Available Facilities

1. Genomics, Epigenomics and Transcriptomics

1) DNA sequencing analysis using next generation sequencers.

The Research Center for Transomics Medicine possesses five types of instruments: Illumina HiSeq2500, HiSeq1500, GAIIx, and MiSeq and Roche GS FLX. The Illumina sequencers are suitable for exome analysis, gene expression analysis, chromatin analysis (ChIP-seq), etc., and the FLX is suitable for *de novo* sequencing, etc. You can use the instrument most suitable for your analysis.

2) DNA sequencing and fragment analysis by Sanger sequencing.

Sequencing and microsatellite marker DNA fragment analysis by Applied Biosystems Model 3130 and 3730 sequencers.

The ABI PRISM® 3730 Genetic Analyzer is a 96 capillary electrophoresis system. It is capable of performing sequencing analysis and fragment analysis, and can be used in various applications by using different lengths of capillaries. The ABI PRISM® 3130 Genetic Analyzer is a 16 capillary system and is capable of performing fragment analysis using Gene Mapper software.

3) Microarray Analysis

Microarray analysis by Affymetrix Gene Chip System.

Affymetrix Gene Chips are the most frequently used technology for genome-wide expression profiling; from the various available microarray platforms. In medical research, expression profiling by microarrays holds great promises for better understanding of diseases, identification of new therapeutic targets and subclassification of diseases to identify individualized treatment strategies.

ʒ Inquiries about available instruments and technology

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Division	Facilitator	E-mail Address	Remarks
Genomics	Hiroki Shibata (Associate Professor)	hshibata@gen.	WGS, Exome-Seq, Amplicon-Seq
Epigenomics	Hidehiro Toh (Lecturer)	toh-h@bioreg.	WGBS, small RNA-Seq
Transcriptomics	Yasuyuki Ohkawa (Professor)	yohkawa@bioreg.	ChIP/mRNAseq, Chromatin analysis
Neurofunctional Genomics	Yusaku Nakabeppu (Professor)	yusaku@bioreg.	Microarray Analysis

2. Proteomics and Metabolomics

○Facilities for proteome analysis (Proteomics)

1) Preparation of Samples for Proteomics

In order to perform identification of proteins using a mass spectrometer, it is necessary to perform pre-processing of protein samples by protease digestion. However, in these processes, it is easy for airborne keratin or chemical substances from plastic equipment etc., to inadvertently contaminate the samples, eventually disturbing the sensitive analysis. Therefore, this kind of operation should be performed in a clean room by specialist personnel.

Fig. 1 Clean room for preparation of samples for proteome analysis

By performing experiments in a clean room equipped with all of the necessary equipment for sample preparation, contamination by keratin and other contaminants are avoidable.

2) Mass Spectrometers

In proteome analysis, various different types of mass spectrometers are used depending on the types of samples, and the purposes of experiments. There are 9 different types of mass spectrometers available.

[Ion Trap Mass Spectrometers]

- LTQ (Thermo Fisher)
  Analysis of complex samples using ultra-sensitive/high-speed scans
  Robust analytical system for many samples using short gradient program.
  Used to identify posttranslational modification (such as phosphorylation) using MS^n analysis.
- LCQ deca (Thermo Fisher)
- LCQ decaXP (Thermo Fisher)
  Used for the identification of proteins from SDS-PAGE bands, in a middle to high protein abundance.

Fig 2 Ion Trap Mass Spectrometers - From left: LTQ, LCQdeca, LCQdecaXP

[Hybrid High-Resolution Mass Spectrometer]

- LTQ OrbitrapVelos (Thermo Fisher)
- Q Exactive (Thermo Fisher)
- TripleTOF5600 (SCIEX)

Fig. 3 Hybrid Mass Spectrometers
From the left: TripleTOF5600, LTQ Orbitrap Velos, Q Exactive

[Triple Quadropole Mass Spectrometers]
- QTRAP5500 (ABSciex)
- QTRAP6500(ABSciex)

Quantification with the MRM method

Fig. 4 From the left: QTRAP6500, QTRAP5500

[MALDI-TOF Mass Spectrometer]
- Autoflex III (Bruker Daltonics)

Used for identifying proteins when there are a large number of specimens with relatively high purity, such as, spot identification of two-dimensional electrophoresis.

Fig. 5 Autoflex III

3) Database Search Engines
- MASCOT server (Matrix science)
- Proteome Discoverer (Thermo Fisher)
- ProteinPilot (Applied Biosystems)

4) Differential analysis employing two-dimensional electrophoresis
- Ettan DIGE system (GE Healthcare)

Includes a standard format 2-dimensional electrophoresis device, Typhoon trio fluorescence scanner, image analysis software (DeCyder 2D), and a spot picker.

-2-dimensional electrophoresis analysis software, Progenesis (PerkinElmer)

Fig. 6 Ettan DIGE system
Typhoon trio (left) and Spot Picker (right)

5) High Performance Liquid Chromatography
- SMART system (GE Healthcare)

○**Facilities for metabolome analysis (Metabolomics)**

1) Sample preparation for metabolomics

Metabolomics research requires proper sample preparation system to obtain high quality metabolome data.

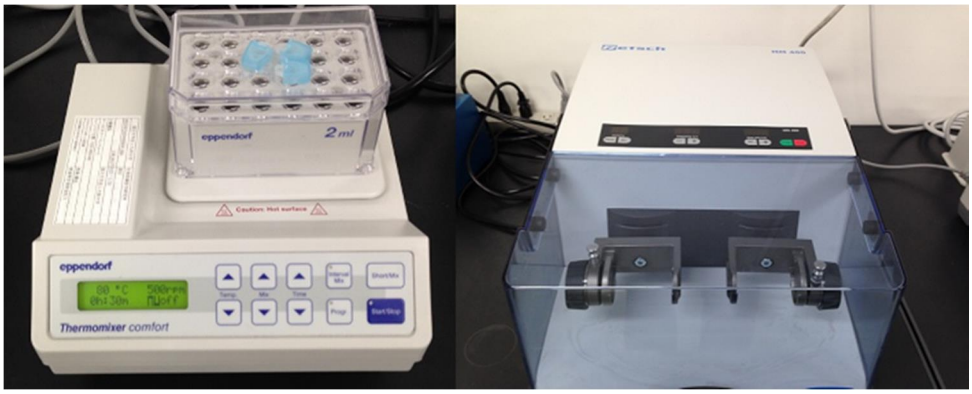

Fig. 7 Sample preparation system for metabolome analysis

2) Mass spectrometers

Different mass spectrometers are needed according to research purposes and target metabolites.
Two types of mass spectrometers are available in this laboratory.

[Triple quadrupole gas chromatography mass spectrometer]

- GC-MS 7000C (Agilent)

For metabolome analysis of low-molecular weight hydrophilic metabolites, including sugars, organic acids and amino acids

Fig. 8 GC-MS system

[Triple quadrupole supercritical fluid chromatography mass spectrometer]

- Nexera UC (Shimadzu)
- LCMS-8060 (Shimadzu)

For targeted lipidomics analysis

Fig. 9 SFC-MS system

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Proteomics	Keiichi Nakayama (Professor)	nakayak1@bioreg.	
Metabolomics	Takeshi Bamba (Professor)	bamba@bioreg.	

3. Structural Biology

1) Automatic setting-up devices for crystallization screening

- Search under 96 different crystallization conditions using a total of 25 µl protein solution (0.2 µl per drop).

Fig. 1 Crystal Gryphon LCP Setting-up

2) NMR analyses of protein structures, protein-ligand interactions using NMR titration experiment, and

dynamics using NMR relaxation (^{15}N etc.)
- Bruker Avance600, Varian Inova600
  A high-performance cryogenic probe is equipped with the two Bruker NMR spectrometers.

3) Single particle analysis and tomography using electron microscopes
- FEI Tecnai G3 Polara (300 kV transmission electron microscope)
  Cryo measurements at liquid helium and nitrogen temperatures,
  Tomography including STEM tomography, STEM(HAADF),
  4K x 4K CCD (UltraScan4000, GATAN),
  Energy filter (GIF Tridiem, GATAN with 2K x 2K CCD)
- FEI Tecnai20 (200 kV transmission electron microscope)
  Tomography, 2K x 2K CCD (Eagle 2k, FEI)

Fig. 2 Cryoelectron microscope (Tecnai Polara)

4) Isothermal titration calorimeter
- VPC-ITC (Microcal)

5) Circular Dichroic Polarimeter (CD)
-JASCO J-820

*1) The *required* conditions or quantities of samples may vary substantially depending on the type of analysis or research aim. Please inquire of the facilitator in the institute beforehand whether the experiment is feasible or not.

*2) *Preliminary* studies need not have been performed on the samples (crystallization, determination of lattice constant, NMR measurement, electron microscopic observation, etc.); however, it is preferable that at least the preparation of the samples or a preliminary study for the collection of the samples has begun.

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Structural Biology	Daisuke Kohda (Professor)	kohda@bioreg.	

4. Embryonic and Genetic Engineering

We can provide the following technological support regarding developmental engineering experiments: ES injection into blastocysts, DNA injection (including Crispr/Cas9) into fertilized eggs, in vitro fertilization/preparation of frozen fertilized eggs, etc.

Service	Reception	Comments
ES cell injection	1 to 2 days p.w. (Tue, Wed, Thu, Fri)	Use of C57BL/6J mice
DNA injection	1 to 2 days p.w. (Tue, Thu)	Use of C57BL/6J mice
Preparation of frozen fertilized egg	Arbitrary	Embryo freezing via in vitro fertilization (IVF); cleaning of mice

Bringing in frozen fertilized egg	Arbitrary	Thawing/transplantation of frozen embryo from other institutions; aim of transfer
Carrying out/thawing frozen fertilized egg	Arbitrary	Thawing/transplantation of frozen embryo, transporting frozen embryo
Preparation of frozen sperm	Arbitrary	Preparation and preservation of frozen sperm

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