Review

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Dissemination of Advanced Mouse Resources and Technologies at RIKEN BioResource Center

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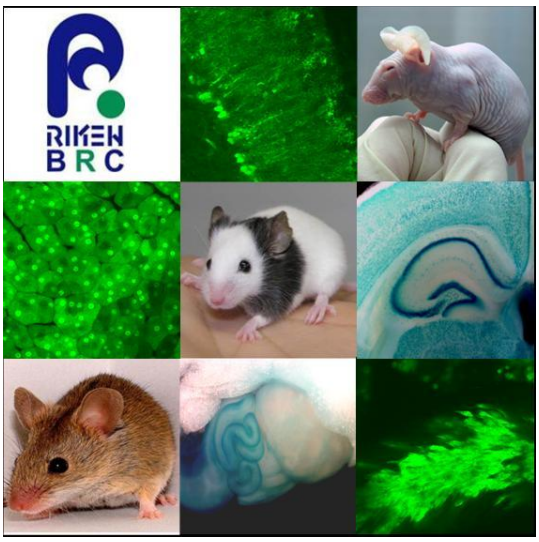
SYNOPSIS

RIKEN BioResource Center (BRC) has collected, preserved, conducted quality control of, and distributed mouse resources since 2002 as the core facility of the National BioResource Project by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. Our mouse resources include over 5,000 strains such as humanized disease models, fluorescent reporters, and knockout mice. We have developed novel mouse strains such as tissue-specific Cre-drivers and optogenetic strains that are in high demand by the research community. We have removed all our specified pathogens from the deposited mice and used our quality control tests to examine their genetic modifications and backgrounds. RIKEN BRC is a founding member of the Federation of International Mouse Resources and the Asian Mouse Mutagenesis and Resource Association, and provides mouse resources to the one-stop International Mouse Strain Resource database. RIKEN BRC also participates in the International Gene Trap Consortium, having registered 713 gene-trap clones and their sequences in a public library, and is an advisory member of the CREATE (Coordination of resources for conditional expression of mutated mouse alleles) consortium which represents major European and international mouse database holders for the integration and dissemination of Cre-driver strains. RIKEN BRC provides training courses in the use of advanced technologies for the quality control and cryopreservation of mouse strains to promote the effective use of mouse resources worldwide.



Keywords: mouse, National BioResource Project, Cre-drivers, optogenetic strain, knockout mouse

Introduction

RIKEN BioResource Center (BRC) was established in 2001 as a comprehensive biological resource center in Japan. The primary mission of the Experimental Animal Division is to collect, preserve, conduct quality control of, and distribute mouse strains that are primarily developed by Japanese scientists[1]. We have been acting as the core facility for mouse resources in the National BioResource Project (NBRP) of the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) since 2002. This paper constitutes an updated review describing the advanced mouse resources at RIKEN BRC and its current activities as an international mouse repository.

Collection and Distribution of Mouse Strains

We have collected over 5,000 strains primarily developed by Japanese scientists as of the end of 2010 (Table 1). Among these strains, the types most popularly requested are genetically engineered mice such as transgenic (Tg) and knockout (KO) mice (Table 2). The humanized disease model designated as "Tsukuba hypertension mouse"[2] has been one of the most frequently requested strains as it is useful for studies on cardiovascular diseases and regulation of blood pressure (Figure 1). Fluorescent reporters such as B6-Tg(GFP-LC3)[3], Tg(Nanog-GFP, Puro)[4], and B6-Tg(Fucci)504/596[5] mice are powerful genetic tools to monitor autophagy, isolate stem cells with a Nanog-driven GFP marker, and visualize every phase of the cell cycle *in vivo*, respectively. Knockout mice and Cre/Flp-drivers are also important to elucidate the functions of genes *in vivo*.

In collaboration with the Cell Engineering Division[6], we have collected and made available 713 gene-trap embryonic stem cell clones generated by an unbiased poly-A (UPA)-trap method[7]. Over 500 strains of *N*-ethyl-*N*-nitrosourea (ENU)-induced mutant mice are also available as models for studying human diseases and gene function[8,9]. Up-to-date resources and relevant information

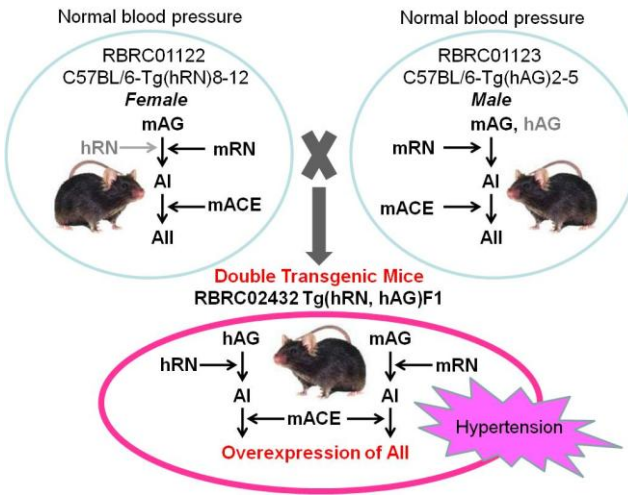


Figure 1. The Tsukuba hypertension mouse model. The Tsukuba hypertension mouse model can be generated by crossing female humanized renin (hRN) and male angiotensinogen (hAG) transgenic mice. Mouse renin (mRN) can only convert mouse angiotensinogen (mAG) to angiotensin I (AI); it cannot convert hAG. Furthermore; hRN can only convert hAG to AI. Therefore, in F1 double transgenic mice, AG and RN of the same species interact and simultaneously generate AI, which is converted by mouse angiotensin converting enzyme (mACE), leading to the overexpression of AII and significant hypertension[2]. In the reciprocal cross (i.e. female hAG x male hRN transgenic mice), the pregnant females exhibit a transient hypertension in late pregnancy due to secretion of placental hRN into the maternal circulation[10].

can be obtained through the websites of RIKEN BRC, NBRP[11], and the RIKEN integrated database of mammals[12]. To date, our mice have been distributed as live mice or frozen embryos to over 30 countries throughout Asia, North America, Europe, Oceania, Latin America, and Africa.

Development of Novel Mouse Resources

RIKEN BRC has developed cutting-edge mouse resources that are in high demand by the research community. These resources

Table 1. Collection of various mouse strains at RIKEN BRC

Strain type	No. of strains	Proportion (%)
Tg	1,688	33.6
KO + GT	1,267	25.2
UPATrap clones	713	14.2
Spontaneous mutant	520	10.3
ENU-induced mutant	502	10.0
Inbred	151	3.0
Recombinant inbred	84	1.7
Wild-derived	67	1.3
Consomic	37	0.7
Total	5,029	100.0

Tg: transgenic, KO: knockout, GT: gene trap, UPATrap: unbiased poly-A trap, ENU: ethylnitrosourea, Consomic: chromosome substitution inbred.

Table 2. Strains most frequently requested from RIKEN BRC as of the end of 2010

BRC no.	Strain name	Phenotype/Use	Depositor	Ref.
02432	Tg(hRN, hAG2)F1	Hypertension, cardiovascular deficiency	Akiyoshi Fukamizu	[2]
00806	B6-Tg(GFP-LC3)	Monitoring of autophagy	Masaru Okabe	[13]
02290	Tg(Nanog-GFP, Puro)	Isolation of stem cells	Shinya Yamanaka	[4]
02706	B6-Tg(Fucci)504	Visualizing S/G2/M cell cycle	Amalgaam	[5]
02707	B6-Tg(Fucci)596	Visualizing G1 cell cycle	Amalgaam	[5]
01420	B6-IRF-7 KO	Homozygote vulnerable to viral infections with decreased interferon serum levels.	Tadatsugu Taniguchi	[14]
01361	B6-p53 KO	High incidence of T-cell-derived lymphoma	Tsuranuki Niwa	[15]
01390	B6-Nrf2 KO	Increased apoptosis, chemically-induced tumor incidence	Masayuki Yamamoto	[16]
01834	B6-Tg(CAG-FLPe)36	Delete frted genes	Shigeyoshi Itohara	[17]
01828	B6-Tg(CAG-cre)	Delete floxed genes	Masaru Okabe	[18]

BRC no. is the strain registration number at RIKEN BRC. Strain names are abbreviated. Tg and KO indicate transgenic and knockout mice, respectively. Phenotypes are according to Mouse Genome Informatics (http://www.informatics.jax.org/).

Table 3. Novel mouse resources developed at RIKEN BRC

Category	Strain name	Specificity/Use
Cre-driver	B6N-Tg(Insl-cre)Utr/Rbrc	Pancreas
	B6N-Tg(Insl-cre/Esr1)Utr/Rbrc	Pancreas, inducible
	B6N-Tg(Krt14-cre)Utr/Rbrc	Skin, coat, nail
	B6N-Tg(Krt14-cre/Esr1)Utr/Rbrc	Skin, coat, nail, inducible
	B6N-Tg(Nes-cre)Utr/Rbrc	Neural tissue
	B6N-Tg(Nes-cre/Esr1)Utr/Rbrc	Neural tissue, inducible
	B6N-Tg(Tagln-cre)Utr/Rbrc	Smooth muscle
	B6N-Tg(Tagln-cre/Esr1)Utr/Rbrc	Smooth muscle, inducible
	B6N-Tg(Tek-cre)Utr/Rbrc	Endothelium
	B6N-Tg(Tek-cre/Esr1)Utr/Rbrc	Endothelium, inducible
	B6N-Tg(Vil1-cre)Utr/Rbrc	Gut epithelium
	B6N-Tg(Vil1-cre/Esr1)Utr/Rbrc	Gut epithelium, inducible
	B6N-Tg(Wap-cre)Utr/Rbrc	Mammary gland
	B6N-Tg(Wap-cre/Esr1)Utr/Rbrc	Mammary gland, inducible
Optogenetics	B6N-Tg(Thy1-ChR2/GFP)Sasa/Rbrc	Activation of green fluorescence protein-positive neurons by blue light
	B6N-Tg(Thy1-ChR2/mCherry)Sasa/Rbrc	Activation of red fluorescence protein-positive neurons by blue light
	B6N-Tg(Thy1-Halo/GFP)Sasa/Rbrc	Inhibition of green fluorescence protein-positive neurons by yellow light
	B6N-Tg(CAG-KO, ChR2/GFP)Sasa/Rbrc	Constitutive expression of bright orange fluorescence protein, Kusabira Orange (KO). When crossed with site-specific Cre mice, conditional activation of green fluorescence- positive neurons by blue light
	B6N-Tg(CAG-KO, Halo/GFP)Sasa/Rbrc	Constitutive expression of bright orange fluorescence protein, Kusabira Orange (KO). When crossed with site-specific Cre mice, conditional inhibition of green fluorescence protein-positive neurons by yellow light
	B6N-Tg(CAG-KO, ChR2/mCherry)Sasa/Rbrc	Constitutive expression of bright orange fluorescence protein, Kusabira Orange (KO). When crossed with site-specific Cre mice, conditional stimulation of red fluorescence protein-positive neurons by blue light

are partly listed in Table 3. Development proposals from Japanese scientists for recombinant genes to be used in these transgenic mice have been selected by our external steering committee members who represent the biomedical scientific community.

Additional Cre-drivers are required for conditional experiments using a Cre-lox system to activate or inactivate gene function in a tissue-specific manner. All of the Cre-drivers listed in Table 3 have been generated in the standard C57BL/6N strain by bacterial artificial chromosome (BAC)-transgenesis with an expectation of higher tissue specificity[19]. The use of Cre recombinase fused with a mutant form of the mouse estrogen receptor ligand binding domain (Cre/Esr1) enables the induction of Cre-mediated targeted deletion by the administration of tamoxifen[20].

Recently, transgenic mice carrying microbial opsin genes, "optogenetic strains," have proven useful in *ex vivo* and *in vivo* studies to elucidate the functions of complex mammalian brain circuits[21]. For example, the brain-specific expression of channelrhodopsin-2 (ChR2), a light-activated cation channel from *Chlamydomonas reinhardtii*, can be used to activate neurons with a blue flashlight at 470 nm[22]. Conversely, halorhodopsin (NpHR), a light-driven chloride-pump from *Naotronomonas pharaonisfunctions*, can be used to suppress neurons with a yellow flashlight at 589 nm[23]. Six of these optogenetic strains have been generated for immediate dissemination to the research community.

Health Quality

We import mice from developer scientists via rederivation by fostering them with BALB/cAJcl-*nu*/+ mothers to ensure that they are specific-pathogen free[1]. Since over 20% of the mice we receive are contaminated with pathogenic or undesirable microbes, such as mouse hepatitis virus, *Pasteurella pneumotropica*, *Helicobacter hepaticus,* intestinal protozoa, pinworm, and ectoparasites, this rederivation procedure is one of the most important tasks for our center prior to disseminating high-quality mouse resources to researchers for their refined animal experiments.

Regular health monitoring in the barrier facility is performed by using dirty bedding sentinels to maintain the excellent health status of our mice[24]. The health quality of our mice has been certified since 2001 by over 700 academic and for-profit organizations around the world.

Genetic Quality

The genetic quality of mouse resources is becoming increasingly important for their use in comprehensive functional genomics studies. Since animal experiments increasingly use multiple genetically modified mouse strains and their crosses, it is necessary to thoroughly maintain those genetic modifications to meet scientific and legal requirements. For this purpose, we established a simultaneous PCR test to detect the presence of multiple transgenes[25]. This PCR test, the "KO-survey," provides users with mouse strains of the highest genetic quality and accurate information on their genetic modifications.

Single nucleotide polymorphisms (SNPs) among different strains are useful to detect genetic contamination and to clarify their

genetic backgrounds. We have demonstrated that the most popular C57BL/6 substrains which differ at a few but critical SNP loci have been distributed by several commercial breeders[26].

International Collaborations

RIKEN BRC is a founding member of the Federation of International Mouse Resources (FIMRe) together with the Jackson Laboratory and European Mouse Mutant Archive[27]. We are participants in the International Mouse Strain Resource (IMSR)[28], a one-stop database for the dissemination of mouse resources generated by Japanese scientists to the international scientific community. We are also a founding member of the Asian Mouse Mutagenesis and Resource Association (AMMRA), whose mission is to promote mouse mutagenesis projects and to facilitate access to mouse resources in Asia.

We have contributed over 700 unbiased gene-trap clones to the International Gene Trap Consortium (IGTC)[29], and are working together with IGTC to generate a public library of mutated mouse ES cell lines. We also participate as an advisory member to the CREATE (Coordination of resources for conditional expression of mutated mouse alleles) consortium, which represents major European and international mouse database holders involved in conditional mutagenesis to develop a strategy for the integration and dissemination of Cre-driver strains.

Training Courses and Summer School

RIKEN BRC has provided training courses to facility managers and senior technicians on the use of advanced technologies for the quality control of mouse resources and the cryopreservation of mouse embryos and sperm to promote the best use of distributed mouse strains. Each course includes theory and practice sessions regarding mouse facility management and quality control programs such as microbial and genetic monitoring tests. Trainees from academic, for-profit, and overseas institutions are all welcome and encouraged to participate in our training courses.

The 1st BRC summer school was held in August 2010 at our Tsukuba campus to provide graduate students with 3 days of intensive lectures that were presented by our division heads and team leaders on mouse resources including the history of developing such resources, NBRP's mission and role, and advanced knowledge and technologies. This summer school aimed to provide graduate students with an opportunity to inspire one another, as well as to understand the importance of biological resources and the necessary infrastructure for their own research.

Conclusion and Prospects

The mouse has been used as an experimental animal for over 100 years since the generation of the inbred DBA strain in 1909 by Dr. Clarence C. Little[30]. International efforts have being made to generate knockout mice for every coding gene using gene-targeting technologies[31]. Recently, the International Mouse Phenotyping Consortium was launched to annotate the function of coding genes by measuring the comprehensive phenotypes of approximately 20,000 knockout mice[32]. The Japan Mouse Clinic has actively collaborated with international efforts and has generated its own high-standard phenotype data[33]. With the increased availability of mouse resources and associated data, the international mouse community is thus growing rapidly and is progressing toward the establishment of integrated large-scale resources and data that will be shared among the international scientific community[34]. Finally, RIKEN BRC keeps itself immersed in the rapid current of the life sciences and is vigilant for innovative findings that can be used to establish valuable mouse resources over the next 100 years.

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Relevant URLs

- RIKEN BioResource Center
  http://www.brc.riken.jp/
- Experimental Animal Division, RIKEN BRC
  http://www.brc.riken.jp/lab/animal/en
- National BioResource Project
  http://www.nbrp.jp/index.jsp
- Cell Engineering Division, RIKEN BRC
  http://www.brc.riken.jp/lab/cell/english
- Deisseroth Lab Resources "Optogenetics", Stanford University
  http://www.stanford.edu/group/dlab/optogenetics/
- Federation of International Mouse Resources (FIMRe)
  http://www.fimre.org/
- The Jackson Laboratory
  http://www.jax.org/
- European Mouse Mutant Archive (EMMA)
  http://www.emmanet.org/
- International Mouse Strain Resource (IMSR)
  http://www.findmice.org/
- Asian Mouse Mutagenesis and Resource Association (AMMRA)
  http://www.ammra.info/
- International Gene Trap Consortium (IGTC)
  http://www.genetrap.org/
- CREATE
  http://www.creline.org/home
- The Japan Mouse Clinic, RIKEN BRC
  http://www.brc.riken.jp/lab/jmc/mouse_clinic/en/index.html
- Amalgaam
  http://www.amalgaam.co.jp/
- Mouse Genome Informatics
  http://www.informatics.jax.org/

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