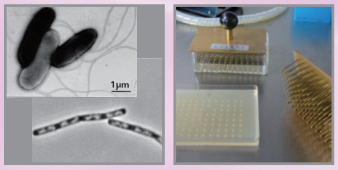
BioResource Now !

Issue Number 7 December 2011



Introduction to Resource Center〈NO.39〉

BioResource Center for *E. coli* and *B. subtilis*, model prokaryote organisms

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Ten years have passed since the inauguration of the National BioResource Project (NBRP). At the beginning of the project, our primary bioresources were mutant *Escherichia coli* strains at the Genetic Stock Research Center, National Institute of Genetics (NIG). Since then, we have obtained resources in the post-genome era that were developed using genomic information. These resources include a collection of *E. coli* strains that contains a mutant for each of its genes. In addition, we have collected knockout *Bacillus subtilis* strains and are now send more than 200,000 samples to researchers across the globe every year (Fig. 1). NIG has been primarily responsible for preserving and distributing the resources, while Kyushu University has been preserving a set of the resources as a protective backup.

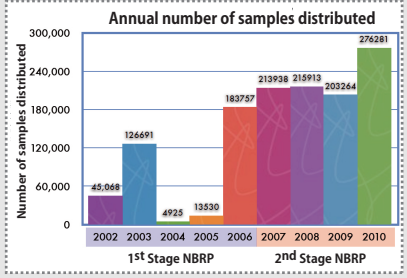


Fig. 1: Number of Samples Distributed Each Year

Genome-wide bioresources of *E. coli* and *B. subtilis*

Development of deletion mutants for all *E. coli* and *B. subtilis* genes began immediately after completion of the sequencing of their genomes. As estimated from the gene sequences, each bacteria has over 4,000 genes. By deleting the genes one by one, we have been able to determine which genes are essential for growth.

There are 2 collections of *E. coli* knockout strains. In one collection, each gene region was replaced by drug-resistance genes (KEIO collection). In the other collection, transposons were inserted into each gene. Obviously, knockout strains of essential genes cannot be developed by replacing them with drug-resistance genes.

In order to resolve this issue, transposon insertion–deletion mutant collections have been developed by partially duplicating a chromosome region and inserting transposons into a gene in the duplicated region. The duplicated region can then be eliminated by recombinant reactions. This method is useful in that the recombinant strains can be selected by culturing the strains at a temperature of 37 °C or more, and the effect of the gene deletion can be evaluated.

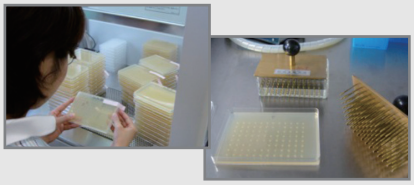
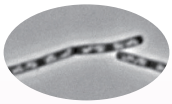


Photo: Replication of the collection of mutant strains for all genes. For delivery, 96 deletion mutant strains are simultaneously inoculated on plates by using a pinholder.

B. subtilis knockout strains have been developed by artificially inserting drug-resistance genes into each target gene by using a recombination technique. However, only about 2,000 mutant strains are currently available. Mutant strains for about 1,900 genes are not yet included in the collection. Completion of this collection is one of the important future tasks for the resource center.



Is *E. coli* so different from *B. subtilis*?

E. coli and *B. subtilis* are both prokaryotes, but they are significantly different from each other. *E. coli* normally inhabits the colon, whereas *B. subtilis* is a soil bacterium that belongs to the same species as *B. natto*. *B. subtilis* forms spores under nutrient deprivation. Both species are closely associated with human life, but they exhibit completely different biological life cycles. In fact, the genetic composition of these bacteria indicates that there are only a few genes that are common to both species and that most of the genes are unique to one species or the other (Fig. 2).

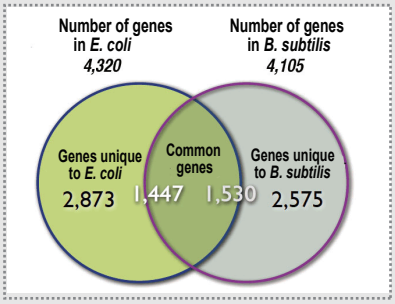
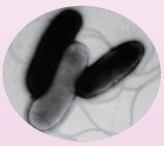


Fig. 2: Common genes in *E. coli* and *B. subtilis* The number of common genes in *B. subtilis* is higher than that in *E. coli* because of the duplication of *B. subtilis* genes.

Prokaryotes are not so simple that the gene functions of all prokaryotes can be determined by analyzing these 2 bacteria. Nevertheless, prokaryote genome research has progressed because of research conducted on these 2 prokaryotes, as their gene functions have been empirically studied more than those of any other species. Therefore, *E. coli* and *B. subtilis* bioresources are indispensible research materials for many prokaryote researchers.

Well-developed collections of protein expression vectors

Many researchers who do not necessarily research *E. coli* itself use *E. coli* as research material. *E. coli* is frequently used to express proteins. Multitudes of expression vectors for *E. coli* have been developed. More than 400 types of vectors, a fraction of the developed vectors, are distributed by our resource center. Readers of this newsletter may be able to find a vector that is useful for their experiments.



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Enhancement of new resources

One of our major goals is the enhancement of *B. subtilis* resources by developing more knockout strains (Table 1). In addition, we have been collecting *E. coli* and *B. subtilis* strains with the minimal gene sets and are preparing for the release of these strains. These strains have been developed by eliminating unnecessary gene regions in the genome so that introduced genes can be expressed more efficiently. We have developed both *E. coli* and *B. subtilis* strains in which more than 1 Mbp of nucleotides were eliminated from the genome. These strains will soon be available for distribution. We have been developing a resource center that distributes *E. coli* and *B. subtilis* bioresources, which are representative species of the gram-negative*1 and gram-positive*2 bacteria, respectively. We sincerely hope that the readers will find useful information from our website and use it in their research.

Website for *E. coli* bioresources
http://www.shigen.nig.ac.jp/ecoli/strain/
Website for *B. subtilis* bioresources
http://www.shigen.nig.ac.jp/bsub/

*1 Gram-negative bacteria: Bacteria that stain red or pink-red (negative) on Gram staining (a method to distinguish bacteria into 2 large groups by staining with a dye). Gram-negative bacteria have double-layered thin cell walls and include approximately 80 genera, such as *E. coli*, *Vibrio cholera*, *Neisseria gonorrhoea*, and root nodule bacteria.

*2 Gram-positive bacteria: Bacteria that stain indigo-blue or blue-purple (positive) on Gram staining. Gram-positive bacteria have single-layered thick cell walls without outer membranes and include approximately 60 genera, such as staphylococci, *Clostridium botulinum*, *B. subtilis*, and lactobacilli.

NBRP prokaryote genetic resources
(*E. coli* and *B. subtilis*)

Mutant strains
- Auxotrophic strains (amino acids and nucleic acids)
- Cell growth mutant strains (cell division mutants, etc.)

Knockout mutant strains
- KEIO collection (deletion mutants with gene displacement)
- Transposon disruption strains (insertion mutants)
- Wide deletion strains (several to tens of kbp deletion mutants)
- Collection of *B. subtilis* deletion mutants (insertion mutants)

Cloned genes
- Mobile plasmid collection
- ASKA library

Hosts and vectors for recombinant DNA
- Host *E. coli* strains
- Expression vectors for *E. coli*

Table 1: Overview of prokaryote resources

From Internet Explorer 6 to a New Browser

Ongoing Column [No. 64] 10 min

Ten years have passed since the release of the Internet Explorer 6 (IE6) in 2001. I wonder how many of the readers still use IE6 and I recommend that those readers think about switching over to a new browser.

Microsoft, the developer of IE, opened a campaign site called "The Internet Explorer 6 Countdown" (http://www.ie6countdown.com/) that aims to reduce the number of IE6 users to 1% of the total number of IE users. The website shows the number of IE6 users in the world.

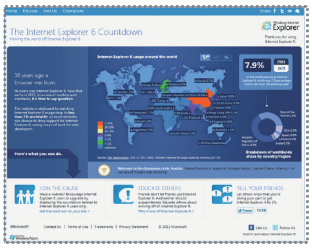

Fig. 1: The Internet Explorer 6 Countdown

According to the website, as of October 2011, IE6 was still used by an average of 7.9% users across the globe. In Japan, 6.8% of users have IE6, a lower percentage than the global average. However, the percentage of IE6 users is relatively high in East Asia compared to America and Europe.
Update program support for IE6 Service Pack (SP) 3 is scheduled to continue until April 8, 2014. However, the National Information Security Center instructed government agencies to facilitate the transition from IE6 to IE8 in their report, "Activities regarding the transition from old to new browsers.

Reference: http://www.nisc.go.jp/press/pdf/browser_transition_press.pdf (Japanese only)

In addition, the new browser is more user-friendly, has higher security than IE6, and runs significantly differently than IE6. Moreover, because supporting IE6 is costly, support for IE6 will soon be terminated, and various services are unavailable in Yahoo! JAPAN and Google to users of IE6.

The new browser has a tab function that prevents computer screens from becoming inundated by many newly opened windows. The new browser can also block users' access to phishing websites or to sites that let users download malicious software.

Fig. 2 : Attempting to access YouTube (http://www.youtube.com/) with IE6 will prompt the warning message, "Your browser is no longer supported. Please upgrade to a modern browser."

Windows XP users can upgrade their browser to IE8 using the following procedure:
① First, ensure the operation system of the computer is Windows XP SP2 or SP3.
② Download the file for IE8 from the Windows IE8 for Windows XP website (http://windows.microsoft.com/en-US/internet-explorer/downloads/ie-8)
③ Run the downloaded file to install IE8.

The websites released by our information center will load faster if the new browser is used. Therefore, I highly recommend that IE6 users take this opportunity to switch to the new browser.

(Gaku Kimura)

Announcements

(Details are available at http://www.nbrp.jp/)

10th anniversary of the NBRP
National BioResource Project achievement report open session

Date and Time: 1300–1700 on January 20 (Friday), 2012
Place: Main auditorium B at 5F, Tokyo Conference Center Shinagawa
Fees: Free of charge
Pre-registration: Required

Download the PDF version of this newsletter at
http://www.shigen.nig.ac.jp/shigen/news/

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BioResource Information

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(JGR) www.shigen.nig.ac.jp/wgr/jgr/jgrUrlList.jsp

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Editor's Note

The distribution of *E. coli* resources has increased rapidly since its genome resources have been added to the collection. It is not surprising that *E. coli* attracts more attention than other prokaryote species, given the number of people who use our Resource Integrated Search Site (BRW) and the number inquiries at our information center. The courteous service of *E. coli* staff members allows us to appreciate the spirit of good service from a resource center that has been maintained over the years. Fortunately, the final newsletter of 2011 was released without any trouble. Although we felt as if time had stopped after the March 11 disaster this year, let us hope for the best in coming year. (Y. Y.)

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