

Confirmation of assassin from exhibits left at crime scene and its comparison with control sample of suspects using APoB primer

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Abstract:

DNA finger printing is a powerful technology for individualization of a person. In Forensic Science it has been used since three decades giving absolute results. In present study an effort has been made to extract and isolate DNA from exhibits like chewing gum and socks that were left at assumed crime scene and consequently the control samples were taken from the suspects. The DNA has been isolated and then amplified by using APoB primer, resulted in the formation of specific band pattern which was supportive in interconnecting the link of suspects with murder. It has been concluded from the present paper that the evidences collected from scene of crime containing saliva and sweat even in trace amount can be utilized as reliable sources for the identification through PCR-VNTR analysis.

Keywords: PCR-VNTR Forensic Science, DNA Isolation, Polymerase Chain Reaction, APoB primer, Hyper Variable Region.

Introduction:

DNA fingerprinting is the most often used technology in the identification of an individual or living organism as used by Forensic Individuals. Every living creature is genetically different. Each individual contains a unique sequence which is specific to that organism. Unlike traditional fingerprints which can be surgically altered or self mutilated, the DNA sequence cannot easily be changed once the material is left at a crime scene, thus increasing its effective use in forensics, and the probability of finding an exact match.

It has been evident that Variable Number of Tandem Repeats (VNTRs) is highly polymorphic markers. Each VNTR locus since associated with a large number of different allele, each allele corresponding to a specific number of repeated sequences in any population. Each individual carries two allele only – one on each of two homologous chromosome. The important methodological merits of VNTRs are that its pattern is unique for every individual. [1-3]. Due to

highly polymorphic character VNTR can be used as an important tool in forensic identification and comparison. They also constitute preferred system for 'DNA Fingerprinting', the direct practical application of which includes criminal and forensic examination, in solving the problem of identification amongst a large number of suspects.

VNTRs have been described as 73 base pair (b.p) 3' to the second polyadenylation signal of the human 'Apolipoprotein B, (APoB) gene (which is located on chromosome 2) and is commonly referred to as APoB 3'-HVR (Hyper Variable Region) [4-6]. APoB 3'-HVR alleles and genotype have been much studied in numerous population. DNA isolated from biological materials can be analyzed by DNA profiling to help establish the source of deoxyribonucleic acid (DNA) [7-8]. It has been demonstrated that blood, semen, hair root, tissues, and bones are a good source of DNA for identity testing [9-11]. Sweat is also a useful material in this respect. These biological materials recovered from crime scene rely primarily upon the identification of blood group, antigens and rarely on iso-enzyme and polymorphic protein. These approaches have several inherent limitations, especially their limited detectability because of the low concentrations of antigens, isoenzymes and proteins in the samples. In addition to these, drying, aging, and contamination associated with this sample limit the number of markers that can be typed. The potential for discrimination using these markers are also less sensitive than DNA analysis.

This paper is focused on the present well known methods of identification based on VNTR analysis. The exhibit i.e. chewing gum and the sock bearing the saliva and sweat recovered from the crime scene was used to isolate DNA. The characteristic banding pattern obtained has been utilized for identification of offender involved in the murder case.

Materials and Methodology:

Isolation of DNA from chewing gum

The chewing gum recovered from the crime scene, which was suspected to be containing saliva, left for air dry at room temperature. To isolate DNA chewing gum was cut into small pieces. The pieces were poured in 0.9% saline solution for 10 minutes and shaken slowly. The extract was collected into ependroff tube and centrifuged at 6,000rpm for 5 minute. The supernatant was discarded and 1 ml of lysis buffer (10mM tris HCl, 10mM EDTA, and 2% SDS) was added to the pellet. The mixture vortexed for 1 minute and then incubated at 65⁰C for 10 minutes. Poured 500 µl of 5 M NaCl to the solution mixed gently for 1 minute and then centrifuged at 4,000rpm for 10 minutes. The supernatant (500µl) was transferred into clean ependroff tube and 1ml of 95% cold ethanol was added to it. Mixed it well and incubated it at 4⁰C for overnight.

After overnight incubation it again centrifuged at 4000rpm for 10 minutes. The supernatant was discarded and 1ml of 70% ethanol was added to the pellets. The mixture vortexed for 1 minute and centrifuged at 10,000 rpm for 5 minutes. The supernatant was discarded and pellet was allowed to air dry. The pellets contain DNA finally dissolved in 50 µl of TE-Buffer and preserved at 4⁰C.

Isolation of DNA from socks

The socks recovered from crime scene, suspected for sweat was allowed to air dry at room temperature. To isolate DNA from sock, 1cm² suspected areas was removed and cut into fine pieces. The pieces were soaked in 0.9% saline solution for 10 minutes. The extract was taken into ependroff tube, 1ml of 1.5% of SDS solution was added and it incubated at 37⁰C for 1 minute. The mixture was centrifuged at 10,000 rpm for 10 minutes. The supernatant (0.5ml) was taken into another clean tube; with an addition of equal volume of 95% ethanol and it allow to incubate at 4⁰C for overnight.

The overnight material centrifuged at 12,000 rpm for 10 minutes, the supernatant was discarded and 650 µl of ethanol was added to the pellets and then centrifuged at 10,000 rpm for 5 minutes. The supernatant was discarded and pellet was allowed to air dry. The pellet contain DNA was dissolved in 50 µl TE buffer and stored at 4⁰C.

Isolation of DNA from buccal cell taken from three suspects

The cotton swabs were soaked in 0.9% saline solution for 10 minutes. The extract was collected into ependroff tube and centrifuged at 6,000rpm for 5 minute. The supernatant was discarded and 1 ml of lysis buffer (10mM tris HCl, 10mM EDTA, and 2% SDS) was added to the pellet. The mixture vortexed for 1 minute and then incubated at 65⁰C for 10 minutes. Poured 500 µl of 5 M NaCl to the solution mixed gently for 1 minute and then centrifuged at 4,000rpm for 10 minutes. The supernatant (500µl) was transferred into clean ependroff tube and 1ml of 95% cold ethanol was added to it. Mixed it well and incubated it at 4⁰C for overnight.

After overnight incubation it again centrifuged at 4000rpm for 10 minutes. The supernatant was discarded and 1ml of 70% ethanol was added to the pellets. The mixture vortexed for 1 minute and centrifuged at 10,000 rpm for 5 minutes. The supernatant was discarded and pellet was allowed to air dry. The pellets contain DNA finally dissolved in 50 µl of TE-Buffer and preserved at 4⁰C.

Amplification of VNTR region (ApoB) sequence by PCR

PCR is a powerful technique in molecular biology to amplify specific DNA sequence (a single or few copies of pieces of DNA) across several orders of magnitude, generating thousands to million copies of a particular DNA sequence. Its success depends on design of optimal primer pair, appropriate primer concentration, optimization of PCR condition.

The method relies on thermal cycling, consisting of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of DNA. Almost all PCR application employ a heat stable DNA polymerase, such as TAQ polymerase, as enzyme originally isolated from the bacterium *Thermus aquaticus*.

For the amplification PCR was done using universal APoB-VNTR primers (5'ATGGAAACGGAGAAATTATG3' and 3'CCTTCTCACTTGGCAAATAC5'). Each DNA amplification reaction used a total reaction volume of 35.013 µl (genomic DNA with TE-Buffer=2 µl, 10X PCR Buffer=4µl, 25mM MgCl₂=1µl, 2.5 mM dNTP = 2µl, 10pm/ µl 20 strand forward primer =2µl, 10pm/ µl ,20 strand reverse primer =2µl, 1µg/ml Taq = 0.013 µl, Nuclease free water =22µl.)

The reactions were done in a Labnet DNA thermal cycler (model: Multigene II) and involved pre-denaturation for 5 min at 95 °C, Denaturation for 1 min at 95 °C, Annealing for 1 min at 55 °C, Amplification for 2 min at 72 °C and proof reading for 5 min at 72 °C. The number of cycle used for the purpose was 32.

Agarose Gel Electrophoresis:

The amplified products were analyzed by electrophoresis using 0.8% agarose gels in TE-Buffer (4 hrs. at 100 V) and detected by Ethidium Bromide staining. The fragment patterns of individuals were analyzed on the basis of presence of bands at particular distance.

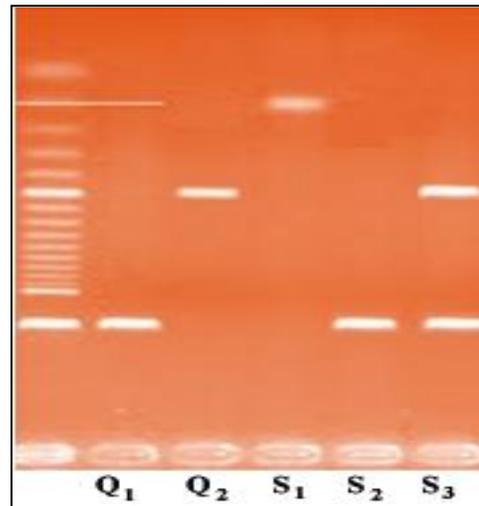
Experimental Results:

Samples were collected from crime exhibits i.e. chewing gum (for saliva) and sock (for sweat) left at crime scene. For forensic identification of DNA the exhibits of the samples were extracted and isolated properly with unique labeling Q1 (from saliva) and Q2 (from Sweat) along with three control sample (buccal swab) from suspects S1, S2 and S3 respectively.

Amplification was done using universal APoB-VNTR primer through Labnet DNA thermal cycler (model: Multigene II). To run the DNA sample 0.8% agarose gel was prepared and poured in the gel tray. After solidification the combs were removed and the gel tray was transferred into the tank containing TAE-Buffer.

5µl of DNA sample was mixed well with 2 µl loading dye on cellophane tape, then the samples were loaded carefully into the well by using micro pipette and samples were allowed to run at 100V for 3 hours. After examination of the bands under UV trans illuminator it was found that The VNTR band pattern of Q1 matched with that of S2 and band pattern of Q2 matched with that of S3, while the band pattern of S1 did not match with the band pattern of Q1 or Q2. [Fig.]

Fig.1 Band pattern of suspected and control sample.



Discussion:

The amplicons were directly visualized by staining on agarose gel electrophoresis. PCR was successfully performed on trace amount of DNA on exhibits had accurately typed the APoB VNTR from their amplification products, which would have been impossible through traditional Southern blot analysis.

VNTR polymorphisms were performed by *Brinkmann et al*^[12] for the possible individualization of human urine samples with reference to doping cases in sport. Investigations were carried out with the RFLP single locus system YNH24/Hinf I and the PCR-VNTR systems Apo B and COL2A1 (AMPFLLs) as well as SE 33 and TC 11 (STRs). The AMPFLP systems gave positive results in 4 out of 8 (Apo B) and 5 out of 8 (COL2A1) samples and YNH24 was successful in 1 out of 3 samples. Negative results were obtained for the AMPFLP systems and YNH24 after longer storage periods whereas the STRs were positive. Using PCR based method, *Chakraborty et al*^[13] genotyped at two hypervariable loci (3' to the Apo-B-structural gene and at the ApoC-II gene). Genotyped data at the Apo-B locus (Apo-B VNTR) were obtained on 240 French Caucasians; the sample size for the ApoC-II VNTR was 162. For 160 individuals two-locus genotype data were available. Applications of some recently developed statistical methods to these data indicate that both of these loci are at Hardy-Weinberg equilibrium (HWE) and there is no indication of allelic associations between these two unlinked loci. In addition, the observed numbers of alleles (12 for

the Apo-B and 11 for the ApoC-II VNTR loci) are also consistent with their respective expectations based on the observed heterozygosities (76.9% for the Apo-B and 85.9% for the ApoC-II loci) suggesting genetic homogeneity of this population-based sample. The multimodal distribution of allele sizes observed for both loci indicate that the production of new alleles at such VNTR loci may be caused by more than one molecular mechanism. The utility of such highly polymorphic loci for human genetic research and forensic applications are discussed in the context of these findings.

These above finding reveal the maximum possibilities of positive result in identification and individualization process by using highly informative hypervariable loci such as the ApoB 3' VNTR locus in population genetic research.

Conclusion:

Biological fluid play a vital role in the field of forensic science for identification and comparison of suspected and control sample. The biological fluid normally left at the scene of crime during committing crime. DNA isolation from such fluids is a major challenge for Forensic biologist since these evidences contain tiny amount of biological material. It is necessary to use a suitable and appropriate method for extraction and isolation of DNA from different biological source using reliable methods, because once the sample utilized wrongly it can't be used again. The DNA sample are very much sensitive to cross contamination, hence the use of appropriate primer for DNA typing is necessary to form a definite interpretation of result. APoB is a universal primer found in almost every individual but with different base pair length. In the present study APoB primer was used to solve a murder case which gave a trustworthy result. From this study it was concluded that APoB locus can be used in forensic science for identification of criminals in almost every cases.

References:

1. Jeffreys, A. J., Wilson, V. and Thein, S.L. "Hyper-variable "minisatellite" regions in human DNA. *Nature*" 1985;314:67-73.
2. Nakamura, Y. Leppert, M. O'Connell, P. Wolff, R. Holm, T. and Culver, M. "Variable number of tandem repeats (VNTR) markers for human gene mapping." *Science* 1987;235:1616-22.

3. National Research Council, "Mapping and sequencing the human genome." Washington, DC: National Academy Press, 1988.
4. Knott, T. Wallis, C. Pease, R. Powell, L. and Scott, J. "A hypervariable region 3' to the human apolipoprotein B gene." *Nucl Acids Res* 1986;.
5. Boerwinkle, E. Xiong, W. Fourest, E. and Chan, L. "Rapid typing of tandemly repeated hypervariable loci by the polymerase chain reaction: application to the apolipoprotein B 3' hypervariable region." *Proc Natl Acad Sci USA* 1989; 86:212-216.
6. Ludwig, E.H. Friedl, W. and McCarthy, B. J. "High-resolution analysis of a hypervariable region in the human apolipoprotein B gene." *Am J Hum Genet* 1989; 45:458-64.
7. "Genetic Witness: Forensic Uses of DNA Tests", Congress of the United States, Office of Technology Assessment, U.S. Government Printing Office, Washington, DC, 1990.
8. Gill, P. Jeffreys, A. J. and Werret, D. J. "Forensic Applications of DNA Fingerprints," *Nature*, Vol. 318, Dec. 1985, pp. 577-579.
9. Kanter, E. Baird, M. Shaler, R. and Balazs, I. "Analysis of Restriction Fragment Length Polymorphisms in Deoxyribonucleic Acid (DNA) Recovered from Dried Bloodstains," *Journal of Forensic Sciences*, Vol. 31, No. 2, April 1986, pp. 403-408.
10. Giusti, A. Baird, M. Pasquale, S. Balazs, I. and Glassberg, J. "Application of Deoxyribonucleic Acid (DNA) Polymorphisms to the Analysis of DNA Recovered from Sperm," *Journal of Forensic Sciences*, Vol. 31, No. 2, April 1986, pp. 409-417.
11. Hopkins, B. Morten, J. E. N. Smith, J. C. and Markham, A. F. "The Development of Methods for the Analysis of DNA Extracted from Forensic Samples," *Technique*, Vol. 1, No. 2, Oct. 1989, pp. 96-102.
12. Brinkmann, B. Rand, S. and Bajanowski, T. "Forensic identification of urine samples," (*International Journal of Legal Medicine*) Volume 105, Issue 1, 1992 pp. 59-61
13. Chakraborty . R. Fornage, M. Gueguen, R. and Boerwinkle, E. "Analysis of PCR Based VNTR Polymorphism Within a Population." (*DNA Fingerprinting: Approaches and Applications, Experientia Supplementum*), Volume 58, 1991, pp 127-143.