Multiplex mRNA quantification using CE-SSCP

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DNA microarray, a major gene expression analysis tool, gives an expression profile of thousands of genes at one experiment. The product of a DNA microarray experiment is a set of genes which is differentially expressed in a certain condition; the set is called DEG. The screened set of genes can be used for studies such as drug screening and multiplex expression analysis. Currently, Northern blot and real-time PCR are the most popular methods for mRNA quantification. However, Northern blot is known to be laborious and real-time PCR is not suitable for multiplex analysis because of the limitation on fluorescence dye.
Here, we report a novel gene expression analysis method using capillary electrophoresis-single strand conformation polymorphism (CE-SSCP) coupled with multiplex PCR. There are three major steps which are template modification, multiplex amplification, and simultaneous detection. Firstly, mRNA targets are modified for multiplex amplification step. After the first step, all targets have same sequences at their both ends, and the sequences are the sites on which the universal primers hybridize. Second step is multiplex amplification of target using PCR with the universal primer pair. Finally, the amplified targets are separated and quantified by CE-SSCP analysis.