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Genus- and Species-Specific Identification of Mycoplasmas by 16S rRNA Amplification

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Volume 58, no. 8, p. 2606–2615. In this paper, we have described a mycoplasma genus-specific primer set (primers GPO-1 and MGSO), which specifically amplifies a 715-bp fragment with all mycoplasmal species investigated but not with other species. The specificity of this primer set, as is demonstrated in Fig. 7 in our article, was proven on the rDNA level.

Very recently we have found that when the mycoplasma genus-specific primer set is used for the amplification of rRNA sequences, predominantly a 350-bp product is formed instead of the 715-bp product. Since this unusual phenomenon is observed only on the rRNA level, not on the rDNA level (as can be seen in Fig. 7), it is most probably due to some unusual features or (length) restrictions of the reverse transcription step.

To avoid this problem, we have replaced primer GPO-1 with a new 5′ primer (GPO-3), which is closer to the 3′ primer MGSO. We have demonstrated that amplification with primer GPO-3 in conjuction with primer MGSO results in a polymerase chain reaction product of 270 bp on both the rDNA and rRNA level (see Fig. 1 in our article) and that these primers display the same specificity as was described previously for the primers GPO-1 and MGSO (see Fig. 2 in our article). For confirmation of the polymerase chain reaction with the primers GPO-3 and MGSO, we have used probe GPO-4.

Thus, for amplification of rRNA sequences with the genus-specific primer set, we advise replacement of 5′ primer GPO-1 and probe GPO-2 with the following oligonucleotides, GPO-3 (new 5′ primer) and GPO-4 (new probe).

GPO-3	5′-GGGAGCAAACAGGATTAGATACCCT-3′ IUB *E. coli* 16S rRNA positions 774–798
GPO-4	5′-CTTAAAGGAATTGACGGGAACCCG-3′ IUB *E. coli* 16S rRNA positions 910–933