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Paternity Disputes – Importance of Y DNA Profiling in Mutation Cases

Deepak Y Kudekar[1]*, Vaishali B Mahajan[2]*, Bhausaheb P More[3] and Krishna V Kulkarni[4]

[1,2]*Assistant Chemical Analyser, India*

[1,2,3]*Deputy Director of Regional Forensic Science Laboratory,Nashik*

[4]*Director of Forensic Science Laboratory ,Maharashtra, India*

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***Corresponding author:** Deepak Y Kudekar, Assistant Chemical Analyser, Regional Forensic Science Laboratory, Maharashtra, India

Abstract

Now a day's DNA fingerprinting is widely used to solve the paternal disputes. Since the application of DNA fingerprinting in forensic science, we see many cases related to paternity disputes where father denies his parentage towards child either in illegal relationship or in pregnancy due to rape. There is one more scenario where married man declines to accept his own child to seek divorce from his wife alleging her cheating on him. In such civil matters, court orders to do paternity testing for verification of the truth.

While DNA profiling, mutation is one factor that sometimes encounters. When there is a male child and mutation observes, Y DNA profiling is useful technique to establish the progeny and paternity as the Y DNA is only carried through male to male and it is same for child, father, uncle, brother which are from the same progeny.

In present scenario, two such cases received at forensic science laboratory, Nashik where in nuclear DNA profiling using AmpFlSTR®Identifiler® PCR amplification kit, mutation observed in one allele between father and child and as the child was male, we carried out Y STR DNA analysis using AmpF*l*STR®Yfiler™ PCR amplification kit, that resulted to the conclusion that the progeny was same for child and suspected Father.

Keywords: PCR; Short tandem repeat; Identifier kit; Y-filer kit; Mutation; Paternity Dispute; Progeny

Introduction

DNA fingerprinting is worldwide used technology in the field of forensic to solve crimes. It is valuable tool to solve paternity disputes including illegal relationships, pregnancy due to rape, suspicion on own child in civil matter and to prove maternity in which mother throws foetus to hide illegal relationship from society. Few million samples are run for purpose of paternity testing in country every year. While performing DNA analysis, mutation can be seen. There are several reasons for mutation. In view of mutation, paternity could not be established due to exclusion at single DNA locus. Generally, minimum 12 autosomal STR markers situated at least ten different chromosomes have to be examined, which is enough to reach a paternity probability of more than 99.9% in normal paternity cases or to find out more than three exclusions [1]. Further, 'two exclusion' rule stated that if two genetic loci do not match between an alleged father and child, the alleged father cannot be excluded as being true father of child [2,3].

Now, routinely, 15 STRs are used to obtain exclusion or strong probability of paternity. In few cases, this number can be inadequate to solve the case particularly when the mother is unavailable for the test or when the biological father is a close relative of the legal one or where only one or two exclusions are found [4]. The number of different Y-chromosome markers were evaluated which gained a significant role in paternity testing with male children [5,6]. We analysed two such cases in which Y DNA analysis using AmpFlSTR®Yfiler™ PCR amplification kit, proved the same paternal progeny of son and father in spite of mutation in Nuclear DNA analysis using AmpF*l*STR®Identifiler® PCR amplification kit [7].

In case number 1, accused and victim were in 'live- in' relationship. Victim gave birth to male baby. Baby was only 8 days old. Accused father of the baby told victim that baby is ill. I will show him to Doctor. He took the baby and killed him, thrown his body in the river. Complaint was lodged by mother of baby in Police station. Our laboratory received sternum, femur bone and scalp hair of baby including blood sample of both parents. Nuclear DNA analysis shows mismatch of father with son at only one locus.

In case number 2, five accused persons took advantage of girl in their village and established sexual relationship with her

at various times threatening to her. She became pregnant and gave birth to baby boy, but everyone denied their crime. Initially victim told name of one of them and later on she told names of another four who took advantage of her. Police authority sent the blood samples of baby and his mother including five accused to forensic Laboratory. Out of five, four accused excluded at multiple loci, but one accused excluded at only one locus out of 15 loci.

In above both cases, to ascertain the progeny, we performed Y DNA profiling of baby and accused father. Y DNA profile of both son and father matched in both cases. This confirmed that they belonged to same paternal progeny.

Materials and Methods

a) Prep filer Express Kit (applied biosystem)

b) Prep filer Express BTA kit

c) Amp F*I*STR Identifier kit (applied biosystem)

d) Amp F*I*STR Y-filer kit (applied biosystem)

e) Forensic Buffer pH 8

f) Proteinase K

g) Phenol: Chloroform: Isoamyl Alcohol 100/100/4

h) Isopropanol

i. Automate Express Forensic DNA System

Kit Used – Prep filer Express

ii. PCR Thermal Cycler Machine, Capacity -96wel l x 0.2ml PCR Tubes

Capable of testing Temperatures - Denaturation, anneling and extension steps

Heating/ Cooling – Peltier based Temperature accuracy-(+-)2

Temperature accuracy+(+-)2

iii. Genetic analyser, Fragment size -600bp

Number of markers – 16 for I- filer 17 For Y-filer, Polymer-POP4

Oven Temp -60^{0}C, Column Size-36cm

Software- Gene Mapper

Steps used in analysis

Extraction of DNA from Blood: Routine organic extraction i.e. Phenol/Chloroform extraction approach is a sensitive method for the recovery of DNA from wide variety of forensic samples [8]. This method was used for extraction of DNA from blood samples. In this method, samples were lysed using Forensic Buffer (pH8), Proteinase K and Sodium Dodecyl Sulphate. It was incubated for 2 hrs at 56^{0}C and Phenol: Chloroform: Isoamyl alcohol added. The aqueous layer containing DNA separated and treated with 2 M Sodium Acetate and the DNA was precipitated using chilled Isopropanol. Precipitated DNA finally dissolved in TE buffer (pH7). This DNA was further used for PCR and STR Genotyping. Simultaneous amplification of 16 STR Loci was completed and analyzed [9,10]. DNA profiles were interpreted by comparing with each other.

We had seen mutation at one allele among son and father in above mentioned both cases. To confirm profiles re-extraction carried on Automate Express machine using Prep Filer TM Express DNA extraction kit. The Prep Filer TM Forensic DNA extraction Kit (Applied Biosystems, Foster City, CA) enables the isolation of DNA from a variety of biological samples that contain small quantities of biological mater4ial in such a way that substances that interfere with PCR are removed. The Prep Filer TMK it was designed specifically to support both manual and automated extraction of DNA from forensic samples [11,12].

Extraction of DNA from Bone: Extraction of DNA from sternum and femur bone was carried out on Automate express Machine using Prep Filer Express BTA kit. Lysis of sternum and femur bone powder (50mg) was performed using 220µl of PrepFilerBTATM Lysis buffer. Then 1 M DTT and 7 µl Proteinase K is added. The Lysis mixture was incubated at 560C for 18 hrs at 1100 rpm using thermoshaker. After lysis the tube containing bone sample was centrifuged for 2 min at 10,000 x g and then the supernatant was transferred to the PrepFiler™ sample tube. Then DNA was extracted on Automate Express machine using the Prep Filer BTATM instrument protocol.

Quantification of DNA

Extracted DNA was quantified using Quantifier human DNA kit on 7500 Real Time PCR System (Applied Biosystems) according to the protocol. Proper diluted DNA sample was used for further PCR reaction.

Polymerase chain reaction

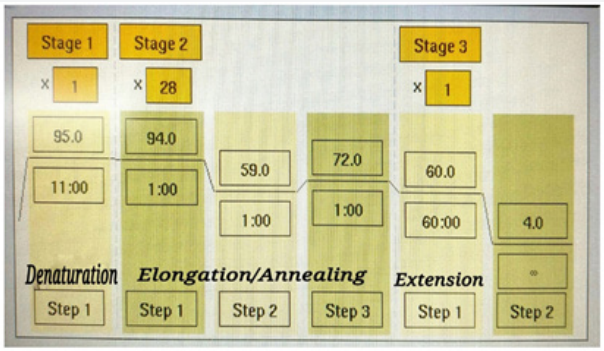

Figure 1: PCR Protocol for AmpFlSTR®Yfiler® PCR amplification kit.

Quantified DNA of all the blood and bone samples from both the cases was processed for PCR using AmpF*I*STR®Identifiler® PCR amplification kit [13] and AmpF*I*STR®Yfiler™ PCR amplification kit on Veriti Thermal Cycler of Applied Biosystems (Figure 1 & 2). AmpF*I*STRTMIdentifiler™ primers amplify

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the STR loci CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX, vWA and gender marker Amelogenin. Yfiler enables simultaneous amplification of 16 Loci on the Y-chromosome, namely DYS456, DYS3891, DYS390, DYS38911, DYS458, DYS19, DYS385a/b, DYS393, DYS391, DYS439, DYS635, DYS392, Y GATA H4, DYS437, DYS438 and DYS448.

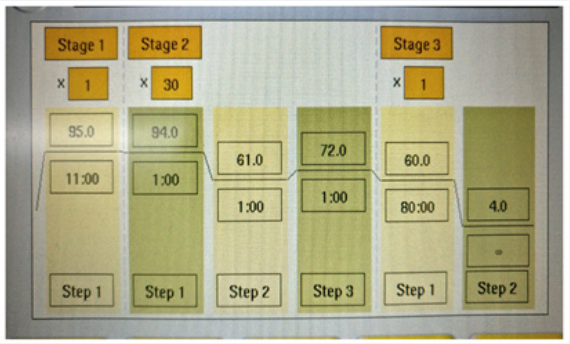

Figure 2: PCR Protocol for AmpF/STR®Yfiler® PCR amplification kit.

Master mix used for Polymerase Chain Reaction was-

a) AmpF*I*STR PCR reaction mix: 10.5 µl

b) AmpF*I*STR Primer Set: 5.5 µl

c) Polymerase: 0.55 µl and 0.8 µl for Y DNA profiling.

d) Volume of Master mix used: 15 µl

e) Volume of sample: 10 µl

f) After PCR amplification Denaturation carried out using HiDi Formamide and Liz 600 size Standard.

STR genotyping

PCR produces millions of DNA fragments of different sizes. Amplified products were separated and detected using 3500 Genetic Analyzer [14] and analyzed using GeneMapper® ID-X Software V 1.5 according to manufacturer recommended procedure. The separation of different fragments of DNA molecules on the basis of their size was achieved by capillary electrophoresis. Simultaneous amplification of 16 STR Loci was completed and analyzed [9,10]. DNA profiles were interpreted by comparing with each other.

Results and Discussion

In case one The DNA extracted from blood sample and bone was typed at 15 STR LOCI and gender specific Amleogenin locus using PCR Amplification technique.

Table 1: STR Genotyping by using Identifier kit in case number 1.

STR LOCUS	Genotype		
	Blood Mother/ Victim	Femur bone of Baby of Victim	Blood Accused (Putative Father)
D8S1179	13,16	13,13	13,13
D21S11	29,30	29,29	29,32.2
D7S820	10,11	11,12	11,12
CSF1PO	7,12	7,12	12,12
D3S1358	15,18	15,16	15,16
THO1	9,9	9,9.3	9.3,9.3
D13S317	9,12	9,10	10,13
D16S539	8,13	8,11	11,13
D2S1338	19,22	18,19	18,23
D19S433	12,15	15,15.2	12,15.2
vWA	15,17	15,16**	18,19**
TPOX	8,9	8,9	9,11
D18S51	16,17	16,17	14,16
AMELOGENIN	X, X	X, Y	X, Y
D5S818	11,11	11,12	11,12
FGA	19,25	19,24	24,25

Table 1 Shows the Nuclear DNA profile of accused, victim and femur bone of baby. Out of 15 different genetic systems analyzed with PCR, accused matched the obligate paternal alleles present in femur bone of baby at 14 STR loci except one locus 'vWA' which may be due to mutation. As per two exclusion rules in parentage testing, we cannot exclude father of child as true father if he excludes at two genetic loci. However, for mother matched at all 15 STR Loci.

In Table 2, case number 2, out of 15 different genetic systems analyzed with PCR accused matched the paternal alleles with baby at 14 STR Loci except 'vWA'. Here also chances of mutation cannot be ruled out. Mother matched maternal alleles at all 15 STR Loci.

In above both cases, to rule out the paternity, we had taken support of Y DNA profiling to prove whether the progeny of child

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and father is same. This type of analysis can establish paternal lineage though mutation observed in nuclear DNA profile. In both the cases (Table 3 & 4) Y DNA profile (male haplotypes) obtained from biological sample of baby matched with Y DNA profile (male haplotypes) obtained from blood sample of father.

Table 2: STR Genotyping by using Identifier kit in case number 2.

STR LOCUS	Genotype		
	Blood Victim	Blood of Baby	Blood Accused (Putative Father)
D8S1179	10,14	12,14	9,12
D21S11	28,29	29,31.2	28,31.2
D7S820	11,12	12,12	10,12
CSF1PO	10,12	10,12	12,12
D3S1358	15,15	15,15	15,16
THO1	7,9.3	7,9	8,9
D13S317	13,14	11,13	8,11
D16S539	10,12	10,10	10,11
D2S1338	18,19	19,23	18,23
D19S433	12,15.2	15.2,16.2	14.2,16.2
vWA	14,16	14,18**	17,19**
TPOX	8,8	8,8	8,8
D18S51	12,18	12,16	13,16
AMELOGENIN	X, X	X, Y	X, Y
D5S818	11,13	11,11	10,11
FGA	19,23	19,21	20,21

Conclusion

Table 3: Y DNA profile in case number 1.

YSTR LOCUS	Haplotypes	
	Femur Bone of Baby	Blood (Putative Father)
B_DYS456	16	16
B_DYS3891	13	13
B_DYS390	22	22
B_DYS38911	31	31
G_DYS458	17	17
G_DYS19	13	13
G_DYS385	11,18	11,18
Y_DYS393	14	14
Y_DYS391	10	10
Y_DYS439	12	12
Y_DYS635	23	23
Y_DYS392	14	14
R_Y_GATA_H4	12	12
R_DYS437	14	14
R_DYS438	11	11
R_DYS448	19	19

As in both the cases, mutation was observed at one locus between baby and father, that locus was same in both cases which was 'vWA' (Table 1 & 2). Being baby was male, we had an option of Y-DNA profiling to confirm the mutation and it was found valuable technique to come over the conclusion that the baby belongs to same progeny of the father (Table 3 & 4). It means the father cannot be excluded as biological father of baby. Analysis of 15 autosomal STR markers in parentage issues may not be sufficient for conclusive results in all the cases. So, the analyst should either take the support of kits having more than 15 Loci or should use the kits like Y-STRs to ascertain the results.

Table 4: Y DNA profile in case number 2.

YSTR LOCUS	Halotypes	
	Blood Baby (DNAnk-175/18	Blood (Putative Father)
B_DYS456	15	15
B_DYS3891	14	14
B_DYS390	23	23
B_DYS38911	31	31
G_DYS458	18	18
G_DYS19	14	14
G_DYS385	14,16	14,16
Y_DYS393	14	14
Y_DYS391	10	10
Y_DYS439	12	12
Y_DYS635	Off ladder	Off ladder
Y_DYS392	11	11
R_Y_GATA_H4	12	12
R_DYS437	14	14
R_DYS438	10	10
R_DYS448	19	19

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