Precise multiplex RNA quantification method based on MLPA-CE-SSCP

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Quantification of RNA provides information crucial for various biological studies. Real-time PCR is known to be the most accurate method for quantifying nucleic acids, and thus represents the state-of-the-art for RNA quantification. However, the use of real-time PCR for RNA 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 RNA quantification method. However, CE-SSCP has not been widely used for multiplex RNA quantification 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 initial amount of RNA could be quantified precisely. We have demonstrated that MLPA-CE-SSCP could be used to monitor expression of 38 metabolic genes of Escherichia coli and 20 Arabidopsis response regulators.