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A rapid, non enzymatic method for genomic DNA extraction from whole blood and mammalian tissues

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Abstract

Although several methods have been exist for DNA extraction from blood or animal tissues samples, traditionally most of these methods consume long time and using expensive chemicals such as proteinase K or toxic organic solvent such as phenol. On the other hand, there is no rapid, simple one method for the extraction of genomic DNA from blood and animal tissues samples in the same time. Since the objective of this study was to development easy modified method for DNA extraction from difference mammalian tissues such as fresh or frozen whole blood, kidney, liver, heart, muscles. The description method have many advantages, reducing the time, using inexpensive materials, no phenol, in addition to small amount of mammalian tissue is required (100-200 mg and 2 ml from whole blood). Genomic DNA was obtained having high molecular weight and good quality, shown by agarose gel electrophoresis and spectrophtometric analysis. These results shown that the modified method is simple, fast, safe, most economical, resulting in a high molecular genomic DNA of good quality from several mammalian tissues and can be used in medical laboratories and research centers.

Keywords: DNA Extraction, Blood, Mammalian Tissues

Introduction

In order to perform a PCR diagnostic test, a protocol for genomic DNA extraction from several mammalian tissues that is easy to perform, fast and that generates contaminant-free product is required to prevent unspecific amplifications (Loffler et al., 1997; Veloso et al., 2000). Therefore, a technique to obtain enough quantity, purity and integrity of nucleic acids from mammalian tissues is essential to molecular biology procedures (Mesquita et al., 2001; Loffler et al., 2002; Gonzalez-Mendoza et al., 2010).

A number of methods have already been described for the isolation of genomic DNA, some methods of DNA isolation require large amount of tissue and are not suitable for the small tissues samples. Protocols that yield DNA in quality from small amounts of tissue frequently involve organic solvents like phenol or enzyme treatment (e.g. proteinase k) in specialized buffer systems, whose preparation is often time consuming and expensive (Bahi and Pfenninger, 1996).

One of the problems faced when extracting DNA by standard methods is the requirement of deproteinizing cell digests with hazardous organic solvents like phenol, (Debomoy, 1991), because of efficiency in the DNA extraction method using phenol, requires long time and toxic solution manipulation, due to the organic solvents that may be hazardous to the environment and to the technician, and also several washing and centrifugation steps increasing the risk of sample contamination (Fernandes et al., 2004). Several methods have been proposed as an alternative to the use of phenol, such as commercial kits for DNA extraction. The use of kits offers a low risk of manipulation and they are faster than conventional protocols, but the amount of DNA recovered from the commercial kits is highly variable and these kits may be expensive (Loffler et al., 1997). New methodologies for DNA extraction include a single step of proteinase K digestion (without the use of organic solvents), DNA adsorption in cilica membrane or simple methods of sequential precipitation of proteins and DNA (Cler et al., 2006).

Due to the importance of having good quality genomic DNA extracted from blood and animals tissues, the need for a universal, rapid, simple procedure is urgent especially when large number of samples need to be analyzed. Since, the aim of the present study was to developed a new, easy and fast genomic DNA extraction method by modifying the original method (Roulston and Bartlett, 2004), that became suitable for genomic DNA extraction from whole blood and some other mammalian tissues such as liver, kidney, heart, muscles tissues etc. and may completed in three hours.

Materials and Methods

The original method is used for genomic DNA extraction from blood only, the modify of this method was for give high quality and quantity of genomic DNA, developed an easy new method suitable for genomic DNA extraction from whole blood and animal tissues consume only three hours. The modification of the original method included change the volumes of some solutions, applied the method for extraction of genomic DNA from some mammalian tissues especially there is no method for genomic DNA extraction without using expensive chemicals such as proteinase K or toxic organic solvent such as phenol., all these conditions where optimized and the protocol is suggested.

Eight blood samples collected in EDTA-tube by venipuncture from healthy persons. Four kinds of animal tissues (kidney, liver, heart and muscle tissues) were collected in labelling sterile containers. All samples transferred to a laboratory and stored at -20°C.

The frozen whole blood samples is thawed and mixed well by inverting several times, 2 ml from each sample placed in a 10-ml sterile stopper glass tube, 6 mL of R.B.Cs lyses buffer (0.01M Tris-HCL, 320 mM sucrose, 5 mM MgCl2, 1% Triton X-100, adjust pH to 8.0) was added and mixing well on a rolling, rotating blood mixer or by inverting many times for 5 min at room temperature, then the tubes centrifugation at (4000 rpm) for 20 min at room temperature. Discard supernatant without disturbing cell pellet, remove remaining moisture by inverting the tube and blotting onto tissue paper. Add a further 4 ml of lysis buffer, mix vigorously and centrifuge at (4000 rpm) for 20 min (this step is necessary when the cell pellet remains red or brown after the first spin). Discard supernatant to obtain a cell pellet.

The frozen animal tissue (kidney, liver, heart and muscle tissues) was thawed. Put 100-200 mg of each tissue in a Petri dish containing 1ml of cell lysis buffer and chop it into very small pieces with sterile scalpel blade, then transfer the mixture to a 10-mL sterile stopper glass tube**.**

For all tubes 4 ml of cell lysis buffer (0.4M Tris-HCl, 150 mM NaCl, 0.06M EDTA, 10 % sodium dodecyl sulfate (SDS), adjust pH to 8.0) was added, vortex briefly for one min to resuspend the cell extract, then 500 µL of 5 M sodium perchlorate was added, the tubes mixed well by inverting several times and by gently pipeting. The tubes placed in water bath for 90 min at 65ºC, gently invert the tubes a few times during incubation. The tubes removed and leave for 5 min to cool to room temperature. 3 ml from ice-cold chloroform was added to each tube, mix on a rotating mixer for 30 min and centrifuge at 4000 rpm for 20 minutes. Transfer upper phase into a clean sterile stopper glass tube using a sterile pipet, repeat centrifugation and transfer the supernatant to a clean tube if the supernatant contains unprecipitated debris.

For purification and precipitation of DNA 2 volume of ice-cold absolute ethanol was added. The tubes mixed by inverting several times then a white cotton-like precipitate was formed, use a sterile glass hook, spool the precipitated DNA onto the hooked end. If no precipitate is visible, then centrifuge tubes at (4000 rpm) for 10 min. Decant supernatant being careful that the DNA pellet does not slip out. Wash the spooled DNA twice in 2 ml of cold 70% ethanol by invert gently, centrifuge tubes at (4000 rpm) for 10 min, carefully decant supernatant and allow the white precipitate to air-dry inverted on a paper towel to until the precipitate becomes clear, usually about 5–10 min – ensure pellet does not slip out.

Finally dissolved the DNA precipitate in an appropriate volume of TE buffer (10mM Tris-HCl, pH 8.0; 1mM EDTA) typically about 100–500 µL according to the volume of pellet, transferred the mixture to a 1.5-ml Eppendorf tubes and stored at 4°C.

Spectrophotometric determination of nucleic acids were obtained by added 20 µl of each DNA sample to Quartz cuvete contain 1980 µl of TE buffer, mixed thoroughly, then optical density (OD) is measured in a spectrophotometer at wavelengths of (260 nm and 280 nm).The ratio between the reading at 260nm and 280nm (OD260/OD280) provides an estimation of the purity of nucleic acid, DNA concentration in the solution is calculated and the quantities were using for the analysis of the results (Maniatis et al., 1989).

Agarose gel electrophoresis**,** Agarose gels (1%) are made by adding (1g) of the agarose to 100ml of 1X TBE buffer (89 mmol of Tris-borate, 89 mmol of boric acid and 2 mmol of EDTA pH 8.0) solubilize by heating at boiling temperature, then the agarose is left to cool at 55^{o}C before pour in plastic plate to solidify, a comb is placed near one edge of gel, and gel is left to harden.1X TBE buffer is pour into gel tank and gel plate is place horizontally in electrophoresis tank, 3µl of loading buffer is mixed with 5 µl DNA for each sample, and then samples are added carefully to individual wells. Power is turned on at 45V and 90 mili- amber for 15 minutes and 45 V and 90 mili-amber for one hours to run DNA samples. Agarose gels are stained with ethidium bromide by immersing them in distill water containing the dye of final concentration of 0.5 µg/ml for 30–45 minutes, DNA bands are visualized on U.V transilluminator. Then photo- graphing is done using digital camera (Maniatis *et al*., 1982).

Results

The modified procedure was efficient in extracting genomic DNA from mammalian tissues including

whole blood**,** liver, kidney, heart and muscles tissues.Our results show that high molecular weight DNA successfully extracted from several types of mammalian tissues, the purity of the DNA determined from the A260/A280 ratio averaged 1.8 for all samples, the yield of DNA ranged from 1078 to 4034 ng/µl (Table 1). Agarose gel electrophoresis of genomic DNA extracted by this method show there was no RNA contamination in all samples nor any sigh of degraded DNA during preparation (Figure 1).

For checking genomic DNA extraction in this method, some genomic DNA samples were subjected to PCR amplification using 1 random primer (Operon Technologies) under the following conditions: 1X PCR buffer with $Mgcl_2$, 200µM dNTPs, 10 pmol Primer, 25-50 ng DNA sample, the final volume make up to 25 µl with Sterile Deionized Distilled Water. The amplification program in thermal cycler system was run as follow: 1cycle 94°C for 2 minutes, 40 cycles (92 °C for 1 minutes; 37°C for 1 minute; 72°C for 1 minutes) and 1 cycles 72°C for 7-10 minutes (Figure 2). When the amplified genomic DNA is run in 1.2% agarose gel electrophoreseis show clear and sharp bands for all samples (Weigand et al., 1993).

Discussion

Many genomic DNA extraction methods have been described for prokaryotes and eukaryotes, from cell sample to specific tissues. Molecular diagnostics are performed by using DNA from different mammalian tissues. However, it is necessary to obtain intact genomic DNA of good quality (Sambrook et al., 1989). This new modified method was tested by comparison with a DNA isolation method successfully used for several animal tissues in our laboratory Gaaib et al. (2011) and AL-Allawi (1987), no significant differences in quality and yield of isolated DNA could be detected between either method (data no shown). Higher concentrations were obtained from the whole blood, liver and kidney tissues, similar to those obtain by Gaaib et al. (2011) and Atmadja et al. (1995).

Spectrophotometer measurements indicated differences DNA concentration and purity, according to the tissue origin (Table 1).The variability in DNA quality and purity can be explained by tissue specific structural complexity. The blood is liquid tissue consist of R.B.Cs, W.B.Cs, and platelets, W.B.Cs are separated from a specimen of whole blood by mixing the specimen with R.B.Cs lysis buffer that lyses R.B.Cs but leaves W.B.Cs intact. W.B.Cs are separated by Spectrophotometer measurements indicated differences DNA concentration and purity, according to the tissue origin (Table 1).The variability in DNA quality and purity can be explained by tissue specific structural

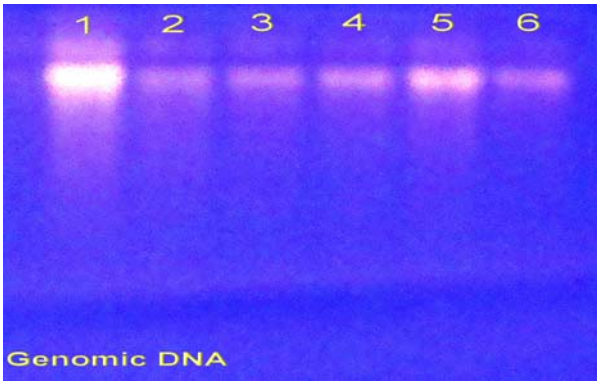

Fig. 1: Genomic DNA sample submitted to electrophoresis in 0.8% agarose gel. 1-(Muscle tissues); 2- (Heart); 3- (Liver); 4- (Kidney); 5, 6- (Blood)

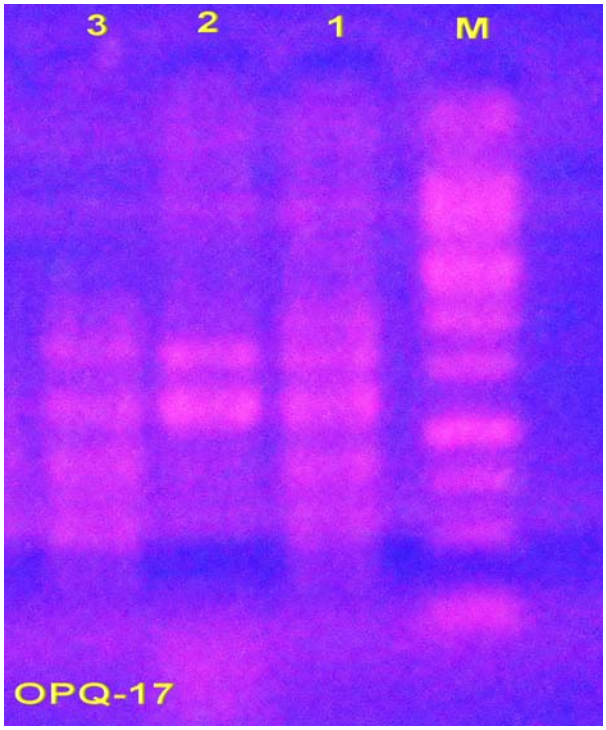

Fig. 2: Amplification Products of Primer OPQ-17 from DNA extracted samples (1. Blood, 2 Liver, 3 Kidney) that Resolve in 1.2 Agarose Gel Stain with Ethidium Bromide. M- λ DNA is digested with *EcoR I* and *Hind III* marker

complexity. The blood is liquid tissue consist of R.B.Cs, W.B.Cs, and platelets, W.B.Cs are separated from a specimen of whole blood by mixing the specimen with R.B.Cs lysis buffer that lyses R.B.Cs but leaves W.B.Cs intact. W.B.Cs are separated by centrifugation, forming a W.B.Cs pellet in the bottom

of the centrifuge tube. The supernatant, contain hemoglobin, plasma proteins and other soluble components from the lysed R.B.Cs or plasma is poured off leaving a relatively clean W.B.Cs pellet. The W.B.Cs pellet is resuspendedin the same R.B.Cs lysis buffer and the tube is again centrifuged and decanted, in order to further wash away contaminating proteins and hemoglobin. Liver and kidney are composed of delicate membrane cells with few fibrous cells. On the other hand the muscle tissue is constituted by many proteins within the cell (Junqeira and Carneiro, 1995).

Table 1: DNA concentration and OD ratio (A260/ A280) of DNA extracted from mammalian tissues

No.	Sample	Genomic DNA concentration	Purity
1.	Muscle tissues	4034 ng / µL	1.63
2.	Heart	1325 ng / µL	1.78
3.	Liver	1451 ng / µL	1.79
4.	Kidney	1078 ng / µL	1.94
5.	Blood	2976 ng / µL	1.85
6.	Blood	1336 ng / µL	1.86

In this method removing the proteins from the cell lysate by using sodium perchlorate at high concentration that will remove SDS and protein complexed with it and prevent proteins from precipitating with your DNA in your ethanol precipitation step, this can be utilized as efficient, rapid and simple deproteinization procedure during the extraction of DNA (Roulston and Bartlett, 2004), several volumes of this solution were tested to give the final volume (500 µL) that used for removed all proteins from the solution.

The deproteinization action of chloroform is based on the ability of denatured polypeptide chains to partially enter or be immobilized at the water–chloroform interphase. The resulting high concentration of protein at the interphase causes protein to precipitate. Since the deproteinization action of chloroform occurs at the chloroform–water interphase, efficient deproteinization depends on the formation of a large interphase area. To achieve this, one has to form an emulsion of water and chloroform. Since chloroform does not mix with water this can only be done by vigorous shaking (Surzycki, 2003).

This modified method proved to be advantageous because of its simplicity, quickness and affordable reagents, besides the high molecular weight DNA and purity achieved in a variety of tissues. Furthermore, there is no phenol in DNA purification, known as a strong PCR inhibitor (Saiki, 1993).

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