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A Novel Method of DNA Fingerprint Using Seminal Fluid

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Annotation: DNA fingerprinting is a cornerstone of forensic science, particularly in sexual assault investigations, where biological traces like seminal fluid are critical evidence. Despite advances, challenges remain in obtaining reliable DNA profiles from small or aged semen stains without complex extraction processes. This study proposes a novel and simplified approach using direct PCR amplification of TPOX, TH01, and SRY loci for DNA fingerprinting directly from semen samples and semen-stained materials. A total of 48 semen samples were analyzed through dilution methods and paper-based sample preservation. The results demonstrate that direct PCR without prior DNA extraction provides rapid, cost-effective, and high-quality DNA profiles suitable for forensic applications. The method successfully amplified STR markers even from aged and diluted samples, proving its reliability for criminal investigations. The findings imply that this technique could enhance forensic laboratories' capacity to process sexual assault evidence more efficiently and economically in Iraq and beyond.

Keywords: DNA fingerprinting, direct PCR, forensic genetics, sexual assault investigation, seminal fluid, STR markers, TPOX, TH01, SRY.

1. Introduction

As the number of sexual assault cases are increasing day by day in Iraq, so there is a need of detection of sperm and semen even if present in small quantity, no matter how old the stains are, we have to detect these stains precisely. Detection of sperm and semen are the most reliable marker for the investigation in cases of rape, sodomy, sexual murder etc.[1]

There are many techniques to detect criminal in sexual assault, however direct PCR is the best method which is a technique where samples are subjected to amplification without first having to go through the extraction process.

There are many advantages of using Direct PCR are First, better DNA profiles could be obtained by using direct PCR because there is no loss of DNA associated with extraction protocols, so it is better. Also, direct PCR takes 2-3hours to generate a DNA profile, while in the traditional method (which include extraction and quantification) takes 10-12hours, so it is faster. Therefore that very important in cases of national security, abduction with danger of life, risk of repetition by a serial perpetrator or when custody time of suspects is limited. Finely, the costs involved in purchasing expensive extraction and quantification kits can be reduced when using direct PCR, so it is cheaper [2, 3].

Short tandem repeats(STR): loci consist of simple tandem repeated sequences of 1-6 bp in length[4, 5].Forensic genetics developed from protein-based techniques a quarter of a century ago and became famous as “DNA fingerprinting,” this being based on restriction fragment length polymorphisms (RFLPs) of high-molecular-weight DNA.[6, 7]At the present time, STRs are applied as best markers of choice in forensic, paternity examination and person identification studies [6, 8].

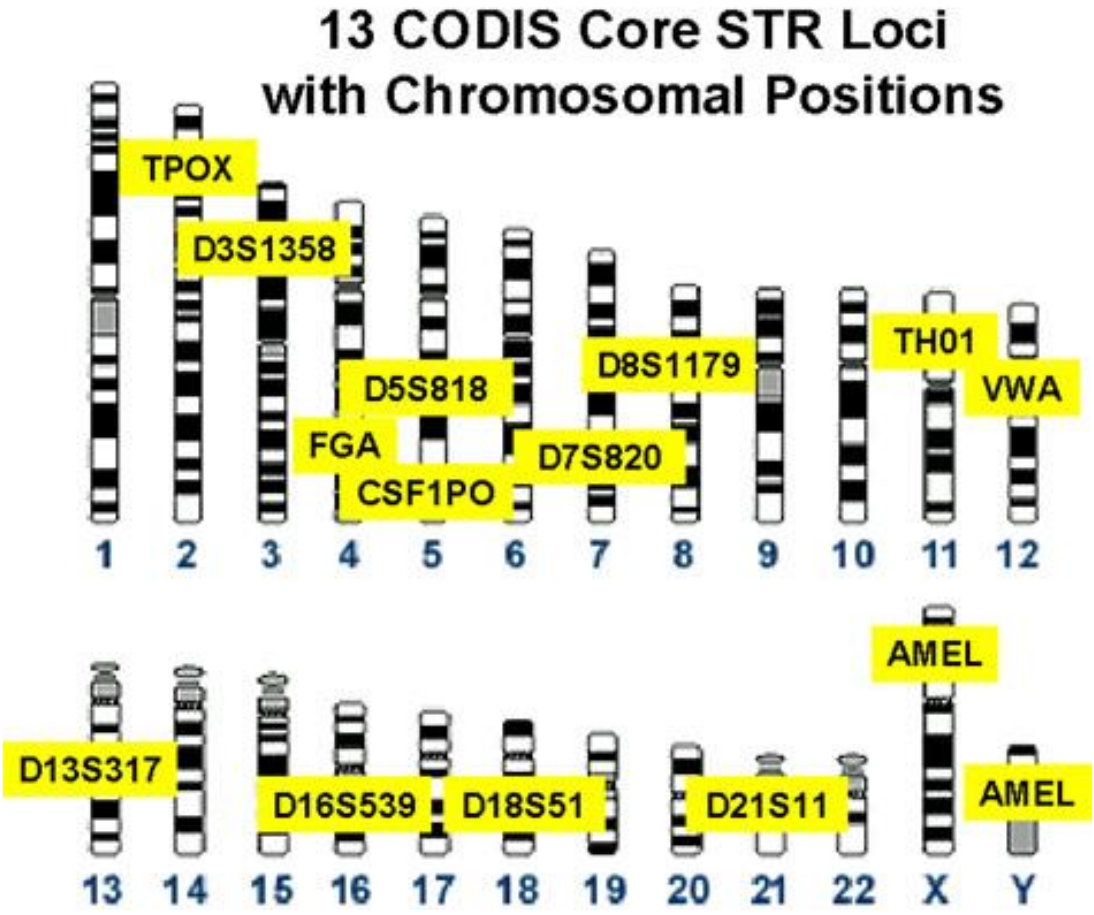

Figure 1: Position of forensic STR markers on human chromosomes

TPOX is (AATG)n, intron 10 of the thyroid peroxidase gene (2p23-2pter)[9, 10].TPOX has been studied in some neighboring countries [4, 11-13].

TH01 is a tetrameric short tandem repeat locus located in intron 01 of the tyrosine hydroxylase gene [14, 15].

SRY (sex-determining region, Y) is the gene responsible of gonadal differentiation in the male and it is essential for the regular development of male genitalia [16-19] it encoded by the human testis, contains a high mobility group (HMG) box [20] and located on short arm of sex chromosome [21].

- This study aims to investigate the DNA fingerprinting using TPOX and TH01 loci and the possibility of applying of it in the forensic applications in Iraq by means of simple, easy and cheap method (direct PCR).

2. Materials & Methods

2.1 Materials

2.1.1 Equipment and Apparatus

Different equipment and apparatuses have been used throughout the study as shown in table 1

Table 1: Equipment used in the study

Equipment	Company
Cooled centrifuge	Labnet (USA)
Thermocycler(PCR)	Labnet (USA)
Hot plate magnetic stirrer	Stuart scientific (U.K.)
Sensitive balance	Sartorius (Germany)
Vortex mixer	Buchi (Switzerland)
DNA –Gel Electrophoresis	Labnet (USA)
Micropipettes	Witeg (USA)
Gel documentation system	Labnet (USA)

2.1.2 Chemicals and buffers

TBE buffer 10X

It is composed of:-

Tris-Borate 890 mM

EDTA 20 mM

Agarose gel: Agarose 1% concentration was used, dissolved in TBE 1X using hotplate.

2.2 Methods

We take 48 semen sample of volunteers from fertility center in Najaf.

2.2.1 Dilution of primer

Take 10µl of each primers (Forward & Reverse) by micropipette and put each one into eppendrof (microcentrifuge tube) and completed with 90µl ddH2O to dilute primers.

- ✓ After that centrifugation.

2.2.2 Semen

We work experiment at two ways:

1. **Fresh Semen (Dilution)**

a) 1:10 tube, take 10µl of semen and completed with 90µl ddH2O.

b) 1:20 tube, take 10µl of semen and completed with 190µl ddH2O.

2. Semen on paper

a) After 1 hour

b) After 24 hours (one day)

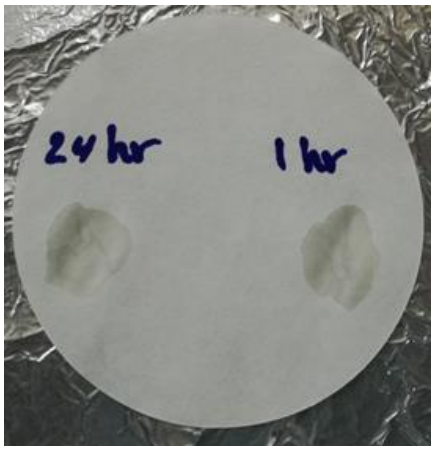

Figure 2: Semen on paper (after 1 & 24 hour)

2.2.2.1 (First – Fresh semen)

1. Add 1.5µl from diluted **forward**

a) (TPOX) primer into (1 to 4) master-mix tubes.

b) (TH01) primer into (5 to 8) master-mix tubes.

2. Add 1.5µl from diluted **reverse**

a) (TPOX) primer into (1 to 4) master-mix tubes.

b) (TH01) primer into (5 to 8) master-mix tubes.

3. Add 2µl of

a) fresh semen into (1,5) tubes.

b) 1:10 diluted semen into (2,6) tubes.

c) 1:20 diluted semen into (3,4,7,8) tubes.

4. Add 15µl of ddH2O into all master mix tubes

Table 2: Fresh semen experiment addition (TPOX & TH01)

Primer type	TPOX				TH01			
No. of master-mix tube	1	2	3	4	5	6	7	8
Forward primer	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl
Reverse primer	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl
Rates of semen	S*	1:10	1:20	1:20	S*	1:10	1:20	1:20
ddH2O	15µl	15µl	15µl	15µl	15µl	15µl	15µl	15µl

* "S" refer to fresh semen.

Table 3: Fresh semen experiment's addition (SRY)

Primer type	TH01							
No. of master-mix tube	1	2	3	4	5	6	7	8
Forward primer	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl
Reverse primer	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl
Parts of paper	-	-	-	-	-	-	-	-
ddH2O	17µl	17µl	17µl	17µl	17µl	17µl	17µl	17µl

2.2.2.2 (Second – semen on paper)

1. Add 1.5µl of forward (TH01) primer into all master-mix tubes.
2. Add 1.5µl of reverse (TH01) primer into all master-mix tubes.
3. Put the DNA sample (semen) into all master-mix tubes.
4. Add 17µl of ddH2O into all master-mix tubes.

Table 4: Semen on paper experiment's addition

Primer type	SRY							
No. of master-mix tube	1	2	3	4	5	6	7	8
Forward primer	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl
Reverse primer	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl	1.5µl
Rates of semen	S*	1:10	1:20	1:20	S*	1:10	1:20	1:20
ddH2O	15µl	15µl	15µl	15µl	15µl	15µl	15µl	15µl

2.3 PCR

Amplification conditions were 40 cycles of

94°C / 4 minutes,

94°C / 30 seconds,

58°C / 50 seconds,

72°C / 2 minutes,

72°C / 5 minutes,

- Important Step! Adjust the time of first step of PCR process (denaturation) into 4 minutes rather than 2 minutes in conventional process.

*Note: this step will help denature a group of protein called PROTAMINES that is found in sperm head with the DNA, while the DNA molecules does not denature because of it is double strand structure.

PCR primers that used are shown in table 2according to[4]

DNA amplifications were repeated three times using the same conditions to confirm the results with negative controls.

Product sizes for TPOX genotyping could be varying between 216bp-256bp.

Table 5: specific primers applied for polymorphism determination of TPOX genotyping

Primer name	Sequence
TPOX F	5'ACTGGCACAGAACAGGCACTTAGG 3'
TPOX R	5'GGAGGAACTGGGAACCACACAGGT 3'
TH01 F	5'GTGTGGGTCTCTGTGTCTTGTTTCATC 3'
TH01 R	5'GTGTGGGTCTCTGTGTCTTGTTTCATC 3'
SRY F	5'CGCATTCATCGTGTGGTCTCG'3
SRY R	5'ATTCTTCGGCAGCATCTTCGC'3

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