Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Babylon

College of Science

Department of Biology

Random amplified polymorphic DNA polymerase chain reaction (RAPD PCR) fingerprints in forensic species and Relation with Environmental parameters

A Research Project

Submitted to Council of the College of Science / University of Babylon in Partial Fulfillment to the Requirements for the Degree of High Diploma in Science /Forensic Evidence

By

Muthanna Jawad Sahar

B.Sc. Biology, 2007

Supervised by

Prof .Dr. Ayad M.J.Almamoori

2021 A.D **1443 H.D**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

صدَقَ الله العليُّ العظيم

سورة ابراهيم، الآية ٤١

Certification

I certify that research is prepared under my supervision at the Department of Biology, College of Sciences, University of Babylon, as partial requirements for the Degree of Higher Diploma in Forensic Evidence.

Signature:

Name : **Prof .Dr .AyadM.J.Almamoori**

Data: / /2021

In view of available recommendation, I forward this research for debate by the Examination Committee.

Signature:

Name: Assistant Professor Dr. Adi Jassim Abdul Razzaq

Title: Chairman of Biology Dept.

Data: / /2021

Committee Certification

We, the examining committee, certify that we have read the research entitled (Random amplified polymorphic DNA polymerase chain reaction (RAPD PCR) fingerprints in forensic species and Relation with Environmental parameters) and examined the student (Hussein ali yammer) in its contents at / /2021, and that in our opinion it is accepted as a research for the degree of High Diploma in Forensic Evidence with) estimation.

Signature:

Data: / / 2021

(Chairman)

Signature:

Data: / / 2021

(Member)

Signature:

Prof .Dr. Ayad M.J.Almamoori

Professor

Depart of Biology

College of Science

University of Babylon

Data: / / 2021

(Member and Supervisor)

Approved by the College Committee on Graduate Studies

Signature:

Dean of Collage of Sciences –University of Babylon

Data: / /2021

Dedication

THE LAST OF THE PROPHETS AND MESSENGERS

MUHAMMAD, PEACE BE UPON HIM AND HIS FAMILY

TO THE SPIRIT OF MY FATHER

TO THE SPIRIT OF MY MOTHER

TO MY SISTERS, MY COMPASSION, AND MY FRIENDS ALL

MUTHANNA 2021

Acknowledgments

Praise be to Allah and peace and blessings be upon his Messenger the Prophet Muhammad peace be upon him and his family and followed them until the Day of religion.

I would like to thank my advisor :Prof .Dr .AyadM.J.Almamoori , University of Babylon for the supervision of the construction of this scientific effort that has been a great asset to me and the length of the continuous research of overseeing and careful supervision and valuable advice.

Muthanna2021

Summary

The aim of this study to detect the effect of Environmental parameters on RAPD PCR analysis in Suspected person on proposed crime scene.

10 Samples were collected and directly divided into two groups , the first groups under 25 C˚ and the Second Group under 4C˚.CBC analysis , heavy metals detection and RAPD were done for all Samples.

some parameters showed the significance differences such as WBC, RDW % CV, MPV, Neutrophil, Eosinophil. Most of concentrations of measured metals were fluctuated during study, the highest concentration of Cu (41.4 ppm) found in Second Group(4C˚) of Samples

The RAPD PCR fingerprints produced by primer A and primer B, respectively. The PCR products ranged in size from 300 to 1517 bp, and each sample had four to ten main bands amplified. The RAPD PCR fingerprints of 10 different human DNA samples under different conditions.

We concluded that temperature and heavy metals have a little effect on RAPD Analysis and RAPD is very significance in Blood analysis through Crime Scene.

Table of Contents

List of Figures

List of Tables

1. Introduction

For most purposes, random amplified polymorphic DNA (RAPD) is the simplest, most cost-effective, and can be done in a modest laboratory. Furthermore, RAPDs can touch a large portion of the genome and have the benefit of requiring no prior information of the genome under study. Improvements in the RAPD method, such as arbitrarily primed polymerase chain reaction, have been made recently (AP-PCR), Random amplified microsatellite polymorphism (RAMPO), and random amplified hybridization microsatellites (RAHM) can supplement the limitations of RAPDs and have increased the usability of this simple method for certain applications(Babu etal.,2021)

The forensic biological samples were identified using the random amplified polymorphic DNA (RAPD) technique. There is no need for a genomic DNA sequence or two polymerase chain reaction (PCR) cycle scripts. There is only one 10-nt primer and one PCR software utilized(Lee&Chang, 1994)

DNA analysis has become "the new type of scientific evidence" in forensic science, drawing public scrutiny and a need for competence. DNA-based evidence is being accepted by a growing number of courts. We think that this technology will be widely recognized in the judicial system in the near future. In forensic medicine, DNA analysis is used for two purposes: criminal investigation and paternity testing. In this article, we cover the basics of DNA, human genetics, and the application of DNA analysis to legal issues, as well as the most often used mathematics(Primorac& Schanfield 2000)

(Woller etal., 1997) look at how polymerase chain reaction (PCR)-based DNA polymorphisms are used in forensic practice in Hungary. The combined use of the 17 PCR-based sequence- or length-polymorphic DNA systems described to

criminal cases provides the forensic scientist the capacity of individualization.

Recombinant DNA technology has recently given several innovative and strong forensic science approaches. On the basis of differences in its sequence, human genomic DNA may be examined directly for individual identification and paternity testing.. Restriction fragment length polymorphism (RFLP) testing generates a DNA fingerprint or profile utilizing a combination of single locus probe (SLP) that varies greatly across people. (Misawa, 1994).

Mitochondrial DNA RFLPs and 9-bp deletion type of mtDNA may suggest the characteristics of the human races. For the purpose of resolving the genetic basis of human uniqueness, we reported the isolation of human-specific sequences using the technique of genome subtraction (HS5, 2282 bp).

Aim of study

Application of Random amplified polymorphic DNA polymerase chain reaction (RAPD PCR) fingerprints in Analysis of Blood Samples in Crime Scene, through following objectives:

1-Collect Blood samples from proposed crime scene

2-Achieve CBC analysis for Blood samples and then Extract DNA from the same samples.

3-Divide the samples in two groups , first Group (25C˚), the second group (45C˚)

4- Measure the DNA polymorphisms by RAPD test .

Review of Literatures

2-1 RAPD PCR in Forensic

In forensic genetics, a revolution has occurred with the advent of minisatellites for DNA analysis in the mid-1980s. Following the discovery of the PCR, forensic genetics methods were developed that allow for both extremely informative regular investigations and more complex, specific investigations in situations involving crime, paternity, relationships, and disasters victim identification etc.(Morling , 2007).

Randomly amplified polymorphic DNA, or RAPD, marker analysis utilizes short PCR primers consisting of random sequences usually in the size range of 8 to 15 nucleotides in length. Complex patterns of PCR products are generated as these random sequence primers anneal to various regions in an organism's genome. RAPD suffers from poor reproducibility between laboratories largely because of the requirement of consistent PCR amplification conditions including thermal cycler ramp speeds. The complex patterns of RAPD also prevent mixture interpretation and provide challenges in consistent scoring of electrophoretic images even in single-source samples.(Yuji&Kozo,1999)

The idea to work on DNA-specific recommendations was born after a round table discussion dealing with the 2004 Tsunami disaster in south east Asia during the 21st congress of the International Society for Forensic Genetics on the Azores, Portugal, in September 2005. The ensuing discussion between scientists and pathologists that had been involved in the International Center in Khao Lak, Thailand, revealed the need for the scientific community to be better prepared to answer the local authorities' questions by formulating generally acceptable scientific standards for the most efficient use of DNA-based victim identification

methods. These recommendations, as well as the many cited references, are intended to provide guidance on establishing preparedness for the forensic genetics laboratory, on collecting and storing ante-mortem and post-mortem samples suitable for DNA analysis, on DNA extraction and genetic typing strategies, on data management, and on issues related to the biostatistical interpretation and reporting of results(Prinz etal.,2007)

DNA evidence sample processing typically involves DNA extraction, quantification, and STR amplification; however, DNA loss can occur at both the DNA extraction and quantification steps, which is not ideal for forensic evidence containing low levels of DNA. Direct PCR amplification of forensic unknown samples has been suggested as a means to circumvent extraction and quantification, thereby retaining the DNA typically lost during those procedures. Direct PCR amplification is a method in which a sample is added directly to an amplification reaction without being subjected to prior DNA extraction, purification, or quantification. It allows for maximum quantities of DNA to be targeted, minimizes opportunities for error and contamination, and reduces the time and monetary resources required to process samples, although data analysis may take longer as the increased DNA detection sensitivity of direct PCR may lead to more instances of complex mixtures. ISO 17025 accredited laboratories have successfully implemented direct PCR for limited purposes (e.g., high-throughput databanking analysis), and recent studies indicate that direct PCR can be an effective method for processing low-yield evidence samples. Despite its benefits, direct PCR has yet to be widely implemented across laboratories for the processing of evidentiary items. While forensic DNA laboratories are always interested in new methods that will maximize the quantity and quality of genetic information obtained from evidentiary items, there is often a lag between the advent of useful

methodologies and their integration into laboratories. Delayed implementation of direct PCR of evidentiary items can be attributed to a variety of factors, including regulatory guidelines that prevent laboratories from omitting the quantification step when processing forensic unknown samples, as is the case in the United States, and, more broadly, a reluctance to validate a technique that is not widely used for evidence samples. The advantages of direct PCR of forensic evidentiary samples justify a re-examination of the factors that have delayed widespread implementation of this method and of the evidence supporting its use(Cavanaugh& Bathrick,2017)

In random-amplified polymorphic DNA (RAPD) analyses a short primer (10 n in length) is designed and added to a PCR reaction with the target DNA. This arbitrary primer is designed without previous knowledge of the target DNA sequence and randomly amplifies segments of DNA in a PCR reaction, The PCR amplicons are then analyzed using gel electrophoresis. RAPD analysis on different species results in unique patterns of DNA fragments due to variations in the genetic code. If the resulting band patterns are species-specific, the DNA fingerprint for that species is established. Unknown samples can be analyzed using the same primer and their band patterns compared to the DNA fingerprints for known samples to verify the species(David etal., 2013)

Multiplex PCR with fluorescently labeled primers has been an essential method for the amplification of short tandem repeats used in human identify testing. Within the STR workflow of extraction, quantitation, amplification, separation, and detection, multiplex PCR is commonly identified as the bottleneck in the process. The time requirement of up to three hours to complete 28-30 cycles of multiplex PCR for STR genotyping is the greatest amount of time required for a single step within the process. The historical use of commercially available thermal cyclers

and heat stable polymerases may have given the impression that large multiplex will always require long PCR cycling times to ensure that all of the varying sized targets (typically 100-400bp) can be amplified in a balanced manner throughout the multiplex. However, with the advent of improved polymerases and faster thermal cyclers the time required for the amplification of large STR multiplexes is no longer on the order of three hours, but as little as 14min. Faster amplification times can be performed while retaining the balance and integrity of large multiplex PCRs by implementation of alternate polymerases and thermal cyclers. With the reduction in PCR cycling times there has also been an impact on the development of integrated and microfluidics devices which employ the use of reduced or rapid thermal cycling protocols as part of their integration.

Similarly, PCR inhibitor resistant polymerases can also reduce overall STR processing times for reference samples by eliminating the need for DNA extraction and purification that is additionally implemented within the development of integrated DNA typing devices.(Romsos&Vallone,2015). it is also envisaged that these assays (i.e. RAPD and related techniques), which reflect effects at whole genome level, would continue to complement the use of emerging technologies (e.g. microarrays which aim to quantify expression of individual genes).

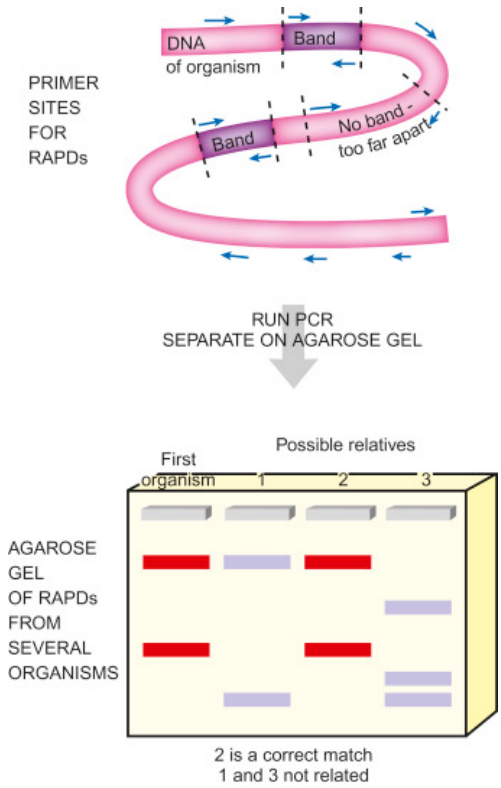

Figure 1: *Randomly Amplified Polymorphic DNA*

Randomly amplified polymorphic DNA, or RAPD, marker analysis utilizes short PCR primers consisting of random sequences usually in the size range of 8 to 15 nucleotides in length. Complex patterns of PCR products are generated as these random sequence primers anneal to various regions in an organism's genome. RAPD suffers from poor reproducibility between laboratories largely because of the requirement of consistent PCR amplification conditions including thermal cycler ramp speeds. The complex patterns of RAPD also prevent mixture interpretation and provide challenges in consistent scoring of electrophoretic images even in single-source samples. Randomly, or RAPD, marker analysis utilizes short PCR primers consisting of random sequences usually in the size

range of 8 to 15 nucleotides in length. Complex patterns of PCR products are generated as these random sequence primers anneal to various regions in an organism's genome. RAPD suffers from poor reproducibility between laboratories largely because of the requirement of consistent PCR amplification conditions including thermal cycler ramp speeds. The complex patterns of RAPD also prevent mixture interpretation and provide challenges in consistent scoring of electrophoretic images even in single-source samples(John, 2012).

RAPD is a technique adapted for rapid detection of genomic polymorphism. The technique is based on the amplification of the genomic DNA with either a single or multiple short oligonucleotide primers of an arbitrary or random sequence. The number and location of these random primer sites varies for different strains of a bacterial species. What results from the separation of the amplification products by agarose gel electrophoresis is therefore a pattern of bands which, in theory, is characteristic of the bacterial strain in question. In most papers, the high discriminatory power of the RAPD pattern is described, though PFGE has been reported as being more useful than RAPDs in distinguishing among *S. aureus* strains. In most cases, the sequences of the RAPD primers, which generate the best DNA pattern for differentiation, must be determined empirically. Another problem is both the inter- and intralaboratory reproducibility of the method, and it has been demonstrated that, for typing *S. aureus* strains, the successful use of RAPD heavily depends on the optimal use of PCR protocols (Beata& Józef,2018).

Random amplified polymorphic DNA (RAPD) is a kind of PCR, but the DNA fragments that are amplified are haphazard ,The arbitrary and short primers (8–12 nucleotides) are used in the PCR, which uses a large template of genomic DNA. By resolving the resulting patterns, a semi unique profile can be garnered from a RAPD reaction. There are 59 species and 24 varieties in 6 sections of 3 subgenera

in Yunnan, 56 of which are endemic to China and 16 of which are only found in Yunnan. RAPD was used to study 12 Clematis species of Yunnan (Pu et al., 2008). Selected ten RAPD primers were used in the random amplification and 89 polymorphic DNA bands were detected. The twelve species can be unambiguously identified. More importantly, the cluster tree largely reflected the phylogenetic relationship of the studied species. (Da Cheng etal.,2015)

In the RAPD method originally described in 1990, genomic DNA (template) is incubated together with an arbitrarily chosen primer that binds to complementary sites on the template DNA, an enzyme, nucleotides, and a suitable buffer system. Depending on where the primer binds to the sample DNA, various stretches of the sample DNA are copied by the action of the polymerase enzyme when submitted to a temperature cycle in a thermocycler. The resulting DNA amplification products are then size-separated by electrophoresis in agarose gels, and the bands are detected using, for example, ethidium bromide and ultraviolet (UV) illumination. Usually short (10 bp), commercially available primers are used.

The inter-simple sequence repeat (ISSR) method is quite similar to RAPD but the primers have been designed to guide amplification mainly to regions with repetitive and therefore highly polymorphic DNA. RAPD and ISSR are relatively quick and easy-to-use, and require only standard laboratory equipment. Although especially RAPD has often been criticized for poor reproducibility, this method is still often used for discrimination of rose genotypes(Nybom etal., 2017)

2-2 Significance of RAPD PCR in Crime scene

Another variation of PCR, called RAPD, amplifies target sequences of different species in order to gauge relatedness. During this process, an arbitrary, rare but not unique, sequence is chosen. Primers are designed to recognize the arbitrary

sequence. The total genomic DNA of the organism is used as a template. The reaction is based upon the likelihood of two rare sites being within a relatively short distance of each other and facing in opposite directions so that the DNA between them can be amplified. However, closely related species have similar DNA sequences, and give similar results for RAPD. The results of the RAPD reactions are analyzed by agarose gel electrophoresis. Each species has a distinct banding pattern and closely related species have similar patterns((David etal., 2013).

Reverse transcriptase PCR (RT-PCR) amplifies processed mRNA by first converting the mRNA into complementary DNA with reverse transcriptase, and then amplifying the cDNA with regular PCR. The technique identifies what regions of a gene are exons, and what regions are introns.

Eukaryotic genes contain non-coding regions called introns. During gene expression, the introns are removed and the exons (coding regions) are spliced together to generate the gene's final mRNA transcript. PCR on eukaryotic genes is difficult because of the length and also the intervening intron sequences.

A technique called RT-PCR utilizes a viral enzyme called reverse transcriptase to make a complementary DNA strand using the mRNA as a template. The mRNA has already been processed (i.e., natural removal of the introns), and the resulting cDNA (complementary DNA) copy is the DNA sequence without the intervening introns. The cDNA is then amplified by normal PCR to obtain many more copies.(Mohammad, 2015)

Differential display PCR compares the mRNA expression patterns of two different organisms or the same organism in two different conditions. The mRNA is amplified by using an oligo(dT) primer and a mixture of random primers. The expression of many mRNA molecules is often investigated using differential

display PCR, another variation of PCR. RNA processing in eukaryotes not only involves removing introns and splicing together the exons, but also the addition of a 3′ poly(A) tail. Oligonucleotide primers containing only thymine are utilized to begin the RT-PCR reaction at the 3′ poly(A) tail. These primers are called oligo(dT) primers. The result is a cDNA of the mRNA transcript. Then RAPD is employed using two different primers to produce a banding pattern that is then assessed by agarose gel electrophoresis.

Rapid amplification of cDNA ends uses a modified PCR reaction to amplify the 5′ and 3′ ends of an mRNA molecule.(LeeAnn etal.,2016)

Variant PCR techniques using reverse transcriptase often fall short in the ability to generate full-length cDNA corresponding to the entire mRNA. Usually this occurs because of mRNA secondary structures that block the reverse transcriptase. Another method called RACE is used to amplify the ends of a cDNA molecule.

In RACE, primers are designed to the internal cDNA/mRNA sequence. Anchor sequences are added to each end of the cDNA to help with PCR and other primers are designed that recognize anchor sequences. Depending upon which end primer is used, cDNA can be amplified from the 3′ end or the 5′ end. Reverse transcriptase extends the end primers to generate an mRNA:DNA hybrid molecule. The mRNA is removed and the second DNA strand is made using the internal primer.(Yuji& Kozo ,1999)

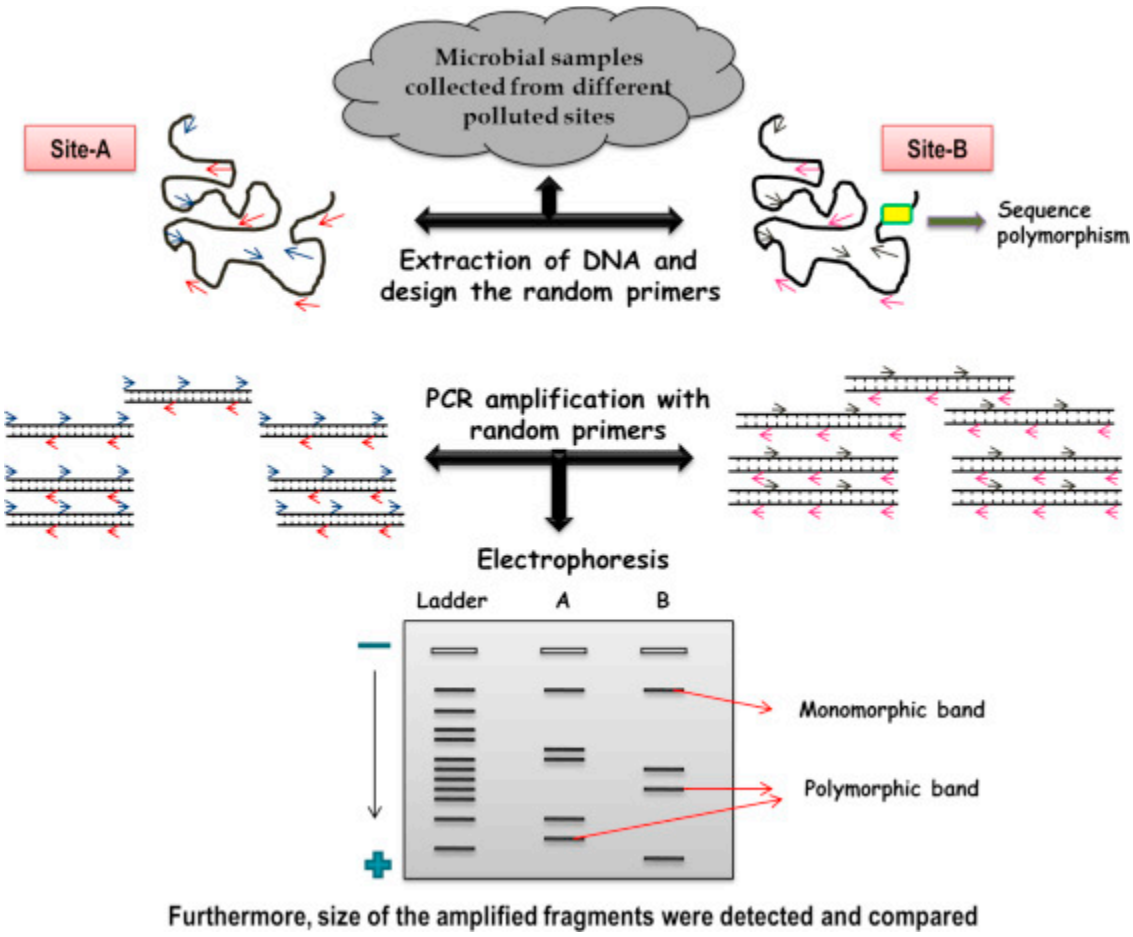

Figure 2: Schematic illustration of random amplified length polymorphism analysis.(Satyanarayan etal., 2019)

2-3-Interaction between RAPD PCR and Environmental factors

RAPD does not require any specific knowledge of the DNA sequence of the target organism. The identical 10-mer primers will or will not amplify a segment of DNA, depending on positions that are complementary to the primers sequence. If a mutation has occurred in the template DNA at the site that was previously complementary to the primer, a PCR product will not be produced, resulting in a different pattern of amplified DNA segments on the gel. RAPD/DAF has been used extensively in fingerprinting of microbial community, since the process is

rapid and user friendly ,Both the processes are highly sensitive to annealing temperature, MgCl2 concentration, and quality and quantity of template DNA and primers as well, hence laboratory dependent and needs carefully developed laboratory protocols to be reproducible. Almost all RAPD markers are dominant and it is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous (one copy) or homozygous (two copies). Codominant RAPD markers, observed as different-sized DNA segments amplified from the same locus, are detected only rarely. Further, mismatches between the primer and the template may result in the total absence or decreased amount of the PCR product which become difficult to interpret results. RAPD profiling was used with 14 random primers to assess microbial diversity in soil samples treated with pesticides (triazolone) and chemical fertilizers (ammonium bicarbonate). RAPD fragment richness exhibit similar DNA diversity in the control and treated samples(Satyanarayan etal., 2019)

(Calvo etal., 2001) found that Because some fraudulent or unintentional mislabeling occurs that can be undetected, resulting in lower quality pâté, and because some population groups, for philosophical or religious reasons, do not wish to eat meat from certain species, a new procedure was developed and evaluated to detect pâté species composition by randomly amplified polymorphic DNA (RAPD). The RAPD method was used to generate fingerprint patterns for pork, chicken, duck, turkey, and goose meats. Ten DNA samples from pork, chicken, turkey, and duck meats were tested to confirm the effectiveness and specificity. Specific results for each species were obtained by the RAPD method. Sensitivity of the method was studied by DNA dilution in each species, detecting as little as 250 pg of DNA. Isolations of DNA from 30 pâtés (tinned and untinned) were carried out, and an optimal DNA was obtained for using as template DNA in

polymerase chain reaction (PCR). The RAPD-PCR pattern was useful to identify species composition of pork, duck, duck-pork, goose, and poultry pâtés. This study demonstrates the usefulness of RAPD fingerprinting to distinguish between species in pâtés.

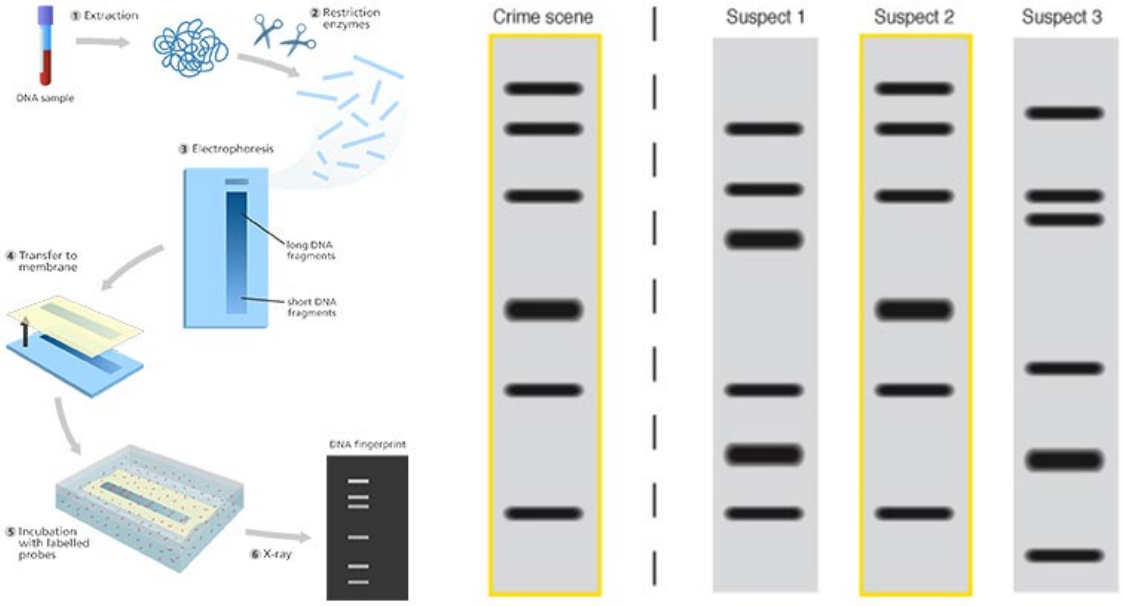

Figure 3: Significance of RAPD PCR in Crime scene

3. Materials and Methods

3-1Samples Collections

Human blood samples (n = 10) were taken intravenously from the proposed suspected persons. About 100 µL of the blood was dropped onto gauze to make a bloodstain After drying,

For the clarify the Environmental effect , Samples was divided into two groups , first group in 25 C˚ and the second group in Refrigerator 4C˚ .

3-2 CBC analysis for blood Samples

All blood Samples were analyzed by automated hematological analyzer after preservation conditions

3-3 RAPD PCR Fingerprint

3-3-1 Genomic DNA extraction

Favor PrepTM Genomic DNA Mini Kit was used to extract genomic DNA from 25 blood samples following the manufacturer's protocol. Transfer up to 200 µl sample (whole blood, serum, plasma, body fluids, buffy coat) to a microcentrifuge tube , Add 20 µl Proteinase K and 200 µl FABG Buffer to the sample. Mix thoroughly by pulse-vortexing, Incubate at 60 ºC for 15 minutes to Iyse the sample. During incubation, vortex the sample every 3-5 minutes. Briefly spin the tube to remove drops from the inside of the Iid , Add 200 µl ethanol (96- 100 %) to the sample. Mix thoroughly by pulse-vortexing for 10 sec. , Briefly spin the tube to remove drops from the inside of the Iid, Place a FABG Mini Column to a Collection Tube. Transfer the mixture (including any precipitate) carefully to the FABG Mini Column. Centrifuge at 6,000 x g for 1 min then place FABG Mini Column to a new Collection Tube , Add 400 µl W1 Buffer to the FABG Mini

Column and centrifuge at full speed (18,000 x g) for 30 sec then discard the flow-through , Add 750 µl Wash Buffer to the FABG Mini Column and centrifuge at full speed for 30 sec then discard the flow-through , Centrifuge at full speed for an additional 3 minutes to dry the column, Place the FABG Mini Column to a Elution Tube , Add 50 µl of Elution Buffer to the membrane center of FABG Mini Column. Stand FABG Mini Column for 3 minutes , Centrifuge at full speed for 1 minutes to elute total DNA and then stored at -20ºC until use.

3-3-2 Agarose gel electrophoresis

Agarose sheet was prepared by dissolving agarose powder in 1X TBE buffer. The amount of agarose which can be dissolved depending upon the purpose in which agarose sheet used. 0.7% agarose sheet used for visualization the DNA after extraction while 1.5%-2% agarose sheet visualization of PCR product (amplicon).

Simply safe (alternative for ethidium bromide) stock solution concentration was 10 mg/ml. Only 5µl of simply safe stock solution were added to 100ml of melting agarose gel to get final concentration 0.5µg/ml (Green and Sambrook, 2012).

3-3-3 Primer pairs preparation

The primer pair used in this study was dissolved using TE Buffer, (pH 8.0) composed of 10mM Tris-HCl containing 1mM EDTA-Na2. Firstly the primer stock tube prepared and then the working solution would prepared from primer stock tube.

According to the instruction provided by primer manufacturer (Bioneer / Korea) the TE buffer were added to get 100 picomole/microliter concentration of primer stock solution. The working solution prepared from stock by dilution with TE buffer to get 10 picomole/microliter .

Sequences of primers (5' to 3'): arbitrary designed primer A: ACGACCCACG and Primer B: CACCACGCCT to generate RAPD fingerprint.

3-3- 4-Reaction mixture

Amplification of DNA was carried out in a final volume of 50 µl reaction mixture as mentioned in table (2)

Table (1): Contents of the Reaction Mixture

No.	**Contents of reaction mixture**	**Volume**
1.	Green master mix	25 µl
2.	Upstream primer (10pmol/µl)	3µl
3.	Downstream primer (10pmol/µl)	3µl
4.	DNA template	5 µl
5.	Nuclease free water	14µl
Total volume		50 µl

3-4 heavy metals measurement

Heavy metals (pb,Cd, Cu, Fe) were measured in each sample by FAAS (Shimadzu 7000) after serum samples digestion by The blood sample (5 ml) that had been kept at 4C for analysis was given a shot of concentrated nitric acid. The blood samples were digested using the microwave. 4.0 ml of the sample and 10.0 ml of a combination of strong hydrochloric and nitric acids in (5 ml conc. HCl + 5 ml conc. HNO3) were used to digest the samples.(Awad etal., 2013), The solution was diluted to 25.0 ml with deionized water.

3-6 Statistical Analysis

SPSS 20 programs used for least significance differences (LSD $\leq$ 0.05), Analysis of variance test (ANOVA) between sites and different Studies parameters

4. Results and Discussion

4-1 CBC parameters

The measurements done for all CBC parameters in two conditions for 25C˚ and for 4C˚, some parameters showed the significance differences such as WBC, RDW % CV, MPV, Neutrophil, Eosinophil.(Table 2)

Table (2): CBC parameters for sample in two conditions.

Groups / Parameters	25C˚	4C˚	p-value
	Mean±S.D		
WBC x10^9/l	8.62±0.09	9.85±0.16	**0.007***
RBC x10^{12}/l	5.22±4.8	5.32±7.3	**0.018**
Hemoglobin g/l	13.22±0.7	13.45±0.9	**0.820**
Htc l/l	39.4±5.6	39.45±3.6	**0.396**
MCV pg	93.86±3.5	93.55±10.1	**0.100**
MCHC g/l	36.20±7.6	36.10±1.9	**0.044**
RDW % CV	13.62±0.0	13.85±0.1	**0.007***

	9	6	
PLT x109/l	279.22±4.8	266.32±7.3	**0.019**
MPV	9.22±0.7	13.45±0.9	**0.820***
Neutrophil x109/l	4.4±5.6	4.45±3.6	**0.39***
Lymphocyte x109/l	3.75±3.5	3.85±10.1	**0.110**
Monocyte x109/l	0.67±7.6	0.66±1.9	**0.045**
Eosinophil x109/l	0.33±0.09	0.36±0.16	**0.017***
Basophil x109/l	0.05±4.8	0.06±7.3	**0.022***

Previous research have produced contradictory results on the findings of WBC, RBC, and HGB, however our study found that these parameters were stable throughout the trial at all temperatures. The effects of storage on most blood count parameters can be reduced if the storage temperature is kept at 4° C and the analysis is completed within 24 hours(Kunz,2001).

Similar findings were obtained in other investigations, which indicated that cooling samples (4–6°C) reduced the variability in CBC parameters.(Zini ,2014)

4-2 Heavy metals Concentrations

Most of concentrations of measured metals were fluctuated during study, the highest concentration of Cu (41.4 ppm) found in Second Group(4C˚) of Samples (Table 3),. Increasing some of concentrations of heavy metals are relatively related with uptake and elimination rates, and metabolism of heavy metals according to the Environmental Conditions (Teh etal., 1999) ,

Table (3):Heavy metals concentration (ppm) in Exposed Workers

Groups / Heavy metals	25C˚	4C˚	p-value
	Mean±S.D		
Pb	0.32±0.05	0.17±0.11	**0.004****
Cd	0.04±0.003	0.05±0.004	**0.043***
Cu	61.5±2.7	41.4±3.5	**0.01***
Fe	0.33±0.01	0.23±0.02	**0.763**

Earlier research showed that when the temperature rises, the extracellular concentration rises due to a shift in the balance between the two the metal in solution and the cell wall exchange sites(Fritioff etal,2005).

4-3 RAPD Results

The findings of a research on the effects of lead, copper, iron, and cadmium on human blood DNA integrity When humans are utilized to evaluate the impacts of heavy metals, they can be employed as biological indicators to quantify the potential consequences of pollutants. Heavy metal genotoxicity was investigated using RAPD (random amplified polymorphic DNA) The DNA damage in with two heavy metals using a RAPD 'fingerprinting' approach. The presence and/or absence of DNA fragments in treated samples compared to untreated ones revealed polymorphism and showed that at higher concentrations (350 mg x l(-1)) of the studied heavy metals, a qualitative measure indicating alterations in RAPD patterns was considerably impacted. Six random primers (decamers) were used to discover 467 RAPD fragments in RAPD profiles, with 224 of these fragments showing polymorphism.(Enan , 2006),

The interpretation of the changes in RAPD profiles is difficult since many factors can affect the generation of RAPD profiles. It is therefore important that these factors are identified and taken into account while using these assays. On the other hand, further analyses of the relevant bands generated in RAPD profile allow not only to identify some of the molecular events implicated in the genomic instability but also to discover genes playing key roles, particularly in the initiation and development of malignancy. Finally, to elucidate the potential genotoxic effects of environmental contaminants, a powerful strategy could be firstly to use the RAPD assay as a screening method and secondly to apply more specific methods measuring for instance DNA adducts, gene mutations or cytogenetic effects. It is

also envisaged that these assays (i.e. RAPD and related techniques), which reflect effects at whole genome level, would continue to complement the use of emerging technologies (Atienzar& Jha,2006)

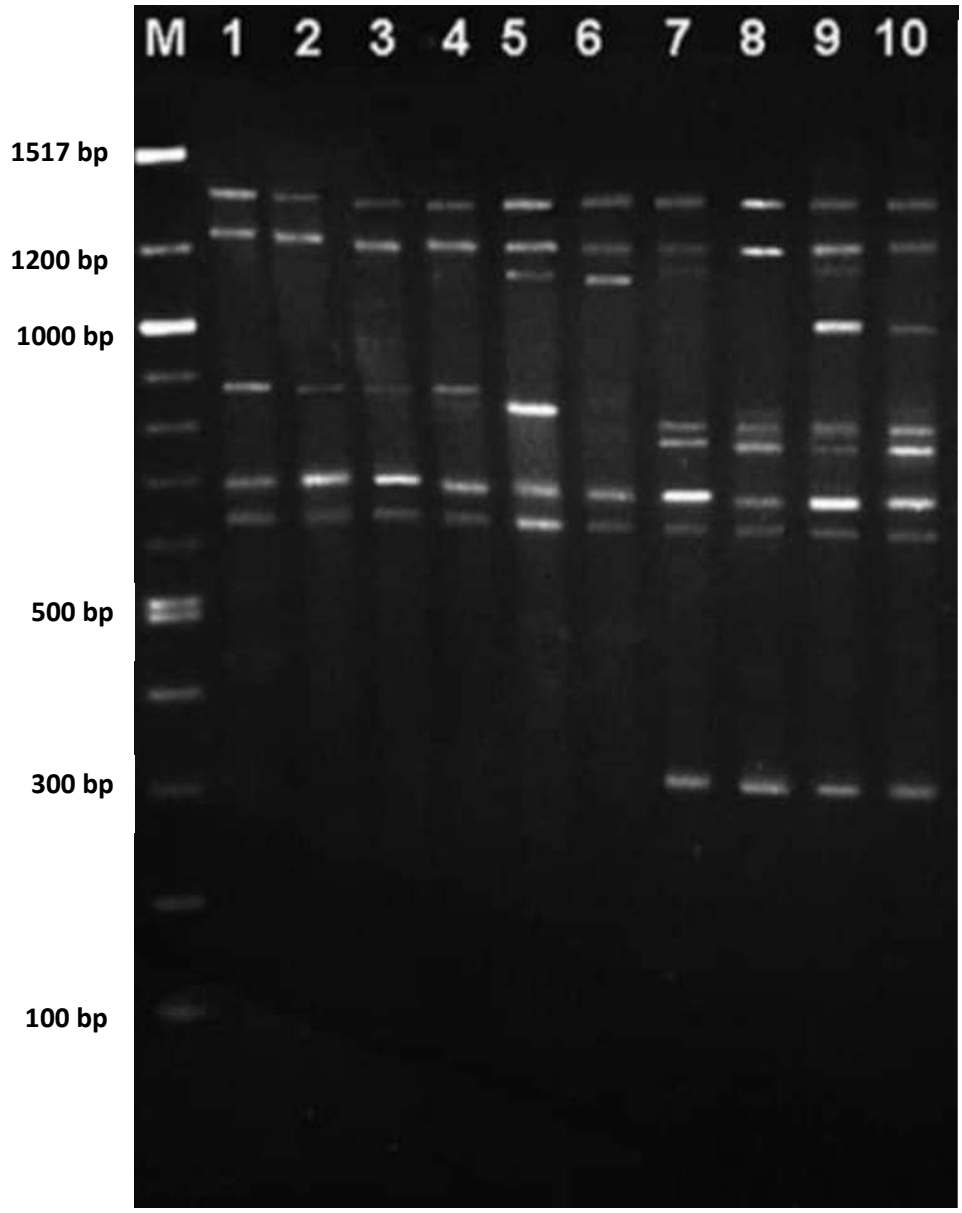

Figure 4:RAPD Analysis for Blood Sample

The RAPD PCR fingerprints produced by primer A and primer B, respectively, are shown in Fig.4. The PCR products ranged in size from 300 to 1517 bp, and

each sample had four to ten main bands amplified. The RAPD PCR fingerprints of 10 different human DNA samples under different conditions.

Statistical tools and parameters, as used in other RAPD studies, should be applied in ecotoxicological research as well in order to fully exploit the potential of this technique. Finally, due to their random nature, RAPD data often must be considered as preliminary until they are further documented by cloning, sequencing and probing techniques (De Wolf etal., 2004).

(Congiu etal.,2000)found that because of economical involvements, the molecular approach was added to the more traditional morphological examination in a double-blind test. All plants belonging to the patented variety were unambiguously identified (13 plants among a total of 31 plants examined). The results were accepted as evidence in the court. This study confirms that the RAPD technique is especially suitable for identification of asexually reproduced plant varieties for forensic or agricultural purposes.

Mobile Rapid DNA technology is close to being incorporated into crime scene investigations, with the potential to identify a perpetrator within hours. However, the use of these techniques entails the risk of losing the sample and potential evidence, because the device not only consumes the inserted sample, it is also is less sensitive than traditional technologies used in forensic laboratories. Scene of Crime Officers (SoCOs) therefore will face a 'time/success rate trade-off' issue when making a decision to apply this technology. (Mapes etal.,2019)indicated in his study we designed and experimentally tested a Decision Support System (DSS) for the use of Rapid DNA technologies based on Rational Decision Theory (RDT). In a vignette study, where SoCOs had to decide on the use of a Rapid DNA analysis device, participating SoCOs were assigned to either the control group (making decisions under standard conditions), the Success Rate (SR) group

(making decisions with additional information on DNA success rates of traces), or the DSS group (making decisions supported by introduction to RDT, including information on DNA success rates of traces).

(Kayser, 2015) determined that the Forensic DNA Phenotyping refers to the prediction of appearance traits of unknown sample donors, or unknown deceased (missing) persons, directly from biological materials found at the scene. "Biological witness" outcomes of Forensic DNA Phenotyping can provide investigative leads to trace unknown persons, who are unidentifiable with current comparative DNA profiling. This intelligence application of DNA marks a substantially different forensic use of genetic material rather than that of current DNA profiling presented in the courtroom. Currently, group-specific pigmentation traits are already predictable from DNA with reasonably high accuracies, while several other externally visible characteristics are under genetic investigation.

The salt concentration, primer annealing temperature, template concentration, primer length, and RAPD PCR fingerprint repeatability were all influenced by these variables. and Simple and reproducible fingerprints of complex genomes can be generated using single arbitrarily chosen primers and the polymerase chain reaction (PCR). No prior sequence information is required (Welsh& McClelland,1990)

(Shiono , 1996) was performed personal identification by DNA polymorphism using forensic specimens. DNA was purified by the potassium iodine method instead of the phenol extraction method. When DNA is amplified by PCR, there are inhibitors of PCR such as melanin in hairs or blood and inorganic salts in bone. This problem was solved by the potassium iodine method. The genotype of the ABO blood groups could be determined by the polymerase chain reaction-

restriction fragment length polymorphism (PCR-RFLP) method in all 29 old blood-stain specimens (obtained 11 months-15 years before) and 14 fingerprint specimens on a glass plate. In sexual assaults against women, one key to identifying the suspect is ABO phenotyping or the typing of other polymorphic markers of the seminal fluid in the victim's vagina.

All three forensic DNA phenotyping methods—the prediction of externally observable features, biogeographic ancestry, and age estimate from crime scene DNA—require a suitable regulatory framework and should be applied in tandem. Before forensic DNA phenotyping may be utilized in practice, efforts must be made to reduce the danger of privacy violations and to guarantee that these technologies are used in a transparent and proportional manner(Schneider etal.,2019)

Conclusions

1-In temperate climates, CBC parameters are more prone to alter, therefore caution should be exercised while storing them at 4°C when a delay of more than 6 hours is expected.

2-The suggested technique has a lot of promise for identifying blood in heavily polluted samples.

Recommendations

1-In a future work, the effects of the preservation time and conditions will be studied and discussed.

2-Differentiate between human blood stain and non-human blood stain by RAPD PCR Analysis .

3-Use other Molecular techniques in crime scene such as RFLP and AFLP.

References

Atienzar FA, Jha AN. The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. Mutat Res. 2006 Nov-Dec;613(2-3):76-102. doi: 10.1016/j.mrrev.2006.06.001. Epub 2006 Sep 18. PMID: 16979375.

Awad A., D.M.H. Ali, M.A. Khalid, M.A. Elsheikh, Assessment of digestionprocedure for determination of trace elements by ICP-OES. Open ScienceRepository Chemistry Online (Open access), e70081933. 2013; D.O.I:10.7392/Chemistry.70081933.

Babu KN, Sheeja TE, Minoo D, Rajesh MK, Samsudeen K, Suraby EJ, Kumar IPV. Random Amplified Polymorphic DNA (RAPD) and Derived Techniques. Methods Mol Biol. 2021;2222:219-247. doi: 10.1007/978-1-0716-0997-2_13. PMID: 33301097.

Beata Krawczyk, Józef Kur, Chapter 16 - Molecular Identification and Genotyping of Staphylococci: Genus, Species, Strains, Clones, Lineages, and Interspecies Exchanges, Editor(s): Vincenzo Savini, Pet-To-Man Travelling Staphylococci, Academic Press, 2018,

Calvo JH, Zaragoza P, Osta R. Random amplified polymorphic DNA fingerprints for identification of species in poultry pâté. Poult Sci. 2001 Apr;80(4):522-4. doi: 10.1093/ps/80.4.522. PMID: 11297293.

Cavanaugh SE, Bathrick AS. Direct PCR amplification of forensic touch and other challenging DNA samples: A review. Forensic Sci Int Genet. 2018 Jan;32:40-49. doi: 10.1016/j.fsigen.2017.10.005. Epub 2017 Oct 18. PMID: 29059581.

Congiu L, Chicca M, Cella R, Rossi R, Bernacchia G. The use of random amplified polymorphic DNA (RAPD) markers to identify strawberry varieties: a

forensic application. Mol Ecol. 2000 Feb;9(2):229-32. doi: 10.1046/j.1365-294x.2000.00811.x. PMID: 10672167.

Da Cheng Hao, Xiao-Jie Gu, Pei Gen Xiao, 9 - Chemical and biological research of Clematis medicinal resources, Editor(s): Da Cheng Hao, Xiao-Jie Gu, Pei Gen Xiao, Medicinal Plants,

David P. Clark, Nanette J. Pazdernik, Chapter 6 - Polymerase Chain Reaction, Editor(s): David P. Clark, Nanette J. Pazdernik, Molecular Biology (Second Edition), Academic Press, 2013, Pages 163-193,

De Wolf H, Blust R, Backeljau T. The use of RAPD in ecotoxicology. Mutat Res. 2004 May;566(3):249-62. doi: 10.1016/j.mrrev.2003.10.003. PMID: 15082240.

Enan MR. Application of random amplified polymorphic DNA (RAPD) to detect the genotoxic effect of heavy metals. Biotechnol Appl Biochem. 2006 Mar;43(Pt 3):147-54. doi: 10.1042/BA20050172. PMID: 16296987.

Encyclopedia of Food Microbiology, Elsevier, 1999, Pages 1907-1913,

Fritioff A, Kautsky L, Greger M. Influence of temperature and salinity on heavy metal uptake by submersed plants. Environ Pollut. 2005 Jan;133(2):265-74. doi: 10.1016/j.envpol.2004.05.036. PMID: 15519457.

John M. Butler, Chapter 16 - Non-human DNA, Editor(s): John M. Butler, Advanced Topics in Forensic DNA Typing: Methodology, Academic Press, 2012, Pages 473-495,.

Kayser M. Forensic DNA Phenotyping: Predicting human appearance from crime scene material for investigative purposes. Forensic Sci Int Genet. 2015 Sep;18:33-48. doi: 10.1016/j.fsigen.2015.02.003. Epub 2015 Feb 16. PMID: 25716572.

Kunz D. Possibilities and limitations of automated platelet counting procedures in the thrombocytopenic range. Semin Thromb Hemost. 2001 Jun;27(3):229-35. doi: 10.1055/s-2001-15252. PMID: 11446656.

Lee JC, Chang JG. Random amplified polymorphic DNA polymerase chain reaction (RAPD PCR) fingerprints in forensic species identification. Forensic Sci Int. 1994 Jul 4;67(2):103-7. doi: 10.1016/0379-0738(94)90325-5. PMID: 7927092.

Mapes AA, Stoel RD, de Poot CJ, Vergeer P, Huyck M. Decision support for using mobile Rapid DNA analysis at the crime scene. Sci Justice. 2019 Jan;59(1):29-45. doi: 10.1016/j.scijus.2018.05.003. Epub 2018 May 4. PMID: 30654966.

Misawa S. [Application of DNA polymorphism to forensic medicine]. Rinsho Byori. 1994 Jun;42(6):636-42. Japanese. PMID: 7914244.

Mohammad Sorof Uddin, Qi Cheng, 12 - Recent application of biotechniques for the improvement of mango research, Editor(s): Palmiro Poltronieri, Yiguo Hong, Applied Plant Genomics and Biotechnology, Woodhead Publishing, 2015, Pages 195-212,

Morling N. PCR in forensic genetics. Biochem Soc Trans. 2009 Apr;37(Pt 2):438-40. doi: 10.1042/BST0370438. PMID: 19290877.

Primorac D, Schanfield MS. Application of forensic DNA testing in the legal system. Croat Med J. 2000 Mar;41(1):32-46. PMID: 10810166.

Prinz M, Carracedo A, Mayr WR, Morling N, Parsons TJ, Sajantila A, Scheithauer R, Schmitter H, Schneider PM; International Society for Forensic Genetics. DNA Commission of the International Society for Forensic Genetics (ISFG): recommendations regarding the role of forensic genetics for disaster victim identification (DVI). Forensic Sci Int Genet. 2007 Mar;1(1):3-12. doi: 10.1016/j.fsigen.2006.10.003. Epub 2006 Nov 28. PMID: 19083722.

Romsos EL, Vallone PM. Rapid PCR of STR markers: Applications to human identification. Forensic Sci Int Genet. 2015 Sep;18:90-9. doi: 10.1016/j.fsigen.2015.04.008. Epub 2015 Apr 23. PMID: 25937291.

Satyanarayan Panigrahi, Parthiban Velraj, Toleti Subba Rao, Chapter 21 - Functional Microbial Diversity in Contaminated Environment and Application in Bioremediation, Editor(s): Surajit Das, Hirak Ranjan Dash, Microbial Diversity in the Genomic Era, Academic Press, 2019, Pages 359-385,

Schneider PM, Prainsack B, Kayser M. The Use of Forensic DNA Phenotyping in Predicting Appearance and Biogeographic Ancestry. Dtsch Arztebl Int. 2019 Dec 23;51-52(51-52):873-880. doi: 10.3238/arztebl.2019.0873. PMID: 31941575; PMCID: PMC6976916.

Shiono H. [Personal identification using DNA polymorphism--the identification of forensic biological materials]. Nihon Hoigaku Zasshi. 1996 Oct;50(5):320-30. Japanese. PMID: 8952331.

Teh, S.J.; Clark, S.L.; Brown, C.L.; Luoma, S.N.&Hinton, D.E.(1999) Enzymatic and histopathologic biomarkers as indicators of contaminant exposureandeffectinAsianclam(Potamocorbulaamurensis).Biomarkers,4(6): 497–509.

Welsh J, McClelland M. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 1990 Dec 25;18(24):7213-8. doi: 10.1093/nar/18.24.7213. PMID: 2259619; PMCID: PMC332855.

Woller J, Füredi S, Pádár Z. Polimeráz láncreakción alapuló DNS-vizsgálatok a magyar igazságügyi gyakorlatban [Use of polymerase chain reaction DNA studies in Hungarian legal practice]. Orv Hetil. 1997 Dec 21;138(51):3223-8. Hungarian. PMID: 9454100.

Woodhead Publishing, 2015, Pages 341-371, H. Nybom, DNA Fingerprinting, Reference Module in Life Sciences, Elsevier, 2017,

Yuji Oda, Kozo Ouchi, SACCHAROMYCES | Introduction, Editor(s): Richard K. Robinson,

Zini G; International Council for Standardization in Haematology (ICSH). Stability of complete blood count parameters with storage: toward defined specifications for different diagnostic applications. Int J Lab Hematol. 2014 Apr;36(2):111-3. doi: 10.1111/ijlh.12181. Epub 2013 Dec 27. PMID: 24373157.

الخلاصة

الهدف من هذه الدراسة هو الكشف عن تأثير المقاييس البيئية على تحليل RAPD PCR في الشخص المشتبه به في مسرح الجريمة المفترض.

تم جمع 10 عينات وقسمت مباشرة الى مجموعتين , المجموعة الاولى تحت 25 درجة مئوية والمجموعة الثانية تحت 4 درجة مئوية حيث تم أجراء تحليل CBC والكشف عن العناصر الثقيلة وتحليل RAPD لجميع العينات.

أظهرت بعض النتائج اختلافات بين المجموعتين مثل MPV,RDW, WBC,Basophile,Eosionphile مع وجود تغايرات في بعض تراكيز العناصر الثقيلة خصوصا النحاس في المجوعة الثانية.

أظهرت نتائج تحليل RAPD بوجود حزم وتغايرات تزواحت بين 300 الى 1517 زوجا قاعديا حيث تبين عدم وجود تأثير لدرجة الحرارة والعناصر الثقيلة على التحليل المذكور.

نستنتج الى ان درجة الحرارة والعناصر الثقيلة لها تأير ضئيل على تحليل ال RAPD وهذا التحليل له اهمية في تحليل الدم خلال مسرح الجريمة.

.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل/ كلية العلوم
قسم علوم الحياة

تقنية ال RAPD في الأدلة الجنائية وعلاقتها بالعوامل البيئية

مشروع بحث مقدم الى

مجلس كلية العلوم – جامعة بابل

كجزء من متطلبات نيل درجة الدبلوم العالي في العلوم/ أدلة جنائية

من قبل

مثنى جواد ساهر

(بكالوريوس علوم حياة، 2007)

إشراف

الاستاذ الدكتور أياد محمد المعموري

2021 م **١٤٤٢ه**