近畿大学工学部研究報告 No. 37, 2003年, pp. 53-57
Research Reports of the School of Engineering,
Kinki University No. 37, 2003, pp. 53-57

深海二枚貝シロウリガイの遺伝子ライブラリーの作製

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Construction of genomic library for deep-sea bivalve, *Calyptogena soyoae*

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The search for drugs from the deep-sea has become one of most exciting branches of marine sciences, combining the fields of biology, chemistry and pharmacology. In order to search genomic resources from deep-sea animals for medical and biotechnological materials, we have started constructing several genome libraries of deep-sea animals. In this study, we present construction and analysis of genomic library of deep-sea bivalve, *Calyptogena soyoae*. In addition, we discuss the methodology of the constructing the library. The library constructed in this study will give the useful resources and information in not only application such as biotechnological or medical fields, but also basic studies on adaptation of the animals to deep-sea environment.

Keywords Genomic library, deep-sea bivalve, *Calyptogena soyoae*

1. Introduction

Chemical compounds obtained from marine organisms or marine natural products, have potential for use in both medicine and biotechnology. For instance, the medical use of marine natural products by Eastern area such as Chinese, may go back a few thousand years, but such use in the developed countries has been limited. Recent years, however, have seen a dramatic upsurge in the systematic collection, analysis and investigating new material. In addition, the search for drugs from the deep-sea has become one of most exciting branches of marine sciences, combining the fields of biology, chemistry and pharmacology.

In order to search genomic resources from deep-sea animals for medical and biotechnological materials, we have started constructing several genome library of deep-sea animals. In this study, we present construction and analysis of genomic library of deep-sea bivalve, *Calyptogena soyoae*.

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2. Materials and methods

2.1. sampling sites

The survey of chemosynthetic communities was conducted at hydrothermal vents in the northern knoll of Iheya Ridge (within two miles radius from 27° 47.00'N, 126° 54.00E, approximately 1,000m in depth) and around so-called "Clam site" [1, 2] in Iheya Ridge, which has been known and studied since 1988, (within two miles radius from 27° 33.00'N, 126° 58.00E, approximately 1,400m in depth) [x, y]. The chemical composition of hydrothermal fluid at North Knoll of Iheya Ridge has been reported by Chiba et al [2]. The observation and sampling of chemosynthetic communities were carried out by using the manned submersible "Shinkai 2000". Five dives of "Shinkai 2000" were conducted during the cruise of the support vessel "Natsushima" (NT97-14) from September 20 to 28, 1997 (Table 1). Animals in chemosynthetic communities were sampled by manipulator of "Shinkai 2000" directly and the samples were frozen at –80°C on "Natsushima".

2.2. Isolation of chromosomal DNA of the deep-sea bivalve

High molecular weight chromosomal DNA of the deep-sea bivalve (Fig. 1), *Calyptogena soyoae*, was isolated using G NOME DNA isolation kit (BIO 101, La Jolla, CA) according to manufacture's instructions.

2.3. Construction of a lambda phage library of the deep-sea bivalve

A genomic library was constructed by ligating *Sau*3AI partially digested deep-sea bivalve, *Calyptogena soyoae* genomic DNA (10–20kb) into the *Bam*HI site of lambdaDASHII (Stratagene Co., La Jolla, CA). Then, *in vitro* packaging of the ligated DNA was performed using GIGAPACK III XL packaging extracts (Stratagene Co., La Jolla, CA) according to the modified version of manufacture's instructions [3]. The lambda phage genomic library was screened for plaque hybridization with the probe and several positive clones were obtained. The positive clones were each purified by several single plaque isolation steps. Each of the inserts in lambda phage was amplified by long PCR and was subcloned into the pCR-Blunt vector (Invitrogen Co.). For sequencing of these cloned fragments, the random shotgun sequencing method was used with a DNA sequencer model 377 (Perkin-Elmer/applied Biosystems Co.). Assembling and editing of the determined DNA sequences were performed with AutoAssembler Version 2.0 (Perkin-Elmer/Applied Biosystems Co.). GENETYX-MAC version 10.1 from software Development (Tokyo, Japan) was used for sequence analysis.

Table 1 Dive list of "Shinkai 2000" at Iheya Ridge (from Sep. 21 to 26, 1997)

Dive No.	Dive areas	Depth (m)	Deepest (m)
975	North Knoll in Iheya Ridge	1,027	1,061
976	North Knoll in Iheya Ridge	988	990
977	Clam sites	1,396	1,406
978	North Knoll in Iheya Ridge	992	1,008
979	North Knoll in Iheya Ridge	1,072	1,073

3. Results and discussion

3.1. Sampling the deep-sea bivalve, *Calyptogena soyoae* from North Knoll of Iheya Ridge

At least 27 species were sampled in North Knoll of Iheya

Ridge during four dives of "Shinkai 2000" (Table 1). Out of several species, the deep-sea bivalve, *Calyptogena soyoae* are obligate species of chemosynthetic communities and thought to have chemosynthetic symbiont bacteria [4]. In order to search genomic resources from deep-sea animals for medical and biotechnological materials, we have started constructing several genome libraries of deep-sea animals (Fig. 1). Prior to constructing the genomic library, high molecular weight chromosomal DNA of the deep-sea bivalve (Fig. 1), *Calyptogena soyoae*, was isolated using G NOME DNA isolation kit (BIO 101, La Jolla, CA) according to manufacture's instructions.

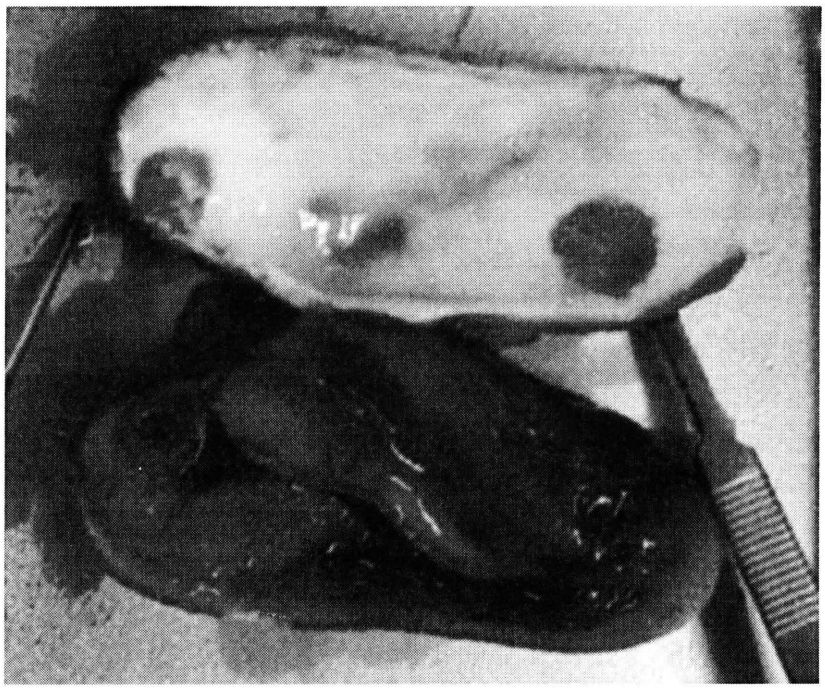

Fig. 1 Anatomy of deep-sea bivalve, *Calyptogena soyoae* .

3.2. Construction of a lambda phage library of the deep-sea bivalve

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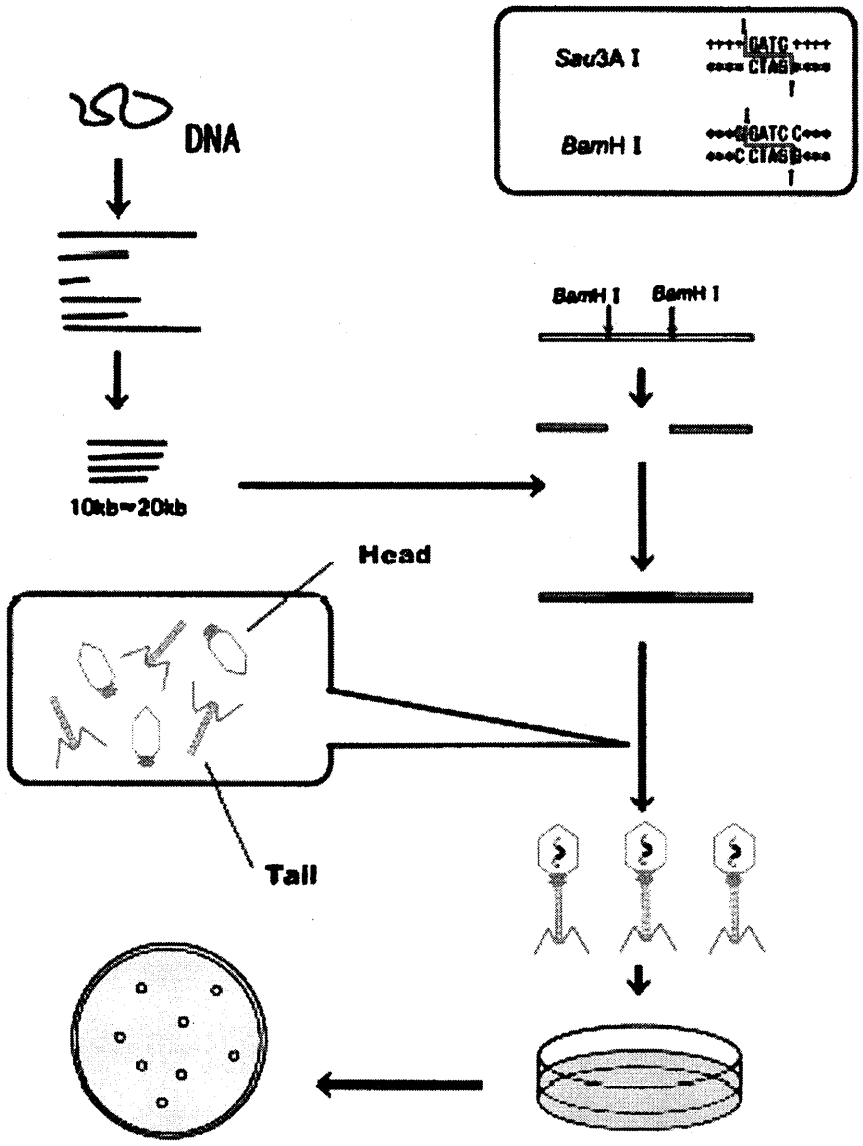

Fig. 2 Principle of constructing genomic library of the deep-sea bivalve, *Calyptogena soyoae*

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