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The Efficiency of the *Alu* Insertion Sequence in Discrimination Among some Individuals

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ABSTRACT

Background:

The *Alu* element is a widely distributed short interspersed nuclear element (SINE) in the human genome and has important applications in forensic science. The current study focused on assessing the effectiveness of Alu insertion polymorphism in forensic DNA profiling to identifying samples of some individuals living in the Babylon Governorate.

Materials and methods:

DNA was extracted From frozen blood samples (60) individuals were collected from Babylon - Al-Hilla Governorate from 8/8/2022 until 8/9/2022.

Results:

purified then a PCR technique *Alu* insertions were A(2q21.1)(111-115bp) 0.75, *Alu* deletion (0.25), B(8q23.1) (0.375) (0.625) C(13q34) (0.5)(0.5), D(15q23) (0.775) (0.225), E(16q23.3) (0.692) (0.308) and F(19q13.12)(0.616)(0.384) respectively.The alleles with *Alu* insertions at (2q21.1) were the most prevalent, whereas the *Alu* insertions at (8q23.1) were the least common. The similarity coefficient among individuals varied from 0.4 to 1, based on the proportion of genetic relatedness between them.

Conclusions:

Alu elements have efficacy in families' discrimination it can be used more than one site, set of *Alu* insertion sequences should be used to accurate results.

Keywords, *Alu* elements, individual discrimination, forensic identification, families discrimination, PCR technique

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INTRODUCTION

The human nuclear genome consists of 23 chromosomes, and has over 3 billion base pairs (bp). The human genome is still being sequenced with analyzed Genes and intergenic non-coding sequences [1, 2].

Retrotransposons and transposons in DNA. its sequences that are inserted into a new regions of the genome by transfer elements. This is a process called translocation. DNA transposons are transferred using the "cut and paste" method. Translocation is the process by which DNA transposons move from one location in the genome to another. Retrotransposons, on the other hand, copy and insert their DNA during transposition, spreading throughout the genome[3, 4]

The *Alu* family of repeated elements was first recognized as a subset of repetitive DNA that exhibited specific cleavage patterns when exposed to the restriction enzyme *Alu*I.[5, 6]. *Alu* elements are derived from the 7SL RNA gene, which is responsible for producing an essential component of the signal recognition particle in the endoplasmic reticulum. *Alu*s share around 90% sequence similarity with the 7SL.[7-9]. They constitute approximately 11% of the human genome and exist in over 1,000,000 copies per haploid genome, with an average distance of one copy per 4 kb[9].

Alu elements consist of two identical sequences [10, 11]. with the left half including the characteristic internal RNA polymerase III split promoter [12, 13]. SINEs are characterized by a short A-rich linker connecting the two dimers, as well as a 3-oligo-dA-rich tail that varies in length, potentially reaching up to 100 base pairs, contingent upon the particular site [14]. *Alu* repeats are commonly flanked by direct repeats that arise from the replication of target sequences during the integration process. The movement of these components occurs through retroposition, facilitated by an intermediary transcript generated by RNA polymerase III.[15, 16]. Their location on chromosomes tends to favor R bands or places rich in AT base pairs [17]. *Alu*s are thought to undergo retrotransposition by utilizing an L1-encoded reverse transcriptase [18, 19].The *Alu* elements originated during the primate radiation approximately 65 million years ago[20]. [20] . Currently, a limited number of source genes, referred to as 'master genes,' are undergoing amplification at a rate of around $8x10^3$ new *Alu* insertions per year [21, 22].

Therefore, *Alu* insertions that exist in several individuals not only share similar properties but also share a common lineage. Thus, if two individuals possess an identical insertion, it is highly likely that they share a common ancestor in whom the insertion originated. Furthermore, there is yet no established technique for completely and specifically eradicating an ingredient. Therefore, the lack of insertion suggests that the original state is more probable [23, 24].

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Allele Frequency Overview

The allele frequency considers all alleles, including recessive alleles that are "hidden" inside carrier species, in contrast to the phenotypic ratio. The phenotypic ratio only describes the actual physical features, or phenotypes, that exist within a population. To calculate the allele frequency, scientists must account for heterozygous people who could be hidden a recessive geneThe Hardy-Weinberg equation, which defines the connection between two alleles within a population, is most frequently used to compute allele frequency. The true allele frequency must be calculated using advanced methods when there are more than two alleles. The frequency of an allele may change over time as a population adjusts to evolution by having more or less copies of a particular allele [16].

THE AIM OF THIS STUDY

focused on assessing the effectiveness of Alu insertion polymorphism in forensic DNA profiling to identifying samples of some individuals living in the Babylon Governorate

MATERIALS AND METHODS

Subject

The sample of this study consist of (60) individuals were collected from Babylon - Al-Hilla city from 8/8/2022 until 8/9/2022, where the number of males was (33), at a rate of 55%, and females were 28, at a rate of 45%. In the sitting posture, individuals were given disposable 3mL syringes to take venous blood samples. Each individual had a vein punctured to retrieve three milliliters of blood, which were then placed into EDTA tubes. To be utilized in the study, blood in EDTA tubes was kept in a deep freezer at - 4 °C.

DNA extraction

Genomic DNA from white blood cells (WBCS) for 60 individual were extracted DNA extraction kit (Favorgen FavorPrep™ Blood Genomic DNA Extraction / Taiwan).

PCR Amplification

The primers:

Primer Name	Sequences	Reference
2q21.21	F5'- CGCCTCTTCTCCTTCTGTTT-'3	[25]
	R-5' CCCGGCCTCGTCTCTTTT-'3	
	R1- 5'GTTCTCCTCACTCGTCTCTT-'3	
8q23.1	F-5'GTTTGACAGTCTTCACAGGC-'3	
	R- 5'AACCCGGCCAGCCCATTTT-'3	
	R1-5' AATGTAGAAGTCAAGCCCATTTT-'3	
19q13.12	F-5'AAATAACTAAACGTTGGAATATAATTAAGGCT-'3	
	R- 5' AACGGCCGCGATAAAGTTTT-'3	
	R1-5' TTATTACTTCCAGCGATAAAGTTTTTAGT-'3	
16q23.3	F-5'AATTTTGAAAAGAAAGGACAAAGTTGG-'3	

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	R-5'CAGGTGTGACATATGTCCAT-'3	
	R1-5'TGCTCCATTTATCCATGTGTC-'3	
15q23	F-5'ACTGAGCAAAATATGACTTAGGAA-'3	
	R-5'AACCGGCCCCGATATGATTTT-'3	
	R1-5'AACAGTATGAATCCGATATGATTTT-'3	
13q34	F-5'TGTACTTAATGCCACTACACTG-'3	
	R-5'AAACGGCCCCCTATGAATTTTT-'3	
	R1-5'AATCATCCCTGCCCCTATGAATTTTT-'3	

First, PCR optimization was carried out utilizing a gradient temperature between 54°C, 57, and 59°C with 2°C step changes in the PCR wells. This was crucial in figuring out the ideal annealing temperature.

The program described herein was implemented within a thermocycler system for the purpose of amplifying certain DNA segments, The initial amplification PCR's other conditions (94 C° DNA denaturation ,57 C° primer annealing ,72 C° elongation and 4 C° incubation) as established by gradient PCR, remained the same, and the annealing temperature was (57 C°).

Determine the Allele Frequency

In order to accurately determine the overall number of alleles within a given population, it is imperative to consider all existing phenotypes. The expression of phenotypic traits linked to a specific allele is frequently masked when dominant and recessive alleles interact synergistically. The examination of allele frequency within a population is conducted by researchers through the utilization of the Hardy-Weinberg (HW) equation. The subsequent mathematical expression is employed within the framework of the Hardy-Weinberg equation..:

$p^2 + 2pq + q^2 = 1$

- The variables P and q are used to denote the frequency of alleles within a population.
- The variable "p" denotes the frequency of the homozygous dominant genotype.
- The variable "q" in this context denotes the frequency of the homozygous recessive genotype [16].

Polymorphic Information Content (PIC) is a measure used in genetics to quantify the amount of polymorphism in a population. Polymorphism refers to the occurrence of two or more distinct phenotypes or genetic variants within a population. PIC is often used in the context of genetic markers, such as Single Nucleotide Polymorphisms (SNPs) or microsatellites.PIC is calculated based on the frequency of different alleles at a given genetic locus. It provides an estimate of the informativeness of a genetic marker. The higher the PIC value, the more informative the marker is for distinguishing between individuals or populations.The formula for calculating PIC varies depending on the type of genetic marker being analyzed, but it generally involves the allele frequencies at a locus. PIC values range

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from 0 to 1, with higher values indicating greater polymorphism and informativeness of the marker[26].

https://gene-calc.pl/hardy-weinberg-page

This web allows examining the compliance between observed and expected number of genotypes based on Hardy-Weinberg law.

RESULTS AND DISCUSSION

The process of extraction or purification The DNA fragments were obtained by extracting and purifying the genomic DNA from blood samples of the individuals and their family members. Subsequently, agarose gel electrophoresis was performed as a first step to confirm the presence of DNA samples following the extraction process. The DNA band of the blood samples appeared in Figure 1.

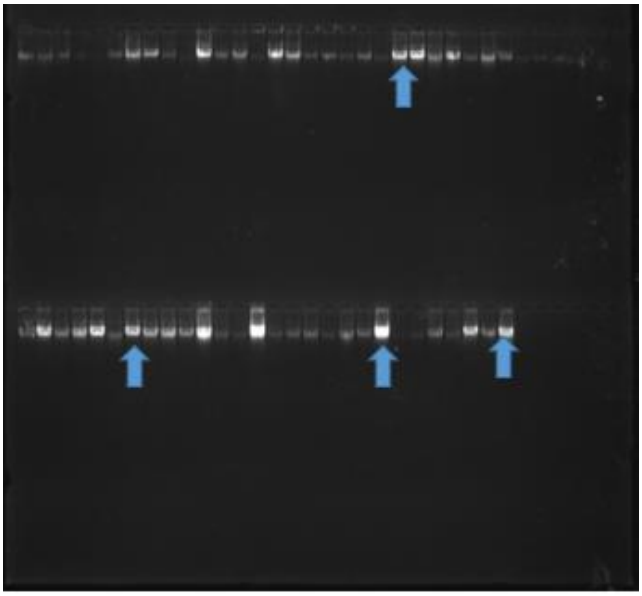

Figure (1) The process involved in this experiment includes the isolation of whole DNA from a blood sample, followed by the utilization of 1% agarose gel electrophoresis. The electrophoresis was conducted using a TBE 1X buffer solution, with a voltage of 75 volts and a current of 20 mA, for a duration of 1 hour. Each well contained a volume of 10 µl

***Alu* elements amplification:**

PCR technique was used to allow DNA amplification. the *Alu* elements region was targeted using primers ((2q21.1)(111-115bp), (8q23.1)(130-134bp), (13q34)(116-120bp), (15q23)(98-102bp), (16q23.3)(120-124bp) and (19q13.12)(104-109pb)) fig.(2)

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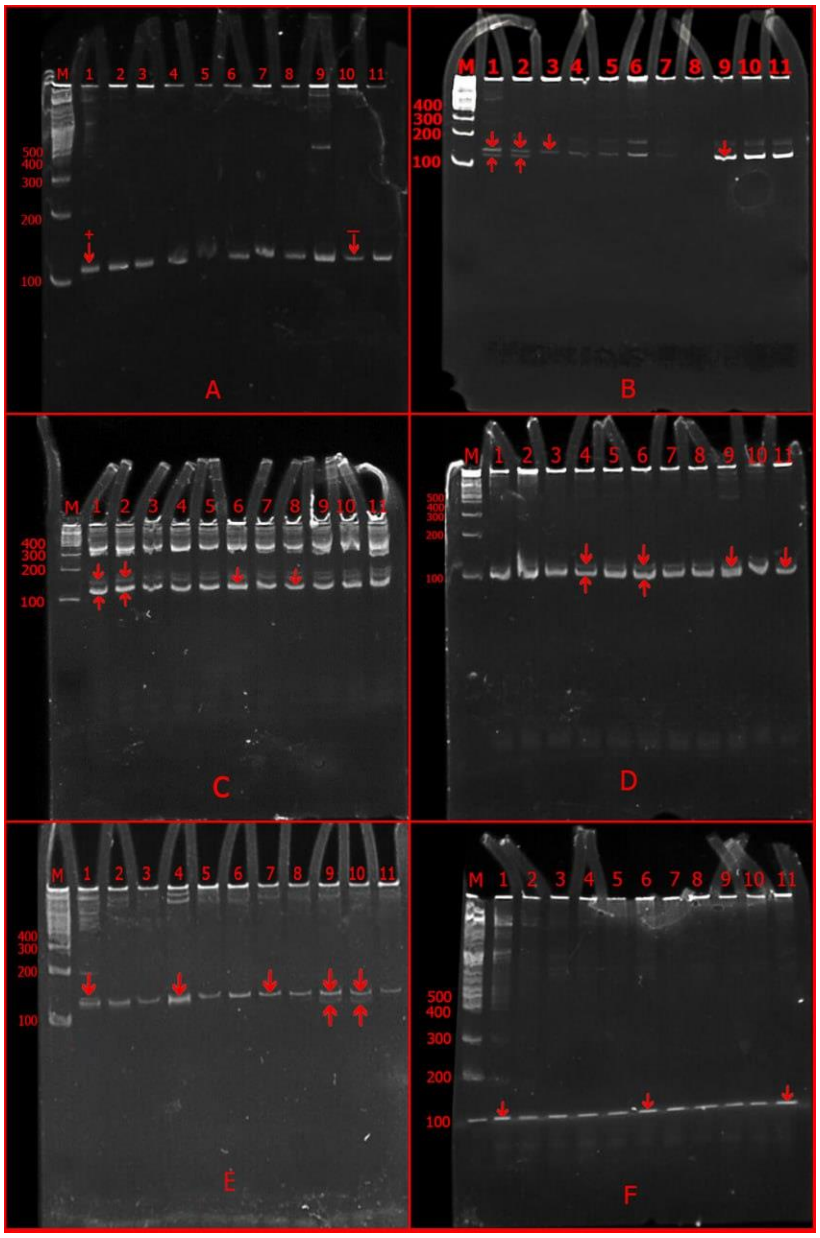

Figure (2) The polyacrylamide electrophoreses pattern of PCR product for *Alu* elements region A(16q23.3) (120-124bp) , B (19q13.12) (104-109pb) C(13q34) (116-120bp), D(15q23)(98-102bp), E(16q23.3)(120-124bp) and F (19q13.12) (104-109pb), This amplification produced bands 100-160 bp under condition annealing 57 C°, M: Promega ladder, Lane 1-11 PCR product of *Alu* elements region.

In Table 1. Show Allele frequency, observed, expected, chi-square, p-v*Alu*e, and fixation index for *Alu* elements region(A((2q21.1) (111-115bp), B(8q23.1)(130-134bp),C(13q34)(116-120bp),D(15q23)(98-102bp), E(16q23.3)(120-124bp) and F(19q13.12)(104-109pb)). The frequencies of alleles containing *Alu* and without *Alu* varied between 0.225 and 0.775, with a ratio of 0.616:0.384, in the group of 60 people. indicating that certain populations possess distinct genetic traits and exhibit an intricate nature in the

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establishment of ethnic groups. The observed chi-square v*Alu*e is statistically significant at a significance level of P<0.05. Two autosomal *Alu* markers exhibit Hardy-Weinberg disequilibrium in a sample of 60 individuals. The B (8q23.1) region has a length of 130–134 base pairs and a frequency of 0.096. The D (15q23) region has a length of 98–102 base pairs and a frequency of 0.59. The remaining regions have a chi-square v*Alu*e that is statistically significant at a level of P<0.05. The genetic loci A (2q21.1) span 111–115 base pairs, C (13q34) spans 116–120 base pairs, E (16q23.3) spans 120–124 base pairs, and F (19q13.12) spans 104–109. If, at the chosen level of significance, there are statistically significant changes, the application will calculate the fixation index (Fis). Positive v*Alu*es may indicate a decrease in heterozygosity, which can be produced by the Wahlund effect, inbreeding, or genetic drift. A negative Fis value indicates an abundance of heterozygotes [27].

No statistically significant disparities were seen in pairs of polymorphic *Alu* insertions situated on the same chromosome. Afterwards, the *Alu* profiles obtained from each family were thoroughly analyzed, marker by marker. Surprisingly, all six markers displayed perfect correspondence between the father, mother, and children.

In figure (3) show the allele frequency for All *Alu* elements region.The comparative efficiency of present results for overall study population are summarized in table (1), If statistically significant differences exist at the chosen level of significance, the application will calculate the fixation index (Fis). Positive v*Alu*es of Fis may indicate a decrease in heterozygosity, which could be caused by factors such as the Wahlund effect, inbreeding, or genetic drift. Negative Fis v*Alu*es indicate an abundance of heterozygotes resulting from either selective breeding or a bottleneck effect. The formula for calculating the fixation index (FIS) is 1 minus the ratio of observed heterozygosity to anticipated heterozygosity. Significant results observed in A (2q21.1), C (13q34) and F(19q13.12) in study population than others *Alu* sites.

Table (1): Allele frequency, observed, expected, chi-square, p-v*Alu*e, and fixation index for *Alu* elements region A((2q21.1) (111-115bp), B(8q23.1)(130-134bp), C(13q34)(116-120bp), D(15q23)(98-102bp), E(16q23.3)(120-124bp) and F (19q13.12)(104-109pb))

Alu	Observed			Allele frequency.		Expected			PIC	p-v*Alu*e	Chi-square	Yate`s chi-square v*Alu*e	Yate`s p-v*Alu*e	*fis
	Alu insertion	*Alu* deletion	*Alu* insertion /*Alu* deletion	*Alu* insertion	*Alu* deletion	*Alu* insertion	*Alu* deletion	*Alu* insertion /*Alu* deletion						
A (2q21.1) (111-115)	45	15	0	0.75	0.25	33.75	3.75	22.5	**0.375**	0	60	55.751	0	1
B(8q23.1)(130-134)	9	24	27	0.375	0.625	8.4375	23.4375	28.125	**0.5339**	0.95313	**0.096**			
C (13q34)(116-120)	1	1	58	0.5	0.5	15	15	30	**0.64**	0	52.26667	49.5083	0	0.9333
D(15q23)(98-102)	35	2	23	0.775	0.225	36.0375	3.0375	20.925	**0.4106**	0.7445	**0.59**	0.2216	0.8950	
E(16q23.3)(120-124)	34	11	15	0.692	0.308	28.73184	5.69184	25.57632	**0.5169**	0.00587	10.27739			0.4139
F(19q13.12)(104-109)	37	23	0	0.616	0.384	22.76736	8.84736	28.38528	**0.4728**	0	**60**	56.81781	0	1

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Allele frequency represented by observed and absence alleles clarified in fig (3) For *Alu* elements region 1((2q21.1) (111-115bp), 2(8q23.1)(130-134bp), 3(13q34)(116-120bp), 4(15q23)(98-102bp), 5(16q23.3)(120-124bp) and 6 (19q13.12)(104-109pb)), the observation alleles were high in 1, 4, 5 and 6 than alleles absence, while equal percent was found in 3 and high absence allele in 2.

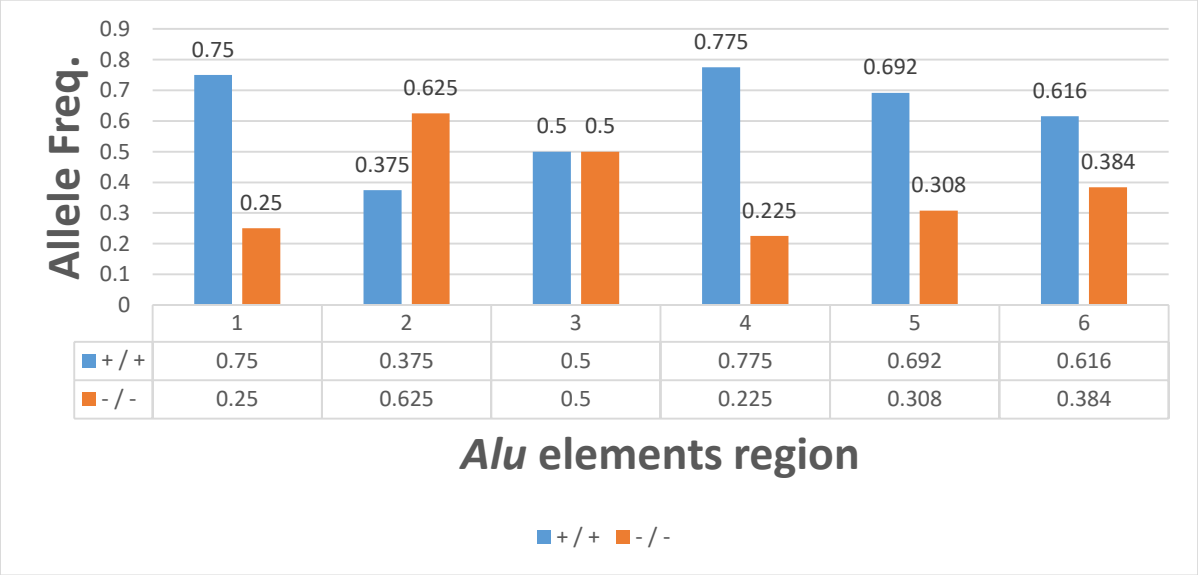

Fig. (3):Allele frequency for *Alu* elements region1((2q21.1) (111-115bp), 2(8q23.1)(130-134bp),3(13q34)(116-120bp),4(15q23)(98-102bp), 5(16q23.3)(120-124bp) and 6 (19q13.12)(104-109pb)).
+/+ : *Alu* insertion,
-/- : *Alu* deletion

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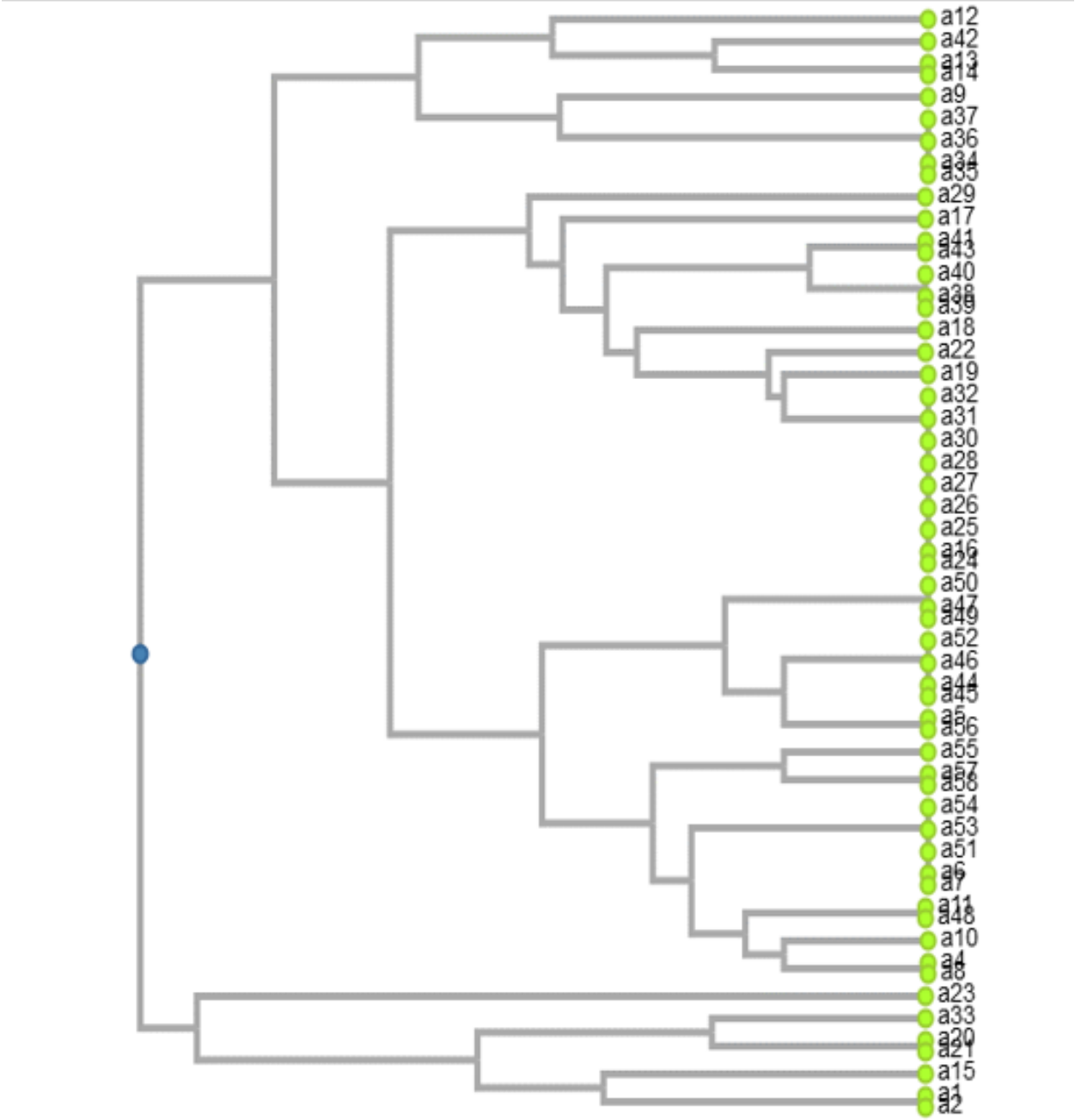

Figure (4) the phylogenetic tree of *Alu* elements of the study population.

In the figure (4), it is shown that there are three basic clusters and under a branching cluster from the main cluster, as these three clusters represent three brothers and their children, as the kinship relationship between these families is evident, and there is a great match between the individuals in the positions of the six *Alu* elements, due to the presence of high marriage between these individuals, reaching up to Three generations, where, for example, sample No. (34,35), (38,39), and (44,45) show a complete match, as the similarity v*Alu*e between individuals equals

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one according to figure. (4) and samples (36,37) and (31,32). (53,54) Less matching, where the similarity v*Alu*e between individual's ranges between (0.7 - 0.9) according to figure No. (5) from the previous samples comparing people with their cousins, and matching is less for samples (28,30), where the similarity v*Alu*e is Between individuals, it ranges between (0.4-0.6) according to figure (5) due to the occurrence of mating of some individuals outside the scope of these families (strangers).

This work employed the multiplex genotyping technique to analyze 6 polymorphic *Alu* loci. The analysis involved three separate amplifications and capillary electrophoresis. This technique enabled differentiation of numerous genetic loci using minimal quantities of DNA and would be appropriate for personal identification purposes.

While the majority of *Alu* insertions remain unchanged in the genome, a certain group of them display variation across the human population. Mamedov [28] analyzed polymorphic *Alu* loci in population samples and provided a set of markers derived from polymorphic *Alu* insertions for the purpose of individual identification.. *Alu* insertions primarily function as a tool for analyzing ancestry. and correlation with specific diseases[29] the studies [30, 31]. emphasized the capacity of several *Alu* loci. Furthermore, the utilization of an *Alu* loci marker set can provide valuable information, especially when genotyping STR profiles proves to be fruitless. polyacrylamide electrophoresis is a cost-effective approach for genotyping *Alu* fragments. It can be used for many *Alu* loci without the need for complex tuning of PCR settings, as described by[32]. However, this approach would pose challenges in identifying longer segments (>500 bp) for handling limited or degraded DNA templates. In contrast, the three amplification methods employed in this investigation yielded fragments of limited length, specifically 150 base pairs or less. Reducing the size of the amplicons can be advantageous for *Alu* genotyping in fragmented DNA. Fluorescence-based detection generally exhibits higher sensitivity compared to agarose-based techniques. The PCR for multiplexing 10 loci required an ideal amount of DNA ranging from 0.5 to 1 ng. Conversely, a standard agarose-based technique necessitates 1–10 ng of DNA for a solitary *Alu* locus. Utilizing an automated sequencer equipped with a fluorescence detection system is the prevailing practice in forensic genetic laboratories. However, this technique necessitates more improvement in order to effectively identify PCR results. Moreover, this technique is beneficial for doing high-throughput genotyping using multi-capillary formats. So, using multiplex PCR along with fluorescence-based capillary electrophoresis detection is a good way to figure out the genotype of many *Alu* sequences. Complete profiles of *Alu* loci were detected from a DNA sample of 0.25 nanograms. As expected, the amplification process using less than 0.25 ng of DNA left out genetic markers and variations that were expected. This suggests that the amplification genotyping was affected by random factors[33].

Other than capillary electrophoresis-based detection, there are several technologies that utilize high-resolution melting analysis. dHPLC or Microchips have been developed for the purpose of detecting *Alu* insertions. The most suitable genotyping method should be chosen

based on the number of samples or *Alu* loci. This approach enables the differentiation of six *Alu* loci by utilizing FAM-labeled smaller fragments, while also allowing for the simultaneous analysis of larger loci using other fluorescent dyes. Increasing the size of the data sets, including polymorphic *Alu* insertions, would improve the ability to differentiate between individuals. Therefore, it is necessary to conduct a more thorough screening of polymorphic *Alu* insertions in order to advance this method[34].

The relevance of mass human genotyping is growing. Human genotyping databases may be used to identify victims of catastrophic catastrophes, identify suspects using biological evidence collected from the site of a crime, determine paternity, and address a host of other issues. Human genotype database initiatives for the entire population or specific demographic groups are being proposed in a number of nations. Numerous molecular genetic markers (MGMs) of various kinds, including as STRs and SNPs, are included in human genetic passports. Despite the fact that these large-scale undertakings seem labor-, money-, and time-intensive, they may have significant benefits in many facets of life. Within the next ten to twenty years, individual genome sequences might be possible due to significant advancements in human genome sequencing technology [35].

Every person's whole genome will inspire medical genetic research and yield a wealth of unique DNA markers. However, it would be helpful to have a small, universal collection of neutral markers to generate "genomic fingerprints." Thus, the development of a genetic marker collection that enables quick, simple, and affordable identification of humans is a crucial and pressing problem. Since the publication of the human genome structure and the accumulation of data on the diversity of the human genome, Multiple genetic methods for human identification, utilizing Short Tandem Repeats (STRs) and Single Nucleotide Polymorphisms (SNPs), have been developed and implemented. Short tandem repeats (STRs) formed the basis for most early human genotyping systems, each of which featured multiple alleles with diverse population frequencies. Despite their higher mutation rate, these markers possess a highly effective discriminatory ability [36].

Systems based on these kinds of MGMs have been created as a consequence of the collection of human SNP data. Most of the time, a single SNP is biallelic and has less discriminating power than a STR. On the other hand, using more polymorphic loci yields a high enough degree of discriminating that is equivalent to STR-based systems.7. Furthermore, compared to STRs, the mutation rate of SNP markers is lower. For the purpose of detecting SNP alleles, a variety of methodological techniques have been developed, including mass spectrometry and allele-specific PCR. Most of the time, locus-specific amplification using a set of primers is necessary for STR and SNP typing, and allele detection using capillary electrophoresis or allele-specific real-time PCR follows[37].

A novel class of MGMs based on RE insertion polymorphism has gained popularity in population research within the last ten years. These markers are promising candidates for human genetic identification because they have many properties that make them belong to a form of

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biallelic indel (insertion/deletion) polymorphism. Numerous investigations aimed at discovering novel polymorphic RE insertions have yielded a growing quantity of potential MGMs dispersed throughout the whole human genome. Two available databases include information on the population distribution and chromosomal location of several polymorphic RE insertions. The use of RE-based genetic markers for cell line fingerprinting has recently gained traction[38, 39].

Alu were employed to genotype several cell lines, yielding distinct multilocus profiles for every cell line. the developed a straightforward and affordable human genetic identification method using highly polymorphic and equally dispersed *Alu* insertions. findings were compared with previously published data, it became clear that all of the chosen polymorphic *Alu* insertions were found in genetically distinct groups with comparable allele frequencies. These results are positive Owing to limited amounts of DNA in many crime cases, it is important to perform genetic tests on samples with only traces of genomic DNA [40].

A minimum input template of only 32 nanograms is adequate for the identification of all variable *Alu* insertions, and even small quantities of DNA do not hinder the accurate determination of alleles. Furthermore, it was determined that a single buccal scrape provides adequate DNA for accurate *Alu* typing, eliminating the necessity of drawing blood to get human genetic profiles. The impact of ultrasonic DNA degradation on the accuracy of human genotyping using the polymorphic *Alu* set was found to be minimal. Nevertheless, extensive DNA degradation can impede the amplification of alleles containing *Alu* sequences, and this factor must be considered when examining specific types of human samples, such as those impacted by fire[41].

In a study in Morocco[42] Forensic DNA typing incorporated *Alu* inserts in its analysis of individuals residing in southern Morocco. The article presents a thorough investigation of seven specific locations that have experienced the addition or removal of human *Alu* pieces. The investigation was carried out on a sample of 215 unrelated adults who were in good health. This sample included five distinct Berber sous populations and one Sahrawi sous community. We acquired precise data on genotype frequency and other genetic factors that are relevant in forensic investigations. These findings align with the results of our research.

The *Alu* element is a highly prevalent short interspersed nuclear element (SINE) found in the human genome, possessing significant forensic applications. An investigation was conducted to assess the usefulness of *Alu* insertion polymorphism in forensic DNA typing within a sample of the Jordanian population located in central Jordan. Polymerase Chain Reaction (PCR) was used to amplify *Alu* insertions in seven distinct genomic loci[43].

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Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with individual verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number B220902 (including the number and the date in 28/9/2022) to get this approval.

CONCLUSIONS

Alu elements have efficacy in families' discrimination it can be used more than one site, set of *Alu* insertion sequences should be used to accurate results.

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Conflict of interests.

There are no conflicts to declare.

References

[1] R. M. Bruce-Chwatt, "A brief history of forensic odontology since 1775," *Journal of forensic and legal medicine,* vol. 17, no. 3, pp. 127-130, 2010.

[2] L. Florea, L. Payer, C. Antonescu, G. Yang, and K. Burns, "Detection of Alu exonization events in human frontal cortex from RNA-seq data," *Frontiers in Molecular Biosciences,* vol. 8, p. 727537, 2021. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8460874/pdf/fmolb-08-727537.pdf.

[3] C. Howard, S. Gilmore, J. Robertson, and R. Peakall, "A Cannabis sativa STR genotype database for Australian seizures: forensic applications and limitations," *Journal of forensic sciences,* vol. 54, no. 3, pp. 556-563, 2009.

[4] M. L. Zattera and D. P. Bruschi, "Transposable elements as a source of novel repetitive DNA in the eukaryote genome," *Cells,* vol. 11, no. 21, p. 3373, 2022.

[5] C. M. Houck, F. P. Rinehart, and C. W. Schmid, "A ubiquitous family of repeated DNA sequences in the human genome," *Journal of molecular biology,* vol. 132, no. 3, pp. 289-306, 1979.

[6] J. R. Loureiro, A. F. Castro, A. S. Figueiredo, and I. Silveira, "Molecular mechanisms in pentanucleotide repeat diseases," *Cells,* vol. 11, no. 2, p. 205, 2022.

[7] E. Ullu and C. Tschudi, "Alu sequences are processed 7SL RNA genes," *Nature,* vol. 312, no. 5990, pp. 171-172, 1984.

[8] H. Yang, M. E. Morales, and A. M. Roy-Engel, "Alu Elements and Human Disease," in *Retrotransposons and Human Disease: L1 Retrotransposons as a Source of Genetic Diversity*, 2023, pp. 77-113.

[9] M. R. Vollger *et al.*, "Improved assembly and variant detection of a haploid human genome using single-molecule, high-fidelity long reads," *Annals of human genetics,* vol. 84, no. 2, pp. 125-140, 2020.

[10] P. L. Deininger, D. J. Jolly, C. M. Rubin, T. Friedmann, and C. W. Schmid, "Base sequence studies of 300 nucleotide renatured repeated human DNA clones," *Journal of molecular biology,* vol. 151, no. 1, pp. 17-33, 1981.

[11] L. Doronina, O. Reising, and J. Schmitz, "Gene conversion amongst Alu SINE elements," *Genes,* vol. 12, no. 6, p. 905, 2021.

[12] G. Paolella, M. A. Lucero, M. H. Murphy, and F. E. Baralle, "The Alu family repeat promoter has a tRNA-like bipartite structure," *The EMBO Journal,* vol. 2, no. 5, pp. 691-696, 1983.

[13] K. A. Tatosyan, D. V. Stasenko, A. P. Koval, I. K. Gogolevskaya, and D. A. Kramerov, "TATA-like boxes in RNA polymerase III promoters: requirements for nucleotide sequences," *International Journal of Molecular Sciences,* vol. 21, no. 10, p. 3706, 2020.

[14] E. P. Economou, A. W. Bergen, A. C. Warren, and S. E. Antonarakis, "The polydeoxyadenylate tract of Alu repetitive elements is polymorphic in the human genome," *Proceedings of the National Academy of Sciences,* vol. 87, no. 8, pp. 2951-2954, 1990.

[15] J. H. Rogers, "The origin and evolution of retroposons," *International review of cytology,* vol. 93, pp. 187-279, 1985.

[16] A. Khan, G. Nanjundan, D. Basvarajaih, and M. Azharuddin, "Statistical Model Derivation and Extension of Hardy-Weinberg Equilibrium," *International Journal of Current Microbiology and Applied Sciences,* vol. 7, no. 10, pp. 2402-2409, 2018.

[17] J. R. Korenberg and M. C. Rykowski, "Human genome organization: Alu, lines, and the molecular structure of metaphase chromosome bands," *Cell,* vol. 53, no. 3, pp. 391-400, 1988.

[18] S. L. Mathias, A. F. Scott, H. H. Kazazian Jr, J. D. Boeke, and A. Gabriel, "Reverse transcriptase encoded by a human transposable element," *Science,* vol. 254, no. 5039, pp. 1808-1810, 1991.

[19] S. Fukuda *et al.*, "Cytoplasmic synthesis of endogenous Alu complementary DNA via reverse transcription and implications in age-related macular degeneration," *Proceedings of the National Academy of Sciences,* vol. 118, no. 6, p. e2022751118, 2021.

[20] P. L. Deininger and G. R. Daniels, "The recent evolution of mammalian repetitive DNA elements," *Trends in Genetics,* vol. 2, pp. 76-80, 1986.

[21] P. L. Deininger, M. A. Batzer, C. A. Hutchison III, and M. H. Edgell, "Master genes in mammalian repetitive DNA amplification," *Trends in Genetics,* vol. 8, no. 9, pp. 307-311, 1992.

[22] R. Castanera, P. Vendrell-Mir, A. Bardil, M. C. Carpentier, O. Panaud, and J. M. Casacuberta, "Amplification dynamics of miniature inverted-repeat transposable elements and their impact on rice trait variability," *The Plant Journal,* vol. 107, no. 1, pp. 118-135, 2021.

[23] M. C. Edwards and R. A. Gibbs, "A human dimorphism resulting from loss of an Alu," *Genomics,* vol. 14, no. 3, pp. 590-597, 1992.

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[24] L. M. Payer *et al.*, "Alu insertion variants alter gene transcript levels," *Genome research,* vol. 31, no. 12, pp. 2236-2248, 2021.
[25] M. Asari *et al.*, "Multiplex PCR-based Alu insertion polymorphisms genotyping for identifying individuals of Japanese ethnicity," *Genomics,* vol. 99, no. 4, pp. 227-232, 2012.
[26] C. M. L. Serrote, L. R. S. Reiniger, K. B. Silva, S. M. dos Santos Rabaiolli, and C. M. Stefanel, "Determining the Polymorphism Information Content of a molecular marker," *Gene,* vol. 726, p. 144175, 2020.
[27] J. Graffelman, D. Jain, and B. Weir, "A genome-wide study of Hardy–Weinberg equilibrium with next generation sequence data," *Human genetics,* vol. 136, pp. 727-741, 2017.
[28] I. Z. Mamedov, "A new set of markers for human identification based on 32 polymorphic Alu insertions," *European Journal of Human Genetics,* vol. 18, pp. 808–814, 2010.
[29] J. Kulski, A. Shigenari, T. Shiina, and H. Inoko, "Polymorphic major histocompatibility complex class II Alu insertions at five loci and their association with HLA-DRB1 and-DQB1 in Japanese and Caucasians," *Tissue Antigens,* vol. 76, no. 1, pp. 35-47, 2010.
[30] C. Phillips *et al.*, "Evaluation of the Genplex SNP typing system and a 49plex forensic marker panel," *Forensic Science International: Genetics,* vol. 1, no. 2, pp. 180-185, 2007.
[31] R. Pereira, C. Phillips, C. Alves, A. Amorim, Á. Carracedo, and L. Gusmão, "A new multiplex for human identification using insertion/deletion polymorphisms," *Electrophoresis,* vol. 30, no. 21, pp. 3682-3690, 2009.
[32] T. Lappalainen, A. J. Scott, M. Brandt, and I. M. Hall, "Genomic analysis in the age of human genome sequencing," *Cell,* vol. 177, no. 1, pp. 70-84, 2019.
[33] B. Budowle, A. J. Eisenberg, and A. v. Daal, "Validity of low copy number typing and applications to forensic science," *Croatian medical journal,* vol. 50, no. 3, pp. 207-217, 2009.
[34] J. Radvansky, P. Resko, M. Surovy, G. Minarik, A. Ficek, and L. Kadasi, "High-resolution melting analysis for genotyping of the myotonic dystrophy type 1 associated Alu insertion/deletion polymorphism," *Analytical biochemistry,* vol. 398, no. 1, pp. 126-128, 2010.
[35] S. Teama, "DNA polymorphisms: DNA-based molecular markers and their application in medicine," *Genetic Diversity and Disease Susceptibility,* vol. 25, 2018.
[36] S. Parthipan, M. Sandrasagran, and S. M. Ishar, "A Review of Mitochondrial SNP Determination using Allele-Specific PCR in Forensic Identification," *Sains Malaysiana,* vol. 51, no. 12, pp. 4019-4030, 2022.
[37] Y. D. Kelkar, S. Tyekucheva, F. Chiaromonte, and K. D. Makova, "The genome-wide determinants of human and chimpanzee microsatellite evolution," *Genome research,* vol. 18, no. 1, pp. 30-38, 2008.
[38] S. V. Ustyugova, A. L. Amosova, Y. B. Lebedev, and E. D. Sverdlov, "Cell line fingerprinting using retroelement insertion polymorphism," *Biotechniques,* vol. 38, no. 4, pp. 561-565, 2005.
[39] S. K. Njoroge, M. A. Witek, M. L. Hupert, and S. A. Soper, "Microchip electrophoresis of Alu elements for gender determination and inference of human ethnic origin," *Electrophoresis,* vol. 31, no. 6, pp. 981-990, 2010.
[40] G. N. Stacey, E. Byrne, and J. R. Hawkins, "DNA profiling and characterization of animal cell lines," *Animal Cell Biotechnology: Methods and Protocols,* pp. 57-73, 2014.

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[41] S. Lanciano *et al.*, "Locus-level L1 DNA methylation profiling reveals the epigenetic and transcriptional interplay between L1s and their integration sites," *Cell Genomics,* 2024.

[42] S. Chadli, M. Wajih, and H. Izaabel, "Analysis of Alu insertion polymorphism in South Morocco (Souss): Use of markers in forensic science," *The Open Forensic Science Journal,* vol. 2, no. 1, 2009.

[43] Z. A. Al-Mazaydeh, S. R. Yasin, and L. H. Tahtamouni, "The implementation of Alu insertion polymorphism as a genetic marker for forensic investigation in a Jordanian population sample," *African Journal of Biological Sciences,* vol. 2, no. 3, pp. 62-71, 2020.

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خلاصة

خلفية:

عنصر Alu هو عنصر نووي قصير متناثر منتشر على نطاق واسع (SINE) في الجينوم البشري وله تطبيقات مهمة في علوم الطب الشرعي. ركزت الدراسة الحالية على تقييم مدى فعالية تعدد أشكال إدخال الو في تشخيص الحمض النووي الشرعي لتحديد عينات من بعض الأفراد الذين يعيشون في محافظة بابل.

المواد والأساليب:

تم استخلاص الحمض النووي من عينات الدم المجمدة وتم جمع (60) فرداً من بابل – محافظة الحلة للفترة من 2022/8/8 ولغاية 2022/9/8 .

نتائج:

تمت تنقيته ثم تم إدخال تقنية0.75 PCR Alu A(2q21.1)(111–115bp) ، حذف(0.25) Alu ، B(8q23.1) (0.375) (0.5)(0.5) C(13q34) (0.625)، (0.225) (0.775) D(15q23)، (0.308) (0.692) E(16q23.3) و F(19q13.12)(0.616)(0.384)على التوالي. كانت الأليلات مع إدخالات Alu عند (2q21.1) الأكثر انتشارًا، في حين كانت إدخالات Alu عند (8q23.1) هي الأقل شيوعًا. وتراوح معامل التشابه بين الأفراد من 0.4 إلى 1 حسب نسبة القرابة الجينية بينهم.

الاستنتاجات:

عناصر الو لها فعالية في تمييز العائلات حيث يمكن استخدامها في أكثر من موقع ويجب استخدام مجموعة من تسلسلات إدخال الو للحصول على نتائج دقيقة .

الكلمات المفتاحية: عناصر الو، التمييز الفردي، التعريف الشرعي، التمييز الأسري، تقنيةPCR

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