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Optimization of Short Tendem Repeats (STR) Typing, PCR, Electrophoresis Based methods in the Field of Forensics

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Abstract

Microsatellites, also known as short tandem repeats (STRs), account for 3% of the human genome and have been associated with a number of disorders. More de novo mutations occur in each generation because of them than from any other variation, although their importance in naturally occurring diseases like autism and neuro developmental disorders has not yet been investigated. The core of forensic DNA profiles are STRs. The sterile nature of PCR necessitates the use of gloves, autoclaving, sterile settings, and DNA and D Nase-free reagents. An experiment's negative control aids in the detection of contamination. DNA sequences with short tandem repeats (STR) have become commonplace, emphasizing the importance of examination. Although validation is crucial for forensic samples that comprise mixtures, the introduction of multiplexed reactions has made STR analysis easier in case work. Genetic analysis of STR loci has proven to be the most accurate way to identify biological components in forensic investigations and human remains. The utilization of primer sequences to differentiate between sequences and the capacity to distinguish across over lapping size ranges are two advantages of STR amplicons. Although contamination within farmed cell lines continues to be an issue, contamination across species and within individual species happens in considerable percentages.

Keywords: Neuro Development Disorders; Human Genome; PCR; DNA; Sterile.

INTRODUCTION

The human genome contains 1-3 base-pair repeating motifs known as STRs, or microsatellites. High mutation rates are a result of frequent DNA replication slippage events. STRs make a considerable contribution to the genetic variety of humans, yet studies on medical sequencing frequently ignore their function in disease.[1-3] Although NGS can profile over a million STRs, genotyping is difficult because of bad calls Based on a standardized set of polymorphic short tandem repeat loci with 15 studies in the European Union and 5 in the North American CODIS system, forensic DNA profiles are created. STR loci, which define human forensics, are multiple repetition units of four nucleotides placed in tandem. By utilizing fluorophores in multiplex PCR evaluations of forensic STR loci, distinct identification based on size and color is made possible.[4,5] Allele assignment due to analytical noise or technological anomalies is prevented by the inclusion of sizing and allele

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standards in commercial reagent kits by utilizing fluorophores in multiplex PCR evaluations of forensic STR loci, distinct identification based on size and color is made possible. Allele assignment due to analytical noise or technological anomalies is prevented by the inclusion of sizing and allele standards in commercial reagent kits.[6]

A standard set of polymorphic short tandem repeat (STR) loci in the human population is used to construct forensic DNA profiles. The North American CODIS system adds five additional loci to the 15 examined by the European Union. With very few exceptions, STR loci are composed of a number of tandemly repeated units of a few nucleotides. Due to trinucleotide expansions, STRs greatly contribute to human genetic variation, including Fragile X Syndrome. In respect to common alleles, repetition units are represented by allele names.[7,8] After PCR, electrophoresis can be used to count the repeat units, which are comparable to base pairs. There can be nine to more than forty different alleles in a locus, producing a statistically unique STR profile. Because size is required to discriminate between loci in the electropherogram, this facilitates the construction of a reduced size STR amplicon multiplex by removing the restriction that not all amplicons can have the smallest size. Commercial STR typing reagent kits make use of CE hardware, such as size and allele standards.[9,10] Using these kits, electropherogram evaluation can be done semi-automatically while assigning allele numbers to the peaks for each locus. Threshold settings stop allele assignment for peaks brought on by technical or analytical noise.[11] The research article presents a comprehensive analysis of various studies, emphasizing the vital role of short tandem repeat (STR) DNA sequences in forensic science for creating national databases and identifying biological samples.[12] Multiplexed reactions enable simultaneous analysis of multiple loci but require thorough validation for reliability.[13] Genetic analysis of STR loci is the most effective technique for sample identification across diverse substrates.[14] Single gene sequencing (SGS) facilitates the creation of STR multiplexes with overlapping amplicon sizes, overcoming size restrictions.[15] Cross-contamination among cell lines, especially HeLa, poses challenges to research reliability.[16,17] Accurate cell line authentication is crucial for research validity.[18] These findings enhance our understanding of STRs and their significance in forensic science and cell line research.

METHODOLOGY

Materials Required: Blood samples, phenol, Chloroform, 70% Ethanol, Proteinase K, Rnase K, Microcentrifuge tubes, centrifuge, vortex mixer.

Sample Collection: Blood was collected from the healthy individuals with informed consent.

Sample Pretreatment: In order to extract DNA using the phenol-chloroform technique, 200–500 l of blood was transferred into a microcentrifuge tube, diluted with TE buffer, and then cell lysed. For 15 to 30 minutes, RNase A was introduced and incubated at 37°C. Three layers upper aqueous, interphase, and lower organic emerged from the combination. DNA was precipitated using 2.5 volumes of chilled ethanol or isopropanol and 0.1 volumes of sodium acetate. Without disturbing the DNA pellet, the DNA was washed, and proper TE buffer was used for resuspension of the DNA. PCR and sequencing are two molecular biology methods that can be performed on the extracted DNA.

Isolation of DNA from Blood Samples using Phenol-Chloroform DNA Extraction

To extract DNA from fresh blood, 2 volumes of reagent A was added to a polypropylene tube and mixed gently until the solution becomes cleared. It was centrifuged at 2500 rpm for 10 minutes to obtain a pellet free of red blood cells (RBCs). The pellet was disturbed thoroughly and half the volume of reagent B was added, along with Proteinase-K, and SDS to make a 1% concentration. It was mixed gently and inverted for 3–4 minutes until the solution became viscous. Incubated at 56°C or over night. After digestion, reagent C was added and mixed gently for 3–4 minutes. DNA was separated into three layers: aqueous, protein, and solvent. The aqueous layer was transfered to another centrifuge tube, ensuring the protein layer was not disturbed. Equal volumes of chloroform and isoamyl alcohol was added to the supernatant and mixed gently for 3–4 minutes. The aqueous phase was transfered to a fresh tube and chilled absolute alcohol was added to precipitate the DNA.

Spooled out the DNA lump in a fresh 1.5-ml tube and alcohol was decanted. The DNA was washed twice with 70% alcohol and the pellet was dried. The pellet was dissolve in an appropriate amount of TE and stored at 4°C, -20°C, or -80°C according to the storage period.

RESULTS AND DISCUSSIONS

The success of DNA extraction can be evaluated through various methods, including visual inspection, quantification, agarose gel electrophoresis, PCR amplification, and DNA sequencing. Visual inspection checks for impurities or degradation, while quantification measures the extracted DNA's concentration. Agarose gel electrophoresis visualizes the size and integrity of DNA fragments, while PCR amplification amplifys specific target regions. Successful PCR amplification indicates the presence of amplifiable DNA. DNA samples have been amplified using primer for STR marker D13S3.[17] Desired gene fragments were obtained and as identified using ladder and confirmed under gel documentation system.

The study examined the STR marker D13S317 from three distinct human genomes using PCR amplification and gel visualization. The findings demonstrated that the STR marker has special properties that make it valuable in forensic investigation and parentage verification, including a wide variety of sequence variances among human populations. Along with VNTRs, these markers are commonly employed in DNA testing for purposes ranging from forensic investigation to confirming parent child ties. The high polymorphism in STR loci offers a solid foundation for identifying people and forming connections. The modest band sizes of the PCR procedure, which conserves time and resources, enable a cost-effective method of employing STR markers for forensic applications. Overall, the findings show that STR markers are appropriate for use in a variety of DNA testing applications, including forensic analysis and research sample authentication.

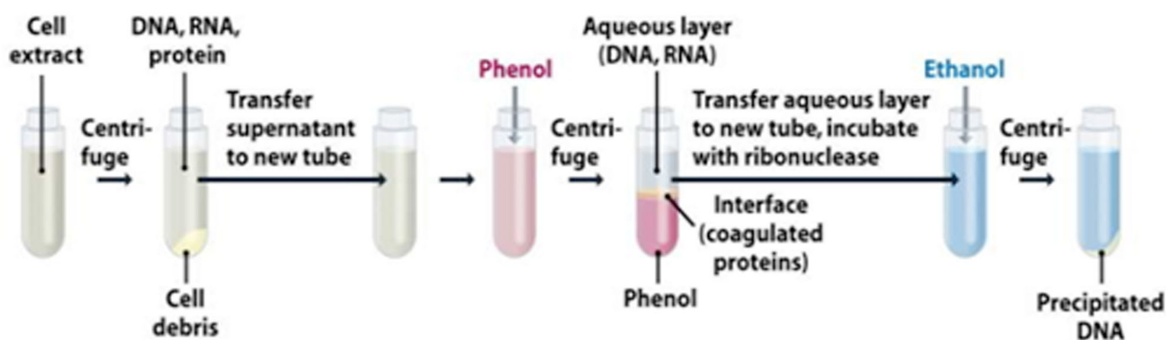

Fig. 1: Schematic diagram of DNA Extraction from different Organisms

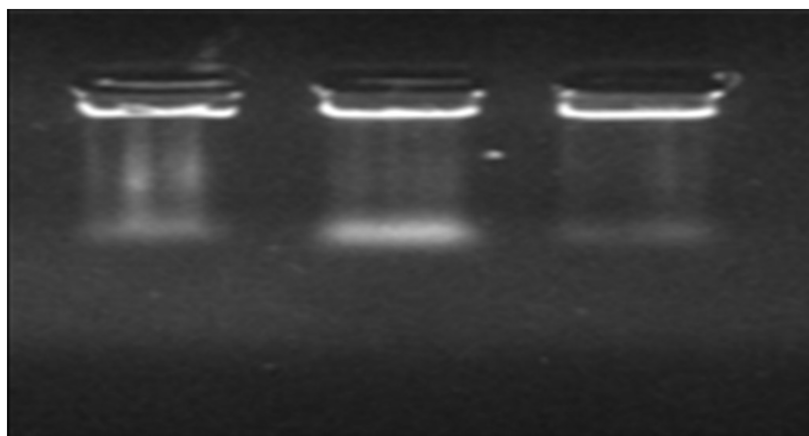

Fig. 2: Representative image showing the genome on agarose gel

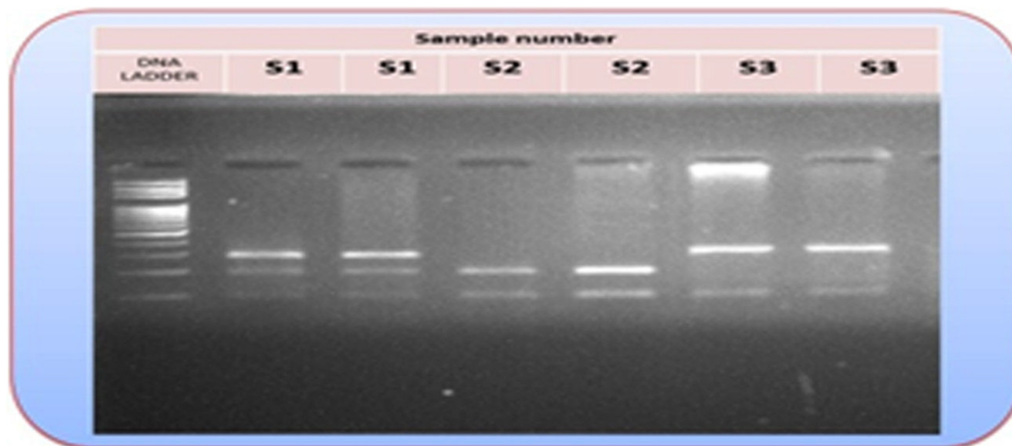

Fig. 3: Image showing PCR product for the STR marker D13S317 in three sample (each were in duplicate)

CONCLUSION

The STR marker D13S317 was effectively amplified by PCR in the current work, and the PCR was validated for this marker. This improved methodology can be used for forensic investigations including paternity testing and individual identification. Due to the large variety of sequence variations seen in human populations, the STR marker D13S317 is a potent DNA testing tool for forensic study and parentage confirmation. In this work, the STR region from three separate human genomes was amplified using blood samples and a D13S317 primer set, providing crucial baseline data for forensic applications and authenticating human research materials in compliance with the ASN-0002 standard.

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