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Comparative Study of Genomic DNA Extraction Protocols from Whole Blood for *P53* Gene Polymorphism in Persons with and without Prostate Cancer

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Abstract

In latest decades, genetic methods have developed into a potent tool in a number of life-attaching applications. In research looking at demographic genetic diversity, QTL detection, marker-assisted selection, and food traceability, DNA-based technologies like PCR are being employed more and more. These approaches call for extraction procedures that provide efficient nucleic acid extraction and the elimination of PCR inhibitors. The first and most important stage in molecular biology is the extraction of DNA from cells. For a molecular scientist, the high quality and integrity of the isolated DNA as well as the extraction method's ease of use and affordability are crucial factors. The present study was designed to establish a simple, fast and inexpensive method for DNA extraction from human peripheral blood (normal male n=2, age 24 years old, patient male (prostate cancer) n=2, age 65 years old) by comparing between them, and aimed to standardize a protocol of DNA extraction using five extraction protocols. The first method was the modified organic method by using sodium perchlorate instead of organic solvent (phenol, chloroform), sodium perchlorate advantage comes from its cheap price and low storage and shipping requirements, the second was the enzymatic method by using proteinase K, third method was done by using detergent, the fourth used phenol-chloroform; finally fifth one was salting out method. The result showed that the organic method gives a good DNA yield and needs relatively short time while the enzymatic method gives an excellent DNA purity which are more suitable for PCR by comparing five protocols using the spectrophotometer and Nanodrop technetium in addition to electrophoresis. Through the use of the five suggested procedures, the PCR multiplication of the *P53* gene with the isolated DNA was effectively carried out. This indicates that, with the exception of the detergent approach, there were no significant inhibiting substances for Taq polymerase in the final solution.

Keywords: DNA extraction, DNA purification, electrophoresis, enzymatic method, P53, PCa, PCR.

Introduction

The physical, chemical, and physiological makeup of the prostate varies greatly between species. The prostate's job is to emit a milky or white fluid that is slightly alkaline and accounts for around 30% of the amount of semen in humans, together with sperm cells and seminal vesicle fluid [1]. The releases generated by the other participating glands, at particular the seminal vesicle fluid, helps to make

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semen which is generally alkaline [2]. The sperm's longevity is increased by the alkalinity of semen, which serves to balance the vaginal tract's acidity. The majority of the spermatozoa as well as the prostatic fluid are discharged in the initial ejaculate fractions. The few spermatozoa released in prostate fluid have greater motility, longer lifespan, and better genetic material preservation than the few spermatozoa discharged in conjunction with mostly seminal vesicular fluid [3].

Older men can develop benign prostatic hyperplasia (BPH). Urination frequently gets challenging when the prostate enlarges to this extent. Frequent urination (frequency) or difficulty starting a urination are two signs (hesitancy). Urination may become uncomfortable, difficult, or even impossible if the prostate becomes too big because it may restrict the urethra and obstruct the passage of urine [4]. Meanwhile, prostate cancer (PC), a type of cancer that develops in the prostate gland in the male reproductive system, is one of the most prevalent cancers influencing older men in developed world and a major induce of mortality for elderly men. While most cases of PC are slow growing, there are some cases that are more gnarly and dangerous [5].

Cancer of prostate can spread to the body, especially the bones, contract lymph, causing pain, difficulty in urination, problems during sexual contact or twice the erection, other symptoms can evolve through advanced phase of disease [6]. The consequences and potential advantages of testing and treatment, which should be reviewed with a doctor at age 50, or at age 45 if the patient is black or has a parent or brother who developed PC before age 65, should be examined as possible candidate for developing PC [7].

Surgery is generally the primary treatment option for PC, although recovery is not always optimum [8]. The possibility of the tumor protein p53 in the treatment of tumors has emerged as a fresh viewpoint on malignancy studies thanks to gene-targeted therapies [9, 10]. It is generally known that p53 is a suppressor of tumors and that 50% of cancerous tissues have p53 mutations [11].

Processing the many samples necessary for linkage studies calls for a quick, consistent procedure that yields a fair production of large molecular mass DNA from clinical specimens [12]. For a molecular scientist, the excellent integrity and purity of the isolated DNA as well as the extraction technique's ease of use and affordability are crucial factors. Suitable human genomic DNA extraction techniques stay away from intense shaking and high degrees of centrifugation [13]. DNA may be extracted using a variety of techniques, such as boiling [14], salting out [15], phenol-chloroform [16], and isopropanol precipitation procedures [17].

The researcher must devise defenses against cell-derived DNAase enzymes assault on the DNA. Thus, buffers containing nuclease activity inhibitors are used in the first phase of the DNA extraction procedure [18]. Finally, it is necessary to separate the liberated DNA from other biological molecules and substances including proteins, lipids, RNA, carbohydrates, amino acids, and fatty acids. The quality of the DNA is crucial since even little impurities can prevent other studies from working, including polymerase chain reaction (PCR) [19].

For many molecular biological procedures to be successful, there must be an enough supply of good quality genomic DNA. Therefore, molecular genetic research has always been interested in the isolation of high purity DNA with the least amount of time and money. Many DNA extraction techniques have been developed to meet these requirements. These techniques, which differ in various ways, all consist of three primary steps; cell lyses, selective genomic DNA release from cellular matrix, and contamination removal to extract genomic DNA [20]. The available procedures vary greatly in terms of the initial blood volume, isolation duration, necessary reagents, and, most importantly, the accuracy of the approach with regard to the quantity and quality of isolated DNA [21].

For DNA analysis, the white blood cells (WBC) of blood samples are often the most practical source of human genomic DNA. Good amount (~250 µg) of DNA may be extracted from 10 ml of whole blood, which is more than enough for a thorough investigation of any gene [22].

Among the issues with traditional methodologies of isolating DNA include the need for the deproteinizing cell digest with risky organic solvents

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like phenol, chloroform, and isoamyl alcohol and the lengthy incubation time for deproteinization. This technique reduces incubation time, which consequently reduces protocol duration time [23]. The procedure outlined in this article does not involve the consumption of any toxic organic solvents. This is accomplished by dehydrating the cells and precipitating the proteins using a concentrated salt solution.

Materials and Methods

Patients and Control

Human peripheral blood obtained from four volunteers at Al Yarmouk hospital, Baghdad province (n=2 normal, n=2 patients with PC). Blood samples 5 ml were processed fresh and were kept in an EDTA tube served as the subjects' DNA isolation reference.

DNA extraction processes were performed in five protocols, and repeated three times for each method to exclude the mistake of handling samples:

Methods

Protocol 1: Perchlorate extraction method

The extraction method was done according to Roulston and Barlett [24], the frozen blood samples were dissolved by turning over several times, an amount of 2 ml of the sample was withdrawn, and added into a sterile glass tube and then 6 ml of lysis solution (1%Triton X-100, 5 mM $MgCl_2$, 0.32M sucrose, 0.01 M Tris-HCL, pH 7.4) was added, the mixture was incubated in the refrigerator for 15 minutes with mixing and rolling every 5 minutes, the mixture was centrifuged for 20 minutes at a rotational speed of 4000 rpm, supernatant was discarded and sediment was dried by turn the tube on a dry paper. Then other 3 ml of lysis solution was added to red blood cells and the mixture again was centrifuged for 20 minutes at a rotational speed of 4000 rpm, supernatant was discarded and sediment was dried by turn the tube on a dry filter paper, 5 ml of cell lysis solution (1% SDS, pH 8.0, 0.06 M EDTA, 150 mM NaCl, 0.4 M Tris-HCl) was added and mixed in the tube several times. Then 1 ml of 5M $NaClO_4$ was added with mixing. The tube was incubated in a water bath for 60 minutes at 65 ˚C with turning the tube from time to time, the samples were raised and left for 5 minutes to cool at room temperature, an amount of 4 ml of 6M NaCl was added and mixed for two-minutes, the samples were centrifuged for 20 minutes at 4000 rpm. The top supernatant was transferred to the sterilized glass tube using a sterile pipette and then re-centrifuged and transferred again to a new sterile tube, for purification of DNA and deposited (0.5 ml of 7.5M Ammonium acetate and 3 ml of absolute ethanol) was added and the tube was left at 0 ˚C for 10 minutes and mixed cautiously several times till the formation of white cotton mass. A sterile glass hook was used to stir the precipitate by the end of the hook, and then washed using 2 ml of 70% cooled ethanol, the tube was centrifuged for 10 minutes 4000 rpm, the precipitate was isolated and left to dry on a filter paper, the precipitate of DNA was dissolved in an appropriate size of an TE buffer (10 Mm Tris pH 7.8 and 1 mM EDTA) then transferred and stored in the Eppendorf tube.

Protocol 2: Enzymatic Extraction Method

DNA was extracted by adding 6 ml of lysis solution (155mM NH_4Cl ,10mM $KHCO_3$, 1mM Na_2EDTA) to 2 ml of whole blood in the glass tube 10 ml, sample was placed on the shaking device for 5 minutes and then placed 15-30 minutes at a temperature 0˚ C (freezer), then centrifuged at 4000 rpm for 20 minutes, supernatant was discarded and sediment was dried by turn the tube on a dry filter paper, 4 ml of lysis solution (155mM NH_4CL ,10mM $KHCO_3$, 1mM Na_2EDTA) was added. For washing and disposal of the remnant's hemoglobin, samples were incubated 15-30 minutes at 0 ˚C and again were centrifuged at 4000 rpm for 20 minutes, the sediment was mixed by vortex for 40 seconds to break down the cell contents, 5 ml of cell analysis solution (4mM EDTA, 100 mM NaCl, 20mM Tris-HCl pH 8.0) were added by a pipette followed by adding 500 µl of 10% SDS. Sample was mixed for 2 minutes to form a foam dense, 50 µl of proteinase K (20mg/ml) was added also, then sample was incubated in a water bath for 90 minutes at 65 ˚C with agitated stirring from time to time every 10 minutes. The samples were placed on ice container for 3 minutes until reaching to room temperature, for precipitate the

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protein content; 4 ml of deposition protein solution (5.3 M NaCl) were added and mixed for a period of one minutes. The sample was centrifuged at 4000 rpm for 20 minutes and was transferred into a new clean tube, then a similar size of isopropanol was added to the size of sample, shaken tightly by hand up to 50 times, white strings appeared then pulled of DNA which was taken by a glass hooked rod end and put in a Eppendorf tube container, then 2 ml of washing solution (70% ethanol) were added and left for 10 minutes after which the sample was centrifuged at 3000 rpm for 5 minutes (to precipitate the DNA). The DNA was left to dry and turned the tube on a filter paper towel for 10 minutes, 2 ml of 70% ethanol solution were added with stirred the tube by hand , and then the precipitate (DNA) was dissolved in the appropriate size of an TE buffer 10 Mm Tris (pH 7.8) and 1 mM EDTA then transferred and stored in the Eppendorf tube [25].

Protocol 3: Detergent Extraction Method

Arial (washing powder) was purchased from a local store; the other chemical compounds were purchased from Promega in the United States. For the best DNA extraction level, one fraction of washing powder (Arial, powder comprises a blend of cleaning agents, enzymes, and chelating complexes) (30% w/v) was used. The next stages were included in the DNA extraction technique [26]:

Buffer for lysis is Sucrose (0.3 M), 0.01 M pH 7.5 TrisHCl, 5 mM magnesium chloride hexahydrate, 1% to get a final volume of 45 ml, 5ml of EDTA-anticoagulated blood were added to a 50 ml centrifuge tube with Triton X100. The tubes were subsequently centrifuged for 5 minutes at 2700 xg. The supernatant was removed and the debris was treated with 1 ml of 10 mM Tris pH 8. By spinning the tube, the material was dislodged from the bottom and swiftly transferred into a 2ml tube. After strong mixing, the material was reconstituted and centrifuged for 1 minute at 675 xg. The supernatant was removed, and the WBCs were reconstituted in 1340 µl of 10 mM TrisCl pH 8 solution. WBCs were separated into two 660 µl (2 ml) tubes. Each tube received 660 µl of washing powder (Ariel D3 active) at one of the optimum levels (30% w/v; which was prepared previously) and a watch glass bead (2 mm diameter). Bead-filled tubes were forcefully agitated to homogenize the mixture (1 min). 500 µl of 5M NaCl were incorporated; tubes were violently shaken for 10 seconds before being centrifuged for 5 minutes at 14000 xg. The supernatant was put into two fresh (2ml) tubes and centrifuged at 14000 xg for 3 minutes. The supernatants were mixed into a single tube (10ml), and DNA was isolated with 3 ml of 96% ethanol. The DNA precipitate was collected with a glass pipette with a heat-sealed narrow end and rinsed in two (1.5ml) tubes with 0.5 ml of 70% ethanol. DNA was treated with 0.5 ml of 10 mM TrisCl pH 8 after the evacuation of ethanol by pressing into the sides of the second test tube. DNA was incubated at 70 °C for 5 minutes. Since DNA was still in large amounts, it was homogenized by up-and-down pipetting with a 1 ml pipette that had a filter tip. DNA was kept at -20 °C for maximum durability.

Protocol 4: Organic Extraction Method

Each sample was taken separately and added in 4 ml extraction buffer and was kept ready in 15 ml sterilized tubes. The extraction buffer was composed of 100 mM Tris-Cl buffer containing 0.15 M Nacl, 0.1M EDTA, 2.5% SDS, with pH adjusted to 7.8. These tubes were incubated at 65 °C for 90 minutes by the addition of 20 µl of proteinase K (20mg/ml). After incubation, the samples were centrifuged at 12000 rpm for 12 minutes at 4°C and collected supernatant in 2ml microfuge tubes. In each tube, 500µl of membrane lysis buffer containing 1 M Tris, 1 M KCl, 1 M $MgCl_2$, 5 M NaCl, 20% SDS, 0.5 M EDTA, were added, mixed on vortex, centrifuged at 12000 rpm for 12 minutes at 4 °C. Since there was no pellet, therefore, an equal volume of phenol, chloroform, and isoamylalcohol (25:24:1) solution were added to the microfuge tubes and mixed the contents on vortex. Tubes were centrifuged at 12000 rpm for 12 minutes at 4 °C to obtain clear aqueous phase. The aqueous phase was carefully transferred to fresh micro centrifuge tubes and added double volume of chilled absolute alcohol. The tubes were swirled gently to precipitate DNA. For further precipitation, the tubes were incubated at -20°C for overnight. After incubation overnight, the contents of the tubes were allowed to stand at room temperature, then centrifuged for 12 minutes at 14000 rpm, by discarding the supernatant, the precipitate was washed with 70 % ethanol twice, then

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by using air drying the DNA pellet was dried, and then suspended in 200 µl TE buffer and store at -20 °C for future use [27].

Protocol 5: Salting out Extraction Method

In 2.5 ml EDTA tubes, vein blood was collected from mixed-age individuals with and without PC. After red blood cells were lysed in an equivalent volume of sterilized ACT solution (7.47g ammonium chloride + 2.06g of tris, applied D.D.W to 1000 ml; pH=7.2), WBCs were extracted from whole blood.

Erythrocyte lyses revealed a shift in the solution's color from blood crimson to a deep clear red after a gentle shaking. WBCs were collected 5 minutes following addition of the ACT solution by rotating for 5 minutes at a speed of 4000 rpm. WBCs that had been pelleted were resuspended by adding 5 ml of PBS solution (8 g of NaCl + 0.2 g of KCl + 1.44 g of Na_2HPO_4 + 0.24 g of KH_2PO_4 added D.D.W to 1000 ml; pH + 7.4) and spinning two to three times at a speed of 4000 for an extra 5 minutes. Resultant liquid was then disposed.

The WBCs solution was given a lyses buffer 5 ml, comprising (100 mM tris HCl, pH=8.5 + 0.5 M of EDTA + 10% SDS + 5 M of NaCl) (2ml). 200 µg of proteinase K are added to the mixture. For digestion, the tubes were placed at 50 °C in a shaking incubator for an entire night. A few hours can be saved by increasing the proteinase K concentration in the mixture.

Following digestion, one liter of ethanol -20 ºC was provided to the lysate, and the specimens were combined or swirled for 20 to 30 minutes, or until the precipitate was fully clear. By removing the accumulated precipitate from the mixture with a disposable tip, the DNA is retrieved. In accordance with the size of the precipitate comprising 500 ml of TE (10 mM of tris HCl + 0.1 mM of EDTA, pH=7.5), supernatant liquid was tapped off and the DNA was spread in a pre-labeled vial.

After washing the DNA in TE solution and adding 500 l of TE, the DNA must be stirred vigorously at 37 °C for 20 hours, ideally overnight. To verify reproducible aliquot discharge for analysis, it is crucial that the DNA is dissolved completely [28].

Determination of DNA Concentration and Purity

The level and purity of the isolated DNA were determined by measuring the UV absorbance of DNA specimens at wavelengths (260 and 280 nm), as follows; a 260 nm, the spectrophotometer's wavelength was set. Using TE buffer (pH 7.6) in a quartz cell with a 2 cm path length, the spectrophotometer was calibrated to zero. TE buffer was used to dilute the sample before the optical density (OD) at 260 nm was measured. The spectrophotometer was rebalanced to zero and adjusted to a wavelength of 280 nm. The DNA level was determined using the following formula after the OD was determined at 280 nm:

$$DNA\ (\mu g/ml) = OD\ \ at\ 260\ nm \times 50\ \mu g/ml \times D$$

A measurement of 1 on the OD scale, where DF is the dilution factor, represents 50 ng/ml of double-stranded DNA. DNA purity is calculated using the OD260/OD280 ratio, which compares measurements at 260 and 280 nm [29]. Additionally, Thermo Scientific's NanoDrop 2000 and an ethidium bromide-stained electrophoresis gel were used to assess the DNA's quality and quantity.

Agarose Gel Electrophoresis and Visualization of DNA Fragments

Each technique's DNA extraction result was fed into a TBE (pH 8.2) glucose gel [30]. The gel was coated with a 3 µl solution of 0.5 mg/ml ethidium bromide. For one hour, the electrophoresis conditions were set up at 80 voltages. The gel was directly shot by a cell phone camera while the bands were visible under a UV transmitter (Gel deco mentation/Applied Bio systems) as shown in Fig.1.

Polymerase Chain Reaction (PCR)

Amplification reactions were performed with the components of the PCR ($MgCl_2$ 4 mM, DATP 400 µM, dGTP 400 µM, dCTP 400 µM, Dttp 400 µM, Taq polymerase 2.25 unit) in a reaction mixture containing (Taq PCR PreMix5µl, Forward primer10 picomols/µ, Reverse primer10 picomols/µ, DNA 1.5µl Distill water 16.5 µl, Final volume25µl). One pair of primer was used (Table 1).

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Table 1. The specific primer of gene p53 used for PCR.

Primer	Sequence	Tm (°C)	Product Size
Forward	5'- AAGACATGCCCTGTGCAGTT -'3	60.18	365 base pair
Reverse	5'- GAGTCTTCCAGCGTGATGAT -'3	57.41	

After conducting numerous experiments, the best conditions for initial denaturation and annealing were discovered. To select the best conditions, the temperature was changed for all samples using gradient PCR, and the DNA template concentration was changed to be between 1.5-2 µl (Table 2, and Table 3). These two factors are thought to be crucial in primer annealing with complement.

Table 2. The Components of the PCR.

Material	Volume
$MgCl_2$	4 mM
DATP	400 µM
dGTP	400 µM
dCTP	400 µM
Dttp	400 µM
Taq polymerase	2.25 unit

Table 3. Combination of the precise gene interactions for identification.

Components	Concentration
Taq PCR PreMix	5µl
Forward primer	10 picomols/µ
Reverse primer	10 picomols/µ
DNA	1.5µl
Distill water	16.5 µl
Final volume	25µl

Results and Discussion

Table 4, displays the concentration and purity of the DNA that was obtained using the five extraction techniques. When compared to other protocols, the spectrometric assay (Cecil, CE10N spectrophotometer, England) showed that protocols 2 and 4 contained DNA with a high quantity and purity. The five protocols yielded purities of nearly (0.97-2.07) in this DNA separation process are signs of effective deproteinization.

Table 4. Readings of OD, purity, and DNA levels after extraction.

Sample No.	Protocol No.	DNA extracted methods	Source	O.D. at 260 nm	O.D. at 280 nm	Ratio260/280 DNA purity	Concentration (µg/ml)
1	1	Organic method	Healthy male	0.066	0.068	0.970	185.13
2			Male with PC	0.336	0.342	0.982	285.6
1	2	Enzymatic method	Healthy male	0.683	0.470	1.453	170.75
2			Male with PC	1.039	0.838	1.239	259.75
1	3	Detergent method	Healthy male	0.387	0.251	1.54	96.75
2			Male with PC	0.737	0.463	1.8	184.25
1	4	organic method	Healthy male	0.636	0.370	2.07	159
2			Male with PC	0.973	0.515	1.88	243.25
1	5	Salting out method	Healthy male	0.867	0.716	1.2	86.7
2			Male with PC	0.173	0.127	1.36	43.25

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Nanodrop reading demonstrated that the high purity and concentration of DNA extracted from whole blood samples was found in Protocol 4 and 3. The five protocols yielded purities of nearly (0.818-2.3) which are indicatives of good deproteinization in this DNA isolation methods, Table 5.

Table 5. Nanodrop measurements, isolated DNA purity and level.

Sample No.	Protocol No.	DNA extracted methods	Source	Ratio260/280 DNA purity	Concentration (µg/ml)	Intensity of DNA band
1[a]	1	Organic method	Healthy male	0.921	122.5	Faint
2[b]			Male with PC	0.818	94.15	Negative
1[c]	2	Enzymatic method	Healthy male	1.026	38.76	strong
2[d]			Male with PC	1.330	24.18	strong
1[e]	3	Detergent method	Healthy male	1.9	116.7	Faint
2[f]			Male with PC	1.8	255.2	Faint
1[g]	4	organic method	Healthy male	2.3	210	strong
2[h]			Male with PC	2.2	266.1	strong
1[i]	5	Salting out method	Healthy male	1.8	29.65	strong
2[j]			Male with PC	1.6	19.56	Faint

From these results, we have noticed differences between nanodrop and spectro-reading. Additionally, DNA extracted from patient sample was varied in purity and yield compare to control subject.

The purpose from the present study is to find a suitable procedure for DNA extraction with low cost, time and hazards as well as high in yield, purity and excitable for PCR amplification. Among the primary strategies for obtaining DNA is from whole blood specimens, and there are a variety of protocols known to extract nucleic acids from such specimens. These techniques range from extremely straightforward manual processes to more complex ones used in automated DNA extraction programs. It would be excellent to identify the solutions that operate best in respect of cost and duration efficiency based on the large variety of possibilities that are accessible. The most popular techniques for obtaining DNA from whole blood specimens, outlining each technique's benefits and drawbacks. The goal of the study was to compare several nucleic acid extraction techniques and identify the top option. The findings suggest that all five protocols might be regarded as ideal methods for DNA extraction. First protocol (organic method) use sodium hypochlorite for salting out process considered simple method (Pfenninger) for DNA extraction and gave a good yield and purity in spite of disappearance of control band in electrophorese which may contribute to mistake in handling of sample. Protocol 2 (enzymatic method) gave a good yield and purity and sharp band as Fig.2 and appear to be suitable for routine PCR. These findings were in agreement with Khosravinia *et al.* [29] and Al-Azawy [31].

Protocol 3 representing salting out by NaCl and replaced proteinase K with one tested concentration of laundry detergent. The purity and yield of DNA extracted by laundry detergent were acceptable but did not show any results by PCR due reason to the possibility of reaction inhibition. On the other hand, Nasiri [32] showed that DNA isolated using detergent was not damaged and did not prevent PCR using primers specific for a certain sequence or stringent digestions.

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Despite the use of phenol, chloroform and amyl alcohol in Protocol 4, results demonstrated high quantity, quality and a good molecular weight of DNA with sharp band in agarose gel. Protocol 4 was common, simple and quick to extract genomic DNA and suitable for PCR but there is some critical about it toxicity. Protocol 5 was found to be easier, less expensive, and more widely applicable than previous techniques. Results revealed good yields and purity as compared to other procedures, and agarose gel electrophoresis revealed high molecular weight DNA. The samples are appropriate for standard PCR as demonstrated by amplifying the extracted DNA. These results are in keeping with a previous study which demonstrated that the salting out approach yields high quality, heavy molecular mass DNA and that it is a straightforward, quick, and inexpensive laboratory procedure [15, 33].

The visual estimation of DNA determined on 0.8% agarose gel Fig.1 was very good in quality for all protocols except sample b which are hidden due to misleading in handling sample or contamination. An essential criteria for determining DNA quantity is the electrophoresis of isolated DNA on gel matrix [34]. Figure 1 illustrates the gel electrophoresis findings from this investigation, which demonstrated that the isolated DNA's quality and quantity were adequate and that there had been no stain on the test lane (no degradation of nucleic acids). In the two isolated samples, the existence of bands with significant molecular mass and intensity suggested good stability and no degradation throughout this extraction procedure (Fig.1). Indeed, the inclusion of Ethylenediaminetetraacetic acid in lysis buffer 2 (which is used to release the genomic DNA of WBCs by lysing the cellular membranes and nuclear membranes) aids in the preservation of DNA stability. This is due to the fact that the majority of enzymes engaged in the digestion of nucleic acids require divalent ion cofactors to increase activity (typically magnesium) [35].

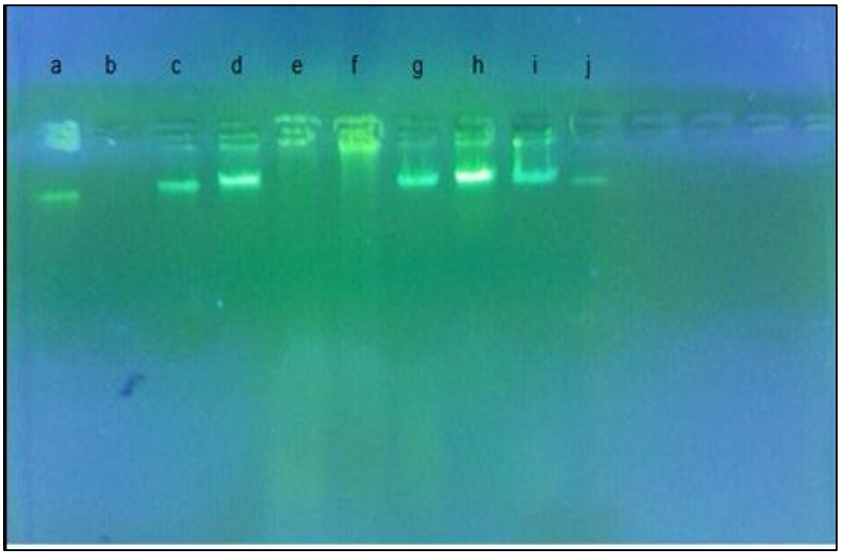

Figure 1. Gel electrophoresis of genomic DNA extraction from whole Blood, 1% agarose gel at 5 vol /cm for 1:15 hours. The ladder is represented from a to j.

The present study found that the use of the organic protocol is absolutely low cost and toxicity but in other hand high purity based on results of the spectrophotometric and nanodrop assay compared with enzymatic method which showed a good DNA purity also. Furthermore, the purity of DNA isolated by these protocols varied from 0.97-2.07, representing good values comparing with the ideal of 1.8 (pure DNA) by using kit method, lesser values reflect contamination by protein and RNA. Similarly, extraction with proteinase K, although yielding a good DNA concentration value, produced a DNA purity below 1.8. Therefore, it is likely that increased exposure time to proteinase K may reduce DNA contamination by proteins and RNA, respectively [36].

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The investigators' handling techniques have an impact on the productivity and accuracy of extracted DNA. When the substance wasn't promptly added to cell lysis buffer for more treatment, a decline in DNA quality and quantity was seen. Also observed that nanodrop findings are more precise than those from spectrophotometers. The quantity and quality of the DNA extracted might be affected by the physical and chemical processes used in the process [37].

The objective of the current investigation was to improve the amount and quality of DNA isolated from blood of healthy and sick people using five different procedures, including lysis buffer, proteinase K, NaClO4, laundry detergent, phenol, chloroform, and isoamyl alcohol. A wide variety of lysis buffer (LB): start blood volume (SBV) ratios were explored. According to Khosravinia et. al. [38], LB:SBV ratio and incubation duration of blood and lysis buffer combination both substantially impacted DNA yield and purity. According to the study's findings, a 6:2 LB:SBV ratio might be used to accept the quality and amount of extracted DNA. It was discovered in this study that incubation times of 60 and 90 minutes are insufficient for lysis, and that incubation times of blood lysis and buffer mixture had a substantial impact on the total amount of extracted DNA and extraction efficiency. Total extracted DNA was considerably influenced by the presence of proteinase K, in contrary to the quantity and quality criteria. According to Khosravinia et al[38]., raising proteinase K levels caused both a larger efficiency of DNA isolation and a greater extraction of total DNA. This can be attributed to the protein degradation activity of the enzyme that can free the DNA from attached proteins (i.e., histones) [39].

According to Jikuzono *et al.*[40], proteinase k is generally employed with 0.5% SDS at 400 mg/ml in nucleic acid production at pH 7.5–8.0 and 55 °C incubation [40]. The study's findings demonstrated that using 20 µl of proteinase K (20 mg/ml) in 10% SDS at pH 7.8 and 65 °C for 90 minutes will raise the extraction yield. This was particularly evident in Protocol 5, which demonstrated a good yield and purity in comparison to Protocol 2, which relied on proteinase K for DNA extraction because the incubation time was extended from 90 minutes to overnight. This process produces high-quality, sufficient amounts of DNA that may be used in a variety of molecular biological procedures. According to results, this procedure can extract DNA from blood in less time. The outcomes were tallied, and the concentration was determined (Tables 4 and 5). The purification of the DNA was evaluated using the 260/280 nm ratio, which has maximum values at 2.07 and lowest values at 0.97, indicating that the level of DNA isolated using this approach is sufficient to run additional PCR reactions, (Tables 4 and 5).

The findings of this investigation demonstrated that phenol, chloroform, and isoamyl alcohol extraction of digested material produced greater quality and quantity of DNA with a significant molecular mass acceptable for genomic DNA testing. The process effectively isolated DNA for PCR testing, and it took around three and a half hours to completion. This reduced the time required for operation. The enzymatic approach, however, required a longer step of specimen digestion with Proteinase K (overnight). It is not cost-effective to utilize these enzymes even though they produce a sufficient DNA extraction technique.

The goal of this work was to discover a rapid, simple, and affordable DNA extraction technique that could be used in any Lab without easy access to commercial kit supplies. When processing several samples containing few organisms, commercial kits can be used effectively. Although using kits to acquire nucleic acid is rapid and simple, they can be costly and hard to get in some places. As a result, this practical method made effective DNA isolation achievable in even modest facilities.

Conventional PCR results from DNA isolated from whole blood that was treated after collected and kept at a deep freeze -20 ºC temperature are shown in Fig. 2. By utilizing five distinct methods, decent size results (100 bp) may be effectively acquired by PCR amplification. The 1 kb ladder is loaded in order to determine the molar mass of the DNA extracted. As shown in Fig. 2, the PCR amplification of the P53 gene with all cellular DNA specimens was effective, indicating the absence of inhibitor for the Taq polymerase enzyme in the ultimate solution, with the exception of the detergent technique, which demonstrates the presence of inhibitor.

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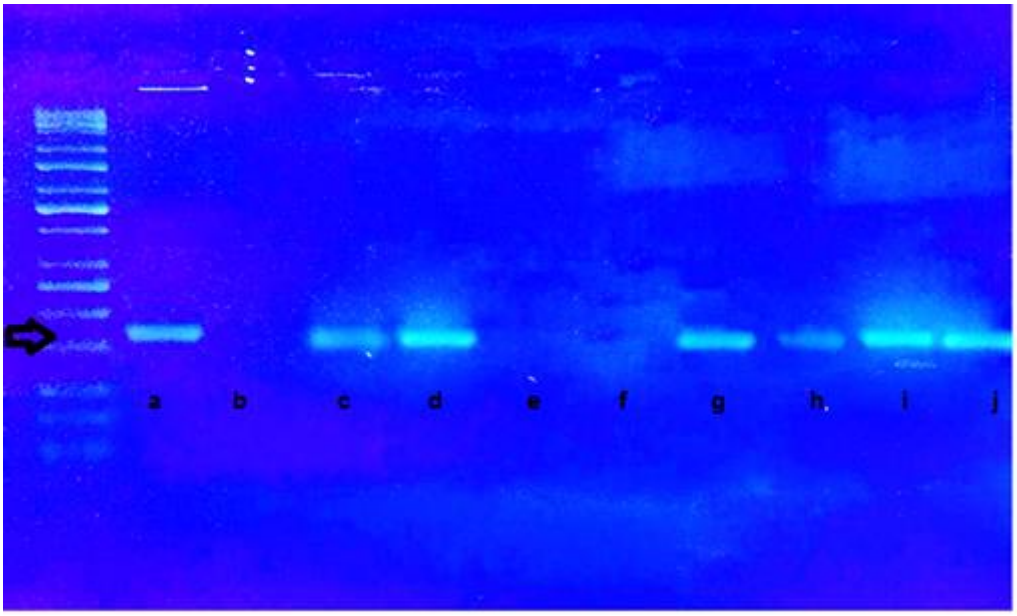

Figure 2. The agarose gel of the p53 gene in the individuals with and without prostate cancer, (100bp). The ladder is represented from a to j.

Amplification products were visualized by electrophoresis. The PCR products Gradient were separated by 1.5% agarose gel electrophoresis and visualized by exposure to UV light after Red Safe stain (Intron Korea). The product was electrophoresis on 1.5% agarose at 5 volt/cm^2. TBE 1X buffer for 01:30 hours. N: DNA ladder (100). The optimum condition of detection p53 that obtained in this experiment is shown below:

Table 6. The optimum condition of detection p53.

No.	Phase	Tm (^{o}C)	Time	No. of cycle
1	Initial Denaturation	94 ^{o}C	3 min	35 cycles
2	Denaturation	94 ^{o}C	1 min	
3	Annealing	60 ^{o}C	1 min	
4	Extension-1	72 ^{o}C	1 min	
5	Extension -2	72 ^{o}C	7 min	

The primary criteria for evaluating the isolated DNA is DNA efficacy or PCR status. The PCR amplification findings showed whether or not the isolated DNA had undergone degradation throughout the extraction procedure. Our proposed models effectively carried out PCR multiplication of the P53 gene with the isolated DNA, demonstrating that there was no significant inhibitory substance for Taq polymerase in the completed solution (fatty acid, protein, and elevated calcium levels are prospective PCR inhibitors) and extracted DNA was of decent quality, with the exception of the detergent method (e,f lane). Conversely, Nasiri et al. demonstrated that DNA generated using a quick and easy approach may be utilized for PCR-based operations with confidence. Additionally, laundry detergents are widely accessible and comprised harmless materials. As a result, this process is a suitable alternative to currently employed standard techniques for isolating genomic DNA and because it satisfies the majority of criteria used in the adoption of an acceptable

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method, it may be utilized often in many fields of molecular biology [32].

Genetics, molecular biology, and biochemistry are all fields that benefit from the application of DNA-based approaches in taxonomic at the area of genera species and subspecies characterization [41]. When using such techniques, pure DNA preparation frequently calls for significant consideration. There are several methods for isolating and purifying DNA. A suitable DNA extraction process should be quick, efficient, inexpensive, devoid of contaminants and toxic effects, and generate DNA of sufficient quality and quantity to be utilized in PCR. The accessibility and affordability of the approach should also be taken into consideration when choosing a method [35] with minimal fragmentation. Conversely, these features are absent from many standard DNA extraction methods [42], therefore reducing their value for creating PCR DNA templates [43].

For the identification of viral Genome in peripheral blood leukocytes (PBLs), the PCR is a very accurate technique [44]. It is also a highly effective technique in the laboratory confirmation of infectious disorders, for which peripheral blood is typically the preferred collection [45].

The slow, intricate, and expensive phenol-chloroform (PhChl) and Proteinase K (PK) processing and extraction techniques for DNA have never been found to significantly impair Taq polymerase-driven DNA amplification. The most popular specimen sources in the diagnostic sector are whole blood, plasma, and sera. It is well acknowledged that rigorous isolation of separated nucleic acids from blood samples is necessary prior PCR process can be conducted since PCR inhibitors in blood specimens have been identified. Numerous steps and reagents used in traditional techniques for genomic purification from clinical specimens, such as organic solvents, denaturing entities, detergents, centrifugation, and precipitation, are incompatible with heat-stable DNA polymerases (Taq polymerase) and are not preferred for use in upcoming systems with automatically generated PCR devices. But due to intrinsic restrictions such limited input volume, levels of DNA amplification inhibition, or poor sample stability, PCR sample handling has to be upgraded. As a result, a quick, easy, and affordable sample preparation technique that enables whole blood DNA analysis (with high yield & purity) by PCR would be useful.

PC has been identified as the most prevalent kind of malignancy in men worldwide during the last 10 years [7]. Although the primary etiology of PC is unclear, it has been linked to a number of variables, including genetics, environment, and levels of sex hormones. Hormone desensitization has hampered the effectiveness of hormonotherapy despite its advancement [46, 47]. Therefore, it is pressingly necessary to find new therapeutic targets.

Mutations in p53 cause the cell cycle to be dysregulated, which leads to aberrant proliferation and neoplastic transformation since p53 is a repressive regulator of cell growth [48]. Researches have shown that aberrant p53 expression is directly related to tumor lymphovascular invasion, clinical stage, and clinicopathology in malignant cells [49, 50]. But it is still unknown how aberrant p53 expression influences PC cells' malignant growth, metastasis, and differentiation.

In conclusion, the FAK-Src-MAPK pathway plays a critical impact in cell proliferation, migration, and adhesion, which are all closely correlated with high levels of protein expression of p53 in PC cells. P53 could serve as a useful anti-cancer target for preventing PC cells from proliferating malignantly and for prostatic tumor gene therapy.

Conclusion

The cost of DNA extraction is significantly reduced by the five suggested techniques for extracting DNA since they use just basic and readily accessible laboratory supplies and equipment and don't require pricy components like K and RNase proteins. Additionally, our findings suggest that the high-quality DNA generated using these modified techniques might be employed for PCR-based investigations, particularly for research on gene polymorphism in a human species. The result showed that the organic method gives a good DNA yield and needs relatively short time while the enzymatic method gives an excellent DNA purity which is more suitable for PCR by comparing five

protocols using the spectrophotometer and Nanodrop technetium in addition to electrophoresis. Through the use of the five suggested procedures, the PCR multiplication of the P53 gene with the isolated DNA was effectively carried out. This indicates that, with the exception of the detergent approach, there were no significant inhibiting substances for Taq polymerase in the final solution.

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Authors' Declaration

- Conflicts of Interest: None.
- I hereby confirm that all the Figures and Tables in the manuscript are mine. Furthermore, any Figures and images, that are not mine, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- The author has signed an animal welfare statement.
- Author signs on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee in University of Mustansiriyah.

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دراسة مقارنة لبروتوكولات استخراج الحمض النووي الجينومي من الدم الكامل لتعدد الأشكال الجيني في جين P53 لدى الأشخاص الطبيعيين والمصابين بسرطان البروستات

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الخلاصة

في العقود الأخيرة ، تطورت الأساليب الجينية لتصبح أداة فعالة في عدد من التطبيقات الملامسة للحياة. في الأبحاث التي تبحث في التنوع الجيني الديموغرافي ، واكتشاف QTL ، والاختيار بمساعدة الواسمات ، وتتبع الطعام ، يتم استخدام التقنيات القائمة على الحمض النووي مثل PCR أكثر فأكثر. تستدعي هذه الأساليب إجراءات الاستخراج التي توفر استخلاصًا فعالًا للحمض النووي والتخلص من مثبطات تفاعل البوليميراز المتسلسل. المرحلة الأولى والأكثر أهمية في البيولوجيا الجزيئية هي استخراج الحمض النووي من الخلايا. بالنسبة للعالم الجزيئي ، تعد الجودة العالية وسلامة الحمض النووي المعزول بالإضافة إلى سهولة استخدام طريقة الاستخراج والقدرة على تحمل التكاليف من العوامل الحاسمة. صُممت الدراسة الحالية لتأسيس طريقة بسيطة وسريعة وغير مكلفة لاستخراج الحمض النووي من الدم المحيطي البشري (ذكر طبيعي ن = 2 ، عمر 24 سنة ، ذكر مريض (سرطان البروستات) ن = 2 ، عمر 65 سنة) من خلال المقارنة بينهما ، وتهدف إلى توحيد بروتوكول استخراج الحمض النووي باستخدام خمسة بروتوكولات استخراج. كانت الطريقة الأولى هي الطريقة العضوية المعدلة باستخدام فوق كلورات الصوديوم بدلاً من المذيب العضوي (الفينول ، الكلوروفورم) ، وتأتي ميزة فوق كلورات الصوديوم من سعرها الرخيص ومتطلبات التخزين والشحن المنخفضة ، والطريقة الثانية كانت الطريقة الأنزيمية باستخدام بروتينيز K ، والطريقة الثالثة باستخدام المنظف ، و الطريقة الرابعة باستخدام الفينول كلوروفورم ، وأخيرًا الطريقة الخامسة كانت طريقة التمليح. أظهرت النتيجة أن الطريقة العضوية تعطي إنتاجية جيدة من الحمض النووي وتحتاج إلى وقت قصير نسبيًا بينما تعطي الطريقة الأنزيمية نقاءًا ممتازًا للحمض النووي وهو أكثر ملاءمة لـ PCR من خلال مقارنة خمسة بروتوكولات باستخدام مقياس الطيف الضوئي وتقنية Nanodrop بالإضافة إلى الترحيل الكهربائي. من خلال استخدام الإجراءات الخمسة المقترحة ، تم تنفيذ مضاعفة PCR للجين P53 مع الحمض النووي المعزول بشكل فعال. يشير هذا إلى أنه ، باستثناء طريقة المنظفات ، لم تكن هناك مواد مثبطة كبيرة لـ Taq polymerase في المحلول النهائي.

الكلمات المفتاحية: استخلاص الدنا، تنقية الدنا، الترحيل الكهربائي، الطريقة الإنزيمية، P53، سرطان البروستاتا، PCR.