Short communication

PCR amplification of genomic DNA from a 24 year old lysate derived from washed human erythrocytes

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Application of the polymerase chain reaction (PCR) has transformed molecular biology and revolutionised the diagnosis of genetic and infectious diseases.¹ The PCR technique even permits the isolation of sufficient DNA for gene analysis from buccal epithelial cells by simple mouthwash² or from a single hair.³ Isolation from whole blood, as opposed to purified mononuclear cells, however, presents certain problems since some blood components, such as haem breakdown products emanating from lysed red cells, appear to inhibit the PCR process.^{4,5} Indeed, haematin has been found to inhibit PCR at as low a level as 0.8 $\mu\text{mol dm}^{-3}$.⁵

We now report the isolation and amplification, using the PCR technique, of human genomic DNA from a haemolysate which had been twice washed with saline, removing the buffy layer each time, extracted with toluene, and stored at -20°C for 24 years. Since human erythrocytes do not contain DNA, success depended both on contamination of the sample with DNA released from white cells and retained during the original preparation of the sample and on efficient removal of the inhibitory components.

A sample of 0.4 ml haemolysate was incubated with proteinase K (100 $\mu\text{g/ml}$) in 0.02 mol dm^{-3} EDTA, 0.5% SDS, and 0.1 mol dm^{-3} Tris, pH 8.3, in a final volume of 0.45 ml for two hours at 55 to 60°C . After storage overnight at 4°C the sample was centrifuged and the precipitate re-extracted with 0.4 ml sterile water. The pooled supernatants were then extracted twice with an equal volume of phenol, once with an

equal volume of phenol/chloroform (1:1), and once with an equal volume of chloroform. After washing three times with an equal volume of ether, discarding the upper ethereal layer each time, the sample volume was reduced to 0.2 ml. Further purification was then achieved using GENE CLEANTM (Bio 101 Inc, La Jolla, CA 92038-2284, USA) according to the manufacturer's instructions, except that the glassmilk was very carefully washed five times with the NEW solution and the DNA was eluted successively with 40 μl and 20 μl sterile water.

Carbonic anhydrase I exons⁶ (unpublished data) were amplified from 1 μl DNA sample using the GeneAmpTM DNA Amplification Reagent Kit (Perkin Elmer Cetus, Norwalk, CT 06859, USA) for 30 cycles (1.3 minutes, 95°C ; 1.3 minutes, 55°C ; 3.0 minutes, 72°C ; final extension 10 minutes, 72°C) with 1 μg of each primer in a total volume of 100 μl .

The products were identified by electrophoresis in a gel comprising 1% agarose and 3% Nusieve agarose in TBE (90 mmol dm^{-3} Tris, 90 mmol dm^{-3} borate, 2.5 mmol dm^{-3} EDTA) run for 10 hours at 100v (figure).

PCR amplification using primer sets [exons 1a (GGAATTCATCCACACCCCAACCACTTC; GGAATTCACAGCTCTGAATGAGAGAAGG) and 3 (GGAATTCGCAAGATAAGCTAGAGTTTG; GGGATCCAGGGTAATTATCTCTCACTTAC)] from human CAI gene in the presence and absence of DNA extracted from stored human haemolysate samples. Lane 1 DNA markers in bp (pBSM13, cut with HpaII). Lane 2 exon 1a primer set without DNA. Lane 3 exon 1a primer set with DNA. Lanes 4 and 5 exon 3 primer set without DNA. Lane 6 exon 3 primer set with DNA.

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The method was attempted on eight different haemolysate samples, which had been similarly prepared by four different workers in three different laboratories. DNA was successfully amplified from six of these samples, one of which had been stored at -20°C for 17 years. Great care was exercised over acknowledged precautions necessary to avoid false 'positives' through contamination,⁷ as is borne out by the negative controls in which sterile water was substituted for the DNA sample.

This method was developed in order to permit characterisation of an interesting genetic variant from haemolysate samples which predate modern DNA technology.

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