

NIBB BIORESOURCE CENTER



Head
IGUCHI, Taisen

Vice 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*, makes it possible to produce genetically controlled organisms with markers placed by 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

Associate Professor: **WATANABE, Eiji**
TANAKA, Minoru
NARUSE, Kiyoshi

Technical Staff: **HAYASHI, Kohji**
NOGUCHI, Yuji

Technical Assistant: **TAKAGI, Yukari**
SUZUKI, Kohta
SUGINAGA, Tomomi
INADA, Yosuke
FUJIMOTO, Daiji
MATSUMURA, Kunihiko
ATSUMI, Miho



Figure 1. Mouse (strain C57BL/6J)

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 staff 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 then. 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, birds, and insects.

In 2014 (from January 1 to December 31), 2,962 fertilized eggs (*in vitro* fertilization; 2,767 eggs of 10 lines in which 2,197 eggs of 10 lines were frozen for long-term storage,



Figure 2. Equipment for manipulating mouse eggs.

frozen eggs: 195 of 3 lines) and 4,948 mice were brought into the facility in the Yamate area, and 59,517 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 supports the activities of 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 2014 (from January 1 to December 31), 53 mice were brought into the facility in the Myodaiji area, and 3,759 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, birds, and insects)

The first floor of the center facility building in the Yamate area provides space and facilities to maintain small fish, chick embryos, and insects. 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. In addition to the rooms mentioned above, a room for insects is also available. All the rooms are qualified to meet the criteria for transgenic animals, allowing

researchers to generate and maintain these important biological tools.

In 2014 (from January 1 to December 31), 11,002 medaka adults were brought to the facility and 94,135 medaka and zebrafish (88,592 fertilized eggs and 5,543 adults, 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 2014 we shipped 207 independent medaka strains, 233 cDNA/BAC/Fosmid clones, and 120 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. 44, 52, and 33).

Model Plant Research Facility

● Plant Culture Laboratory

Assistant Professor: HOSHINO, Atsushi
TSUGANE, Kazuo

Technical Staff: MOROOKA, Naoki
Technical Assistant: SUZUKI, 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, 4 phytotrons, and 12 rooms with the P1P physical containment level for established and emerging model plants including the thale cress *Arabidopsis thaliana*, several carnivorous plants, the rice *Oryza sativa*, the moss *Physcomitrella patens*, green alga *Chlamydomonas reinhardtii* and several other flowering plants. Most culture space is fully used the whole year by more than 60 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 light environmental simulators (max 120,000 lux by using xenon lamp) and 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. 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.

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 green houses (44, 44, and 45 m²) with heating are used for the sensitive carnivorous plants, and wild-type strains of medaka fish *Oryzias* sp. Seven green houses (4, 6, 6, 6, 6, 9, and 9 m²) with air-conditioning are provided for the cultivation of a rice *Oryza* sp., *Lotus japonica* and related legume species, as well as mutant lines of the Japanese morning glory. Two green houses (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.

In 2014, several plant culture facilities came into use. Of these, the multiple precise environment control plant incubator system is composed of 10 incubators. Users can control all the incubators synchronously. A tissue culture rack with dimming LEDs and pulse-width modulation controllers is used for algae culture under precise light control.



Figure 5. A tissue culture rack with dimming LEDs and pulse-width modulation controllers.

● 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 10,000 BAC clones, and provided 3 DNA clones and 28 *Ipomoea* lines to both local and foreign biologists this year.

Research activities of the assistant professor A. Hoshino are shown on the laboratory page (p. 57).

Cell Biology Research Facility

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.

Research activities of assistant professor Y. Hamada, the principal investigator of the Laboratory of Cell Sociology, is shown on the laboratory page (p.17).



Figure 6. Equipment for tissue and cell culture.