海底下堆積物メタゲノム中の遺伝子機能探索へ向けた基質誘導性遺伝子発現法 (SIGEX) の改良

Improvement of substrate-induced gene expression (SIGEX) method for exploring gene function in subseafloor sedimentary metagenomes

*溝渕 早紀[1]、若松 泰介[1]、森澤 高至[1]、西川 奈七[2]、寺田 武志[3]、石井 俊一[4]、稲垣 史生[4]、芦内 誠[1]、諸野 祐樹[4]
*Saki Mizobuchi[1], Taisuke Wakamatsu[1], Takashi Morisawa[1], Nana Nishigawa[2], Takeshi Terada[3], Shun’ichi Ishii[4], Fumio Inagaki[4], Makoto Ashiuchi[1], Yuki Morono[4]

1. 高知大学大学院総合人間自然科学研究科農学専攻、2. 高知大学土佐さきがけプログラム、3. マリン・ワーク・ジャパン、4. 国立研究開発法人海洋研究開発機構高知コア研究所
1. Graduate School of Integrated Arts and Sciences, Kochi Univ., 2. Tosa Sakigake Programs, Kochi Univ., 3. Marine Works Japan, 4. Kochi Institute for Core Sample Research, Japan Agency for Marine-Earth Science and Technology

The substrate-induced gene expression (SIGEX) method is a gene-screening approach that uses a promoter trap-type vector based on the assumption that general gene expression is induced by substrates and metabolites of catabolic enzymes and that regulatory elements are often positioned in proximity to catabolic genes [1]. The gene induction response of inserted genome fragment is detected by the co-expression of the gene of green fluorescence protein located at downstream of the inserted sequence, and the induction-positive clone can be isolated by fluorescence-activated cell sorting in high throughput manner. One of the advantages of this SIGEX method is that we can assess the potential function of the gene independently of the sequence (or database) information, and thus can explore unknown function in environmental metagenomes. For maximizing the effectiveness of SIGEX method, constructing largest possible genomic library is critically important.

In this study, we attempted to improve the steps in constructing SIGEX library to obtain genome library with a larger number of inserted sequences and longer fragment size. The environmental DNA extracted from subseafloor sediment sample taken at offshore Shimokita Peninsula, Japan, was inserted into the SIGEX vector conferring evoglow gene that can form matured green fluorescent form of protein in both aerobic and anaerobic condition [2]. By using topoisomerase adapted vector and optimizing ratio of vector to insert, as well as the conditions for electroporation, we could obtain SIGEX library with approximately 1×10^6 clonal variation with >800 bp of inserted DNA sequences. We performed gene-induction incubation by adding various organic compounds including halogenated phenol mixture, which showed induction of 0.078% of clones at 0.5 mM in concentration. In the presentation, we will show the detail of methodological improvements together with the sequence analysis results obtained from induction-positive clones.

[1] Uchiyama *et al.* (2005) *Nat. Biotechnol.*, **23**, 88-93 [2] Drepper *et al.* (2007) *Nat. Biotechnol.*, **25**, 443-445

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