

PCR Amplification of DNA Extracted from Paraffin-Embedded Tissue †

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= Abstract =The polymerase chain reaction(PCR) DNA amplification method is a powerful new tool in the field of individual identification or examination of medicolegal evidences. Usually DNA is extracted from fresh tissue, blood, semen or dried specimen. We try to find suitable way to obtain DNA from paraffin-embedded tissue(PET), the most common preparation in pathological archives. Since PET processing conditions vary in their suitability for amplification, variable methods 1)simple long incubation 2) xylene method 3)heating method 4)ether method were tried. Two VNTR loci of D17S30 and apolipoprotein B gene were used for PCR amplification. The deparaffination process using ether shows the best result in the DNA extraction and PCR amplification.

Key Words: PCR, Paraffin-embedded tissue(PET), Ether

INTRODUCTION

A limitation of DNA fingerprint using either PCR or RFLP has been its reliance on fresh tissue as a source of DNA, and this makes DNA fingerprint invalid in retrospective study. The most common method for preserving human tissue is fixation in formalin followed by paraffin embedding. Several research groups have performed Southern blot analysis on the DNA extracted from such specimen(Goelz *et al.* 1985, Dubeau *et al.* 1986). While this approach can provide invaluable information, it is extremely laborious and therefore not suitable for the examination of large numbers of samples. Furthermore, DNA from the old or improperly fixed samples is often degraded and

cannot be analysed by Southern blotting.

The use of PCR for analysing DNA profile extracted from the fixed, paraffin-embedded tissue provides a relatively simple and extremely sensitive method for examining large numbers of samples(Impraim *et al.* 1987, Shibata *et al.* 1988). Additionally PCR does not require high-molecular-weight DNA and therefore allows the analysis of deteriorated specimens that may be inappropriate for Southern blot analyses.

However, several authors used different methods for extracting DNA from PET with variable results. In this study, the author tried to find appropriate method for DNA extraction in PCR amplification of two VNTR loci, D17S30 and apolipoprotein B gene.

MATERIALS AND METHODS

1. Samples

Twenty fresh placentae obtained from normal full-term spontaneous delivery was sectioned into 2×2×0.5 cm blocks and fixed

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using 10% buffered neutral formalin. After fixation, samples were processed for paraffin embedding. Preparation for paraffin embedding included one 80% (v/v), two 95% (v/v), and three 100% absolute ethanol washes, followed by two xylene and paraffin changes. Samples were embedded with an Autotechnicon (Technicon, Tarry town, NY) paraffin embedder.

2. Preparation of samples for PCR

Dry sections, 5 μm thick, were sliced from each sample and were deparaffinized as follows. The microtome and blade were carefully cleaned with xylene between each block to prevent sample-to-sample contamination.

a) Simple long incubation

Tissue sections without dewaxing were directly suspended in digestion buffer (100 mM NaCl, 10 mM Tris-HCl, 25 mM EDTA, 1% SDS, 0.1 mg/ml proteinase K) using about 1.2 ml/100mg tissue. Incubation was done for one hour at 37°C, up to 5 days. DNA was extracted by conventional phenol-chloroform extraction method (Maniatis *et al*, 1989) and two times of spun column were treated for the extract.

b) Xylene method

The paraffin block was diced with blade, placing in a processing cassette, and rehydrated by immersion for 30 min in each of the following: xylene, three changes; 99 per cent alcohol, two changes; and one change each in 95 per cent alcohol and running tap water. Rehydrated sections were transferred directly to the digestion buffer described as the above, followed by phenol-chloroform DNA extraction.

c) Heating method

The sliced section in digestion buffer without proteinase K was heated at about 65°C till the tissue fragment was sunk. Then, the tissue was transferred directly to digestion buffer.

d) Ether method

After transferring the sliced PET to digestion buffer without proteinase K, about same volume of ether was added and mixed well. After incubation at 56°C for one hour, ether was removed with pipette. This process was repeated two to three times till the tissue was

sunk. Residual ether was evaporated by incubation at 56°C for 30 min. After this, proteinase K of 200 $\mu\text{g}/\text{ml}$ was added and incubation was done at 37°C for up to 3 days with daily addition of proteinase K.

After DNA extraction, 1 μg of DNA were electrophoresed on 0.8% agarose gels, stained with ethidium bromide, and photographed under ultraviolet light.

3. PCR amplification

Amplification was done for two DNA loci, D17S30 and apolipoprotein B gene as described by Lee (1991). Briefly describing, the primers in amplification of D17S30 locus were 5'-CGAAGAGTGAAGTGCACAGG-3' and 5'-CACAGTCTTTATTCTTCAGCG-3'. The PCR was carried out in 25 μl of reaction mixture containing 20 ng of genomic DNA, 0.5 μM of each primer, 300 mM of each dNTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM magnesium chloride and 1.25 unit of Taq polymerase. After denaturation for 5 min at 94°C, denaturation for 1 min at 95 and annealing for 1 min at 55°C were carried out, followed by extension for 8 min at 72°C and repeated for 30 cycles.

The primers in the amplification of the 3' hypervariable region of the apolipoprotein B gene were 5'-ATGGAAACGGAGAAATTATG-3' and 5'-CCTTCTCACTTGGCAAATAC-3'. The PCR was carried out in 25 μl of a reaction mixture containing 25 ng of genomic DNA, 200 μM each dNTP, 25 μg of each primer, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM magnesium chloride, and 1.25 units of Taq polymerase. Denaturation for 1 min at 94°C, and annealing and extension for 6 min at 58°C were repeated for 26 cycles.

The polymorphism of the amplified products was analysed by silver staining after electrophoresis on acrylamide gels.

RESULTS

The four methods extracting DNA from PET demonstrated variable degree of results. The ether extraction method for deparaffination shows

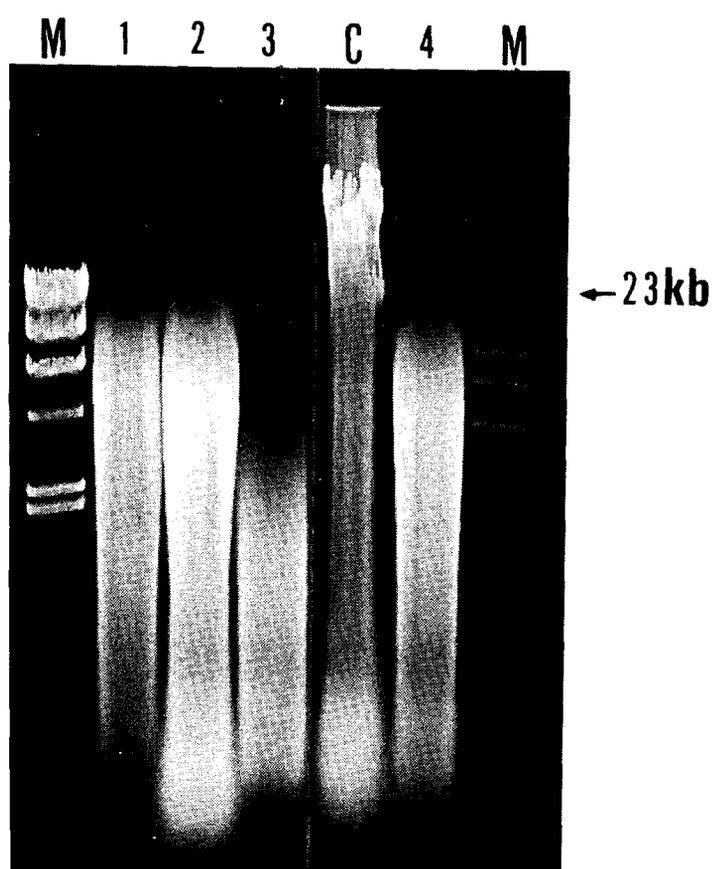


Fig. 1. DNA pattern isolated from paraffin-embedded tissue by simple long incubation (lane 1), ether extraction (lane 2), xylene extraction (lane 3), and heat treatment (lane 4). C; control DNA isolated from fresh placenta, M; size standards from lambda DNA digested with Hind III.

the best DNA quality as illustrated in Fig. 1 and satisfiable results in the yield of DNA extracted and PCR amplification. The DNA quality in simple digestion method was similar to that of the ether method, but the quantity of DNA extracted was quite low. The amount of the DNA extracted by the heating method was larger than that of the simple digestion method, but smaller than that of either ether or xylene extraction. There was no significant difference in the amplified DNA profile between the simple digestion and ether extraction comparing to that of the fresh tissue, but the background of the electrophoresis tracks was hazier and dirtier than that of the fresh tissues.

In the D17S30 profile amplified with those of heat and xylene extraction, the upper band (high molecular weight band) in heterozygote

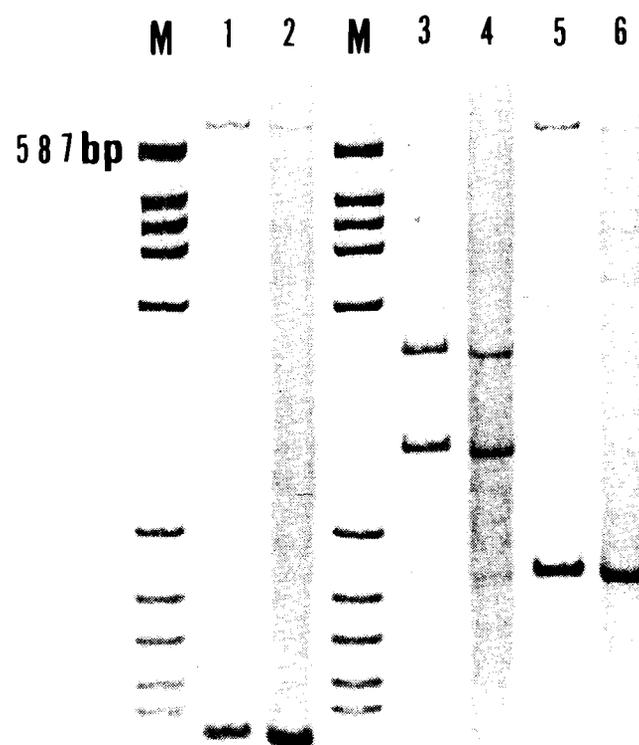


Fig. 2. Expression of D17S30 amplified fragments between fresh tissue (lane 1, 3 and 5) and paraffin-embedded tissue (lane 2, 4 and 6). M; size standards from pBR322 DNA digested with Hae III.

sometimes disappeared with advent of the lowest band, especially in the cases of marked DNA degradation.

Using ether and simple digestion methods, the products for PCR amplification presented the same electrophoresis band pattern between from PET and from fresh tissue. Large amount of DNA extracted from paraffin embedding tissue seemed to be entrapped around the well in agarose gel electrophoresis comparing to that of fresh tissue.

DISCUSSION

After fixation, heating process seems to play a role in differentiating formalin embedded tissue from PET in the point of DNA extraction (Jackson *et al.* 1991). As well known, formaldehyde is not a good fixative for nucleic acids itself, and DNA in its native state does not react to any extent with formaldehyde. Heating, necessary for impregnation of wax, destroys the

hydrogen bonds that hold the two strands of DNA together and allows the reaction of formaldehyde with amino groups on the bases now exposed by the uncoiling of the double-stranded DNA molecule. The poor fixation of DNA by formaldehyde at room temperatures causes loss of DNA into the fixative solution by leaching. Such leaching obviously increases with the length of time that the tissue spends in fixative, and a prolonged period of fixation can lead to a dramatic reduction in the yield of DNA (Lee and Lee 1992). With this fact we checked the lapping time from fixation to paraffin embedding in PET processing.

Several authors used different method for PET. Shibata *et al.* (1988) have reported a simple method allowing amplification of 100-bp products from sections that were deparaffinized and boiled before PCR. Manos *et al.* (1989) developed improved methods providing increased product yield with products of over 800bp. He used octane or mixed xylene for deparaffination. In this study, we cannot obtain satisfiable results with xylene. Sometimes the upper high molecular weight band fail to amplify compared to fresh DNA. This discordant result with other study may be related with either different PCR target or the degree of DNA degradation. Xylene and heating process seem to be related with tissue hardening causing enzyme hindering in digestion of tissue with proteinase K. During digestion, the solid particle of tissue floated even with prolonged digestion. To avoid this, we used ether in deparaffination process yielding good results.

Large amount of DNA is retained in the well of agarose gel. This may be related with the conformational change, denatured DNA, which cannot be used as DNA template.

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