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DNA FINGERPRINTING IN POPULATION OF AL-QADISIYAH PROVINCE, IRAQ USING TPOX AND TH01 LOCI TOWARDS FORENSIC APPLICATIONS

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Abstract

This study aimed to study the possibility of using Short tandem repeat (STR) loci which are the most useful DNA genetic markers for attempting to individualize biological material for application in parenthood and forensic cases. Fifty individuals were volunteers and blood samples were collected from them. Polymerase chain reaction (PCR) was done with specific designed primers for typing TPOX and TH01 str loci. The results revealed an obvious variation among the individuals covered by this study which at TPOX and TH01str loci and these data could be used for building up the database for forensics purposes for the Iraqi population.

Introduction

Short tandem repeats loci consist of simple tandem repeated sequences of 1–6 bp in length (Foroughmand *et al.,*2014) 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 (Parson, 2 018; Freire-Aradas *et al.,*2017). At the present time, STRs are applied as best markers of choice in forensic, paternity examination and person identification studies.(Parson, 2018; Zhang,2006; Soltyszewski *et al.,* 2006; Yunis *et al.*, 2005).

STR (or microsatellite) loci build up of simple tandem repeated sequences of 1–6 bp in length (Meraz-Rios *et al.,* 2014). 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 (Silva and Moura-Neto, 2004). 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 (Hochmeister *et al.,*1995; Bayoumi *et al.,*1997).

Because of their abundance, polymorphic nature, and amenability to amplification by PCR, STRs are ideal markers for genomic mapping and genetic linkage analysis (Budowle *et al.,*1997; de Pancorbo *et al.,*1998).

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 (Vural *et al.,* 1998). 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 (Jiang *et al.,*1999).

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[Parson, 2018;Butler, 2015] as in Fig. 1.

TPOX is (AATG)n, intron 10 of the thyroid peroxidase gene (2p23-2pter) (Zhang, 2006; Meraz-Rios *et al.,* 2014). TPOX has been studied in some neighboring

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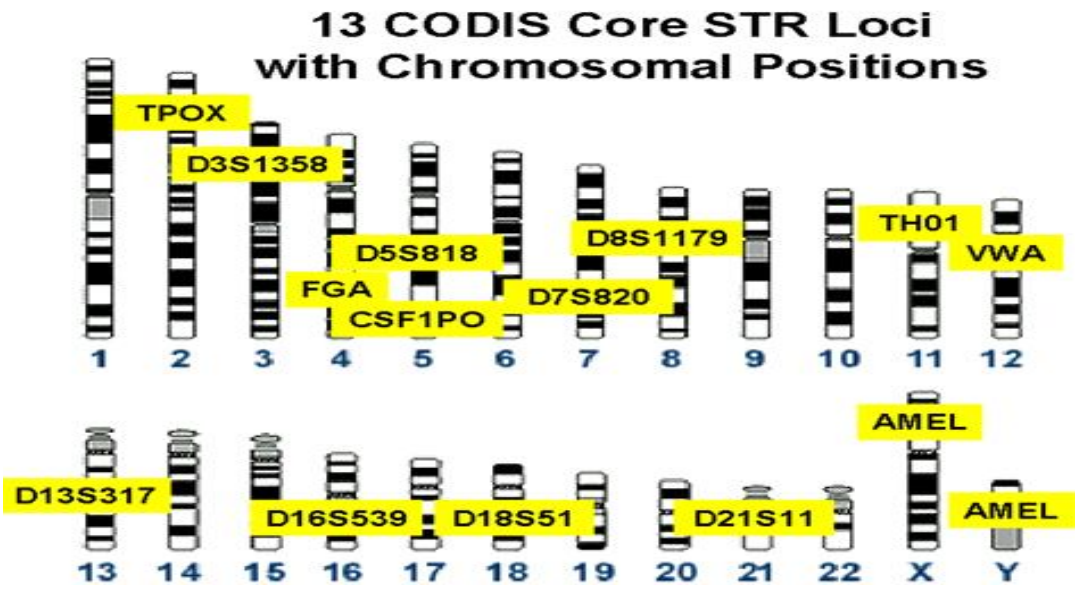


Fig. 1: CODIS system in human (Butler, 2006).

countries (Foroughmand *et al.,* 2014; Bayoumi *et al.,*1997; Shimada *et al.,* 2002; Hadi Cakir *et al.,* 2001).

TH01 is a tetrameric short tandem repeat locus located in intron 01 of the tyrosine hydroxylase gene (Yin, 2018; Yao *et al.,* 2018; Messina *et al.*, 2018)

This study aims to investigate the DNA fingerprinting using TPOX and TH01loci and the possibility of applying of it in the forensic applications in Iraq by means of simple, easy and cheap method with safe DNA dye.

Materials and Methods

Materials

Chemicals and buffers

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

Safered Solution (10 mg/ml)

Safered (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.

Agarose gel

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

Methods

Study individuals

This study was carried out on 50 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.

DNA 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.

Genotyping

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 2% Agarose gel and stained with safered 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 (Foroughmand *et al.,* 2014).

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

Product sizes for TPOX,TH01 genotyping could be varying between 216bp-256bp.

Table 1: Specific primers applied for polymorphism determination of TPOX,TH01 genotyping.

Primer name	Sequence
TPOX F	5'ACTGGCACAGAACAGGCACTTAGG 3'
TPOX R	5'GGAGGAACTGGGAACCACACAGGT3'
TH01 F	5'GTGTGGGTCTCTGTGTCTTGTTTCATC3'
TH01 R	5'GTGTGGGTCTCTGTGTCTTGTTTCATC3'

Results and Discussion

TPOX and TH01 are 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 Figs. 2, 3, 4 and 5 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 tables 2 and 3.

Table 2: Allelic frequency and percentage of genotyping in the individuals using TPOX str.

Allele No.	Frequency (n=50)	Percentage
1	14	28
2	48	96

Table 3: Allelic frequency and percentage of genotyping in the individuals using TH01 str.

Allele No.	Frequency (n=50)	Percentage
1	44	88
2	32	64

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,TH01 locus.

The results of our study are in harmony with findings of previous studies (Soltyszewski *et al.,* 2006; Yunis *et al.,* 2005; Picanco *et al.,* 2015; Lane, 2008).

Our study could prove the discriminative power of using TPOX,TH01 as STR through multiple DNA bands and inequality among the people that could be also supported by other studies (Parson, 2018; Zhang, 2006; Zhou *et al.,* 2004).

Conclusion

Our results powerfully support the application of TPOX,TH01 genetic markers for personal identity testing in the Iraqi population in forensics.

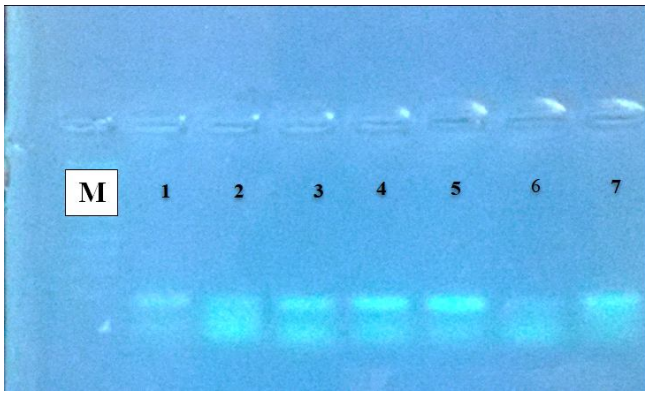
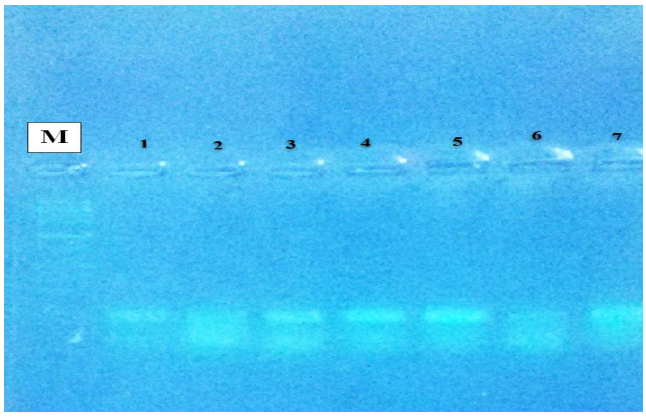


Fig. 2: Genotyping of TOPX STR marker. The PCR products were analyzed on an 2% Agarose gel and visualized by safered.



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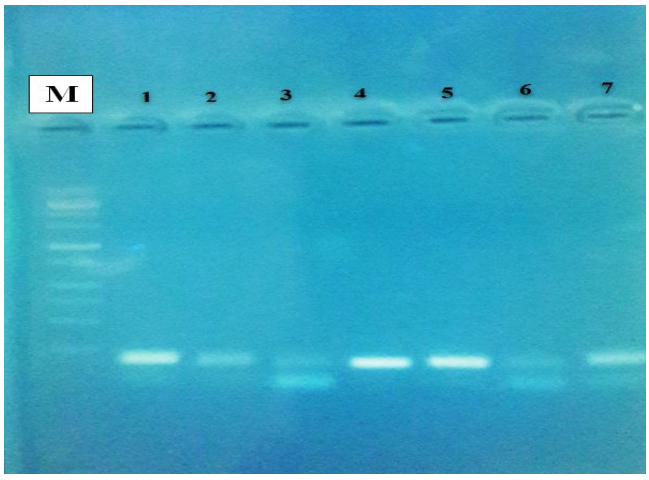

Fig. 4: Genotyping of TH01 STR marker. The PCR products were analyzed on an 2% Agarose gel and visualized by safered.

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