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Extraction And Purfication Of DNA From Blood Samples

A RESEARCH PROJECT

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{ وَأَن لَّيْسَ لِلإِنسَانِ إِلاَّ مَا سَعَى }

صدق الله العلي العظيم

[آية 39 :سورة النجم]

“Dedicated to my beloved

parents & family”

For their love, endless

support, encouragement

and sacrifices

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Summary:

Determination of the type and origin of the body fluids found at a crime scene can give important insights into crime scene reconstruction by supporting a link between sample donors and actual criminal acts.
Blood samples were collected from volunteer , samples were used to feign the different possible blood samples obtained for forensic medical purpose. The following feign were created ten fresh blood samples , six dried blood stain on the carpet , five blood stain on top soil.
Isolating DNA was attempted from favorgen kit. After extraction DNA ,electrophoreses gel has been done ,then measuring purification and concentration of DNA by Nano drop . higher purity and concentration was extracted from fresh blood .

INTRODUCTION

History of Forensic DNA

Forensic science aims to help judges and juries in solving legal issues, not only in criminal but also in civil cases, the main aspects of forensic science are the identification, individualization and classification of physical evidence found at a crime scene, the ultimate goal of forensic science, in the identification process, is individualization which gives information on the origin of a particular piece of evidence, for many years only few types of evidence, such as fingerprints, could be unequivocally individualized, all other types of evidence could only be said to be consistent with originating from a particular location or individual. However, in 1980s, one particular biological tool revolutionized forensic investigation - DNA analysis . [1]

Forensic DNA analysis has greatly evolved since it was first used over 30 years ago and has become an indispensable and routine part of forensic casework. Employing extremely sensitive PCR-based techniques to analyze biological material has enabled forensic scientists to link suspects to crime scenes or one crime scene to another using biological evidence left behind in form of saliva, hair, skin cells or various body fluids .

The evolution of forensic genetics has been driven by the analysis of human genetic variation, beginning more than a century ago with Karl Landsteiner's discovery of the human ABO blood group polymorphisms and his early realization that this variation was applicable to solving crime DNA is the genetic code of most organisms. The DNA of humans and many other organisms such as cats, dogs, sheep, cattle, tigers, horses, plants, and bacteria, has been used in forensic work. Most human DNA is present in the nucleus of the cell. It is packaged in the 46 chromosomes of most cells. This DNA is termed nuclear DNA. However, a small portion of the DNA complement of

each cell is housed in the mitochondria. This mitochondrial DNA is inherited by a different mechanism and is treated differently in the forensic context [2]

The DNA revolution began in 1984 with the discovery by the 34- year old Professor Sir Alec Jeffreys from Leicester University, U.K. of hypervariable loci known as minisatillites. These were detected by hybridization of probes to Southern blots of restriction-enzyme-digested genomic DNA. Shared „core sequences" between different minisatellite loci allowed probes to detect many independent minisatellites simultaneously, yielding the hypervariable multi-band patterns known as DNA fingerprints [3]

Modern forensic DNA begins with the first DNA case that was processed also by Alec Jeffreys from Leicester University, this case involved the murders of two 15-year-old girls, Lynda Mann and Dawn Ashworth, that was similar stories found naked from the waist down (Seton,1988).

DNA EXTRACTION

DNA extraction is an important and the first step in DNA analysis for biochemical and forensic purpose the extracted DNA from biological sample must be free from contaminated such as protein and RNA (wang et al., 2003) Although DNA may have to be extracted from different sources for forensic purpose such as sliva, semen, stain, hair, blood sample are the most common objects for forensic analysis. The method for genomic DNA extraction from whole blood usually involve disruption and lysis of blood sample by deter gent that leads to solubilization of membrane lipids and proteolytic removal of protein. A final step involve precipitation of the DNA with ethanol or isopropanol or its adsorption to a filter or matrix [4]

Blood sample are remarkably valuable to research; forensic genetic laboratories

have to take genetic profile from blood that can deposited on a wide range of supports Likewise, genetic, hematology, and biochemistry studies employed in human clinic, demand blood sample in order to recognize and prevent human

serious disorders (Phillips et al,2000; wang et al 2003) -Pure genomic DNA extraction from biological sample is the vital primary step to succeed in various molecular biological techniques such as (PCR)polymerase chain reaction, restriction enzyme analysis, mutation detection genotyping as well as linkage analysis (Phillips et al,2000; wang et al 2003) -Moreover; DNA extraction from blood sample is the most important requirement for the determination of genetic abnormalities; epigenetic studies and various diagnostic and preventive tests (Angelini et al, 2000; Lewis et al, 2005) -DNA extraction demand a rapid and economical procedure with minimal co-extraction of inhibitors of downstream process Furthermore; it needs to be flexible enough to be apply to frozen and clotted sample. In recent years some innovative method has been introduced by using chematography clumns or absorbing DNA on silica and coated magnetic bead matrix which are easy to perform, however they are not economized when a high through put amount of extracted DNA is required more over; the physical

separation of DNA from these matrixes mast be done to eliminate the inhibitory

effect of matrix in downstream processes. Such as PCR Blood sample were freshly collected from healthy volunteers and handled in different ways that mimicked, the range of conditions for instance; the fresh and frozen sample were treated by EDTA .[5]

-DNA quality is a critical issue for the most amplification based analysis. since the DNA amplification is influenced by the presence of CO-Purified inhibitors from matrix or extraction reagent, which can reduce sub sequent polymerase chain reaction (PCR) efficiency.

-DNA damage may also occur during the procedure of extraction DNA extraction is the removal of the deoxy ribonucleic Acid (DNA) from the cell or viruses in which its normally resides.

-the extraction of DNA is often an early step in many diagnostic process that used to detect bacteria and viruses in many environments as well as diagnosing disease and genetic disorders. DNA isolation is a process of purification of DNA from sample by using a combination of physical and chemical the first isolation of DNA was done in1869 by Fredrich Miescher [1] currently it's a routine procedure in molecular biology or forensic analysis. for the chemical method, there are many different kit used for extraction and selecting the correct one will save time on kit optimization and extraction procedure 1(Dahm R) (January 2008) -DNA extracted is a routine procedure that used to isolated DNA from the nucleus of cell. The extracted DNA can be used for mole qular analysis including PCR, electrophoresis, sequencing, finger printing and also cloning .

There are many methods of extraction include organic extraction, solid -phase extraction and ion- exchange fast technology for analysis of nucleic Acid(FTA) that can be used in DNA extraction especially when the sample is blood or sliva [6]

-DNA extraction involves many steps which they are

1. Breaking cell to release the DNA.
2. Separating DNA from protein and other cellular debris
3. precipitating DNA with Alcohol
4. Cleaning the DNA
5. confirming the presence and quality of the DNA

-DNA extraction and purification from human blood sample occurs by using different method.

while people committing crimes and others trying to salve them have always existed, the history of forensic genetics is quite short. DNA typing was first introduced in forensic analysis in the middle of the 1980s but the DNA molecules was discovered long before that...

Modern forensic DNA being with the first DNA case that was processed also by Alec Jeffreys from Leicester university. this case involved the murders of two 15 years -old girls, Lynda man and dawn Ashworth. That was similar stories found naked the waist down.

Blood as source of DNA

Blood is an excellent source for human DNA.

DNA is present in white blood cell of human, but not in red blood approximately some in volume is enough DNA for atypical VNTR analysis -DNA from sperm head is usually the most important source of DNA evidence for sexucel assault cases We need a special extraction method that required to release DNA form sperm head. -Blood is the most common body fluid encountered at crime scenes .it is a very complicated liquid tissue and serves as the transporting medium for all the substances in the body [7], If blood stain collected from crime scene, there are several presumptive tests that used to identify blood as well as confirmatory tests. -There are many pros and cons for blood sample -fast and quick procedure -Blood sample are visible-needles puncture to skin so patient will feel some degree of pain. Blood sample are fast to collect though not quick as swabs sample. Blood is the main source of DNA for genotype related studies in human. A rapid, efficient and cost- effective method for the isolation of genomic DNA from whole blood is needed for screening a large number sample Blood is the most common body fluid that encountered at crime scenes, it's a very complicated liquid tissue and serves as the transporting medium for all the substances in the body. if a blood sample and slain is collected from crime scene, there are several presumptive tests to identify blood as well as confirmatory tests. there are many published protocols (1,3-7) these protocols used toxic organic.[8]

chemical (SDS- proteinase -k phenol isolation method or its modification).

Blood is often related to wards including hemo or hemato from the Greek stem

“haima” Blood is a circulating tissue composed of fluid plasma (wafer, minerals, hormones, and others) and cells (Red blood cell. White blood cells and platelets)

Structure and Function of DNA

Deoxyribonucleic acid (DNA) is a biopolymer composed of two antiparallel strands of deoxyribose molecules connected by phosphate groups, referred to as the sugar phosphate backbone. Connecting each strand to one another are complementary, nitrogenous bases called nucleosides, which interact with one another via hydrogen bonding from opposing anti-parallel strands. The nitrogenous bases that make up DNA include two purines, adenine (A) and guanine (G), which interact via hydrogen bonding with two pyrimidines, thymine (T) and cytosine (C), respectively . In mammalian cells, DNA is found in the nucleus of a cell. It is supercoiled into linear structures called chromosomes .[9]

In addition to nuclear DNA, animal cells contain mitochondrial DNA (mtDNA), present in the mitochondria of a cell .

Materials and Methods:

Materials:

Generalized Chemicals

Table (3-1) Generalized Chemicals

Item	Manufacturer
Agarose	Pronadisa
Methanol	EDUCEC/ India
Ethedium Bromide Biotech/UK Ladder 100bp	iNTRON
Na2EDTA	Scharlau Chemise S.A/European Union
Sodium Chloride NaCl	BDH/England
Sodium Dodecyl Slphate SDS	Bio Basic Canada
Tris-Hcl	Bio Basic/Canada
X6 Loading Dye	Thermo Scientific™ /Eurex
Proteinase K	BG317165

Table (3-2) Devices and Equipment

Devices	Manufacturer
Balance	Kern/ Germany
Centrifuge	USA
Disposable syringes (5ml) Medico	United Arab Emirates
Disposable tips	Gilson/France
Electrophoresis power Supply	KOREA

Flask (250) ml	OXFORD
Gel documentation Vision	JAPAN
Gel Electrophoresis	KOREA
Graduated Glass Cylinder (250), (50)	Superior(Germany)
Hot plat	
Water bath	

Gel Electrophoresis

Gel Electrophoresis according .

Agarose 0.5%

(0.5) of agarose was dissolved in 100ml 0.5x TBE buffer by heating until the solution became clear. After the solution cool, add 0.5 gl of ethidium bromide and mix well.

Photo of result of Electrophoresis

By using gel documentation system Electrophoresis result was identified. according to ladder base pairs of DNA bands were measured, if the DNA band was equal to target product size the result that mean positive result. lastly, the gel was photographed using gel documentation saving picture.[10]

Collection of Human Blood:

(30) thirty sample of blood was collected on 21/2/2018. Three milliliters of blood are collected by vein puncture in EDTA tubes from all persons and are transmitted direct to lab, for DNA extraction and molecular analysis.

30 eyes were divided into three groups. The first groups were extracted DNA from fresh blood, the second groups were extracted DNA from dried blood stains on carpet, and last groups Extract DNA from blood stains from soil.[11]

Method of Extraction DNA

Genomic DNA of (fresh, frozen and dried blood stains on carpet and soil) whole blood collected Genomic DNA of (fresh, frozen and dried blood stains on carpet and soil) whole blood collected in EDTA tube, was isolated according to (name of kit):

1- 200gl sample was transfer up to a microcentrifuge tube.

2- Proteinase K 20 gl and FABG 200 gL buffer was added to the sample
3- then incubate at 60 °C for 15 minutes to lyse the sample during incubation, vortex the sample every 3-5 minutes.
4- Brifly the tube was spin to remove the drops from inside of the lid.
5- 200 gl ethanol (96-100) was added to the sample. vortex the sample for 10 secs, then spin the tube to remove drops from inside the lid
6- A FABG mini Column was placed of collection tube then transfer the mixer to FABG mini Column. Then centrifuge at 6000xg for one min
7- 400 gl W1buffer was added to FABG mini Column and centrifuge at 18000xg foe 30 sec then discard the flow-through.
8- 750 gl wash buffer was added to FABG mini Column and centrifuge at full speed for 30 secs then discard the flow-through.
9- then centrifuge at full speed for 3 minutes to dry the column.
10- FABG mini Column was placed to Elution tube.
11- 50-200 gl heated Elution buffer was added to the membrane center of FABG mini Column stand FABG mini Column for 3 min.
12- 1 minutes centrifuge to elute total DNA 13- Store the DNA at 4°C or -20°C.

RESULTS

Result:

The result show concentration of DNA in table (1) for fresh blood was between (25530-4420 ng/p l) and purity was between (1.65-1.84). While concentration of DNA of top soil stain in the table (2) was between (396.30-436.40 ng/ p l) and purity was between (1.28-1.69). The DNA concentration was between (255.30-341ng/ p l) and purity was between (1.51-1.94) from bloodstain on carpet.

Figure (2) Blood From Soil
Figure (3) Blood From Carpet

Table (1) concentration and purification of extract DNA from human fresh blood by using favorgen kit.

NODNA	con.	purity(ng/µl)
1	300.70	1.65
2	255.30	1.73
3	320.30	1.84
4	286.50	1.84
5	291.00	1.81
6	310.00	1.76
7	352.21	1.81
8	365.23	1.67
9	442.0	1.67
10	352.21	1.67

Table (2) concentration and purification of extract DNA from human dried bloodstain on top soil after half hours by using favorgen kit

NODNA con.	purity(ng/µl)	
1	407.70	1.52
2	396.30	1.69
3	406.10	1.43
4	426.50	1.37
5	436.40	1.28

Table (3) concentration and purification of extract DNA from human dried blood stain on carpet after half hours by using favorgen kit

NODNA con.	purity(ng/µl)	
1	326.70	1.70
2	255.30	1.51
3		
4		
5	291.50	1.53
6	341	1.94

Discussion:

Blood samples are remarkably valuable to the research forensic genetic laboratories have to take genetic profiles from blood that can he deposited on a wide range of supports.

The details that is show by these results are the samples contamination with top soil component. point out that soil texture and structure are mainly determined by sand. silt. mud and organic matter content through the organization of micro- and macro-aggregates.[12]

"who referred that the yield of DNA per gram of soil depends on the method used and on the properties of the soil", The soil components interact with DNA extraction and affected DNA yield. mentioned that environmental samples such as soil and sediments often contain high levels of organic matter. especially hemic acids and phenolic compounds.

mentain that " ln order to obtain a good yield and purity of DNA from soil. collecting of samples must be clean much it could. this agreed with who mentioned that extraction of DNA from soils requires no culturing or growth of the organisms" [13]

The Nano drop analysis for A260/280 resulted in an average of about 1.8 that indicates the extracted DNA was free from protein contamination.

Conclusion

In conclusion, this study improves dependable approach for DNA extraction not only from fresh blood samples but also from dried stain carpet and soil.

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