Direct PCR from whole blood, without DNA extraction

B.Mercier, C.Gaucher, O.Feugeas and C.Mazurier

Centre Régional de Transfusion Sanguine 19-21, rue Camille Guérin, BP 2018, 59012 Lille Cédex, France

Submitted August 23, 1990

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 MgCl_2 , 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 denaturing 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 totally inhibited the amplification reaction. We therefore recommend the use of 1 or 2 μl of whole blood in a 100 μl PCR reaction in order to prevent such inhibitory effects. This rapid and convenient procedure provides an alternative to tedious DNA purifications and is especially of interest in family studies since it prevents possible errors inherent to the DNA extraction procedure such as samples inversion or contamination. However, this method may not be sensitive enough for some PCR studies like viral DNA detection or chimerism detection after grafts involving the amplification of 'marginal' sequences.

REFERENCES

1. Gross Bellard *et al.* (1973) *Eur. J. Biochem.* **36**, 32–38.
2. Miller *et al.* (1988) *Nucl. Acids Res.* **16**, 1215.
3. Ferre *et al.* (1989) *Nucl. Acids Res.* **17**, 2141.

Figure 1. PCR amplified fragments electrophoresed on a 2% agarose gel. Lane 4: pBR322 Hae III digest. Lanes 1a and b: amplification of HLA DR B alleles (271 bp) 35 cycles of 1 min at 94°C , 1 min at 62°C and 2 min at 72°C .

Lanes 2a and b: amplification of intron 17 BcII polymorphic site of the FVIII gene (142 bp) 40 cycles of 1 min at 94°C , 1 min at 50°C , 2 min at 72°C . Lanes 3a and b: amplification of exon 18 of the von Willebrand factor gene (289 bp) 35 cycles of 1 min at 94°C , 1 min at 55°C , 2 min at 72°C . Superscript a: 1 μl of whole blood in 100 μl of PCR reaction with special denaturation: 3 min at 94°C , 3 min at 55°C , repeated 3 times. Superscript b: 0.3 μg of purified DNA in 100 μl of PCR reaction denaturation: 5 min at 94°C . 2.5 U of Taq polymerase (Amersham) were added in 100 μl of PCR reaction after the initial denaturation step.

Figure 2. Blood sample volume as a factor of the PCR reaction efficiency. All PCR are performed on HLA DQ A (242 bp). Lane 1: pBR322 Hae III digest. Lanes 2 to 5: respectively 1, 2, 3 and 4 μl of whole blood. Special 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.