A rapid and efficient method for DNA extraction from paraffin wax embedded tissue for PCR amplification

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Abstract
DNA from archival, formaldehyde fixed, paraffin wax embedded human tissue, suitable for amplification by the polymerase chain reaction (PCR), was obtained using a microwave method based on the capture of DNA by magnetic beads. Fragments of the α-1-antitrypsin gene (AAT) and the apolipoprotein E gene (APOE) were amplified successfully from human liver and brain tissue, respectively. This procedure provides a more rapid, simple and efficient method for reproducibly obtaining DNA from preserved tissue that has been kept in storage for up to 30 years.

Keywords: PCR, DNA extraction, paraffin wax sections.

PCR has proven to be a rapid and extremely sensitive method for examining DNA from formaldehyde fixed, paraffin wax embedded tissue.\(^1\)\(^2\) Exist ing methods consist of a deparaffinising step using organic solvents\(^3\)\(^4\) or are microwave based\(^5\) followed by proteinase digestion\(^6\) or use of a chelating agent prior to obtaining DNA suitable for use as PCR template. These methods are time consuming and involve repeated manipulations. The method we describe is very rapid (takes less than 15 minutes) and as the procedure is carried out in a single tube, the likelihood of cross-contamination is minimised.

Methods
Human liver samples (kindly provided by Dr P Williams, Department of Gastroenterology, Llandough Hospital, Cardiff) and human brain tissue samples (kindly provided by Professor J Lowe, Department of Pathology, Queen's Medical Centre, Nottingham) were obtained by cutting 20 μm thick serial sections from archival paraffin wax embedded blocks. The brain tissue samples had all been collected within the past five years, whereas some of the liver samples had been in storage for up to 30 years. Three sections from each sample were transferred to a sterile 1.5 ml microcentrifuge tube, to which was added 200 μl Dynabeads DNA DIRECT (Dynal (UK) Ltd). This product consists of magnetic beads capable of binding DNA suspended in lysis buffer (composition not disclosed by vendor). The tubes were capped, placed in a microwave oven (Sanyo EM-S150) and irradiated at 80 watts for 10 seconds (split into two 5 second treatments). During this time the samples were monitored closely to ensure that they did not boil over. Following microwave treatment the samples were incubated at room temperature for five minutes. This resulted in a solid paraffin wax ring forming above the beads/lysis buffer mixture and permitted the DNA released from lysed cells to be adsorbed onto the Dynabeads. The DNA was washed twice with 200 μl of washing buffer as supplied (proprietary mix of salts in aqueous solution) using a magnetic particle concentrator (Dynal (UK) Ltd). As the paraffin wax pellet was solidly attached to the wall of the microcentrifuge tube above the aqueous layer, it was pierced easily with a yellow pipette tip thereby allowing washing buffer to be added/removed. After the final wash, the DNA/Dynabeads complex was resuspended in 30 μl TE buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA) and transferred to a fresh tube. The DNA was eluted from the beads by heating at 65°C for five minutes and stored at 4°C until use; 5 μl of eluted DNA was used per 50 μl PCR.

The DNA obtained was used for amplification of a 227 base pair (bp) fragment of the apolipoprotein E (APOE) gene\(^6\) and a 237 bp fragment of the α-1-antitrypsin (AAT) gene.\(^7\) PCRs were carried out in a total volume of 50 μl consisting of 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl₂, 0.1% Triton X-100, 200 μM dNTPs, 25 pmoles of each primer, and 2.5 units of Taq DNA polymerase (Promega, Madison, Wisconsin, USA). The primers for the AAT PCR were 5′AAGCT-GTCCTCTGTTCTCTC3′ and 5′TTGAG-GAGGGAGAGGAGTGT3′; those for the APOE PCR were 5′TCCAAGGAGCTG-CAGGCAGGCACA′ and 5′ACAGAAATTCG-CCCCGGCCCTGTACAGGCA3′. The APOE PCR mix also contained 10% dimethyl sulphoxide. All reactions were overlaid with 20 μl mineral oil. Amplification conditions were 30 seconds at 94°C, 30 seconds at 60°C (AAT PCR) or 30 seconds at 65°C (APOE PCR) and one minute at 72°C for 35 cycles.

Results and Discussion
The AAT and APOE products generated from DNA prepared from paraffin wax embedded human liver and brain tissue were analysed (5 μl) by electrophoresis in 2.5% agarose gels. The gel was visualised by staining with ethidium bromide and photographed under ultraviolet light (fig 1). A single band of the expected size was obtained in all cases.
Postmortem diagnosis of Factor V Leiden from paraffin wax embedded tissue

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Abstract

Activated protein C resistance resulting from Factor V Leiden is an important inherited thrombophilia disorder which is found in 3.5% of people in the UK. The genetic defect can be detected using the PCR and the diagnosis can be made postmortem from paraffin wax embedded tissue. The presence of Factor V Leiden should be sought in all cases of unexplained sudden death resulting from venous thromboembolism.

Keywords: Factor V Leiden, thromboembolism, thrombophilia disorder.

Activated protein C resistance has been recognised recently as an important risk factor predisposing to venous thromboembolism. The disorder results from a specific missense mutation (G1691A) in exon 10 of the coagulation factor V gene, which is located in the sequence encoding the activated protein C cleavage site. Recent studies have shown that the mutant factor Va (Factor V Leiden) is 10 times less susceptible to deactivation by this natural anticoagulant. Factor V Leiden can be detected by means of the PCR as the nucleotide deletion is obtained matched with those detected for the restriction enzyme, MnlI. Recently, we have demonstrated the importance of diagnosing this disorder retrospectively from postmortem paraffin wax embedded tissue.

Case report

A 24 year old man sustained a soft tissue laceration to the right leg following a road traffic accident. The wound was debrided and sutured and the patient was prescribed diclofenac sodium (Voltarol) as analgesia. One week later, he presented with lower abdominal pain and appendicitis was diagnosed provision-