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A potent inhibitor of *Taq* polymerase copurifies with human genomic DNA

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The polymerase chain reaction (PCR) is a rapid method for amplifying a defined sequence from small amounts of input DNA (1). We have encountered a potent inhibitor of *Taq* polymerase in some samples of genomic DNA extracted from human blood (2). Such DNA fails to amplify but is nevertheless a good substrate for restriction enzymes. We have been unable to attribute the effect to a specific blood anticoagulant. The inhibition is not overcome by banding the DNA on CsCl gradients or by fractionating restriction enzyme digests on agarose gels. Furthermore, amplification of a sample that performed well was blocked when DNA containing the inhibitor was added.

The inhibitor however can be efficiently removed from single stranded DNA by gel filtration. To prevent entrapment, the DNA is first digested with a restriction enzyme that cuts outside the region to be amplified. The sample is then boiled for 5 minutes and immediately centrifuged through a 1ml Sephadex G50 column, preequilibriated in 1mM Tris pH8.0, 0.1mM EDTA. The eluted DNA is suitable for amplification. Neither boiling the DNA nor passage through the column alone abolishes the inhibitor.

This technique has enabled us to obtain high efficiency amplification from all our previously recalcitrant DNA samples. The method is also useful for improving the yield of amplified material from crude blood lysates (3) - in which case predigestion with a restriction enzyme is unnecessary because the DNA is extensively nicked.

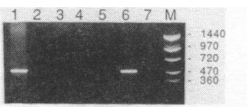

Figure 1. PCR amplification of human DNA samples. Reactions were performed using Cetus *Taq* polymerase and a homemade thermocycling apparatus (4). The conditions used were as recommended by the manufacturers using oligonucleotides to amplify aldolase B exon 2 (5). One third of the reaction product is shown after electrophoresis through a 1.5% agarose gel. The DNA samples used for amplification were as follows :

1. Genomic DNA that was found to amplify efficiently. 2. Genomic DNA that was found to contain the inhibitor. 3. Inhibited DNA digested with Xba1. 4. Inhibited DNA boiled for 5 minutes. 5. Inhibited DNA digested with Xba1 and passed through a Sephadex G50 column. 6. Inhibited DNA digested with Xba1, boiled for 5 minutes and passed through a G50 column. 7. Mixture of inhibited and uninhibited DNAs.

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