Direct Sequencing of
PCR Products Using
Unlabeled Primers

INTRODUCTION

Directly sequencing polymerase chain reaction (PCR) products without any additional cloning steps shortens processing time and minimizes potential errors due to imperfect PCR fidelity. Although, many direct methods have been described, none are widely accepted because they do not always produce a clean sequence pattern.

We improved the method for producing single-stranded DNA from double-stranded PCR products using lambda exonuclease (2) in four ways. First, double-stranded DNAs with a 5'-phosphoryl terminus on only one strand are produced not only by PCR with the 5'-phosphoryl primer and the 5'-hydroxyl primer, but also by restriction endonuclease digestion of PCR products synthesized with a pair of 5'-hydroxyl primers attached by a restriction sequence. This is useful if amplified samples are to be cloned into other DNAs, and primers with restriction sites have already been synthesized. Second, PCR amplification conditions are optimized to produce clean sequence ladders. Third, the procedure excludes phenol extraction steps, which are the main causes of DNA loss. This greatly increases reliability and reduces the processing time. Fourth, the concentration of 5'-Phosphoryl primer is lowered to reduce the amount of T4 polynucleotide kinase used and eliminate the need for concentrating the primer by ethanol precipitation.

We also performed direct sequencing of double-stranded DNA by the typical snap-cooling procedure (1,3) and the typical asymmetric PCR procedure (4) using the same DNA samples and the same unlabeled primers. The improved lambda exonuclease method was the most reliable and produced the best results.

MATERIALS AND METHODS

DNA Sample

The vicinity of the mouse myelin basic protein (MBP) gene promoter region (5) is used as sample. Three

| A1 5' | TCTGAGTGTGCTACTGCTAC 3' |
| A2 5' | CACGGATCCCTGCTGTTCTGCAGCTACTGCTAC 3' |
| A3 5' | ACAGCAAGTACCATGAGCCA 3' |
| B1 5' | TGCTAGACCCCCATCTCTCT 3' |
| B2 5' | TTACAGAGAGCAGATCCA 3' |

Figure 1. Primers used

Figure 2. Sequence pattern examples produced by the improved lambda exonuclease digestion method. Samples A and B were amplified with 5'-phosphoryl primers (5'-phosphoryl A1 and A3 for A, 5'-phosphoryl B1 and B2 for B). Sample C was amplified with A2 with the BamH1 sequence attached and A3. Amplification conditions were 20 cycles for sample A, 15 cycles for sample B and 20 cycles for sample C. Each amplified sample was digested to single-stranded DNA by the method described in the text and sequenced with 10 pmol of unlabeled A1 for A, B1 for B and A2 for C using 35S-dCTP and Sequenase.