

Isolation and Quantification of DNA from different evidences and role of RFLP in DNA fingerprinting

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Abstract:

Over the past twenty years, DNA analysis has been revolutionized forensic science and has become a dominant tool in law enforcement. The DNA is unique for each and every person. This application is used in forensic science to identify the criminals.

The DNA is isolated from the biological samples collected from the crime scene. DNA can be extracted from saliva, blood, hairs, semen or any body fluid. The various materials like cigarette, chewing gum or any material containing body fluids may contain DNA as it is used by the person. The person is then identified by various methods like STR, RFLP and VNTR. The estimation of the DNA is done by DPA method.

Introduction :

DNA forensic is the bed rock tool for crime investigation. It is one of the very accurate and reliable measures that have become the main source in the law enforcement. The DNA is being fundamental structure of the life and always unique for the individual. 99.99% of the human genome is similar and contains exons and introns. Only 0.1% of the DNA is different and gives the specificity to each person. During any case investigation various physical, testimonial and biological kinds of evidences may be found at the crime scene. Out of these biological evidences are very important. These include saliva, blood, semen or any biological fluid. These fluids contains variable amount of DNA and can ensure the CSIOs that the source person of DNA was present at the crime scene. However it doesn't confirm the person as the culprit or his direct involvement in the crime, but he can't refuse his presence on the crime scene. DNA forensic involves collection of samples with proper care and fingerprinting of DNA. The various techniques in the DNA forensic include PCR, STR, VNTR and RFLP etc. Restriction Enzyme Length polymorphism and Short Tandem Repeats gives the band pattern on the gel which is very specific. Using these technologies the individual can be identified. The DNA forensic is also used in the paternity identification and the missing cases. The qualification of DNA can be done by the Agarose gel electrophoresis method. DNA can be estimated by DPA method.

Materials and Methods:

Isolation of DNA: Samples were dipped in lysis buffer and incubated for 1 hour. 500 μ l NaCl was added. Then centrifuged at 4000rpm for 10 minutes and treated with ice cold isopropanol. Incubated at 4⁰ C for overnight. Performed ethanol wash and centrifuged centrifuged at 10000 rpm for 10 minutes. DNA was stored in TE buffer.

Running on gel: using 0.8 % agarose and TAE buffer the gel was prepared and the samples were run on the gel. DNA was observed under UV transilluminator.

Quantification of DNA: the vials were kept in boiling water bath and 4ml of DPA reagent was added with 10 μ l of samples and water. The O.D. of the samples is measured at the 595nm and graph is plotted. Using the graph, the conc. of DNA is estimated.

RFLP: two enzymes EcorI and Hind III were used for RFLP. 7 μ l of samples and 2 μ l of enzymes 2.5 μ l of assay buffer were added and volume was made 25 μ l by water. The band patterns were observed under UV transilluminator.

Results:

Fig1: Qualitative analysis of DNA by gel electrophoresis:-



Fig 1: showing DNA bands on gel electrophoresis
1st band = DNA from cigarette butt
2nd band = DNA from chewing gum
3rd band = DNA from lipstick mark

Key words: DNA, Extraction of DNA, evidence, RFLP

Estimation of DNA by DPA method:

The deoxyribose of DNA in presence of acid dihydroxylevulin-aldehyde which reacts with diphenylamine to give a blue colour.

Standard solution: 1mg/ml of Calf thymus DNA in distilled water.

Table1: showing the Estimation of DNA by DPA method

Fig2: The Graph showing the Concentration of DNA in the unknown samples

Concentration of DNA in the samples calculated by graph:

1. Cigarette butt \longrightarrow U1= 125 μ g
 10 μ l \longrightarrow 125 μ g
 12.5 μ g/ μ l, **1.11mg/ml**
2. Lipstick mark \longrightarrow U2= 110 μ g
 10 μ l \longrightarrow 110 μ g
 11 μ g/ μ l, **0.11mg/ml**
3. Chewing gum \longrightarrow U3= 220 μ g
 10 μ l \longrightarrow 220 μ g
 22 μ g/ μ l, **0.22mg/ml**

RFLP:-

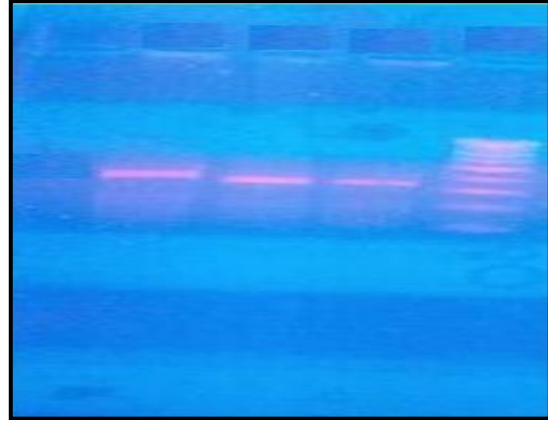


Fig 2: showing different band pattern on Agarose gel after RFLP

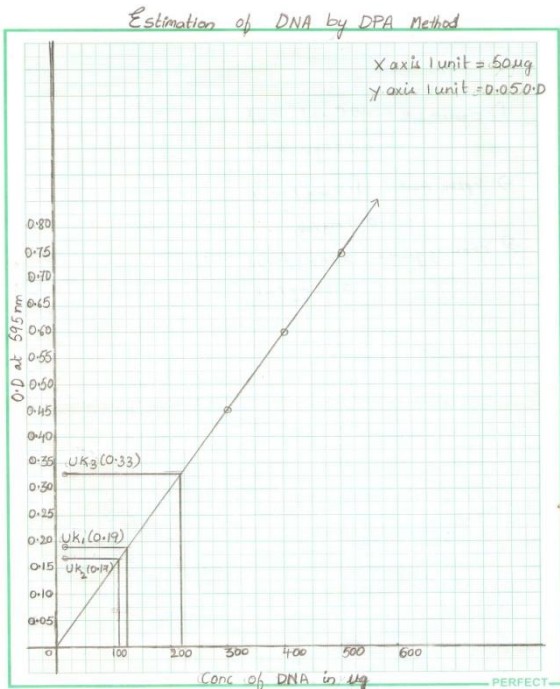
The different band pattern on the gel was obtained as the samples were collected from different sources and not from the same individual.

Conclusion:

DNA forensic technique is highly authentic and very much accurate. Each person has similarities and differences in DNA sequences. DNA forensic works on the basic like extraction, isolation and identification. The source person of the DNA can be identified by using RFLP and STR.

DNA can be isolated from the various materials like cigarette, lipstick print or chewing gum used by the person. The quantity of the DNA however differs.

By above studies and results, we can conclude that, the evidences concern with direct contact with saliva like chewing gum are most important as they can provide considerable amount of DNA. Such samples should be collected with prime priority and preserved.



From the Graph the following information can be obtained:

References:

1. Autosomal Short Tandem Repeat Analysis of Ancient DNA by Coupled Use of Mini- and Conventional STR Kits* Oh CS, Lee SJ, Park JB, Lee SD, Seo SB, Kim HY, Kim J, Kim YS, Shin DH.
2. Performance of two 17 locus forensic identification STR kits-Applied Biosystems's AmpF(STR(®) NGMSelect™ and Promega's PowerPlex(®) ES17 kits. Tvedebrink T, Mogensen HS, Stene MC, Morling N
3. Inter-laboratory evaluation of the ISO standard 11063 "Soil quality - Method to directly extract DNA from soil samples". Petric I, Philippot L, Abbate C, Bispo A, Chesnot T, Hallin S, Laval K, Lebeau T, Lemanceau P, Leyval C, Lindström K, Pandard P, Romero E, Sarr A, Schloter M, Simonet P, Smalla K, Wilke BM, Martin-Laurent F.
4. Extraction platform evaluations: A comparison of Automate Express™, EZ1® Advanced XL, and Maxwell® 16 Bench-top DNA extraction systems. Davis CP, King JL, Budowle B, Eisenberg AJ, Turnbough MA.
5. A method for DNA and RNA co-extraction for use on forensic samples using the Promega DNA IQ™ system. Bowden A, Fleming R, Harbison S.
6. Automated genomic DNA extraction from saliva using the QIAextractor. Keijzer H, Endenburg SC, Smits MG, Koopmann M.
7. Maxim G. Brevnov,1Ph.D.; Hemant S. Pawar,1Ph.D.; Janna Mundt,1Ph.D.; LisaM.Calandro, 1M.P.H.; Manohar R. Furtado,1Ph.D.; and Jaiprakash G. Shewale,1Ph.D. Developmental Validation of the PrepFiler™
8. Forensic DNA Extraction Kit for Extraction of Genomic DNA from Biological Samples*
9. A Simple Automated Instrument for DNA
10. Extraction in Forensic Casework*
11. Recovery of DNA for Forensic Analysis from Lip Cosmetics*
12. REFERENCE: Webb LG, Egan SE, Turbett GR. Recovery of DNA for forensic analysis from lip cosmetics. J Forensic Sci 2001;46(6):1474-1479.
13. David J.Walsh,1 M.S.;Amy C.Corey,1 B.S.; Robin W.Cotton, Ph.D.;Lisa Forman, ~ Ph. D. ; George L. Herrin, Jr. 2 Ph. D. ; Charlotte J.Word,1 Ph.D. ; and Daniel D. Garner, 1 Ph. D . Isolation of Deoxyribonucleic Acid (DNA) from Saliva and Forensic Science Samples Containing Saliva.
14. REFERENCE : Walsh, D. J. , Cor ey, A.C .,Cotton , R.W. , Forman , L . ,Herrin, G. L. ,Jr . ,Word , C J .and Garner , D. D . , "Isolation of Deoxyribonucleic Acid (DNA) from Saliva and Forensic Science Samples Containing Saliva, "Journal of Forensic Sciences, JFSCA, Vol . 37,No. 2, March 1992, pp.387-395.
15. Extraction of DNA from cigarette butts using proteinase forensicGEM™
16. Charis Shepherd, Sallyann Harbison² and David J. Saul ¹ ZyGEM Corporation Ltd, Waikato Innovation Park, Ruakura Road, Hamilton, New Zealand. ² Forensic division, Institute of Environmental Science and Research Ltd, Mt Albert Science Centre, Private Bag 92021, Auckland, New Zealand.
17. Laboratory experiments in Microbiology by M .Gopal Reddy, D.V.R. Saigopal, K.V. Mallaiiah.
18. Crime Scene Management by Dr. M.S. Rao, Dr. B.P. Maithil, K.V. Ravi kumar.
