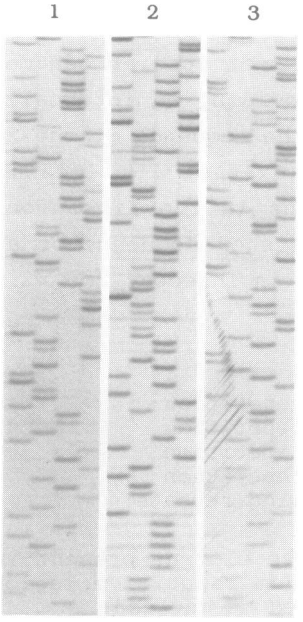
Volume 17 Number 14 1989 Nucleic Acids Research

Direct sequencing from low-melt agarose with Sequenase®

Keith A.Kretz, Geoffrey S.Carson and John S.O'Brien

Department of Neurosciences and Center for Molecular Genetics, University of California, San Diego, La Jolla, CA 92093, USA
Submitted June 6, 1989

The polymerase chain reaction (PCR) technique (1) for the amplification of specific segments of DNA has provided researchers with a powerful tool in the characterization of disease-causing mutations. Direct sequencing of PCR products is preferred and many methods have been described (2,3). These methods are useful but often require time-consuming purification of the PCR product and/or end-labeling primers with ^{32}P. Recently a method was described in which PCR products are sequenced directly using Taq polymerase (4). Here, we describe a method in which PCR products purified in low-melt agarose can be sequenced directly using Sequenase. This allows the PCR product to be prepared for direct sequencing in a single step.



A 20 µl aliquot of the PCR reaction product was purified in 1% NuSieve GTG agarose (FMC) and the band was excised. The DNA-containing gel slice was melted by heating to 70°C for 10 min. 10 µl of DNA were annealed to 5 µl of sequencing primer (10 ng/µl) by heating to 95°C (or placing in boiling water) for 10 min and cooling to 37°C. Sequencing was then performed using the Sequenase sequencing kit (US Biochemicals) according to the manufacturer's instructions except that the labeling reactions were performed at 37°C to prevent the gel from solidifying. Electrophoresis was performed on 6% polyacrylamide/8 M urea (Fig. 1). While overnight exposure is often sufficient, longer exposures are sometimes necessary.

This method is fast, simple, and generates up to 200 bp of readable sequence. In addition to the original PCR primers, degenerate oligonucleotides can be used as sequencing primers (data not presented). We have also sequenced plasmid and phage DNA by this method (Fig. 1).

Fig. 1. Lane 1, a 1 kb PCR product from prosaposin cDNA (5) was sequenced using the 5' PCR primer as the sequencing primer; lane 2, pBluescript (KS II -) with KS sequencing primer (Stratagene); lane 3, M13mp19 with the M13 universal primer (US Biochemicals).

References
1. R.K. Saiki, et al. (1988) Science 239:487-491.
2. D.R. Engelke, et al. (1988) Proc. Natl. Acad. Sci. USA 85:544-548.
3. U.B. Gyllensten and H.A. Erlich (1988) Proc. Natl. Acad. Sci. USA 85:7652-7656.
4. K.B. Gorman and R.A. Steinberg (1989) Biotechniques 7:326-331.
5. J.S. O'Brien, et al. (1988) Science 241:1098-1101.
Sequenase® is a registered trademark of US Biochemicals.