Use Markers of Short Tandem Repeats (STR) for Samples of Bone in the Forensic Diagnosis of the Human Being in the in Kerbala Governorate

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Abstract

STR's analysis of DNA from bone samples plays an important role in identifying missing persons. We present a method for DNA extraction from bone samples that involve complete demineralization and digestion of the sample, followed by purification by silica binding. This method, along with the multiple STR typing approach, has also proven highly successful in recovering DNA profiles from bone samples from a wide range of contexts. These methodological steps include bone cutting and grinding, DNA extraction, Re-purification in case of highly inhibited samples, quantification, amplification multiplex STR, Data Analysis of the DNA Typing and The guidelines for the preparation of personal reports. the total number of study samples reached 48, whose ages ranged between (14 to 33) years divided into 3 groups, group (1) in which (16) bone samples from the son, group (2) in which (16) blood samples from the father and group (3) It contains (16) blood samples from the mother. Conformity was carried out in the identification tests for persons in the forensic evidence laboratories of the Iraqi Ministry of the Interior, according to the protocols in the Kits used and by forensic experts.

Keywords: *Forensic Sciences, Identifcation Human, STR typing.*

Introduction

Forensic science is an area that solves legal cases in civil and criminal cases [(1)]. Forensic is a mixture of different branches of science that can provide information to the legal courts that are imposed by investigative bodies in the criminal justice system worldwide[(2)].

Forensic science is a multidisciplinary field, which helps rebuild a crime scene based on the collection, analysis, and interpretation of scientific evidence. Each crime is unique in its own way. This is because the nature of the crime that was carried out, the location of the crime scene, the people involved in the crime, and the things that were used, all differ from one crime scene to the next[(3)].

The availability of DNA samples is absolutely essential to determine the DNA pattern in a forensic study. DNA pattern determination is a method in which genetic differences at the DNA level are used to determine an individual[(4)].

The DNA samples present at the crime scene or mass disasters may be insufficient in quality and quantity, because they may often contain very small amounts of DNA or may be very degraded due to prolonged exposure to various environmental conditions such as heat, light, moisture, and microorganisms[(5)]. It is common in the forensic study to have highly degraded DNA samples from a variety of sources. Therefore, it is preferable for the collection of samples to be proper, and to handle carefully during the identification of the DNA pattern to reduce the chances of external contamination[(6)].

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Bones are often the primary source of samples in person identification and criminal investigations. Bones are often used in forensic study because they have the ability to demonstrate resistance to extreme and severe conditions such as high temperature, humidity, and bacteriological action[7][8].

Bones is one of the important evidence that is often found at the crime scene related to mass disasters, terrorism, human trafficking and missing persons. Morphological observations can be used by forensic scientists to determine the age, gender, residence, and race of a human individual[9].

Defining a forensic DNA pattern has a significant impact on society by providing reliable evidence to convict violators or exonerate innocent suspects. Another area for applying DNA pattern determination is paternity testing using autosomal, mitochondrial or Y chromosome markers[10][11].

DNA is often referred to as the "genetic blueprint for life" as the individual whose genetic makeup is coded into the DNA. This is intended to mean that DNA is passed from parents to their offspring[12].

The signs currently used are known as Short Tandem Repeat (STR), and they are length polymorphs. STR are short repeating sequences that are mostly found in the not encoded areas of DNA, either within or between genes[13].

Materials and Method

Sample collection: Samples were collected from Al-Shaheed Ghazi Hospital and Al-Kindi Teaching Hospital from sick and injured people from Kerbala governorate, after their approval was obtained. The total of 16 bone samples obtained was as follows (femur: 4, hip bone: 2, tibia: 2, ulna: 1, instep: 2, hand comb: 1, radius bone: 2, Humerus: 2). 16 blood samples were obtained from the father and mother of the person.

Samples were obtained directly from the operating theaters and placed in the (Cup Tube) and saved directly in the storage box, which contains ice bags, so that they can be kept cool until they are transferred to the laboratory.

Blood samples were collected and stored on FTA cards and kept at room temperature.

Sample preparation: A portion of the calcium was removed using an emery machine (Dremel, Racine, WI, USA) to ensure the sample was cleaned properly and that there were no other tissues on the bone. Then cut the large bones into small pieces and wash twice with 10% sodium hypochlorite for 15 minutes, then wash twice with deionized water for 15 minutes, once rinsed in 96% ethanol. Allow samples to dry overnight at room temperature. It is placed in marmeric slurry and liquid nitrogen is poured on it until the sample is immersed and begins to move the slurry stick in a continuous circular motion until the nitrogen dries and this process is repeated until a fine-grained powder is obtained because the more accurate the flour, the more pure the DNA is. Each bone sample is prepared separately using sterile instruments in a specially designed room[15].

DNA extraction: The extraction of bone samples was performed using the EZ1 DNA Investigator Kit (48) by the EZ1 Advanced XL device and extracted according to the protocol for the EZ1 DNA Investigator Kit (48) DNA extraction kit supplied with the kit and as approved by the forensic laboratories.

As for blood samples collected by FTA™ Mini Card, they are used directly with the process of DNA amplification (PCR), which will be mentioned later.

DNA quantification: The quantification of DNA was estimated using a 7500 REAL-TIME PCR using the Quantifier ™ Trio DNA Quantification Kit with the HID Real-Time PCR software for v1.2 analyses to determine the isolated DNA. The samples were diluted according to the amplitude of the relevant short tandem frequency group (STR) amplified, while the samples that specified a small quantity of the recommended concentration were amplified using the maximum size of the DNA extract.

This group was used to evaluate the quantification of human DNA, the presence of PCR inhibitors and the level of DNA degradation (via the degradation index (DI) for each sample at one time. Data were accepted with R2 values from 0.99 or higher.

PCR amplification

Bone samples: PowerPlex® Fusion 6C System (PC) was used to amplify bone samples according to the Promega Corporation (2017) manual and according to the method used in forensic laboratories. A mixture of reaction solution was prepared for each sample in a 1.5 ml PCR reaction tube:

Master Mix = 5.0µl; Primer Pair Mix = 5.0µl; template DNA= 15.0µl "Total reaction volume = 25.0µl"

The Thermal Reaction and cycling setup was performed according to the procedures described in the PowerPlex® Fusion 6C System user manuals. Reaction products were kept at 4°C until use.

Blood samples: A small piece (punch) with a diameter of 1.2 mm was taken from the dried spots from each of the samples saved on the FTA™cards. The punch was cleaned with alcohol to prevent cross contamination. The punch was processed according to the manufacturer's instructions. The punch was washed three times using the FTA™ purification reagent for 5 minutes at room temperature, followed by two washes with the TE (10 mm Tris pH 8.0, 0.1 mm EDTA). Washed punches were dried for 30 minutes at 60 ° C.

FTA™ punches are placed in PCR tubes, with the following components added and final volume adjusted to 50µL with sterile dual distilled water: 200 nM per primer, 800µM DNTPs, 1.5 mM MgCl2 and 2.5 U Taq polymerase. The Thermal Reaction and cycling setup was performed according to the procedures described in the PowerPlex® Fusion 6C System user manuals. Reaction products were kept at 4 ° C until use.

Capillary electrophoresis separation: PCR products were detected and separated by capillary electrophoresis on 3550 Applied Genetic Analyzer (Thermo Fisher Scientific, Oyster Point, CA). A mixture of 0.5 µl of GeneScan™ 600 LIZ® Size Standard v2, 9.5 µl of Hi-Di™ Formamide, was dispensed and 1.0 µl of PCR products was added to each well. The Capillary electrophoresis was performed with run settings as indicated by the manufacturer for PowerPlex® Fusion System - Promega Corporation.

Data analysis: Genotypes and electrical diagrams obtained after DNA separation were obtained using the Gene Mapper ID-X version 1.4 (Applied Biosystems, USA). Data were statistically analyzed using GenoProof 3 SoftwareKinship Examination (Qualitype GmbH, Dresden, Germany) (Dumache, 2017).

Results and Discussion

The paternity test is based on the alleles matching at STR 24 loci between the child, mother and father (Trio Cases). In this case, no genetic discrepancy was observed for the alleged relationship between the father and the child in any of the STR 24 loci. Results are shown in Table 1.

The combined parenting index was calculated (CPI) and Paternity Probability (W) for standard trio cases using GenoProof3 software. The paternity index (PI), the Combined paternity index (CPI), is then calculated by multiplying the individual PIs across, obtained from each loci tested. Using a CPI and a prior probability of 0.5, the parenting probability (W) is calculated according to the following equation;□(CPI/(CPI+1))

Table 1: The value of the paternity index father-son and the maternity index mother-son for each locus

Locus	Mother	Son	Father	PI	PE
AMEL	X,X	X,Y	X,Y		
D3S1358	15/18	15/17	16/17	2.3320	61.7167%
D1S1656	11/15	13/15	12/13	7.4626	87.0489%
D2S441	11	11/14	14	3.4364	50.2681%
D10S1248	13/16	14/16	13/14	1.4044	41.4736%
D13S317	12	12/14	12/14	12.9533	92.429%
Penta E	10	10/18	17/18	17.2413	94.2841%
D16S539	11	11/12	11/12	1.6880	49.5334%
D18S51	16	16	13/16	3.6576	74.5286%
D2S1338	24	24	24/25	5.5803	82.8828%
CSF1PO	10/12	10/12	11/12	0.8903	19.2194%
Penta D	10	10	9/10	5.6179	82.9921%
TH01	7/9	7/9	9	3.0684	45.441%

Locus	Mother	Son	Father	PI	PE
vWA	17/18	17	17	3.6023	52.1861%
D21S11	32.2/33.2	30/32.2	28/30	2.2026	59.7529%
D7S820	12	10/12	10/12	1.8601	53.4653%
D5S818	11/12	11/12	11/12	1.4308	9.0661%
TPOX	8/11	11/12	11/12	12.9533	92.429%
DYS391	--	--	--		
D8S1179	13	13	13	3.1152	46.1041%
D12S391	20/21	20	20	8.8495	78.6769%
D19S433	13/13.2	13.2/14	14/14.2	1.5192	45.0106%
FGA	20/26	22/26	22/23	2.5549	64.6898%
D22S1045	16/17	16/17	16	2.2471	30.8025%

Definition: PI (typical paternity index), PE (power of exclusion)

Where was combined parenting index was Calculated (CPI) 652,559,360,682 and Paternity Probability (W) 99.9999999998% and also (CPE) calculated 99.999999999% (Paternity practically proven). In the second case, the results were as follows:combined parenting index was Calculated (CPI) 4,551,267,998,683 and Paternity Probability (W) 99.9999999999% and also (CPE) calculated 99.9999999999% (Paternity practically proven). In the third case, the results were as follows: combined parenting index was Calculated (CPI) 305,372,452,857 and Paternity Probability (W) 99.9999999996% and also (CPE) calculated 99.9999999973% (Paternity practically proven).In the fourth case, the results were as follows: combined parenting index was Calculated (CPI) 2,008,537,679 and Paternity Probability (W) 99.9999999502% and also (CPE) calculated 99.9999999868% (Paternity practically proven).In the fifth case, the results were as follows: combined parenting index was Calculated (CPI) 3,362,894,053 and Paternity Probability (W) 99.9999999702% and also (CPE) calculated 99.999999966% (Paternity practically proven).In the sixth case, the results were as follows: combined parenting index was Calculated (CPI) 33,542,176,411 and Paternity Probability (W) 99.999999997% and also (CPE) calculated 99.9999999922% (Paternity practically proven).In the seventh case, the results were as follows: combined parenting index was Calculated (CPI) 1,093,931,389,095 and Paternity Probability (W) 99.9999999999% and also (CPE) calculated 99.9999999998% (Paternity practically proven).In the eighth case, the results were as follows: combined parenting index was Calculated (CPI) 25,017,963,856 and Paternity Probability (W) 99.999999996% and also (CPE) calculated 99.9999999925% (Paternity practically proven).In the ninth case, the results were as follows: combined parenting index was Calculated (CPI) 453,038,745 and Paternity Probability (W) 99.9999997792%and also (CPE) calculated 99.9999999632% (Paternity practically proven).In the tenth case, the results were as follows: combined parenting index was Calculated (CPI) 4,837,584,758,232 and Paternity Probability (W) 99.9999999999%and also (CPE) calculated 99.9999999999% (Paternity practically proven).In the eleventh case, the results were as follows: combined parenting index was Calculated (CPI) 3,068,923,208 and Paternity Probability (W) 99.9999999674% and also (CPE) calculated 99.9999999494% (Paternity practically proven).In the twelve case, the results were as follows: combined parenting index was Calculated (CPI) 76,047,184,748 and Paternity Probability (W) 99.9999999986% and also (CPE) calculated 99.9999999986% (Paternity practically proven).In the thirteenth case, the results were as follows: combined parenting index was Calculated (CPI) 194,517,436,486 and Paternity Probability (W) 99.9999999994% and also (CPE) calculated 99.9999999974% (Paternity practically proven).In the fourteenth case, the results were as follows: combined parenting index was Calculated (CPI) 176,512,622,425 and Paternity Probability (W) 99.9999999994% and also (CPE) calculated 99.9999999995% (Paternity practically proven)[10-12].In the fifteenthcase, the results were as follows: combined parenting index was Calculated

(CPI) 51,637,272,228,769 and Paternity Probability (W) 99.9999999999% and also (CPE) calculated 99.9999999999% (Paternity practically proven).In the sixteenth case, the results were as follows: combined parenting index was Calculated (CPI) 69,762,623,876 and Paternity Probability (W) 99.9999999985% and also (CPE) calculated 99.9999999974% (Paternity practically proven).

The current study has shown that 24 loci of STR technology have sufficient discriminatory ability to exclude or include the alleged father in disputed parenting cases. This is consistent with many reports describing the strength of interest and discrimination of STR markers. In the paternity and identity test. The calculated paternity index values and the probability of paternity that are important in applying the paternity test DNA typing method, were high with the current STR typing, and the paternity index measures the strength of the genetic match between the alleged father and a specific child. If the alleged father and child share a low frequency allele, they will be considered strong and will give a high parenting index[13-15].

When the child's biological parent is questionable, the PCR-based STR test is the accurate choice available for paternity testing, in relation to crime issues in the current study, STR's analysis was very useful for personal identification.

Data from this study leads to the conclusion that STR genotyping is a powerful tool for analyzing conflicts between parents. From a practical point of view, standardized methodology with high reproducibility and low cost plus the availability of STR loci in the human genome is easy to implement, making it ideal genome markers for parenting/maternity evaluation.

Specifically, in all cases of trio paternity, 24 forensic loci were effective exclusion of paternity or to provide sufficient positive evidence (strong evidence) on paternity, and to provide a high discriminatory power at a rate ranging between W 99.999999996% to 99.9999999999%. by Using a database qualified well.

Conclusion

Successful, STR typing is a tool genetic reliable and powerful have an important central role in the community to solve the problems of family relationships and judicial studies.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq.

Conflict of Interest: Non

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