Direct PCR from CVS and blood lysates for detection of cystic fibrosis and Duchenne muscular dystrophy deletions

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Inadequate blood samples or chorionic villus samples (CVS) are occasionally submitted for diagnostic DNA studies. Extraction of such samples by routine means yields small amounts of DNA, frequently not enough to perform all the tests required. We describe here two modifications of a simple method (1) which avoids having to extract DNA and can be performed in a few hours.

A single strand (containing ~ 1 ng DNA) from a CVS sample was placed in a 1.5 ml eppendorf tube and centrifuged briefly before removing the tissue culture medium. The strand was resuspended in 50 μl of ‘PCR buffer with non-ionic detergents’ (50 mM KCl, 10 mM Tris- HCl (pH 8.3), 2.5 mM MgCl₂, 0.1 mg/ml gelatin, 0.45% NP40, 0.45% Tween 20) and 2 μl of 5 mg/ml proteinase K was added. Incubation proceeded for 1—2 hours at 50°C. The lysate was boiled for 5 minutes and 20 μl (~0.2 μg DNA) of the lysate was added to the PCR reaction in a 100 μl volume.

Alternatively, a 500 μl aliquot of thawed blood was mixed with 500 μl of ‘lysis buffer’ (0.32 M sucrose, 10 mM Tris- HCl (pH 7.5), 5 mM MgCl₂, 1% Triton X-100) in a 1.5 ml eppendorf tube. The sample was centrifuged for 20 seconds and the pellet was resuspended in 1 ml of lysis buffer, by vortexing. This was repeated twice, with resuspension in 500 μl of ‘PCR buffer with non-ionic detergents’ plus 6.0 μl of 5 mg/ml proteinase K. The sample was incubated for 1—2 hours at 50°C, with occasional inversion to help disrupt the sample, then stored at ~20°C. An aliquot of the lysate (20 μl is ~1 μg of DNA) was boiled prior to addition to the PCR reaction, as described above. The procedure described by Erlich (1) is for fresh blood while all our work has been on frozen blood stored at ~20°C.

Both the CVS and blood lysates were tested for the 3 bp ΔF508 deletion (2) which is the most frequent cause of cystic fibrosis (fig. 1). These results were confirmed on DNA extracted from the samples by our routine procedure. In addition, the blood lysate was tested in the Duchenne muscular dystrophy multiplex PCR system (3) with successful amplification of all 9 products (data not shown).

This procedure gives a simple and very rapid way of amplifying directly by PCR from a sample without extracting DNA, thereby reducing time and workload significantly. Another major advantage is that only 25—30 cycles are needed compared to the 40 cycles specified by Mercier (4). The reduced number of cycles reduces errors which can occur during the amplification process and diminishes the risk of amplifying contaminating material (personal experience).

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REFERENCES


Figure 1. Screening for the 3 bp ΔF508 deletion in cystic fibrosis. A 56 bp band is seen if the deletion is not present and a 53 bp band is seen if the deletion is present. Samples are from genomic DNA (tracks 1, 3—5) or blood lysates (tracks 2, 6). Tracks 2 and 3 are from the same individual. Track 7 is a CVS lysate. Final PCR conditions were 0.2 mM dNTP mix, 1 μM each primer, 3 units of Replinase (Du Pont) and 1×PCR buffer (50 mM Tris- HCl, pH 9.0, 20 mM (NH₄)₂SO₄, 2.5 mM MgCl₂). Reaction times were 2' @ 95°C, 2' @ 57°C, 2' @ 70°C for 30 cycles. Thirty μl samples were analysed on a 10% non-denaturing polyacrylamide gel.

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