Technical report

A spin cartridge system for DNA extraction from paraffin wax embedded tissues

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Abstract

A simple and efficient method of DNA extraction from paraffin wax embedded tissues using a spin cartridge system is described. Such DNAs were shown to be suitable for amplification by the polymerase chain reaction, which targeted two human papillomavirus genes and one globin fragment giving rise to products of 450, 150, and 110 base pairs, respectively. Different human tissues, stored for up to 20 years, were successfully amplified, demonstrating the usefulness of this very simple procedure for retrospective studies.
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Keywords: paraffin wax embedded tissues; DNA extraction; polymerase chain reaction; human papillomavirus

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Virtually every tissue removed from the human body for diagnostic purposes is fixed, paraffin wax embedded, and stored for years in pathology departments. These specimens are an endless source of material for research. The polymerase chain reaction (PCR) has the ability to amplify short fragments of DNA or RNA from biological specimens. The use of these approaches permits a great number of molecular biology and genetic analyses.

A general method of extraction of DNA from paraffin wax embedded tissues uses multiple washes with xylene to deparaffinise the sections, and digestion with a protease, usually followed by phenol-chloroform-isoamyl alcohol treatment for DNA purification.[1] The last step is important to reduce protein contamination that could interfere with the PCR assay.[2] We have used this method in our laboratory for years. It proved to be very efficient but is laborious, time consuming, and requires several centrifugations and wash steps that increase the risk of sample contamination. Recent publications have proposed modifications to this method. Microwave based DNA extraction methods have been suggested as a replacement for the organic solvents used in the deparaffinisation of sections,[3] or in combination with other strategies.[4] The use of the Sephadex G-50 minicolumn[5] and the GlassMAX system[6] were proposed as substitutes for phenol-chloroform-isoamyl alcohol treatment. Based on these modifications, we sought an alternative procedure, described here.

Materials and methods

The specimens tested were derived from three different pathology departments in São Paulo, Brazil, and consisted of two biopsy specimens of cervical intraepithelial neoplasia grades I and III, three normal breast tissues, 10 squamous cell carcinomas of the conjunctiva, 32 squamous cell carcinomas of the vulva, and 312 lymph nodes, with or without metastases, obtained from radical vulvectomies. Fixation was performed in non-buffered formalin, except for 22 lymph nodes that were fixed in buffered formalin. Tissue blocks were 2–20 years old. Two slices, 12 μm each, of paraffin wax embedded tissue (approximately 9 cm^2) were collected in 1.5 ml microcentrifuge tubes containing 100 μl TEP solution (10 mM Tris, EDTA 10 mM, 200 μg/ml proteinase K; Gibco/BRL, Gaithersburg, Maryland, USA). Without a dewaxing step, samples were digested for 2–3 days at 55°C. After digestion, the tubes were boiled for 10 minutes to denature residual proteinase and contaminating proteins, followed by addition of 455 μl of 6 M sodium iodide and vigorous shaking for 20 seconds. The solution was added to the GlassMAX Spin Cartridge System (Gibco/BRL) and spun for 20 seconds. The cartridge was washed with 400 μl of cold washing buffer (provided with the kit) and spun for 20 seconds. This step was repeated twice more, followed by centrifugation for one minute. The DNA adherent to the cartridge was eluted with 40 μl of water or TE (10 mM Tris-HCl pH 7.6, 1 mM EDTA) at 65°C by centrifugation for 20 seconds.

The amplification of a 110 base pair β globin gene fragment was obtained by a 50 μl PCR containing 5 μl of DNA extracted from PET. The primers were PCO3 (5'–ACACAACTG TGTTCACTAGC–3') and PCO4 (5'–CAAC TTCATCCACGTTCACC–3')[7] in a mixture containing 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 200 μM of each deoxyribonucleotide triphosphate (dNTPs), 50 nM of each primer, and 2.5 U of Taq DNA polymerase (Cenbiot, Porto Alegre, RS, Brazil). Reaction mixtures were overlaid with mineral oil and 40 cycles of amplification were performed in a DNA thermal cycler (Perkin-Elmer Cetus Instruments, Norwalk,

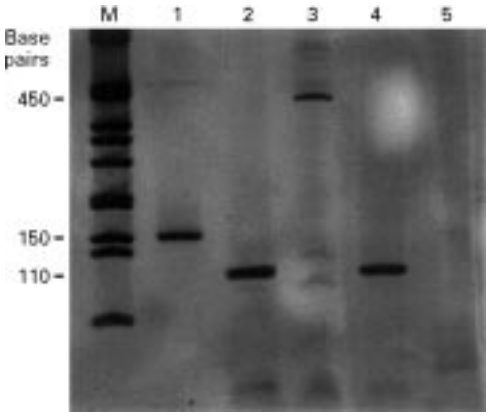

Figure 1 Polyacrylamide gel electrophoresis of PCR products obtained from DNAs extracted from paraffin wax embedded tissues. Samples were amplified with HPV consensus primers GP5+/GP6+ (lane 1), MY09/11 (lane 3), and β globin primers PCO3/PCO4 (lanes 2 and 4). Lanes M and 5 contained molecular weight marker (1 kb DNA ladder, Gibco/BRL) and negative control (no DNA added to PCR reaction mix), respectively.

Connecticut, USA) with the following cycling profile: denaturation at 95°C for one minute, annealing at 55°C for one minute, extension at 72°C for two minutes, preceded by one cycle at 95°C for five minutes. The last cycle was followed by a final prolongation step for seven minutes at 72°C. The same volume of DNA solution used in the β globin PCR was used to amplify fragments of the L1 gene of several human papillomaviruses (HPV). We used the GP5+/GP6+ primers,[8] which amplify a 150 base pair fragment (GP5+ 5'–TTTGT TACTGTGGTAGATACTAC–3'; GP6+ 5'–GAAAAATAAACTGTAAATCATATTC–3'), and the generic primers MY9/11, which generate an amplicon of 450 base pairs from the L1 gene of more than 40 HPV types.[9] The reaction mixture contained 5 μl DNA, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 3 mM $MgCl_2$, 200 μM of each dNTP, 0.05 nM of each GP5+/GP6+ primer or 500 nM of each MY09/MY11 primer, and 2 U of Taq DNA polymerase. PCR with the GP5+/GP6+ primers was performed with the following cycling profile: denaturation at 94°C for one minute, annealing at 40°C for two minutes, and extension at 72°C for 1.5 minutes for 40 cycles. The first cycle was preceded by a denaturation step at 95°C for five minutes, and the last cycle was followed by one cycle at 72°C for 10 minutes. Amplification with the MY9/MY11 primers was performed as follows: one cycle at 95°C for five minutes, one minute at 55°C, two minutes at 72°C, 40 cycles of one minute at 95°C, one minute at 55°C, two minutes at 72°C, followed by an extension step at 72°C for 10 minutes.

PCR products were analysed by electrophoresis on 7% polyacrylamide gels, followed by silver staining.[10]

Results and discussion

Figure 1 shows a representative silver stained gel containing several specific PCR products. Such DNA fragments were further shown to be suitable to HPV type determination by dot-blot hybridisation with specific oligonucleotide probes and restriction fragment length polymorphism (data not shown).

With the procedure described, we were able to amplify efficiently DNA extracted from archival paraffin blocks stored for up to 20 years. As expected, amplification products were more efficiently obtained with primers that amplified a shorter fragment of the viral genome, such as the GP5+/GP6+ primers. This simple procedure, less laborious than other reported methods, should facilitate the molecular analysis of large number of archival specimens in clinical studies.

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