Republic Of Iraq
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DNA Fingerprinting in Population of Al-Qadisiyah Province using TPOX locus for Forensics

A Research
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بسم الله الرحمن الرحيم

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ)

صدق الله العظيم

سورة البقرة: الآية 32

Dedication

To who was present with me at all times in my heart and mind to prophet of peace…

"**Mohammed**"

Peace and prayer be on him and his purified family.

To my great family who encouraged me

To all my friends

Israa Allawei and Aseel Basim

Acknowledgments

*First of all, praise is to "**Allah**", who enabled us to overcome all the difficulties that were associated with this work till I brought it to the present state. Peace and prayer be on the most honourable Mohammed and his purified family.*

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Thank you.

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1.Introduction

Short tandem repeats loci consist of simple tandem repeated sequences of 1–6 bp in length (1). 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(2, 3).At the present time, STRs are applied as best markers of choice in forensic, paternity examination and person identification studies.(2, 4-6).

STR (or microsatellite) loci build up of simple tandem repeated sequences of 1–6 bp in length(7). Due to the larger variable number tandem repeat (VNTR or minisatellite) loci, STRs could reveal a high degree of length polymorphism as a result of variation in the number of repeated units displayed(8). On the other hand, VNTRs, which take place mostly in telomeric areas, STRs appear to be abundant throughout the human genome and occur, on average, every 6–10 kb(9, 10).

Because of their abundance, polymorphic nature, and amenability to amplification by PCR, STRs are ideal markers for genomic mapping and genetic linkage analysis (11, 12).

In addition to their suitability for mapping and linkage analysis, STRs provide a source of highly informative loci for use in the identification of individuals(13). DNA profiling based on PCR amplification of STRs has the advantage of being more sensitive than conventional techniques. Furthermore, because of their small allele sizes (generally < 300 bp), STR systems are preferred to be used(14).

FBI Laboratory's Combined DNA Index System (CODIS) were selected in November 1997 the 13 CODIS loci used in the U.S. are CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11(2, 15).

TPOX is (AATG)n, intron 10 of the thyroid peroxidase gene (2p23-2pter)(4, 7).TPOX has been studied in some neighboring countries (1, 10, 16, 17).

This study aims to investigate the DNA fingerprinting using TPOX locus and the possibility of applying of it in the forensic applications in Iraq by means of simple, easy and cheap method.

2. Materials and Methods

2.1 Materials

2.1.1Equipment 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
Autoclave	**Gallenkamp (England)**
Cooled centrifuge	**Labnet (USA)**
Thermocylcer	**Labnet (USA)**
Distillatory unit	**Kent (England)**
Hot plate magnetic stirrer	**Stuart scientific (U.K.)**
Sensitive balance	**Sartorius (Germany)**
Spectrophotometer	**Labnet(USA)**
Vortex mixer	**Buchi (Switzerland)**
DNA –Gel Electrophoresis	**Labnet (USA)**
Micropipettes	**Witeg (USA)**
Gel documentation system	**Labnet (USA)**
Water bath	**Labnet (USA)**

2.1.2Chemicals and buffers

2.1.2.1 TBE buffer 5X (Maniatis *et al.*, 1982)

It is composed of:-

Tris-Base	54 g
Boric acid	27.5 gm
EDTA 0.5M (pH 8)	20 ml

The volume was brought to up 1 L and autoclaved

2.1.2.2 **Ethidium bromide** solution (10 **mg/ml) (Maniatis *et al.*,,1982)**

Ethidium bromide (0.1 g) was dissolved in 10 ml of D.W and stirred with a magnetic stirrer for six hours to ensure the complete dissolving, then it filtrated and stored in a dark bottle, wrapped with aluminum foil at 4°C.

2.1.2.3 Agarose gel

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

2.2Methods

2.2.1Study individuals

This study was carried out on 40 Iraqi individuals, aged between (14-69) years. Blood samples were collected from subjects attending AD Diwaniyha Teaching Hospital. About three milliliters of blood withdrawal from each individual and placed into Ethylenediaminetetraacetic acid (EDTA)-tubes then transferred to the laboratory in cooling conditions in less than one hour and half.

2.2.2DNA Extraction

DNA was isolated from peripheral blood by means of FavorPrep Blood Genomic DNA Extraction Mini Kit (South Korea) according to the manufacturer's instructions at Department of Medical Biotechnology / College of Biotechnology / University of Al-Qadisiyha and stored at -20˚C for Polymerase Chain reaction.

2.2.3Genotyping

Genotyping was took place on cycler machine (LABNET) using primers table (1). Amplification conditions were 40 cycles of 94°C / 4 minutes, 94°C / 30 seconds, 60°C / 50 seconds and 72°C / 2 minutes with a final extension step of 72°C / 7 minutes, PCR products were run on 1.8% Agarose gel and stained with Ethidium bromide then analyzed using UV transilluminator, standard DNA ladder 100bp (Bioneer, South Korea) was used.

PCR primers that used are shown in table 2 according to (1).

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 (2): specific primers applied for polymorphism determination of TPOX genotyping

Primer name	Sequence
TPOX F	**5'ACTGGCACAGAACAGGCACTTAGG 3'**
TPOX R	**5'GGAGGAACTGGGAACCACACAGGT3'**

3. Results and discussion

TPOX is considered as one of the most reliable locus to be used in the forensic DNA studies. The results raveled a polymorphic DNA bands due to the genetic diversity among the people as shown in figure (1-2) which could be lead to the ability of using it as application for tracing back any genetic discrimination through the forensics.

The variety of DNA bands after amplification was summarized in table (3).

Table (2): Allelic frequency and percentage of genotyping in the individuals

Allele No.	Frequency (n=40)	Percentage
1	**14**	**35**
2	**13**	**32.5**
3	**7**	**17.5**
4	**0**	**0**

The results revealed an obvious genetic diversity among the individuals who covered by this study, this genetic variety being caused by different repeats occurring at TPOX locus.

The results of our study are in harmony with findings of previous studies (5, 6, 18, 19).

Our study could prove the discriminative power of using TPOX as STR through multiple DNA bands and inequality among the people that could be also supported by other studies (2, 4, 20).

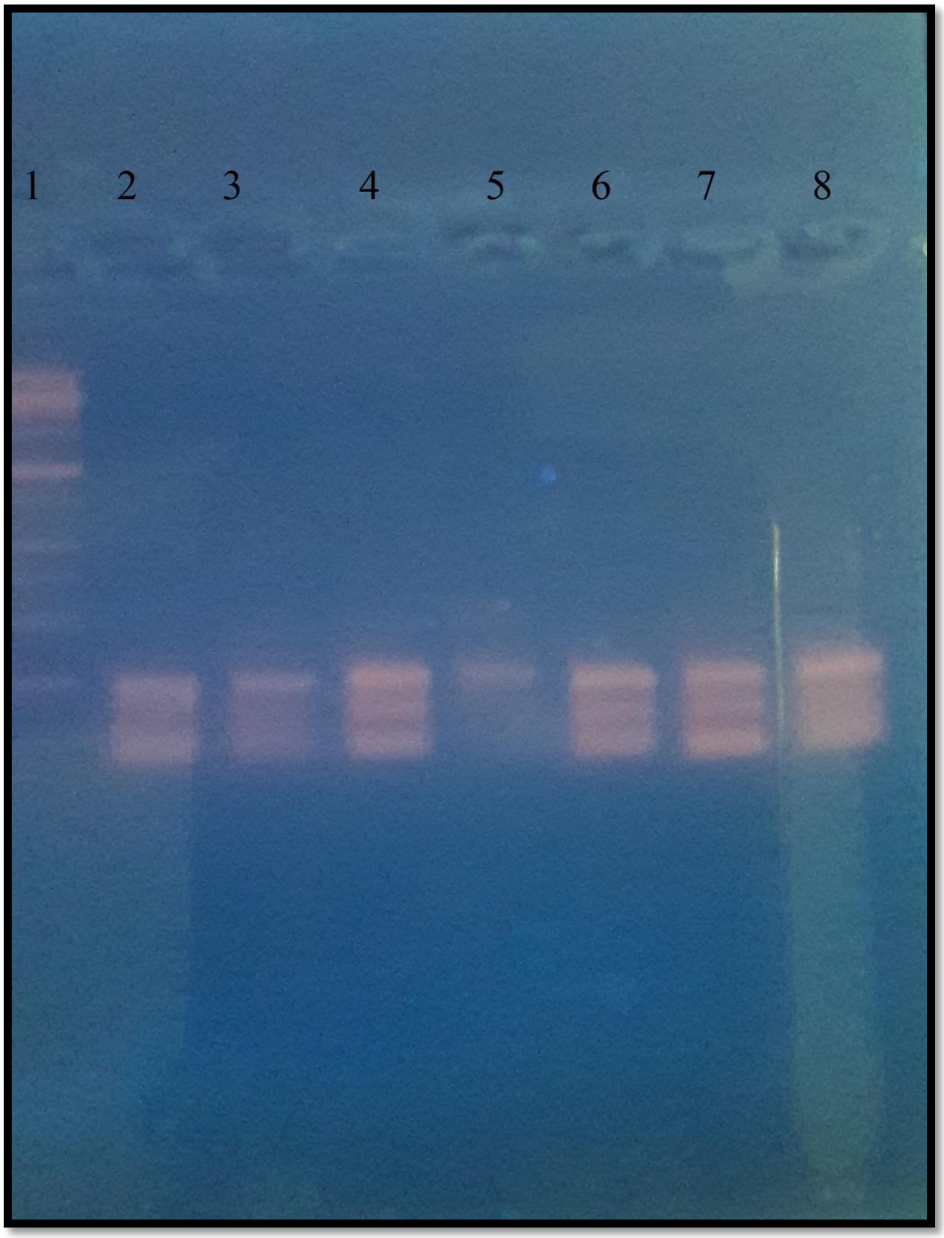

Figure 1: Genotyping of TOPX STR marker. The PCR products were analyzed on an 2% Agarose gel and visualized by EtBr.

Figure 2: Genotyping of TOPX STR marker. The PCR products were analyzed on an 2% Agarose gel and visualized by EtBr

4.Conclusion

Our results powerfully support the application of TPOX genetic markers for personal identity testing in the Iraqi population in forensics.

Authors' note

The manuscript has wrote by Endnote

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