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, which have markers placed on them, 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

Associate Professor:	*WATANABE, Eiji*
Technical Staff:	*OHSAWA, Sonoko*
	NOGUCHI, Yuji
Technical Assistant:	*TAKAGI, Yukari*
	SUGINAGA, Tomomi
	FUJIMOTO, Daiji
	KITAZUMI, Noriaki

The worldwide genome project has almost been completed and basic biological research is now in a post-genome era in which researchers focus 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 was also opened at the end of 2003 in the Yamate area of NIBB.

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) was opened in the Myodaiji area of NIBB. Since then, 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 1. Mouse breeding room in the Yamate area

Figure 2. Breeding cages for replacement in the clean corridor

From April 1, 2020 to March 31, 2021, 2,813 mice (2 transgenic lines and wild-type) were brought into the facility in the Yamate area, and 34,445 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 this area after microbiological cleaning using *in vitro* fertilization-embryo transfer techniques (8 transgenic lines), and stored using cryopreservation (46 transgenic lines). The frozen eggs of 132 mice lines 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, and were able to introduce intended mutations into the genome DNA.

Figure 3. Large sized autoclave in the Yamate 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 provided and set at 37.5 degrees (suitable for chick embryogenesis). The researchers can manipulate these embryos under optimal conditions, thus removing biohazard risks.

For researchers who employ fish as an experimental model, 480 tanks (1 liter) and 450 tanks (3 liter) are available for medaka and zebrafish, respectively. Additionally, water can be maintained to suit the conditions desired for fish breeding. A medaka line that allows gene induction by heat treatment, in combination with a cre/loxP system, has been developed using this facility. All the rooms are qualified to meet the criteria for transgenic animals, allowing researchers to generate and maintain these important biological tools.

Figure 4. Liquid nitrogen tank

In 2020, 0 zebrafish (0 fertilized eggs) were brought to the facility nor were there any fertilized eggs or chicken embryos brought in or taken from the laboratory. The animals housed within the facility were used for research activities in neurobiology and developmental biology.

Figure 5. Vinyl isolators in the quarantine room

III. Research activities

The associate professor of this center, Dr. Eiji Watanabe, is the principal investigator of the Laboratory of Neurophysiology, which studies various mechanisms of the visual system using a psychophysical approach. For more details, please refer to the laboratory's page.

MODEL PLANT RESEARCH FACILITY

Assistant Professor:	*HOSHINO, Atsushi*
	TSUGANE, Kazuo
Technical Staff:	*MOROOKA, Naoki*
Technical Assistant:	*YAMAGUCHI, Chinami*

The Model Plant research facility manages facilities for the cultivation of plants in general, and the rearing of several animal species that do not qualify to be housed in other facilities.

This facility equips and manages around 75 culture boxes or growth chambers, as well as 13 rooms with the P1P

physical containment level required for established and emerging model plants, such as 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 which include several carnivorous plants. The facilities are also used to grow the sea anemone *Exaiptasia pallida*. Most culture space is used throughout the 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 also available. Furthermore, a tissue culture rack with dimming LEDs and pulse-width modulation controllers are used for algae cultures 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. In addition, several analytical instruments, that include two flow cytometry systems and a DUAL-PAM, for DNA content and chlorophyll fluorescent measuring, respectively, are also available. On top of this, 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 NIBB Myodaiji area building is maintained for Japanese morning glories and related *Ipomoea* species, several carnivorous plants, the castor bean, 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 and a periodical mass-flowering plant, *Strobilanthes flexicaulis*. Four air-conditioned greenhouses (4, 6, 9, and 9 m^2) are provided for the cultivation of Japanese morning glories and several carnivorous plants. Two air-conditioned greenhouses (9 and 18 m^2) meet the P1P physical containment level and are available for experiments using transgenic rice plants, Japanese morning glories, as well as carnivorous 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 the rearing of Japanese rhinoceros beetles and the common grass yellow butterfly.

Figure 6. Webcam monitoring of Japanese rhinoceros beetle larvae.

Between April 2020 and March 2021, 4 culture boxes were replaced by newer ones.

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 7. Equipment for P3A experiments