Republic of Iraq
Ministry of Higher Education
and Scientific Research
University of Babylon
College of Science
Department of Biology

Detection the best DNA extraction method for different hair shafts samples

A Research

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

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

بِسْمِ اللَّهِ الرَّحْمنِ الرَّحِيمِ

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

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Dedication

To my creators and commissioner God, who teach humans and guide them to knowledge

To the prophet and messenger Muhammad peace be upon him and his family

To my father the great heart to the source of patience and hope

To my lovely mother who made me what I am now

There are no words enough to thanks you

To my wife who support and encourage me along my study.

To my children flowers of my life

To my brother and sisters who gave me a lot support

THAER

Acknowledgments

Praise be on Allah and peace and blessings be upon his Messenger the Prophet Muhammad peace be upon him and his family and who followed him until the Day of religion. I would like to thank my advisor prof. Dr. Rabhab Emraan Radhi Al-Jilawi, University of Babylon for his supervision of the construction of this scientific effort that has been a great asset for me.

My Thanks are extended to the Deanship of the Faculty of Sciences and the presidency of the Department of Biology / College of Sciences / University of Babylon for helping and guiding me.

THAER

Summary

Hair can be a valuable source of DNA especially in forensic casework and for noninvasive studies of human, when blood samples not available. This study emphasizes the impact of hair dyes on structure of DNA .Two samples group of hair were used, one normal hair group and the another staining hair group. DNA extracted by using two different techniques (spin column and phenol chloroform extraction method), the Nanodrop spectrophotometer and Agarose gel electrophoresis was used in estimation of DNA extraction method. the results of Nanodrop DNA concentrations and DNA purity by Spin column DNA extraction method in normal hair were showed at (0.7-1.2ng/ul) and (0.36-0.52) respectively, whereas, in staining hair group the results were showed were showed at (7.1-162ng/ul) and (1.01-1.18) respectively. the results of Nanodrop DNA concentrations and DNA purity by phenol chloroform extraction method in normal hair were showed at (14.9-23.7ng/ul) and (1.37-1.38) respectively, whereas, in staining hair group the results were showed were showed at (192.4-338.2ng/ul) and (1.22) respectively. The integrity of genomic DNA from normal hair and staining hair samples that extracted by two methods (Spin column and phenol chloroform extraction method) was checked by 0.8% agarose gel electrophoresis method and the results were showed that the normal hair and appeared as excellent extraction and sharp DNA bands with no DNA lysis, whereas, the staining hair and appeared as high density DNA extraction with high DNA lysis. The Phenol Chloroform DNA extraction method was showed that the normal hair as excellent low density extraction and sharp DNA bands with no DNA lysis, whereas, the staining hair was appeared as huge density DNA extraction with high DNA lysis. Our results were demonstrated that staining hair by chemical dyes had a significant influence on the structure of DNA results. The organic method by Phenol Chloroform DNA extraction method was an appropriate method for extraction DNA from hair shaft, since this method used for extracting the old and degraded samples.

Table of Contents

NO.	Subject	No. of Page
	Summary	
	Chapter one Introduction	1
1.	*1. Introduction*	1
	Chapter two *Literatures Review*	2
2.	2.1. DNA Extraction	3
3.	2.2.Organic (Phenol–Chloroform) Extraction	4
4.	2.3.Solid-Phase DNA Extraction Methods	4
5.	2.4.DNA from FTA Spots	5
6.	2.5.Chelating Resins (Chelex)	6
7.	2.6.Differential Lysis	6
8.	2.7.DNA Extraction from Bones and Teeth	7
9.	2.8.Laser Capture Microdissection	7
10.	2.9.Automation of DNA Extraction	8
11.	2.10.Microfluidic DNA Extraction Devices	9
12.	2.11. DNA Extraction from hair	9
	Chapter Three Materials and Methods	12
13.	3- Materials and Methods	12
14.	3-1- Materials	12
15.	3-1-1- Equipments and Instruments	12
16.	3-1-2- kits	13
17.	3-1-3- Chemicals	14
18.	3-2-1- Chemical solutions preparation:	15
19.	3-2-2- Methods	15
20.	3-2-3- Hair DNA extraction by G-spin DNA extraction method:	15
21.	3-2-4- Phenol-Chloroform DNA extraction method:	17
22.	3-2-5- Genomic DNA estimation	18
23.	3-2-6- Agarose gel electrophoresis	18
	Chapter four results and discussion	21
24.	4-1 Nanodrop spectrophotometer results:	20
25.	4-2- Agarose gel electrophoresis results:	21
	Chapter Five: Conclusion and Recommendation	24
	References	25

Table of Tables

NO.	Name of Tables	No. of Page
1.	Table (3-1):Equipment's and Instruments	12
2.	Table (3-2): The kits used in this study with their companies and countries of origin	13
3.	Table (3-3) The Chemicals	14
4.	Table (4-1): represent the DNA concentration and DNA purity by Nanodrop by Spin column DNA extraction method	20
5.	Table (4-2): represent the DNA concentration and DNA purity by Nanodrop by phenol chloroform DNA extraction method	20

Table of Figures

NO.	Name of Figures	No. of Page
1.	Figure (3-1): The type of hair samples that used in this study. The Normal hair and staining hair samples..	19
2.	Figure (4-1): Agarose gel electrophoresis image that showed the Genomic DNA extraction from normal hair and staining hair samples.	21
3.	Figure (4-2): The phenol chloroform DNA extraction method image that showed the DNA separation step with high protein degradation in staining hair samples with low protein degradation in normal hair samples.	22

CHAPTER ONE

INTRODUCTION

1. Introduction

Deoxyribonucleic acid (DNA)[Alberts.*et al*.2014] could be a particle composed of two polynucleotide chains that coil around each other to make a twofold helix carrying genetic information for the advancement, working, development and propagation of all known living beings and numerous viruses. DNA and ribonucleic acid (RNA) are nucleic acids. Alongside proteins, lipids and complex carbohydrates (polysaccharides), nucleic acids are one of the four major sorts of macromolecules that are fundamental for all known shapes of life. The two DNA strands are known as polynucleotides as they are composed of less difficult monomeric units called nucleotides.[James.*et al*.2019] Each nucleotide is composed of one of four nitrogen-containing nucleobases (cytosine [C], guanine [G], adenine [A] or thymine [T]), a sugar called deoxyribose, and a phosphate bunch.

The nitrogenous bases of the two partitioned polynucleotide strands are bound together , with hydrogen bonds to create double-stranded DNA. Both strands of double-stranded DNA store the same biological data. This data is duplicated as and when the two strands isolated. A huge portion of DNA (more than 98% for people) is non-coding, meaning that these sections don't serve as patterns for protein sequences. [Rahane.*et al*.2019] Under the genetic code, these RNA strands specify the sequence of amino acids at proteins in a process called translation. Within eukaryotic cells, DNA is organized into long structures called chromosomes. Before exemplary cell division, these chromosomes are duplicated in the process of DNA replication, providing a full set of chromosomes for each daughter cell.

Eukaryotic organisms (animals, plants, fungi and protists) store most of their DNA inside the cell nucleus as nuclear DNA, and some in the mitochondria as mitochondrial DNA or in chloroplasts as chloroplast DNA.[Masood.*et al*.2020] In contrast, prokaryotes (bacteria and archaea) store their

DNA only in the cytoplasm, in circular chromosomes. Within eukaryotic chromosomes, chromatin proteins, such as histones, compact and organize DNA. These compacting structures guide the interactions between DNA and other proteins, helping control which parts of the DNA are transcribed. The aim of this study was to analyze the MtDNA sequences of hairs subjected to dyes for forensic application.

The aim of this study

This study aimed to determine the best DNA extracted method to extract DNA from hair shafts .

CHAPTER TWO

LITERATURES REVIEW

2. Literature Review

2.1. DNA Extraction

DNA extraction is a method to purify DNA by using physical and/or chemical methods from a sample separating DNA from cell membranes, proteins, and other cellular components.[Friedrich Miescher in 1869] DNA isolation for the first time. The process of isolating purified nuclear and/or mitochondrial DNA from both forensic specimens (blood, semen or saliva stains, hairs, muscle, bones, teeth, etc.) and reference samples (bucal swabs, blood spots on FTA, or liquid blood) is a crucial step to DNA profiling.

Advances in forensic DNA extraction systems have been aimed at increasing the efficiency in the amount of purified DNA recovered (free from polymerase chain reaction (PCR) inhibitors) and automating the process for high-throughput analysis while maintaining a high integrity of the DNA molecule. Currently, the validated methods for DNA extraction most widely used in forensic laboratories can be classified into three groups on the basis of their purification strategies: organic (phenol–chloroform) extraction, solid-phase DNA extraction methods (silica based), and ionic chelating resins (Chelex).

Specific procedures using some of these basic DNA isolation principles (or a combination of them) have been developed depending on the type of sample source. These include the differential lysis procedure for the selective extraction of sperm cells, special procedures for bone and teeth DNA extraction, the procedure for DNA purification on reference biological samples spotted on FTA paper, or previous selection of specific cell types by laser-capture microdissection coupled with DNA extraction. Automated DNA extraction procedures with different robotic have also been implemented in forensic labs for high-throughput sample preparation, avoiding manual errors while improving sample tracking and reproducibility. Quality standards for DNA

extraction in forensic labs include preventive measures against DNA contamination as well as the use of appropriate positive and negative controls for monitorization [Jakubowska.*et al.*2012]

2.2.Organic (Phenol–Chloroform) Extraction

Organic extraction has been one of the DNA extraction methods most used in the forensic field. The first step of any DNA extraction assay is the breakdown of cell membranes and proteolytic digestion in the presence of sodium dodecylsulfate (SDS), and proteinase K. DNA is first purified by mixing thoroughly the cell lysate with a phenol–chloroform solution followed by centrifugation in order to separate the organic phase, where proteins become trapped, from the supernatant aqueous phase, where DNA remains. DNA in the aqueous phase is further purified by precipitation with ethanol and finally resuspended in a low-salt buffer. For maximal DNA recovery and purity, the organic method protocol developed in many forensic labs involves a filtration purification step of the aqueous phase (instead of ethanol precipitation) using Centricon, Microcon, or, more recently, Amicon filter devices for DNA washing and concentration by centrifugation through membranes with different pore sizes (30–100 kDa) (Millipore, Billerica, MA). Although this method is very efficient for the recovery of double-stranded high-molecular-weight DNA free from PCR inhibitors, it is time-consuming, requires multiple tube transfers, and is difficult to automate[Hedman.*et al.*2013].

2.3.Solid-Phase DNA Extraction Methods

This extraction method is based on the ability of DNA to bind to silica in the presence of chaotropic salts such as guanidinium thiocyanate, sodium iodide, and guanidinium hydrochloride. Typically, cells are first lysed with proteinase K to release the DNA and then a binding buffer containing a chaotropic salt is added to prepare DNA for adsorption to the silica at (

pH<7.5). Once DNA binds to silica, undesirable impurities can be swill away after subsequent washing steps ,DNA may be eluted under alkaline conditions and depressed salt concentrations. The silica method can be carried out in two different formats : silica columns and silica-coated paramagnetic beads. In the first state , after DNA binding in the column, washing of impurities and DNA elution are made by centrifugation. In the magnetic beads procedure, washing steps and DNA elution are facilitated simply by applying a magnetic force without the need of centrifugation devices. Magnetic bead-based purification is currently one of the procedures best suited for DNA isolation in the forensic field as it enables rapid DNA purification with very efficient removal of PCR inhibitors, and it is suitable for high-throughput extraction using robotic platforms[Ayoib.*et al.*2017] .

2.4.DNA from FTA Spots

FTA® is an acronym for Fast Technology for Analysis of nucleic acids. It consists of a cellulose-based matrix treated with a weak base, a chelating agent, an anionic surfactant or detergent, and a uric acid (or a urate salt). Biological samples, such as blood or saliva, can be applied to FTA cards whose chemicals lyse cells and the released DNA remains immobilized. This system provides DNA preservation avoiding nuclease damage and microbial development, allowing a long-term storage at ambient temperature under dry conditions. FTA is at present a procedure widely implemented by several forensic laboratories for DNA collection of reference saliva or blood samples. There are two main strategies for DNA extraction from FTA paper. One is to wash out proteins and cellular debris from the FTA spot, keeping the DNA bound to the FTA, and then use a clean paper punch to perform the PCR analysis. Alternatively, DNA can be eluted from FTA by a Chelex extraction or other procedures using the eluted DNA for further analysis. The main advantages of FTA are the feasibility

of automation and its long-term preservation due to its storage capabilities under ambient temperature.[Park.*et al.*2008]

2.5.Chelating Resins (Chelex)

A rapid and inexpensive procedure for DNA extraction that has become popular in the forensic field is the use of chelating resins, such as Chelex 100 (Bio-Rad Laboratories, CA). These resins can bind divalent ions such as Ca2þ and Mg2þ deactivating unwanted nucleases and, therefore, protecting DNA molecules from cleavage. In most protocols, forensic samples are added to a 5% Chelex suspension, boiled for several minutes, and then centrifuged to remove the resin leaving DNA in the supernatant. Unfortunately, the boiling procedure of chelating resins denatures DNA and yields single-stranded DNA that can be analyzed only by PCR-based methods. On the other hand, the DNA purity is not as good as that obtained with the organic extraction or the solid-phase procedures[Singh.*et al.*2018].

2.6.Differential Lysis

A specific protocol for the selective separation of epithelial cells DNA from sperm DNA in sexual assault cases was developed in 1985 by Peter Gill. The procedure is a modified version of the organic extraction method based on the resistance of sperm nuclei to be lysed in the absence of a reducing agent such as dithiothreitol (DTT). The protocol involves a first lysis step in the presence of SDS and proteinase K aimed to release the female epithelial cells DNA in the supernatant. The washed pellet of sperm cells are subsequently lysed by treatment with SDS, proteinase K, and DTT and the sperm DNA is recovered from the supernatant of this second lysis fraction. The success of this method to separate sperm DNA from vaginal cell DNA depends on the relative number of each cell type and the conditions of preservation of the forensic

evidence. Failure to separate the male and female fractions by this procedure results in a mixed DNA profile[Butler.*et al*.2011].

2.7.DNA Extraction from Bones and Teeth

Several specific protocols have been described for DNA extraction from bones and teeth. All of them entail two primary steps. Previous preparation of compact bone tissue or teeth's dentine powder by pulverization in liquid nitrogen using a freezer mill, and the use of high concentrations of ethylenediaminetetraacetic acid (EDTA) to demineralize the hydroxyapatite matrix making osteocytes or odontocytes accessible to lysis. Early forensic protocols performed demineralization of bone samples by extensive EDTA washes before the lysis step with the subsequent loss of cellular material during different washing steps and high risk of sample contamination. More recently, a number of protocols have been developed for complete demineralization during the lysis step (using a lysis buffer containing 0.5 M EDTA), resulting in full physical dissolution of the bone sample and maximal recovery of DNA. Bone or teeth powder lysates are then submitted to organic extraction followed by Amicon filtration or purified by silica solid-phase procedures[Higgins.*et al.*2013].

2.8.Laser Capture Microdissection

Laser capture microdissection (LCM) is a technique that allows to select and collect specific cell types. It is of particular interest in the forensic field for specific sperm cell separation from mixtures of biological fluids in sexual assault cases. It combines existing light microscopic instrumentation with laser beam technology. There are two general methods of LCM: ultraviolet (UV) cutting systems and infrared (IR) capture systems. While in the IR system, after visualization of the cells of interest via microscopy, they are isolated by transfer of laser energy to a thermolabile polymer with formation of a polymer–cell

composite. In the UV system, cells can be selectively captured by photovolatilization of cells surrounding the target cells. In both methods, captured sperm cells are transferred to a vial for DNA extraction. The LCM technique is used particularly in unbalanced mixtures in which very low levels of sperm cells are mixed with a high content of epithelial cells of the victim. As the number of captured cells is usually very small, the DNA extraction process is usually done by cell lysis in a small volume in the presence of proteinase K and a nonionic detergent such as Tween 20. Subsequent inactivation of the proteinase by a heat shock in the same vial of capture is carried out to finally obtain the DNA for downstream PCR analysis, minimizing the possibility of contamination and preventing the loss of DNA that could occur during the procedures for DNA purification[Golubeva.*et al.*2012].

2.9.Automation of DNA Extraction

Development of robotic platforms for the extraction of DNA has been fundamental in ensuring a high-throughput processing of both reference samples and forensic evidences as well as guaranteeing reproducibility and sample tracking. Automation has been implemented in many forensic labs dealing with DNA profiling from large batches of reference samples for inclusion in national DNA databases. Automation has also taken great interest in disaster victim identification cases, enabling laboratories to speed up the process of DNA identification of missing persons. Most of the DNA extraction robots are based on solid-phase procedures with paramagnetic beads. There are several robotic stations for both small-scale and high-throughput processing as well as validated specific protocols for automated extraction of reference samples (blood, saliva, and FTA) and forensic samples (semen stains, blood, saliva, hair, bones, etc.). EZ1 (Qiagen), Maxwell 16 (Promega), and Automated Express (Life Science) are examples of small-scale platforms for automated DNA extraction of 6–16 samples simultaneously using paramagnetic beads,

which have been validated for forensic samples. While the Tecan Freedom EVO automated liquidhandling workstation and the Beckman 2000 robot workstation are high-throughput platforms that can handle up to 96 samples at a time and are also validated for forensic analysis.[Holmberg.*et al.*2013]

2.10.Microfluidic DNA Extraction Devices

The development of miniaturized devices for DNA preparation, manipulation, and analysis at the micron (microtechnology) or submicron level (nanotechnology) has become one of the most active research areas in molecular biology. They offer several advantages over conventional techniques that include reduced sample and reagent consumption, high-throughput and high-speed analysis, and easy automation and integration of different molecular analysis in a single biochip. In addition, microfabrication enables labs to increase the detection limit with the potential to manipulate DNA at the level of individual molecules with very important implications for the analysis of traditional challenges (DNA mixtures, low copy number, etc.) in forensic genetics. In respect to this technology, silica solidphase microchips have been developed for DNA extraction from forensic samples as well as some prototype microdevices for the differential lysis procedure.[Emily.*et al.*2013]

2.11. DNA Extraction from hair

The first reported use of forensic human-hair comparison was by Rudolf Virchow in 1861 . Of course, at that time the investigations relied on the microscopic appearance of hair only and could never gain human identification . Nowadays it is indispensable of use human hair in DNA forensic tests analysis for identification because human hair sample is considered as one of the most common biological evidence that can be found in crime scene and it is not easily destroyed because of the tough outer coating of hair . Even with exposure to moisture and decomposition of accompanying tissue . The tools of molecular

biology have qualified the scientists to analyze hair from crime scene, if hair sheath material contains a root material, this hair should subjected to nuclear DNA analysis (STR profile) which is best for the comparison of a questioned sample (crime scene or human remains) with a reference sample (suspect) . Mitochondrial DNA (MtDNA) has several features that can make it useful marker for human identification; usually resort to its marker when nuclear DNA marker fails to give a reliable result , such as the cases of the degraded samples or skeletal remains or hair without root . It exhibits some advantages over nuclear DNA analysis in such cases like hundreds to thousands of copies of MtDNA present in a cell (depend on the type of cell) compared to 2 copies of nuclear DNA, and its maternally inherited [Halah.*et al*.2017]. The high copy number of the mitochondrial genome often means that mtDNA data can be reliably generated even when attempts to type nuclear DNA markers fail to produce a profile, but still this test has low power of discrimination, labor intensive and expensive. MtDNA was a closed circular molecule with a certain region related to forensic analysis and located in control region of mtDNA were highly variable among individuals . This sequence variation was within two hypervariable (HV) regions HV1 and HV2 . Hairs contain extremely small quantities of DNA making the methods used extract the DNA of major importance .

In 1984, Dr. Alec Jeffreys developed DNA fingerprinting technique used to identify the individuals. He found that certain regions of DNA contained repeated DNA sequences. The regions with repeat units that are 2-6 base pair in length are called Short Tandem Repeats. The first STR multiplexes developed was quadraplex created by Forensic Science Services (FSS) that comprises four STR Loci. Now-a-days, there are different multi locus commercial kits available and used by researchers to solve the crimes so as to give justice to the society (Vaishali.*et al*.2019).

Hair shafts as a potential source of DNA is valuable for noninvasive study of human and nonhuman populations. It can be used in genetic analysis to identify individuals and breed in animal husbandry traceability, wild animal germplasm resources protection, forensic medical study and paleontological research, etc. The characteristic of mitochondrial DNA (mtDNA) extracted from hair shafts has already been well described previously [Jakubowska.*et al*.2012] In addition, James Robertson et al. [Hedman.*et al*.2013] described that mtDNA has limited value because mtDNA profiles cannot be compared with national and international databases of Short Tandem Repeat (STR) genotypes. Furthermore, mtDNA cannot discriminate maternal relatives and lack the discriminating power of STR profiles. However, fragmentation of the nuclear DNA (nuDNA) in the hair shafts was considered as a result of the keratinisation process [Ayoib.*et al*.2017] and nuDNA seldom been successfully analyzed. Thus, a major concern in the hair shafts DNA extraction and amplification today is trying to find an efficient, stable and easy way to extract DNA and amplify nuDNA targets.(Zheng Guan.*et al*.2013)

In most studies about molecular genetics, molecular diagnostics, DNA extraction is considered as the very first step that will give a significant effective. The material used for DNA extraction is vary from fresh blood to dried blood, tissue, oral mucosa and hair root. The dried blood and hair root are the two materials that easy to collect and can store for long term, which leads to more advantages for many time extractions to have fresh DNA and enough amount DNA for further studies**.(** Nguyen.*et al*.2012).

hair specimens containing the root portion can serve as a reliable and renewable source of DNA.(Suzanne.*et al*.2007) ,In conclusion, since the adoption of agarose gels in the 1970s for the separation of DNA, it has proven to be one of the most useful and versatile techniques in biological sciences research.(Pei Yun Lee.*et al*.2012).

CHAPTER THREE

MATERIALS AND METHODS

3- Materials and Methods

3-1- Materials

3-1-1- Equipment's and Instruments

That used in this study

Table (3-1)Equipment's and Instruments

No.	Instrument / equipment	Company / Country
1.	Camera	Nikon/Japan
2.	Electrophoresis	Bioneer/ Korea
3.	Eppendorf tubes	Biobasic/ Canada
4.	Exispin vortex centrifuge	Bioneer/ Korea
5.	High speed Cold Centrifuge	Eppendorf/ Germany
6.	Incubator	Memmert (Germany)
7.	Micropipettes (differentvolumes)	Eppendorf / Germany
8.	Nanodrop	Thermo Scientific/ UK
9.	Refrigerator	Concord/ lebanon
10.	Sensitive Balance	Ohaus /USA
11.	U.V transilluminator	Wised/Korea
12.	Vortex	CYAN/ Belgium
13.	Water bath	Kottermann (Germany)

3-1-2- kits

That used in this

Table (3-2): The kits used in this study with their companies and countries of origin:

No.	Kit	Company	Country
	G-spinTM Total DNA Extraction Kit	**iNtRON**	**Taiwan**
	Proteinase k 22mg		
	RNase 3mg		
	Buffer CL lysis buffer		
	Buffer BL DNA binding buffer		
	WA buffer protein degradation		
	Wash buffer		
	Buffer CE DNA Elution		
	Spin Column / Collection Tube		

3-1-3- Chemicals

Table(3-3) The Chemicals

No.	Chemical	Company and Origin
1.	10X TBE buffer	iNtRON (Korea)
2.	Absolute Ethanol	CHEM-LAB (Belgium)
3.	Agarose	iNtRON (Korea)
4.	Beta-mercaptoethanol	THOMAS BAKER(India)
5.	Boric acid	THOMAS BAKER(India)
6.	Chloroform alcohol	THOMAS BAKER(India)
7.	EDTA	BIO BASIC (Canada)
8.	Ethidium Bromide 10mg/ml	BioBasic (Canada)
9.	Isopropanol	LOBA-Chemie (India)
10.	Loading dye	Geneaid (Taiwan)
11.	NaCl	THOMAS BAKER(India)
12.	NaoH	THOMAS BAKER(India)
13.	Nuclease free water	BioLabs/ UK
14.	Phenol	HiMedia (India)
15.	SDS	BIO BASIC (Canada)
16.	Tris-base	THOMAS BAKER(India)
17.	Tris-Cl	BIO BASIC (Canada)

3-2-1- Chemical solutions preparation:

1- Hair lysis Buffer: The buffer was prepared according to (Wong *et al*., 2016) by mixed NaoH (0.2 M), and sodium dodecyl sulfate (SDS 2%), beta-mercaptoethanol (β-ME, 2%) and ethylenediaminetetraacetic acid (EDTA, 0.01 M).

2- Phenol chloroform DNA extraction reagent: The buffer was prepared according to (Sambrook *et al*., 2012) by mixed Buffer-Saturated Phenol and chloroform (1:1).

3-1X TBE buffer : The buffer was prepared according to (Sambrook *et al*., 2012) dissolve 10.8 g Tris base ,5.5 g Boric acid and 0.5g EDTA (pH 8.0) in 1000 ml distilled water.

3-2- 2 Methods

In this study two types of extraction method was used to extracted DNA from hair samples. As following:

3-2-3- Hair DNA extraction by G-spin DNA extraction method:

This method was used for extracted DNA from hair by using **G-spin DNA extraction** and done according to company instruction (**Hair DNA extraction protocol**) as following steps:

1. The 10 hair pieces from hair root were cut into1 cm length, then carefully transferred into a new 1.5 ml tube.
2. The hair sample were Crashed 10 ~ 20 times using micropestle with 200µl Buffer CL lysis.
3. A 20µl Proteinase K and 5µl RNase A Solution was added into sample tube and mixed well by vortexing vigorously.

4. The lysate samples was incubated at 56℃ in water bath for 30 min. inverting the tubes every 2 min during the incubation until hair lysis completely.

5. A 200µl of Buffer BL DNA binding buffer was added into upper sample tube and mix thoroughly, then incubated the mixture at 70℃ for 5min.

6. The sample tubes were placed in centrifuge at 13,000 rpm for 5 min. Then carefully transfer 400µl the supernatant into a new 1.5 ml tube.

7. A 200µl absolute ethanol was added into the lysate, and mix well by pulse vortex.

8.The mixture was transferred into Spin Column (in a 2 ml Collection Tube) without wetting the rim, and centrifuged at 13,000 rpm for 1 min.

9. The filtrate was discarded and place the Spin Column in a new 2 ml Collection Tube.

10. A 700µl Buffer WA (Buffer WB) was added to the Spin Column without wetting the rim, and centrifuged for 1 min at 13,000 rpm.

11. The filtrate was discarded and place the Spin Column in a new 2 ml Collection Tube.

12. A 700µl Buffer WB was added into the Spin Column without wetting the rim, and centrifuged for 1 min at 13,000 rpm.

13. The filtrate was discarded and place the Spin Column in a new 2 ml Collection Tube.

14. The empty Spin Column with collection tubes were centrifuge again for additional 1 min to dry the membrane and the flow-through discarded.

15. The Spin Column was placed into a new 1.5 ml tube, and 50µl Buffer CE (DNA elution buffer) was added directly onto the membrane.

16. The Spin Column was incubated for 1 min at room temperature and then centrifuge for 1 min at 13,000 rpm to elute the DNA.

3-2-4- Phenol-Chloroform DNA extraction method:

The phenol-chloroform extraction of DNA extraction was done according to (Sambrook *et al*., 2012) as following steps:

1. The hair samples from hair root were cut into1 cm length, then carefully transferred into a new 1.5 ml tube.

2. The hair sample were Crashed 10 ~ 20 times using micropestle with 200µl **hair lysis buffer (**NaoH 0.2 M, SDS 2%, beta-mercaptoethanol (β-ME 2% and EDTA 0.01 M).

5. A 1 ml of phenol: chloroform (1:1). Mix gently for 2-3 minutes and centrifugation at 10000 rpm at 4°C for 15 minutes.

6. The upper layer was transferred into new tubes and equal volume isopropanol was added to all tubes.

7. The tube was placed in centrifuge to precipitated the hair DNA at 10000 rpm on 4°C for 15 minutes.

7. The supernatant was discarded 1ml 80% ethanol was added to wash the hair DNA.

volume of 95% of ethanol and leave it till the precipitation is settle down, minimum for 3 hrs to overnight.

8. Then centrifuged at 10000 rpm on 4°C for 5 minutes to decant the supernatants and dry the pellet.

9. A 50 µl TE buffer was added tore-suspension of DNA and incubated the DNA at 70°C in a water bath for 10 min for the inactivation of DNA degrading enzymes.

3-2-5- Genomic DNA estimation

The extracted hair DNA was checked by using Nanodrop spectrophotometer (THERMO. USA), that check and measurement the purity of DNA through reading the absorbance in at (260 /280 nm) as following steps:

1. After opening up the Nanodrop software, chosen the appropriate application (Nucleic acid, DNA).
2. A dry wipe was taken and cleaned the measurement pedestals several times. Then carefully pipette 2µl of free nuclease water onto the surface of the lower measurement pedestals for blank the system.
3. The sampling arm was lowered and clicking OK to initialized the Nanodrop, then cleaning off the pedestals and 1µl of DNA was added to measurement.

3-2-6- Agarose gel electrophoresis

The extracted DNA from two method was analyzed by agarose gel electrophoresis following steps:

- Agarose gel (0.8%) was prepared in using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C.
- Then 3µl of ethidium bromide stain were added into agarose gel solution.
- Agarose gel solution was poured in tray after fixed the comb in proper position after that, left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray.
- The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer.

- A10µl of extracted DNA was mixed with loading dye (1µL loading dye: 4µL DNA samples)were added in to comb wells. Then electric current was performed at 100 volt and 80 AM for 1hour.
- DNA products were visualized by using UV Transilluminator.

Figure (3-1): The type of hair samples that used in this study. The Normal hair and staining hair samples.

CHAPTER FOUR

RESULTS

AND

DISCUSSION

4-1 Nanodrop spectrophotometer results:

The DNA concentrations and purity that extracted from normal hair and staining hair samples by spin column and phenol chloroform extraction method were checked by Nanodrop spectrophotometer (Thermo Scientific. USA) check the DNA concentration at (ng/µl) and measured the DNA purity by reading the absorbance in at (260 /280 nm) and the results were showed as following tables and figures:

Table (4-1): represent the DNA concentration and DNA purity by Nanodrop by Spin column DNA extraction method:

Type of DNA samples	DNA concentration ng/µl	DNA purity 260/280nm
N1	**0.7**	**0.36**
N2	**1.2**	**0.52**
S1	**7.1**	**1.01**
S2	**162**	**1.18**

N1 and N2: Normal hair samples, S1 and S2: Staining hair samples

Table (4-2): represent the DNA concentration and DNA purity by Nanodrop by phenol chloroform DNA extraction method:

Type of DNA samples	DNA concentration ng/ul	DNA purity 260/280nm
N1	**14.9**	**1.37**
N2	**23.7**	**1.38**
S1	**338.2**	**1.22**
S2	**194.5**	**1.22**

N1 and N2: Normal hair samples, S1 and S2: Staining hair samples

4-2- Agarose gel electrophoresis results:

The integrity of genomic DNA from normal hair and staining hair samples that extracted by two methods (Spin column and phenol chloroform extraction method) was checked by 0.8% agarose gel electrophoresis method and the results were showed as following figure:

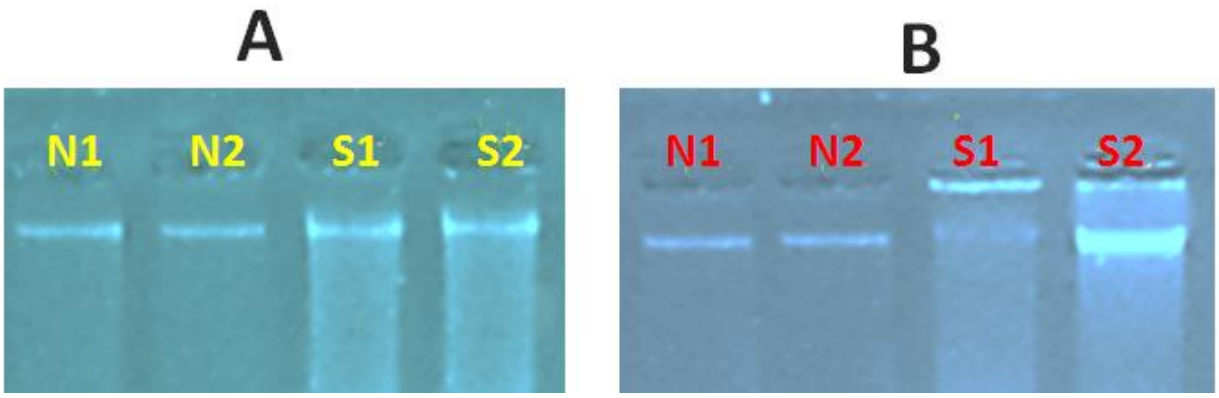

Figure (4-1): Agarose gel electrophoresis image that showed the Genomic DNA extraction from normal hair and staining hair samples.

Where, the figure (A) showed spin column DNA extraction method, lane (N1-N2) represent the normal hair and appeared as excellent extraction and sharp DNA bands with no DNA lysis, whereas, the lane (S1-S2)were represent the staining hair and appeared as high density DNA extraction with high DNA lysis. The figure (B) showed Phenol Chloroform DNA extraction method, lane (N1-N2) represent the normal hair and appeared as excellent low density extraction and sharp DNA bands with no DNA lysis, whereas, the lane (S1-S2)were represent the staining hair and appeared as huge density DNA extraction with high DNA lysis.

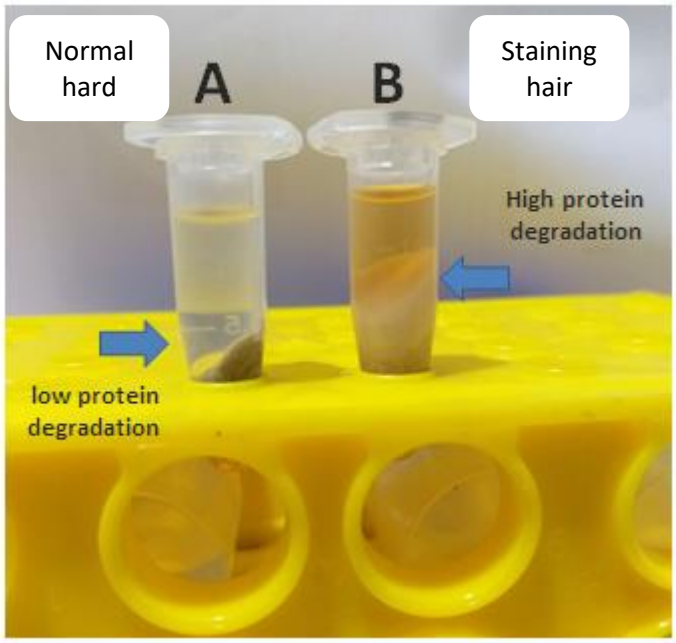

Figure (4-2): The phenol chloroform DNA extraction method showed the DNA separation step with high protein degradation in staining hair samples with low protein degradation in normal hair samples, such that A:Low protein degradation(normal hair); B: High protein degradation(staining hair)

Using phenol-chloroform mtDNA extraction protocol isolated from 50% of hair samples included follicles and this percentage was not far from the results of the previous study [Linda Vigilant.*et al*.2008].

Naturally, the hair was rich in protein (keratin) [Xiong.*et al*.2012], and required additional steps to the shaft (no trace left) and this would give time to release DNA from the keratinized tissue [Barbara.*et al*.2013]. In order to edit DNA from hair tissue, detergents were used [Paula.*et al*.2012]. The amount of DNA extracted from hair shafts was very low with reducing agents (eg, SDS, DTT) and proteinase K in the lysis solution. There were faint and sometimes unclear bands that appeared after DNA extraction using the phenol-chloroform method due to the low concentrations . An adequate and integrity bands pattern appeared on the agarose gel after DNA extraction, and thus these results affected the bands pattern during gel electrophoresis that appear as faint bands,

and both methods revealed the inconsistency in yield and purity between the categories of treated hair samples. Hair (bleached) showed very low concentration results in contrast to untreated and graying hair. It may be because hydrogen peroxide (specific to hair dye factor) attacks the bonds within DNA, as happens in protein disulfide bonds [Daniell.*et al.*2003]. This result not only affected the result of the PCR product by the appearance of the intensity of the bands during gel electrophoresis, but also affected the sequencing results. This result may show that DNA from dyed hair is more degraded so we get lower production of DNA in contrast to untreated hair, these results agree with (Baker *et al*.2004)

The samples, which did not represent a good band density on the agarose gel, reverted to a lower DNA template [Nguyen.*et al*.2012] especially in the hair shaft samples even if more extracted DNA was added in the amplification step. However, the use of hair dyes can also be an influencing factor, it contains polymerase chain reaction inhibitors such as melanin and with the effect of coloring treatments (hydrogen peroxide), converting water-insoluble melanin into water-soluble melanin and this works in the same way as DNA During the purification process, it remains in DNA solution as a PCR inhibitor [Paula.*et al.*2012].

Chapter Five
Conclusion and Recommendation

Conclusion :

Our results were demonstrated that staining hair by chemical dyes had a significant influence on the structure of DNA results. The organic method by Phenol Chloroform DNA extraction method was an appropriate method for extraction DNA from hair shaft, since this method used for extracting the old and degraded samples.

Recommendation:

This study was recommended to performed new other molecular identification study Basel STR gene for treated and normal hair samples

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الخلاصة:

يمكن أن يكون الشعر مصدرًا قيمًا للحمض النووي خاصةً في حالات الطب الشرعي والدراسات غير الباضعة للإنسان ، عندما لا تتوفر عينات الدم. تركز هذه الدراسة على تأثير صبغات الشعر على بنية الحمض النووي ، حيث تم استخدام عينتين من مجموعة الشعر ، إحداهما طبيعية ومجموعة أخرى للشعر المصبوغ. تم استخلاص الحمض النووي باستخدام طريقتين مختلفتين (طريقة استخلاص الفينول الكلوروفورم والعمود الدوراني) ، مقياس الطيف الضوئي النانوي والرحلان الكهربي لهلام Agarose وقد تم استخدامهما في تقدير طريقة استخلاص الحمض النووي. أظهرت نتائج تراكيز DNA Nanodrop ونقاء الحمض النووي بطريقة استخلاص الحمض النووي لعمود Spin في الشعر العادي عند (0.7–1.2 نانوغرام / مايكرولتر) و (0.36–0.52) على التوالي ، بينما أظهرت النتائج في مجموعة صبغ الشعر عند (7.1–162ul / ng) و (1.01–1.18) على التوالي. أظهرت نتائج تراكيز DNA Nanodrop ونقاوة الحمض النووي بطريقة استخلاص الفينول كلوروفورم في الشعر الطبيعي عند (14.9–23.7 نانوغرام / مايكرولتر) و (1.37–1.38) على التوالي ، بينما أظهرت النتائج في مجموعة صبغ الشعر عند (192.4–338.2ul / ng) و (1.22) على التوالي. تم التحقق من سلامة الحمض النووي الجيني من الشعر الطبيعي وعينات الشعر المصبوغة التي تم استخلاصها بطريقتين (طريقة استخلاص الفينول الكلوروفورم والعمود المغزلي) باستخدام طريقة الفصل الكهربائي لجيل Agarose بنسبة 0.8٪ وأظهرت النتائج أن الشعر الطبيعي وظهر على أنه استخلاص ممتاز و أشرطة DNA حادة بدون تحلل الحمض النووي ، بينما ظهر الشعر المصبوغ على شكل استخراج DNA عالي الكثافة مع تحلل عالي للحمض النووي. أظهرت طريقة استخلاص Phenol Chloroform DNA أن الشعر الطبيعي هو استخراج ممتاز منخفض الكثافة وشرائط DNA حادة بدون تحلل الحمض النووي ، بينما ظهر الشعر المصبوغ على شكل استخراج DNA عالي الكثافة مع تحلل DNA عالي. أظهرت نتائجنا أن صبغ الشعر باستخدام الأصباغ الكيميائية كان له تأثير كبير على بنية نتائج الحمض النووي. كانت الطريقة العضوية باستخدام طريقة استخلاص DNA Phenol Chloroform طريقة مناسبة لاستخراج DNA من جذع الشعرة ، حيث استخدمت هذه الطريقة لاستخراج العينات القديمة والمتحللة.

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الكشف عن أفضل طريقة لاستخراج الحمض النووي منقوص الاوكسجين لعينات مختلفة من جذور الشعر

مشروع بحث مقدم الى

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

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