BioResource Now !

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Introduction to Resource Center〈NO. 50〉

National BioResource Project Mice (ENU-mutagenesis)

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The mouse has been used as an experimental animal for more than 100 years and, accordingly, an enormous amount of information about its genes and diseases has been accumulated. Since many genetically homogeneous inbred mouse lines have been reared, animal experiments can now be performed with high reproducibility. In addition, owing to its small size and well-characterized genome, advanced genetic manipulation techniques can be applied to this mammal model at an individual level, and experiments and research carried out from the initial to the final stages of disease development can be tracked at the molecular level, which cannot be accomplished using human subjects. Thus, mice have been widely used worldwide as typical model animals for humans for both fundamental research and direct medical applications.

The RIKEN BioResource Center (BRC) was established in 2001. Since then, the Experimental Animal Division has collected, preserved, and provided mice for research purposes. In 2002, the RIKEN BRC launched its activities as a core institution of the National BioResource Project. To date, the RIKEN BRC has collected more than 7,000 mouse strains and has provided strains developed in Japan to 950 organizations within Japan and 36 other countries. When an article on the BRC was first published in BioResource Now! in December 2005, the number of mouse strains maintained was 1,600. Therefore, the number of strains has increased by more than four times during these past eight years. Many genetically modified mouse lines have been produced for research on cancer, the brain, allergies, development, and tissue regeneration in Japan, and high-quality studies have been performed using these mice. Owing to the support and contribution of these high-level research communities, in the past decade, the RIKEN BRC has collected approximately the same number of mouse strains as those contained in similar resource organizations in Europe and the United States (Fig. 1, Fig. 2).

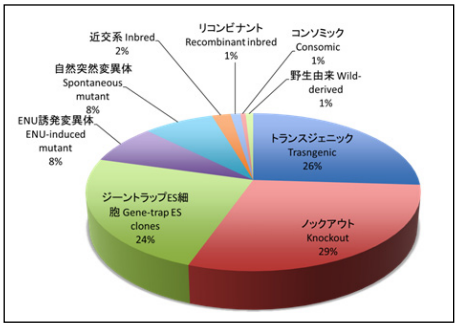

Fig. 1: Types of collected strains

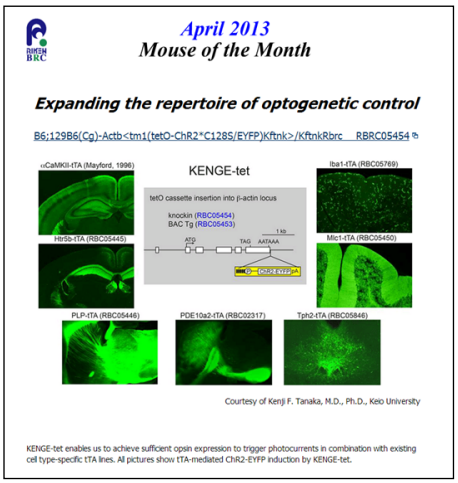

Fig. 2: Mouse of the Month, an article introducing collected strains

The quality of materials used for research is one of the most important factors for ensuring reproducibility of the research results. The quality control procedures for mouse resources consist mainly of microbiological monitoring and genetic monitoring. With respect to microbiological monitoring, 24 items, including documenting the main pathogenic microorganisms that could affect the experimental results, have been regularly examined using decoy mice, and the results of these evaluations have been published on the homepage of the Experimental Animal Division.

Until 2013, mouse hepatitis virus and *Mycoplasma pulmonis* were occasionally detected during monitoring of mice that were deposited from universities and research institutions. Such contaminations were not observed in 2014. However, intestinal protozoa and threadworms are still detected in nearly 50% of mice. Therefore, we are concerned about the spread of intestinal protozoa and threadworms among research colonies in Japan. The BRC has performed caesarean operations and embryo transfer to eliminate microbial contaminations and can therefore provide high-quality specific pathogen-free (SPF) mice with confidence.

Experiments involving genetic manipulation for specific cells, tissues, or organs at arbitrary time periods in mice have been successfully conducted using Cre-loxP*1 or TET*2 technology. From the standpoints of the reproducibility of animal experiments, 3R*3, and legal compliance regarding the handling of genetically modified organisms, the genetic quality of genetically modified mice must be precisely controlled. However, it is difficult to maintain the quality of genetically modified mice at the high level required for research. At sites where research is performed by breeding multiple transgenic and knockout mice, the genetic quality of many types of genetically modified mice must be quickly and exhaustively controlled. The KO-survey polymerase chain reaction (PCR) method [1] (Fig. 3) is used to exhaustively examine various transgenes, and the loxP/Frt-survey method is used for structural examination of the gene construct of a conditional knockout mouse; thus, the BRC can accurately judge the genotype of a genetically modified mouse and provide accurate information about genetically modified organisms.

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*1 Cre is a recombinase (a recombination enzyme) that recognizes a specific DNA sequence and recombines it. When two loxP sites are present in the same direction, Cre can cut the region between these two sites. Thus, the expression of a gene can be manipulated by exploiting this characteristic and determining the control regions of Cre and loxP.

*2 By administering tetracycline, the expression of a gene can be freely controlled.

*3 Replacement, reduction, and refinement (3Rs) are the main factors required internationally when performing an animal experiment.

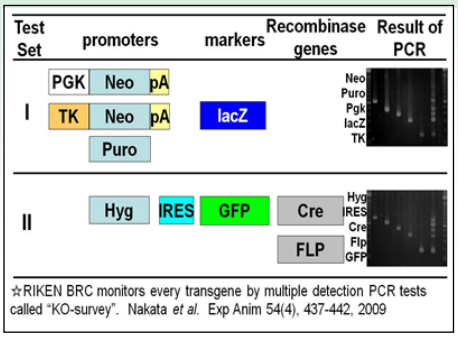


Fig. 3: KO-survey PCR method

The specific strain used for genetic manipulation is known to affect the experimental results. With recent advances in analytical methods for phenotypes and improvements in the measurement accuracy of equipment, phenotypic differences between some substrains that are genetically similar, such as C57BL/6J and C57BL/6N, have been revealed. Therefore, substrains must be appropriately discriminated to ensure accurate experimental results. Single nucleotide polymorphism (SNP) markers have been used to effectively discriminate substrains. At present, the genetic differences among inbred C57BL/6 mouse strains supplied from breeders from all over the world can be readily discriminated using these SNPs [2] (Fig. 4).

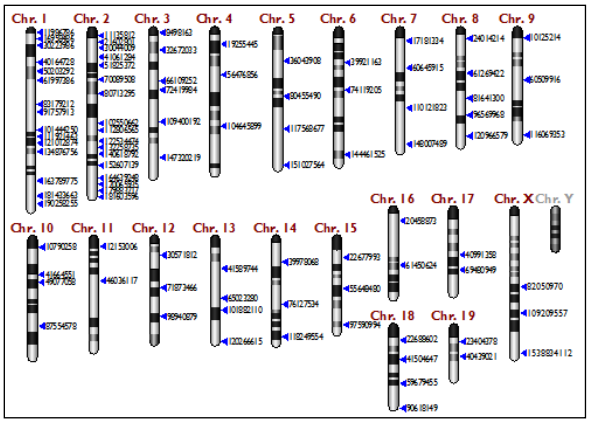


Fig. 4: SNPs that can discriminate genetic differences among C57BL/6N substrains

The RIKEN BRC participated in the establishment of the Federation of International Mouse Resources (FIMRe), and has registered strains developed by Japanese researchers in the International Mouse Strain Resource (IMSR), a one-stop-shop database of the FIMRe. Thus, the RIKEN BRC has made an international contribution to experimental mouse resources. The IMSR is a website through which 235,972 mouse resources maintained across 20 international core organizations can be searched. Almost all mouse resources are cryopreserved in the form of frozen embryos, frozen sperm, or knockout embryonic stem (ES) cells. For a researcher who is a user of frozen strains preserved in the FIMRe and who has no technology available for cryorecovery, the RIKEN BRC will perform cryorecovery services and provide live mice to the researcher.

In 2011, the International Mouse Phenotyping Consortium (IMPC) was launched. In Japan, the RIKEN BRC, the Experimental Animal Division, the Japan Mouse Clinic, and the Technology and Development Unit for Knowledge Base of Mouse Phenotype collaboratively participated in the IMPC. The IMPC has obtained knockout ES cells of the standard strain C57BL/6NTac from repositories of the Knockout Mouse Project (KOMP) in the United States and from the European Conditional Mouse Mutagenesis Program (EUCOMM) in Europe, produced chimera and knockout mice, and analyzed their phenotypes. Knockout mouse projects for whole genes that were initiated in Europe and the United States have been integrated into the IMPC, and international-standard phenotypic analyses are currently underway at the IMPC.

Japan, China, South Korea, Taiwan, and Singapore have participated in the Asian Mouse Mutagenesis and Resource Association (AMMRA) and the Asian Mouse Phenotyping Consortium. In 2013, the Australian Phenomics Network (APN) joined the AMMRA. The AMMRA has thus strengthened the cooperation among Asian countries and has pursued collaboration with the IMPC.

The year 2013 was a time of great change for mouse resources because of progress in genome-editing technology. In particular, genetic modification can be accomplished using the CRISPR/Cas9 system, which eliminates the need for ES cells. In other words, high-efficiency gene disruption can be performed simply by inducing mRNA expression of the genome-editing enzyme Cas9 and guiding RNA that determines the genome-editing site into a fertilized egg. This process is less expensive and more efficient than conventional knockout mouse production using ES cells, and multiple genes can be disrupted simultaneously. Therefore, the new genome-editing technology will likely be diffused quite rapidly. At present, the preservation, handling, and quality control of a large number of mouse strains created using genome-editing technology have also brought forth new challenges. In the future, mouse resources, including genetically modified mice created using new technologies, will be developed and diffused further as these animals are indispensable for research in the life sciences.

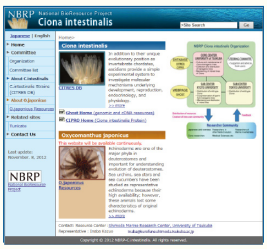
References

[1] Nakata H *et al*. Simultaneous detection of multiple transgenes for genetically-modified mouse strains. *Exp Anim* 58(4): 437-442, 2009.

[2] Mekada K *et al*. Genetic differences among C57BL/6 substrains. *Exp Anim* 58(2):141-149, 2009.

Database of this Month

National BioResource Project *"Ciona intestinalis"*



CITRES
- Number of strains：95
- Number of genes：165

As of February 2014

Natural-population species (wild type)

DB name：NBRP Ciona intestinalis
- CITRES (http://marinebio.nbrp.jp/ciona/)
- Ordering site of natural-population species (wild type) (http://w-ciona.lab.nig.ac.jp/cgi-bin/ghost_order_top.cgi)

Language：Japanese, English

Contents of CITRES :
- Strain resources of *Ciona intestinalis* (transgenic, enhancer-trap, mutant, various tissue GFP marker, and neuron Kaede strains): sperm, juvenile, and adult
- DNA Constructs
- GFP-expressed images, document information

Contents of natural-population species : juvenile and adult

Features :
- Resources can be ordered from the website.
- Strains can be accessed from a large number of GFP-expressed images, and expression information can be searched by narrowing down the expression stages and sites (tissues) of interest.

Cooperative DB : Ghost, CIPRO, RRC

DB construction group : NBRP Ciona intestinalis, NBRP Information

Management organization: Genetic Resource Center, NIG

Year of first DB publication : 2008 Year of last DB update: 2013

Comment from a practicing developer : Our database does not contain a large number of resources. However, our resources have been developed by researchers in Japan and we have prepared a sufficient number of resources to be used for advanced research in the life sciences. Currently, our database is generally used by specialists rather than by ordinary researchers. In the future, we will publish explanatory articles in our database to help users in other fields. We will also link our database with external genomic data to create a substantial and easy-to-use web site. The ordering site of wild-type species appears to be simple at first glance. However, the order form, including the classification of species providers and the schedule of ordering resources, is relatively complicated.

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Editor's Note

At present, the RIKEN BRC is a world-famous resource center such that almost all mouse researchers are familiar with the mice preserved in the center. In fact, novel resources have been rapidly established in the center in the past decade or so by a group led by Dr. Atsushi Yoshiki. I believe that, owing to its highly knowledgeable, specialized, and international management team, the Experimental Animal Division has become a solid and reliable organization (Y. Y.).

BioResource Information

(NBRP) www.nbrp.jp/
(SHIGEN) www.shigen.nig.ac.jp/
(WGR) www.shigen.nig.ac.jp/wgr/
(JGR) www.shigen.nig.ac.jp/wgr/jgr/jgrUrlList.jsp

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