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—Review—
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National BioResource Project-Rat and Related Activities

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Abstract: In order to establish a system to facilitate the systematic collection, preservation, and provision of laboratory rats (*Rattus norvegicus*) and their derivates, the National BioResource Project-Rat (NBRP-Rat) was launched in July 2002. By the end of 2008, more than 500 rat strains had been collected and preserved as live animals, embryos, or sperm. These rat resources are supplied to biomedical scientists in Japan as well as in other countries. This review article introduces NBRP-Rat and highlights the phenome project, recombinant inbred strains, BAC clone libraries, and the ENU-mutant archive, named the Kyoto University Rat Mutant Archive (KURMA). The future direction of rat resources are also discussed.
Key words: bioresource, ENU mutagenesis, NBRP-Rat, phenome project, RI strains

History of Laboratory Rats

In Japan, the first appearance of rat (*Rattus norvegicus*) domestication was seen in two guides for breeding fancy rodents, rats and mice, published around 1780. The works *Yosotamanokakehashi* from 1775 (see Fig. 1) and *Chingansodategusa* from 1787 [24, 26] introduced coat color variants of fancy rodents and explained their breeding, feeding, and housing. In Europe, rat domestication began in the 1800s when rat baiting was developed as a sport, and albino rats were observed occasionally and utilized for entertainment or breeding purposes [3].

The rat began its career as a laboratory animal in Europe in the 1850s, and became the first mammalian species to be domesticated for scientific purposes [12]. Their suitable size and easy handling might have been the major selective points in early animal experiments in the studies of breeding, behavior, psychology, nutrition, endocrinology, genetics, and others. Rats are now well known as the most important animals for safety or toxicological testing of chemical compounds, including drug candidates. Laboratory rats derived from 'outbred' or 'closed colony' animals, like Wistar or Sprague-Dawley rats, are usually supplied by commercial breeders and used for such testing, although inbred strains, such as Fischer (F344) or Long Evans (LE), are also popularly used for similar purposes. Some 700 different rat strains that comprise the above mentioned inbred and outbred strains of various natures (spontaneous mutant, congenic, recombinant inbred, consomic, transgenic, etc.) have recently become available through resource centers, and the majority have been developed from only a few founder strains, of which the Wistar strain plays

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Talented bear-like fancy rodents with a crescent spot could jump through a loop of the moon…

In any age, both Mt. Fuji and fancy rodents can be seen. In particular, precious rodents with speckled or spotted coat colors are said to bring wealth…

Fig. 1. Rodents (rats and mice) in *Yosotamanokakehashi*.

Box 1. Domestication and experimental use of rats

around 1780	Guides to fancy rodents (rats and mice) in Japan[1]
around 1800	Domestication of rats in Europe
~1860	Beginning of experimental use of rats in Europe
1892 ~	Introduction of laboratory rats into USA from Europe
1901	Foundation of a fancy rat association in France[2]
1906 ~	Establishment of the Wistar rat colony in USA
1916	Foundation of a fancy mouse and rat club in UK[3]

1: *Yosotamanokakehashi* (1775), *Chingansodategusa* (1787)
2: APRAC, Association de Promotion du Rat comme Animal de Compagnne
3: LSCMRC, the London & Southern Countries Mouse & Rat Club

by far the most important role.

The origin of the first albino rats from the famous Wistar Institute can be traced to two possible sources. One track leads to Adolf Meyer, a Swiss neuropathologist, who invented the term 'psychobiology' in later years. Meyer emigrated in 1892 from Europe and joined Henry Donaldson at his department at the University of Chicago. Meyer also introduced albino rats from the University of Geneva. He persuaded Donaldson of the advantages of the rat in studies of the nervous system and in the early 1890s, the Japanese scientist Shikishi Hatai and other members of Donaldson's department performed the first neuroanatomical experiments using albino rats in the US. The second path by which albino rats were brought into Donaldson's department was the development of spontaneous mutants captured from wild rats in North America. Donaldson and Hatai moved to the Wistar Institute together with four pairs of albino rats from the colony of Chicago University in 1906 [5]. The original albino rat colony in the Wistar institute is therefore considered to have been established either with Europe-derived laboratory rats or captured wild mutants, since Hatai at the Wistar Institute reported the first time that albino mutants were obtained from common Norway rats (the non-albino laboratory rats that were used in North America or Europe in those days) under laboratory conditions in 1912 [7]. This history is supported by the record, that so-called 'Wistar rats' were not only experimentally used at the Wistar Institute but were also

supplied to other institutions, beginning in 1911.

Many reference strains and model rats have been developed in Japan from imported outbred rats from the USA, especially from the Wistar Institute or commercially available Wistar rats and Sprague-Dawley rats or Long-Evans rats. Spontaneously hypertensive rats (SHR) [19] and stroke-prone hypertensive rats (SHRSP) [20] were developed from Wistar rats at Kyoto University (Kyo:Wistar rats) and are representative rat models of human diseases. Furthermore, many genetic models of human diseases have been developed by selective breeding methods in Japan and other countries; for instance, Helen Dean King not only developed the Wistar-derived inbred strain King Albino (PA or WKA), but also captured wild rats to produce inbred strains and succeeded in developing the BN strain. Many inbred strains were also independently developed at Columbia University: F344, AUG, COP, and at other universities and institutes [12].

The laboratory rat is now widely used for translational research, because it is a suitable animal for medical experiments due to its well-characterized physiology and not least because it is large enough for many surgical manipulations and examinations that cannot be performed on mice. To understand the genetic base of rat models, the first whole genetic map of the rat was drawn using simple sequence polymorphism (SSLP) markers in 1992 [23] and the whole draft sequence of the rat genome was reported in 2004 [6]. Recent developments, such as the establishment of rat ES or iPS cells or the generation of gene-knockout rats using Zinc Finger Nucleases (ZFN), will boost the utilization of rats, especially in the field of functional genomics [4, 10, 11, 16].

Rat Bio Resource Centers

Important rat strains for various research fields in life science have been maintained for more than 100 years on the basis of individual efforts by many scientists. Such effort is intrinsically inefficient and susceptible to unexpected changes in funding and local interests. The NIH rat model repository workshop was held in August 1998, and 58 scientists from the USA and elsewhere discussed the needs, use, opportunities, and parameters for optimal importation, standardization, maintenance, and distribution of genetically defined rat strains. The group of internationally recognized scientists strongly encouraged the NIH to establish a national rat genetics resource center. As a result, the Rat Resource Research Center (RRRC) was established in 2001. A similar approach on a larger scale was undertaken in Japan in 2002 with the National BioResource Project-Rat, (NBRP-Rat), addressing the need of collecting, preserving, and supplying unique rat strains that resemble human diseases or are of other value for biomedical research, and which have mainly been developed in Japan. These two large rat repositories, RRRC and NBRP-Rat, are operating internationally [2].

National BioResource Project-Rat (NBRP-Rat)

The aims of the National BioResource Project-Rat (NBRP-Rat) are the collection of rat strains, phenotypic and genetic characterization, the maintenance of live stock under specific-pathogen free conditions, the cryopreservation of embryos and sperm [8, 18], the preparation and maintenance of a publicly accessible database on deposited rat strains (http://www.anim.med.kyoto-u.ac.jp/NBR/), and the global supply of these rat strains. The NBRP-Rat is a very timely project in this field and we could elevate the potential of rat resources by performing this fundamental project as described below [24].

By the end of 2008, more than 500 rat strains had been deposited in the NBRP-Rat. Inbred strains, substrains, and congenic strains are major collections. In the genetic categories, animal models for human diseases or mutant strains developed from rats with spontaneous mutations or by selective breeding for particular phenotypes are included. Recombinant inbred strains and consomic strains are also preserved in the NBRP-Rat repository. Reporter gene transgenic rats are useful tools for investigating the physiological or pathological process in animal models. Transgenic rats with GFP, LacZ, and DsRed are particularly valuable for examining cellular fate in living animals [22], and have great potential utility for cell trafficking studies after organ and cell transplantations. Tissue organogenesis and trans-differentiation of stem cells are expected to be targets of study

using these reporter gene transgenic rats. In addition to the rat strains, a set of sperm and DNA of 5,000 ENU mutagenized F344 G1 animals have been placed in the NBRP repository [15].

Rat strains or their DNA samples have been supplied to 466 institutions in Japan and 22 institutions in the USA, Canada, UK, Germany, Sweden, Thailand, Indonesia, Malaysia, China, and Taiwan. To support the standardization and ease the exchange of cryopreserved rat bioresources among research and resource laboratories worldwide, two DVDs (Japanese and English versions), which contain protocols and movies of the cryopreservation of rat embryos and sperm, intracytoplasmic sperm injection, and re-derivation techniques, have been produced and supplied to the research community.

Phenome Project

To re-evaluate the collected strains at NBRP-Rat, male rats of 163 inbred strains and female rats of 40 inbred strains have been phenotypically characterized for 109 parameters under the Rat Phenome Project [13]. This systematic characterization is the first large-scale strain survey of many phenotypes of biological importance in the rat. In this project, 179 rat strains (including wild rats) have also been genotyped with 357 microsatellite markers [14]. In the European STAR project, 96 rat strains from NBRP characterized in the phenome project have been genotyped with approximately 20,000 SNPs [21].

Phenotype data have been collected for 7 categories, functional observational battery (neurobehavior), behavior studies, blood pressure, biochemical blood tests, hematology, urology, and anatomy, which include 109 parameters. A major feature of this phenome project is the development of phenotypic 'Strain Ranking', which allows visual data scoring, shows the biological range of various phenotypic parameters, and reveals normal and abnormal values for various rat strains. 'Strain Ranking' at NBRP-Rat provides an opportunity to easily and simultaneously compare phenotypic values for multiple rat strains, and could reveal unique and unknown characteristics even in well-characterized strains. For instance, strain ranking in the passive avoidance test identified the unique learning ability of BN strains, whose DNA was used for the Rat Genome Project.

Female data have recently been added and it is now possible to display gender differences (Fig. 2). The examples impressively demonstrate that glucose, triglyceride, and hematocrit values are significantly higher in males. In contrast, MCHC, prothrombin, plasma chloride, and relative adrenal weights show significantly higher values in females. Although comparative evaluations of these gender differences in rats and humans or other laboratory animals have not been completed yet, important rats were identified by this project. Phenome data are publicly available from our website (http://www.anim.med.kyoto-u.ac.jp/NBR/phenome.aspx).

NBRP-Rat is also genotyping selected rat strains and has developed a pedigree which integrates all major rat strains as well as several mutants and even wild rats from different continents (http://www.anim.med.kyoto-u.ac.jp/NBR/phylo.aspx). The genotyping comprises 357 microsatellite markers and includes animals from some 180 different origins. Furthermore, a charting tool utilizes these data to draw strain-specific phylogenetic charts that refer to a freely selectable rat strain. These useful tools and data support investigators in elucidating the phylogenic relationships of rat strains and to select suitable strains for genetic experiments.

Functional Polymorphisms

An allelic variation that cause changes in the appearance of any possible phenotype, such as enzyme activity, disease susceptibility etc. is called a functional polymorphism. NBRP-Rat has recently surveyed 140 rat strains for gene mutations reported in particular rat strains that are responsible for functional polymorphisms (Fig. 3) [9]. Forty-nine rat strains show the insertion type of *Cdkn1a/p21* [29], suggesting their resistance to prostate cancer; 79 rat strains have the duplication type of *Fcgr3* [1], suggesting their resistance to experimentally induced nephritis; and 63 and 29 rat strains have point mutations of *Lss* and *Fdft1* [17], respectively, suggesting their susceptibility to cataracts. Point mutations of *Gpr10* type [28] could be detected in 45 strains, suggesting their tendency toward overeating; certainly, body weight values at 10 weeks of age were significantly higher in this group. Such information is very helpful

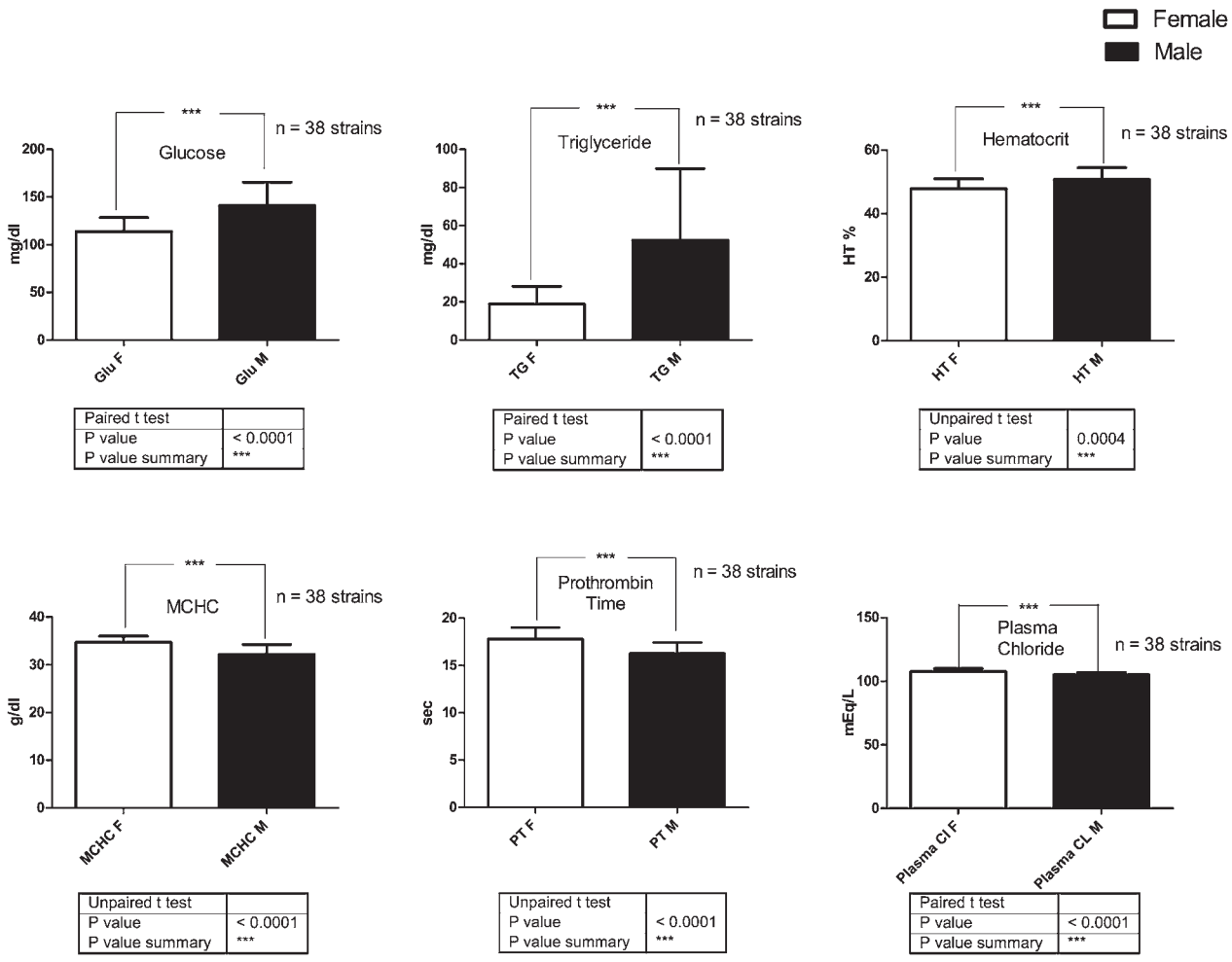

Fig. 2. Gender differences of serum biochemical and hematological parameters in rat inbred strains.

for selecting suitable rat strains for particular experiments. Since functional polymorphisms were also found in outbred or closed colonies at different rates among rat colonies [9], we recommend that testing or experiments with rats should be planned after giving careful consideration to this issue.

Recombinant Inbred Strains

Recombinant inbred (RI) strains are produced from an outcross between two well-characterized inbred stains followed by at least 20 generations of inbreeding to create several new inbred lines with a genome that is a mosaic of the parental genomes. The resulting RI strains are independent inbred strains with unique phenotypes due to the allelic mixture of the parental genes. Once the fully inbred status has been achieved, the results of a single genotyping of strain distribution patterns (SDPs) of polymorphic genetic markers, can be utilized in all future experiments. In subsequent projects, only the phenotype of the RI strains needs to be determined and can be linked to the stable genomic information. Furthermore, RI strains provide a suitable genetic platform for quantitative trait locus (QTL) analysis by reducing individual, environmental, and measurement variability.

The largest rat RI strain panel is available from NBRP-Rat. The LEXF/FXLE RI set (n=34), derived by reciprocal crossing of F344/Stm and LE/Stm [25], has been genotyped for approximately 20,000 SNPs and pheno-

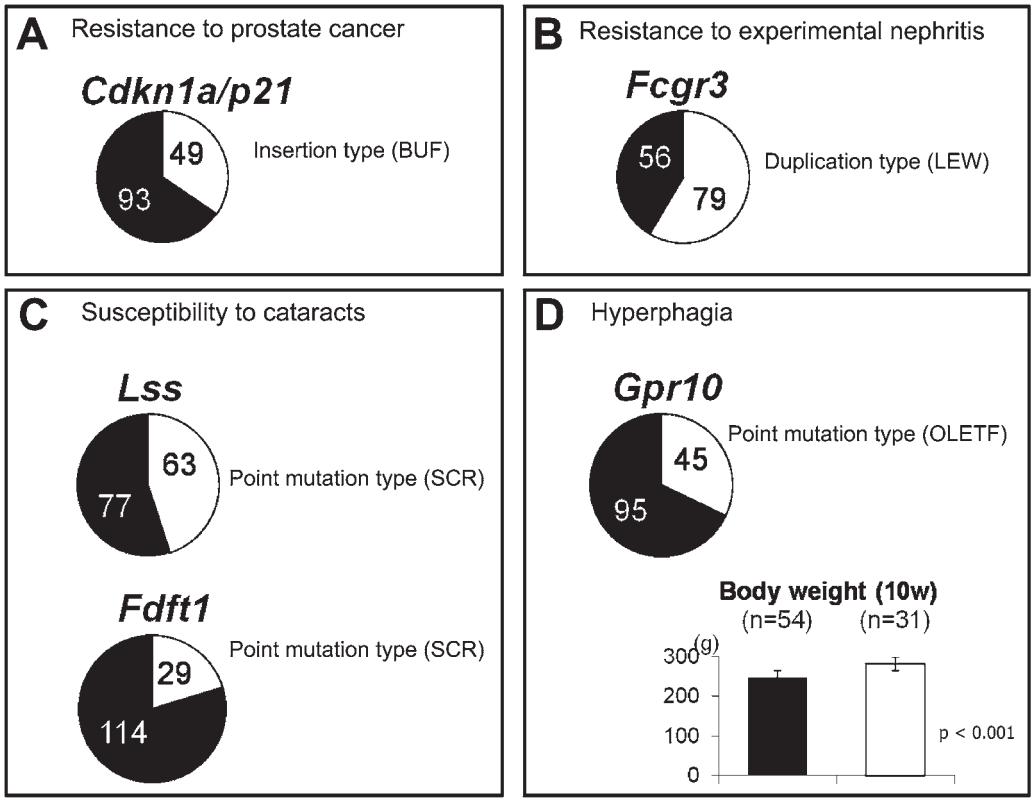

Fig. 3. Distribution of disease-related functional polymorphisms in rat inbred strains. (A) Insertional mutation in the promoter region of the *Cdkn1a* gene is associated with resistance to prostate cancer. (B) Duplication of the *Fcgr3* gene is associated with resistance to experimentally induced nephritis. (C) Missense mutations of *Lss* and *Fdft1* genes are associated with susceptibility to cataracts. (D) Missense mutation at the translation initiation codon of the *Gpr10* gene is associated with hyperphagia. The average body weight of the mutant-type strains (n=31) is significantly higher than that of wild-type strains (n=54).

typed for 74 quantitative traits. QTL analysis has already detected 250 QTLs for these traits, indicating the usefulness of the LEXF/FXLE to identify QTLs [21, 27].

Rat BAC Clone Libraries

To strengthen the power of the LEXF/FXLE RI strain, BAC libraries with ten-fold coverage were constructed for the parent F344/Stm (238,080 clones, average length 116 kb) and LE/Stm strains (259,968 clones, average length 129 kb), as a collaboration with RIKEN Genomic Sciences Center and the National Institute of Genetics. All BAC clones were deposited at the Gene Engineering Division, RIKEN BioResource Center to enable their worldwide distribution. BAC ends were sequenced and mapped to the reference genome sequence of the BN strain. In the F344/Stm BAC library, 155,144 clones were mapped, of which 138,800 (89%) clones were mapped with both ends and 16,344 (11%) with one of either ends. The mapped F344/Stm BAC clones cover ~96% of autosomes and ~92% of the X chromosome. In the LE/Stm BAC library, 103,062 clones were mapped, of which 55,821 (54%) clones were mapped with both ends and 47,241 (46%) with one of either ends. The mapped LE/Stm BAC clones cover ~82% of autosomes and ~52% of the X chromosome. Mapping data can be accessed publicly using the GBrowser at the homepage of NBRP-Rat (http://analysis2.lab.nig.ac.jp/ratBrowser/cgi-bin/index.cgi?org=rn).

Table 1. Generation of gene-targeted rats from KURMA

Gene	Screening on KURMA by MuT-POWER					Recovery by ICSI			
	Number of G1 samples screened	Mbp screened	Mutations found	Mutation type	Mutations per Mb	Injected oocytes	Transferred oocytes (%)*	Born (%)	Mutated
Gene A	1,536	12.50	A → C	E → A (Missense)	6.25	36	30 (83.3)	9 (25.0)	6 (♂4, ♀2)
			A → C	N → H (Missense)		38	35 (92.1)	15 (39.5)	5 (♂2, ♀3)
Gene B	1,536	4.22	C → A	S → X (Nonsense)	4.22	37	30 (81.1)	10 (27.0)	3 (♂1, ♀2)
Gene C	1,536	6.45	A → T	Q → L (Missense)	2.15	282	240 (85.1)	22 (7.8)	9 (♂4, ♀5)
			A → T	K → M (Missense)					
			T → C	Silence					
Gene D	4,608	16.92	T → G	L → R (Missense)	8.46	97	75 (77.3)	11 (11.3)	7 (♂2, ♀5)
			T → C	Silence					
Gene E	4,608	14.32	T → A	L → Q (Missense)	7.16	203	156 (76.8)	17 (8.4)	10 (♂4, ♀6)
			G → A	Intronic					
Total 5 genes	–	54.11	10	–	5.41	693	566 (81.7)	84 (12.1)	40 (♂17, ♀23)

*: The 2-cell cleaved embryos were transferred into the oviducts of pseudopregnant F344/NSlc rats.

Kyoto University Rat Mutant Archive (KURMA)

Although the laboratory rat is an increasingly used mammalian model in biomedical research, there was no simple technology for producing *in vivo* genetically engineered mutations equivalent to knockout or knock-in mice for several decades. To overcome such a limitation, we have generated a large repository of ENU-induced mutations, called the Kyoto University Rat Mutant Archive (KURMA) [15]. DNA mutations in the repository can be efficiently screened with a high-throughput and low-cost assay based on the Mu-transposition reaction (MuT-POWER). Animals carrying any mutations can be recovered from frozen sperm by intracytoplasmic sperm injection (ICSI). Table 1 shows the results of screening by MuT-POWER for particular target genes and the recovery of mutated rats by ICSI, which indicates the reliability of these technologies for establishing gene-targeted rat models from the KURMA. There are estimated to be 2.5–3.5 millions mutations in the KURMA, which means that at least one mutation of the particular gene will be acquired from 5,000 samples. Expansion up to 10,000 male G1 samples would guarantee mutations in any gene of interest.

The KURMA has enabled the production of several rat models for human diseases, including epilepsy, cancer, hypertension, or diabetes, a series of diseases for which mice have proved less interesting. With such a variety of mutations in different genetic contexts, the teaching of comparative pathology and functional genomics will increase.

Future Aspects of Rat Resource Center

The major aim of rat resource centers is the supply of bio materials to the research community. Progressing technologies require existing repositories to preserve and maintain not only animals or materials of traditionally derived rat strains but also genetically engineered rats. ENU- or transposon-mediated mutagenized archives with thousands of individual samples have to be stored and made available in the interests of biomedical research. Powerful genetic manipulations of rat ES cells [4, 10], iPS cells [11], or other techniques enable the generation of knock-out models and will greatly expand the scope of present experimental limitations with new rat resources. Such repositories would ideally be managed across core centers in the USA, EU, and Japan to share the resources and standardize cryopreservation technologies. In these centers, laboratory space and equipment along with rat strains and related materials, should be provided to interested researchers to perform preliminary experiments and for archive screening. The advantage of bioresources in combination with sophisticated technologies, such as MRI, other bio-imaging technologies, behavioral measure stations, etc. is the

acquisition of fast and accurate results as prerequisites for larger projects.

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