

NIBB BIORESOURCE CENTER



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To understand the mechanisms of living organisms, studies focusing on each gene in the design of life (the genome) are required. The use of model animals and plants, such as mice, Medaka (*Oryzias latipes*), zebrafish, *Arabidopsis*, *Lotus japonicus*, and *Physcomitrella patens*, make it possible to produce genetically controlled organisms with markers placed using genetic and cell engineering technology. Such marking allows detailed studies of genes and cell functions. The model organisms mature in a short period of time; therefore, changes in cells, organs, and individuals can be totally and efficiently observed. The NIBB BioResource Center has equipment, facilities, and staff to maintain such organisms safely, efficiently, and appropriately.

Model Animal Research Facility

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Figure 1. Mouse breeding room in the Yamate area

The worldwide genome project has almost been completed and research on basic biology has arrived at a post-genome era in which researchers are focusing on investigating the functions of individual genes. To promote the functional analysis of a gene of interest it is essential to utilize genetically altered model organisms generated using genetic engineering technology, including gene deletion, gene replacement and point mutation.

The NIBB Center for Transgenic Animals and Plants was

established in April 1998 to support research using transgenic and gene targeting techniques at NIBB. The NIBB Center for Transgenic Animals and Plants was integrated into the NIBB BioResource center in April 2010, and was renamed “The Model Animal Research Facility”.

Technical and supporting staff develop and promote research-supporting activities. A state-of-the-art facility for transgenic animals opened at the end of 2003 in the Yamate area.

The activities of the model animal research facility are as follows:

1. The provision of information, materials, techniques, and animal housing space to researchers.
2. The use of various kinds of instruments to analyze mutant, transgenic, and gene-targeted animals.
3. The development of novel techniques related to transgenic and gene targeting technology.
4. Cryopreservation and storage of transgenic mice strains.
5. Generating genetically-engineered mice using the CRISPR/Cas9 method.

I. Research support activities (mouse)

In 2001, the NIBB mouse facility (built under specific pathogen free (SPF) conditions) opened in the Myodaiji area and the production, breeding, analysis, cryopreservation and storage of genetically manipulated mouse strains has been conducted there since. The new center facility building in the Yamate area strengthened research activities using genetically altered organisms. The building has five floors and a total floor space of 2,500 m² in which we can generate, breed, store and analyze transgenic, gene targeting, and mutant mice under SPF conditions. The mouse housing area was constructed based on a barrier system. This building is also equipped with breeding areas for transgenic small fish and birds.



Figure 2. Large sized autoclave in the Yamate area.

In 2017 (from January 1 to December 21), 3,940 mice (7 transgenic lines and wild-type) were brought into the facility in the Yamate area, and 40,428 mice (including pups bred in the facility) were taken out.

A number of strains of genetically altered mice from outside

the facility were brought into the mouse housing area by microbiological cleaning using *in vitro* fertilization-embryo transfer techniques (12 transgenic lines), and stored using cryopreservation (28 transgenic lines). Frozen eggs of 33 mice lines were taken out of the facility.

Genome editing experiments were performed on three kinds of target genes. We generated gRNAs of the target genes, which were transferred into fertilized eggs with Cas9 protein. We were able to introduce intended mutations into the genome DNA.

A new mouse facility in the Myodaiji area opened at the beginning of 2005. The facility provides research-supporting activities to researchers in the Myodaiji area. In March 2009, we expanded the facility which includes areas for breeding, behavioral tests, and transgenic studies using various kinds of recombinant viruses. In 2017 (from January 1 to December 21), 0 mice were brought into the facility in the Myodaiji area, and 1,246 mice (including pups bred in the facility) were taken out.



Figure 3. Equipment for gene transfer.

II. Research support activities (small fish and birds)

The first floor of the center facility building in the Yamate area provides space and facilities to maintain small fish and chick embryos. In the laboratory room for chick embryos, a large incubation chamber is equipped and set at 42 degrees (suitable for chick embryogenesis). The researchers can manipulate chick embryos under optimal conditions, removing biohazard risks. For researchers who employ fish as an experimental model, 480 tanks (1 liter) and 450 tanks (3 liters) are available for medaka and zebrafish, respectively. Water circulates and can be maintained to suit the conditions desired for fish breeding in the aquarium systems. Currently, over three mutant lines and over fifteen transgenic lines of medaka and zebrafish are maintained in our facility. A medaka line that allows gene induction by heat treatment, in combination with a cre/loxP system, has been developed in this facility. All the rooms are qualified to meet the criteria for transgenic animals, allowing researchers to generate and maintain these important biological tools.

In 2017 (from January 1 to December 21), 75 zebrafish (75

fertilized eggs) were brought to the facility. In the laboratory for chick embryos there were no fertilized eggs or chicken embryos brought in or taken out this year. These animals were used for research activities in neurobiology and developmental biology.



Figure 4. Liquid nitrogen tank.

III. Research activities

The associate professor of this center - E. Watanabe - is the principal investigators of the Laboratory of Neurophysiology. The Laboratory of Neurophysiology is studying mechanisms of the visual system using a psychophysical approach. For details, please refer to the page of the laboratory (p. 44).

Model Plant Research Facility

● Plant Culture Laboratory

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The Plant Culture Laboratory manages facilities for the cultivation of plants in general and also for the rearing of several animal species that do not fit in other facilities.

The Plant Culture Laboratory equips and manages 73 culture boxes or growth chambers, and 13 rooms with the P1P physical containment level for established and emerging model plants, for example the thale cress *Arabidopsis thaliana*, the rice *Oryza sativa*, the moss *Physcomitrella patens*, the liverwort *Marchantia polymorpha*, the green alga *Chlamydomonas reinhardtii* and several other flowering plants including several carnivorous plants. Most culture space is fully used the whole year by more than 70 researchers from both outside and inside groups.

As well as regular culture conditions, extreme environmental conditions for light and temperature are available for various types of experiments. Three chambers (3.4 m² each) that can control CO₂ and humidity in addition to temperature and light (max 70,000 lux) conditions are available. A tissue culture rack with dimming LEDs and pulse-width modulation controllers are used for algae culture under precise light control. Autotrophic and heterotrophic culture devices are

also available for researchers using cyanobacteria, algae, and cultured flowering plant cells. Aseptic experiments can be performed in an aseptic room with clean benches and a safety cabinet. Several analytical instruments including a flow cytometry system and a DUAL-PAM, for DNA content and chlorophyll fluorescent measuring, respectively, are also available.

Next to the institute building of the Myodaiji area, a 386-m² experimental farm is maintained for Japanese morning glory and related *Ipomoea* species, several carnivorous plants and other flowering plants necessary to be cultivated outside. Three greenhouses (44, 44, and 45 m²) with heating are used for the sensitive carnivorous plants. Four greenhouses (4, 6, 9, and 9 m²) with air-conditioning are provided for the cultivation of rice *Oryza* sp., *Lotus japonica* and related legume species, as well as mutant lines of the Japanese morning glory. Two greenhouses (9 and 18 m²) with air-conditioning meet the P1P physical containment level and are available for experiments using transgenic plants. The Plant Culture Laboratory also maintains a 46 m² building with storage and workspace. Part of the building is used for rearing of the orchid mantis and the Japanese rhinoceros beetle.

The building renovation work was completed in May 2017, and the temporally closed facilities including the three chambers that can produce extreme environmental conditions were renewed and opened.

Cell Biology Research Facility

Associate Professor: WATANABE, Eiji

The Cell Biology Research Facility provides various equipment for tissue and cell culture. This laboratory is equipped with safety rooms which satisfy the P2 physical containment level, and is routinely used for DNA recombination experiments.



Figure 5. Equipment for tissue and cell culture.