR Kit in Solving a Paternity Case with one Mismatch on D21S11 Locus

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Abstract

Genetic markers are very frequently used in human identification of paternity, maternity, and kinship cases. Establishing the kin relationships between a child and his biological father or biological mother involves many statical facts. DNA fingerprinting technology has been proven a useful tool worldwide to solve the crime. Before the discovery of DNA fingerprinting technology, other technologies such as dactyloscopy, anthropology, odontology, and medico-legal examinations were used for identification of human remains. These technologies provide limited information in comparison to DNA finger printing which provides the genetic information of the unknown human remains. The human remains may be soft tissue, bones, teeth, or hairs from which the genetic information can be extracted from these wide variety of biological materials. In conditions where the biological material mainly soft tissue is degraded due to climatic conditions, presence of scavengers, and rate of bacterial growth but skeletal remains (bone and teeth) are found intact in such situations. In these circumstances, hard tissues have been observed as the most suitable source for forensic application. In this study, we present a paternity testing case of a Very Rare Mutating Marker D21S11 between father and child. The average mutational rate of the D21S11 marker is estimated at 0.19%.

Keywords: Genetic markers, Paternity, DNA fingerprinting, D21S11, Mutation.

Introduction

Short tandem repeats (STRs), also known as microsatellites or simple sequence repeats (SSRs), are short tandemly repeated DNA sequences that are present in all eukaryotic cells as well prokaryotic cells. STRs involve a repetitive unit of 2-7 bp (base pair) forming series with lengths of up to 100 nucleotides (nt)[1]. In Forensic DNA Fingerprinting, microsatellites are important genetic markers and it plays a major role in human identification. The short tandem repeats comprise 2 to 7 base pairs in length. It is the blessed method of DNA fingerprinting that is widely applied in forensic casework, paternity or maternity disputes, immigration processes, disaster victim identification, population studies, identification of missing persons, and various types of forensic cases where the query is to generate genetic or STR Profile. DNA extraction is the first and perhaps most important step in any Forensic DNA analysis,

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human DNA can be extracted from all the nucleated cells present in hair, tissue, blood, etc.

According to Mendel's laws of inheritance, the genetic information is transmitted in an equal percentage (50%) from each of the biological parents (50% by mother and 50% by father) to the offspring but in some cases, the phenomenon of heredity is not done like mendelian theory, the mutation is one of the best examples of this. Mutations are changes in the genetic sequence in such a way as to alter the genetic message carried by that gene, In STRs the mutational rate influences the structure of the tandem unit, and the STRs represent an important part of the mutations in the genome that heredity from one generation to another[2,3]. Paternity or maternity testing consists of comparing the genetic profiles between the child and the Alleged Father or Mother that is based on the analysis or examination of the STR loci between the child and the alleged father or mother. Paternity or Maternity tests are carried out by the analysis of family groups at the hypervariable loci. Microsatellite loci have a high degree of variability because of a high rate of mutations that alter microsatellite length[4]. A mutation event is often seen as a shift in allelic change or slippage in the comparison of parent and off-spring[5]. The average mutational rates of the core STR loci are estimated at 0.01% to 0.64%. The average mutational rate of the D21S11 marker is estimated at 0.19% and D21S11 is a very complex STR marker with six sub-repeat sections and short interconnecting sequences. The known allele range D21S11 STR marker is from 12 to 41.2 repeats and the variants are of equal lengths but they are different sub-repeat compositions[6].

In this study, we present a case of paternity testing by autosomal STR markers. In this case, an unknown skeleton was recovered from a forest by an investing officer on the information given by a resident. After the registration of the case, police started the investigation for identification of "the unknown deceased and for other information," the skeleton was sent for postmortem, In the meantime, a local family informed to police that their daughter aged 25 years been missing since last 3 months. As per postmortem, human remains were more than a month old and femur bone of an unknown skeleton was seized for forensic DNA analysis. To settle/verify the claim of the family, reference blood samples of the missing child's probable parents were collected for DNA analysis. Therefore, investigating officer (IO) sent reference samples of both husband and wife for the DNA test to the State Forensic Science Laboratory, Jaipur, Rajasthan 302016, India.

Material and Methods

Isolation of DNA

The seized femur bone of an unknown deceased was found intact inside the packet. For the processing of femur bone, the whole surface of bone was first cleaned to remove major dirt material then the bone surface was slightly scraped off using a sterile surgical knife on the shaft (body) portion of the femur using double gloves. After wiping off of peeled material on the shaft, the shaft was broken into less than 0.5 inches pieces using hammer and broken pieces of the shaft were converted into powdered form. The powdered form of the femur bone was subjected to DNA isolation using the bone DNA extraction kit (Promega, CA, USA) for processing and DNA IQ™ chemistry for purification of DNA on Maxwell® RSC 48 instrument (Promega, CA, USA) according to the recommendations of the manufacturer.

Quantification of DNA

Quantification of the DNA from an unknown bone sample was performed by using the Investigator Quantiplex Pro Kit (Qiagen, Germany) according to the recommendations of the manufacturer. We prepared a master mix with a standard volume of 18 µL for every sample and the master mix consisted of 9 µL of Quantiplex Pro Reaction Mix and 9 µL of Quantiplex Pro Primer Mix. After preparation, we dispensed 18 µL of the master mix into the wells of the PCR plate. Further, 2 µL of standard solutions, 2 µLQuantiTect Nucleic Acid Dilution Buffer to the NTC tubes, and 2 µL of unknown sample DNA was dispensed individually into the wells of the PCR plate. The quantification was performed on a 7500 Real-Time PCR System (Thermo-Fischer, USA), using the HID Real-Time PCR Analysis Software v 1.1/v1.2. The blood sample of the suspect on the FTA card was directly subjected to amplification[7].

Amplification of the Autosomal STR Markers (PowerPlex® Fusion 5C System)

Amplification of the 24 autosomal STRs locus which included 22 CODIS (D3S1358, D1S1656, D2S441, D10S1248, D13S317, PENTA-E, D16S539, D18S51, D2S1338, CSF1PO, PENTA-D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, FGA, D22S1045) and two sex determining loci AMELOGENIN and DYS391) was performed using PowerPlex® Fusion 5C system kit (Promega) by Verti™ Thermal Cycler (Thermo Fisher Scientific, CA, USA-Thermo) according to

recommendations of the manufacturer except for half-reaction volume (Table-1)[8].

Table 1: PowerPlex® Fusion 5C PCR cycling conditions

Temperature	Time	Number of Cycle
96°C	1 minute	01 cycle
94°C	10 seconds	–
59°C	1 minute	30 cycles
72°C	30 seconds	–
60°C	10 minutes	01 cycle
04°C	∞	-

Amplification of the X-STR Markers (Investigator Argus X-12 QS System)

Amplification of the Investigator Argus X-12 QS Kit contains primers for one gender determining loci AMELOGENIN (AM) and 12 X-STR markers (DXS7132, DXS7423, DXS8378, DXS10074, DXS10079, DXS10101, DXS10103, DXS10134, DXS10135, DXS10146, DXS10148 and HPRTB). These markers are clustered into 4 linkage groups. One autosomal STR marker D21S11 is included as a consistent marker. Amplification was performed by Verti™ Thermal Cycler (Thermo Fisher Scientific, CA, USA-Thermo) according to recommendations of the manufacturer (Table-2).

Table 2: Investigator Argus X-12 QS PCR cycling conditions

Temperature	Time	Number of Cycle
98°C	1 minute	
61°C	100 seconds	03 cycle
72°C	5 seconds	
96°C	10 seconds	
61°C	100 seconds	27 cycles
72°C	5 seconds	
68°C	2 minutes	-
10°C	∞	-

Electrophoresis and Data Interpretation

For the separation and detection of amplified fragments, capillary electrophoresis took place on an ABI 3500xL Genetic Analyzer (Thermo Fischer, Scientific, CA, USA). 1 µL of the PCR product or allelic ladder (AL) was diluted in 10 µL of the mixture of 0.5 µL of WEN ILS (Internal Lane Standard)–500 Size Standard and 9.5 µL of Hi-DiTMFormamide (Thermo Fischer, Scientific, CA, USA).

The X-STR's amplified fragments are also detected in ABI 3500xL Genetic Analyzer (Thermo Fischer, Scientific, CA, USA). In this scenario, 1 µL of PCR product or allelic ladder (AL) was diluted in the mixture consisting 0.5 µL of DNA Size Standard 550 (BTO) (Qiagen, Germany) and 12 µL of Hi-DiTMFormamide (Thermo Fischer, Scientific, CA, USA).

We used 36cm capillary and POP-4 (polymer) for the separation and allelic ladder for the detection of fragments. The obtained raw data were analyzed using the Gene Mapper ID-X version- 1.6. The analytical threshold for STR and X-STR markers was 100 RFU(Relative Fluorescence Unit).

Results and Discussion

In Forensic DNA Fingerprinting Laboratories, it is a standard procedure to exclude a man from paternity when there are more than 2 mismatches present between the genetic (STR) profiles of the Questioned child and the alleged father (AF). In the presented case, a mutation was detected at the D21S11 locus thus additional X-STRs (Investigator Argus X-12 QS System) amplification was performed. The genetic profiles on STR markers of the tested persons are presented in table 3 & 4. The mutation on the D21S11 locus between the child and the "father" is presented in figure-1. The confirmation of the paternity was made by the X-STR markers. On the STR markers, the probability of paternity (PP) was 99.9999475% and the combined paternity index (CPI) was 1.908 x 109. For the statistical analysis, we used the GenoProf-3 software (Dresden, Germany).

In some cases, analysis of 27 Autosomal STR markers in paternity testing is not sufficient for conclusive results regarding the confirmation or exclusion from paternity. Thus in these situations depending on the gender of the child, haploid markers X-STR or Y-STR are needed to complete the paternity testing. In kinship analysis where one of the parents is deceased the haploid markers can also be used to complete the establishment of a kinship relationship[10–13]. The International Society of Forensic Genetics (ISFG) published the guidelines in 2017 which established the use of X-STR markers in kinship analysis.

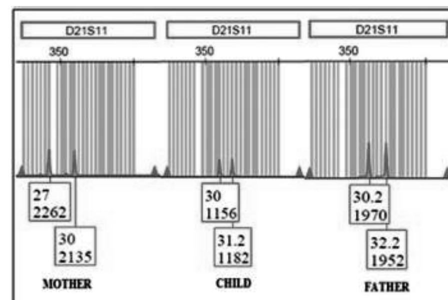

Fig. 1: Genetic profile of Mother, Child and Father on D21S11 STR marker.

Table 3: Genetic profile obtained by 24 STR markers (PowerPlex® Fusion 5C system kit)

Locus	DNA Profile obtained from Reference Blood sample of Mother	DNA Profile obtained from FemurBone	DNA Profile obtained from Reference Blood sample of Father
Amelogenin	X,X	X,X	X, Y
D3S1358	15,18	15,18	15,15
D1S1656	11,15	11,19.3	17.3, 19.3
D2S441	11,11	11, 12	10, 12
D10S1248	15,15	15,16	16, 16
D13S317	7,10	7,8	8, 8
PENTA-E	16,16	16,18	18, 21
D16S539	11,12	9,12	9, 10
D18S51	15,19	14,19	14, 17
D2S1338	19,25	19,20	20, 20
CSF1PO	10,12	12, 12	11, 12
PENTA-D	10,10	10,11	11, 11
TH01	6,9	6,6	6,9
vWA	17,18	16,18	16,17
D21S11	27,30	30,31.2	30.2,32.2
D7S820	8,11	10, 11	10,11
D5S818	10,11	11,13	13,13
TPOX	11,11	8,11	8, 11
DYS391	-	-	10
D8S1179	12,16	12,15	11, 15
D12S391	22,22	22,22	17, 22
D19S433	13,14	13, 15	13, 15
FGA	19,22	19,23	20, 23
D22S1045	14,16	14, 15	15,15
Probability of paternity (PP) = 99.9999475%			
Combined paternity index (CPI) = 1.908 x 109			

Table 4: Genetic profile obtained by Investigator®Argus X-12 QS kit

Locus	DNA Profile obtained from Reference Blood sample of Mother	DNA Profile obtained from Femur Bone	DNA Profile obtained from Reference Blood sample of Father
QS1	Q	Q	Q
AMELOGENIN	X,X	X,X	X, Y
DXS10103	19	18,19	18
DXS8378	10,12	12	12
DXS10101	29.2,32	28.2,32	28.2
DXS10134	35	35,38	38
DXS10074	16,17	16,17	17
DXS7132	14,15	14,15	14
DXS10135	25, 33	19,33	19
DXS7423	15	13,15	13
DXS10146	23,30	30,31	31
DXS10079	19,21	19	19
HPRTB	13	13	13
DXS10148	24.1,25.1	25.1,26.1	26.1
D21S11	27,30	30,31.2	30.2,32.2

Conclusion

In cases of paternity or maternity with one or two mismatches/mutations on the autosomal STR markers between the child and the alleged parent father/mother, it is necessary to use supplementary or additional haploid markers X-STR or Y-STR in addition to autosomal markers, depending on the gender of the child, to solve the inconclusive paternity/maternity case.

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Conflict of Interest

Authors declared that they have no conflict of interest.

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