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VIEWPOINT

Simple Preparation Method of PCR Fragments for Automated DNA Sequencing

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Abstract In an effort to find a simple and inexpensive purification method of polymerase chain reaction (PCR) reaction before cycle sequencing reaction, we compared a commercial system with a precipitation protocol performed in our laboratory. We found that, particularly with small PCR products, our method works with greater success than the method compared. Our precipitation method may be used on a larger PCR fragment before cycle sequencing reaction as well. Furthermore, it has the advantage of being simple as the well-known dilution method; in contrast to the dilution method, the precipitation method removes excess primers as well as possible primer dimers. J. Cell. Biochem. 73:433–436, 1999. © 1999 Wiley-Liss, Inc.

Key words: PCR; sequencing; purification; precipitation

Direct automated DNA sequencing of polymerase chain reaction (PCR) fragments is a procedure used increasingly in basic research as well as in clinical biochemistry and microbiology. In order for this method to be useful, it is desirable to have access to a fast, easy, efficient procedure for purification of the PCR product before the actual sequencing reaction.

A number of high-quality commercial systems are available for purifying PCR products from agarose gels. These systems are fairly expensive and time consuming, however, and require a considerable amount of PCR product. In addition, small PCR products (shorter than about 150 base pairs in length) prepared in this way often do not provide readable sequence data, possibly because of loss of product. We have devised a simplified PCR fragment purification method based on isopropanol precipitation which is inexpensive, fast, and run efficient for PCR fragments of any length.

The PCR reactions were carried out in a total volume of 50 µl containing template DNA, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 0.2 mM of each deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP), 0.4 mM of each primer, and 1 U Dynazyme™ DNA polymerase (Finnzymes, Finland). Subsequently, 20 µl of the PCR product was precipitated with isopropanol in the following manner: 80 µl of water, 6 µl of 5 M NaCl, and 100 µl of isopropanol were added, and incubated for 5 min at room temperature, centrifuged for 15 min at 10,000*g*; after removal of the supernatant, the pellet was washed in 100 µl of 80% ethanol. The tube was inverting several times and centrifugated for 15 min at 10,000*g*. The supernatant was removed, the pellet dried briefly at 37° C for 10 min, and resuspended in 10 µl of water. From another 20 µl of the reaction mixture, the PCR product was recovered by electrophoresis through a 1% agarose gel, followed by excision of the relevant band from the gel, and subsequent purification using a Prep-a-Gene Kit (Bio-Rad Laboratories,

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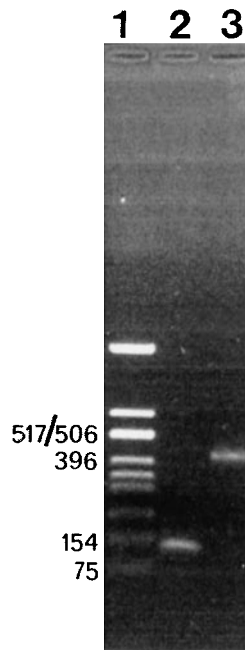

Fig. 1. Lane 1, DNA size markers (pBR322 digested with *Hin*dIII and *Hin*fI). **Lane 2**, an aliquot of 20 µl polymerase chain reaction (PCR) product, using human genomic DNA as template, and the primers (+): 5′-TGTTGCTGCAGATCC-GTGGGCGT-3′ and (−): 5′-GAGGTCACTCACCTGGAGT-GAGC-3′ (nt. 17,561–17,583 and nt. 17,690–17,668 of the human P53 gene). The amplification conditions were 95°C for 30 s, 66°C for 45 s, and 72°C for 90 s for a total of 35 cycles. The last cycle was followed by a 7-min extension at 72°C. **Lane 3**, an aliquot of 10 µl of the PCR-amplified product using mycobacterial chromosomal DNA as template, and the primers (+): 5′- GGATTAGATACCC(C/G/T)(C/G/T)G-TAGTTC-′ and (−): 5′-GACGTC(A/G)TCC(A/C/G/T)C(A/G/T)CCTTCCTC-3′. The amplification conditions were 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, for a total of 25 cycles.

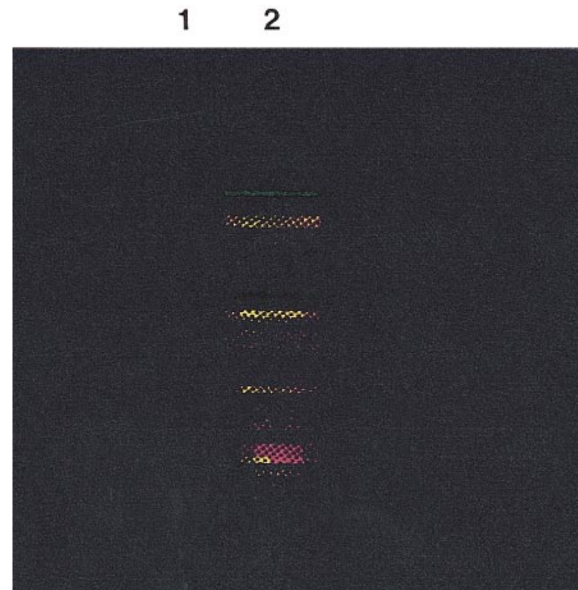

Fig. 2. Sequencing gel image of sequencing reactions performed on a fragment obtained by polymerase chain reaction (PCR) amplification of exon 10 of the human *p*53 gene. **Lane 1**, Prep-a-Gene purified PCR product; **lane 2**, isopropanol-precipitated PCR product.

USA) according to the manufacturer's instructions.

The total amount of DNA from the two preparations made as described above were subjected to cycle sequencing reactions using a Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, USA). The sequencing reaction products were extracted twice with an equal volume of phenol/chloroform (1:1) and analyzed in 6% acrylamide gel containing 7 M urea with an Applied Biosystems 373A Automated DNA Sequencer.

For one of the analyses shown here, 200 ng of human genomic DNA was used as template in combination with primers flanking exon 10 of the *p53* gene [Murakami et al., 1991], resulting in a 131-base pair (bp) DNA fragment (Fig. 1). For another analysis, a colony of mycobacterium obtained from a clinical sample was lysed in 100 µl of TE-buffer (10 mM Tris-HCl (pH 7.6), 1 mM EDTA, pH 8.0). For the 16S rDNA primers, 1 µl of the supernatant was used for PCR in combination with primers [Borre et al., 1994], giving rise to a 411-bp product (Fig. 1).

The sequencing gel images (Fig. 2) show that the Prep-a-Gene purified human PCR product did not give rise to any useful sequence data, whereas the corresponding PCR product purified by isopropanol precipitation resulted in a clearly readable sequence (Fig. 3). Both purification methods gave a mycobacterial PCR product suitable for sequencing (data not shown).

We have used the isopropanol precipitation method described here for different DNA fragments from a number of different organisms and have found it to be generally applicable for fragments up to 5,000 bp in length. The procedure described has the advantages of simplicity, speed, reproducibility, and low cost and is now routinely used in our laboratory. It is suitable only for PCR reactions that do not contain any major fragments other than the expected product. Recently, a procedure com-

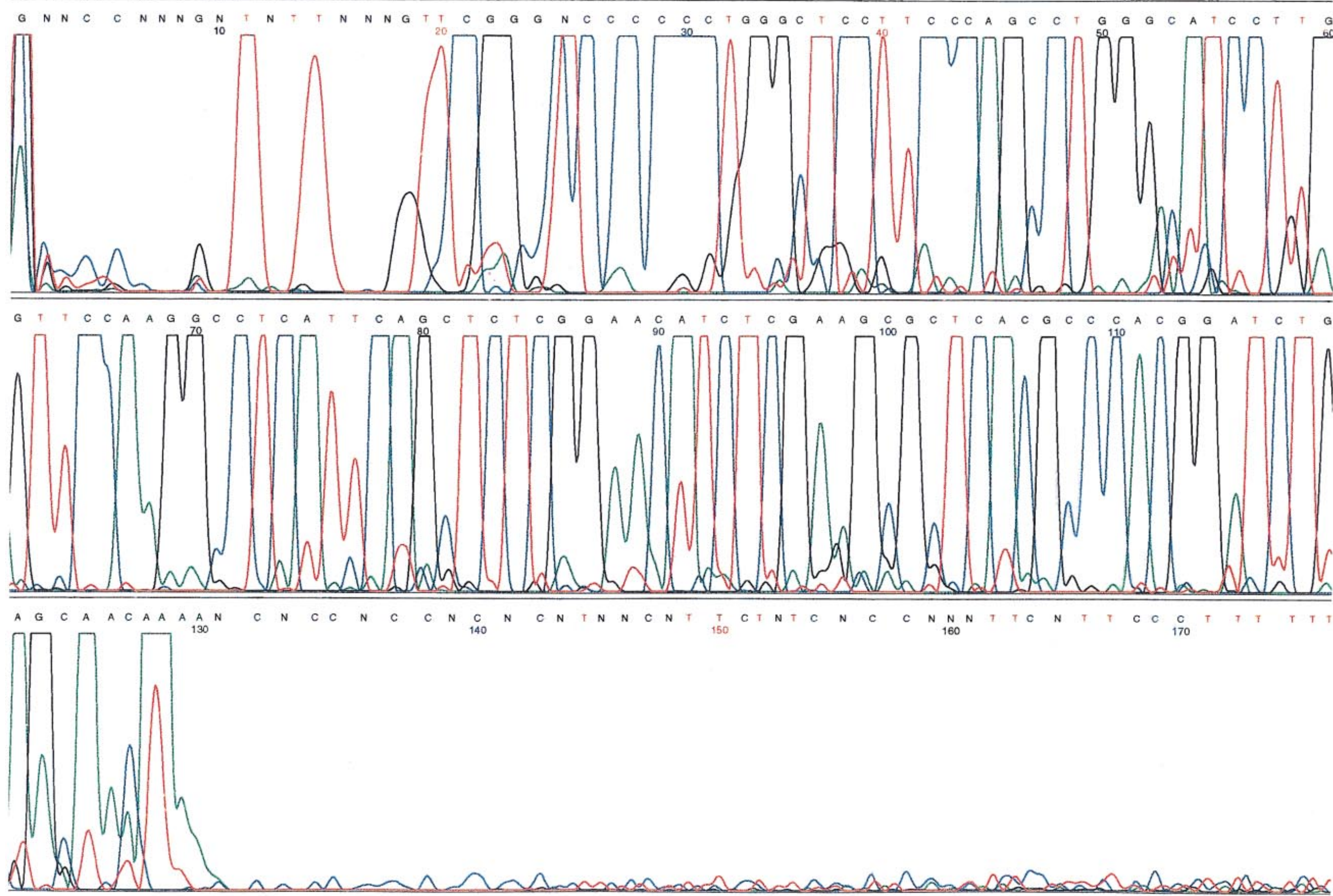

Fig. 3. Chromatogram showing data obtained from a sequencing reaction performed on an isopropanol precipitated fragment obtained by polymerase chain reaction (PCR) amplification of exon 10 of the human *P53* gene.

prising simple dilution of the PCR product as the sole preparation was designed [Kronick, 1995] and is clearly as simple as the isopropanol precipitation. In our experience, however, the latter procedure is superior since it will remove possible primer dimers, unlike the dilution method.

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