DNA amplification fingerprinting using 1 0 x polymerase chain reaction buffer with ammonium sulfate for human identification

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ABSTRACT

Objective: The polymerase chain reaction (PCR) - based DNA amplification fingerprinting (DAF) or randomly amplified polymorphic DNA (RAPD) is based on a strategy using a single arbitrary oligonucleotide primer to generate anonymous amplification of genomic DNA. On this basic strategy, in this study, we aimed to test individual differences and usefulness of 2 basic primers (5'-CGCGCCGG-3' and 5'-TGCCGAGCTG-3') and examined whether there is a positive effect on results of 10 x PCR buffer with ammonium sulfate.

Methods: A new approach in DNA fingerprinting, 10 x PCR buffer with ammonium sulfate, is presented in the study. Primers with single 8 and 10 nucleotides in length and 2 different PCR buffers with or without ammonium sulfate were used to identify 135 volunteers with no blood relationship. This study was carried out at the Pharmacology Laboratory, University of Gaziantep, School of Medicine, Turkey between 1999 and 2000.

Results: An average of 10 major bands representing 500-1500 base pair (bp) in length was determined as amplified DNA products on standard agarose gels for these volunteers. The use of ammonium sulfate in 10 x PCR buffers has increased to 92% success ratio of individual difference obtained from the 8 nucleotides primer.

Conclusions: With this study, more reliable results can be obtained by using ammonium sulfate in 10 x PCR buffers.

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Many methods and strategies are developed to distinguish individuals in forensic medicine. Deoxyribonucleic acid fingerprinting, a powerful and reliable method has been a great importance in forensic identification, and it is widely used as molecular markers in practice.[1-19] Introducing polymerase chain reaction (PCR) to laboratories, PCR-based DNA typing methods have been recognized with considerable successes; parentage testing, relationship, criminal investigation, group accidents and postmortem cases can be given as application examples.[3,4,6,9,11,13,15,16,18,20-31] Polymerase chain reaction is an analytic method, which can certainly achieve required accuracy with limited amounts of samples, old or degraded DNA molecules in forensic specimens.[3,6,10-12,15,18,20,22,29,32-34] Deoxyribonucleic acid amplification fingerprinting or randomly amplified polymorphic DNA (RAPD) have also been used in a wide variety of species, including many investigations in population biology, genetics and ecology[13,16,17,25,35-40] When little or no sequence data is available for the organism of

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interest, this method can be applicable to any organism without having no need for an extensive background research.[13,16,17,25,40] The applicability of DNA amplification fingerprinting (DAF) for forensic purposes such as human identification is presented in this study. While studying DAF, identification of 135 volunteers is planned and performed using PCR buffers with ammonium sulfate or not. All results are given comparatively herein.

Methods. The study was performed on 135 volunteers without any family relationship and not belonging to one ethnic group living in Gaziantep City, Turkey. This study was carried out at the Pharmacology Laboratory, Gaziantep University School of Medicine, Turkey between 1999 and 2000. Deoxyribonucleic acid was extracted by the salting out procedure[41] from whole blood samples. It was amplified by using 8 and 10 base short arbitrary oligonucleotide primers (MBI Ferments, Lithuania), and 2 different buffers in the PCR. A very commonly used 10 x PCR buffer includes only KCl, Tris HCl. Chamberlain et al[42] examined the efficiency of more complex buffers in supporting the activity of the Taq polymerase in 1988. For this reason in our study 10 x PCR with ammonium sulfate (NH4)2SO4) was used as a complex buffer besides 10 x PCR without ammonium sulfate. In this study, Taq polymerase and buffer are recommended by the manufacturer of the polymerase (MBI Fermentans, Lithuania). Four different mixtures were prepared to improve the test results. They were named as protocol 1, 2, 3 and 4. An 8 nucleotides primer (5'-CGCGCCGG-3') was used in protocol 1 and 2, similarly a 10 nucleotides primer (5'-TGCCGAGCTG-3') was used in protocol 3 and 4. In protocol 1, the final concentration for amplification in PCR were performed in amount of 100mM Tris-HCl (pH8.8), 500mM KCl, 0.8% Nunnated P40, 25mM MgCl2, 2mM each of the deoxyribonucleotide triphosphates (dNTPs), 1mM 8 nucleotides primer (MBI Fermentas, Lithuania), 0.75U of Taq polymerase (MBI Fermentas, Lithuania), 1.25µl (5%) Dimethyl sulfoxide (DMSO)[3,30,42] and the mixture was prepared. Negative controls containing water only were included in every reaction set. In Protocol 3, like first mixture, instead of 8 nucleotides primer same amount 10 nucleotides primer. In protocols 2 and 4, like first PCR mixture, instead of 10 x PCR buffer, same amount of 10 x PCR buffer (MBI Fermentas, Lithuania) with 200 mM ammonium sulfate (NH4)2SO4) were used.[18,26,29,32] All PCR mixtures (25 µl) were put in 0.2 ml Eppendorf tubes, and 1µl purified DNA was added to these mixtures in all tests. The protocols where DMSO was not used instead of 1.25µl DMSO, same amount of dH_2O was added. The thermal cycler (Genius, Cambridge) was set as: 94ºC for 2 minutes (one cycle); 94ºC for one minute, 40ºC for one minute; 72ºC, 2 minutes for 35 cycles; and lastly at 72ºC for 7 minutes (one cycle). Polymerase chain reaction products with 15µl, obtained from completion of cycles was mixed with Orange-G solution, and samples were loaded to wells in 2% agarose gels. Marker 5 (100 bp DNA Ladder Plus, MBI Fermentas, Lithuania) was used to identify bands. Polymerase chain reaction products were run by using agarose gel electrophoresis apparatus including 1% ethidium bromide trisporic acid (, TBE) buffer (0.9mol/L Tris, 0.9mol/l borate, 0.025mol/L ethylenediaminetetraacetic acid (EDTA) (pH: 8.3) at 100 V for 80 minutes. Photographs of bands were taken with ultraviolet light.

Results. Bands obtained from each 4 protocols were between 500-1500 bp in length. The arithmetic mean of all classified data were calculated, and the observation was that of average 9 definite different bands, and identification of 83 individuals (61.5%) from 135 was performed in protocol 1. When an ammonium sulfate application buffer (Protocol 2) was used, average 10 band patterns were observed, thus 117 individuals (86.7%) might be identified. Comparing products of protocol 1 and 2, (total success of method) 124 individuals (92%) were identified (**Figure 1**). Bands with similar patterns on protocol 2 were compared with PCR products obtained from protocol 1 by running in same agarose gel, identification of 7 individuals was achieved with different band patterns. Deoxyribonucleic acid fingerprints of all volunteers were taken with this method, only 11 individuals (8%) were not exactly identified at the end of essays. A significant individual identification was not obtained from 10 nucleotides primer unlike 8 nucleotides primer (**Figure 2**). Although bands were not identified which is good enough in the mixtures without DMSO (data not shown), better band patterns were observed 5% DMSO in the PCR reaction mixtures.

Discussion. Since properties such as length of primers and contents of nucleotide are quite effective on the results found, the most important factor can be considered as proper selection of primers in PCR studies.[3,13,16,20,34,35,37,40,44] Primer chosen are amplified sequences having polymorphic properties in PCR. There is some evidence that high primer guanine/cytosine (G/C) content generates detectable levels of amplification products.[25,37] G/C and CG bp are more stable than AT and TA bp.[26] Caetano-Anolles et al[16,17,] and Bassam et al[39] have revealed the genetic differences between organisms by using different oligonucleotide primers. The

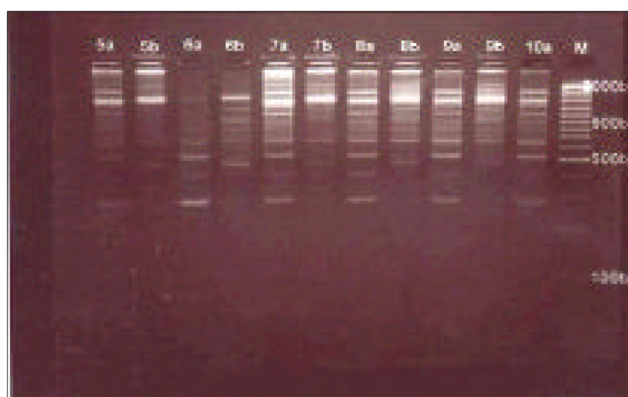

Figure 1 - Polymerase chain reaction products obtained from Protocol 1 (without ammonium sulfate buffer and 8 bp primer) and 2 (with ammonium sulfate and 8 bp primer). Also "a" means (5a-10a) with ammonium sulfate, and "b" means (5b-9b) without ammonium sulfate. (Line numbers 5-10 were sample numbers). Each number represents one person. Letters a and b also represent the type of buffer (with or without ammonium sulfate buffer) on the same person.

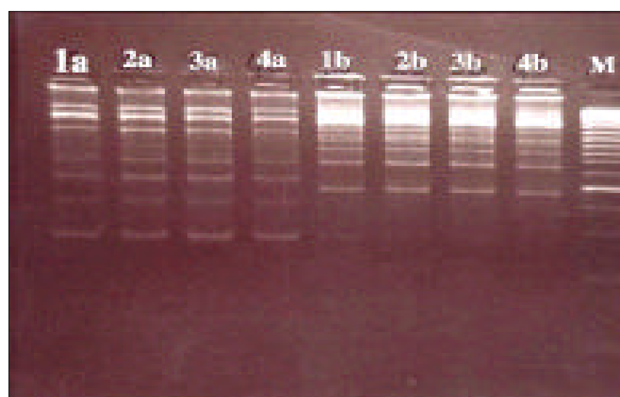

Figure 2 - Polymerase chain reaction products obtained from Protocol 3 (without ammonium sulfate buffer and 10 bp primer) and 2 (with ammonium sulfate and 10 bp primer). Also "a" means (1a-4a) with ammonium sulfate, and "b" means (1b-4b) without ammonium sulfate. (Line numbers 1-4 were sample numbers). Each number represents one person. Letters a and b also represent the type of buffer (with or without ammonium sulfate buffer) on the same person.

highest performance in human genome has been shown applicability of tested primers among a reference to selection of primers. In addition, Dulger et al[34] and Tokdemir et al[35] have presented satisfactory results with all G/C 8 nucleotides primer in individual identification. In this study, same 8 nucleotides primer is chosen, and 70% G/C with content 10 nucleotides primer is additionally used. When short DNA sequences are compared with 8 nucleotides primer, exact and distinct bands patterns are certainly generated giving a positive result (**Figure 1**). Due to its high GC content, cosolvents in the PCR reaction, like 1-10% DMSO or glycerol are applied to increase the amount of amplified DNA.[3,30,43] Thus, 8 nucleotides primer with all GC and 10 nucleotides primer are rich GC content, DMSO cosolvent is used in PCR reactions and better band patterns are observed (**Figure 1**). Considerably well steps are taken on product efficiency and specific productivity result in PCR, such new PCR buffers involving different components (for example ammonium sulfate) are tested in different studies.[18,26,29,32] A new approach in DNA fingerprinting, 10 x PCR buffer with ammonium sulfate is used in the study presented. The interesting point is that band patterns and level differences seen in standard 10 x PCR buffer and 10 x PCR buffer with ammonium sulfate are different. Identification of 83 individuals from 135 is achieved with protocol 1. When an ammonium sulfate application buffer (Protocol 2) is used, average 10 band patterns are observed and 117 individuals may be identified (**Figure 1**). In additionally, ammonium sulfate buffer can be increased the activity of Taq DNA polymerase while changing its function, and also providing new bands generation produced without standard buffer. However, high-density bands are obtained as an auxiliary help of ammonium sulfate buffer in Taq polymerase activity. Thus, with only ammonium sulfate, better and more reliable results are obtained for individual identification. Although primer and other reaction conditions are the same, only products obtained from buffer solutions with and without ammonium sulfate have been shown different band patterns. Thus, an observation can be made in using ammonium sulfate in 10 x PCR buffer for human identification. Conclusively, ammonium sulfate in PCR reactions has an active role in results. However, only 11 individuals (8%) are not identified both PCR Protocol 1 and 2, 124 individuals (92%) have been identified. Deoxyribonucleic acid products are then amplified by using 10 nucleotides primer in Protocols 3 and 4 mentioned. Although optimum conditions are provided while performing studies with 10 nucleotides, specific and clear band patterns are not observed like 8 nucleotides, resulting in 11 unidentified individuals (**Figure 2**).

In conclusion, DAF is easily applicable when large populations are concerned[17,34,35,40] and it represents a conceptual and practical advance in DNA fingerprinting.[16,25] Experiments are performed by using 2 different primers and PCR buffers to distinguish individuals. The products obtained from buffer mixtures with and without ammonium sulfate have realized different band patterns on the condition that all primers and other reaction mixtures are the same. Thus, the use of ammonium sulfate in 10 x PCR buffers has a definite and

important role in human identification. Although specific band patterns can be seen with the 8 nucleotides primer, same satisfaction does not come into existence with the 10 nucleotides primer. An overall great success of 92% was obtained. The method applied requires careful optimization of amplification parameters.[13,36] All tests, and mixtures for DNA fingerprinting are to be performed with special care and in a standardized manner. Even changing of only one parameter can certainly cause different results. The method can be carried to further works with the help of more sensitive methods rather than using agarose in forensic medicine. Further studies are also needed to improve applicability of this method by ensuring reproducibility within laboratories in routine cases.

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References

1. Gill P, Jeffreys AJ, Werrett DJ. Forensic applications of DNA 'fingerprints'. ***Nature*** 1985; 318: 577-579.
2. Nichols RA, Balding DJ. Effects of population structure on DNA fingerprint analysis in forensic science. ***Heredity*** 1991; 66: 297-302.
3. Bej AK. Amplification of nucleic acids by polymerase chain reaction (PCR) and other methods and their applications. ***Crit Rev Biochem Mol*** 1991; 26: 301-334.
4. Erlich HA, Gelfand D, Sninsky JJ. Recent advances in the polymerase chain reaction. ***Science*** 1991; 252: 1643-1650.
5. Herrera RJ, Tracey ML. DNA fingerprinting: basic techniques, problems, and solutions. ***J Crim Justice*** 1992; 30: 237-248.
6. Akane A, Shiono H, Matsubara K, Nakamura H, Hasegawa M, Kagawa M. Purification of forensic specimens for the polymerase chain reaction (PCR) analysis. ***J Forensic Sci*** 1993; 38: 691-701.
7. Nakamura Y, Leppert M, O'Connell P, Wolff R, Holm T, Culver M et al. Variable number of tandem repeat (VNTR) markers for human gene mapping. ***Science*** 1987; 235: 1616-1622.
8. Georgiou M, Hatzaki A, Koutselinis A. Identification of an alleged offender of murder by VNTR analysis. ***Am J Forensic Med Pathol*** 2000; 21: 162-165.
9. Sullivan KM, Gill P, Lingard D, Lygo JE. Characterization of HLA DQa for forensic purposes. Allele and genotype frequencies in British Caucasian, Afro-Caribbean and Asian populations. ***Int J Leg Med*** 1992; 105: 17-20.
10. Ludes B, Pfitzinger H, Mangin P. DNA fingerprinting from tissues after variable postmortem periods. ***J Forensic Sci*** 1993; 38: 686-690.
11. Romero RL, Juston AC, Ballantyne J, Henry BE. The applicability of formalin-fixed and formalin fixed parffin embedded tissues in forensic DNA analysis. ***J Forensic Sci*** 1997; 42: 708-714.
12. VanOorschot RAH, Gutowski SJ, Robinson SL. HUMTH01: amplification, species specificity, population genetics and forensic applications. ***Int J Leg Med*** 1994; 107: 121-126.
13. Gene M, Fuentes M, Huguet E, Pique E, Bert F, Corella A, et al. Quechua amerindian population characterized by HLA-DQa, YNZ22, 3'APO B, HUMTH01, and HUMVWA31A polymorphisms. ***J Forensic Sci*** 1998; 43: 403-405.
14. Ülküer MK, Ülküer Ü, Kesici T, Menevse A. Data on the PCR Turkish population based loci: LDLR, GYPA, HBGG, D7S8, and Gc. ***J Forensic Sci*** 1999; 44: 1258-1260.
15. Tsopanomichalou M, Sourvinos G, Arvanitis D, Michalodimitrakis M. Analysis of eight polymorphic human genetic markers in a well-defined Greek population. ***Am J Forensic Med Pathol*** 2000; 21: 172-177.
16. Caetano-Anolles G, Bassam BJ, Gresshoff PM. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. ***Bio Technology*** 1991; 9: 553-557.
17. Caetano-Anolles G, Bassam BJ. DNA amplification fingerprinting using arbitrary oligonucleotide primers. ***App Biochem Biotechnol*** 1993; 42: 189-200.
18. Richie KL, Goldsborough MD, Darfler MM, Benzinger EA, Lovekamp ML, Reeder DJ, et al. Long PCR for VNTR analysis. ***J Forensic Sci*** 1999; 44: 1176-1185.
19. Gillings M, Holley M. Amplification of anonymous DNA fragments using pairs of long primers generates reproducible DNA fingerprints that are sensitive to genetic variation. ***Electrophoresis*** 1997; 18: 1512-1518.
20. Strom CM, Rechitsky S. Use of nested PCR to identity charred human remains and minute amounts of blood. ***J Forensic Sci*** 1998; 43: 696-700.
21. Dimo-Simonin N, Grange F, Brandt-Casadevall C. PCR-based forensic testing of DNA from stained cytological smears. ***J Forensic Sci*** 1997; 42: 506-509.
22. Anderson TD, Ross JP, Roby RK, Lee DA, Holland MM. A validation study for the extraction and analysis of DNA from human nail material and its applications to forensic casework. ***J Forensic Sci*** 1999; 44: 1053-1056.
23. Urquhart AU, Kimpton CP, Downes TJ, Gill P. Variation in short tandem repeat sequences: A survey of twelve microsatellite loci for use as forensic identification markers. ***Int J Leg Med*** 1994; 107: 13-20.
24. Jorquera H, Budowle B. Chilean population data on ten PCR-Based loci. ***J Forensic Sci*** 1998; 43:171-173.
25. Lee JCI, Chang JG. Random amplified polymorphic DNA polymerase chain reaction (RAPD PCR) fingerprints in forensic species identification. ***Forens Sci Int*** 1994; 67: 103-107.
26. Steighner RJ, Tully LA, Karjala JD, Coble MD, Holland MM. Comparative identity and homogenity testing of the mtDNA HV1 region using denaturing gradient gel electrophoresis. ***J Forensic Sci*** 1999; 44: 1186-1198.
27. Tokdemir M, Dulger HE, Akin H, Doymaz MZ. A study on polymerase chain reaction-based HLA DQa locus in Elazig, Turkey. ***Am J Forensic Med Pathol*** 2000; 21: 184-188.
28. Dulger HE, Tokdemir M. HLA-DQA1 and polymarker locus allele frequencies for Elazig, Turkey. ***J Forensic Sci*** 2000; 45: 942.
29. Matsumoto H, Tsuruya S, Tsuda R, Orihara Y, Kubo S. Japanese population study of a Y-linked dinucleotide repeat DNA polymorphism. ***J Forensic Sci*** 1999; 44: 588-591.
30. Desmarais D, Zhong Y, Chakraborty R, Perreault C, Busque L. Development of a highly polymorphic STR marker for identity testing purposes at the human androgen receptor gene (HUMARA). ***J Forensic Sci*** 1998; 43: 1046-1049.
31. Nellemann LJ, Moller A, Morling N. PCR typing of DNA fragments of the short tandem repeat (STR) system HUMTH01 in Danes and Greenland Eskimos. ***Forensic Sci Int*** 1994; 68: 45-51.

32. Khatib H, Ezzughayyar M, Ayesh S. The distribution of the vWF alleles and genotypes in the Palestinian population. ***J Forensic Sci*** 1997; 42: 504-505.
33. Evison MP, Smillie DM, Chamberlain AT. Extraction of single-copy nuclear DNA from forensic specimens with a variety of postmortem histories. ***J Forensic Sci*** 1997; 42: 1032-1038.
34. Dulger HE, Tokdemir M, Erbag B, Atalay EO, Doymaz MZ. A study on DNA fingerprinting using various human samples. ***Bulletin Leg Med*** 1997; 2: 15-20.
35. Tokdemir M, Dulger HE, Doymaz MZ. Demonstration of differences between individuals by DNA fingerprinting. ***Firat Univ J Health Sci*** 1998; 12: 59-65.
36. Caetano-Anolles G, Gresshoff PM. Generation of sequence signatures from DNA amplification fingerprints with mini-hairpin and microsatellite primers. ***Biotechniques*** 1996; 20: 1044-1056.
37. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. ***Nucleic Acids Res*** 1990; 18: 6531-6535.
38. Bassam BJ, Caetano-Anolles G, Gresshoff PM. Fast and sensitive silver staining of DNA in polyacrylamide gels. ***Anal Biochem*** 1991; 196: 80-83.
39. Bassam BJ, Caetano-Anolles G, Gresshoff PM. DNA amplification fingerprinting of bacteria. ***Appl Microbiol Biotechnol*** 1992; 38: 70-76.
40. Welsh J, McClelland M. Fingerprinting genomes using PCR with arbitrary primers. ***Nucleic Acids Res*** 1990; 18: 7213-7218.
41. Miller SA, Dykes DD, Polesky HF. A Simple salting out procedure for extraction DNA from human nucleated cells. ***Nucleic Acids Res*** 1988; 16: 1215.
42. Chamberlain JS, Gibbs RA, Ranier JE, Nguyen PN, Caskey CT. Deletion screening of the Duchene muscular dystrophy locus via multiplex DNA amplification. ***Nucleic Acids Res*** 1988; 16: 11141-11156.
43. Bonner G, Klibanov AM. Structural stability of DNA in nonaqueous solvents. ***Biotechnol Bioeng*** 2000; 68: 339-344.
44. Vincent J, Gurling H, Melmer G. Oligonucleotides as shorts as 7-mers can be used for PCR amplification. ***DNA Cell Biol*** 1994; 13: 75-82.