

Direct PCR from solid tissues without DNA extraction

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One of the major limitations in the routine use of PCR for diagnosis has been the inability to apply PCR directly to clinical material. We have previously described a technique, termed FoLT (Formamide Low Temperature) PCR, which allows direct PCR from blood without any preparative steps (1, 2). It is based on the use of formamide and reduced incubation temperatures. Here we describe a modification of FoLT PCR which allows direct amplification from solid tissue samples without the need for any DNA purification steps.

Two oligonucleotide primer combinations directed against the murine β_2 microglobulin gene were used in this study. Using a standard PCR protocol it was not possible to amplify the appropriate DNA fragment directly from a 1 mm^3 slice of murine liver tissue using a number of modifications of a standard PCR protocol, as illustrated in Figure 1. Instead of the expected amplification products of 300 bps and 460 bps respectively only DNA smears were evident. A similar result was also obtained for samples where the tissue was teased to give rise to a single cell suspension.

No PCR products were observed when the standard FoLT PCR protocol was applied to solid tissue or cell suspension. Heating the samples in formamide for 10 minutes prior to amplification allowed the target DNA fragments to be amplified. However, non specific bands were observed. The best amplification signals were obtained when the samples were cycled in 100% formamide between 95 and 72°C 30 times prior to FoLT PCR. We believe that cycling of the samples in formamide prior to PCR amplification allows the DNA on the edges of the tissue samples to be solubilised and thus be accessible for amplification. Mercier *et al.*, (1991) have described a precycling protocol for PCR amplification from blood using a standard PCR protocol (4). The precycling protocol described here is far more extensive and employs higher temperatures.

The method described here is generally applicable since it has been successfully used on a range of tissues including brain, heart, testis, ovaries, kidney, skin and skeletal muscle using a range of different primer sets (Data submitted but not shown). We have also used this technique to amplify DNA from parasite tissue. In particular the lack of an intron within a cathepsin gene from the trematode parasite, *Fasciola hepatica*, was determined using this technique (Data submitted but not shown).

An important point to note is that it is necessary to use *Tth* polymerase instead of *Taq* polymerase in FoLT PCR since *Tth* polymerase seems to be less sensitive to the presence of organic material (3) and more tolerant to the presence of formamide (1). Adding too much tissue to a PCR reaction may lead to inhibition.

This is the first description of a direct PCR protocol from solid tissues. The modified FoLT PCR protocol described has applications in forensic science, genetic analysis of tissue biopsy samples, determining the origin species of meat samples and screening transgenic animals. It obviates the need for proteinase K digestion of tissue samples and DNA purification, procedures that may take up to a day to perform. As such, the modified FoLT PCR protocol described here is faster and less expensive than current methodologies for PCR from solid tissues.

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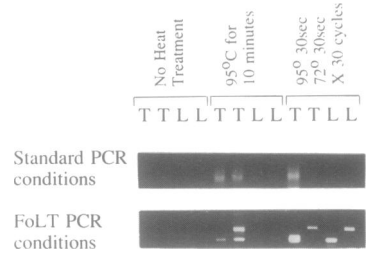

Figure 1. Comparison of standard PCR and FoLT PCR for amplification directly from liver tissue samples. The target was murine liver either as an intact piece of 1 mm^3 (T) or teased to give a single cell suspension (L). The first sample of each pair contained the following primers, β_2-mF1 (5′ ATACTCACGCC-ACCCACCGG 3′) β_2-mR1 (5′ TCCACACAGATGGAGCGTCCA 3′). In the second sample of each pair, primer β_2-mR2 (5′ TTATACTACGACATATTAA 3′) replaced β_2-mR1. For FoLT PCR the tissue or teased tissue was mixed with 18 µl of 100% formamide and subjected to the pre-treatments as described. For standard PCR the PCR mixture minus the *Tth* polymerase was subjected to the pre-treatments. The PCR reactions were performed as follows: Standard PCR: 50 µl PCR reactions containing 200 µM of dATP, dCTP, dTTP and dGTP (Pharmacia, Uppsala, Sweden), 0.2 µM of forward and reverse primers, 10 mM Tris pH 9.0 (at 25°C), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% Gelatin, 0.1% Triton X-100 and 2.5 units of *Tth* polymerase (Toyobo, Japan) were performed in a robotic arm Gene Machine (Innovonics, Melbourne, Australia) for 30 cycles of 30 seconds for each of the following temperatures 95, 50 and 72°C. FoLT PCR: For 100 µl reactions, 1 mm^3 of murine liver was mixed with 18 µl of deionised formamide. To this a 82 µl mix containing the rest of the PCR reaction components was added. The final concentrations of the other components were as follows 200 µM of dATP, dCTP, dTTP and dGTP (Pharmacia, Sweden), 0.2 µM of forward and reverse primers, 10 mM Tris-pH 9.0 (at 25°C), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% Gelatin, 0.1% Triton X-100 and 2.5 units of *Tth* polymerase (Toyobo, Japan). The samples were overlayed with liquid paraffin and subjected to 30 cycles of PCR amplification using a robotic arm Gene Machine (Innovonics, Australia). The incubation temperatures and times for FoLT PCR were 85°C 30′, 40°C 30′ and 60°C 30′. For the Perkin Elmer 9600 PCR machine, the incubation temperatures were as follows 80°C 20′, 40°C 20′ and 60°C 20′.