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Introduction to Resource Center No.17

National BioResource Project "C. elegans"

Shohei Mitani School of Medicine,
Tokyo Women's Medical University

Ongoing Column No.24

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Cell Technology initiated a new series entitled "Let's Use! BioResource". Bioresources in Japan will be introduced in a series of 14 volumes from November 2006 to December 2007.

Introduction to Resource Center No.17

National BioResource Project "C. elegans"

Shohei Mitani
School of Medicine, Tokyo Women's Medical University



 1

The history of *Caenorhabditis elegans* bioresources shows the history of researchers' community. Most of the *C. elegans* mutant strains that Dr. S. Brenner initially used for his research at Cambridge University, England, are also available at the *Caenorhabditis* Genetics Center (CGC). The CGC is located in the US and presently cryopreserves the mutant and transgenic strains whose data were published in journal articles. The strains requested through means such as emails to the persons in charge will be shipped within 2 to 3 weeks.

 2

As the Core Institute of the National BioResource Project at the School of Medicine, Tokyo Women's Medical University, we collect, preserve, and provide *C. elegans* strains. Researchers who do not handle *C. elegans* may wonder why there is a need to establish another resource center since the CGC already exists. With other species, it would be favorable to establish stock centers at multiple locations to back up the strains. However, in this case, the idea of redundantly preserving CGC strains at other institutes is unheard of. We independently collect, preserve and provide mutant strains to the research community while taking into consideration their requests. In other words, the CGC focuses on accumulating the results of past achievements, while the National BioResource Project in Japan focuses on supporting the initiation of future researches on *C. elegans*.

 3

The project was initiated by genome sequencing. The whole genome sequencing of *C. elegans* was completed before any other species in 1998. However, the *C. elegans* research community was already planning for post-genome analysis before that. Genome sequencing teams at the Sanger Centre and Washington University were publishing the sequenced data in the order of precedence, and many research groups began using the sequence information even before the completion of sequencing. They were exploring the means of conducting their researches after the completion of sequencing. One idea was to isolate mutants using reverse genetics and elucidate gene functions. Numerous research groups competed fiercely to develop a technology to isolate mutant strains. Our group successfully developed a technology to isolate the deletion mutants of *C. elegans* in our laboratory. This was considered a promising means to supply useful mutant strain resources to researches all over the world; this achievement led to the initiation of the project in the summer of 2004. Subsequently, we have been collecting and preserving deletion mutants at the rate of approximately 500 mutants/year and releasing the mutant strain database on the website of the National BioResource Project.

Mutant strain database of NBRP *C. elegans*
<http://shigen.lab.nig.ac.jp/c.elegans/>

The number of mutant strains whose data is released by us is the highest in the world for a single laboratory; this number exceeds the total number of strains whose data is released by the groups in the US and Canada that had initiated the project approximately 5 years ahead of us. In this issue, we would like to briefly introduce some of the experimental methods (collection and preservation) that we actually follow in our laboratory.

 4

First, I will describe the cryopreserved stock of the *C. elegans* mutant strains that we already possess. Dr Brenner was able to cryopreserve *C. elegans* in the early stages of his research, which is a characteristic of *C. elegans*. Since then, it has been known that the larvae of *C. elegans* can be semi-permanently preserved if they are suspended in a cryopreservation liquid containing glycerol and frozen with the same methods as cell lines. We developed a method that uses trehalose instead of glycerol. The use of this cryopreservation liquid further improved the preservation conditions. This method helps in achieving a higher recovery rate and is significantly advantageous for research on mutant strains. *C. elegans* can be grown on agar media with *Escherichia coli* as food. Figure 1 shows that a large number of individual organisms can be cultured in a low-temperature incubator by preparing a large amount of media. After culturing, the mutated *C. elegans* strains are washed with M9 buffer and the samples from a plate distributed to each tube of a 96-well format and cryopreserved (Fig. 1 (2)). *C. elegans* can be stably preserved for several years



(1) Cultivation



(2) Collection



(3) Cryopreservation

Fig. 1



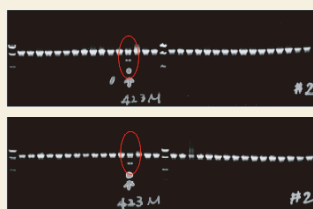
Fig. 2



(1) Processing by a dispensing robot

(2) Agarose electrophoresis for 96 samples

5 Small amounts of DNA are retained from each tube, and DNA mixtures are prepared stepwise for the collection of mutant strains. These DNA mixtures are used to prepare templates for polymerase chain reaction (PCR) by using a dispensing robot. This helps in efficiently accomplishing the process of PCR to electrophoresis by using a 96-hole format (Fig. 2). Often, when searching for deletion mutations by using a highly sensitive PCR, only faint bands of mutant strains are first observed in addition to the amplified bands of wild-type strains since the samples contain DNAs from numerous tubes (Fig. 3 (1)). We always conduct each experiment twice in order to distinguish the targets from artifacts. When we found positive results, we go back to the original DNA solution before mixing, and rerun the PCR again (Fig. 3 (2)). The second time, the bands of wild-type strains appear faint, whereas those of mutant strains are rather distinct. In this manner, the original culture plates can be correlated.



(1) The first screening



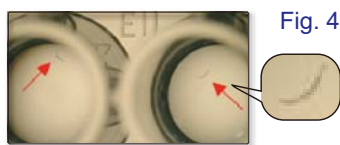
(2) The second screening

Fig. 3

6 Once culture plates are obtained, the tubes are thawed and the *C. elegans* culturing initiated. The *C. elegans* strains include many wild-type and a few mutant strains. A deletion mutation is first introduced only in a single chromosome and thus the mutants are diluted during culturing. We culture *C. elegans* in a liquid medium in a 96-hole format by using a nematode sorter (Fig. 4). In this manner, subsequent manipulations can also be carried out by using multichannel pipettes and robots. Once *C. elegans* is proliferated by liquid culturing for approximately a week, a fraction of the culture is extracted and solubilized to search for mutant strains by PCR by using the DNA as template. After the extraction of individual mutant strains, quality checks such as determining the sequence of the deletion sites are performed, and the data on these strains are released on the websites of the National Institute of Genetics.



(1) Extraction of individual by using a nematode sorter



(2) Individual cultivation with 96-hole plate

Fig. 4

7 We aimed to acquire data from living organisms at as high a throughput as possible. The completion of genome sequencing realized the acquisition of a dramatically greater amount of information in the field of life science. In order to further accelerate the speed of data acquisition, it is necessary to store the actual phenotypes of organisms and scientific truths that are experimentally confirmed via gene modification. In this regard, *C. elegans* is a sufficiently simple, multicellular organism of which many strains are available. Further, *C. elegans* harbors numerous orthologs of human genes. Thus, we hope to play a role in advancing the most fundamental subset of medical research and wish to distribute mutant strains and information regarding them on a daily basis.

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Let's subscribe to RSS!

Recently, websites that provide information through the internet has started distributing RSS (Really Simple Syndication). Since last summer, SHIGEN has also started distributing RSS to deliver information regarding new resources and services efficiently. In this issue, I would like to introduce how to subscribe to RSS feeds using Internet Explorer 6 (IE6).

- Installing the Plug-in -

IE6 does not have RSS functionality and you will need to install a plug-in to use RSS. We will be installing a free software called RSS bar, which is developed by Darksky.

- Click on "RSS (bar)," which appears in the left menu of "Darksky Website" (<http://darksky.biz/>) and click on "Download" in the upper tab. Download "RSS Bar for Internet Explorer 1.30" (as on 5/25/2007) and unzip the zip file.
- If your IE6 browser is in use, please close it. Run the file named "Setting.exe," which is located within the unzipped folder. Once the file is run, the installer is initiated and a panel is displayed; press "Setup" to complete the task. Please also refer to the "Darksky Website," which explains the installation method.
- Restart IE6 and click on "View→Explorer Bar→RSS Bar" to display the RSS side bar on the left.

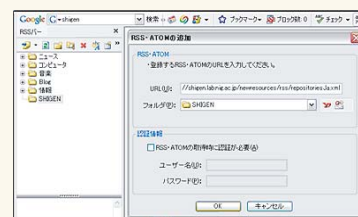
- Subscribing to RSS -

Next, let us subscribe to SHIGEN's RSS feeds.

- Open the web page of New Resources of SHIGEN. (<http://shigen.nig.ac.jp/newresources/newResourcesListFormAction.do>) The "RSS:SHIGEN-R" and "RSS:SHIGEN-S" logos are displayed next to the "New repositories" and "New services/sites" on that page. Please click on the "RSS:SHIGEN-S" logo.

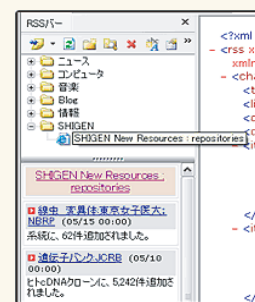


- Copy the address of the displayed page to register it with the RSS bar. Click on "Add RSS-ATOM" on the top left of the RSS bar. Paste the previously copied address of "RSS:SHIGEN-R" onto the URL textbox in the "Add RSS-ATOM" window.



Select "Root" from the pull-down menu of the folder and click on "OK."

- A "SHIGEN New Resources" file will appear on the RSS bar. If the RSS page is displayed at the bottom when you click on the file, you have successfully subscribed to our RSS feeds. To check for new feeds, you can either click on the file or click on the "renew" button on the RSS bar.
- You can use the same procedures to subscribe to "RSS-SHIGEN-R". In addition, please refer to the website below to learn how to subscribe to RSS with IE7, Firefox2.x, Safari and Google Reader.



URL : <http://www.shigen.nig.ac.jp/shigen/about/rss.jsp>
(Japanese only)

(Tohru WATANABE)

Editor's Note: *C. elegans* is an excellent model in the age of high-throughput science, isn't it? The consensus for commoditizing bioresources and information is widespread among the communities of *C. elegans* researchers, possibly due to the frequent use of *C. elegans* for basic researches. The discussion of MTA(2) by Dr. Suzuki, which was supposed to be covered this month, will be introduced next month. (Y.Y.)

Contact Address:

1111 Yata, Mishima-shi, Shizuoka 411-8540, Japan
Center for Genetic Resource Information, National Institute of Genetics
Tel: 055-981-6885 (Yamazaki)
E-mail: BRnews@chanko.lab.nig.ac.jp

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