Direct PCR from whole blood, without DNA extraction

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Typically DNA used in PCR assays is usually extracted according to the phenol-chloroform method (1) or by an alternative ‘salting-out’ rapid purification (2). Moreover partially purified DNA obtained with rapid procedures have been reported to be suitable for PCR amplification (3).

We describe here a more simple and efficient method to amplify DNA directly from whole blood samples without any purification. This method proved to work not only on fresh blood samples but also on frozen blood samples stored several months at −20°C. According to our procedure, a small volume of blood (1 or 2 μl) is introduced directly in the PCR reaction (100 μl PCR reaction: 50 mM KCl, 10 mM Tris HCl pH 8.0, 1.5 mM MgCl₂, 0.1 mg/ml gelatin, 200 μM each dNTP) and the amplification is performed as on purified DNA with only a modification of the initial denaturation step. Instead of heating the samples for 5 min at 94°C as usual on genomic DNA template, the whole blood PCR reactions were heated for 3 min at 94°C then cooled for 3 min at 55°C and this two-step incubation was repeated 3 times. In fact, the efficiency of the whole blood PCR relies on this alternating heating-cooling steps since a single longer heating incubation resulted in very poor amplification. In our experiments we successfully amplified fragments up to 900 bp from frozen whole blood samples. Aliquots of amplified fragments from PCR, performed either on purified DNA or blood, were analysed directly on agarose gels by ethidium bromide staining: no difference was noticed between purified DNA and blood samples for all the different amplified sequences tested (Figure 1). These data suggest that using whole blood is as sensitive and specific as using purified DNA as starting material for PCR assays, providing the initial denaturating step modification. However, because of the potential Taq polymerase inhibitors (such as hemoglobin) it contains, whole blood used in too large amount may turn to be detrimental to the PCR reaction, as shown on Figure 2 where as little as 4 μl of blood in 100 μl PCR reaction denaturation (3 min at 94°C, 3 min at 55°C, repeated 3 times) before the addition of 2.5 U of Taq polymerase (Amersham) and 40 cycles of amplification (1 min at 94°C, 1 min at 60°C, 2 min at 72°C) were performed.

REFERENCES