The use of exonuclease III for polymerase chain reaction sterilization

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Submitted January 28, 1991

The carryover of previously amplified sequences (amplicons) into new PCR reactions is a serious problem. Recently Furrer et al. reported a ‘pre-PCR’ sterilization technique using DNase I or restriction enzymes for contamination control (1). Unfortunately, these methods require the reaction tube to be re-opened to add target DNA and Taq polymerase following the enzymatic treatment (providing an opportunity for subsequent contamination), and correspondingly, do not address possible carryover contamination from these materials. Furthermore, our results indicate that the endonucleolytic activity of DNase I severely degrades primers.

To overcome these problems, we have developed two alternative protocols for pre-PCR sterilization which utilize exonuclease III (exo III). Exo III catalyzes the sequential cleavage of 5' mononucleotides from the 3' hydroxyl end of duplex DNA. In Protocol I, double-stranded target DNA and all PCR reagents (including Taq polymerase) are incubated with exo III (30°C/30 min) followed by the heat inactivation of exo III (95°C/5 min). Protocol I is based on the size difference between the carryover amplicon and the target DNA, with the much smaller amplicon being more readily digested by the exo III treatment. In Protocol II, the target DNA is first denatured in the presence of the PCR reaction components (100°C/5 min) then quick-chilled on ice. Exo III and Taq polymerase are then added and the mixture incubated (30°C/30 min) followed by heating (95°C/5 min). Protocol II works because exo III does not degrade single stranded target DNA. Following denaturation, the DNA consists primarily of hybrids between the primers and 1) any contaminating amplicon and 2) the genomic target DNA. Upon treatment with exo III, the 3' ends of the amplicons, and the primers which are hybridized to either amplicon or genomic DNA, are degraded; however, the genomic DNA remains largely intact. Since excess primer is used, the small quantity degraded by exo III is negligible. With both protocols, the PCR tubes remain closed between sterilization and the end of the PCR. 30°C is used for the exo III digestion since Taq polymerase is essentially inactive at this temperature. Reciprocally, 95°C inactivates exo III but not Taq polymerase. By selective activation and inactivation, both enzymes can be present during the procedure.

Figure 1A shows that while $5 \times 10^5$ copies of the HIV amplicon were completely degraded by the exo III treatment (lane 2), the same sequence remained amplifiable when integrated into the genomic DNA of HIV-infected cells (lanes 4/6). Fig. 1B shows the results using a linearized plasmid target. Again, $5 \times 10^5$ amplicon copies were degraded by the exo III treatment (lane 4), while the plasmid target (lane 2) remained amplifiable (signal slightly reduced).

The advantage of the exo III sterilization is that it destroys both amplicon and primer dimer carryover. Furthermore, the reaction tubes are not opened until the end of PCR. The success of this method does, however, depend on the quality of exo III (no contamination with endonuclease) and on the integrity of the target DNA (high single strand molecular weight).

REFERENCES


Figure 1. Sterilization with Protocol II. Amplifications were performed for 30 cycles with primer pair SK145/SK431 which provides a 142 base-pair amplicon (2). Cellular target was obtained from HIV-infected H9 cells (provided by Dr. C. Hanson). The infected cells were lysed with proteinase K (120 $\mu$g/ml; 55°C/1 hr) in 10 mM Tris (pH 8.0), 1 mM EDTA, 0.5% Tween 20 and 0.5% NP-40 followed by amplification of the lysates. Plasmid target was pBKH10S (a gift from Dr. J. Rossi), which contains most of the HIV genome. Amplicon target was provided by amplification of the plasmid. PCR products were analysed on a 3% NuSieve/1% agarose gel. A. Results with cellular target. Lanes 1/2: amplicon target ($5 \times 10^5$ copies); lanes 3/4: cellular target (20 HIV infected H9 cells in $10^4$ uninfected H9 cells); lanes 5/6: amplicon ($5 \times 10^5$ copies) plus cellular target. Lanes 1/3/5 were controls; lanes 2/4/6 were treated with 10 units of exo III (BRL). 'M' is the standard 123 base pair ladder. B. Results with plasmid target. Lanes 1/2: amplicon ($5 \times 10^5$ copies) plus plasmid target ($10^5$ copies); lanes 3/4: amplicon target ($5 \times 10^5$ copies). Lanes 1/3 were controls; lanes 2/4 were treated with 10 units of exo III.

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