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

서상우[1], 박영섭[1], 정규열[1,2,*]
[1]포항공과대학교 화학공학과; [2]I-BIO program
(gyjung@postech.ac.kr*)

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 p68 RNA helicase gene as a template. Various deletion mutants showed different enzymatic activity including ATPase, RNA unwinding, and RNA binding. Consequently, functional domains of each enzymatic function were easily identified and compared with the previous studies. The results also showed that this method allows a rapid and simple route for the functional genetics.