Construction of promoter/operon-trap vector systems for screening of environmental metagenome library

Won-Ho Kim, Jin-Young Lee, Su-Jin Lee, Byung-Ki Hur, and Geun-Joong Kim

Institute of Biotechnological Industry, College of Engineering, Inha University

TEL: +82-32-860-8676, FAX: +82-32-872-4046

The enormous diversity of uncultured microorganisms in soil and other ecosystems provides a potentially rich source of genetic materials, which is critically important for finding new metabolites and enzymes. Screening of useful genes and enzymes from metagenomic library mainly relied on a selection route among nucleotide sequence-based, function-based or substrate-induced gene expression screening. Despite the advance in high-throughput screening technology and selection methods, the discovery of novel gene and natural products remains difficult. Here we introduce a novel route for the isolation of functional genes, expecially promoter and operon, by promoter/operon-trap vector systems. The strategy is based on the knowledge that metabolic genes are generally clustered and thus controlled by its own elements, such as promoter, regulatory genes and proteins. As for efficient screening in high throughput mode, we constructed a double selection system oppositely located and spanned the cloning site by green and red fluorescent protein, available for shotgun cloning that allows for the selection of positive clones in vast quantity of colonies by one or double fluorescence according to the direction of promoter/operon inserted. More sensitive monitorings were realized by introducing a concept polycistronic mode of expression. The feasibility of trap systems was tested for the isolation of novel promoter/operon from metagenome library, resulted in potential pool for practical application. These observations strongly suggested that the trap system can be effectively used as an approach for accessing to novel biocatalysts in natural genetic resources.

Acknowledgements

This work was supported by the Korea Research Foundation Grant (KRF-2002-005 -D00005) and Ministry of Science & Technology Grant (MG02-0301-001-1-0-2).

References

1. Oh, H.J., K.W. Cho, I.S. Jung, W.H. Kim, B.K. Hur and G.J. Kim. (2003), Expanding functional spaces of enzymes by utilizing whole genome treasure for library construction. *J. Mol. Catal. B: Enzymatic.* **26**, 241-250.
2. Handelsman, J., (2004), Metagenomics: Application of genomics to uncultured microorganisms. *Microbiol. Mol. Biol. Rev.*, **68**(4), 669-685.