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Comparison of different methods of DNA extraction from paraffin-embedded tissues

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Abstract

The most common human archival specimens are formalin-fixed and paraffin-embedded tissues. PCR-based techniques have been coupled with new developments in the extraction of DNA from FFPE. Herein, we report the results of a comparison of different methods of DNA extraction from FFPE specimens, including phenol-chloroform, salting-out, and silica-based commercial kits. Results showed no significant differences between the amounts of DNA obtained from each of the extraction methods studied; however, the salting-out DNA extraction method described is much easier and less toxic than the phenol–chloroform method.

INTRODUCTION

Formalin-fixed, paraffin-embedded (FFPE) tissues are valuable for both therapeutic applications and research. FFPE is a specific technique used to prepare and preserve tissue specimens utilized in research, examination, diagnostics, and drug development (1-2). Tissues are first collected from both diseased and non-diseased donors. Tissue specimens are then preserved through a process called formalin fixing. This step helps to preserve the vital structures and proteins within the tissue (3). Each specimen is then embedded into a paraffin wax block and sliced, mounted on a microscopic slide, and examined (4). Paraffin samples can be applied in pathological and molecular diagnoses of cancerous malignancies. Recent years have brought us great advances in medical diagnostic technologies, such as next-generation sequencing and gene expression assays (5). This technological progress contributed to the increased involvement of personal medicine in many clinical areas, including anti-cancer therapies (6). Identification of the mutation predisposing the patient to a particular treatment option is one key factor impacting the result of therapy (7). Therefore, molecular oncological diagnostics is one of the fastest growing areas of laboratory medicine. Nonetheless, the application of molecular DNA-based techniques to FFPE tissues suffers from challenges. Fixation with formalin, the most widely used fixative in histopathology, has many advantages such as the ease of tissue handling, the possibility of long-term storage, an optimal histological quality, and its availability in large quantities at low prices. Unfortunately, formalin fixation induces DNA-tissue protein cross-links which can prevent amplification. Additionally, nucleic acid fragmentation may occur in formalin-fixed tissue due to aging of the specimen or the pH of the fixative (8).

In the present study, different methods of DNA extraction from FFPE specimens were compared so as to determine a protocol involving xylene-ethanol deparaffinization on slides followed by a silica-based, phenol-chloroform, or salting-out extraction method that would allow for the extraction of high quality nucleic acid from FFPE tissues and high rates of amplification.

METHODS AND MATERIALS

In this study, ten paraffin blocks of colonic cancer tissue that had been stored for more than five years were selected. Using a microtome, sections of five to ten microns of these blocks were prepared. Three methods were used to extract DNA: phenol-chloroform, the salting-out method, and commercial kits based on silica columns. Samples were extracted using all three methods followed by a PCR reaction for the GAPDH gene, and the results were compared.

For deparaffinization, 1 ml of xylene solution was

added to the samples, which were then incubated at 55 °C for 10 min, followed by centrifugation at 10,000 rpm for 5 minutes, and the supernatant was discarded, followed by 95%, 75%, and 50% ethanol rinses as previously described. Briefly, the tissue pellets were dried at 37 °C. All pellets were digested overnight with 20 µl proteinase K (20 mg/ml and 180 µl digestion buffer (10 mM Tris-HCl, pH 8.0.100 mM EDTA, pH 8.0.50 mM NaCl, and 0.5% SDS) at 55 °C. Proteinase K was inactivated the next day by incubation at 95 °C for 15 minutes. The reaction product was used as a sample for extraction.

For phenol-chloroform extraction, an equal volume of phenol was added and vortexed. After spinning for 3 minutes at 14,000 rpm, the aqueous layer was transferred to a new tube. An equal volume of phenol- chloroform-isoamyl alcohol (25:25:1) was added, and the product was vortexed and then spun for 5 minutes at 14,000 rpm in a microcentrifuge. The aqueous layer was transferred to a new tube. For DNA precipitation, an equal volume of cold isopropanol was added to the aqueous phase. After mixing thoroughly, the tube was placed in a freezer for 30 minutes. The tube was then spun at maximum speed for 10 minutes at 4 °C in a microcentrifuge. The supernatant was discarded, and the pellet was washed with 1 ml 70% cold ethanol and spun at maximum speed for 10 minutes at 4 °C. The supernatant was discarded carefully, and the pellet was dried; care was taken to avoid over-drying. The pellet was then re-suspended with 40 µl of biological grade dH2O.

For the salting-out method, after proteinase K inactivation, 500 ul of ammonium acetate stock solution was added to the samples. The microtubes were vortexed for 20 s at high speed, incubated on ice for 5 min, and centrifuged at 13,000 g for 3 min. The supernatant was transferred to another tube, 600 ml of isopropanol was added, and the solution was centrifuged at 16,000 g for 5 min. The DNA pellet was washed in 70% ethanol and centrifuged at 16,000 g for 1 min. Then, the supernatant was discarded. DNA was dissolved in 40 ul biological grade dH2O.

When using a commercial DNA isolation kit, the manufacturer's instructions were followed for the NucleoSpin DNA FFPE XS, Microkit (MACHEREY-NAGEL, Germany) for extracting DNA from FFPE. The final elution volume of dH2O was 50 µl.

The quality of the extracted DNA was evaluated using an absorbance ratio of 260 nm to 280 nm (A260/A280). Samples with the A260/A280 ratio falling within the range of 1.8–2.0 were considered to be of good quality.

The DNA concentrations in all obtained samples were evaluated using spectrophotometric measurement of absorbance at 260 nm wavelength.

With all samples, PCR reaction was performed by GAPDH primer 5'-GACAGAGAGCCTGGTGGAAAA-3' and 5'-CCGGCAGATGTCTTAGCCAG-3', and the results were analyzed on agarose gel 2%.

RESULTS

The total amounts and purity of DNA for each method are shown in Table 1. All three extraction methods produced good yields of DNA, which were adequate for the PCR reactions. Variance analyses showed no statistically significant differences among the amounts of DNA extracted in each method studied. GAPDH fragment (474bp) was amplified from the DNA extracted using all methods and cases.

DISCUSSION

The most common human archival specimens are formalin-fixed and paraffin-embedded tissues. PCR-based techniques coupled with new developments in the extraction of DNA from FFPE now enable pathologists to use such archival material for a variety of purposes (9). Multiple factors affect the molecular profiles of cells. Such factors affecting cell profiles in FFPE tissues include pre-fixation time, the properties of the fixatives, the conditions of the fixative, and the post-fixative storage parameters (10). Although FFPE are commonly regarded as presenting damage incurred during the fixation and embedding processes, it has been demonstrated that the DNA obtained from FFPE is suitable for use in PCR. The goal of the current study was to determine the optimal method of extracting DNA from FFPE tissues by comparing the phenol-chloroform, salting-out, and commercial kit methods (11). The method most frequently used to separate protein from DNA is the phenol–chloroform method (12). The basis of this separation is the difference in solubility of the nucleic acids, proteins, and lipids in these organic solvents. This method has the advantage that high-purity DNA can be obtained, although one disadvantage of it is that laborious procedures are required, including several centrifugations (13). This extra manipulation greatly increases the potential for carryover contamination. Another problem is the toxicity of these solvents, in particular phenol. When analyzing PCR amplification, however, it was observed that the salting-out extraction method described is as efficient as the phenol–chloroform and the commercially available DNA isolation kit methods (14-15). The results showed no significant differences between the amounts of DNA obtained from each of the extraction methods studied; however, the ammonium acetate DNA extraction method described is much easier and less toxic than the phenol–chloroform method.

Table 1. Comparison of DNA purity and yield with three methods: PC: phenol-chloroform, SO: salting-out, and kit: silica-based commercial kit

Sample	Method	Purity 260/280	Yield of DNA ug
1	PC	1.58	45
	SO	1.84	24
	Kit	1.75	37
2	PC	1.62	43
	SO	1.77	33
	Kit	1.80	41
3	PC	1.32	32
	SO	1.76	28
	Kit	1.91	31
4	PC	1.65	46
	SO	1.74	33
	Kit	1.84	38
5	PC	1.55	38
	SO	1.79	26
	Kit	1.75	27
6	PC	1.62	39
	SO	1.84	25
	Kit	1.79	30
7	PC	1.54	54
	SO	1.85	23
	Kit	1.79	31
8	PC	1.62	39
	SO	1.71	19
	Kit	1.84	24
9	PC	1.58	36
	SO	1.77	28
	Kit	1.84	25
10	PC	1.62	39
	SO	1.85	27
	Kit	1.76	31

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