



## Application of DNA Typing Technologies in Forensic Medicine

### Adli Tıpta DNA Tipleme Teknolojilerinin Uygulanması

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#### ABSTRACT

Nuclear DNA markers are widely used for crime investigation and paternity testing. Parentage testing interpretation relies on the fact that Short Tandem Repeats are inherited in a true Mendelian fashion and express a codominant nature of allelic variants. DNA microsatellites or Short Tandem Repeats are short, tandemly repeated sequences of a bi-, tri- or tetranucleotide unit with a random distribution throughout the genome. They have been used extensively in applications as diverse as diagnosis of inherited diseases and forensic medicine for DNA fingerprinting and parentage testing. The success of this last application is due to the fact that Short Tandem Repeats are highly polymorphic and, at the same time, they are sufficiently stable to be inherited unaltered from one generation to the next.

**Key words:** Paternity, forensic medicine, short tandem repeats.

#### ÖZET

Nükleer DNA markırları suç arařtırmaları ve babalık testlerinde sıkça kullanılmaktadır. Babalık testinin yorumlaması kısa bitişik tekrarların (Short Tandem Repeats) gerçek Mendeliyan düzeninde kalıtıldığı ve allelik değışkenlerin eş baskın doğasını tanımladığı gerçeğine dayanır. DNA mikrosatellitleri ya da kısa bitişik tekrarlar genom boyunca rastgele dağılmış ikili, üçlü, ya da dörtlü nükleotid ünitelerinin birbiri ardısına tekrarlayan kısa serileridir. Bunlar kalıtılmış hastalıkların tanısından DNA parmak izi tespiti ve babalık testi gibi adli tıp uygulamalarına kadar geniş bir uygulama alanı bulmuştur. Babalık testi uygulamalarındaki başarı kısa bitişik tekrarların yüksek oranda polimorfik olmakla birlikte aynı zamanda yeterince istikrarlı olup bir nesilden diğerine değışmeden kalıtılmasına bağlıdır.

**Anahtar Kelimeler:** Babalık, adli tıp, kısa bitişik tekrarlar.



## Introduction

Human leukocyte antigen (HLA) is the major histocompatibility system in man, and has an extreme diversity of antigens in several closely linked serologically and molecular detectable genetic loci. The practical use of HLA in paternity cases is confined to typing of two loci, HLA-A and HLA-B. The system is one of codominant genes, so two antigens may be present for each locus. A tissue-typing laboratory that can detect most or all of these specificities can type for about 300 haplotypes with as many as 100,000 genotypes.

Tissue typing is recognized throughout the world as the most discriminating test for determination of nonpaternity. Because of the large number of antigens involved in one genetic system of closely linked loci and the scattered distribution of these antigens in the population, the chance of exclusion with HLA typing alone is at least 91% and is 95% in combination with ABO and Rh typing. Utilization of HLA typing in paternity cases in the world will continue to increase rapidly as education about the extreme usefulness of such tests becomes more widespread and legislation is altered accordingly<sup>1</sup>.

Serological HLA typing and other testing yielded consistent results in 37 of these 39 cases, exclusion of paternity could be confirmed only by serological HLA typing in 1 case and only by other testing in 1 case. Even in cases where exclusion could not be confirmed, it was possible to increase the probability of paternity. Blood stains with known HLA types were then subjected to lymphocytotoxic inhibition tests to detect HLA antigens using one of the following methods: a two-stage method using an antibody and a bloodstain extract; a one-stage method where a bloodstain was allowed to react directly with an antiserum; or a washing method where washing was combined with one of the two above methods. Some antisera yielded false-positive reactions, which limited the range of usable antisera. With the exception of cross-reactions, the washing method eliminated false-positive reactions. Antigens were detectable in bloodstains that were left to stand for up to 42 days<sup>2</sup>.

DNA typing was investigated by determining DR and DQB1 types using the Hot Start PCR-SSP method using various samples of known HLA types and tissue samples collected from unidentified corpses. The samples could be bloodstains had been stored for 10 to 20 years, saliva stains, hair samples (root samples and hair shaft samples), cigarette butts and lipstick did not affect the results of DNA typing. Although DNA typing was possible in many cases

using tissue samples collected from unidentified corpses, the results were affected by factors such as status of corpse, postmortem interval, postmortem change and tissue type<sup>2</sup>.

This review is shedding a light on the task of DNA and its important application in forensic medicine and determining individual identity in paternity testing and crime scene. It also showed the development in this field.

## **Role of DNA in Forensic Medicine**

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. Thousands of cases have been closed and innocent suspects freed with guilty ones punished because of the power of a silent biological witness at the crime scene. 'DNA fingerprinting' or DNA typing (profiling) as it is now known, was first described in 1985 by an English geneticist named Alec Jeffreys<sup>3</sup>.

Dr. Jeffreys found that certain regions of DNA contained DNA sequences that were repeated over and over again next to each other. He also discovered that the number of repeated sections present in a sample could differ from individual to individual<sup>4</sup>. By developing a technique to examine the length variation of these DNA repeat sequences, Dr. Jeffreys created the ability to perform human identity tests. These DNA repeat regions became known as VNTRs, which stands for variable number of tandem repeats. The technique used by Dr. Jeffreys to examine the VNTRs was called restriction fragment length polymorphism (RFLP)<sup>5</sup>.

The past 15 years have seen tremendous growth in the use of DNA evidence in crime scene investigations as well as paternity testing. Technologies used for performing forensic DNA analysis differ in their ability to differentiate two individuals and in the speed with which results can be obtained. In addition to that tiny amounts of sample, as little as a single cell in some cases, can yield a useful DNA profile<sup>6</sup>. DNA can be extracted from minute or degraded material because DNA is physically much more resistant to degradation than proteins. In addition, the same DNA genotype can be obtained from any tissue (i.e., blood, saliva, semen, hair, skin, bones)<sup>2</sup>.

## **DNA Polymorphisms**

Within each nucleated human cell there are two complete copies of the genome. The genome is 'the haploid genetic complement of a living organism' and in humans contains

approximately 3 200 000 000 base pairs (bp) of information, which is organized into 23 chromosomes. Humans contain two sets of chromosomes – one version of each chromosome inherited from each parent giving 46 chromosomes. Great advances have been made in our understanding of the human genome in recent years, in particular through the work of the Human Genome Project that was officially started in 1990 with the central aim of decoding the entire genome. It involved a collaborative effort involving 20 centers in China, France, Germany, Great Britain, Japan and the United States. A draft sequence was produced in 2001 that covered 90% of the euchromatic DNA <sup>7</sup>. The genome can be divided into different categories of DNA based on the structure and function of the sequence; first is the “noncoding” parts that represent the great majority (more than 90%) of the human genome because they do not contain genetic information directly relevant for protein synthesis. The second part is “coding”, the genetic variation in this part is rather limited with the exception of the HLA region. This is the result of the fact that expressed genes are subjected to evolutionary or selection pressure during evolution to maintain their specific function <sup>8</sup>. In contrast, the noncoding part of the genome is not mainly controlled by selection pressure, and thus mutations in these regions are usually kept and transmitted to the offspring, leading to a tremendous increase in genetic variability<sup>7</sup>.

With advances in molecular biology techniques, it is now possible to analyze any region within the 3.2 billion bases that make up the genome. DNA loci that are to be used for forensic genetics should have some key properties, they should ideally be highly polymorphic, be easy and cheap to characterize, give profiles that are simple to interpret and easy to compare between laboratories, not be under any selective pressure and have a low mutation rate<sup>9</sup>.

An important percentage of the noncoding DNA (30%) consists of repetitive sequences that can be divided into two classes: tandemly repetitive sequences and interspersed elements. Tandemly repeated sequences can be found in satellite DNA, but from the forensic point of view, regions of repetitive DNA much shorter than satellite DNA are much more interesting. These regions can be classified into minisatellites and microsatellites or short tandem repeat (STRs) <sup>10</sup>. Minisatellites, otherwise known as variable number of tandem repeats (VNTR) loci<sup>11</sup>, are composed of sequence motifs ranging from around 15 to 50 bp in length, reiterated tandemly for a total length of 500 bp to 20 Kb. The repeat unit ranges from 2 to 6 bp for a total length between 50 and 500 bp. In addition, minisatellites and STRs have differences in their distribution in the human genome and probably in their biological function. Thus,

minisatellites are more common in subtelomeric regions, whereas STRs are widely distributed throughout the human genome, occurring with a frequency of one locus every 6–10 kb<sup>12</sup>. Although unequal crossing over and even gene conversion<sup>13</sup> are involved in the variability of minisatellites, replication slippage is mainly involved in the origin of the variability in microsatellites<sup>14</sup>.

VNTRs were the first polymorphisms used in DNA profiling and they were successfully used in forensic casework for several years. Interpreting VNTR profiles could also be problematic. Their use in forensic genetics has now been replaced by short tandem repeats<sup>15</sup>.

### **Single Nucleotide Polymorphisms (SNPs)**

One of the most significant outcomes of the Human Genome Project has been the identification of large numbers of single nucleotide polymorphisms (SNPs)<sup>16</sup>. The simplest type of polymorphism is the SNP; single base differences in the sequence of the DNA. SNPs are formed when errors (mutations) occur as the cell undergoes DNA replication during meiosis<sup>17</sup>. However, SNPs are so abundant throughout the genome that it is theoretically possible to type hundreds of them. This will make the combined power of discrimination very high<sup>18</sup>. SNPs have not been used widely in forensic science to date, and the dominance of tandem repeated DNA will continue for the near future<sup>19</sup>. The great advantage STRs have over SNPs is their frequency of occurrence once every 15 Kb, typical rate of mutation  $10^{-3}$ , typical number of alleles usually between 5 and 20, a maximum of 15 STR loci examined at one time and method of detection is capillary gel electrophoresis<sup>9</sup>. Therefore, the application of SNP in forensic medicine is conserved.

The vast majority of forensic DNA analysis involves the characterization of biological material recovered from the scene of a crime. Several panels of SNPs have been developed that are designed to provide maximum discrimination powers for forensic identification<sup>20</sup>. The application of SNPs to specialized applications, for example, SNP based blood groupin<sup>21</sup> and molecular autopsy (looking for mutations that can explain sudden death is likely to become more widespread<sup>22,23</sup>).

### **Epigenetics Applications in Forensic Medicine**

Epigenetics is the study of heritable changes in gene expression other than changes in the underlying DNA sequence. Such changes include DNA methylation, histone modification,

chromatin remodeling, genomic imprinting, X chromosome inactivation and non-coding RNA regulation. Recent progresses on epigenetics open new possibilities in tackling these challenging problems in forensic science, including identification of fetal paternity testing in embryonic period, determination of the necessary allele in paternity testing, discrimination of identical twins, origination analysis of micro tissue, verification of forged DNA<sup>24</sup>.

Genome-wide epigenetic modification plays a pivotal role in regulating gene expression through chromatin structure and stability, tissue-specific and embryonic developmental specific gene regulation, and genomic imprinting. Mechanisms include chromatin remodeling through histone modification and DNA methylation, RNA associated gene silencing and chromosome inactivation, and genomic imprinting. These epigenetic mechanisms provide an added layer of transcriptional control of gene expression beyond those associated with variation in the sequence of the DNA. Variation in epigenetic regulation helps explain genetic diversity between individuals that used in forensic medicine<sup>25</sup>.

### **DNA-Typing Technique**

Technologies used for DNA typing for forensic purposes differ in their ability to differentiate two individuals and in the speed and sensitivity with which results can be obtained. The speed of analysis has radically improved for forensic DNA analysis. DNA testing that previously took more than 1 week can now be performed in a few hours<sup>6</sup>.

The first technique was used is Southern blotting with multi-locus and single-locus (SLP) DNA probes have been used for paternity testing, and they are still used especially SLPs by a few laboratories working in paternity testing<sup>26</sup>. The disadvantages of this method are require several days for sufficient radioactive decay to produce a visible band on the film and multilocus probes were proposed for forensic genetic analysis. However, this type of probe was not very successful in the forensic field because despite its informativeness, statistical problems of evaluation of the evidence in cases of match and standardization problems arose. For these reasons, this probe was substituted in the forensic field by the analysis of VNTRs using SLPs under high-stringency conditions<sup>27</sup>. The main advantage of SLP analysis is the enormous variability of some of the minisatellites and the adequate knowledge of the mutation rate in some of them. The main disadvantages are the time needed for the analysis and especially the need for the relatively large amount of non degraded DNA required for SLP

typing. Because DNA extracted from forensic specimens is often degraded because of environmental conditions, these techniques have often failed to produce reliable results<sup>6</sup>.

PCR has overcome these difficulties, and it has strongly enhanced the usefulness of DNA profiling techniques in forensic science. PCR is a technique for the *in vitro* amplification of specific DNA sequences by the simultaneous primer extension of complementary strands of DNA. The PCR method was devised and named by Mullis and colleagues at the Cetus Corporation<sup>28</sup>. PCR technique, it is more likely to be successful in analyzing badly degraded material mainly because of the small size of some of the DNA polymorphisms (SNPs and STRs) susceptible to analysis by PCR<sup>29</sup>. A number of creative methods for PCR product detection have been described. The first one was the use of sequence-specific primer (SSP). It is simple, fast and reliable and has high exclusion probability of paternity (66.3%)<sup>30</sup>. In this method gel electrophoresis was done after PCR amplification then followed by sequence specific oligonucleotide (SSO) method<sup>31</sup>. This method used to detect variation in HLA-class II genes, especially in the HLA DQA1 system<sup>32</sup>. An SSO probe is usually a short oligonucleotide (15–30 nucleotides in length) with a sequence exactly matching the sequence of the target allele. The SSO probe is mixed with dissociated strands of PCR product under very stringent hybridization conditions such that the SSO and the PCR product strand will be hybridized if there is a perfect sequence complementary but will not be if there are different sequences. The classical format for the use of SSO probes is to spot dissociated PCR product strands onto a nitrocellulose or nylon membrane and to probe the membranes with labeled SSO; because of the fact that the samples are spotted as a “dot” on the membrane, this format is known as dot blotting. A reverse dot-blot format is much more commonly used<sup>33</sup>. Several genetic loci can be analyzed by this technology using commercially available kits. The AmpliType PM PCR amplification kit (Perkin-Elmer, Foster City, CA) was very popular in forensic laboratories some years ago. With this kit, the loci HLA DQA1, LDLR, GYPA, HBG, D7S8, and GC are amplified in a multiplex fashion. The last five loci listed are typed simultaneously in a single reverse dot-blot strip containing ASO probes; HLA DQA1 must be typed in a separate strip<sup>27</sup>.

The efforts of forensic scientists have mainly addressed the amplification of fragment-length polymorphisms. The minisatellite D1S80 (pMCT118) was the first to be applied to the forensic routine<sup>34</sup>, but these systems have been substituted by STRs. Dinucleotide STRs are the most common STRs in the human genome and are the genetic markers most commonly used for linkage analysis, although they are not being used in forensic science. The reason is that

analysis of these STRs has been affected by strand slippage during amplification, producing artifactual stutter bands<sup>35</sup>. Nevertheless, tetra and pentanucleotide repeats appear to be less prone to slippage and are more suitable for forensic purposes. STRs were firstly analyzed in manual electrophoretic systems. Denaturing polyacrylamide gels are recommended for standardization purposes, given that with native gels sequence variation can also be detected making the typing prone to errors. STR electrophoretic mobility under native and denaturing conditions should also be checked since some STRs (especially AT-rich ones) have been shown to have anomalous mobility in polyacrylamide gels<sup>36</sup>.

The introduction of fluorescent-based technology and the use of DNA sequencers have revolutionized the field, allowing the typing of large multiplexes as well as the automation of the typing. The use of sequence reference allelic ladders is essential for STR typing. In general, the reference allelic ladders comprise most of the alleles of the system, but intermediate alleles are always possible even in the simplest STRs. Interpretation guidelines have been produced<sup>37</sup> to distinguish these intermediate alleles and can be easily implemented in automatic sequencers. There are many multiplexes commercially available. A very popular one is the SGM Plus (Applied Biosystems), which comprises 10 loci- HUMFIBRA/FGA, HUMVWFA, HUMTH01, D18S51, D21S11, D6S477, D8S1179, D16S539, D19S433, and amelogenine. However, the extremely discriminative 15-plexes are becoming more and more popular and, among these, the Poweplex16 (Promega) and the Identifiler (Applied Biosystems) are the more commonly used by forensic labs. STR typing is a reliable and robust tool for analyzing the forensic practice as well as for paternity testing. Short tandem repeats are widespread throughout the human genome and are a rich source of highly polymorphic markers, which can be detected by PCR. To gain a better appreciation for how the polymorphism at a particular locus affects the individual identity, the polymorphism was in 15 STR loci in forensic investigation and paternity testing. Multiplex STR typing was used to study the 15 STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA) in addition to a gender identification marker, amelogenin, by capillary electrophoresis on 310 Genetic Analyzer<sup>38</sup>. These techniques have brought about a revolution in DNA typing methods through increased efficiency and the application of multiplex fluorescence detection. The development of new STR based typing methods utilizing mini- and Y-STR PCR multiplexes has increased the flexibility of the investigator, permitting the analysis of inhibited and degraded DNA<sup>39</sup>.



Using STR genetic markers is effective to identify the paternity<sup>40</sup>. DNA was extracted and typing was possible even when blood stains had been stored for 10 to 20 years. With saliva stains, typing was possible in all cases by purifying DNA using a Microcon. With hair samples, typing was possible with all hair root samples and about half of hair shaft samples. With cigarette butts, as was the case with saliva stains, typing was possible using purified DNA. Lipstick did not affect the results of DNA typing. In addition to that, teeth should be considered for DNA analysis as they are rich sources of quality DNA which can be utilised in all forensic investigations<sup>41</sup>. Although DNA typing was possible in many cases using tissue samples collected from unidentified corpses, the results were affected by factors such as status of corpse, postmortem interval, postmortem change and tissue type. The results of typing were also favorable using bone samples<sup>42</sup>. Advances in ability to dissect the human genome and the availability of platforms for genome-wide analysis and whole-genome sequencing are expected to develop new tools for both biomedical and forensic DNA analyses<sup>43</sup>. DNA typing can individualize single cells left at the crime scene or analyze ancient human remains. Such advances in genetics, genomics and molecular biology are likely to improve human forensic casework<sup>44</sup>.

### **Role of RNA in Forensic Medicine**

With the development of molecular biology, the evidences of genetics has been used widely in forensic sciences. DNA technology has played an important role in individual identification and paternity testing, RNA technology is showing more and more wide application in prospect. The application and progress of RNA in forensic science including estimation of postmortem interval, bloodstain age, wound age, as well as determination of cause of death and the source of body fluids<sup>45</sup>.

### **Conclusions**

DNA is an important tool to identify identity of individuals in crime scene even from degraded specimens and paternity testing by different methods considering time , speed, sensitivity and cost of method that used. With the development of molecular biology, epigenetic and RNA technology were used in forensic science.

## Kaynaklar

1. Mayr WR. The HL-A system in paternity testing (Das HLA System in der Paternitatsserologie). *Z Rechtsmed.* 1974; 75:81-103.
2. Kiuchi M. Application of the HLA system to forensic medicine--from serology to DNA polymorphism. *Nihon Hoigaku Zasshi.* 2002; 56:229-35.
3. Jeffreys AJ, Wilson V, Thein SL. Individual specific finger prints of human DNA. *Nature.* 1985; 316:76-9.
4. Jeffreys AJ, Wilson V, Thein SL. Hypervariable mini satellite regions in human DNA. *Nature.* 1985; 314:67-73.
5. Jeffreys AJ, Wilson V, Thein SL, Weatherall DJ, Ponder BA. DNA finger prints and segregation analysis of multiple markers in human pedigree. *Am J Hum genet.* 1986; 39:11-24.
6. Butler JM. *Forensic DNA Typing Biology, Technology and Genetics of STR Markers*, 2nd edition, London, Academic Press, 2005.
7. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG et al. The sequence of the human genome. *Science.* 2001; 291:1304-51.
8. Waterston RH, Lindblad-Toh K, Bimney E, Rogers J, Brent FS, Collins FS et al. Initial sequencing and comparative analysis of the mouse genome. *Nature.* 2002; 420:520-62.
9. Goodwin W, Linacer A, Hadi S. *An Introduction to Forensic Medicine.* England, Wiley, 2007.
10. Litt M, Luty JAA. hypervariable minisatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Am J Hum Genet.* 1989; 44:397-401.
11. Nakamura Y, Leppert M, O'Connell P, Wolff R, Holm T, Culver M et al. Variable number of tandem repeats (VNTR) markers for human gene mapping. *Science.* 1987; 235:1616-22.
12. Beckman JS, Weber JL. Survey of human and rat microsatellites. *Genomics.* 1992; 12:627-31.
13. Jeffreys AJ, Tamaki K, MacLeod A, Monckton DG, Neil DL, Armour JAL. Complex gene conversion events in germline mutation at human minisatellites. *Nat Genet.* 1994; 6:136-45.
14. Di Rienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, Freimer NB. Mutational processes of simple-sequence repeat loci in human populations. *Proc. Natl. Acad. Sci. U S A.* 1994; 91: 3166-70.
15. Jeffreys AJ, Brookfield JF, Semeonoff R. Positive identification of an immigration test-case using human DNA fingerprints. *Nature.* 1985; 317:818-9.
16. Miller RD, Phillips MS, Jo I, Donaldson MA, Studebaker JF, Addelman N et al. High-density single-nucleotide polymorphism maps of the human genome. *Genomics.* 2005; 86:117-26.
17. Thorisson GA, Stein LD. The SNP Consortium website: past, present and future. *Nucleic Acids Res.* 2003; 31:124-7.

18. Gill P. An assessment of the utility of single nucleotide polymorphisms (SNPs) for forensic purposes. *Int J Legal Med.* 2001; 114:204-10.
19. Gill P,Warrett DJ, Budowel B, Guerrieri R. An assessment of whether SNPs will replace STRs in national DNA databases. *Sci Justice.* 2004; 44:51-3.
20. Dixon LA, Dobbins AE, Pulker HK, Butler JM,Vallon PM, Coble MD et al. Analysis of artificially degraded DNA using STRs and SNPs – results of a collaborative European (EDNAP) exercise. *Forensic Sci Int.* 2006; 164:33-44.
21. Doi Y, Yamamoto Y, Inagaki S, Shigeta Y, Miyaishi S, Ishizu H et al. A new method for ABO genotyping using a multiplex single-base primer extension reaction and its application to forensic casework samples. *Legal Med.* 2004; 6:213-23.
22. Ackerman MJ, Tester DJ, Driscoll DJ. Molecular autopsy of sudden unexplained death in the young. *Am J Forensic Med Pathol.* 2001; 22:105-11.
23. Levo A, Koski A, Ojanpera I, Vuori E, Sajantila A. Post-mortem SNP analysis of CYP2D6 gene reveals correlation between genotype and opioid drug (tramadol) metabolite ratios in blood. *Forensic Sci Int.* 2003; 135:9-15.
24. Yang YR, Wang PX, Fang XD, Yan JW. Progress on epigenetics applications in forensic science. *Fa Yi Xue Za Zhi.* 2012; 28:366-70.
25. Waggoner D. Mechanisms of disease: epigenesis. *Semin Pediatr Neurol.* 2007; 14:7-14.
26. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol.* 1975; 98:503-17.
27. Carracedo A, Sanchez-Diz P. Forensic DNA-typing technologies: a review. In *Methods in Molecular Biology*, vol. 297: *Forensic DNA Typing Protocols* (Ed A Carracedo). Totowa, NJ, Humana Press, 2012.
28. Mullis K, Faloona F. Specific synthesis of DNA in vitro via polymerase- catalyzed chain reaction, in *Methods in Enzymology* (Ed R Wu):335-50. New York, Academic Press, 1987.
29. Hagelberg E, Sykes B, Hedges R. Ancient bone DNA amplified. *Nature.* 1989; 342:485.
30. Liu LM, Liang J, Wang BJ, Ding M, Li JP, Li CM et al. HLA-DRB1 typing by PCR-sequence specific primers for paternity determination. *Fa Yi Xue Za Zhi.* 1999; 15:201-54.
31. Conner BJ, Reyes AA, Morin C, Itakura K, Teplitz RL, Wallace RB. Detection of sickle cell beta S-globin allele by hybridization with synthetic oligonucleotides. *Proc Natl Acad Sci U S A.* 1983; 80:278-82.
32. Saiki RK, Bugawan TL, Horn TG, Mullis KB, Erlich HA. Analysis of enzymatically amplified beta-globin and HLA-DQ alpha DNA with allele-specific oligonucleotide probes. *Nature.* 1986; 324:163-6.
33. Saiki RK, Walsh PS, Levenson CH, Erlich HA. Genetic analysis of amplified DNA with immobilized sequence-specific oligonucleotide probes. *Proc Natl Acad Sci U S A .* 1989; 86:6230-4.

34. Budowle B, Giusti AM, Allen RC. Analysis of PCR products (pMCT118) by polyacrylamide gel electrophoresis. In *Advances in Forensic Haemogenetics* (Eds HF Polesky, WR Mayr):148-50. Berlin, Springer, 1990.
35. Hauge XY, Litt M. A study of the origin of "shadow bands" seen when typing dinucleotide repeat polymorphisms by the PCR. *Hum Mol Genet.* 1993; 2:411-5.
36. Lareu MV, Pestoni C, Phillips C, Barros F, Synder Combe-Court D, Lincoln P et al. Normal and anomalous electrophoretic behaviour of PCRbased DNA polymorphisms in polyacrylamide gels. *Electrophoresis* .1998; 19:1566-73.
37. Gill P, Whitaker J, Flaxman C, Brown N, Buckleton J. An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. *Forensic Sci. Int.* 2000; 112:17-40.
38. El-Alfy SH, Abd El-Hafez AF. Paternity testing and forensic DNA typing by multiplex STR analysis using ABI PRISM 310 Genetic Analyzer. *J Genet Eng Biotechnol.* 2012; 10:101-12.
39. Thompson R, Zoppis S, McCord B. An overview of DNA typing methods for human identification: past, present, and future. *Methods Mol Biol.* 2012; 830:3-16.
40. Li J. Chromosome STR genetic markers in paternity identification .*Zhong Nan Da Xue Xue Bao Yi Xue Ban.* 2004; 29:432-4.
41. Manjunath BC, Chandrashekar BR, Mahesh M, Vatchala Rani RM. Review-DNA Profiling and forensic dentistry: a review of the recent concepts and trends. *J Forensic Legal Med.* 2011; 18:191-7.
42. Kiuchi M. Application of the HLA system to forensic medicine--from serology to DNA polymorphism.*Nihon Hoigaku Zasshi.* 2002;56:229-35.
43. Kayser M, de Knijff P. Improving human forensics through advances in genetics, genomics and molecular biology.*Nat Rev Genet.* 2011; 12:179-92.
44. Giardina E, Spinella A, Novelli G. Past, present and future of forensic DNA typing. *Nanomedicine (Lond).* 2011; 6:257-70.
45. Gao LL, Li YY, Yan JW, Liu YC. Application and progress of RNA in forensic science. *Fa Yi Xue Za Zhi.* 2011; 27:455-9.

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