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Evaluation and Optimization of Genomic DNA Isolation Protocols from Human Solid Tissues

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Abstract

Molecular diagnostics are performed by using DNA isolated from different body tissues. However, it is necessary to obtain genomic DNA of good quality. The objective of this study was to describe the efficient protocol of DNA extraction from human breast, adipose, brain, liver, kidney, prostate, lung, larynx, endometrium and muscle tissues. We obtained high molecular weight DNA of good quality, shown by agarose gel and DNA fragments. Spectrophotometric analysis of DNA concentration showed variation among the DNA from different tissues, with the liver, breast, endometrium and adipose tissues presenting the greatest and the smallest concentration, respectively. The described protocol has proven to be advantageous due to its simplicity, quickness, affordable reagents and absence of phenol, resulting in a high molecular weight DNA of good quality from several tissues. Genomic DNA extraction from solid tissues of patients with cancer risks was carried out by using two different procedure including manuel and automatically isolation. The QIAamp spin columns (QIAGEN, Hilden, Germany) were used for extraction and purification of genomic DNA from different tissues by Tissue Kits and Tissue Card or automatically, EZ1 automatic DNA isolation system. Furthermore, we used Phenol-Chloroform protocol for manuel isolation in this study.

Keywords: nDNA, solid tissue

INTRODUCTION

In clinical practice and molecular diagnosis, it is well established that patients with tumors of the same histotype and with similar clinical and pathological features differ greatly in prognosis. The polymerase chain reaction (PCR) is a method for the in vitro amplification of specific nucleic acid sequences. The technology provides a powerful means for the rapid detection of infections of gene rearrangements in lymphoproliferative disorders and of inherited diseases. Furthermore, the technique can serve as an initial step for PCR based investigations. An attractive feature of PCR is that, unlike other molecular biological procedures, high molecular weight DNA is not required for successful amplification. As minute quantities of degraded DNA can also serve as the substrate for the reaction, the method is ideally suited to a template extracted from sample material. The combined advantages of exquisite sensitivity and the ability to use routinely processed materials allow large scale, retrospective studies to be carried out. The most widespread protocols for DNA extraction from solid tissues utilise shorter or longer proteolytic treatment-ranging from 20 min or without additional organic solvent purification and ethanol precipitation. For the large scale, routine processing of archival material, DNA extraction should be simple and rapid, with no influence on the success of the PCR [6]. Moreover, as few steps as possible should be involved to minimise the possibility of contamination. The laborious and hazardous phenol-chloroform extraction should be particularly avoided.

We performed a qualitative study, comparing several reported rapid DNA extraction procedures which take less than 1 hours. The ability of these methods to provide DNA suitable for the PCR was investigated. For a more precise comparison, various sets of primers were chosen to amplify a single copy human gene. Several practical aspects of the PCR were also examined, taking into consideration the size of the processed tissue, previous dewaxing, target sequence length, and the reuse of extracts. Genomic DNA extraction from solid tissues of patients with cancer risks was carried out by using two different procedure including manuel and automatically isolation. The QIAamp spin columns (QIAGEN, Hilden, Germany) were used for extraction and purification of genomic DNA from different tissues by Tissue Kits and Tissue Card or automatically, EZ1 automatic DNA isolation system. Furthermore, we used Phenol-Chloroform protocol for manuel isolation in this study.

MATERIALS AND METHODS

DNA was extracted from patients with cancer risks. The samples were unselected for age or family history. Clinical and pathological records were rewieved to confirm the diagnosis of cancer risks in all subjects.

DNA Isolation using Phenol-Chloroform Method

Samples from all the tissues were stored at -20 °C, from which DNA was extracted. A sample consisted of a 200 mg biopsy. Samples were ground separately with mortar and

pestle in liquid nitrogen. The frozen powder was transferred to a 2 ml eppendorf tube and 800 µl of extraction solution (50 mM Tris-HCL, pH 8.0; 25 mM EDTA and 400 mM NaCl), 100 µl 10% SDS, and 20 µl Proteinase K (10 µg/µl) were added. The extract was homogenized and incubated at 65 °C for 3 h. After incubation, proteins and cellular debris were precipitated by adding a 300 µl 6 M NaCl, kept at 4 °C for 15 min. Centrifugation was done at 25.000 g for 20 min. 500 µL of the supernatant were transferred to a new eppendorf, with 500 µL 8 M guanidine hydrochloride (pH 8.0), and 0.49 M ammonium acetate solution, and kept in mild agitation for 90 min. Nucleic acids were precipitated by adding 800 µL of cold 100% isopropyl alcohol, followed by centrifugation at 8.000 g for 5 min. Pellets were washed with 400 µL of 70% isopropyl alcohol. After drying, pellets were resuspended in 150 µL TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA and 50 µg/µL RNAse). DNA samples were stored at 4 °C. [1, 2].

DNA Isolation using EZ1 Nucleic acid isolation analyser

Most samples were extracted with the Tissue Kit (QIAGEN, ATQ, Biotechnology) and author's modified methods were also employed. Extractions of biopsy material were done with a modification of the QIAGEN protocols and included the addition of 570 mg (30 µl) of PCR grade proteinase K (MBI, Fermentase), 6.5% (30 µl) ß-mercaptoethanol (BME) and incubation at 42 °C for 12-24 hours on a rocking platform. Then DNA isolation was done using tissue kit with Nucleic acid isolation equipment (QIAGEN, Bio Robot EZ1) from solid cancer tissue.

Electrophoretic and Spectral Analysis

Five microliter of each DNA was analyzed on a 1% agarose gel (TAE buffer), including a molecular weight marker (Figure 1) and then stained with ethidium bromide (0.5 µg/ml) for 30 min and then agarose gel washed in double-distilled and UV-irradiated H_2O. Analysis of DNA fragmentation was performed by ethidium-bromide stained agarose gel electrophoresis. The ethidium bromide luminescence from the CCD camera is integrated for 1-2 s into the computer memory directly from the gel on the UV Transilluminator using Gel Doc. 1000 system (Bio Rad). One of the most common methods for nucleic acid detection is the measurement of solution absorbance at 260 nm (A260) due to the fact that nucleic acids have an absorption maximum at this UV wavelength. Although a relatively simple and time-honored method, A260 suffers from low sensitivity and interference from nucleotides and single-stranded nucleic acids. Furthermore, compounds commonly used in the preparation of nucleic acids absorb at 260 nm leading to abnormally high quantitation levels. However, these interference and preparation compounds also absorb at 280 nm leading to the calculation of DNA purity by performing ratio absorbance measurements at A260/ A280 .

RESULTS AND DISCUSSION

The protocol was efficient in extracting genomic DNA from all solid tissues. Analysis of whole genomic DNA in agarose gel (Figure 1) and amplified fragments by PCR (Figure 2) demonstrated that the extracted DNA had high molecular weight, one of the most important aspects for successful amplifications of larger fragments. When genomic DNA was extracted from tissue the DNA solutions by Phenol-Chloroform method were of a sufficient purity (A_{260}/A_{280} = 1.7 – 2.0). In addition, the amplification products from the genomic DNA solutions by Phenol-Chloroform method were not detected. Thus, these results suggested that the desirable genomic DNA was not extracted from human tissue samples by Phenol-Chloroform method, although it was possible to extract it by EZ1 nucleic acid DNA isolation method were of a sufficient purity (A_{260}/A_{280} = 1.7 – 1.8). Genomic DNA EZ1 Nucleic acid isolation methods were found that of material human solid tissues can be extracted by rapid and simple methods. Therefore, the tissue DNA extraction procedure is sufficiently efficient and yields adequate amounts of genomic DNA with a sufficient level of repeatability. A DNA extraction method has been developed and applied successfully to the detection solid tissues. This study has shown that DNA can be extracted efficiently from processed samples using different protocols [3, 4, 5]. The study reported confirm that the extraction method, produces DNA of suitable quantity and quality for subsequent PCR based detection applications. The procedures are reliable and reproducible, typically displaying a success rate of over 90%. In summary, we developed a fast and reliable genomic DNA extraction protocol for solid tissues of patients with cancer risks.

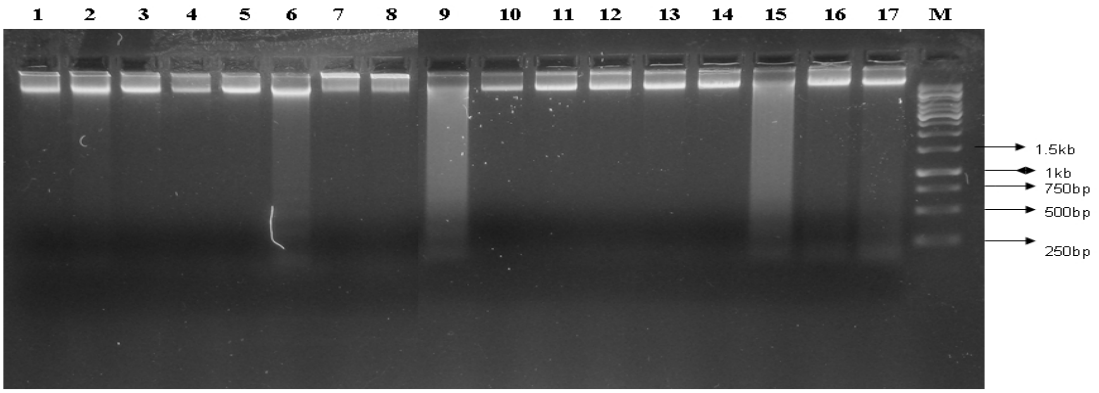

Figure 1. Genomic DNA s were loaded in a %1 agarose gel and seperated by electrophoresis for visualised by ethidium bromide staining with transillumination. Respectively, Lane 1, 2, 6-17 genomic DNAs isolated from breast tumor tissues with Bio Robot EZ1. Lane 3, 4, 5 genomic DNAs isolated from lung tumor tissues with Bio Robot EZ1.

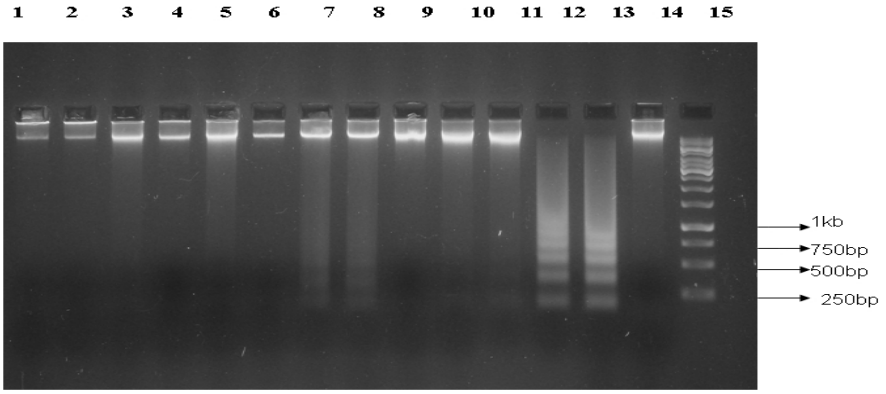

Figure 2. Genomic DNA s were loaded in a %1 agarose gel and seperated by electrophoresis then visualised by ethidium bromide staining with transillumination. Respectively, Lane 1, 2, 3, 4-10 Genomic DNAs isolated from breast tumor tıssues with Bio Robot EZ-1. Lane 11, 12, 13 and 14 Genomic DNAs isolated from lung tumor tıssues with Bio Robot EZ1.Lane 15 1 kb ladder size standard

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