

NIBB CORE RESEARCH FACILITIES



Head
KOBAYASHI, Satoru

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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| <i>Technical Staff:</i> | <i>MORI, Tomoko</i> <i>MAKINO, Yumiko</i> <i>YAMAGUCHI, Katsushi</i> |
| <i>Postdoctoral Fellow:</i> | <i>KITAZUME, Tatsuya</i> |
| <i>Technical Assistant:</i> | <i>ASAO, Hisayo</i> <i>FUJITA, Miyako</i> <i>WAKAZUKI, Sachiko</i> |
| <i>Secretary:</i> | <i>ICHIKAWA, Mariko</i> |

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.

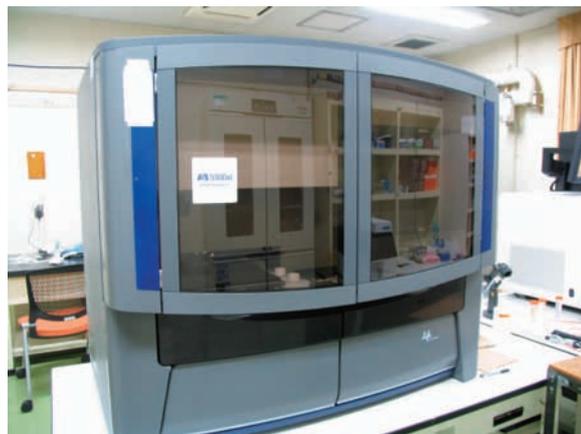


Figure 1. Next-generation sequencer SOLiD5500xl

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

Associate Professor (Specially appointed)
SHIGENOBU, Shuji

NIBB Research Fellow: MAEDA, Taro
Technical Assistant: SUZUKI, Miyuzu
Visiting Scientist: OGAWA, Kota

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.

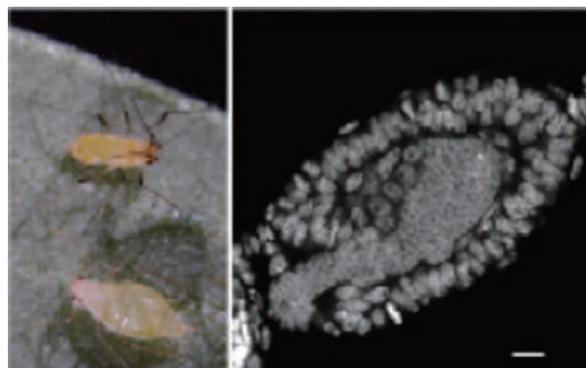


Figure 1. Pea aphids and the bacterial symbiont, *Buchnera*. Adult aphids (Left). A developing viviparous embryo which symbionts are infecting (Right). Scale bar = 20um.

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)
 KAMEI, Yasuhiro

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| Technical Staff: | HIGASHI, Sho-ichi TANIGUCHI-SAIDA, Misako |
| Technical Assistant: | ICHIKAWA, Chiaki |
| Secretary: | ISHIKAWA, Azusa |

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

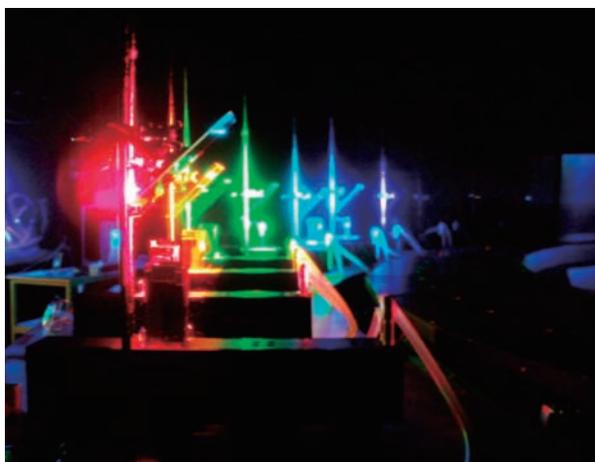


Figure 1. An example of experiments using the Large Spectrograph. Various color rays (monochromatic light from right side and reflected by mirrors) were irradiated simultaneously to samples in cooling chambers.

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.

Publication List on Cooperation

[Original papers]

- Moritoh, S., Eun, C-H., Ono, A., Asao, H., Okano, Y., Yamaguchi, K., Shimatani, Z., Koizumi, A., and Terada, R. (2012). Targeted disruption of an orthologue of DOMAINS REARRANGED METHYLASE 2, OsDRM2, impairs the growth of rice plants by abnormal DNA methylation. *Plant J.* 71, 85-98.
- Satoh, C., Kimura, Y., and Higashijima, S. (2012). Generation of multiple classes of V0 neurons in zebrafish spinal cord: Progenitor heterogeneity and temporal control of neuronal diversity. *J. Neurosci.* 32, 1771-1783.
- Suzuki, T., Yano, K., Ito, M., Umehara, Y., Suganuma, N., and Kawaguchi, M. (2012). Positive and negative regulation of cortical cell division during root nodule development in *Lotus japonicus* is accompanied by auxin response. *Development* 139, 3997-4006.
- Takeda, N., Maekawa, T., and Hayashi, M. (2012). Nuclear-localized and deregulated calcium- and calmodulin-dependent protein kinase activates Rhizobial and Mycorrhizal responses in *Lotus japonicus*. *Plant Cell* 24, 810-822.
- Watakabe, A., Kato, S., Kobayashi, K., Takaji, M., Nakagami, Y., Sadakane, O., Ohtsuka, M., Hioki, H., Kaneko, T., Okuno, H., Kawashima, T., Bito, H., Kitamura, Y., and Yamamori, T. (2012). Visualization of Cortical Projection Neurons with Retrograde TET-Off Lentiviral Vector. *PLoS ONE* 7, e46157.

[Original paper (E-publication ahead of print)]

- Suzuki, T., Kim, C.S., Takeda, N., Szczygłowski, K., and Kawaguchi, M. TRICOT encodes an AMP1-related carboxypeptidase that regulates root nodule development and shoot apical meristem maintenance in *Lotus japonicus*. *Development* 2012 Dec. 18.

● Research activity by Y. Kamei

Associate Professor (Specially appointed)
KAMEI, Yasuhiro

Technical Assistant: KANIE, Yuta

To investigate a gene function in each cell we have to express the gene in the cell *in vivo*, and ideally the expression must be limited only to the single cell. Tissue or cell specific promoters were used to reveal gene functions, however promoter-driven gene expression was governed by cell fate or environment, therefore we could not control the timing of gene expression. To achieve timing-controlled gene expression we employed one of the stress responses, the heat shock response. The heat shock promoter is the transcription regulation region of heat shock proteins and all organisms have this mechanism. Positioning the target gene downstream of the promoter, we can induce the target gene expression by heating.

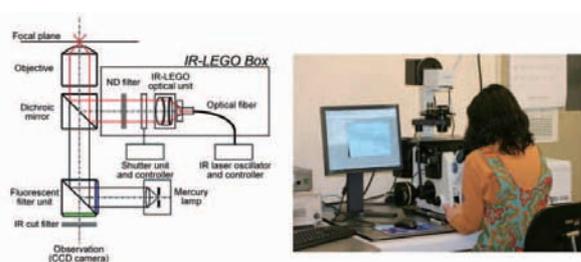


Figure 1. An infrared laser-evoked gene operator (IR-LEGO) microscope system in NIBB.

Infrared (IR) beams can heat water molecules, which are the main constituent of cells, hence, we can heat a single cell by irradiating IR to a target cell using a microscope. We have developed a microscope, IR laser evoked gene operator (IR-LEGO), specialized for this purpose (Figure 1). The IR-LEGO microscope can irradiate an IR laser to a single cell *in vivo* such as *C. elegans*, medaka and *Arabidopsis*, to induce the heat shock response at a desired timing.

Optimal heating induces the heat shock response and subsequent gene expression, while an excess results in cell death. Hence, we must precisely control laser heating; however, there was no way to measure temperature in a microenvironment under microscopic observation. To achieve this we employed green fluorescent protein (GFP) as a thermometer. Since fluorescent matter has the common property of temperature dependent decrease of emission intensity, we can estimate temperature shift by emission intensity change. GFP expressing *E. coli* was used to measure temperature as a micro thermometer. Using this probe, we evaluated heating properties of IR-LEGO such as speed of temperature rise and 3-dimensional distribution of temperature during IR irradiation. In a model tissue which contained GFP expressing bacteria in polyacrylamide gel, temperature rose rapidly with IR irradiation and kept a constant level dependant on IR laser power (Figure 2 left). On the other hand, the heated area was limited to a small volume about as large as a typical cell (Figure 2 right).

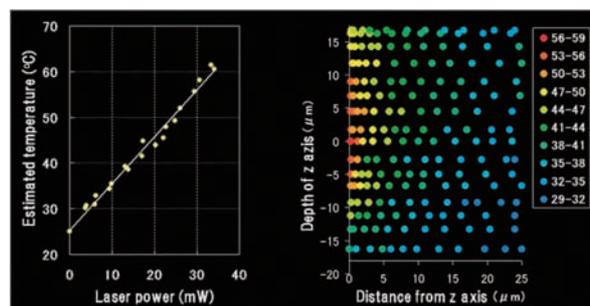


Figure 2. Heating profiles (laser power dependency of focus temperature and 3-D temperature map) of IR irradiation.

With this in mind, we tried to induce gene expression in various species. At first, we reported an IR-LEGO experiment in living *C. elegans*. Target gene expression in a target cell could be induced with only 1 s-IR irradiation. Whereas the optimal power range which can induce gene induction without cell damage was limited. Excess laser power resulted in cell death or cessation of cell division. We confirmed that an optimal irradiation, e.g. 11 mW for 1 s, induced physiological gene expression in the target cell and subsequent cell division or morphogenesis underwent normal development. Next, we tried the experiment in animals, medaka, zebrafish and xenopus, and the higher plant, *Arabidopsis*, since all organisms have a heat shock response system. We succeeded in local gene induction in the species as expected.

Studies of cell fates, cell-cell interaction, or analysis of non-cell autonomous phenomena require a fine control system of gene expression in experiments. IR-LEGO will be a powerful

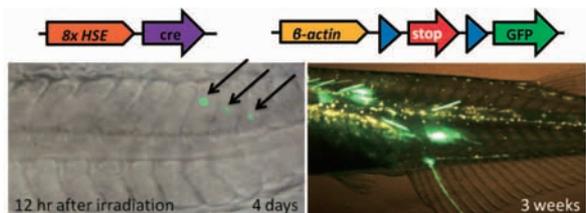


Figure 3. Cre-loxP mediated long-term GFP marking in a living medaka individual for lineage tracing.

tool for these studies in combination with molecular biological techniques, such as the cre-loxP system. By Applying IR-LEGO to a mutant and its rescue transgenic strain; using hsp-cre with a rescue gene which is sandwiched by loxP sequences, we will achieve single-cell knockout experiments in living organisms, and reveal fine interaction between the cells. We are now testing this system using medaka. We have already constructed a medaka TILLING library and a screening system for reverse genetic mutant screening, furthermore we have confirmed a system operation of a cre-loxP system in medaka using IR-LEGO (Figure 3).

Publication List

[Original papers]

- Ansai, S., Ochiai, H., Kanie, Y., Kamei, Y., Gou, Y., Kitano, T., Yamamoto, T., and Kinoshita, M. (2012). Targeted disruption of exogenous EGFP gene in medaka using zinc-finger nucleases. *Dev. Growth Differ.* 54, 546-556.
- Kitano, T., Hayashi, Y., Shiraishi, E., and Kamei, Y. (2012). Estrogen rescues masculinization of genetically female medaka by exposure to cortisol or high temperature. *Mol. Reprod. Dev.* 79, 719-726.
- Masuyama, H., Yamada, M., Kamei, Y., Fujiwara-Ishikawa, T., Todo, T., Nagahama, Y., and Matsuda, M. (2012). Dmrt1 mutation causes a male-to-female sex reversal after the sex determination by Dmy in the medaka. *Chromosome Res.* 20, 163-176.
- Yasuda, T., Oda, S., Li, Z., Kimori, Y., Kamei, Y., Ishikawa, T., Todo, T., and Mitani, H. (2012). Gamma-ray irradiation promotes premature meiosis of spontaneously differentiating testis-ova in the testis of p53-deficient medaka (*Oryzias latipes*). *Cell Death Dis.* 3, e395

[Original paper (E-publication ahead of print)]

- Kobayashi, K., Kamei, Y., Kinoshita, M., Czerny, T., and Tanaka, M. A heat-inducible cre/loxP gene induction system in medaka. *Genesis* 2012 Nov 3.

Data Integration and Analysis Facility

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|----------------------|---|
| Assistant Professor: | UCHIYAMA, Ikuo |
| Technical Staff: | MIWA, Tomoki NISHIDE, Hiroyo NAKAMURA, Takanori |
| Technical Assistant: | YAMAMOTO, Kumi OKA, Naomi |

The Data Integration and Analysis Facility supports research activities based on large-scale biological data analysis, such as genomic sequence analysis, expression data analysis, and imaging data analysis. For this purpose, the facility maintains high-performance computers with large-capacity storage systems. On the basis of this system, the facility supports development of data analysis pipelines, database construction and setting up websites to distribute

the data worldwide. In addition to computational analysis, the Data Integration and Analysis Facility supports NIBB's information infrastructure, the maintenance of the network system in the institute and 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 shared memory parallel computer (DELL PowerEdge R905; 4 nodes/16 cores, 256GB memory), a high-performance cluster system (DELL PowerEdge M1000e+M610; 32 nodes/256 cores, 768GB memory) and a large-capacity storage system (DELL Equallogic; 35TB SAS, 26TB SATA, 750GB SSD). All subsystems are connected via a high-speed InfiniBand network so that large amounts of data can be



Figure 1. Biological Information Analysis System

processed efficiently. Some personal computers and color/monochrome printers are also available. 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. Especially, we have supported the construction and maintenance of published databases of various model organisms including XDB (*Xenopus laevis*), PHYSCObase (*Physcomitrella patens*), DaphniaBASE (*Daphnia magna*), The Plant Organelles Database, and MBGD (microbial genomes).

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 network ORION connecting the three research institutes in Okazaki. Many local services, including sequence analysis services, file sharing services, and printer services, are provided through this network. We also maintain a public World Wide Web server that hosts the NIBB home page (<http://www.nibb.ac.jp/>).

Research activity by I. Uchiyama

Assistant professor I. Uchiyama is the principal investigator of the Laboratory of Genome Informatics, which currently focuses on microbial comparative genomics studies. For details, please refer to the laboratory page (p. 65).