

Simplified method for confirmation of PCR products

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The products of polymerase chain reaction (PCR) (1) are usually visualized by agarose or polyacrylamide gel electrophoresis followed by ethidium bromide staining. However, depending on the purpose of experiment, it sometimes needs to be shown whether the amplified DNA originated from target region or not. In that case a labelled oligonucleotide probe complementary to an appropriate internal sequence between the two normal primers is prepared and used as a probe for Southern hybridization (2). Another method uses a second PCR with another primer in the target sequence (3). The product is verified by restriction endonuclease digestion. However, confirmation by these methods is time-consuming, especially the Southern hybridization analysis. A new method of PCR using three primers is demonstrated in the present report. Amplification of DNA was performed based on the method described by Saiki *et al.* (1). Template DNAs were isolated from *Mycoplasma hominis*, *M.pneumoniae*, and *Escherichia coli* by the method of Marmur (4). Primers were obtained from 3 regions in 16S ribosomal RNA (rRNA) sequence of *Mycoplasma* species (Fig. 1). Two primers, B-1 and Mp-7, were demonstrated to be well-conserved regions among many bacterial 16S rRNAs (5), and used as outer primers in the present study. An inside primer, Mp-6, was shown to be specific for *M.pneumoniae*. A 50 μl of reaction buffer (10 mM Tris-Cl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, pH 8.3) containing 200 μM dNTPs, 100 ng of template DNA, 2.5 units Taq polymerase (Stratagene, California, USA) and 1 μM outer primers (B-1 and Mp-7) was incubated in thermal cycler (Iwaki glass Co. Ltd., Tokyo, Japan). The concentration of the inner primer, Mp-6, in the reaction mixture was 0.1 μM when PCR using three primers was performed. Samples were denatured at 94 C for 2 min. Subsequent rounds of amplification consisted of 1-min denaturation at 94 C, 1-min annealing at 62 C, and 1.5-min extension at 74 C. After 25 rounds of the amplification, samples were incubated for an additional 5 min at 74 C. Two μl of the amplified samples was analyzed by electrophoresis on a 1.5% agarose gel. Amplification with primers B-1 and Mp-7 yielded the expected 952 bp DNA fragment in all templates of *M.pneumoniae*, *M.hominis*, and *E.coli* (Fig. 2, lanes 2–4). In contrast, amplification using 3 primers presented here yielded two DNA fragments (268 bp, 952 bp) in *M.pneumoniae* but in neither *M.hominis* nor *E.coli* (Fig. 2, lanes 8–10). When B-1 and Mp-6 were used in PCR amplification as a control, 268 bp fragment was detected in only *M.pneumoniae* template (Fig. 2, lanes 5–7). This was due to specificity for *M.pneumoniae* of the primer Mp-6. This result indicates that the PCR using 3 primers is useful method for the confirmation of amplified DNA.

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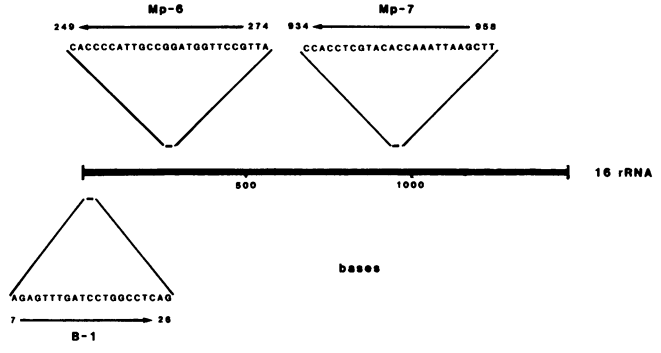

Fig. 1. Location of three primers, B-1, Mp-7 and Mp-6, for PCR amplification on 16S rRNA of *M.pneumoniae*.

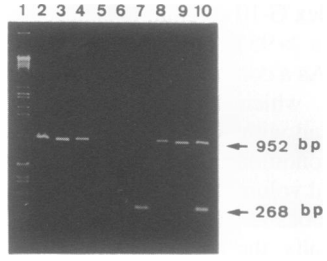

Fig. 2. Analysis of the PCR products by agarose gel electrophoresis. Lane 1 is a molecular weight marker. PCR were performed using B-1 and Mp-7 primers (lanes 2–4), B-1 and Mp-6 primers (lanes 5–7), or three primers (lanes 8–10). DNA extracts from *E.coli* (lanes 2, 5 and 8), *M.hominis* (lanes 3, 6 and 9), and *M.pneumoniae* (lanes 4, 7 and 10) were used as templates.