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The Estimation of Genome Potential for STR Analysis in bloodstain collected at different temperatures

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Abstract

DNA analysis is used in forensic investigations to identify individuals and solve crimes. Important pieces of evidence are frequently found at crime scenes, including bloodstains. However, both the quantity and the quality of the extracted DNA can vary depending on the conditions in which bloodstains are exposed before analysis, such as different temperatures.

This study, to evaluate the genome potential for performing short tandem repeat (STR) analysis on bloodstains collected from three different temperature settings: -20^0, 4^0, and room temperature. The study will evaluate how temperature affects DNA extraction and typing. Previous studies have demonstrated that burns and high heat can cause DNA to degrade and lower DNA quantities in bloodstains. Additionally, research has shown that even after being heated to specific temperatures, cleaned bloodstains can still produce sufficient DNA for analysis. This research will help to clarify the challenges and limitations involved in conducting STR analysis on bloodstains exposed to a range of temperatures by examining the effects of various temperatures on DNA quantity and quality in bloodstains. The results will help investigators in the forensic field increase DNA recovery techniques and raise the accuracy of DNA profiling in criminal investigations.

Keywords: Short Tandem Repeat (STR analysis on bloodstain); Temperatures; DNA extraction; Criminal investigations.

INTRODUCTION

Forensic DNA analysis is crucial in criminal investigations, with Short Tandem Repeat (STR) analysis being a widely used method. However, temperature changes can affect the quality and quantity of DNA extracted from blood stains. This thesis aims to investigate the impact of temperature variations on STR analysis in blood stains and develop reliable estimation models. The research will involve collecting samples under controlled temperatures, aiming to improve the accuracy and reliability of forensic DNA analysis in criminal investigations.[1,2] Blood's history spans millions of years, with scientific understanding evolving from ancient beliefs to modern discoveries. Microscopy in the 17th century revolutionized blood circulation, while Karl Landsteiner's 1901 discovery revolutionized transfusion treatment. Hematology advanced in the late 19th and early 20th centuries, with specialized magazines like "Blood" promoting interdisciplinary collaboration. Blood has significantly impacted medical practices,

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particularly in transfusion medicine.[3,4,5] Blood is a crucial biological evidence found at crime scenes, serving various functions such as transporting vital materials, removing waste, protecting the body from pathogens, regulating body temperature, transporting hormones, and preventing excessive bleeding. It is composed of cells, enzymes, proteins, water, and inorganic substances. Blood stains provide information on the circumstances surrounding violent crimes, driving criminal investigations and supporting the conclusion of criminal cases. Blood detection in forensic science can help determine the source of blood, such as foetal blood, pregnancy and abortion blood, menstrual blood, and arterial blood. Bloodstain analysis from clothing is a crucial technique in forensic science for extracting valuable information from bloodstains in criminal investigations.[6,7,8] It helps in reconstructing details, identifying patterns, and developing conclusions by examining the size, shape, distribution, and characteristics of bloodstains.[9,10,11] Proper documentation, careful collection, and storage of garments are essential for maintaining the integrity of evidence. Ageing of blood can be determined by colour changes over time. Bloodstain analysis provides valuable data for criminal investigations and judicial actions. Factors affecting genomic potential for STR analysis include DNA degradation, detection rate, and quantitative PCR assays. Experimental data and analysis are required to determine the impact of temperature on DNA degradation and the success rate of STR analysis.[12-15]

METHODOLOGY

Material Required

Chemicals: 1x TAE buffer, Sol. B (pH 8.0), 20% SDS, Proteinase K, Phenol-chloroform-Isoamyl alcohol mixture (25:24:1), 5M Sodium Acetate (pH 5.2), Chilled Isopropanol (IPA), 70% ethanol, Nuclease free water, Primers, dNTPs + Buffer etc.

Instruments: Microcentrifuge, Autoclave, Refrigerator, PCR Machine, RT-PCR Machine, Pipette/Tips, Eppendorf tubes 2ml, Scissors, Forceps etc.

Sample Collection: Blood collected from a single person after taking consent, a total of 30 samples of bloodstains were spotted in a cotton cloth/fabric. Out of which 10 blood stains were kept at Room temperature (RT), 10 at -20^0, and 10 at 4^0. The samples were incubated at respective temperatures for 11 days before processingfurther.

Sample Pretreatment: The sample was collected on 25th April 2023 and kept each 10 samples at three temperatures Room Temperature (RT), -20^0, 4^0 for 10 days. From 11th day DNA extraction from each sample was done having an interval of one day between three samples.

Procedure

DNA extraction from blood stains

The study involved cutting a stain-free cloth and adding 1.5ml of Solu. B, 50% SDS, and Proteinase K. The mixture was incubated overnight and centrifuged at 10,000 RPM for 15 minutes. The supernatant was transferred to a fresh tube, mixed with Sodium Acetate and PCI, and incubated overnight. The pellet was washed with 70% ethanol, air-dried, and dissolved in nuclease-free water. The dissolved DNA was visualized under UV light using agarose gel electrophoresis.

Agarose Gel Electrophoresis

Agarose gel was created by diluting 10X TAE buffer to 1X and mixing 300 mg of agarose with 30 ml of 1X TAE buffer. The flask was heated, and the agarose was dissolved, while boiling was avoided. A gel-casting tray was sealed with biohazard tape. To the cooled agarose solution, 0.5 µl of ethidium bromide was added. The agarose was poured to a depth of 1 cm and allowed to solidify. A 500 µl DNA sample, 2.5 µl loading dye, and a marker were added to the gel. The gel was placed in a tank filled with TAE buffer. The electrophoresis was run at 80V for an hour until the front dye reached the gel's bottom. The electrodes were checked for bubbling, and the gel was photographed under UV transillumination.

DNA Quantification

The quantification was done using the Qubit 3.0 fluorometer kit and its instructions. The assay tubes for the standards and one assay tube for each user sample were set up. The working solution was prepared by diluting the Qubit reagent 1:200 in Qubit buffer. The assay tube and standard tubes were prepared according to the table given below.

Table 1: Assay tube and standard tubes protocol

Components	Standard Assay Tubes (µl)	Sample assay tubes (µl)
Working solution (from step 2)	190	199
Standard (from kit)	10	-

Table Cont...

Vol. of sample	-	1
Total volume in each tube	200	200

The tubes were vortexed for 2-3 seconds and incubated for 2 minutes at room temperature (RT). The tubes were inserted into the Qubit Fluorometer, and readings were taken. The readings were in ng/µl.

q-PCR

Procedure:Reaction mixture is prepared by adding all the components in one tube except sample and dividing the content equally in all wells and then adding sample in last. Reaction mixture was prepared for the total volume of 10µl.

Table 2: Table Reaction mixture for q-PCR

S/No.	Content	Volume (µl)
1	2X qPCR Mix	5
2	Fwd. Primer NEAT 1	0.5
3	Rev Primer NEAT 1	0.5
4	RNAse free water	3.8
5	Sample (DNA)	0.2

Running of q-PCR: The 2-step q-PCR involved a hot start at 950°C for 5 minutes, followed by denaturation, primer annealing, and extension at 600°C for 20 seconds. The amplification cycle was 45 cycles, culminating in melting.

Table 3: Steps in q-PCR

Sr/No.	Content	Temp (°C)	Duration (sec)
1	Pre-denaturation	95	3 min
2	Denaturation	95	10
3	Annealing &Extension	60	20
4	Melting	------	------

STR Analysis: STR analysis compares allele repeats at specific DNA loci in samples, used in forensic analysis, human sample authentication, and cell line authentication. It detects and measures short tandem repeat sequences, 2-7 base pairs long.[16]

Procedure

DNA Extraction: The DNA is extracted from the biological sample of interest using established protocols.

Primer Design:Primers target specific STR loci for amplification, allowing repeat sequence amplification.

Polymerase Chain Reaction (PCR): PCR amplifies targeted STR loci using primers, generating multiple copies of the STR region through denaturation, annealing, and extension cycles.

Fragment Separation: PCR products are separated using electrophoresis or

capillary electrophoresis to determine STR repeat lengths.

Data Analysis: Using software or genetic analysers, separated fragments are analysed to identify STR locus repeat units and establish links.

STR analysis is utilized in forensic science for human identification and cell line authentication, enabling discrimination and accuracy in unrelated individuals.

RESULTS AND DISCUSSION

Gel-Electrophoresis Results RT-PCR Results

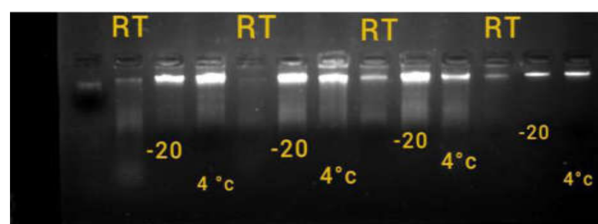

Fig. 1: 1-12 Gel-Electrophoresis samples visible as bands in results

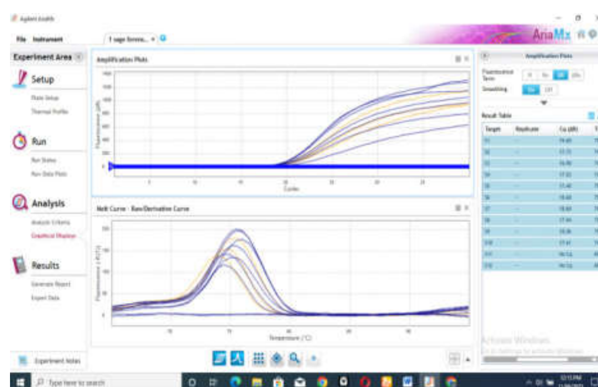

Fig. 2: Melt curve (Fold change) of NEAT 1 gene expression

Note: *CT values range from 17.49 to 19.32, indicating NEAT1 gene expression in all samples, with lower values indicating higher abundance.*

In the given dataset, the CT values for the NEAT1 gene range from **17.49 to 19.32.** Sample B3 (S3) has the lowest CT value of **17.49**, suggesting the highest expression level of NEAT1 among the tested

samples. Sample B1 (S1) and B10 (S10) have the highest CT values of **19.04** and **19.32**, respectively, indicating relatively lower expression levels of NEAT1.

Table 4: Statistical Summary of qPCR Data.

Parameters	CT values	Fold change values
Mode	18.21	74.5
Median	1.245	75.25
Mean	18.25	74.85
Range	17.49 – 19.32	69.5 – 76

STR Analysis Results

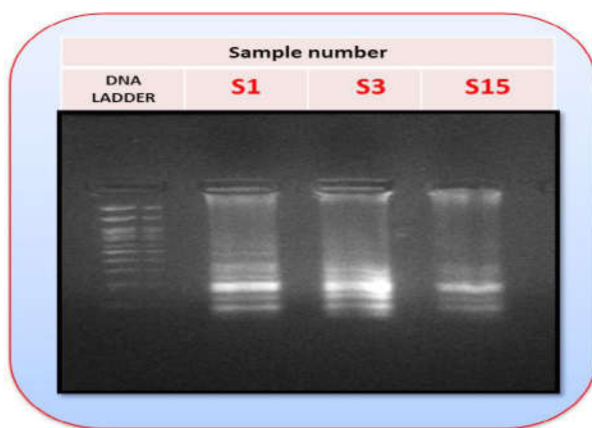

Fig. 3: Amplification of human genome STR region from blood-stained cloth samples using molecular marker-based primer set yields easily distinguishable bands, providing baseline data for forensic purposes

CONCLUSION

The study investigates the application of Short Tandem Repeats (STRs) for temperature-dependent blood stain analysis. For locating genetic variations and mutations in Mendelian illnesses, researchers found STR analysis to be reliable. The effect of temperature on the degradation of DNA and detectable loci was also investigated. The results of these investigations have implications for forensic research, improving methods for diagnosis and treatment, and benefiting society.

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