NIBB Core Research Facilities

Head
YOSHIDA, Shosei

The NIBB Core Research Facilities support basic biological research conducted at NIBB. They consist of three facilities that develop and provide state-of-the-art technologies aimed at increasing the understanding of biological functions through the application of functional genomics, bioimaging, and bioinformatics. The NIBB Core Research Facilities also act as an intellectual hub to promote collaboration among NIBB researchers and other academic institutions.

Functional Genomics Facility

Professor
SHIGENOBU, Shuji

Technical Staff:	*MORI, Tomoko*
	MAKINO, Yumiko
	YAMAGUCHI, Katsushi
	MORI, Syogo
Technical Assistant:	*ASAO, Hisayo*
	AKITA, Asaka
	MATSUMOTO, Miwako
Admin Support Staff:	*ICHIKAWA, Mariko*

The Functional Genomics Facility is a division of the NIBB Core Research Facilities organized jointly by NIBB and NIPS for the promotion of DNA and protein studies. The facility maintains a wide array of core research equipment, ranging from standard machinery (*e.g.* ultracentrifuges) to cutting edge tools (*e.g.* next generation DNA sequencers), which amount to 90 instruments in total. The facility is dedicated to fostering collaborations with researchers both at NIBB and other academic institutions worldwide through the provision of these tools as well as expertise. Our current focus is functional genomics. We also act as a bridge between experimental biology and bioinformatics by providing close consultation and training.

In 2021, we still suffered from the effects of the COVID-19 pandemic that hampered research activities worldwide. While we operated our facility placing the highest priority on users' safety, we provided remote support for the facilities' users and online communication with collaborators to sustain research projects. Such efforts resulted in 11 co-authored papers being published.

Representative Instruments

Genomics

The advent of next-generation sequencing (NGS) technologies is transforming modern biology thanks to ultra-high-throughput DNA sequencing. Utilizing HiSeq, NextSeq and MiSeq (Illumina), Sequel (PacificBio Sciences), and MinION and GridION (Oxford Nanopore Technologies), the Functional Genomics Facility is committed to joint research aimed at exploring new yet otherwise inaccessible fields in basic biology. We have upgraded the Sequel instrument in 2021, which enabled a significant increase of the HiFi long read production.

During 2021, we carried out 53 NGS projects in collaboration with researchers from academic institutions throughout the world. These projects cover a wide range of species (bacteria, animals, plants, and fungi) including both model and non-model organisms, and various other applications such as genomic re-sequencing, RNA-seq, single-cell trnascriptome and ChIP-seq.

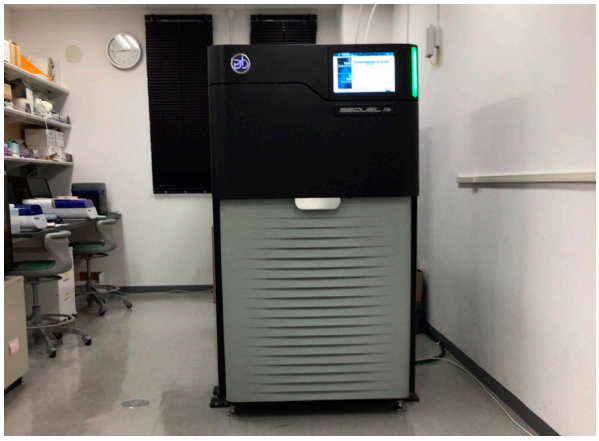

Figure 1. Next-generation sequencer

Proteomics

As is listed below, two types of mass spectrometers and two protein sequencers are used for proteome studies in our facility. In 2021, we analyzed approximately 600 samples with mass spectrometers and protein sequencers.

- LC-MS (AB SCIEX TripleTOF 5600 system)
- LC-MS (Thermo Fisher SCIENTIFIC Orbtrap Elite)

Protein sequencer (ABI Procise 494 HT; ABI Procise 492 cLC)

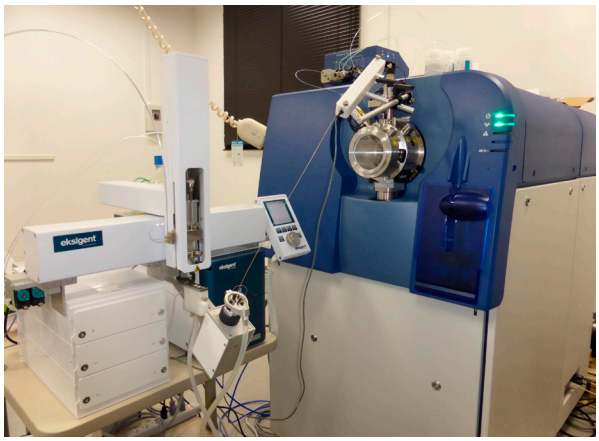

Figure 2. LC-MS/MS system

Other analytical instruments (excerpts)

- Cell sorter (SONY SH800)
- Bioimaging analyzer (BIO-RAD ChemiDoc XRS+ ; Fujifilm LAS 3000 mini; GE FLA9000)
- Laser capture microdissection system (Thermo Fisher Scientific Arcturus XT)
- Real-time PCR machine (Thermo Fisher Scientific ABI 7500, QuantStudio 3)
- Ultracentrifuge (Beckman XL-80XP etc.)
- Microplate reader (PerkinElmer Nivo; Hitachi SH-9000Lab)
- Single-cell analysis system (Fluidigm C1, 10x Genomics Chromium X)

Publication List on Cooperation:

〔Original papers〕

- Goto, T., Soyano, T., Liu, M., Mori, T., and Kawaguchi, M. (2022). Auxin methylation by IAMT1, duplicated in the legume lineage, promotes root nodule development in *Lotus japonicus*. Proc. Natl. Acad. Sci. U.S.A. *119*. e2116549119. DOI: 10.1073/pnas.2116549119
- Hasegawa, Y., Ueno, S., Wei, F.-J., Matsumoto, A., Uchiyama, K., Ujino-Ihara, T., Hakamata, T., Fujino, T., Kasahara, M., Bino Takahiro and Yamaguchi, K., Shigenobu, S., Tsumura, Y., and Moriguchi, Y. (2021). Identification and genetic diversity analysis of a male-sterile gene (MS1) in Japanese cedar (Cryptomeria japonica D. Don). Sci. Rep. *11*, 1496. DOI: 10.1038/s41598-020-80688-1
- Igawa-Ueda, K., Ikuta, T., Tame, A., Yamaguchi, K., Shigenobu, S., Hongo, Y., Takaki, Y., Fujikura, K., Maruyama, T., and Yoshida, T. (2021). Symbiont Transmission onto the Cell Surface of Early Oocytes in the Deep-sea Clam *Phreagena okutanii*. Zoolog. Sci. *38*, 140–147. DOI: 10.2108/zs200129
- Ishida, S., Suzuki, H., Iwaki, A., Kawamura, S., Yamaoka, S., Kojima, M., Takebayashi, Y., Yamaguchi, K., Shigenobu, S., Sakakibara, H., Kohchi, T., and Nishihama, R. (2022). Diminished Auxin Signaling Triggers Cellular Reprogramming by Inducing a Regeneration Factor in the Liverwort Marchantia polymorpha. Plant Cell Physiol. *63*, 384–400. DOI: 10.1093/pcp/pcac004
- Ishishita, S., Kitahara, S., Takahashi, M., Iwasaki, S., Tatsumoto, S., Hara, I., Kaneko, Y., Kinoshita, K., Yamaguchi, K., Harada, A., Ohmori, Y., Ohkawa, Y., Go, Y., Shigenobu, S., Matsuda, Y., and Suzuki, T. (2022). Uterus-specific transcriptional regulation underlies eggshell pigment production in Japanese quail. PLoS One *17*, e0265008. DOI: 10.1371/journal.pone.0265008
- Korgaonkar, A., Han, C., Lemire, A.L., Siwanowicz, I., Bennouna, D., Kopec, R.E., Andolfatto, P., Shigenobu, S., and Stern, D.L. (2021). A novel family of secreted insect proteins linked to plant gall development. Curr. Biol. *31*, 1836-1849.e12. DOI: 10.1016/j.cub.2021.01.104
- Maeda, T., Takahashi, S., Yoshida, T., Shimamura, S., Takaki, Y., Nagai, Y., Toyoda, A., Suzuki, Y., Arimoto, A., Ishii, H., Satoh, N., Nishiyama, T., Hasebe, M., Maruyama, T., Minagawa, J., Obokata, J., and Shigenobu, S. (2021). Chloroplast acquisition without the gene transfer in kleptoplastic sea slugs, *Plakobranchus ocellatus*. eLife *10*, e60176. DOI: 10.7554/eLife.60176
- Miyazaki, S., Fujiwara, K., Kai, K., Masuoka, Y., Gotoh, H., Niimi, T., Hayashi, Y., Shigenobu, S., and Maekawa, K. (2021). Evolutionary transition of doublesex regulation from sex-specific splicing to male-specific transcription in termites. Sci. Rep. *11*, 15992. DOI: 10.1038/s41598-021-95423-7
- Okamoto, S., Kawasaki, A., Makino, Y., Ishida, T., and Sawa, S. (2022). Long-distance translocation of CLAVATA3/ESR-related 2 peptide and its positive effect on roots sucrose status. Plant Physiol. *189*, 2357–2367. DOI: 10.1093/plphys/kiac227
- Okamoto, S., Kawasaki, A., and Makino, Y. (2022). Characterization of Oligopeptides in *Solanum lycopersicum* Xylem Exudates. Life *12*. 592. DOI: 10.3390/life12040592
- Sato, K., Uehara, T., Holbein, J., Sasaki-Sekimoto, Y., Gan, P., Bino, T., Yamaguchi, K., Ichihashi, Y., Maki, N., Shigenobu, S., Ohta, H., Franke, R.B., Siddique, S., Grundler, F.M.W., Suzuki, T., Kadota, Y., and Shirasu, K. (2021). Transcriptomic Analysis of Resistant and Susceptible Responses in a New Model Root-Knot Nematode Infection System Using *Solanum torvum* and *Meloidogyne arenaria*. Front. Plant Sci. *12*, 680151. DOI: 10.3389/fpls.2021.680151
- Shigenobu, S., Hayashi, Y., Watanabe, D., Tokuda, G., Hojo, M.Y., Toga, K., Saiki, R., Yaguchi, H., Masuoka, Y., Suzuki, R., Suzuki, S., Kimura, M., Matsunami, M., Sugime, Y., Oguchi, K., Niimi, T., Gotoh, H., Hojo, M.K., Miyazaki, S., Toyoda, A., Miura, T., and Maekawa, K. (2022). Genomic and transcriptomic analyses of the subterranean termite *Reticulitermes speratus*: Gene duplication facilitates social evolution. Proc. Natl. Acad. Sci. U.S.A. *119*, e2110361119. DOI: 10.1073/pnas.2110361119
- Subhankar, B., Yamaguchi, K., Shigenobu, S., and Aoki, K. (2021). *Trans*-species small RNAs move long distances in a parasitic plant complex. Plant Biotechnol. *38*, 187–196. DOI: 10.5511/plantbiotechnology.21.0121a
- Takeuchi, T., Matsubara, H., Minamitani, F., Satoh, Y., Tozawa, S., Moriyama, T., Maruyama, K., Suzuki, K.T., Shigenobu, S., Inoue, T., Tamura, K., Agata, K., and Hayashi, T. (2022). Newt Hoxa13 has an essential and predominant role in digit formation during development and regeneration. Development *149*, dev200282. DOI: 10.1242/dev.200282
- Tominaga, T., Miura, C., Sumigawa, Y., Hirose, Y., Yamaguchi, K., Shigenobu, S., Mine, A., and Kaminaka, H. (2021). Conservation and Diversity in Gibberellin-Mediated Transcriptional Responses Among Host Plants Forming Distinct Arbuscular Mycorrhizal Morphotypes. Front. Plant Sci. *12*, 795695. DOI: 10.3389/fpls.2021.795695
- Yoshida, Y., Shaikhutdinov, N., Kozlova, O., Itoh, M., Tagami, M., Murata, M., Nishiyori-Sueki, H., Kojima-Ishiyama, M., Noma, S., Cherkasov, A., Gazizova, G., Nasibullina, A., Deviatiiarov, R., Shagimardanova, E., Ryabova, A., Yamaguchi, K., Bino, T., Shigenobu, S., Tokumoto, S., Miyata, Y., Cornette, R., Yamada, T.G., Funahashi, A., Tomita, M., Gusev, O., and Kikawada, T. (2022). High quality genome assembly of the anhydrobiotic midge provides insights on a single chromosome-based emergence of extreme desiccation tolerance. NAR Genomics Bioinforma. *4*, lqac029. DOI: 10.1093/nargab/lqac029

〔Review article〕

- Miura, T., Oguchi, K., Yamaguchi, H., Nakamura, M., Sato, D., Kobayashi, K., Kutsukake, N., Miura, K., Hayashi, Y., Hojo, M., Maekawa, K., Shigenobu, S., Kano, T., and Ishiguro, A. (2022). Understanding of superorganisms: collective behavior, differentiation and social organization. Artif. Life Robot. *27*, 204–212. DOI: 10.1007/s10015-022-00754-x

SPECTROGRAPHY AND BIOIMAGING FACILITY

Specially Appointed Associate Professor
KAMEI, Yasuhiro

Technical Staff:	*KONDO, Maki*
	TANIGUCHI-SAIDA, Misako
Visiting Undergraduate:	*YOSHIKAWA, Ryusei*
Technical Assistant:	*ICHIKAWA, Chiaki*
	ASAO, Momoko
	NAKAGAWA, Mami
	AOYAMA, Chie

The Spectrography and Bioimaging Facility assists both collaborative and core research by managing and maintaining research tools that use **Light**. The facility, under the guidance of Dr. Kamei, also provides technical support through the management of technical staff assisting in the advancement of collaborative and core research projects, as well as academic support to researchers (please refer to the Collaborative Research Group Research Enhancement Strategy Office section for more information). Among the equipment available are advanced biological microscopes, and the Okazaki Large Spectrograph for photobiology. The Okazaki Large Spectrograph is the world's largest wide spectrum exposure mechanism, and is capable of producing a range of wavelengths from 250 nm (ultraviolet) to 1,000 nm (infrared) along its 10-meter focal curve, thus allowing exposure to strong monochromatic light. The facility's microscopes, which include cutting edge devices such as confocal and multi-photon excitation microscopes, are an indispensable part of core and collaborative projects conducted by both internal and external researchers.

Standard Instruments: Okazaki Large Spectrograph (OLS)

The spectrograph runs on a 30 kW Xenon arc lamp and projects a wavelength spectrum ranging from 250 nm (ultraviolet) to 1,000 nm (infrared) onto its 10 m focal curve with an intensity of monochromatic light at each wavelength more than twice as great as that of the corresponding monochromatic component of tropical sunlight at noon (Watanabe *et al*., Photochem. Photobiol. *36*, 491-498, 1982). The spectrograph is dedicated to action spectroscopical studies of various light-controlled biological processes.

In addition to the other action spectroscopical studies concerning various regulatory and damaging effects of light on living organisms, research involving both biological and artificial organic molecules have been conducted since it has been set up. The NIBB Collaborative Research Program for the Use of the OLS supports about 10 projects every year conducted by both visiting scientists, including foreign researchers, as well as members of NIBB.

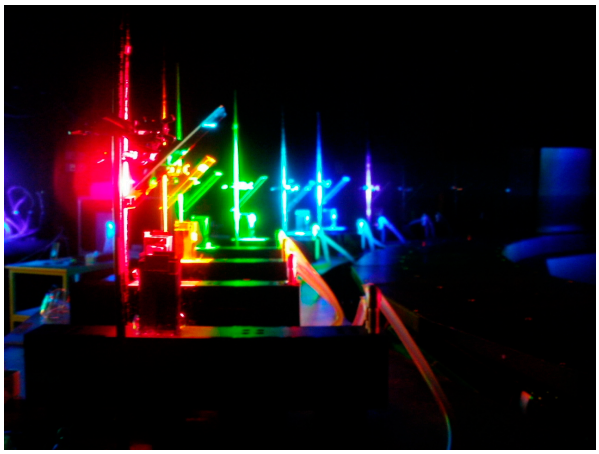

Figure 1. An example of an experiment using the Large Spectrograph. In this photo, various color rays (monochromatic light from right side and reflected by mirrors) are irradiated simultaneously onto samples stored in cooling chambers.

Microscopes

This facility also provides bioimaging machinery (Figure 2), such as wide-field microscopes (Olympus IX-81 and BX-63), confocal microscopes (Leica TCS-SP8, Nikon A1R, Nikon A1Rsi and Yokogawa CSU-X1 with EM-CCD/CMOS cameras), multi-photon microscopes (Olympus FV1000-MP, FV1200-MPs, Leica TCS-SP8 MPs) and other advanced laser microscopes boasting specialized, cutting edge technology (Light-sheet Microscope and Infrared Laser-Evoked Gene Operator microscope: IR-LEGO), which can be utilized by researchers within NIBB, as well as collaborative guest researchers. Starting from 2016, we have commenced two new types of Collaborative Research Programs. One is a new category within the NIBB Collaborative Research for Integrative Bioimaging program using machinery and bioimage processing/analysis techniques, and the other is the Advanced Bioimaging Support Program (ABiS) which operates under the framework of the Grant-in-Aid for Scientific Research on Innovative Areas.

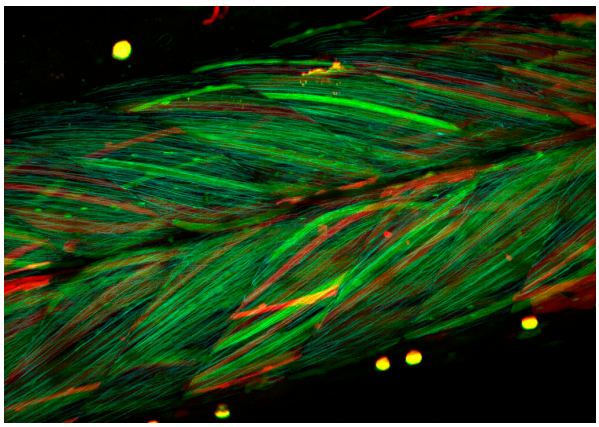

Figure 2. Multi-color confocal microscope image of muscle in a transgenic medaka larva. Each muscle cell expressed randomly various color variants of fluorescent protein, such as Cerulean, GFP, YFP and dsRed. The transgenic line was provided by NBRP Medaka.

The light-sheet microscope was developed by Dr. Ernst Stelzer's group at the European Molecular Biology Laboratory (EMBL). This microscope can realize high-speed z-axis scanning in deeper tissues by illuminating specimens from the side with a light sheet (more information is given in the report submitted by Dr. Shigenori Nonaka's Laboratory for Spatiotemporal Regulations). Subsequently, Dr. Nonaka has conducted and supported roughly 10 Collaborative Research Program projects for Integrative Bioimaging. The IR-LEGO, developed by Drs. Shunsuke Yuba and Yasuhiro Kamei at the National Institute of Advanced Industrial Science and Technology (AIST), can induce a target gene of interest by heating a single target cell *in vivo* with a high efficiency irradiating infrared laser. This microscope technology can be applied to thermal biology through local heating (the details of this are provided in the next section). IR-LEGO was also used for about 10 Collaborative Research projects, including applications aimed at both animals and plants.

Workshop, Symposium and Training course

In 2021, we held the 9th biological image processing training course in cooperation with Drs. Kagayaki Kato, Shigenori Nonaka, Yasuhiro Kamei, Takashi Murata and Hiroshi Koyama. The course was held in an online meeting format due to the COVID-19 pandemic. We have also started a new course "Optical Microscopy Principle Training Course" cooperation with Drs. Joe Sakamoto, Yasuhiro Kamei, Atsushi Taniguchi, Shigenori Nonaka in NIBB and Motosuke Tsutsumi and Kohei Otomo in ExCELLS. In this course, participants learned microscopy principles thorough lectures and built bright-field and fluorescent microscopes by themselves using optical devices, such as lenses, filters, light sources and cameras (Figure 3). We additionally hold some training courses and seminars related to microscopy and image analysis, including their technologies and applications.

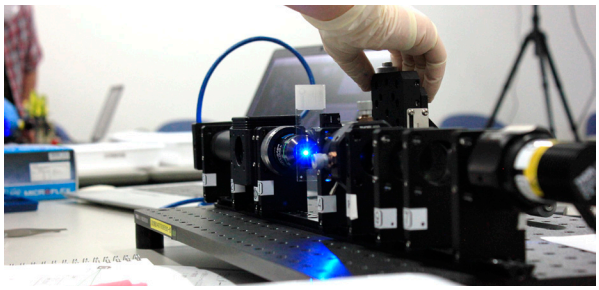

Figure 3. A scene from the 1st Optical Microscopy Principle Training Course. The participants built up a basic microscope by themselves using optical devices.

Publication List on Cooperation

〔Original papers (Selected)〕

- Ansai, S., Mochida, K., Fujimoto, S., Mokodongan, D.F., Sumarto, B.K.A., Masengi, K.W.A., Hadiaty, R.K., Nagano, A.J., Toyoda, A., Naruse, K., Yamahira, K., and Kitano, J. (2021). Genome editing reveals fitness effects of a gene for sexual dichromatism in Sulawesian fishes. Nat. Commun. *12*. 1350. DOI: 10.1038/s41467-021-21697-0
- Beppu, K., Tsutsumi, R., Ansai, S., Ochiai, N., Terakawa, M., Mori, M., Kuroda, M., Horikawa, K., Tomoi, T., Sakamoto, J., Kamei, Y., Naruse, K., and Sakaue, H. (2022). Development of a screening system for agents that modulate taste receptor expression with the CRISPR-Cas9 system in medaka. Biochem. Biophys. Res. Commun. *601*, 65–72. DOI: 10.1016/j.bbrc.2022.02.082
- Goto, T., Soyano, T., Liu, M., Mori, T., and Kawaguchi, M. (2022). Auxin methylation by IAMT1, duplicated in the legume lineage, promotes root nodule development in Lotus japonicus. Proc. Natl. Acad. Sci. U.S.A. *119*, e2116549119. DOI: 10.1073/pnas.2116549119
- Kaneko, E., Sato, H., and Fukamachi, S. (2021). Validation of the three-chamber strategy for studying mate choice in medaka. PLoS One *16*, e0259741. DOI: 10.1371/journal.pone.0259741
- Kinoshita, N., Yamamoto, T.S., Yasue, N., Takagi, C., Fujimori, T., and Ueno, N. (2022). Article Force-dependent remodeling of cytoplasmic ZO-1 condensates contributes to cell-cell adhesion through enhancing tight junctions. iSCIENCE *25*, 103846. DOI: 10.1016/j.isci.2022.103846
- Matsuo, M., Kamei, Y., and Fukamachi, S. (2021). Behavioural red-light sensitivity in fish according to the optomotor response. R. Soc. Open Sci. *8*, 210415. DOI: 10.1098/rsos.210415
- Mohri, T., and Kyozuka, K. (2022). Starfish oocytes of A. pectinifera reveal marked differences in sperm-induced electrical and intracellular calcium changes during oocyte maturation and at fertilization. Mol. Reprod. Dev. *89*, 3–22. DOI: 10.1002/mrd.23544
- Tokanai, K., Kamei, Y., and Minokawa, T. (2021). An easy and rapid staining method for confocal microscopic observation and reconstruction of three-dimensional images of echinoderm larvae and juveniles. Dev. Growth Differ. *63*, 478–487. DOI: 10.1111/dgd.12758
- Wu, D., Arakawa, H., Fujita, A., Hashimoto, H., Hibi, M., Naruse, K., Kamei, Y., Sato, C., and Kitajima, K. (2021). A point-mutation in the C-domain of CMP-sialic acid synthetase leads to lethality of medaka due to protein insolubility. Sci. Rep. *11*, 23211. DOI: 10.1038/s41598-021-01715-3
- Zeng, C.-W., Kamei, Y., Shigenobu, S., Sheu, J.-C., and Tsai, H.-J. (2021). Injury-induced Cavl-expressing cells at lesion rostral side play major roles in spinal cord regeneration. Open Biol. *11*, 200304. DOI: 10.1098/rsob.200304

〔Research activity by Yasuhiro Kamei〕

Specially Appointed Associate Professor Yasuhiro Kamei is the principal investigator of Laboratory for Biothermology. For details, please refer to the laboratory page.

DATA INTEGRATION AND ANALYSIS FACILITY

Associate Professor
UCHIYAMA, Ikuo

Technical Staff: *NISHIDE, Hiroyo*
NAKAMURA, Takanori
SUGIURA, Hiroki
Technical Assistant: *KOTANI, Keiko*

The Data Integration and Analysis Facility supports research activities based on large-scale biological data analysis, such as genomic sequence, expression data, and imaging data analysis. To achieve this, the facility maintains high-performance computers with large-capacity storage systems. It accordingly supports the development of data analysis pipelines and database construction based on these systems, and also sets up websites to distribute data worldwide as well as providing basic technical support. In addition to computational analysis, the Data Integration and Analysis Facility supports NIBB's information infrastructure, the maintenance of the institute's network systems and provides computer/network consultation for institute members.

Representative Instruments

Our main computer system is the Biological Information Analysis System (BIAS) (Figure 1), which consists of a high-performance cluster system (HPE Apollo r2800, 20 nodes/800 cores, 192 GB memory/node), a shared memory parallel computer (HPE ProLiant DL560, 72 cores, 3TB memory; HP ProLiant DL980 G7, 80 cores, 4TB memory), a high-throughput storage system (DDN SFA7700X, 1.52PB+880TB), and a large capacity storage system (DELL PowerEdge R620, 720TB). All subsystems are connected via a high-speed InfiniBand network, so that large amounts of data can be efficiently processed. Some personal computers and color printers are also available for use. On this system, we provide various biological databases and data retrieval/analysis programs, and support large-scale data analysis and database construction for institute members and collaborative researchers. We have provided support in the construction and maintenance of published databases of various model and non-model organisms in particular. These include XDB (*Xenopus laevis*), PHYSCObase (*Physcomitrella patens*), iNewt (*Pleurodales waltl*), The Plant Organelles Database, MBGD (microbial genomes), DB-HABs (harmful algal blooms), and ChaetoBase (*Chaetoceros gracilis*).

Figure 1. Biological Information Analysis System

The facility also provides network communication services. Most of the PCs in each laboratory, as well as all of the above-mentioned service machines, are connected by a local area network, which is linked to the high-performance backbone ORION network connecting the three research institutes in Okazaki. Many local services, including sequence analysis, file sharing, and printer services, are provided through this network. We also maintain a public World Wide Web server that hosts the NIBB home page (https://www.nibb.ac.jp/en).

Research activity by Ikuo Uchiyama

Associate Professor Ikuo Uchiyama is the principal investigator of the Laboratory of Genome Informatics. For more details, please refer to the laboratory page.