

NIBB BIORESOURCE CENTER



Head
FUJIMORI, Toshihiko

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

Specially Appointed Professor:

NARUSE, Kiyoshi

Associate Professor:

WATANABE, Eiji

TANAKA, Minoru

OHSAWA, Sonoko

HAYASHI, Kohji

NOGUCHI, Yuji

Technical Staff:

TAKAGI, Yukari

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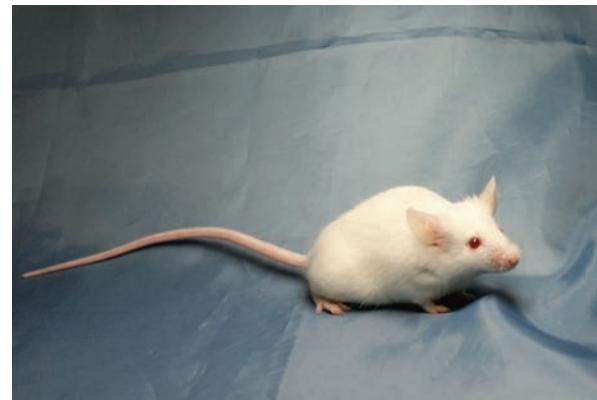


Figure 1. Mouse (MCH(ICR))

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 strains.

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. Equipment for manipulating mice eggs.

In 2016 (from January 1 to December 31), 2,907 fertilized eggs (*in vitro* fertilization; 2,546 eggs of 14 lines in which 1,747 eggs of 9 lines were frozen for long-term storage, frozen eggs: 361 of 7 lines) and 4,175 mice were brought

into the facility in the Yamate area, and 59,019 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, and stored using cryopreservation.

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 2016 (from January 1 to December 31), 25 mice were brought into the facility in the Myodaiji area, and 1,961 mice (including pups bred in the facility) were taken out.



Figure 3. Large sized autoclave in the Myodaiji area.

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 2016 (from January 1 to December 31), 600 medaka (300 adults and 300 fertilized eggs) were brought to the facility and 23,691 medaka and zebrafish (2,796 adults, 90 embryos and 20,805 fertilized eggs, including animals bred in the

facility) were taken out. 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.

In 2007 NIBB was approved as a core facility of the National BioResource Project (NBRP) for Medaka by the Japanese Government. We have supported the activities of NBRP Medaka by providing standard strains, mutants, transgenic lines and organizing international practical courses for medaka. In 2014 we began providing the CRISPR/Cas9 genome editing platform as well as the TILLING library screening system to promote the reverse genetic approach. In 2015 we shipped 220 independent medaka strains, 155 cDNA/BAC/Fosmid clones, and 195 samples of hatching enzyme to the scientific community worldwide.



Figure 4. Quarantine room for medaka and zebrafish.

III. Research activities

The associate professors of this center - E. Watanabe, T. Naruse, and M. Tanaka - are the principal investigators of the Laboratory of Neurophysiology, the Laboratory of Bioresources, and the Laboratory of Molecular Genetics for Reproduction, respectively. The Laboratory of Neurophysiology is studying mechanisms of the visual system using a psychophysical approach. The Laboratory of Bioresources has conducted a genetic and genomic analysis of quantitative traits and Mendelian phenotype variations, as well as evolution of sex determination systems in medaka related species. The Laboratory of Molecular Genetics for Reproduction is studying the molecular mechanisms of reproductive organ development and sex differentiation using mutagenized or transgenic medaka. For details, please refer to the pages of each laboratory (p. 42, 51, and 33).

Model Plant Research Facility

Plant Culture Laboratory

Assistant Professor: HOSHINO, Atsushi
TSUGANE, Kazuo
Technical Staff: MOROOKA, Naoki
Technical Assistant: SUZUKI, Keiko
KOTANI, Keiko

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 62 culture boxes or growth chambers, and 12 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 80 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.

Due to the building renovation work, 4 phytotrons and three light environmental simulators were removed, and some of the facilities are temporarily closed. The closed facilities will be renewed and opened in May 2017.

Morning Glory BioResource Laboratory

Assistant Professor: HOSHINO, Atsushi

The Japanese morning glory (*Ipomoea nil*) is a traditional floricultural plant in Japan, and is studied worldwide, especially in plant physiology and genetics. NIBB collects, develops and distributes DNA clones, mutant lines for flower pigmentation, and transgenic lines as a sub-center of the National BioResource Project (NBRP) Morning glory, and collaborates with the core organization center, Kyushu University. We collected several mutant lines, and provided 47 DNA clones to both local and international biologists this year. A database for the whole genome sequence of *I. nil* was built and opened to the public.

Research activities of the Assistant Professor A. Hoshino are shown on the laboratory page (p. 58).

Cell Biology Research Facility

Associate Professor: WATANABE, Eiji
Assistant Professor: HAMADA, Yoshio
Technical Assistant: SUGINAGA, Tomomi

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.