

Application of DNA Fingerprinting Technology in Forensic Investigation

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Abstract- Every individual in the world can be identified at the molecular level on the basis of an extremely high level of polymorphism in the sequence of his or her DNA, which he or she inherits from his or her biological parents and is identical in every cell of the body. DNA fingerprinting, as this technique of identification is called, can confirm with certainty the parentage of an individual. The application of DNA profiling in the criminal justice system is an important issue in criminal investigations today. The technology is changing rapidly and several new techniques are becoming available. DNA profiling has been described as a powerful breakthrough in forensic science. The Forensic Use of DNA Profiling is a major contribution to a technology which can help not only in including the culprit but also to exclude the innocent. In this article an attempt is made to elaborate the changing scenario of the technology in the recent years as well as to present some real cases where different variants of the DNA fingerprinting technology were successfully applied in solving the criminal cases in our laboratory.

Index Terms- DNA, Genotyping, STR's, Y DNA, mt DNA

I. INTRODUCTION

The human genome with 3 billion base pairs in size harbors genetically relevant information, which is essential for each individual but appears to represent only 10 % of the human genome. This minor part of the gene-coding DNA has been subjected to evolutionary pressures and selection mechanisms ensuring the development of higher organized organisms. The engine driving the process is the non-directed mutations, which are maintained when the generation of a neutral or improved ability is successful while negative mutations normally get lost. The so-called non-coding regions of the human genome are not regulated by these rules of selection and maintenance as long as these are not affecting the survival capacities of the individual. This is the reason for the accumulation of mutations leading to the generation of a genetic diversity within non-coding genomic DNA. The notable exceptions are polymorphisms in gene-coding regions, which reveal a high genetic stability combined with a very low mutation frequency.

A special part of non-coding DNA is comprised of repetitive sequences. Highly polymorphic spots in these non-coding regions are mini- or micro- satellites characterized by repeating DNA blocks, each of which contains up to several hundreds of base pairs in size (1). The single-locus satellites are localized at a specific site of a given human chromosome, while multi-locus satellite elements of short tandem repeats (STRs) are

spread throughout the entire genome. The widespread use of Short Tandem Repeat (STR) technology in forensic caseworks has resulted in the successful DNA typing of a wide range of forensic samples (2-6). This success is partly due to the availability of polymorphic STR loci in the human genome (7,8) and the relatively short (500 base pairs or less) lengths of amplified Polymerase Chain Reaction (PCR) amplicons.

STR markers were first described as effective tools for human identity testing in the early 1990s (9,10). The variable number of tandem repeats (VNTR) defines the length of the individual-specific "alleles", which can be examined now by PCR techniques. STR typing is typically performed using size comparisons with standardized allelic ladders that possess the most common alleles, which have been sequenced to reveal the true number of repeats. Different STR kit manufacturers supply allelic ladders with slightly different allele ranges. **At present sixteen validated forensic markers are used in a forensic DNA report. Multiplex PCR amplifications kits are available from Applied biosystems Foster City, CA and Promega Corpn.**

Amplification of compromised DNA samples including samples exposed to harsh environmental conditions, skeletal remains of missing persons, or human remains from mass disasters can result in a partial or no genetic profile. This loss of signal may be the result of either PCR inhibitors which co-extract with forensic evidence or a fragmented DNA template. These factors can impact the ability to obtain probative information from large multiplexes producing a wide range of PCR products. The AmpFISTR® MiniFiler™ PCR Amplification Kit delivers results when other traditional methods produce little to no results. Casework and missing person laboratories now have a tool that facilitates the analysis of degraded samples and results in fewer allele dropouts, reducing the need for repeat analysis. Degradation of forensic samples occurs over time due to bacterial, biochemical or oxidative processes. The development and introduction of truncated PCR amplicons or "mini STR" technology shows remarkable promise for use in forensic casework applications. In 2007, Applied Biosystems released the first commercially available miniSTR multiplex, the AmpFISTR® MiniFiler™ PCR amplification kit (11). MiniFiler™ has the capability to elucidate genotypes from the eight largest loci (D13S317, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO, and FGA, as well as Amelogenin) contained within the Identifiler™ PCR amplification kit.

The analysis of Y-chromosomal short tandem repeats (Y STRs) is a powerful tool for analyzing mixed forensic stains and for paternity testing. Paternity cases involving the common trio constellation of mother, offspring and alleged father can usually

be solved with STR's alone, and do not seem to require any additional or alternative markers. If a father/son relationship is to be tested, Y str markers are useful .The Y chromosome is found only in males, and therefore genetic markers along the Y chromosome can be specific to the male portion of a male–female DNA mixture such as is common in sexual assault cases. Y chromosome markers can also be useful in missing person's investigations, some paternity testing scenarios and historical investigations, because of the fact that most of the Y chromosome (barring mutation) is passed from father to son without changes. Multiplex PCR amplifications kits are available from Applied Biosystems, Foster City,CA. and Promega Corpn.

However, if a father/ daughter parentage is in question, it may be worthwhile using also X chromosome (ChrX) markers for testing. Fathers transmit their X-chromosome to daughters as haplotypes. Analysis of X-chromosomal loci might be beneficial in deficiency paternity cases, where half-sisters and/or grandmothers are examined. If a father/son relationship is to be tested, ChrX markers are not useful at all. For testing mother–daughter relationships, ChrX markers are similar to autosomal STR markers and do not provide any specific advantage. Testing mother–son kinship, however, is more efficiently performed using ChrX markers. The exclusion chance in such cases is identical to that of ChrX STRs in father/daughter tests (12). The commercially available Mentype Argus kit (Biotype AG, Dresden, Germany) makes it possible now to examine eight different linkage groups in one multiplex reaction.

The mitochondrial DNA (mt DNA) analysis is also being used in forensic investigation. The mt DNA is important because all mothers have the same mt DNA as their daughters, because of the fact that the mitochondria of each new embryo come from mother's egg cell whereas the father's sperm contribute only the nuclear DNA. The mt DNA is present in numerous copies per cell and is applicable when nuclear DNA is extremely degraded as in air crashes where high temperatures degrade the nuclear DNA. The second reason is that mt DNA is 100% maternally inherited; therefore during identification of body the mt DNA of remains of the body can be easily compared with that of the mother or maternal uncles of the victim (13). Mitochondrial DNA kit is available from Applied Biosystems, Foster City,CA.

We provide below some real cases where different variants of the DNA fingerprinting technology were successfully applied in solving the criminal cases in our laboratory.

Case 1

This was the first case solved by the DNA fingerprinting Unit MP proving the fact that the DNA technology is not only to apprehend the guilty but to save the innocent. A physically challenged unmarried girl gave birth to a child. The girl was supposed to have indicated by sign language the alleged father of her new- born child. The child died a few days after birth. The referral blood samples taken from the mother (the complainant) as well as from the alleged father and femur bone with adhering tissues of the dead child were received in our lab. DNA profile of all three samples was generated. The comparison of the DNA profile of the dead child, the mother and the alleged father proved conclusively that the alleged father of the child is not the biological father of the dead child, thereby proving the allegation

of fathering the dead child to be false. The child's profile had an allele from its mother on all forensic STR markers but not so from the alleged father, thus proving this case to be one of exclusion. This finding was further confirmed by the Y (Male) DNA profile of the suspected father and the child, as the child was the male child (confirmed by the XY on Amelogenin loci). The child was found paternally unrelated with the alleged father.

Case 2

Burnt skeletal remains of a young person were found on the roof of a house. There was no other clue available to prove the identity of the deceased. Face and body was found to be burnt only teeth and few bones were seen. A vigilant doctor who examined the remains carefully preserved postmortem blood, burnt tissue, femur bone and a few teeth which were referred to our lab for analysis. The referral blood samples of an elderly couple (who could not identify the remains but suspected the remains to be of their missing son) were also sent to the lab. All the samples received here yielded amplifiable DNA. Comparative analytical studies on the DNA profile obtained from the blood samples of the couple and the profile generated from burnt forensic samples confirmed the remains to be those of the son of the couple (Table 1).

Table 1: Results obtained from the available samples by using AmpflSTR® identifier kit in case 2.

Genetic Markers	DNA profile generated from referral blood sample of Father	DNA Profile Generated from Femur Bone	DNA profile generated from referral blood sample of Mother
D8S1179	14, 15	14,16	15,16
D21S11	30, 30	30, 33.2	30, 33.2
D7S820	8, 12	9, 12	9,12
CSF1PO	9, 10	10, 10	10,12
D3S1358	16,16	17,16	17,17
TH01	8, 9	9, 9	9, 9.3
D13S317	10, 12	8, 10	8,12
D16S539	12,13	13, 12	11,12
D2S1338	20, 23	23, 18	18, 18
D19S433	13, 14	14, 15.2	15.2,15.2
vWA	14, 14	14, 16	16,17
TPOX	8, 11	8, 8	8, 8
D18S51	13,17	17, 15	15,19
D5S818	11,12	11, 12	10,12
FGA	24, 25.2	24, 25.2	22.2 , 24
AMELOGENIN	X Y	X Y	X X

Note- Forensic DNA test is based on the fact that the child shares the allele from his parents. For example on Ist loci tested i.e. on D8S1179 the child is 14,16, one of these two allele has to come from father (14 in this case) and the other one from his mother(16 in this case). This has to be tested on all the 16 loci

in the kit. One or two mismatch in this test shows exclusion. But this needs further confirmation by Y DNA, mt DNA or X DNA kit/markers, as per the requirement or availability of the referral samples.

Case 3

In a case a mandible, scapula and few teeth were recovered near a petrol pump near a highway. A person from the staff was missing since last couple of years. During investigation the referral blood samples of father was sent to DNA lab along with the mandible, scapula and teeth. mandible and scapula were not found suitable for DNA examination. DNA was isolated from teeth but only partial DNA profile could be generated with AmpfISTR® identifier kit. By this time the we got the AmpfISTR® MiniFiler™ kit from Applied Biosystems. The remaining loci were amplified and confirmed by using this kit (Table 2) and it became possible to fix the identity of few teeth.

Table 2: Results obtained from the degraded sample with the AmpfISTR® identifier and AmpfISTR® MiniFiler amplification kit in case 3

Genetic Markers	DNA Profile Generated from Few Teeth	DNA profile generated from referral blood sample of Father
D8S1179	<u>12,12</u>	<u>10,12</u>
D21S11	<u>29,31,2</u>	<u>29,31,2</u>
D7S820	<u>10,12*</u>	<u>8,12</u>
CSF1PO	<u>10,12*</u>	<u>12,12</u>
D3S1358	<u>14,16</u>	<u>15,16</u>
THO1	<u>8,9</u>	<u>6,8</u>
D13S317	<u>11,11</u>	<u>8,11</u>
D16S539	<u>10,13</u>	<u>9,13</u>
D2S1338	<u>19,20</u>	<u>17,20</u>
D19S433	<u>13,16,2</u>	<u>14.2,16.2</u>
vWA	<u>15,18</u>	<u>16,18</u>
TPOX	<u>9,11</u>	<u>11,11</u>
D18S51	<u>15,16*</u>	<u>14,15</u>
D5S818	<u>11,13</u>	<u>11,13</u>
FGA	<u>20,24</u>	<u>24,25</u>
AMELOGENIN	<u>XY</u>	<u>X Y</u>

*low peaks in the profile confirmed by AmpfISTR® MiniFiler™ amplification kit

Note: In this case as the referral sample of mother was not available so for deciding the case conclusively Y DNA profile of the both were also matched to confirm patrilineal relatedness.

Case 4

Another case pertains to an even more complicated & precise forensic DNA profiling examination. A young girl committed suicide. Her postmortem examination revealed that

her uterus was suspected to be gravid (6-8 weeks). The examining doctor had put a question mark against the uterus examination column. The dead girl’s uterus, referral blood samples of the two suspects- a young neighbor and the dead girl’s father were sent to the DNA unit, for examination. On opening the uterus, the tiny fetus could not be discerned as the inner mass had turned pulpy due to the sample not being preserved as per DNA examination guidelines. However, careful sampling from the endometrial lining as also from the outer wall of the uterus was done. But when autosomal STR DNA profile was generated it was found to be the same female. The DNA from the endometrial lining was checked for the presence of Y DNA with AmpfISTR® Y filer amplification kit. A Y profile was generated. Y DNA profiles using the same kit were also generated from the referral blood samples of both the accused. A careful study of the DNA profiles thus obtained revealed that, in fact, the maternal grandfather was the father of this foetus (in his dead daughter’s Uterus). The other person accused of being the father of the unborn fetus was thus exonerated of a crime he never committed.

Case 5

In most of these cases, the victim is from a cast different from that of the perpetrator, and interestingly, most of these allegations have been proved to be false post- DNA profiling examination.

For example, an allegation was made by a lady of a different cast against two Gurjar youths of having raped her. The lady was working as an employee of another Gurjar land-owner. On analysis of vaginal smear samples and clothing of the victim and the referral blood samples of the two accused, it was found that the source of male DNA present in the private part and the clothing of victim was not of the two accused (their STR profile was different from the STR profile of the male found in the samples of the victim).

Interestingly, this male (whose presence has been confirmed in the private parts of the victim) shares a similar Patrilineal Y-chromosome profile as found on the Y chromosome profile of both accused. That is to say, the male fraction found on the person of the complainant is of a male related to the accused patrilinealy.

Case 6

A case of suspected child swapping in the Govt. hospital was received at DNA fingerprinting laboratory, Sagar for establishing the paternity of the questioned child. A woman gave birth to a child in a Government Hospital. Her family members claimed that the child born to the woman was a male but the child handed over to them was female. Referral blood sample of both parents as well as of the female child were referred to DNA Fingerprinting unit to unravel the truth. DNA was isolated by using FTA paper and phenol chloroform extraction procedure, DNA fragments were amplified by using AmpfISTR® identifier kit and the analysis was done by Genemapper software version 3.5 using 3100 Sequencer supplied by Applied Biosystems. The genotyping results showed a complete match of the newborn child with both parents except on vWA locus with mother. At vWA locus the father and daughter were homologous 16, while on the same locus mother was homologous 17, thereby

showing a mismatch. With the data of genotyping it was not possible to decide the paternity of the child conclusively. Therefore to confirm the paternity of the child mitochondrial DNA sequencing of control region was performed. The mitochondrial DNA sequence of mother and child showed complete matching. Thus, the female child was found to be the biological daughter of the couple proving their allegation against the hospital administration to be false.

Thus from the above illustrative cases, it is obvious the DNA profiling is a tool that is not only used to apprehend the guilty but also to exonerate the innocent.

As it often happens in the justice delivery system, conventional evidence can be tempered with, witnesses turn hostile, but DNA evidence remains the same. The Passage of time does not affect it and neither does is change. DNA evidence thus unravels the truth-it never lies.

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