DISTRIBUTION OF THE HIGHEST QUALITY MOUSE RESOURCES FROM THE RIKEN BIORESOURCE CENTER

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Mice are one of the most important model organisms in life sciences to understand human health and the pathogenesis of complex diseases, because there are various inbred strains, information on the complete genome sequence and well find technologies for genetic and embryonic manipulations. RIKEN BRC has been designated as a central core facility for mouse resources in the National BioResource Project of MEXT, in Japan since 2002. With the support of the scientific community, we have collected over 3,000 strains, including unique genetically engineered models, ENU mutants, inbred and wild-derived strains developed mainly in Japan. Our mice are also cleaned-up to a specific pathogen-free state, strictly monitored for their health, accurately tested on genetic modifications and strain backgrounds. Thus, RIKEN BRC is on track to establishing mouse resources of the highest quality by 2010. RIKEN BRC protects the intellectual property rights of the developer of the strains by using MTA, and promotes distribution of the mice to the international scientific community. RIKEN BRC is one of the founding members of the Federation of International Mouse Resources (FIMRe), and has participated in the International Mouse Strain Resource (IMSR), a one-stop web shop of mouse strains held by FIMRe members. Training courses are also provided to disseminate advanced technologies to best use our mouse resources. RIKEN BRC collaborates closely with the international scientific community to contribute to the advancement of life sciences.

Key words: Mouse resources, genotypes, deposition, cryopreservation.

Mouse resources

Since the early 1930's over 400 genetically uniform inbred strains have been established for biomedical studies (Beck *et al*., 2000). The mouse genome sequence has been decoded following the human genome in 2002, and its information is available online (Venter *et al*., 2001; Waterston *et al*., 2002). Approximately 99 % of mouse genes were found to correspond to their human counterparts. Moreover, comprehensive cDNA libraries have been created in mice and become widely available (Okazaki *et al*., 2002). Transgenic and gene-targeting technologies have enabled us to create genetically engineered mice and test a specific gene function *in vivo* (Doetschman *et al*., 1987; Capecchi, 1989; Evans, 1981). Development of pluripotential stem cell lines such as embryonic stem (ES), germline stem and induced pluripotential stem cells have further extended the use of mouse resources in the field of regenerative biology and medicine (Kanatsu-Shinohara *et al*., 2004; Takahashi, Yamanaka, 2006). Cryopreservation of embryos and sperm in mice is also widely used technology in mice for long-term storage of the expanding genetic resources (Glenister, Thornton, 2000).

With the above advantages as experimental animals, mice are one of the most important model organisms in life sciences to understand human health and pathogenesis of complex diseases. In the US, Canada and the European Community large-scale strategic mutagenesis projects in mice are now underway to target every gene for the establishment of research resources to unravel human gene functions (Collins *et al*., 2007).

Establishment of the RIKEN BioResource Center

The RIKEN BioResource Center (BRC, http://www.brc.riken.jp/en) was established in 2001 as a global not-for-profit bioresource center providing biological materials, technical services, and educational programs to private enterprises and academic organizations around the world. RIKEN BRC is the only specialized comprehensive biological resource center in Japan and operates with three principles; «trust», «sustainability» and «leadership». RIKEN BRC aims at: 1) promoting life sciences and the bio-industry in Japan and around the world by facilitating bioresources of the highest quality; 2) making strategic plans and implementing them for developing the bioresources that are the essential infrastructure for sustainable development of mankind, and 3) conducting research and development to provide cutting-edge bioresources with the highest quality. RIKEN BRC acts as a core station for the dissemination of bioresources produced by Japanese scientists.

The mouse has been one of the major resources at RIKEN BRC. The mouse resource is also part of the National BioResource Project (NBRP, http://www.nbrp.jp/) for more than 25 categories including animals, plants, microbes, cell lines and DNA. The NBRP has been funded since 2002 by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan. This dedicated governmental support for the research infrastructure has accelerated the establishment of useful biological resources, resulted in a number of excellent research outcomes and has been highly appreciated by the international scientific community (Aitman *et al*., 2008).

Mouse strains at RIKEN BRC

We have collected over 3,000 strains by focusing on novel mouse strains developed mainly in Japan. These mouse strains can be classified into several categories, such as inbred, spontaneous mutant, transgenic, knockout, conditional knockout, Cre-driver, ENU mutant, recombinant inbred and wild-derived strains (Fig. 1). These strains are useful models to analyze gene functions and disease processes in the fields of cancer, immunology, neurobiology, infectious diseases, developmental and regenerative biology, life style-related diseases and drug discovery (Table 1). The proportion of transgenic and knockout mice has been growing rapidly in recent years. The wild-derived mice, including 42 strains, 4 species of *Mus* and 5 subspecies of *Mus musculus*, are unique and the world's largest mouse resources for genetic diversity (Yoshiki, Moriwaki, 2006). Gene-trap ES cell clones have

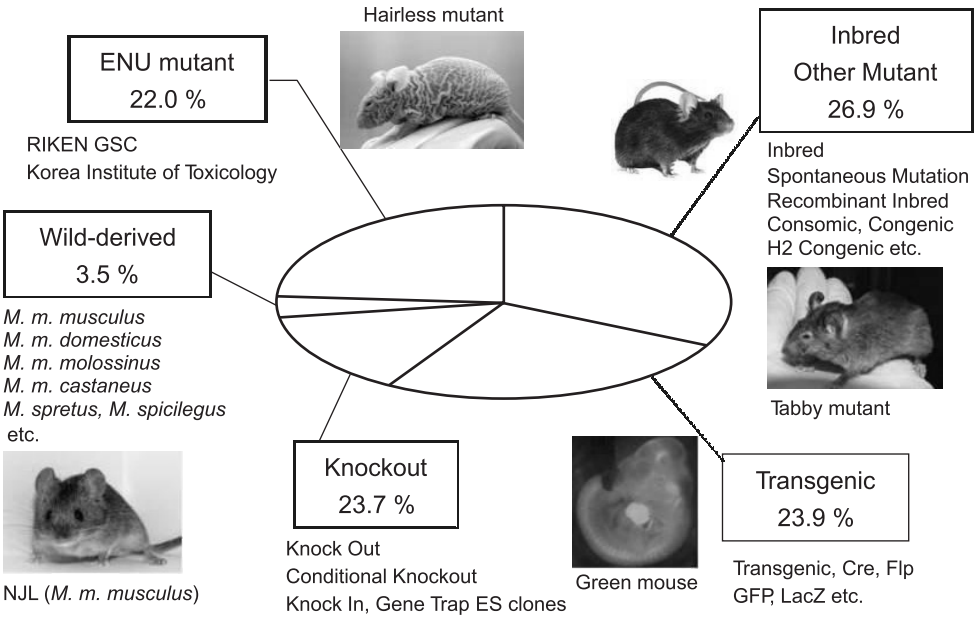

Fig. 1. Collection of various mouse strains at RIKEN BRC. (Notes. See details in the text).

Table 1

Useful mouse models available from RIKEN BRC

BRC No.	Strain name	Research field	Type of strain	Developer scientist	Reference
01088	B6-Hras KO	Cancer	Targeted congenic	Toshikuni Sasaoka	*Oncogene*. 2000. 19. P. 2951–2956
02217	MoMuLV/RET-MEN2A Tg	Cancer	Transgenic	Masahide Takahashi	*Cancer Res*. 2000. 60. P. 5254–5260
01361	B6-p53 KO	Cancer	Targeted congenic	Tsuranuki Niwa	*Oncogene*. 1993. 8. P. 3313–3322
01878	B6-p27 KO	Cancer	Targeted congenic	Keiichi Nakayama	*Cell*. 1996. 85. P. 707–720
00222	SL/Kh	Cancer	Inbred	Hayase Shisa	*J. Natl. Cancer. Inst.* 1987. 79. P. 781–787
01474	B6-Fas KO	Immunology	Targeted congenic	Shigekazu Nagata	*Nat. Genet*. 1995. 11. P. 294–300
01593	bcl-2 KO	Immunology	Targeted congenic	Yoshihide Tsujimoto	*Cancer Res*. 1995. 55. P. 354–359
01721	Bcl-xL Tg	Immunology	Transgenic	Shigeo Koyasu	*J. Exp. Med.* 1996. 183. P. 381–391
00300	RelA KO	Immunology	Targeted congenic	Takahiro Doi	*J. Exp. Med*. 1997. 185. P. 953–961
01498	C57BL/6J-Tg(Camk2-mutPolg)1Bsi	Bipolar disorder	Transgenic inbred	Tadashi Kato	*Mol. Psychiatry*. 2006. 11. P. 577–593
02324 02325	B6.Cg-Disc1<Rgsc1390> B6.Cg-Disc1<Rgsc1393>	Depression and schizophrenia	Gene-driven ENU mutant	Yoichi Gondo and John Roder	*Neuron*. 2007. 5. P. 387–402
00209	MSM/Ms	Neurobiology	Wild-derived inbred	oshihiko Shiroishi	*J. Natl. Cancer Inst*. 1978. 61. P. 1301–1306
02695	Delphin KO	Neurobiology	Targeted congenic	Masayoshi Mishina	*PLoS ONE*. 2008. 3. e2297
GSC0080	$Gdf5^{Rgsc451}$	Osteoarthritis (OA) model	Phenotype-driven ENU mutant	Hiroshi Masuya	*Hum. Mol. Genet.* 2007. 16. P. 2366–2375
00267	CAG-EGFP-Tg (Green mouse)	Developmental biology	Transgenic inbred	Masaru Okabe	*FEBS Lett.* 1997. 407. P. 313–319

been incorporated into our collection. These mouse strains have been distributed as live breeder pairs, frozen embryos and sperm. Recently, mouse ES cells derived from standard inbred strains have become available from the Cell Engineering Division of RIKEN BRC (http://www.brc.riken.jp/lab/cell/). The inbred ES cell lines derived from C57BL/6 are especially valuable for gene-targeting to establish a knockout mouse with a pure genetic background (Tanimoto *et al*., 2008).

Cooperation with the scientific community

In order to cooperate closely with the scientific community, we have invited distinguished Japanese and overseas scientists to be members of the BRC Experimental Animal Steering Committee, BRC Advisory Council, BRC Promotion Advisor and the RIKEN Advisory Council to provide useful advice and suggestions. Their comments, and recommendations have been incorporated into our annual plan and strategy for the operation of the Experimental Animal Division. We have also made continuous efforts to maintain good public relations with scientific societies, local civilian societies, students, children and their parents for their understanding and support of our activities.

Animal welfare and safety operation

Our resource activities must be conducted with special attention to animal welfare, safety, environmental conservation and research ethics. All our operations must be in accord with the following laws, standards and guidelines for animal experiments in Japan. The Law for the Humane Treatment and Management of Animals (Law No. 68, 2005), Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain (Notice No.88 of the Ministry of the Environment dated April 28, 2006), Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions (Notice No. 71 of the MEXT dated June 1, 2006), and Law concerning the Conservation and Sustained Use of Biological Diversity Through Regulations on the Use of Living Modified Organisms, so-called «Cartagena Law» for handling genetically-engineered mice. The so-called 3R principles (Refine, Replace and Reduce) of animal experiments were stipulated in the above «The Law for the Humane Treatment and Management of Animals» in 2005. Our protocols, including animals and recombinant DNA technologies, have been approved by the Tsukuba Institute Animal Experiments Committee and the Tsukuba Institute recombinant DNA Experiments Committee, respectively.

Intellectual property and quality issues

Many scientists in the world have created genetically engineered mice to find a specific gene function and its regulation *in vivo*. These mouse resources are potentially invaluable as models for human complex diseases and useful for drug discovery. Therefore, every organization recognizes the importance of the intellectual property rights associated with these mouse resources. The intellectual property rights of the mouse resources created by a developer scientist are generally considered to belong to the developer scientist's organization, as well as an important property of their nation. At RIKEN BRC, the Material Transfer Agreement (MTA) for the deposition and distribution of mouse resources has been used to protect the intellectual property rights of the developer scientist or its organization, and to clarify the terms and conditions of use requested by the depositor for recipients of the mouse resources.

Another important function of MTA is to control the quality of biological resources. RIKEN BRC prohibits recipients of the biological resources to transfer them to a third party in the MTA. Since we encountered several bitter claims regarding microbial and genetic contamination of biological resources transferred through third parties, we always strongly recommend scientists to obtain the biological resources directly from our center.

Clean-up of mouse strains

We have cleaned up all the imported mice in RIKEN BRC by using three main facilities for quarantine, rederivation and maintenance of specific pathogen-free (SPF) mice. The quarantine facility was set up at the corner of the Tsukuba campus as a P2 laboratory equipped with racks of isolation chambers under negative pressure. All live mice imported from outside organizations must stay in

this quarantine for two days and one night to test for infection of 8 dangerous pathogens by serology and PCR.

Based on the test results, mice are transferred to the second facility, equipped with bioBubble housings of negative or positive pressure (bioBubble Inc., Colorado, USA). Then, the mice are bred to expand the colonies for rederivation by embryo transfer or Cesarean section. After rederivation treatments, the mice are monitored by a 2nd round of microbial tests for 19 pathogens using embryo recipients or foster mothers. If the mice are found to be SPF, they are transferred to the breeding rooms in the barrier at the 3rd and 4th floors of the BRC main building.

Jcl:ICR and BALB/cA-*nu*/+ (CLEA Japan, Inc., Tokyo, Japan) females were used as recipients of embryo transfer and foster mothers, respectively. The BALB/cA-*nu*/+ foster mothers exhibit higher nursing capability over Jcl:ICR and accept babies of various strains including wild-derived strains. Also, BALB/cA-*nu*/+ mice are supplied as free from opportunistic pathogens such as *Staphylococcus aureus*. The clean-up of deposited mice in RIKEN BRC contributes to the high quality standards of animal experimentation in Japan.

Mouse husbandry in the barrier

The air conditioning of each mouse room is regulated at a constant temperature (24 °C ± 2 °C) and relative humidity (55 ± 5 %) with appropriate ventilation. The animal rooms and corridors are supplied with HEPA-filtered fresh air of appropriate pressure to maintain the clean environment within the barrier facility. The mouse rooms are illuminated with a cycle of 12L/12D (08:00 on, 20:00 off). Sensitive strains are supplemented with commercial paper nests (Regular Shack, Shepherd Specialty Papers, Tennessee, USA). The mice are freely given gamma–irradiated food (CE2, CLEA Japan, Inc., Tokyo, Japan) and filtered drinking water. All supplies including caging, paper bedding and water bottles are sterilized by autoclaving. Humans must take a wet shower and wear sterilized clothing, rubber shoes, disposable masks and gloves prior to entry into the barrier. A card key system safeguards the barrier facility and helps to minimize microbial contamination carried by humans.

We use an individually-ventilated microisolation cage system in the barrier facility. Cage exchange is conducted regularly with intervals of one or two weeks by qualified animal technicians. The mice are handled with disinfected forceps covered with silicon rubber caps or gloved hands. The mice are identified by ear punch. Their breeding records are kept on cage cards and digital files of a Filemaker database. The mouse racks, floors and walls are regularly cleaned with a disinfectant solution. A washing room in the main building is equipped with semi-automatic washing lines for cages and water bottles, and three floor-loading autoclaves.

Animal technicians

Our mouse facilities are operated in cooperation with research scientists, veterinary scientists, technical scientists, animal technicians and other members. Qualified animal technicians in particular have played substantial roles in the facilities for maintaining, breeding and preserving various kinds of mouse strains. In Japan we have a certification system for laboratory animal technicians and instructors by the Japanese Society for Laboratory Animal Resources. Animal technicians must pass both written and practical examinations to become a Junior Laboratory Animal Technician (JLAT or the second grade), and a Senior Laboratory Animal Technician (SLAT or the first grade) with a prerequisite of job experiences for more than 1 and 5 years, respectively. RIKEN BRC strongly encourages our animal technicians to obtain these certificates.

Advantages for deposition

A developer scientist of mouse strains can protect and exploit the intellectual property rights of their strains by deposition to RIKEN BRC. The deposited strains are soon submitted to IMSR (http:// http://www.informatics.jax.org/imsr/index.jsp), so that the strains become visible to the international scientific community. Consequently, the deposition may increase citation of the strains and opportunities for collaboration. Moreover, scientists and their organizations can reduce the cost and space just for preserving the mouse strains alive. If the strain is in high-demand in the scientific community, the scientists or organizations must respond to these requests from all over the world. RIKEN BRC can instead respond to those requests and ship the mice worldwide. RIKEN BRC also produces

frozen embryos and sperm of the deposited strains for long-term storage.

Cryopreservation

Mouse strains that are in high demand or of low-survivor after freeze-thawing should be kept alive. Most strains are cryopreserved as embryos or sperm whenever possible. The embryos are produced by *in vitro* fertilization and cryopreserved at the 2-cell stage. Mutant and genetically engineered strains are also stored as frozen sperm. Embryos are frozen in 1.8 ml plastic tubes by vitrification with EFS (ethylene glycol, ficoll and sucrose) solution (Kasai *et al*., 1990). Sperm is collected from the cauda epididymis, suspended in an 18 % raffinose and 3 % skim milk solution (Nakagata, 2000), and embedded in plastic straws. The frozen embryos and sperm are stored in DR-430LM liquid nitrogen tanks in the BRC main building.

Another backup storage facility has been operated since 2007 in the Harima Institute, about 500 km from Tsukuba. Since the ground in Japan is fragile and frequently hit by earthquakes, we need to manage the risk from such disasters. Harima Institute is located on the most stable ground in Japan and is considered to be the best location for backup storage and houses a duplicate inventory of our frozen mouse strains.

Quality control

Health monitoring of mouse strains is one of the most important quality control programs. Periodic monitoring for major pathogens is done in every rack of the facility using sentinel mice exposed to dirty bedding (Ike *et al*., 2007). Pathogens to be examined are classified into four classes as shown in Table 2. All sentinel mice are tested for the class A and B pathogens. The sentinel mice kept in the rack for immuno-deficient mice are examined for the class C pathogens as well. The class D contains pathogens rarely detected in the Japanese facilities, hence mice are sent to the ICLAS monitoring center at the Central Institute for Laboratory Animals, Kawasaki, Japan for class D examination by request. The test results are published regularly as a HEALTH CERTIFICATE on our home page. The environment of the facility is monitored quarterly for bacteria and fungi at every critical point.

Genetic monitoring is another integral part of our quality control programs. Genetically modified strains such as transgenic and knockout mice were genotyped by using PCR with allele specific primers. The genotyping PCR protocols have been made public on our website as downloadable pdf files. Genetic backgrounds of congenic strains were monitored with simple sequence length polymorphism (SSLP) markers covering the genome (Wakeland *et al*., 1997). Inbred and wild-derived strains were also monitored with standard 15 biochemical genetic markers (Nomura *et al*., 1984). Recently, the single nucleotide polymorphisms (SNPs) were screened among inbred and wild-derived strains to clarify subtle and comprehensive genetic differences (Mekada *et al*., 2009). Thus, RIKEN BRC is able to distribute microbiologically and genetically high-quality mouse strains to the international scientific community.

Further characterization of each strain was conducted by flow cytometry for immunological profiling, histopathological examinations and other

Table 2

Classification of pathogens in health monitoring at RIKEN BRC

Class	Pathogens
A	*C. piliforme*, Ectromelia virus, Lymphocytic choriomeningitis virus (LCMV), Mouse hepatitis virus (MHV), *M. pulmonis*, Sendai virus (HVJ)
B	*C. rodentium*, *C. kutscheri*, Dermatophytes, *P. pneumotropica*, Salmonella spp., *H. hepaticus*, *H. bilis*, Ectoparasites, Intestinal protozoa, Pinworms
C	*S. aureus*, *P. aeruginosa*, *P. carinii*
D	Pneumonia virus of mice (PVM), Mouse encephalomielitis virus (TMEV/GDVII), Minute virus of mouse (MVM), Reovirus type 3 (Reo3), Mouse adenovirus (MAV), Mouse rotavirus (EDIM), Mouse cytomegalovirus (MCMV), Lactate dehydrogenase elevating virus (LDHEV)

biochemical tests. This characteristic data of each strain is essential to establish the mouse resources of the highest global standard by 2010.

Distribution

The mouse strains are distributed as live breeder pairs, frozen embryos or sperm. Genomic DNA, tissues and organs of each strain are also available if requested. Users can access the mouse strains available in our center through our website or IMSR (Fig. 2). Users must complete a Material Transfer Agreement for Distribution and an order form. Some strains require permission of use from the Depositor using an approval form. RIKEN BRC starts to prepare the mice upon receipt of the order documents from the users. We have been providing mouse resources not only to scientists in Japan but also to those overseas with minimum fees solely to reimburse the RIKEN BRC for a part of the preparation and handling costs of the requested mouse resources. This reimbursement is indispensable to continue distribution requested by the scientific community. Recipient scientists also pay the shipping cost to the courier company.

Training courses

Our training courses are aimed to disseminate the best advanced technologies to use the mouse resources. The Experimental Animal Division and Bioresource Engineering Division provides training courses for animal facility management, and the latest technologies for cryopreservation of mouse embryos and sperm. The courses include theory and practice of mouse facility management, quality control programs such as microbial and genetic monitoring tests, mouse embryo freezing, thawing and transfer to recipients. We have accepted trainees from domestic and overseas universities, institutions, pharmaceutical and other companies.

International network

RIKEN BRC is a founding member of Federation of International Mouse Resources (FIMRe, http://www.fimre.org). FIMRe is a collaborating group of Mouse Repository and Resource Centers worldwide, including the Jackson Laboratory, European Mouse Mutant Archive (EMMA, http://www.emmanet.org/) and other centers (Davisson, 2006; Hagn *et al*., 2007). We participate in IMSR, a

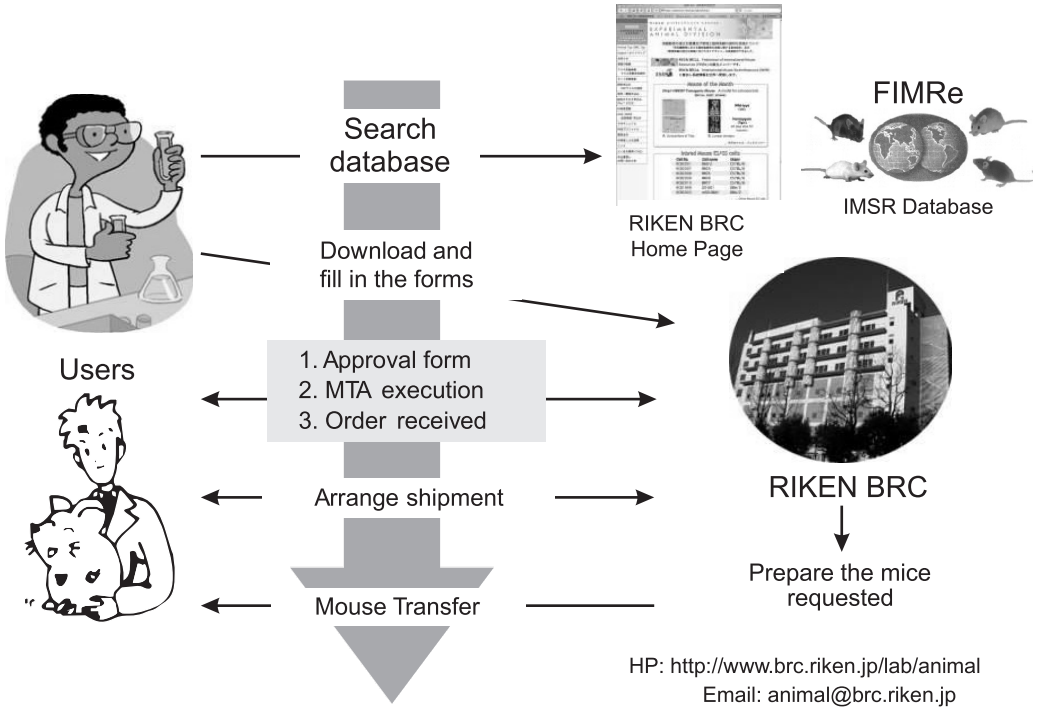

Fig. 2. How to order mouse strains from RIKEN BRC.

Organization	No. of Strains
FIMRe Members	
The Jackson Laboratory (TJL)	4,074
RIKEN BioResource Center (RBRC)	**1,649**
Mutant Mouse Regional Resource Centers (MMRRC)	1,610
European Mouse Mutant Archive (EMMA)	1,027
Mammalian Genetics Units, Harwell	360
Kumamoto Univ. (CARD)	321
Canadian Mouse Mutant Repository (CMMR)	167
Mouse Moldes of Human Cancer Consortium (MMHCC)	96
Australian Phenomics Facility (APF)	14
Others	3,128
Total (Sept 2008)	12,446

Federation of International Mouse Resources
http://www.fimre.org/

International Mouse Strain Resource
http://www.informatics.
jax.org/imsr/index.jsp

Fig. 3. Contribution to the international network of mouse resources.

one-stop web shop of mouse strains available from FIMRe member organizations (Fig. 3). Most of the strains are cryopreserved as embryos, gametes and ES cells. Many biomedical researchers, however, do not have their own technical expertise or facilities to rederive live mice from frozen stock and they have difficulties to use the frozen strains for their research. To improve this condition, RIKEN BRC has recently executed an agreement with other FIMRe members for the international recovery of mouse strains from distant repositories to facilitate the use of frozen strains in the international scientific community.

RIKEN BRC has also participated in the Asian Mouse Mutagenesis and Resource Association (AMMRA, http://www.ammra.info/) as a founding member. We have so far entered into a memorandum of understanding with institutions in Taiwan, Korea and China to promote cooperation in areas of mutual interest in laboratory animal sciences. More recently, we have started mutual visits with the Laboratory Animal Center, Institute of Cytology and Genetics, Siberia Branch of the Russian Academy of Sciences in 2007.

Home page

In collaboration with our Information Division we provide users from our home page the most up-to-date information regarding our activities, novel mouse resources and strains for freeze-down. Research publications by users and relevant literature on the mouse strains have been frequently surveyed by direct email letters and public databases. We have submitted a revised list of our strains monthly to the IMSR and contributed to the international scientific community. Periodic e-mail newsletters are distributed to over 2,000 users worldwide. The e-mail newsletter contains an article entitled «Mouse of the Month», topic news and a report of our activities. We have also disseminated information on forthcoming symposia and academic meetings.

Conclusion

RIKEN BRC has continued its efforts to establish its own unique mouse resources of the highest quality in the international network of scientific community. We strongly hope to promote life sciences and bio-industry in Siberia and the world through the dissemination of these mouse resources. We really appreciate the increasing opportunities to share our knowledge, technology and information on mouse resources with scientists in Siberia.

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References

Aitman T.J., Critser J.K., Cuppen E. *et al*. Progress and prospects in rat genetics: a community view // Nature Genet. 2008. V. 40. P. 516–522.

Beck J.A., Lloyd S., Hafezparast M. *et al*. Genealogies of mouse inbred strains // Nature Genet. 2000. V. 24. P. 23–25.

Capecchi M.R. Altering the genome by homologous recombination // Science. 1989. V. 244. P. 1288–1292.

Collins F.S., Rossant J., Wurst W. A mouse for all reasons. International Mouse Knockout Consortium // Cell. 2007. V. 128. P. 9–13.

Davisson M. FIMRe: Federation of international mouse resources: global networking of resource centers // Mamm. Genome. 2006. V. 17. P. 363–364.

Doetschman T., Gregg R.G., Maeda N. *et al*. Targeted correction of a mutant HPRT gene in mouse embryonic stem cells // Nature. 1987. V. 330. P. 576–578.

Evans M. Origin of mouse embryonal carcinoma cells and the possibility of their direct isolation into tissue culture // J. Reprod. Fertil. 1981. V. 62. P. 625–631.

Glenister P.H., Thornton C.E. Cryoconservation–archiving for the future // Mamm. Genome. 2000. V. 11. P. 565–571.

Hagn M., Marschall S., Hrabe de Angelis M. EMMA–the European mouse mutant archive // Briefings in Functional Genomics and Proteomics. 2007. V. 6. P. 186–192.

Ike F., Bourgade F., Ohsawa K. *et al*. Lymphocytic choriomeningitis infection undetected by dirty-bedding sentinel monitoring and revealed after embryo transfer of an inbred strain derived from wild mice // Compar. Med. 2007. V. 57. P. 272–281.

Kanatsu-Shinohara M., Inoue K., Lee J. *et al*. Generation of pluripotent stem cells from neonatal mouse testis // Cell. 2004. V. 119. P. 1001–1012.

Kasai M., Komi J.H., Takakamo A. *et al*. A simple method for mouse embryo cryopreservation in a low toxicity vitrification solution, without appreciable loss of viability // J. Reprod. Fertil. 1990. V. 89. P. 91–97.

Mekada K., Abe K., Murakami A. *et al*. Genetic differences among C57BL/6 substrains // Exptl. Anim. 2009. V. In press. P.

Nakagata N. Cryopreservation of mouse spermatozoa // Mamm. Genome. 2000. V. 11. P. 572–576.

Nomura T., Esaki K., Tomita T. ICLAS Manual for genetic monitoring of inbred mice. Tokyo: University of Tokyo Press,. 1984. V. P.

Okazaki Y., Furuno M., Kasukawa T. *et al*. Analysis of the mouse transcriptome based on functional annotation of 60,770 full-length cDNAs // Nature. 2002. V. 420. P. 563–573.

Takahashi K., Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors // Cell. 2006. V. 126. P. 663–676.

Tanimoto Y., Iijima S., Hasegawa Y., *et al*. Embryonic stem cells derived from C57BL/6J and C57BL/6N mice // Compar. Med. 2008. V. 58. P. 347–352.

Venter J.C., Adams M.D., Myers E.W. *et al*. The sequence of the human genome // Science. N.Y., 2001. V. 291. P. 1304–1351.

Wakeland E., Morel L., Achey K. *et al*. Speed congenics: a classic technique in the fast lane (relatively speaking) // Immunology Today. 1997. V. 18. P. 472–477.

Waterston R.H., Lindblad-Toh K., Birney E. *et al*. Initial sequencing and comparative analysis of the mouse genome // Nature. 2002. V. 420. P. 520–562.

Yoshiki A., Moriwaki K. Mouse phenome research: implications of genetic background // ILAR J. National Research Council, Institute of Laboratory Animal Resources. 2006. V. 47. P. 94–102.