

NIBB CORE RESEARCH FACILITIES



Head
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The NIBB Core Research Facilities were launched in 2010 to support basic biology research in NIBB. They consist of three facilities that are developing and providing state-of-the-art technologies to understand biological functions through functional genomics, bioimaging and bioinformatics.

The NIBB Core Research Facilities also act as an intellectual hub to promote collaboration among the researchers of NIBB and other academic institutions.

Functional Genomics Facility



Associate Professor (Specially appointed)
SHIGENOBU, Shuji

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Secretary:	ICHIKAWA, Mariko

The Functional Genomics Facility is a division of the NIBB Core Research Facilities and organized jointly by NIBB and NIPS for promoting DNA and protein studies. The facility maintains a wide array of core research equipment, from standard machinery like ultracentrifuges to cutting edge tools such as next generation DNA sequencers, which amount to 60 different kinds of instrument. The facility is dedicated to fostering collaborations with researchers both of NIBB and other academic institutions worldwide by providing these tools as well as expertise. Our current focus is supporting functional genomics works that utilize mass spectrometers and DNA sequencers. We also act as a bridge between experimental biology and bioinformatics.

Representative Instruments

Genomics

The advent of next-generation sequencing (NGS) technologies is transforming today's biology by ultra-high-throughput DNA sequencing. Utilizing the SOLiD5500xl (Applied Biosystems), HiSeq2000 (Illumina), and MiSeq (Illumina) the Functional Genomics Facility is committed to joint research aiming to exploring otherwise inaccessible new fields in basic biology.

During 2012 we carried out 47 NGS projects in collaboration with NIBB laboratories as well as the researchers of other academic institutions. These projects cover a wide range of species (bacteria, animals, plants, and humans) including both model and non-model organisms,

and various applications such as genomic re-sequencing, RNA-seq and ChIP-seq.

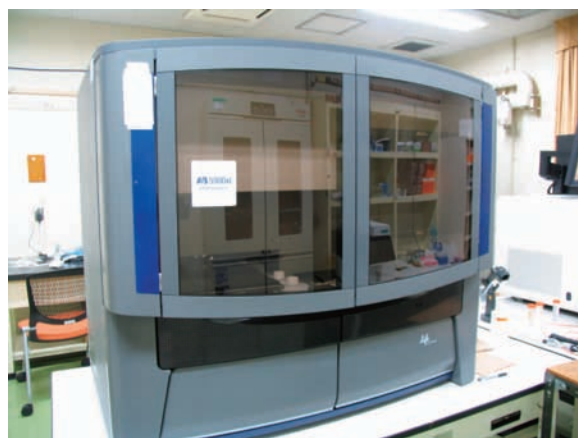


Figure 1. Next-generation sequencer SOLiD5500xl

Proteomics

Three different types of mass spectrometer and two protein sequencers, as listed below, are used for proteome studies in our facility. In 2012, we analyzed approximately 248 samples with mass spectrometers and 37 samples with protein sequencers.

- GC-Mass Spectrometer (JEOL DX-300)
- MALDI-TOF-MS (Bruker Daltonics REFLEX III)
- LC-Q-TOF MS (Waters Q-TOF Premier)
- Protein sequencer (ABI Procise 494 HT; ABI Procise 492 cLC)

Other analytical instruments

- Flow Cytometer (Coulter EPICS XL)
- Bio Imaging Analyzer (Fujifilm LAS 3000 mini; GE FLA9000)
- Laser Capture Microdissection System (Arcturus XT)
- DNA Sequencer (ABI PRISM 310; ABI 3130xl)
- Real Time PCR (ABI 7500)
- Ultra Centrifuge (Beckman XL-80XP etc.)



Figure 2. LC-Q-TOF-MS

Genome Informatics Training Course

We organize NIBB Genome Informatics Training Courses every year. In 2012, we provided two two-day training courses on next-generation sequence data analyses and transcriptome analysis. These courses are designed to introduce the basic knowledge and skills of bioinformatics analysis to biologists who are not familiar with bioinformatics.



Figure 3. NIBB Genome Informatics Training Course

● Research activity by S. Shigenobu

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Symbiosis Genomics

“Nothing, it seems, exists except as part of a network of interactions.” (Gilbert & Epel, 2008)

Every creature on the earth exists among a network of various biological interactions. For example, many multicellular organisms, including humans, harbor symbiotic bacteria in their bodies: some of them provide their hosts with essential nutrients deficient in the host's diet and others digest foods indigestible by the host alone. In spite of numerous examples of symbioses and its intriguing outcomes, the genetic and molecular basis underlying these interactions remains elusive. The goal of our group is to establish a new interdisciplinary science “Symbiosis Genomics”, where we aim to understand the network of biological interactions at the molecular and genetic level. To this end, we take advantage of state-of-the-art genomics such as next-generation sequencing technologies.

I. Genomic revelations of a mutualism: the pea aphid and its obligate bacterial symbiont

Aphid species bear intracellular symbiotic bacteria in the cytoplasm of bacteriocytes, specialized cells for harboring the bacteria. The mutualism is so obligate that neither can reproduce independently. The newly released 464 Mb draft genome sequence of the pea aphid, *Acyrtosiphon pisum*, in

consort with that of bacterial symbiont *Buchnera aphidicola* illustrates the remarkable interdependency between the two organisms. Genetic capacities of the pea aphid and the symbiont for amino acid biosynthesis are complementary. The genome analysis revealed that the pea aphid has undergone characteristic gene losses and duplications. The IMB antibacterial immune pathway is missing several critical genes, which might account for the evolutionary success of aphids to obtain beneficial symbionts. Lineage-specific gene duplications have occurred in genes in a broad range of functional categories, which include signaling pathways, miRNA machinery, chromatin modification and mitosis. The importance of these duplications for symbiosis remains to be determined. We found several instances of lateral gene transfer from bacteria to the pea aphid genome. Some of them are highly expressed in bacteriocytes.

Aphid research is entering the post-genome era. We analyzed the transcriptome of aphid bacteriocytes using RNA-seq technology featuring a next-generation DNA sequencer. We found thousands of genes over-represented in the symbiotic organ in comparison with the whole body. Many genes for amino acid metabolism are found to be over-represented as expected: the plant sap-eating insect depends on the bacterial symbionts to supply essential amino acids. In addition, many kinds of novel secretion proteins that are found only in aphid species are extremely enriched in the bacteriocytes. We also found that bacteriocytes express Distal-less (Dll), a homeodomain-containing transcription factor throughout the life cycle. Future study should focus on dissecting the genetic network of these components, which should allow us to understand the genetic basis on which symbiosis generates evolutionary novelty.

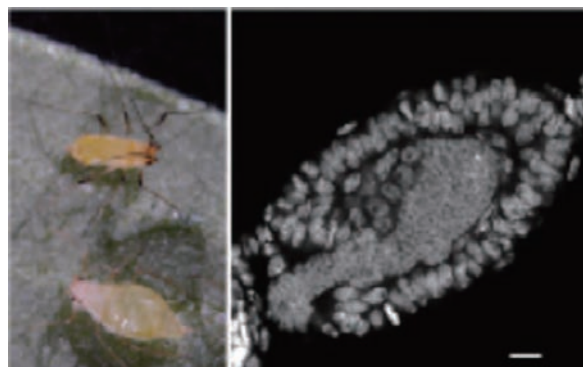


Figure 1. Pea aphids and the bacterial symbiont, *Buchnera*. Adult aphids (Left). A developing viviparous embryo which symbionts are infecting (Right). Scale bar = 20µm.

Publication List

[Original papers]

- Hojo, M., Maekawa, K., Saitoh, S., Shigenobu, S., Miura, T., Hayashi, Y., Tokuda, G., and Maekawa, H. (2012). Exploration and characterization of genes involved in the synthesis of diterpene defence secretion in nasute termite soldiers. *Insect Mol. Biol.* 21, 545-557
- Gallot, A., Shigenobu, S., Hashiyama, T., Jaubert-Possamai, S., and Tagu, D. (2012). Sexual and asexual oogenesis require the expression of unique and shared sets of genes in the insect *Acyrtosiphon pisum*. *BMC Genomics* 13, 76

〔Original paper (E-publication ahead of print)〕

- Shigenobu, S., and Stern, D. Aphids evolved novel secreted proteins for symbiosis with bacterial endosymbiont. *Proc. Royal Soc. B: Biol. Sci.* 2012 Nov 21.

Spectrography and Bioimaging Facility



Associate Professor (Specially appointed)
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The Spectrography and Bioimaging Facility assists both collaborative and core research by managing and maintaining research tools that use “Light”. The facility also provides technical support through management of technical staff assisting in the advancement of collaborative and core research projects, as well as academic support to researchers. Among its tools are advanced microscopes for biology and the Okazaki Large Spectrograph for photobiology. The Okazaki Large Spectrograph is the world’s largest wide spectrum exposure mechanism, capable of producing a range of wavelengths from 250 nm (ultraviolet) to 1,000 nm (infrared) along its 10 meter focal curve; allowing exposure to strong monochromatic light. The facility’s microscopes, which are cutting edge devices such as confocal and multi-photon excitation microscopes, are used by both internal and external researchers as vital equipment for core and collaborative research projects.

Representative Instruments:

Okazaki Large Spectrograph (OLS)

The spectrograph runs on a 30 kW Xenon arc lamp and projects a wavelength spectrum 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

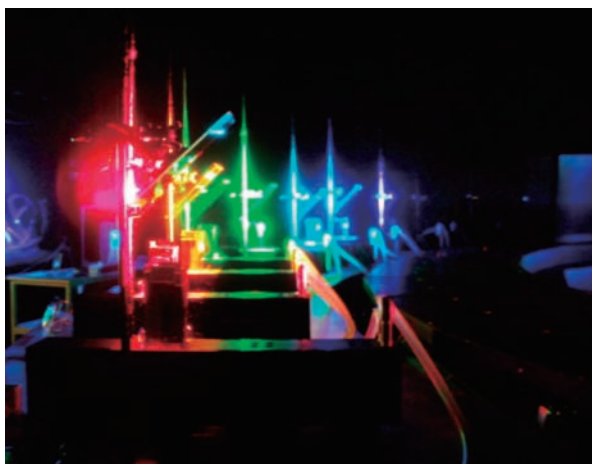


Figure 1. An example of experiments using the Large Spectrograph. Various color rays (monochromatic light from right side and reflected by mirrors) were irradiated simultaneously to samples in cooling chambers.

than twice as much 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.

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 those in NIBB.

Action spectroscopical studies for various regulatory and damaging effects of light on living organisms, biological molecules, and artificial organic molecules have been conducted.

Microscopes

This facility also has Bioimaging machines such as widefield microscopes (Olympus IX-81, BX-63 and KEYENCE BZ-8000), confocal microscopes (Olympus FV1000, Leica TCS SP2, Nikon A1R, Nikon A1Rsi, Carl Zeiss Duo 5 and Yokogawa CSU-X1) and other advanced custom-made laser microscopes for special aims (Digital Scanned Light-sheet Microscope: DSLM and Infrared Laser-Evoked Gene Operator microscope: IR-LEGO) for users in NIBB and collaborative guest researchers. We began Collaborative Research Programs using these machines since 2010.

The DSLM 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 tissue by illuminating from the side of a specimen with a light sheet (more information is described in Dr. Nonaka’s section: Lab. for Spatiotemporal Regulations). Dr. Nonaka conducted and supported about 7 projects of the Collaborative Research Program for the Use of the DSLM. On the other, the IR-LEGO was developed by Drs. Shunsuke Yuba and Yasuhiro Kamei at the National Institute of Advanced Industrial Science and Technology (AIST). This microscope can induce a target gene of interest by heating a single target cell *in vivo* with a high efficiency irradiating infrared laser (Kamei *et al.* *Nat. Methods*, 2009). Details are described in the next section. The IR-LEGO was also used for about 10 Individual Collaborative Research projects, including applications for animals and higher plant.

Workshop and Symposium

In 2012, we held workshops (training course) on IR-LEGO for plants (*Arabidopsis*) and fish (medaka) in Japan and Singapore (as a joint workshop by NIBB, the National University of Singapore, and Temasek Lifesciences Laboratory) respectively. We also have been holding a “Bioimaging Forum” every year which discusses Bioimaging from various directions such as microscopy, new photo-technology, and computer science. In 2012, we held the 6th and 7th forums which focused on all imaging sciences, from astronomy to biology, and optogenetics and adaptive optics, respectively.