Precise multiplex gene expression analysis based on MLPA-CE-SSCP

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Quantification of mRNA provides information crucial for various biological studies. Real-time PCR is known to be the most accurate method for quantifying mRNA, and thus represents the state-of-the-art for gene expression analysis. However, the use of real-time PCR for mRNA quantification is limited to a single target per analytical run because of reductions in quantification power and limitations of fluorescence dyes associated with multiplex applications.

Capillary electrophoresis-based single-strand conformation polymorphism analysis (CE-SSCP is an alternative multiplex analysis method. However, CE-SSCP has not been widely used for multiplex applications due to low resolution problem. In this study, we developed high-resolution CE-SSCP system using PEO-PPO-PEO triblock copolymer solution. Moreover, for the multiplex amplification of RNA, modified multiplex ligation-dependent probe amplification (MLPA) was combined with CE-SSCP analysis so that the amount of mRNA could be quantified precisely. We have demonstrated that MLPA-CE-SSCP could be used to monitor expression of 31 metabolic genes of Escherichia coli and 16 genes of Caenorhabditis elegans.