

One-step quantitative detection system based on CE-SSCP coupled with multiplex asymmetric PCR using common primers via template-tagging

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Rapid diagnosis of bacterial infections has been considered as important criteria for a patient management and an appropriate therapy of bacteria-induced disease in the early phase. Among several techniques, CE-SSCP combined with 16S rRNA gene-specific PCR takes a huge attention due to its benefits such as high sensitivity, resolution and reproducibility. However, as in any other PCR-based technology, multiplex detection and quantification is a major hurdle for this method because of primer dimer formation and non-specific amplification by multiple sets of primers. In this study, we developed a novel technique for multiplex detection and quantification of pathogens by CE-SSCP coupled with multiplex asymmetric PCR using common primers via template-tagging. For linearly amplifying the reversed transcripts of 16S rRNAs from nine septicemia-inducing pathogens, the reverse transcripts were tagged with the common sequence and subsequently amplified by asymmetric PCR using the common primers. After the amplification, the amplicons were separated and quantified by CE-SSCP. The results illustrated the potentials of this method in the diagnosis of the infectious disease.