Attachment

Available Facilities

1. Genomics, Epigenomics and Transcriptomics

1) DNA sequencing analysis using next generation sequencers.

Fig.1 Next Generation Sequencers

The Research Center for Transomics Medicine possesses short-read-type next generation sequencers, mainly Illumina instruments, including Illumina NovaSeq6000, HiSeq2500, HiSeq1500, and MiSeq, which are beneficial for super parallel analysis in single cell transcriptomics or epigenomics (ATACSeq, ChILSeq) adding to regular genome/transcriptome analysis. We are flexible to accept proposals of various other studies that require the use of NGS upon request.

2) DNA sequencing and fragment analysis by Sanger sequencing.

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

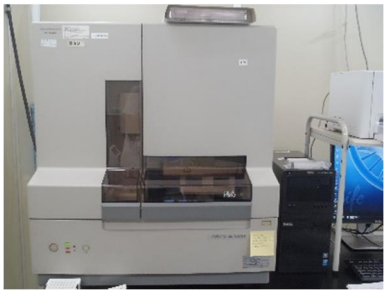

Fig.2 ABI PRISM® 3130

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.

Fig.3 Affymetrix Gene Chip System

Affymetrix Gene Chip System is the most frequently used technology for genome-wide expression profiling among various available microarray platforms. －In medical researches, expression profiling by microarrays holds great promises for better understanding of diseases, identification of new therapeutic targets, and sub-classification of diseases to identify

ƹ Inquiries about available instruments and technology

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Division	Facilitator	E-mail Address	Remarks
Genomics	Hiroki Shibata (Associate Professor)	hshibata@gen.	Whole Genome Seq, Exome-Seq, Amplicon-Seq
Epigenomics	Hidehiro Toh (Lecturer)	toh-h@bioreg.	Whole Genome Bisulfite-Seq, Small RNA-Seq
Transcriptomics	Yasuyuki Ohkawa (Professor)	yohkawa@bioreg.	ChIP/ChILSeq, scRNA/ATAC-Seq
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 mass spectrometry, it is necessary to perform pre-processing of protein samples by protease digestion. During the process, it is easy for airborne keratin or chemical substances from plastic tubes to inadvertently contaminate the samples, eventually disturbing the sensitive analysis. Therefore, the operation should be performed in a clean room by specialist personnel.

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

In a clean room equipped with all of the necessary equipment for sample preparation, contamination by keratin and other contaminants can be avoided.

2) Mass Spectrometers

For proteome analyses, various types of mass spectrometers are available depending on the types of samples,－and the purposes of experiments. There are 6 different types of mass spectrometers available.

[Hybrid High-Resolution Mass Spectrometer]

- LTQ OrbitrapVelos Pro (Thermo Fisher)
- TripleTOF5600 (SCIEX)

Fig.2 Hybrid Mass Spectrometers
From the left: TripleTOF5600,and LTQ Orbitrap Velos Pro

[Triple Quadropole Mass Spectrometers]

- QTRAP5500 (ABSciex)
- QTRAP6500(ABSciex)

Quantification with the MRM method

Fig.3 From the left: QTRAP6500, and QTRAP5500

[MALDI-TOF Mass Spectrometer]

- Autoflex (Bruker Daltonics)

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

Fig. 4 Autoflex

3) Database Search Engines

- MASCOT server (Matrix science)
- Proteome Discoverer (Thermo Fisher)
- ProteinPilot (Applied Biosystems)

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

1) Sample preparation for metabolomics

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

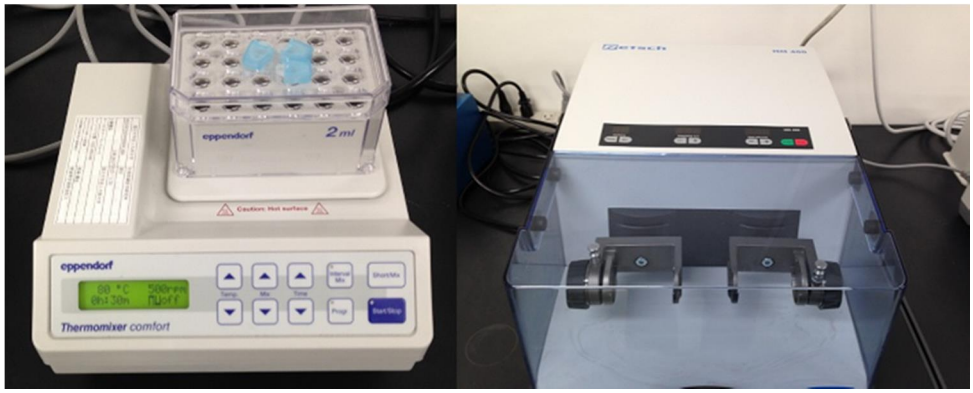

Fig. 5 Sample preparation system for metabolome analysis

2) Mass spectrometers

Different mass spectrometers are required according to research purposes and target metabolites.

Two types of mass spectrometers are now available.

[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. 6 GC-MS system

[Triple quadrupole supercritical fluid chromatography mass spectrometer]

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

For targeted lipidomics analysis

Fig. 7 SFC-MS system

ƹ Inquiries about available instruments and technology

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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.)

- There are two Bruker Avance600 spectrometers.
  A high-performance cryogenic probe is equipped with one of the two spectrometers.

3) Single particle analysis and tomography using electron microscopes

- FEI Tecnai G3 Polara (300 kV transmission electron microscope)
  Cryo measurements at liquid nitrogen temperature,
  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 conditions or quantities of protein samples required for a successful measurement 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, the preparation of the samples or a preliminary study for the collection of data is desirable.

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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.

Fig. Electroporator (left) and Micromanipulator System (right)

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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