Public call for collaborative research for fiscal year 2017 (Proteomics and Metabolomics)

Since having been recognized as a Joint Usage/Research Center for the Multi-Stratified Host Defense System on April 1, 2010, the Medical Institute of Bioregulation, Kyushu University has been promoting collaborative research with researchers involved in host-defense studies. We have established two projects: (1) <u>an instrument usage type project</u> in which collaborative research is conducted using the multi-stratified levels of research facility infrastructure and the technology of this institute (<u>a maximum budget of 500,000 yen</u>, including travel and accommodation expenses, is distributed); and (2) <u>a collaborative research type project</u> (travel and accommodation expenses only) that aim at clarifying the host-defense system using the knowledge accumulated about host defense in this institute. We publicly call for research proposals for fiscal year 2017.

1. Field of research: Proteomics and Metabolomics

Proteomics is a comprehensive analytical method for proteins that employs mass spectrometry. In the past, protein research was performed on individual proteins using such methods as western blot, but proteomics approach makes it possible to obtain information on expression levels, post-translational modifications, and even interactions between many general proteins. By adopting this kind of comprehensive analytical method, it is possible to gain a deeper understanding of life processes and promote researches more efficiently than conventional techniques allow. The following facilities for proteome analysis are available.

Metabolomics, exhaustive analysis of metabolites, has been applied in various fields as precise phenotypic characterization by means multi-component profiling. However, since the targeted metabolites are widely ranging from low to high molecular weight, technology used for simultaneous multi-component analysis has become critical concern. Selection of suitable technology is essential to effectively conduct metabolome analysis. Our laboratory has strong knowledge background and is equipped with numerous sophisticated technologies for high-resolution metabolomics research. The available facilities for metabolomics analysis are listed.

•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 MSⁿ 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.

[MALDI-TOF/TOF Mass Spectrometer] - 4700 Proteome analyzer (ABSciex)





Fig. 5 Autoflex III

3) Database Search Engines

- MASCOT server (Matrix science)
- Bioworks (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 4700 Proteome analyzer



Fig. 7 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. 8 Sample preparation system for metabolome analysis

2) Mass spectrometers

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

[Gas chromatrograph triple quadrupole mass spectrometer]

- GC/MS 7000C (Agilent)

Simultaneous analysis of 17 steroids (evaluation of endocrine disrupting effects, etc.) Metabolome analysis of hydrophilic low molecular metabolites such as sugars, organic acids and amino acids



Fig. 9 GC-QqQMS system

[Liquid chromatrograph triple quadrupole mass spectrometer]

- Nexera X2 (Shimadzu)
- LCMS-8040 (Shimadzu)

Analysis of targeted lipid mix is possible



Fig. 10 LC-QqQMS system

[Supercritical fluid chromatrograph triple quadrupole mass spectrometer]

- UPC^2 (Waters)
- Xevo-TQ-micro (Waters)

Suitable for analysis of targeted lipid mix



Fig. 11 SFC-QqQMS system

2. Qualification of applicants

Researchers belonging to universities, national or public institutions, or equivalent institutions

3. Period of research From April 1, 2017 to March 31, 2018

4. How to apply

Required Documents: Prescribed application form (1 original and 1 copy).

The application form is downloadable at http://www.bioreg.kyushu-u.ac.jp.

Please fill out the application form and send it to the address below by post or e-mail. Please discuss the contents with the facilitator of the institute before sending in your completed application form.

Application deadline: Friday, February 24, 2017.

Send to: Yoichiro Shibata / Mihoko Nakano(contact person) General Affairs Section, Academic Research Cooperation Division, Administrative Office (Medical Sciences, Dental Science and Pharmaceutical Sciences), Kyushu University Address: 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, JAPAN Mail: ijksomu@jimu.kyushu-u.ac.jp Tel: +81-92-642-6672; Fax: +81-92-642-6776

5. Acceptance or rejection

Once the review committee has made a decision to either accept or reject the application, you will be informed of the result as soon as possible.

6. Report of the results

Please submit a report describing the progress and results of the research once the study has been completed. If you have published a paper based on the results of the study, please acknowledge the facilities of our institute that you have used in the paper as follows:

"This work was partly performed in the Cooperative Research Project Program of the Medical Institute of Bioregulation, Kyushu University."

In addition, please submit a copy of the reprint of the paper.

7. Others

(1) Once a research theme has been chosen, the researcher responsible for the experiment must become a collaborator of the Medical Institute of Bioregulation. The researcher who wishes to receive reimbursement for travel expenses as a collaborator should answer "yes" to the "requirement of travel expense" in an application form.

(2) If you wish to conduct an urgent experiment, we may accept your application even after the application deadline. Please contact Yoichiro Shibata / Mihoko Nakano (contact person), the General Affairs Section, Academic Research Cooperation Division, Administrative Office (Medical Sciences, Dental Science and Pharmaceutical Sciences), Kyushu University.

8. Mandatory expenses

Research and travel expenses are provided, as per the budget. Travel expenses are also provided to research collaborators, as per the budget. Please contact the facilitator of the institute before beginning a collaborative research.

9. Handling of intellectual properties

Intellectual properties are handled in accordance with the Intellectual Property Handling Rules of Kyushu University.

10. Inquiries

 Regarding the application for collaborative research: Yoichiro Shibata / Mihoko Nakano (contact person)
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