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 technologies. Such marking allows us to conduct detailed studies of genes and cell functions. Because these model organisms mature in a short period of time, changes in cells, organs, and individuals can be thoroughly and efficiently observed. On this front, the NIBB BioResource Center has the equipment, facilities, and staff to safely, efficiently, and appropriately maintain such organisms.

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 which are generated using genetic engineering technology, and harness techniques such as 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"; a place where technical and supporting staff develop and promote research-supporting activities. Furthermore, 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 spaces 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 ever since. The new center facility building in the Yamate area has strengthened research activities that require genetically altered organisms. The building has five floors and a total floor space of 2,500 m^2 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 small transgenic fish and birds.

Figure 2. Large sized autoclave in the Yamate area.

In 2018, 3,197 mice (8 transgenic lines and wild-type) were brought into the facility in the Yamate area, and 49,376 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 after microbiological cleaning using *in vitro* fertilization-embryo

transfer techniques (11 transgenic lines), and stored using cryopreservation (23 transgenic lines). The frozen eggs of 1 mice line were taken out of the facility.

Genome editing experiments were performed on two 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 was opened at the beginning of 2005. The facility provides research-supporting activities within 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 2018, 8 mice were brought into the facility in the Myodaiji area, and 826 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 provided 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 1 liter tanks and 450 3 liter tanks are available for medaka and zebrafish, respectively. Water can be maintained to suit the conditions desired for fish breeding. 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 2018, 0 zebrafish (0 fertilized eggs) were brought to the facility. There were no fertilized eggs or chicken embryos brought in or taken from the laboratory for chick embryos 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, Dr. Eiji Watanabe, is the principal investigator of the Laboratory of Neurophysiology. The Laboratory of Neurophysiology is studying various mechanisms of the visual system using a psychophysical approach. For more details, please refer to the page of the laboratory.

MODEL PLANT RESEARCH FACILITY

● ***Plant Culture Laboratory***

Assistant Professor:	*HOSHINO, Atsushi*
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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 qualify for housing in other facilities.

The Plant Culture Laboratory equips and manages around 75 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 external and internal 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 m^2 each) that can control CO_2 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 which are exposed to precise light concentrations. 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 two flow cytometry systems and a DUAL-PAM, for DNA content and chlorophyll fluorescent measuring, respectively, are also available. In addition, a liquid handling system for fully automated *in situ* hybridization of sections of up to 60 glass slides simultaneously is also provided.

A 386-m^2 experimental farm next to the institute building of the Myodaiji area is maintained for Japanese morning glory and related *Ipomoea* species, several carnivorous plants and other flowering plants that must be cultivated outdoors. Three heated greenhouses (44, 44, and 45 m^2) are used for the sensitive carnivorous plants. Four air conditioned greenhouses (4, 6, 9, and 9 m^2) with air-conditioning are provided for the cultivation of rice *Oryza* sp., *Lotus japonica* and other related legume species, as well as Japanese morning glory mutant lines. Two air conditioned greenhouses (9 and 18 m^2) 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 m^2 building with a storage area and workspace. Part of this building is used for rearing of the orchid mantis and the Japanese rhinoceros beetle.

In 2018, several culture boxes were replaced and newer ones were introduced in their place. Of these, a culture box can irradiate extremely bright light (max 76,000 lux) via the use of high luminance LED lamps.

The assistant professors of this facility, Dr. A. Hoshino and Dr. K. Tsugane are the principal investigators of each group in the Laboratory of Biological Diversity. For more details, please refer to the page of the laboratory.

Figure 5. A plant culture box with high luminance LED lamps.

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 6. Equipment for tissue and cell culture.