High-throughput Functional Analysis Using Expressional PCR Coupled with PCR-based Site-directed Mutagenesis

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Redesign of the existing natural biological systems requires many components that can be controlled by purpose. Protein functions as well as regulation mechanisms, first of all, should be understood in a systematic manner for well-controlled system. Although many methods are already used to clarify protein functions, they have a limitation on the high-throughput analysis by reason of the labor-intensive and time-consuming process. In this study, we developed a rapid and simple method to analyze protein functions efficiently based on the PCR-based site-directed mutagenesis and the expressional PCR using a coupled in vitro transcription/translation system derived from E. coli and eGFP (enhanced green fluorescence protein) gene as a template.1-2) Various deletion mutants showed different fluorescence activity. The results also showed that this method allows a rapid and simple route for the functional genetics.