Center for Brain Science

RESEARCH RESOURCES DIVISION

CBS Co-Creation of Knowledge Project

RRD

Research and Technology SERVICES GUIDE

- Published in **2024** -

Research Resources Division
RIKEN Center for Brain Science
JULY, 2024

Contents

Research Resources Division (RRD), RIKEN Center for Brain Science

Outline

The RRD is an organization that provides technical services and development primarily to members in the Center for Brain Science (CBS). Currently, the division consists of four support units as shown in the organizational chart on page 4, which cover a wide range of technical fields.

Please refer to the website links below, and this guidebook for details concerning our versatile technical services.

https://cbs.riken.jp/en/faculty/rrd/

RRD Director
Hiroyuki Kamiguchi, M.D., Ph.D.

Grouping of RRD Services

The major services provided by RRD are categorized as follows:

1. Staff-run technical services:

Following a request and samples from a research laboratory, knowledgeable and experienced RRD technical staff perform analysis using optimal conditions or create tangible objects based on samples.

The service contents are listed on the organizational chart on page 4. Most support can be applied for online through the RIKEN Core Facilities Management System (R-COMS).

2. Technical personnel support section (TPSS):

TPSS provides customized expert technical support for labs, such as helping out with lab work when a lab is short-handed or providing technology that a lab itself does not have. TPSS also provides support for the transfer of a lab's newly developed technology to other labs.

3. Management services:

Shared experimental areas and common-use equipment are managed and maintained by the RRD. Lab members can use them on their own by registering as a user and making an advance reservation at R-COMS. A list of common-use equipment for each unit is shown on pages 10.31-33.36.

4. Technical support activities:

RRD also organizes exhibitions of research materials and instructions of equipment to lab members for educating scientific cutting-edge technology especially related to Neuroscience.

5. External cooperation :

Collaborative research and commissioned tests will be used to disseminate some of the RRD's research and technical support outside of RIKEN.

Users of RRD Services

The users of RRD services are primarily CBS members, as the RRD is part of CBS. However, as far as the situation of facilities, equipment and personnel permits, all RIKEN members outside of CBS are eligible. In addition, non-RIKEN researchers also have a chance to use part of staff-run technical services.

Service Fees

The expenses of the RRD are mainly covered by the CBS internal budget. Service fees are charged for the use of RRD support services.

Proprietary Rights of Animals and Analyzed Data

When the RRD stores a lab's experimental animals, samples, materials, or analyzed data as part of its services, ownership of those materials belongs to the lab unless otherwise agreed upon.

Publishing Scientific Data Acquired with RRD

So that we can continue providing technical support services, we ask that you credit RRD's specific contribution in the Materials and Methods and/or Acknowledgements section(s) of your paper when publishing research results obtained with our assistance. If the research results were obtained via collaboration with our technical support, please indicate co-authorship in your publications.

Formal name of RRD
Center: RIKEN Center for Brain Science
Division: Research Resources Division
Units:
Support Unit for Animal Resources Development
Support Unit for Bio-Material Analysis
Support Unit for Functional Magnetic Resonance Imaging
Support Unit for Electron Microscopy Techniques

Acknowledgement Examples

Note: ###) Please enter the above formal name of support unit

1. Materials and Methods section (space dependent):
   *XXX was analyzed using the YYY service at the Support Unit for ### in RIKEN Center for Brain Science, Research Resources Division (RRD).

2. Acknowledgments section (in each paper under three different cases):

   i) Without credit in the Materials and Methods section:
   *We are grateful to the Support Unit for ###, RIKEN CBS Research Resources Division, for technical help with XXX .
   *We thank the Support Unit for ###, RIKEN CBS Research Resources Division, with special thanks to Ms. YY and Mr. ZZ for XXX.

   ii) With some credit in the Materials and Methods section:
   *We thank Ms. XXX for technical assistance with YYY.

3. With assistance from more than two RRD units:
   *We thank the staff of Research Resources Division, RIKEN Center for Brain Science, for generation of AAA mice and analysis of ZZZ.

4. Credit as a co-author:
   *Our affiliation is the Support Unit for ###, Research Resources Division, RIKEN Center for Brain Science.

Number of RRD Staff

(as of July 1, 2024)

Organization	No. of staff*(No. of part-timers)	Note
Research Resources Division	1	
Support Unit for Animal Resources Development		
Animal Care and Management	49	contact employees
Laboratory Animal Health Care	4	
Staff-run Technical Services	4(1)	
Zebrafish Experiment Facility	15 (11)	
Office	3 (1)	
Support Unit for Bio-Material Analysis		
Staff-run Technical Services & Shared Experimental Areas Management	11 (1)	
TPSS	5	
Office	2(2)	
Support Unit for Functional Magnetic Resonance Imaging		
Staff-run Technical Services	4	
Office	1	
Support Unit for Electron Microscopy Techniques		
Staff-run Technical Services	2	
Office	1	

*Not included Director and Unit Leaders

Research Resources Division (RRD)

Director: Hiroyuki Kamiguchi, MD, PhD

Support Unit for Animal Resources Development (ARD)

Unit Leader: Kimie Niimi, DVM, PhD

Laboratory Animal Facilities

- Mammalian Animal Facility (mice, rats, rabbits, macaques, marmosets, and others)
- Aquatic Animal Facility (zebrafish)

Mammalian

Staff-run Technical Services

Complete Services:

- Mouse Embryo Manipulation
- Generation of Transgenic Mice
- Generation of Knockout Mice
- Mouse and Rat Decontamination
- In Utero Electroporation
- Marmoset Supply
- Generation of Congenic Mice
- CRISPR/Cas System
- Establishment of Embryonic Stem (ES) Cell
- Antibody Production
- Animal health control
- Marmoset Perfusion Fixation
- Marmoset Surgical Assistance

Training/Support:

- Guidance and Support for Animal Experiments

Common Use Facilities & Materials

- Behavioral Testing Facilities for Mice and Rats
- Cooperative Use of Mouse Strains
- Operation and Treatment Rooms for Macaques and Marmosets

Zebrafish

Staff-run Technical Services

Complete Services:

- Zebrafish Embryo Manipulation
- Generation of Transgenic Fish
- Generation of Knockout Fish
- CRISPR/Cas System
- Sperm cryopreservation
- Fish Health Care

Common Use Facilities & Materials

- Housing and Breeding Facilities
- Microinjector
- Collection, Preservation and Provision for Zebrafish Strains

Support Unit for Bio-Material Analysis (BMA)

Unit Leader: Nobuhiko Miyasaka, PhD

Staff-run Technical Services

- DNA Sequencing (Plasmid Purification, Sequencing)
- Gene Polymorphism Analysis
- BioArray Analysis
- FANTOM3 Clones Distribution
- Amino Acid Analysis
- Peptide Synthesis
- Labware Washing
- Genetic Quantitative Analysis
- Next Generation Sequencing
- Mass Spectrometry
- Flow Cytometry and Cell Sorting
- Protein purification
- Lab Support Service

Technical Personnel Support Section (TPSS)

- Molecular Biological Experiments
- Histological Experiments
- Bioimaging Experiments
- Virus Production Experiments

Shared Experimental Areas (Self-run Use)

- Radiation Controlled Area, RI Center: CBS Common Use Facility (CBS Common Use Experimental Room)
- Common Use Equipment (CUE) Experimental Rooms :
  - ・Molecular Biology Room
  - ・Biochemistry Room
  - ・P2/Level2 Experimental Room
  - ・Microscopy/Imaging Room #1, #2
  - ・Super Resolution Microscopy Room #1, #2
  - ・Gene Quantitative Analysis Room
  - ・Chromatography Room
  - ・Histological Preparation Room
  - ・Flow Cytometry Room
- Freezer Room (Lending Service)

Technical Support Activities

- Research Equipment and Material Exhibition
- BMA Classes

External Cooperation

- Commissioned Tests

Support Unit for Functional Magnetic Resonance Imaging (fMRI)

Unit Leader: Tomohisa Okada, MD, PhD

Technical Services (Training/Support)

- Human Subject Scans
- Preliminary Experiments and Training
- Monkey Subject Scans
- Ethical Compliance Examinations

External Cooperation

- Collaborative Research
- Commissioned Tests

Support Unit for Electron Microscopy Techniques (EMT)

Unit Leader: Yoshiyuki Kubota, PhD

Technical Services (Training/Support)

- Analysis of EM Data and Image Analysis for 3D Reconstruction
- Analysis of Neuron Ultrastructure with High Pressure Freezing

Available Equipment

- Focus Ion Beam-SEM
- FE-SEM
- Ultramicrotome
- PC for Image Analysis

Center for Brain Science, Office of the Center Director

Services

- Administrative Works for RRD
- RRD system management
- R-COMS support

July 1, 2024

Support Unit for Animal Resources Development

Support Unit for Animal Resources Development (ARD) maintains CBS's well-equipped laboratory animal-housing facilities and supplies reliable high-quality animals. The highly-trained technical staff provide services such as transgenic/knockout mice generation and mouse embryo manipulation. This unit also helps users to understand and follow official procedures for animal experiments.

Facility for Mammalian Species

Animal Housing Capacity

The following table shows the total housing area and capacity for the housing of mammalian species in each facility.

(As of JULY 1, 2024)

Building	CBS Cent. Bldg.	CBS East. Bldg.	CBS West Bldg.& Frontier Life Science	CBS West Bldg. Annex	CBS NCGR Bldg.	CBS Ikenohata Bldg.	Total
Floor space	6,000m² (64,500ft²)	263m² (2,827ft²)	140m² (151ft²)	412m² (4,430ft²)	4,346m² (46,780ft²)	872m² (9,376ft²)	12,033m² (129,355ft²)
Housing capacity							
Mice (5/cage)	6,000	50	-	-	14,000	-	20,050 (100,250 mice)
Rats (3/cage)	-	-	-	-	840	-	840 (2,520 rats)
Rabbits (1/cage)	-	-	-	-	10	-	10
Macaques (1/cage)	-	20	29	5	-	-	54
Marmosets (4/cage)	-	-	-	59	-	-	59 (236 marmosets)
Marmosets (8/cage)	130	-	-	16	-	-	146 (1,168marmosets)

Animal Care and Management

The ARD facilities house SPF mice, rats, macaques, and marmosets, and conventional rabbits. In principle, animals are owned by each research laboratory.
Animal care and management are carried out according to the standard operating procedure for each animal species as defined by the ARD. The animal care technicians working in the facilities are outsourced through professional companies that are contracted after a competitive bidding process.

Animal Health Care

The ARD staff including four veterinarians, carries out the following operations on animal health care.

Veterinary care in the animal facilities

Veterinarians perform the following:

Health monitoring of mice and rats; periodic health examinations of monkeys and marmosets; diagnosis, treatment, and prevention of animal diseases; evaluation of health status of incoming animals from outside institutions and vendors; quarantine of animals procured; and certification of health status for animals exported to outside institutions.

Arrangement, maintenance and management of animal facilities

Veterinarians oversee animal care and management, arrange facilities, coordinate use of facility space, and approve animal facility visitors.

Microbiological monitoring procedures

The microbiological monitoring procedures for maintaining the health of mice/rats conducted by the ARD are described below; the test result information may be provided to outside institutions when mice/rats are shipped from ARD facilities.

Mice:

Three to seven monitoring cages each housing 1 to 3 sentinel mice are placed in each animal room. During cage exchanges (done every two weeks), bedding taken from several rearing cages in the same room is placed in the monitoring cage. Sentinel mice which have been in the monitoring for at least 3 months are used for microbiological testing.

Rats:

Three to six monitoring cages each housing 1 to 3 sentinel rats are placed in each animal room. During cage exchanges (done every week), bedding taken from several rearing cages in the same room is placed in the monitoring cage. Sentinel rats which have been in the monitoring cage for at least 3 months are used for microbiological testing.

Testing of sentinel mice/rats:

Internal testing is performed by A R D veterinarians and staff once every 3 months. External testing is outsourced to the Central Institute for Experimental Animals (International Council for Laboratory Animal Science Monitoring Center) once every 6 months.

The average number of sentinel mice and rats in each animal room is indicated below.

Internal testing

Mice: 2 mice each are selected from 2 monitoring cages which have been kept for at least 3 months in each animal room.
Rats: 1 rat each is selected from 2 monitoring cages which have been kept for at least 3 months in each animal room.

External testing:

1 mouse or rat which has been raised in a monitoring cage for at least 6 months is randomly selected and sent out for external testing.

Evaluation of test results

The test results are analyzed by the veterinarian in charge and compiled in a microbiological test result chart. When issues arise based on the test results, instructions on countermeasures are given to staff and users.

Technicians in the Support Unit for Animal Resources Development provide the following services for researchers.

Mouse Embryo Manipulation

In vitro Fertilization (IVF)

Oocytes collected from superovulated females are fertilized with matured sperm collected from males. The embryos after in vitro fertilization can be cryo-preserved or transferred to pseudo-pregnant females to obtain pups.

Micro-insemination (Intracytoplasmic sperm injection: ICSI)

Immature sperms collected from infant or infertile males are injected into the cytoplasm of oocytes under a microscope to obtain embryos. Sperms with impaired motility collected from sterile males are also available.

Embryo Transfer

Embryos are transferred into the oviduct of recipients. This method can be used to obtain decontaminated mice and a large number of age-matched mice.

Fertilized Egg Freezing and Cryopreservation

Valuable mouse strains are maintained in CBS for future neuroscience research. Fertilized eggs taken from these mice are frozen by vitrification methods and preserved in liquid nitrogen to maximize resources.

Sperm Freezing and Cryopreservation

Valuable mouse strains are maintained in CBS for future neuroscience research. Sperm taken from these mice are frozen and preserved in liquid nitrogen.

Reanimation of Mice from Cryopreserved Fertilized Eggs

Cryopreserved fertilized eggs from ARD stocks or introduced from outside institutions are thawed and used for embryo transfer as described above.

Reanimation of Mice from Cryopreserved Sperms

Frozen sperms from ARD stocks or introduced from outside institutions are thawed and used for in vitro fertilization. Fertilized eggs are then used for embryo transfer as described above.

Generation of Congenic Mice

When you wish to replace the genetic background of genetically modified mice with a B6/J strain or other strain, in vitro fertilization can accelerate in producing the next generation of offspring compared with the natural mating. We are able to perform backcrossing for the desired number of generations.

Generation of Transgenic Mice

Plasmids are purified with a QIAGEN plasmid kit or equivalent, digested with restriction enzymes (to remove the backbone vector), run on an agarose gel, and a gel fragment containing the transgene is excised. The transgene is purified using the QIAGEN QIAquick Gel Extraction kit. The researchers adjusts the transgene to a concentration of 20~50ng/μl and submits 50μl (in the case of BAC transgene, 50μl of 1-10ng/μl solution) for microinjection into fertilized mouse eggs. The injected eggs are transferred at the 2-cell stage into the oviduct of recipient females. Tail samples from a few weeks-old mice are provided to the researchers for genotyping, and created transgenic founder mice are provided to the researchers. It takes approximately two months to obtain transgenic founder mice.

CRISPR/Cas9 Genome-Editing System

The guide RNA complementary to the target sequence and Cas9 mRNA (or protein) are prepared by the researchers. They are microinjected into 250 fertilized eggs, and then the injected eggs are transferred at the 2-cell stage into the oviduct of recipient females. Tail samples from 2-week- old mice are provided to the researchers for genotyping, and created genome-edited founder mice are provided to the researchers. It takes approximately two months to obtain genome- edited founder mice. This service was started from FY2014.

Generation of ES Cell-based Knockout Mice (Service in Suspension)

Targeting vectors constructed by researchers are introduced into embryonic stem (ES) cells using electroporation and approximately 300 ES cell colonies are picked up. Positive clones selected by the researchers are injected into blastocysts. The injected blastocysts are transferred into recipient mice. The researchers receive the chimera mice and examine them for germline transmission. It takes approximately three to four months to obtain chimera mice.

ES Cell Establishment (Service in Suspension)

Embryonic stem (ES) cells can be established from genetically modified mice generated by laboratories. It takes approximately three weeks to establish at least two lines of ES cells (for example mice with a C57BL/6 genetic background).

Mouse Decontamination

Decontamination by embryo transfer

Embryos are transferred into the oviduct of SPF recipients.

Decontamination by hysterectomy

Fetuses at gestational days 18 and 19 are removed from the mother's uterus using sterile techniques and reared by a SPF foster mother.

Rat Decontamination

Decontamination by hysterectomy

Fetuses at gestational days 21 and 22 are removed from the mother's uterus using sterile techniques and reared by a SPF foster mother.

In Utero Electroporation (Service in Suspension)

In utero electroporation is a useful method for transfecting neuronal progenitor cells in vivo. By adjusting the placement position of the electrodes and the developmental time-point of electroporation, specific subsets of neurons can betargeted.

Polyclonal Antibody Production

To produce polyclonal antibodies, researchers provide immunogens to the staff for administration to the desired animal(s): rabbits, mice, or rats. The antibody titers are checked with ELISA tests.

Marmoset Supply

Marmoset breeding started in 2007 to provide marmosets to laboratories in CBS. Currently, the facility is maintaining 25 pairs capable of breeding approximately 50 offspring per year.

Behavioral Testing Facilities for Mice and Rats

The behavioral testing facilities for mice and rats are grouped into two categories as follows.

A. Behavioral testing rooms for common use

These are common-use rooms for all CBS laboratories. The ARD manages the room and maintains the equipment.

Locations: Facilities are located on the 8th floor of the CBS Cent Bldg. and the 2nd and 3rd floor of the CBS NCGR Bldg.

Reservations: Reservations to use the rooms can be made through the R-COMS system. Rooms can be reserved for fixed hours or days. The maximum period that rooms can be reserved is 15 days, not includingholidays.

Long-term use: The term of use is decided between individual laboratories and the ARD.

User fees: The fees are charged per hour or day of use based on the reservation record by the R-COMS system.

Special experiment area: In this area, the researchers can infect mice with viral vectors (P1A/P2A level) and perform behavioral testing of the infected mice.

B. Rental rooms

The ARD lends the rooms to laboratories for a fixed term (ex. 3 months, 6 months or 1 year). Experimental equipment is to be supplied, managed, and maintained by the individual laboratories.

Locations: Facilities are located on the 2nd floor and 3rd floors of the CBS NCGR Bldg.

Use: The term of use is decided between individual laboratories and the ARD.

User fees: CBS extra lab room charge facility use expenses.

Outline of Common Use Behavioral Testing Equipment

Building	Current Room No.	Current Equipment ID (Use this ID when booking)	Former Room No. (CBS West Bldg.)	Original Equipment ID	Behavior Testing Equipment & Facilities	Sound attenuating room
CBS Central	N810-1	WC0535	107-1	WC0535	Mice long-term behavioral rhythms analysis (30ch)	Yes
	N810-2	WC0536	107-3	WC0536	Morris water maze	None
	N810-3	WC0543	205	WC0543	Contextual and cued fear conditioning test (2ch)	None
			206	WC0960	Passive avoidance test (8ch)	None
	N810-4	WC1176	205-1	WC0961	Barnes circular maze	None
			-	-	Eight-arm radical maze	None
	N810-5	WC0539	-	-	Dissection room	None
	N810-6	WC0959	205-A	WC0553	3-chambers analysis	None
			205-C	WC0959	Open field test 600x600 (4ch)	Yes
	N811-1	WC1177	205-D	WC0965	Light/dark transition test (4ch)	Yes
	N811-2	WC0544	205	WC0964	Startle response & Pre-pulse inhibitation test (4ch)	None
	N811-3A	WC0545	205-B	WC0966	Tail suspension & Porsolt forced swim test (2ch)	None
	N811-3B		205-F	WC0545	Elevated plus maze (double)	Yes
			205-F	WC0545	Y-maze	Yes
	N811-4	WC0547	205-B	WC0966	Ultrasonic analysis	None
			206	WC0960	Rotarod test	None
			206	WC0960	Hole-board test	None
	N811-5	WC0548	205-E	WC0548	Open field test 500x500 (4ch)	Yes
			206	WC0960	Contextual and cued fear conditioning test (2ch)	None
	N811-6	WC0550	-	-	(Treatment room)	None
	N812	WC0551	107-4	WC0551	Forced swimming test	None
			107-4	WC0551	Metabolic cage	None
			205-1	WC0961	Five choice serial reaction time test	None
	N814	WC0552	107-1	WC0552	Scanet 12ch	Yes
	N815	WC0554	-	-	(Treatment room)	None
CBS NCGR	301c	WC0528			Startle response & Pre-pulse inhibitation test (4ch)	None
	301c	WC0541			Rotarod test （1 ch） x 2 sets	None
	301c-1	WC0526			Tail suspension & Porsolt forced swim test (2ch)	Yes
	301c-2	WC0531			Morris water maze	Yes
	301c-3	WC0532			Contextual and cued fear conditioning test (2ch)	Yes
	311c-1	WC0530			Elevated plus maze (single)	Yes
					Light/dark transition test (4ch)	
	311c-2	WC0527			Open field test 500x500 (4ch)	Yes
	311e	WC0529			Activity Sensor system	Yes
					Scanet	
	202a	-			Rental room	None
	202c	-			Rental room	None
	202d	-			Rental room	None
	202e	WC1016			Intellicage (4 ch)	None
		WC1017			Intellicage (4 ch)	None
		WC1018			Five choice serial reaction time test （ 12 ch ）	None
	202f	-			Rental room	None
	202h	-			Rental room	None

Rental Rooms for Housing Special Animals

Housing rooms for special animals are equipped in room 506 the CBS East Bldg. These rooms are available for rent by laboratories for housing special animals (e.g., ferrets, prairie voles) and mice/rats. Users are responsible for all animal care, including installing housing equipment. Users can move animals freely between these rooms and their laboratories.

Operation and Treatment Rooms for Monkeys (Macaques and Marmosets)

The ARD is equipped with an operating room for cranial and other surgery, a procedure/autopsy room for formalin fixation, etc., an ICU room for post-operative care or care for animals in poor condition, and a treatment room for drug administration, etc. These rooms can be used by all laboratories, and are managed by ARD.

Locations:

The facilities are located in rooms 310 and 311 of the CBS West Bldg., N905-1/S906-1/ S906-2/S906-3 of the CBS Central Bldg.

Reservations:

Reservations to use these rooms can be made through the R-COMS system from the COMMON website.

User fees:

Fees are charged by time or day of use based on the reservation record by the R-COMS system.

Cooperative Use of Mouse Strains

CBS researchers can cooperatively use JAX Mice purchased from the Jackson Laboratory by CBS labs. The list of JAX Mice is available on the web. http://cbs.intra.riken.jp/mouse/

Zebrafish Experiment Facility

This facility was set up at the CBS Ikenohata Bldg. in March 2004. The breeding area is 872m^2 and the laboratory area is 365m^2. The breeding area has the following kinds of tanks: 40 outsized (extra-large), 4,320 large-scale, 2,160 medium and 4,120 small, with a total breeding capability of about 200,000 zebrafish. A high-quality control of water purity is maintained in this facility, and the water temperature is kept at 28.0~28.5℃. The laboratory area accommodates apparatus and materials for embryonic manipulation, microscopy, and strain storage.

This facility is also a core facility of the National BioResource Project Zebrafish (Dr. Okamoto, Senior Visiting Scientist of CBS). The core facility's goal is to collect, preserve, and provide zebrafish strains as a useful vertebrate model in Japan.

From April 2018, we started the charging system for these support services.

Technical Support Services

The Zebrafish Experiment Facility provides the following support and training services for users.

Zebrafish Maintenance and Breeding

Fish maintenance in the facility is carried out by RRD staff according to the CBS Zebrafish Facility Manual. When using the Aquatic Animal Experiment Facility for the first time, the users must submit an application form and attend facility orientation sessions. A separate application is required to use zebrafish rearing facilities.

Zebrafish Sperm Cryopreservation

Zebrafish sperm is frozen to preserve strains. We recommend using 3-5 male zebrafish per strain.

Generation of Transgenic/Knockout/Knockin Zebrafish

We offer many services, from construction of plasmids for injection to manufacturing transgenic fish, transgenic fish sorting and strain maintenance.

Plasmid Construction: Plasmids for producing transgenic fish are constructed based on the vector containing Tol2 transposon elements. Constructed vectors for transgenic lines with modified bacterial artificial chromosomes (BACs) or synthesis guide RNA/Cas9 mRNA for knock-out, knock-in, etc. also available.

Injection: Plasmids, modified bacterial artificial chromosomes (BACs) or guide RNA/Cas9 mRNA are injected into fertilized eggs; embryos are bred for about 4-5 months in the breeding room and then provided to users.

Screening and Maintenance: After crossing, founder fish are selected by genomic PCR analysis of the DNA from the embryo or by observation of fluorescent marker proteins in embryos using a fluorescent microscope. Guided by the staff, users perform screenings and maintenance of the fish.

Support for Equipment Use

Equipment (Behavioral experiments, electrophysiology, imaging, etc.) in the facility is available for users with support and training by the staff.

Support Unit for Bio-Material Analysis

The Support Unit for Bio-Material Analysis (BMA) provides the Staff-run Technical Services, including bio-material analyses (e.g., nucleic acids, proteins, and amino acids), cell sorting, and peptide synthesis. In addition, customized technical services have been offered by expert technicians of the Technical personnel Support Section (TPSS) since December 2018. The unit also maintains and supervises the Shared Experimental Areas. These areas include the Common Use Equipment (CUE) Experimental Rooms, where both general-purpose and highly technical equipment are available for shared use in various research projects. Additionally, the BMA holds 'BMA Class' and 'RRD Exhibition for Research Materials' for effective utilization of staff-run technical services and CUE in CBS.

BMA internal website: http://common.riken.jp/rrd/ComInstrue/index.html

Staff-run Technical Services for Bio-Material Analysis

For Users

- **Procedure**
  Users (possessing RIKEN ID) wishing to order technical services should first read the guidelines for desired services on the above BMA website and confirm the equipment to be used, content of measurement/analysis, and fees. The experiment plan should also be discussed with the person in charge as necessary.

- **Operating hours**
  RIKEN normal working hours are observed. If an operation must be temporarily shut down for maintenance or other official events, an announcement will be made. These can be found on the "News & Announcements" section of the BMA internal website.

- **Management of analyzed data**
  Analyzed original data will be kept on file at RRD for a period of five years, except the data from human materials of some services (e.g., DNA Sequencing). We will also discard all data when a lab is closed.

- **Publishing Scientific Data Acquired with RRD** *(Also see page 2)*
  We would like to ask users to credit our specific contribution when publishing research results that were obtained with the assistance of our staff-run technical services. Please see more details in page 2 of this service guide.

DNA Sequencing (Plasmid Purification, Pretreatment, Sequencing)

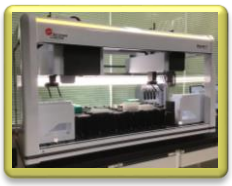
CBS Cent. Bldg., N010/011

1. Support Services

Plasmid Purification

Transformed bacteria with plasmid DNA is received from the user and then incubated in the medium overnight. Plasmids are extracted by alkaline-SDS and silica membrane methods using a Biomek® i7 automated workstation (Beckman Coulter). The purification system is set up in either a 96-well format or an 8-well format.

The 384-well plate Pipetting service is transferred from a 384 well-plate to 4 x 96 well-plate.



FX Robotic workstation
(Beckman Coulter)

Pretreatment for Sequencing (Cycle Sequencing Reaction)

Cycle sequencing is performed using purified plasmids or PCR products as templates. After cycle sequencing, the samples are automatically transferred to the DNA Sequencing Service. The service also accepts GC rich samples and samples that require refined purification of the PCR product (ExoSAP treatment).

DNA Sequencing (Sanger Method)

Redundant terminators in the sample are removed using the CleanSEQ Kit (Beckman Coulter) magnetic beads. The DNA sequencing analysis is conducted with the 3730xl DNA analyzer (Applied Biosystems).

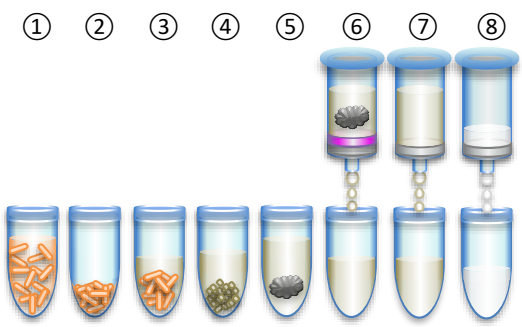
Users are able to order from Plasmid Purification to DNA Sequencing simultaneously.

3730xl DNA Analyzer
(Applied Biosystems)

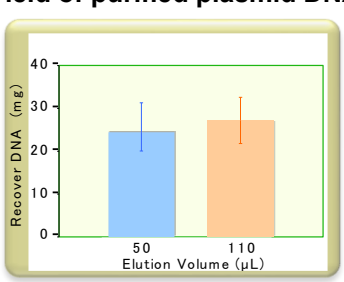
2. Outline (Methods and Results)

Protocol for Plasmid DNA Purification

① Incubate pre-cultured media with bacterial cells at 37℃ for 17 hr.
② Harvest bacterial cells
③ Resuspend bacterial cells with Resuspension Buffer
④ Lyse bacterial cells by adding Lysis Buffer and mixing
⑤ Neutralize by adding Neutralization Buffer and mixing
⑥ Transfer crude lysates onto the filter and clear crude lysates by centrifuge
⑦ Transfer cleared crude lysates onto the DNA binding filter and bind DNA to silica membrane of the filter by centrifuge
⑧ After washing the silica membrane by Wash Buffer, elute plasmid DNA by Elution Buffer



Yield of purified plasmid DNA

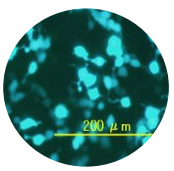


Transfection of cell lines

The fluorescent-expressing plasmid pmCyan-N1 is purified from *E. coli* strain DH5α using Nucleospin Multi-96 Kit.

The right figure shows the transfection efficiency with HEK293 cells using.
Lipofectamine 2000 (Invitrogen).
Transfection of HeLa and N2a cells is also available.

HEK293 cells



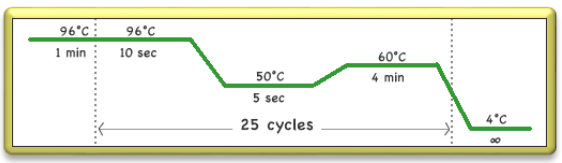
Microscopic Image

Pretreatment for Sequencing

[Reaction Solution]

Sample (Template DNA + Primer)	5.0 μl
BigDye Terminator v3.1	1.0 μl
5 x Sequencing Buffer	3.5 μl
Deionized Distilled Water	10.5 μl
Total	20.0 μl

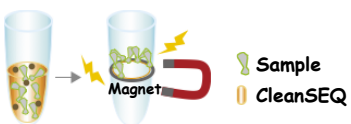
[Cycle Sequencing Reaction]



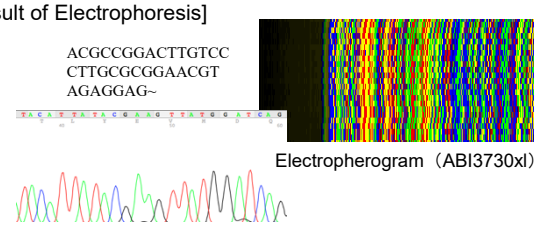
DNA Sequencing

[Cycle Sequencing Product Purification]

Redundant terminators are removed using CleanSEQ Kit magnetic beads.



[Result of Electrophoresis]



Electropherogram（ABI3730xl）

Analysis Data (Software: 4 Peaks)

BioArray Analysis (Bioanalyzer)

CBS Cent. Bldg., S004

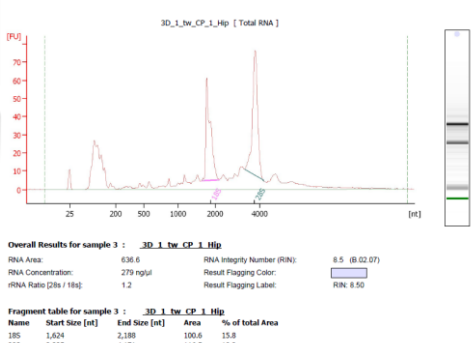
1. Support Services

Total RNA degradation and DNA fragment size are checked by the Agilent 2100 Bioanalyzer.
This equipment is also used for quality check (QC) of the next generation sequencing (NGS) samples.

2. Outline (Methods and Results)

Sample concentration

RNA Nano Chip: 25 – 500 ng/μL
RNA Pico Chip: 200 – 5000 pg/μL
DNA 1000 Chip: 0.1 – 50 ng/μL
High Sensivity DNA Chip: 5 – 500 pg/μL



The RIN is calculated automatically
by the Bioanalyzer for 18S, 28S rRNA.
(RIN: RNA Integrity Number, Index of the RNA quality)
A RIN of more than 7 is usually used for experimentation. (Max10)

Genetic Polymorphism Analysis

CBS Cent. Bldg., N010/011

1. Support Services

Fragment Analysis *

Fluorescence-labeled DNA fragments are subject to capillary electrophoresis using the 3130xl Genetic Analyzer (Applied Biosystems), to detect the size or mobility.

Genotyping

① Genomic DNA Extraction

Mouse or rat genomic DNA is purified from tails or ears using the Agencourt DNAdvance Kit (Beckman Coulter). The genotyping service is also available for other samples (tails of zebrafish, nails of humans and so on) .

② PCR *

Fluorescence-labeled DNA fragments are amplified using fluorescence-labeled primer(s).

③ Analysis of peak size *

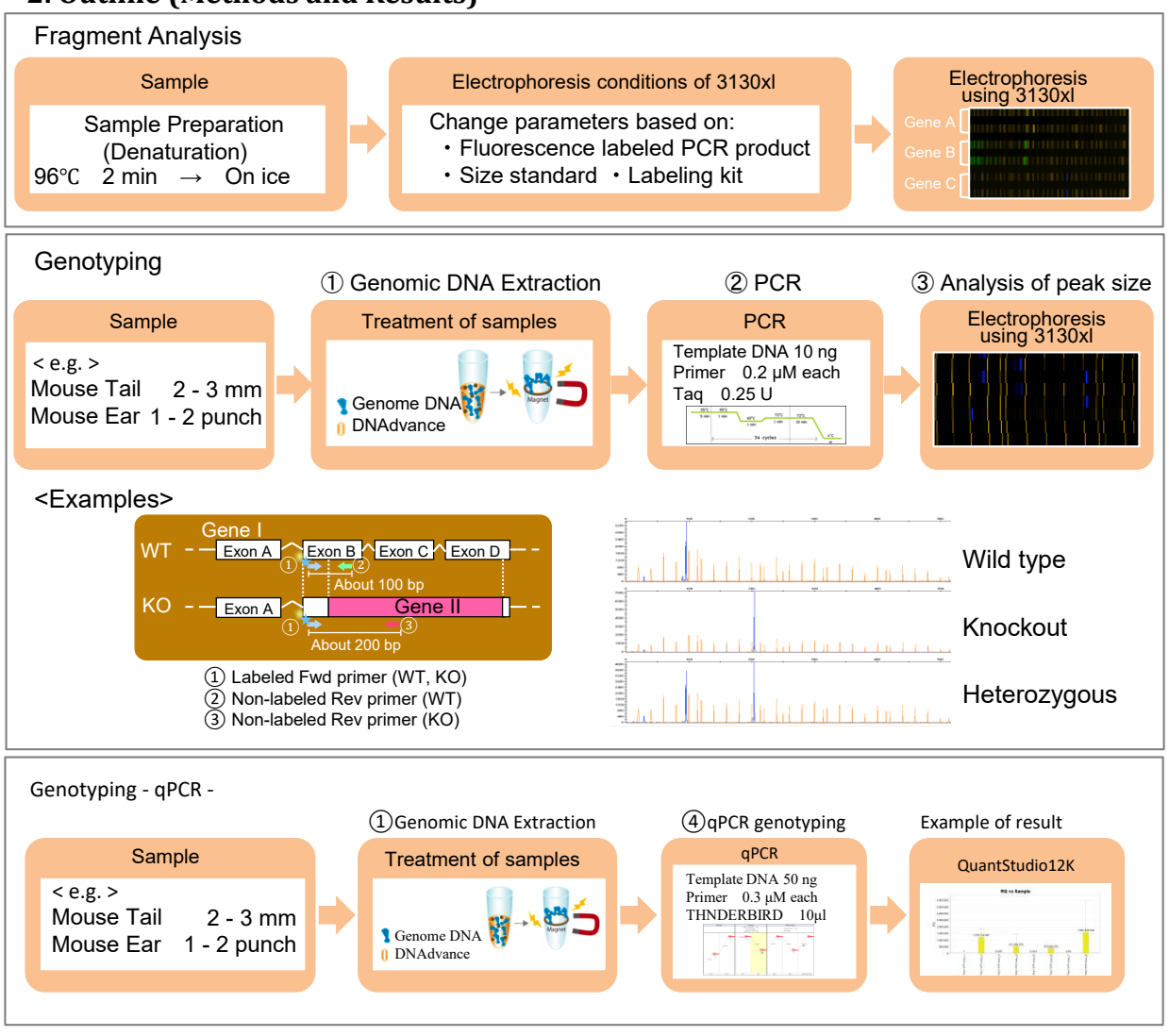
Labeled DNA fragments are electrophoresed using the 3130xl Genetic Analyzer.

④qPCR genotyping

Genotyping using the real-time PCR device for genomic DNA (①) extracted from the biological sample.

* When conditions for the analysis are not decided, we examine the optimum. (additional charge)

2. Outline (Methods and Results)



Gene Quantitative Analysis

CBS Cent. Bldg., C011

1. Support Services

Gene Expression Quantification

TaqMan Assay or SYBR Green Assay: The sample, primer, probe (for TaqMan assay only) and enzyme are mixed and then monitored for fluorescent intensity using the Real-Time PCR machine (QuantStudio 12K or 7900HT, Applied Biosystems).

Genotyping For SNP

TaqMan Assay: The sample, primer, two TaqMan probes and enzyme are mixed. The SNP signal is detected using the Real-Time PCR machine.

Mouse Genotyping

TaqMan Assay or SYBR Green Assay: The sample, primer, probe (for TaqMan assay only) and enzyme are mixed and then monitored for fluorescent intensity using the Real-Time PCR machine.

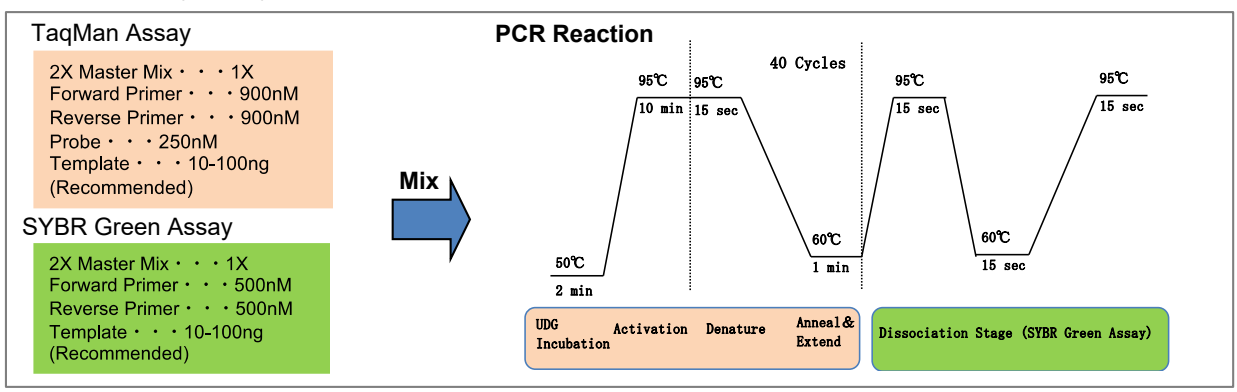
Nonspecific Reaction Check

User-designed primers can be checked for nonspecific reaction activity.

QuantStudio 12K
(Applied Biosystems)

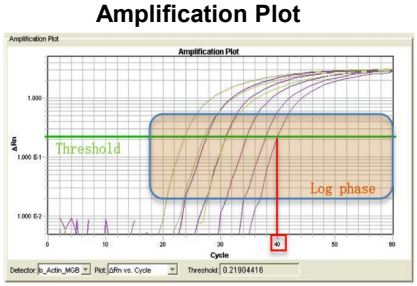
2. Outline (Methods)

PCR Reaction (Default)



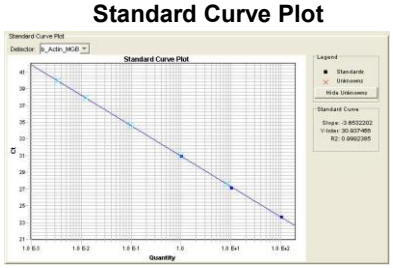
3. Outline (Results)

Amplification Plot



The Ct is set to be within the exponential growth region of the amplification plot. The threshold is the line whose intersection with the amplification plot defines the Ct.

Standard Curve Plot



The standard curve plots the Ct values vs. the quantity of the standard samples.

Results Table

Well	Sample Name	Detector Name	Reporter	Task	Ct	Quantity
1	Control_1/100	GAPDH_MGB	VIC	Standard	25.696	1
2	Control_1/10	GAPDH_MGB	VIC	Standard	28.016	10
3	Control_1	GAPDH_MGB	VIC	Standard	31.682	100
4	Sample A	GAPDH_MGB	VIC	Unknown	28.374	11.016
5	Sample B	GAPDH_MGB	VIC	Unknown	30.268	2.924
6	Sample C	GAPDH_MGB	VIC	Unknown	28.016	10

Quantity of the standard sample

For each sample:
the quantity is calculated using the Ct value of the standard curve.

Example

	GAPDH		Probe A		Relative Value for Probe A
	Result	Relative Value (X)	Result (Y)	Quantity Result (Y/X)	
Sample A	6384	1	0.981	0.981	1
Sample B	8584	1.34	44.2	33	33.6
Sample C	7430	1.16	1.93×10^4	1.66×10^4	1.69×10^4

1.Relative RNA value (X) is normalized among GAPDH values (Internal Control).
2.Result of Probe A (Y) was normalized with the X value.
3. A relative value for Probe A is calculated as the ratio between two sample's Y/X values.

Next Generation Sequencing (Library Pretreatment, Sequencing)

CBS Cent. Bldg., S004

1. Support Services

Library Preparation

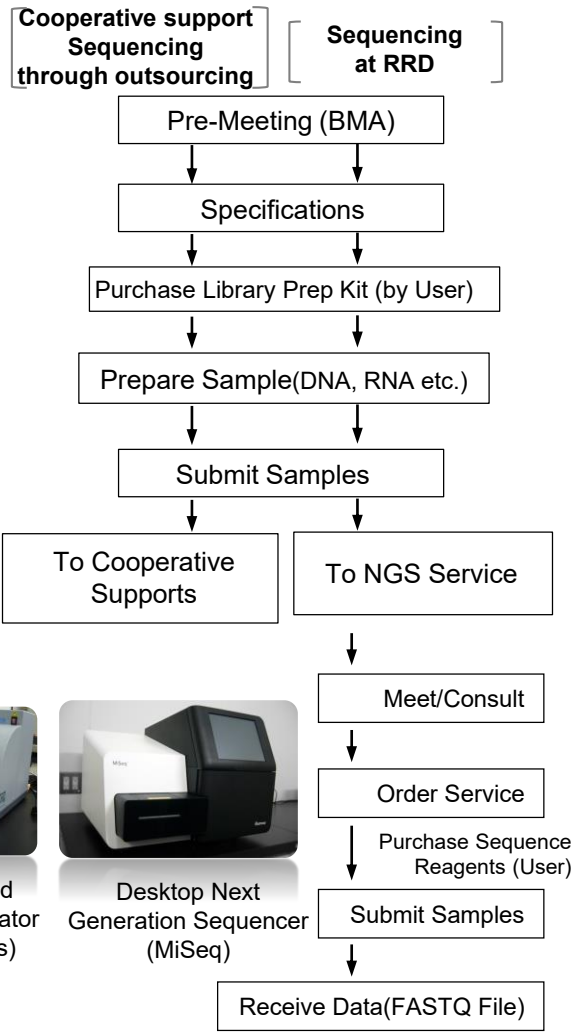
・Genomic DNA enrichment including exons using SureSelect and library preparation for next-generation sequencing (NGS).

- Other support applications:
  e.g. Whole Genome, ChIP-Seq, Methyl-Seq,
  RNA-Seq, Single-cell RNA Seq [Quartz-Seq2].

Sequencing using MiSeq

- Deep sequencing Using MiSeq is performed in BMA.
- Cluster generation, and Sequencing by the MiSeq (Illumina). FASTQ files are generated from the sequence data.

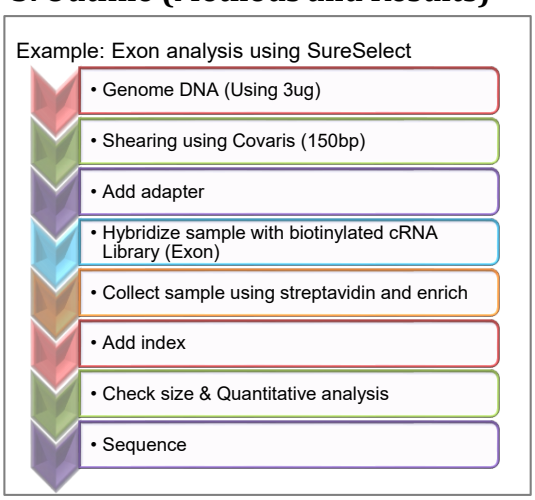
2. Procedure



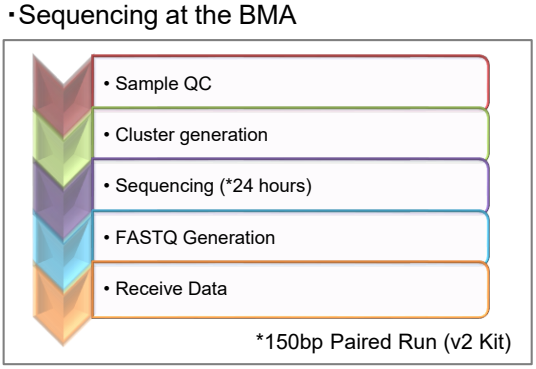
Focused ultrasonicator (Covaris)

Desktop Next Generation Sequencer (MiSeq)

3. Outline (Methods and Results)



・Sequencing at the BMA

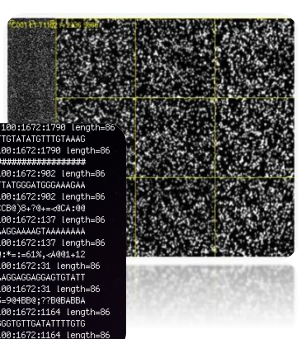


・Sequencing through Outsourcing

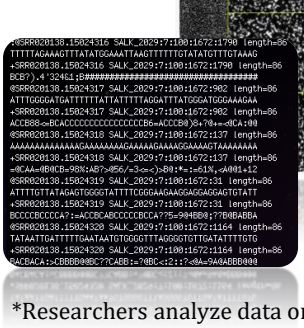
1. At the consultation, decide on a kit to use, etc.
2. BMA coordinates library preparation . The service corresponds to the following applications; Exome, RNA-Seq, ChIP-Seq, Methyl-Seq
3. Quality Checks are conducted by the Bioanalyzer and MiSeq
4. KAPA kit is used for the quantitative analysis
5. Sequencing at the cooperative outsource.

Result

Cluster image

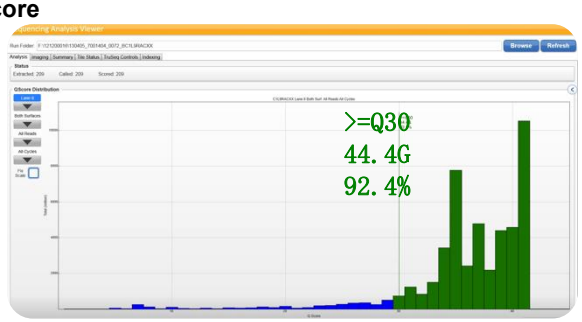


FASTQ image



*Researchers analyze data on the FASTQ files using 3rd party software.

Quality Score



Mass Spectrometry

CBS Cent. Bldg., S002

1. Support Services

Molecular Weight Determination

Electro-spray ionization (ESI)-mass spectrometer (MS) or Matrix-assisted laser desorption ionization (MALDI)-MS are used to determine molecular weights of organic compounds. High resolution and high accuracy measurements are also available using the ESI-MS.

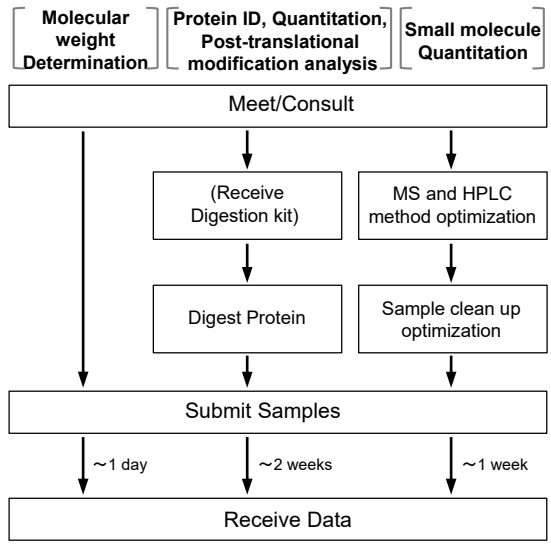
Protein Identification (ID) and Post-Translational Modification Analysis

The protease-digested peptides are analyzed using the LC-ESI-MS/MS. For high accuracy identification and modification analysis, digested peptides are analyzed using the quadrupole-orbitrap MS. Proteome analysis for relative protein quantitation are also available by stable isotope labeling methods among multiple samples and/or label free method (LFQ).

Protein / Small Molecule Quantitation

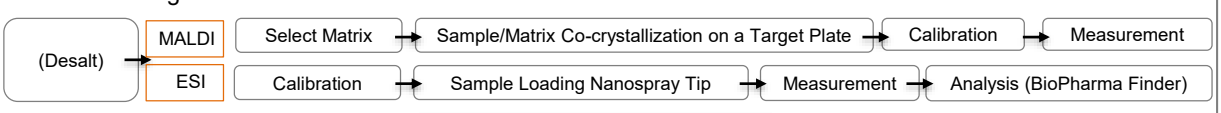
Target proteins or small molecules are quantified by MRM (Multiple Reaction Monitoring) method using the triple-quadrupole MS with a suitable LC. For absolute quantitation, addition of stable isotope labeled internal standards is recommended.

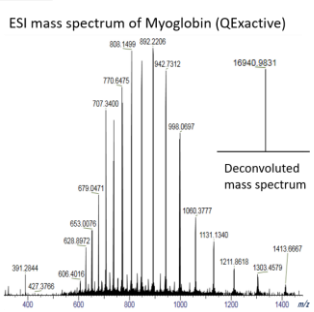
2. Procedure

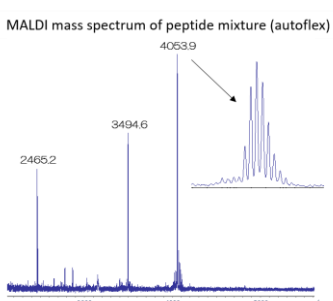


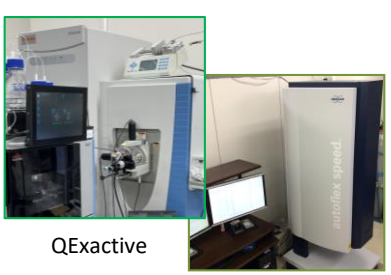
3. Outline (Methods and Results)

Molecular Weight Determination







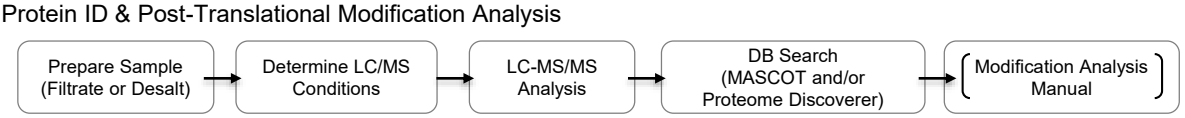


QExactive

autoflex

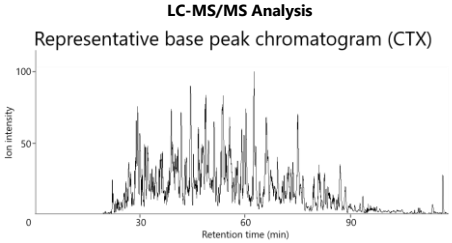
- Detection sensitivity: fmol-pmol range.
- Do not use detergent. If it is necessary, limit the detergent to a minimal amount. Samples may require ZipTip processing to concentrate and/or desalt prior to analysis.

Protein ID & Post-Translational Modification Analysis

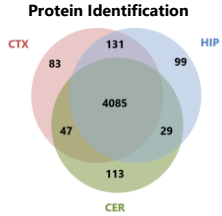


Comparison of Specific and Ubiquitous Protein Expressions among Mouse Brain Regions, Cerebral Cortex (CTX), Hippocampus (HIP), and Cerebellum (CER)

LC-MS/MS Analysis



Protein Identification



Total of **4587** proteins were identified from triplicate samples of the three brain regions.

Vanquish Neo + QExactive

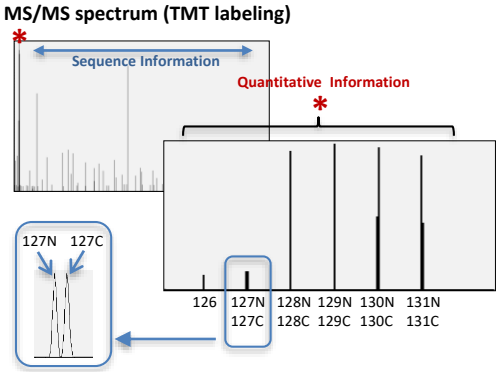
- We recommend performing all protein digestions at the clean bench (N005).
- Taxonomy information is required for a MASCOT database search.

Comprehensive and Relative Protein Quantitation Analysis

Desalt → Determine LC/MS Conditions → LC-MS/MS Analysis → DB Search (MASCOT and Proteome Discoverer) → Data Analysis (Proteome Discoverer)

-Stable isotope labeling method-

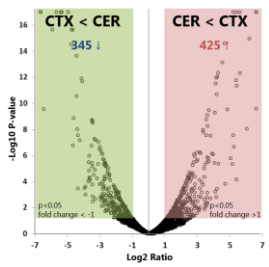
MS/MS spectrum (TMT labeling)



-Label-free method (LFQ)-

Comparison of Protein Expression between CTX and CER

Volcano Plots



Vanquish Neo + QExactive

・ Stable isotope labeling methods (e.g. iTRAQ/TMT, SILAC, and 18O) and label-free method are available for comprehensive and relative protein quantitation among multiple samples.

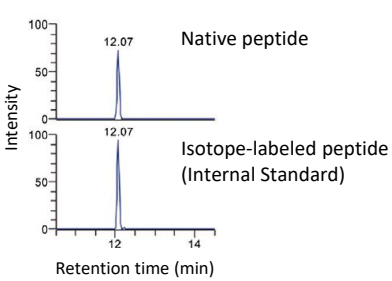
Targeted Protein and Small Molecule Quantitation Analysis

--Multiple Reaction Monitoring (MRM) method-

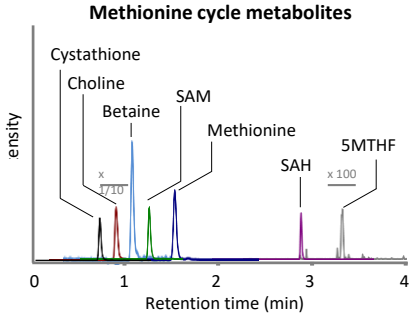
Make the Primary Transition List → Determine LC/MS Conditions → MRM Analysis (1) → Fix the Transition List → MRM Analysis (2) → Data Analysis (Pinpoint or TraceFinder)

- Quantitation using MRM method -

Peptide (angiotensin-II)*



Methionine cycle metabolites



Vanquish UHPLC + TSQ Altis

Easy-nLC 1200 + TSQ Vantage

・ MRM is an analytical method with high sensitivity; it selectively detects only target peptides or small molecules (Maximum transitions: 3,000 MRMs per run).
・ Proteins with MRM transitions list created by the RRD are able to be analyzed without method development.
・ As a first step for quantitation of new compounds, optimization of various protocol conditions such as sample clean-up steps (by user) and analytical methods for UHPLC and TSQ Vantage (by BMA staff) are required.

* Modified from user's publication: *Mol Cell.* 2015 Jun 18;58(6):1015-27.

Peptide Synthesis

CBS Cent. Bldg., S003

1. Support Services

Peptide synthesis:

Peptides are synthesized by the solid phase method using automated peptide synthesizers; quality is determined by HPLC and MALDI-TOF/MS analysis. Crude peptides or purified peptides in lyophilized form are provided to users.

Modifications:

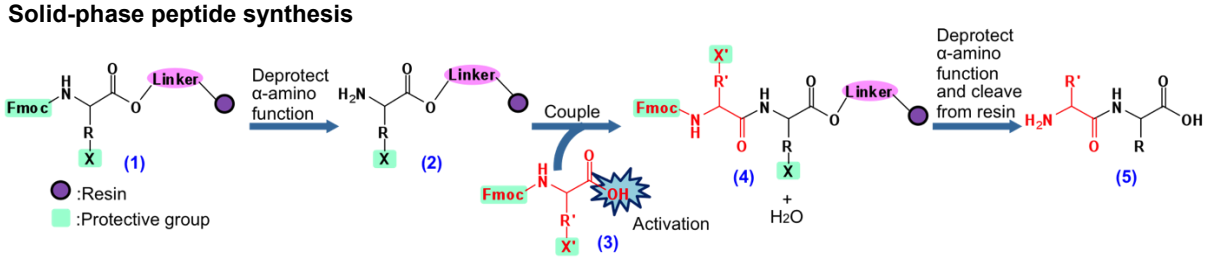
Conjugation to a carrier protein (KLH or BSA) for antibody production.
N-terminal : Free, Ac, Fmoc
C-terminal : Free, NH_2, Resin

Special synthesis:

Long chain peptides (≧31aa), 250μmol scale, biotinylation, cyclization, fluorescent label, peptide synthesis including unusual amino acids and phosphoamino acids.

2. Outline (Methods and Results)

Solid-phase peptide synthesis



Synthesis

Peptides are synthesized by the solid phase method; using a peptide synthesizer, they are built "backwards" from the C-terminal to the N-terminal.
Three different peptide synthesizers are used, suitable for each synthesis scale and sequence.

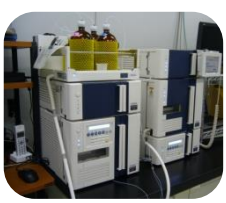
433A

Liberty Blue

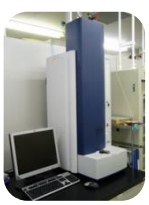
MultiPep CF & MicroColumn

Purification of peptides

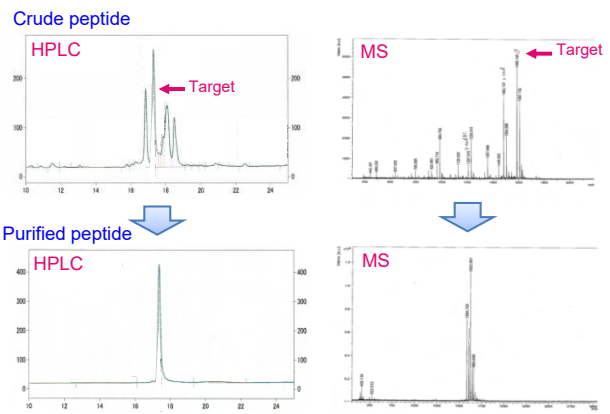
The target peptide is separated and collected by HPLC system. Quality of the crude and purified target peptides is determined by HPLC and MALDI-TOF/MS analysis.



Chromaster



microflex



HPLC conditions:

Column, Inertsil ODS-3 (250 x 4.6 mm I.D.) Column temp., 25℃
Mobile phase, 1-51% CH3CN (contg. 0.1% TFA) Analytical time, 50 min
Flow rate, 1.0 mL/min Detection, UV at 215nm

Protein Purification

CBS Cent. Bldg., C013

1. Support Services

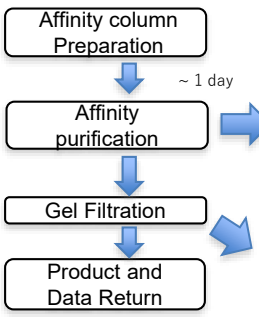
- **Antibody Purification**
  Antibodies are purified from plasma and sera by AKTA (Cytiva).

- **Protein Purification**
  [Biological Proteins]
  Particular proteins are purified from bio-material, such as brain tissue, and cells. Products and data (e.g. chromatograms) are returned to users.
  User-designed purification protocols are welcome.

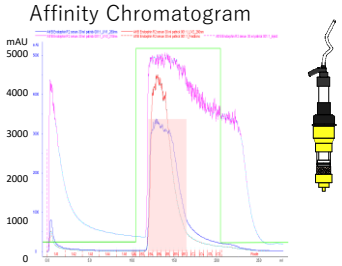
  [Target Proteins]
  Purification of the following proteins are available: Recombinant proteins, His-tag fusion proteins, GST fusion proteins, IgG antibodies, and serine proteases.

2. Outline (Methods and Results)

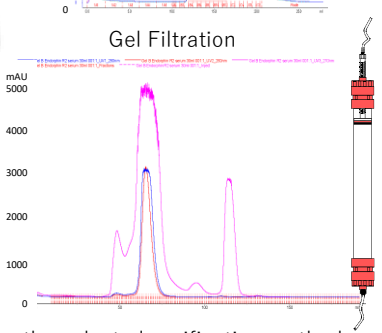
Antibody Purification Flow



Affinity Chromatogram



Gel Filtration



AKTA Explorer 10

Purified by the selected purification method (determined at the preliminary meeting/consultation).

Amino Acid Analysis

CBS Cent. Bldg., C013

1. Support Services

Ninhydrin method

- Amino Acid Composition Analysis

The quantitative analysis of free amino acids, amino sugar and protein hydrolysate is performed by an amino acid analyzer using the post-column method.

Fluorescent method

– Neurotransmitter Amino Acid Analysis

The quantitative analysis of amino acids (glutamate, aspartate, and GABA) in brain tissue and brain microdialysates is accomplished on a high-performance liquid chromatograph (HPLC) with fluorescent detectors (FLD) using the pre-column method.

Electrochemical method

– Monoamine Analysis

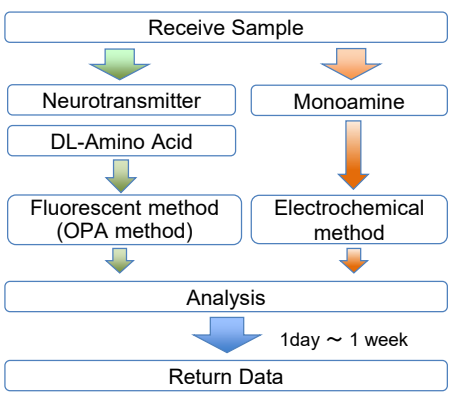
The quantitative analysis of monoamines (noradrenaline, dopamine, serotonin) and its metabolites in the brain tissue extracts or brain microdialysates are performed using HPLC with an electrochemical detector (ECD).

Neuromodulator Analysis

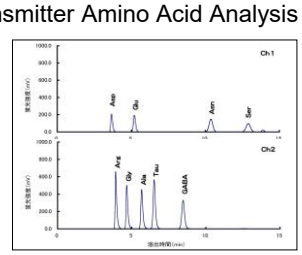
The quantitative analysis of a non-listed small molecule as the above technical service is performed using HPLC with FLD/UV/ECD detector

- Development of analytical method for a small molecule to chose a detector and examining the optimization of the analysis condition.
- The quantitative analysis by HPLC (FLD/UV/ECD)

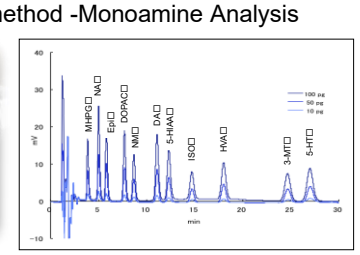
2. Outline (Methods and Results)



Fluorescent method -Neurotransmitter Amino Acid Analysis



Electrochemical method -Monoamine Analysis

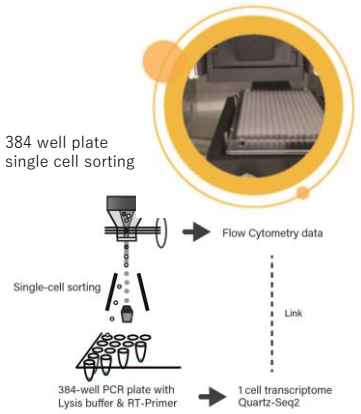


Flow Cytometry and Cell Sorting

CBS Cent. Bldg., S008

1. Support Services

- Cells specifically labeled with fluorescent dyes or expressing fluorescent proteins are separated using a fluorescence-activated cell sorter, FACSAria (Becton Dickinson).
- Able to perform the index sorting that is useful for one cell analysis.
- Isolated cell preparations, together with the property diagram analyzed by software FlowJo (Becton Dickinson) for flow cytometry (FCM) analysis and the microscopic data obtained under an Olympus Fluorescence Microscope (EVIDENCE), are returned to researchers.



Sasagawa et al. Genome Biology 19:299, 2018

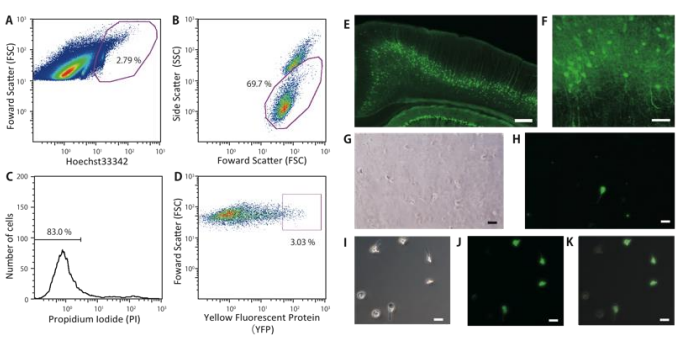
2. Outline (Methods and Results)

Example: Pretreatment

Extract tissue from brain
*if possible, remove the white matter
↓
Cut tissue to 1-2 mm then transfer to 50 mL
↓
Incubate the papain solution containing the tissue at 37℃ with agitation
↓
Triturate with pipette ⟵ 2 times
↓
Filtrate through 40 μm nylon mesh
↓
Triturate with pipette tip
↓
Filtrate through 40 μm nylon mesh
↓
Add propidium iodide and Hoechst33342
↓
Flow Cytometry analysis and cell sorting

Example: Results

An example of flow cytometry analysis and cell sorting with YFP expressing cells from mouse brain (Cited from reference)



The target cells are detected by using several parameters.
(A) High level of Hoechst 33342 detected nuclei (in the polygonal gate),
(B) Doublets or multiplets and debris excluded using FSC/SSC dot plots (outside of the polygonal gate), (C) Histogram plot indicating selected live cells. Dead cell populations are localized in the high PI fluorescent range, (D) FSC and YFP two parameter dot plots indicate the sorted cell population (in the quadrangle gate), (E) Frozen tissue section of the cortex, (F) Dissected cortex, (G and H) Triturated cell mixtures before cell sorting, (I-K) YFP expressing cells purified by cell sorting. Scale bars: 200μm in E, 20μm in F-K .

Reference; Ohtawa and Mataga, SEITAI NO KAGAKU 62(2): 165 - 170, 2011.

Labware Washing

CBS Cent. Bldg., S001

1. Support Services

・CBS Central Bldg.
Labware washing, drying, and sterilization (by heat treatment or autoclave).

・All CBS Bldgs.
Pipet tip packing and sterilization (by autoclave).

2. Outline (Methods and Results)

Order: Bring labware with completed application form to the RRD washing room.

Washing: Using a pH neutral non-phosphate liquid detergent (2% Scat 20X-N) in an ultrasonic cleaning machine.

Rinse: Using tap and pure water in an automatic washing machine.

Drying: The washed labware is dried, and then capped with aluminum foil.

Heat Treatment: Please let us know if this step is necessary for your experimental purpose.

List of Main Equipment for Staff-run Technical Services in the BMA

(as of July 1, 2024)

Equipment	Manufacturer	Model	Location
DNA Sequencing			
DNA Sequencer	Applied Biosystems	3730xl	CBS Cent. N011
Liquid Handling Workstation	Beckman Coulter	Biomek i7	CBS Cent. N010
Gene Quantitative Analysis			
Real-Time PCR System	Applied Biosystems	7900HT, QuantStudio 12K	CBS Cent. C011
Genetic Polymorphism Analysis			
Genetic Analyzer	Applied Biosystems	3130xl	CBS Cent. N011
BioArray Analysis			
Bioanalyzer	Agilent	Agilent 2100 Bioanalyzer	CBS Cent. S004
Next Generation Sequencing			
Next Generation Sequencer	illumina	MiSeq	CBS Cent. C011
Mass Spectrometry			
MALDI TOF/TOF Mass Spectrometer	Bruker Daltonics	autoflex	CBS Cent. S002
Triple Quadrupole Mass Spectrometer	Thermo Fisher Scientific	TSQ Altis/Vantage	CBS Cent. S002/N008
Quadrupole-Orbitrap Mass Spectrometer		Q Exactive	
Nanoflow HPLC		EASY nLC1200/Vanquish Neo	
Ultra Performance Liquid chromatography		Vanquish UHPLC	
Peptide Synthesis			
Peptide Synthesizer	Thermo Fisher Scientific	433A Peptide Synthesizer	CBS Cent. S003
	CEM Corporation	Liberty Blue	
		MultiPep CF	
HPLC	Hitachi High-Technologies	L-7450 and others	
	GL Sciences	PU714M, BG-34-02, UV702 and others	
MALDI TOF Mass Spectrometer	Bruker Daltonics	microflex	
AKTA Protein Purification System	Cytiva	AKTA explorer10S with Frac-950 AKTA prime	CBS Cent. C013
Amino Acid Analysis			
Amino Acid Analyzer	Hitachi High-Technologies	L-8900	CBS Cent. C013
HPLC	Shimadzu	LC-10Avp	
	Eicom	700 Series Sytem	
	Eicom	HITEC-500	CBS Cent. N004
Flow Cytometry and Cell Sorting			
Flow Cytometry System	Becton Dickinson	FACS Aria II SORP, FACSAria SORP, FACSymphony A3	CBS Cent. S008

Technical Personnel Support Section (TPSS)

The BMA set up a new section for providing customized support services termed Technical Personnel Support Section (TPSS) in October 2018 and allocated expert technicians. Purpose of the services is to help labs closely with technical support to meet their requirements. For example, TPSS staff perform routine experiments in the lab according to their experimental protocol. As advanced services, techniques not available in the lab are also provided by expert TPSS staff to do experiments and/or operate state-of-the-art instruments. Furthermore, we actively try to receive technical transfer of a newly developed technique from a lab and then provide it to different labs as an advanced service by TPSS.

http://common.riken.jp/rrd/cominstrue/subaEng/tpss/tpss.html

For Users

- **Procedure:**
  1. At the beginning to use desired service, users should discuss with the person in charge in BMA about experimental plan including service contents and schedule. The BMA unit leader will attend the meeting as necessary.
  2. Application can be done through R-COMS. Application forms for uploading the R-COMS can be obtained from the website above.
- **Operating hours:**
  RIKEN normal working hours are observed and advanced reservation is always necessary.
- **Management of analyzed data:**
  Analyzed original data will be kept on file at RRD for a period of five years, except the data from human materials of some services (e.g., Validation of data). We will also discard all data when the corresponding lab is closed.
- **Publishing Scientific Data Acquired with RRD**
  Please credit our specific contribution in the methods and/or acknowledgments section(s) of your paper (*please see page 3 for details*). As a special notes, when a TPSS staff made a significant contribution to your research, we would appreciate it if you could acknowledge the person or include the person as a co-author of your paper.

Features of Services by TPSS staff:

Molecular Biological Experiment
Plasmid construction and amplification

Histological Experiment
AAV Microinjection under Stereotaxic Conditions
Histological Staining and Imaging
- Perfusion Fixation
- Immunohistochemistry
- In situ hybridization
- LacZ, Nissl, CO, and Hematoxylin (HE) staining
- Embedding
- Imaging by digital scanners/microscopes

Bioimaging Experiment
Multi- Sectioning Image Acquisition using TissueCyte

Virus Production Experiment
Production of AAV vector, lenti virus vector, rabies virus vector e.t.c.

TPSS: Molecular Biological Experiment

CBS Cent. Bldg., C010

1. Support Contents

▸ Plasmid Construction

This support constructs new plasmids. The original plasmid DNA provided by the user are recombined and reconstructed using the method requested by the user. Consultations on design are also available.

▸ Plasmid Amplification

The required amount of plasmid DNA is amplified and purified from E. coli.
A QIAGEN kit is used for purification. The yield estimates for each column size are as follows:
Miniprep: up to 20 μg　Midiprep: up to 100 μg　Maxiprep: up to 500 μg

2. Outline (Method and Results)

Recombine the insert gene and vector provided by the user using the recombination method requested by the user
↓
Select clones using the method requested by the user
↓
Perform sequence analysis as necessary
(Request BMA technical support DNA sequence analysis)
↓
Amplify and purify to the requested yield
↓
Prepare glycerol stock if requested
↓
Measure DNA concentration
↓
Hand over the final product and report

TPSS: Histological Experiment (Histological Staining and Imaging)

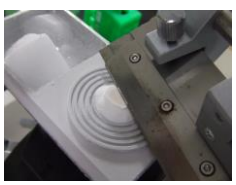
CBS Cent. Bldg., C005

1. Support Contents

- Services from histological preparation using mouse brain tissue to its imaging are provided.
- You are able to order a part of work contents, 1. to 5. below.
  1. Perfusion fixation
  2. Tissue sectioning (freezing sample, non-freezing sample)
  3. Staining by order methods
     (e.g. Immunostaining, In situ Hybridization, Nissl, Hematoxylin/Eosin staining)
  4. Mounting tissue on slide glasses
  5. Image acquisition (By using RRD CUE digital slide scanner)

2. Outline (Method and Results)

<View of Sectioning>



Freezing Microtome
YAMATOREM-700

<Procedure>

Using users' materials (e.g. samples, chemicals) and their experimental protocols, customized support services are performed. Rats and zebra fish are also acceptable to the service with a prior consultation held.

＜Staining example＞

Nissl Staining

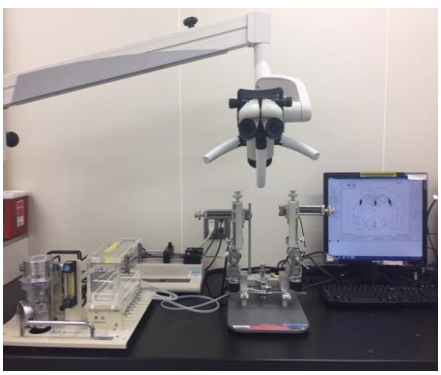
HE Staining

TPSS: Histological Experiment (AAV Microinjection)

CBS Cent. Bldg., N815 , Neural Circuit Genetics Bldg., 311f

1. Support Contents

- Leica Angle Two Dual Stereotaxic/ Mouse is used to perform AAV injection in the mouse brain.
- Other viral vectors, retrograde or anterograde tracers, chemicals are also available to injection.



Leica Angle Two Dual Stereotaxic/ Mouse

2. Outline (Method and Results)

＜Procedure＞

Pull a glass capillary (Sutter, Micropipette puller P-97)
↓
Attach the glass capillary to one side of a Teflon tube
and set it on the Angle Two arm.
Attach the opposite side of the Teflon tube
to the Hamilton syringe and set it to the syringe pump.
↓
Fix anesthetized mouse to stereotaxic apparatus.
↓
Incise the mouse head to expose the skull.
↓
Start up the Angle Two system.
↓
Adjust the height of the brain under the microscope.
↓
Check the coordinate of the target and make a hole in the skull.
↓
Suction up AAV from the tip of capillary and align to the target.
↓
Inject AAV
↓
Pull out the capillary from the brain and suture the skin.
↓
Stop anesthesia, remove the mouse from the stereotaxic
and return it to a cage.

TPSS: Bioimaging Experiment (Multi- Sectioning Image Acquisition using TissueCyte)

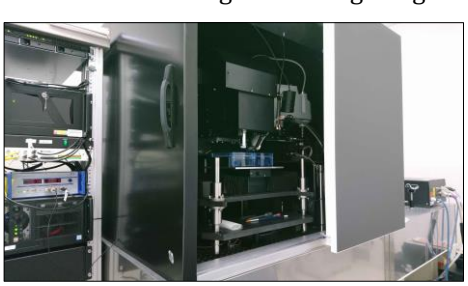
CBS Cent., C005, C107

1. Support Contents

Multi- Sectioning Image Acquisition using TissueCyte

TissueCyte 1400FC (TissueVision) is a device that can image each cross-section as a wide-range fluorescence image with a two-photon laser microscope while automatically sectioning the brain of a small animal such as mice and zebrafish in the XY direction with a high-performance vibratome.

This support includes from embedding of mouse brain tissue to stitching of 2D tiling images.



TissueCyte 1400FC

Ragan, T., et al. (2012). Serial two-photon tomography for automated *ex vivo* mouse brain imaging. *Nat. Methods* 9, 255–258.

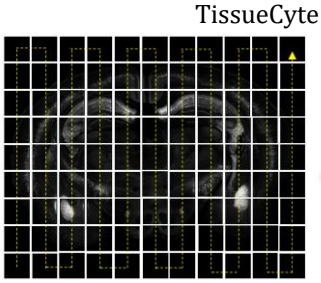
2. Outline (Method and Results)

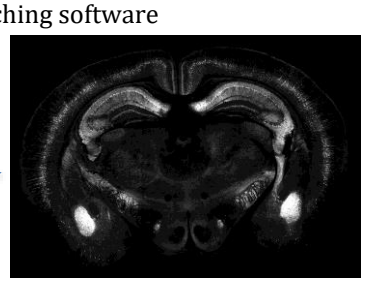
＜Procedure＞

Preparation of brain block (embedding)
↓
Set the brain block to the TissueCyte
↓
Setting of various conditions for imaging
↓
Imaging
↓
Image processing (stitching)
↓
Return data and sample

＜Example： B6.Cg-Tg(Thy1-YFP)HJrs/J ＞

TissueCyte Stitching software





3D construction (Image J)

TPSS: Virus Production Experiment

CBS Cent. Bldg., C010

・Adeno-associated virus (AAV)

1. Support Contents

- Provide custom-made AAV vectors (P1 level)
- Respond technical consultation with users concerning AAV production

The followings are discussed with users at a pre-meeting, and we will produce the ordered AAV.

a. AAV's serotype
b. Expressing gene
c. Titer (gc/mL) & volume (μL)

◆ Please prepare Transfer-plasmid by users.

◆ In some cases, we would like to ask users to prepare serotype-plasmid.

2. Procedure

In the safety cabinet, experiments are done.

Preparation culture cell and plasmids
↓
Transfection of plasmids & AAV production
↓
Harvest AAV from supernatant & cells
↓
Concentration of supernatant by PEG
↓
Ultracentrifuge (purification/concentration)
↓
Titer check（qPCR or dPCR)

・Rabies virus (RV)

1. Support Contents

- Provide custom-made RV vectors (P2 level)
- Respond technical consultation with users concerning RV production

For custom-made RV production, we confirm user's status (whether the user obtain the followings).

A. Plasmids and cells for the RV production
B. Approval for RV experiment

The followings are to be discussed with the user at the pre-meeting, and we will produce the ordered RV.

a. Strain and envelope protein
b. Expressing gene
c. Titer (IU/mL) and volume (μL)

※Please prepare all cells and plasmids for RV by users.

2. Procedure

In the safety cabinet, experiments are done.

Preparation culture cell and plasmids
↓
Transfection of plasmids & RV production
↓
Harvest RV & RV amplification
↓
Exchange glycoprotein of RV (eg. EnvA)
↓
Ultracentrifuge (purification/concentration)
↓
Titer check

・Lentivirus (LV)

1. Support Contents

- Provide custom-made LV vectors (P2 level)
- Respond technical consultation with users concerning LV production

The followings are discussed with users at a pre-meeting, and we will produce the ordered LV.

a. LV's envelope protein
b. Expressing gene
c. Titer (gc/mL) & volume (μL)

※ Please prepare all plasmids for LV by users.

2. Procedure

In the safety cabinet, experiments are done.

Preparation culture cell and plasmids
↓
Transfection of plasmids & LV production
↓
Harvest LV from supernatant
↓
Ultracentrifuge (purification/concentration)
↓
Titer check（qPCR or dPCR)

BMA manages and maintains the Shared Experimental Areas. The Common Use Equipment (CUE) Experimental Rooms are located in the CBS Cent. Bldg. (B1F, 1F). CBS Common Use Facility for unsealed radioisotope experiments is located in the RI Center. These experimental areas are available for use of both CBS and all other labs in RIKEN.

Common Use Equipment (CUE) Experimental Rooms

http://common.riken.jp/rrd/cominstrue/subaEng/indexe/indexcome.html

1. Support Services

Widely use and/or highly technical equipment are organized into groups and installed in separate rooms in the CBS Cent. Bldg. according to the purpose of use (B1F: 10 rooms, 1F: 1 room) .
Upon completion of user registration online, the equipment and working spaces in these rooms are available for use (self-run user).

2. Procedure

- **Users**

A user is any person in RIKEN given permission to access the Experimental Rooms by the Unit Leader of the Support Unit for Bio-Material Analysis (hereinafter referred to as "UL").

- **Applicant**

1) To use the CUE Experimental Rooms in the CBS Cent. Bldg., registration is necessary (The RIKEN Core Facility Management System, R-COMS) will use for the registration from April 1, 2023).
2) Orientation (only for new users) and System Key Box registration (according to the approved application) will take place by a CUE Administrative Officer
3) To conduct experiment that requires careful attention to safety management; All experiment plans including the CUE Experimental Room numbers need to be approved/issued by the Safety Division before initiating the experiments.

- **User registration validity**

Users are permitted to use the facility from the date their application is approved until one day before of approval of annual registration renewal for the following year.
The renewal notice will be sent from BMA to budget managers and users at the early fiscal year.

- **Conditions of use**

1. The user can use all equipment in the Experimental Rooms after UL approval. . However, if users wish to conduct an experiment that requires careful safety management, they must have approved or issued receipt for all registered experiments by the Safety Division before initiating experiments.
2. CUE must be operated by the user. Be aware that these are shared use equipment; read instruction manuals that accompany the equipment and follow instructions. Certain designated equipment requires special instruction for beginners and/or users by the CUE Equipment Adviser or manufacturer. For more information, please contact the adviser.
3. The annual registration user fees are required for the use of the CUE Experimental Rooms. An additional fee is charged for specific equipment requiring special consumables and/or annual contracts for maintenance. Concerning other equipment with free usage fees, consumables such as reagents will be borne by the user's Lab.
4. Workspaces for sample preparation are organized for each experimental room. In-use reagents and solvents can be temporarily stored in the rooms but return all waste to the user's lab (exceptions: liquid waste for chromatograph analysis, autoclaved culture-liquid waste, etc.).
5. Contact the CUE Equipment Adviser promptly if there are any problems with the equipment. In case of any damage to the equipment, whether or not it is the fault of the user, the adviser should be notified immediately. All repairs must be made following the adviser's instructions. If the user is clearly at fault for equipment damage, the user's lab is responsible for repair costs.

- **Hours of use**

There are no restrictions. The key to each laboratory on the B1F is a key box, and the key to each laboratory on the 1F is controlled by the IC-key.

- **Equipment reservation**
  Shared facilities/equipment reservations are available online. Use the R-COMS (RIKEN Core Facility Management System) to make advance reservations (reservations and changes can also be made on the shared PC in the laboratory).
  https://riken.simprent.jp/users/login?locale=en

- **Logbooks**
  Enter the required items (name, lab and etc) in the logbook attached to the piece of equipment.
  Note: CUE equipment that has not been used/reserved more than 1 year may be discontinued.

3. Features of the CUE Experimental Rooms and Main Equipment Lists

(As of April 1, 2024)

- **Molecular Biology Room (N002)**
  This room contains basic equipment for molecular biology such as thermal cyclers and spectrophotometers. The Live Cell imaging and Analysis System and Gene transfection System are installed. Genetic recombination experiment is also available under P1, P1A (only C.elegans) and P2 in this room.

- **Gene Quantitative Analysis Room (C011)**
  The Real-Time PCR systems and desktop next generation sequencer are used for staff-run analysis and self-run use.

- **P2/Level 2 Experimental Room (S009)**
  Sample preparation space is available under P1, P2, Level 1 and Level 2. This room is also available for shared use to maintain cultured cells, including microorganisms such as viruses (Level 1, Level 2), and Living Modified Organisms (P1, P2).

- **Histological Preparation Room (C004)**
  This room contains, all processing equipment needed for histological preparation and observation (from tissue fixation embedding, and sectioning to staining). It is also equipped with cryostats for freezing specimens.

- **Microscopy/Imaging Room #1 (N003)**
  General-purpose all-in-one fluorescence microscopes and slide scanners are installed in this room. There are also imaging systems for chemiluminescence, fluorescence, and colorimetric imaging applications.

- **Microscopy/Imaging Room #2 (C107, 1F)**
  High magnification confocal laser scanning microscopes are installed in this room under a temperature-controlled environment. Neurolucida system is also available in this room.

- **Super-Resolution Microscope Room #1 (N005-1)**
  The room contains a confocal laser microscope with super resolution, time gate, high speed scan, and high-sensitive photon counting functions.

- **Super-Resolution Microscope Room #2 (C012-1)**
  The room contains a spinning disk confocal super resolution microscope for high-speed imaging including a live cell imaging and Lattice Structured Illumination Microscopy System with lattice SIM system, a CO_2 incubator and a clean bench.

- **Chromatography Room (N004)**
  Several chromatography systems for analysis of proteins, peptides, and neurotransmitters are installed in this room. Among these, HPLC conditions have been established for the following methods: simultaneous analysis of monoamines and their metabolites, high-sensitive dopamine & serotonin analysis, and simultaneous analysis of glutamate & GABA.

- **Biochemistry Room (N005)**
  Basic equipment for biochemistry, such as microplate readers, a centrifugal evaporator and freeze dryer are available in this room. One of the key features of this room is the installation of several pieces of special equipment, such as Biacore and NanoSight.

- **Flow Cytometry Room (S008)**

  The Flow Cytometer, the flow cytometry software "Flowjo" and Automated cell isolation system which is available for the pretreatment of FCM's samples are installed in this room; other pieces of equipment are used for Staff-run Technical Service.

- **Other available equipment**

  Besides the CUE, BMA also manages and maintains the CBS Common Use Equipment, Mass Spectrometry Room (C308-2) and equipment for microdialysis sampling.

<< All common Use Equipment are available to use by members of CBS Co-Creation Labs >>

CUE Experimental Room name (Room number)	Equipment ID#	Equipment	Manufacturer	Model
Molecular Biology Room (N002)	WC0735	Live Cell Imaging & Analysis System*	Sartorius	Incucyte® SX5 HD/3CLR
	WC0739	CO2 Incubator	Thermo FisherSCIENTIFIC	Forma Steri-Cycle i160
	WC0730	Safety Cabinet	Thermo Fisher Scientific	Thermo Fisher Scientific 1300
	WC0731	Auto Clave	TOMY	LBS-245
	WC0095	Refrigerated Centrifuge	Thermo Fisher Scientific	Solvall Legend X1R
	WC0072	Low Speed Centrifuge	TOMY	LC122
	WC0031	Thermal cycler	Applied Biosystems	GeneAmp® PCR System9700
	WC0732	Microvolume Spectrophotometer	Thermo Fisher Scientific	NanoDrop One
	WC1174	Fluorescence Microscope (Inverted)	EVIDENT(OLYMPUS)	IX71
	WC0037	Culture Microscope (Inverted)	Leica Microsystems	DMIL
	WC0038	Stereoscopic Microscope (Optical)	Leica Microsystems	MZ95
	WC0043	Laser Microdissection system	Leica Microsystems	LMD7
	WC0096	Multi-Shaker Oven	TAITEC	HB-100
	WC0039	BioShaker (Tabletop)	TAITEC	M.BR-024
	WC0572	BioShaker (Large size)	TAITEC	BR-180LF
	WC0098	Water Bath Shaker	TAITEC	Personal-11 EX set
	WC0040	Sonoporation Gene Transfection System	NEPA GENE	Sonitron2000
	WC0041	MicroPulser™ electroporator	Bio-Rad	MicroPulser
	WC0042	Gene Pulser MXcell Electroporation System	Bio Rad	Gene Pulser Mxcell
	WC0668	ImagPrep*	Bruker Daltonics	ImagPrep
	WC0958	Micro Refrigerated Centrifuge	TOMY	MX-301
	WC0241	SDS-PAGE	Invitrogen, ATTO	Xcell4 SureLock Midi-Cell, AE-6500
	WC1055	Digital PCR system	QIAGEN	QIAcuity One Digital PCR system
	-	walk-up : Ultrasonic Cleaner	AS ONE	VS-100III
	-	walk-up : Microwave	PHC	NE-S30
	-	4°C Freezer	NIHON FREEZER	GS-5203AF311E

CUE Experimental Room name (Room number)	Equipment ID#	Equipment	Manufacturer	Model
Gene Quantitative Analysis Room (C011)	WC0649	Real-Time PCR System (No. 1) *	Applied Biosystems	ABI7900HT
	WC0347	Real-Time PCR System*	Applied Biosystems	QuantStudio 12K
	WC0345	Next Generation Sequencer*	Illumina	MiSeq
	-	walk-up : Tabletop Centrifuge	TOMY	LC121

CUE Experimental Room name (Room number)	Equipment ID#	Equipment	Manufacturer	Model
P2/Level 2 Experimental Room (S009)	WC0158	Safety Cabinet*	DALTON	ClassII TypeA2B (NSE-1500)
	WC0644	Safety Cabinet*	PHC	ClassII TypeA2 (MHE-1301A2-PJ)
	WC0220	Multi Incubator	PHC	MCO-170MUV-PJ
	WC0059	Biohazard Ultracentrifuge	Beckman Coulter	Optima XE-100
	WC0217, WC0710-WC0712	CO2 Incubator Thermo (No.1)	Thermo Fisher Scientific	370 T/C sensor
	WC0705, WC0713-WC0715	CO2 Incubator Thermo (No.2)	Thermo Fisher Scientific	370 T/C sensor
	WC0130, WC0707-WC0709	CO2 Incubator PHC (No.1)	PHC	MCO-170AICUVD-PJ
	WC0704	CO2 Incubator PHC (No.2)	PHC	MCO-170AICUVD-PJ
	WC0140	walk-up : Micro High Speed Centrifuge	TOMY	CAX-571
	WC0142	walk-up : Table Top Low Speed Centrifuge	Sakuma	SL-IV
	WC0141	walk-up : Inverted Fluorescence Microscope	EVIDENT(OLYMPUS)	CKX53
	WC0155	walk-up: Autoclave	TOMY	BS325
	WC0156	Table top water bath shaker	TAITEC	Personal 11-SD set
	WC0208	walk-up : Astrason Ultrasonic Generator	Mizonix	XL2020
	WC0214	walk-up : Ultrasonic Generator Bioruptor	Cosmo Bio	UCD-200TM
	WC0723	walk-up: Ultra Low Temperature Freezer (-80℃)	Revco	RLE50086D-81
	WC0724	walk-up: Bio Medical Freezer (-30℃)	PHC	MDF-MU500H-PJ
	WC0725	walk-up: Bio Medical Cooler (4℃)	Japan Freezer	UKS-5410DHC

CUE Experimental Room name (Room number)	Equipment ID#	Equipment	Manufacturer	Model
Histological Preparation Room (C004)	WC0080	Embedding Console System (No. 1)	Sakura Finetek	TEC-IV
	WC0622	Embedding Console System (No. 2)	Sakura Finetek	TEC-IV
	WC0623	Vacuum Infiltration Processor (No.1) *	Sakura Finetek	VIP6
	WC0081	Vacuum Infiltration Processor (No.2) *	Sakura Finetek	ETP-150C
	WC0182	Sliding Paraffin Microtome	Leica Microsystems	SM2010R
	WC0726	Sliding Paraffin Microtome	Thermo Fisher Scientific	HM400
	WC0332	Sliding Freezing Microtome	YAMATO	ROM-380
	WC0189	Cryostat	Thermo Fisher Scientific	HM525 NX
	WC0163	Biolostic Particle Delivery System (Gene Gun)	Bio-Rad	Helios Gene Gun
	WC0193	Vibratome (for PFA fixed tissue)	D.S.K	DTK-1000
	WC0716	Fume Hood (for histological staining)	DALTON	DFV-11SK-75ALT
	WC0160	Cryostat	Thermo Fisher Scientific	CryoStar NX70
	WC0924	Portable Cleanbench	ASONE	CT-900AD
	-	walk-up : Optical Microscope	Nikon	ECLIPS E200
	-	walk-up : Biological Microscope (Uplight)	EVIDENT(OLYMPUS)	BX50
	-	walk-up : Stereomicroscope	EVIDENT(OLYMPUS)	SZ60
	-	walk-up : Stereoscopic Fluorescence Microscope	Leica Microsystems	MZFLIII
	-	walk-up : Ultrapure Water System	ORGANO	PURELAB Ultra Genetic
	-	walk-up : Ultrasonic Cleaner	VELVO-CLEAR	VS-25

CUE Room (Room number)	SimpRent#	Equipment	Manufacturer	Model
Microscopy/Imaging Room #1 (N003)	WC0045	Digital Slide Scanner*	Hamamatsu Photonics	NanoZoomer Digital Pathology 2.0HT
	WC0574	NanoZoomer*	Hamamatsu Photonics	NanoZoomer S60
	WC0741	Research Slide Scanner	EVIDENT(OLYMPUS)	SLIDEVIEW VS200
	WC0733	Software for imaging analysis /NDP.view2 Plus	Hamamatsu Photonics	NDP.view2 Plus
	WC0573	All-in-one Fluorescence Microscope	KEYENCE	BZ-X700
	WC0928	Lumino-Imaging Analysis System (No. 1)	Bio-Rad	ChemiDoc Touch MP
	WC0626	Lumino-Imaging Analysis System (No. 2)	FUJI FILM	LAS-3000
	WC0641	Variable mode imager	Cytiva	Typhoon9400
	WC0343	MultiNA, Microchip electrophoresis for DNA/RNA (MulitiNA)*	SHIMADZU	MCE-202
Microscopy/Imaging Room #2 (C107)	WC0019	Confocal Laser Scanning Microscope (Upright) *	EVIDENT (OLYMPUS)	FV3000
	WC0020	Confocal Laser Scanning Microscope (Inverted)*	EVIDENT (OLYMPUS)	FV3000
	WC0021	Neuron tracing & analysis system (Fluorescence Microscope + Software	EVIDENT (OLYMPUS)/MBF	IX71 + Neurolucida
	WC0022	Software for imaging analysis	Molecular Devices	MetaMorph
	WC0522	Software for imaging analysis	Leica Microsystems	LAS X
	WC0627	Software for imaging analysis*	MBF	Neurolucida360
	WC0628	Software for imaging analysis*	MBF	BrainMaker
	WC0629	Software for imaging analysis*	MBF	NeuroInfo
	WC0630	Computer for Imaging Analysis	HP	Z240 Tower Workstation
	WC0631	Computer for Imaging Analysis	Supermicro	Viento Xeon Single CPU Model
	WC0632	Computer for Imaging Analysis	DELL	Precision 5820

CUE Experimental Room name (Room number)	Equipment ID#	Equipment	Manufacturer	Model
Super-Resolution Microscope Room #1 (N005-1)	WC0521	Super-Resolution Confocal microscope*	Leica Microsystems	TCS SP8 STED ONE
Super-Resolution Microscope Room #2 (C012-1)	WC0023	Spinning Disk Confocal Super Resolution Microscope *	EVIDENT(OLYMPUS)	SpinSR10
	WC0719-WC0722	CO2 Incubator	PHC	MCO-170AICUV
	WC0035	Bio-Clean Bench	PHC	MCV-B161F
	-	Tabletop Centrifuge	TOMY	LC120
	WC0034	Lattice Structured Illumination Microscopy System*	Carl Zeiss	Elyra 7 type S (Lattice SIM ^{2})

CUE Experimental Room name (Room number)	Equipment ID#	Equipment	Manufacturer	Model
Chromatography Room (N004)	WC0578	HPLC (Fluorescence Detection)*	GL Science, Eicom	EP-700 etc.
	WC0055	HPLC (Electrochemical Detection_ECD500)*	Eicom	HTEC-500 etc.
	WC0625	AKTApurifier10*	Cytiva	AKTApurifier
	WC0102	HPLC-PDA*	JASCO	PU-4180-LPG etc.
	WC0056	Table Top Ultracentrifuge	Beckman Coulter	Optima MAX-TL
	WC0235	Table Top Micro Refrigerated Centrifuge	KUBOTA	3K30C
	WC0240	walk-up : Ultrasonic Homogenizer	SMT	UH-50
	-	walk-up : pH meter	METTLER TOLEDO	SevenEasy
	-	walk-up : Analytical Balance (Max 6,100g)	METTLER TOLEDO	PG6002-S
	-	walk-up : Analytical Balance (Max 220g)	METTLER TOLEDO	AB204-S
	-	walk-up : Ultrasonic Cleaner	BRANSON	5510J-DTH
	-	walk-up : Vapor Pressure Osmometer	Wescor	Vapor5520
CUE Experimental Room name (Room number)	**Equipment ID#**	**Equipment**	**Manufacturer**	**Model**
Biochemistry Room (N005)	WC0579	Blood test (for animal)	NIHON HOHDEN	MEK-6358
	WC0580	Blood biochemistry analysis (for animal)	FUJIFILM	3500V
	WC0520	Molecular Interaction Analysis System*	Cytiva	Biacore X 100 Plus Package
	WC0078	Multilabel Counter	PerkinElmer	Wallac 1420 ARVO MX-2
	WC0079	Multimode Plate Reader	Thermo Fisher Scientific	Varioskan Flash
	WC0790	Multimode Plate Reader	Thermo Fisher Scientific	Varioskan LUX
	WC0057	Freeze Dryer (No. 1) *	EYELA	FDU-830
	WC0624	Freeze Dryer (No. 2) *	EYELA	FDU-830
	WC0581	Centrifuge Concentrator (No. 1)	TOMY	CC-105
	WC0621	Centrifuge Concentrator (No. 2)	TOMY	CC-105
	WC0577	Ultracentrifuge	Beckman Coulter	Optima XL-100K
	WC0859	Nanoparticle Tracking Analysis System	Malvern Panalytical (Fuji Film	NanoSight
	WC0239	Clean Bench	AS ONE	CT-1200AD
		Centrifugal Evaporator	TAITEC	VC-15s
		Multi-Shaker Oven	TAITEC	Multi-Shaker Oven HB
		Aluminum Block Bath	TAITEC	DTU-1B
	WC0240	Microplate Reader	Bio-Rad	Model 550

CUE Experimental Room name (Room number)	Equipment ID#	Equipment	Manufacturer	Model
FlowCytometry Room S008	WC0643	Flow Cytometry Analysis Software	BD	FlowJo
	WC997	Automated magnetic cell separation system	Miltenyi Biotec	autoMACS Pro Separator
	WC2775	High-parameter flow cytometer*	BD	FACSSymphony A3
CUE Experimental Room name (Room number)	**Equipment ID#**	**Equipment**	**Manufacturer**	**Model**
Other available equipment	WC0301	Orbitrap mass spectrometer (QExactive)*	Thermo Fisher Scientific	QExactive
	WC0302	QTRAP LC-MS system with ion-mobility separation device	SCIEX	QTRAP 6500+ and others
	WC0674	Lending service(walk-up) : Equipments for Microdialysis sampling		
		-Micro fraction collector	Univender	820 Microsampler
		-Micro fraction collector	BAS	CMA140
		-Micro syringe pump	BAS	CMA/100
		-Micro syringe pump	BAS	CMA/142
		-Instrument Table	Eicom	
		-Acryl Case	Eicom	
		-Liquid Switch	BAS	CMA/110
		-Liquid Switch	Eicom	Si-60

Support Unit for Functional Magnetic Resonance Imaging

Equipped with a 3-Tesla MRI system and a 7-Tesla MRI system, we support non-invasive functional MRI (fMRI) studies on human subjects and animals conducted by laboratories within RIKEN. Besides fMRI studies which are the primary focus of our MRI facility, we also have the capability to perform DTI, MRA, MRS, and simultaneous EEG/fMRI measurements. We have installed a number of peripheral devices for fMRI studies, such as stimulus presentation devices (video projectors, earphones, an electrical stimulation device, etc.), to meet researchers' various needs for experiments. Subject condition (heartbeat, respiration, eye movement, pupil size, EMG, button press response, etc.), stimulus timing, and the shape of gradient pulses can be recorded together with the acquisition trigger timing signal, enabling this information to be used for post-processing and analysis of the MRI data. We have unique, in-house developed pulse sequences, data analyzing processes and peripheral equipment. Multi-shot EPI for high spatial resolution imaging, software for regressing out noise due to both respiration and cardiac pulsation, and a bite-bar system for minimizing subject motion are all available for users. We have put special emphasis on developing software to process k-space (raw) data so that we are not limited by the vendor's processing stream, which is optimized for clinical applications rather than research. We have developed software for reconstruction, noise reduction, and visualization of data in variety of formats. Using these cutting-edge technologies, users can perform many types of experiments and optimally process the data according to their needs. For researchers outside RIKEN, MRI experiments can be performed as Commissioned Test.

MRI spec

Items	3T MRI	7T MRI
Product name	MAGNETOM Prisma	SIGNA™ 7.0T
Vender	Siemens	GE
Static field(Tesla)	2.89	7
Max Gradient(mT/m)	80	100
Max slew rate(T/m/s)	200	200
Number of RF reception	64	64
Number of RF transmission	2	8
Body RF coil	Yes	No
Helium consumption(L/month)	0	0
Date of introduction	March 2015	April 2023

Peripheral devices

- Projector WUX5000 (Canon)
- LCD Goggle device (NNL)
- Audio stimulation device
- Physiological signal sensors
  （Heartbeat, Respiration, etc.）
- EEG device available under MRI environment
- Response button devices
- Optical fiber thermometer
- Eyeglasses for MRI
- Bite-bar system for head stabilization
- Computer for stimulus device control
- Computer for analog signal recording

Collaborative Research and Commissioned Test

There are two means available to users outside RIKEN. Either they can conduct measurements as part of an internal RIKEN experimental program after signing a joint research agreement with a RIKEN laboratory, or they can conduct measurements using the commissioned exam system. In the latter case, only data acquisition is provided. In the case of commissioned exams on human subjects, in particular, it is necessary to undergo ethical review outside RIKEN. In addition, if you wish to use experimental equipment that is not already available, a safety review will be required.

Collaborative Research Procedures

After discussing the experiment with the RIKEN laboratories, including our unit, and obtaining the agreement of all parties, a joint research plan will be established at both facilities. If the measurement involves human subjects, the research will be subject to RIKEN's internal ethical review. It is important to clarify the payment of fees as this may not be available depending on the type of budget.

Procedure for Using Commissioned Test

First, please consult with our unit. Depending on the measurement details, this service may not be available. If the project is deemed acceptable, you will be asked to apply for contract testing. After paying the fee according to the plan, the experiment will be started. After all the planned experiments are completed, the contract will be terminated when the measurement data is delivered to you.

Support Unit for Electron Microscopy Techniques

The Support Unit for Electron Microscopy Techniques (EMT) provides facilities and technical support for research that requires ultrastructural context, such as synaptic connections, and fine structures of intracellular organelles. The staff has specialized knowledge and skills for EM, and works with individual research laboratories to understand their specific needs, proposes options for research methods to achieve their research goals, and provide the best technical support possible at the Support Unit. We also host seminars to train individual researchers and trainees on various EM technologies available at the Support Unit.

Technical Services Available for Electron Microscopy Techniques

The Support Unit for Electron Microscopy Techniques provides the following technical support with in-depth training on the use of these microscopes to assist researchers to obtain high-quality images required for their research.

Electron Microscopy

Technical Services Provided by the Staff

Preparation of tissue for electron microscopy:

Biological specimens such as brains and organs are chemically fixed, stained with metal, and embedded in resin. The tissue can also be processed with a high-pressure freezing, resin replacement, and embedding for better preservation of its ultrastructure. Before the experiment, we would like to have a good discussion with researchers to understand their research project to select the best tissue processing method for the research project.

Electron microscope:

a) FIB-SEM (Focused Ion Beam-Scanning Electron Microscope)

The block surface revealed by thin (>4 nm) milling by the FIB is imaged using SEM. Repeating this process will produce serial electron micrographs automatically. The effective milling depth is about 100 μm; therefore, we recommend an imaging area of about 100 x 100 μm2. With this technique, the data obtained are in digital form, while physical tissue block portion are lost.

b) FE-SEM (Field Emission-Scanning Electron Microscope)

Serial ultra-thin sections obtained with an ultramicrotome are collected on either wafer or tape, and the region of interest is imaged using the FE-SEM. Relatively larger image areas are possible. Each section can be re-viewed multiple times for higher power or different regions of interest. Section thickness is >40nm thick.

Analysis of the EM data sets:

Instructions are provided on image analysis tools for 3D reconstruction, including NIH-image, VAST-Lite, and Amira.

Available Equipment

The Support Unit for Electron Microscopy Techniques provides the following EM equipment on B1F and 1F of the Brain Science Central Building.

(List of main equipment)

Equipment	Manufacturer	Model
FIB-SEM	ThermoFisher	Helios 5
FE-SEM	JEOL	IT-800
Ultramicrotome	Leica Microsystems	ARTOS-3D
ATUM	RMC	ATUM
Ultramicrotome	Leica Microsystems	UC-6, FC-6
High pressure freeze eq.	Leica Microsystems	EM ICE
Opto/electrical stimulation Unit	Leica Microsystems	

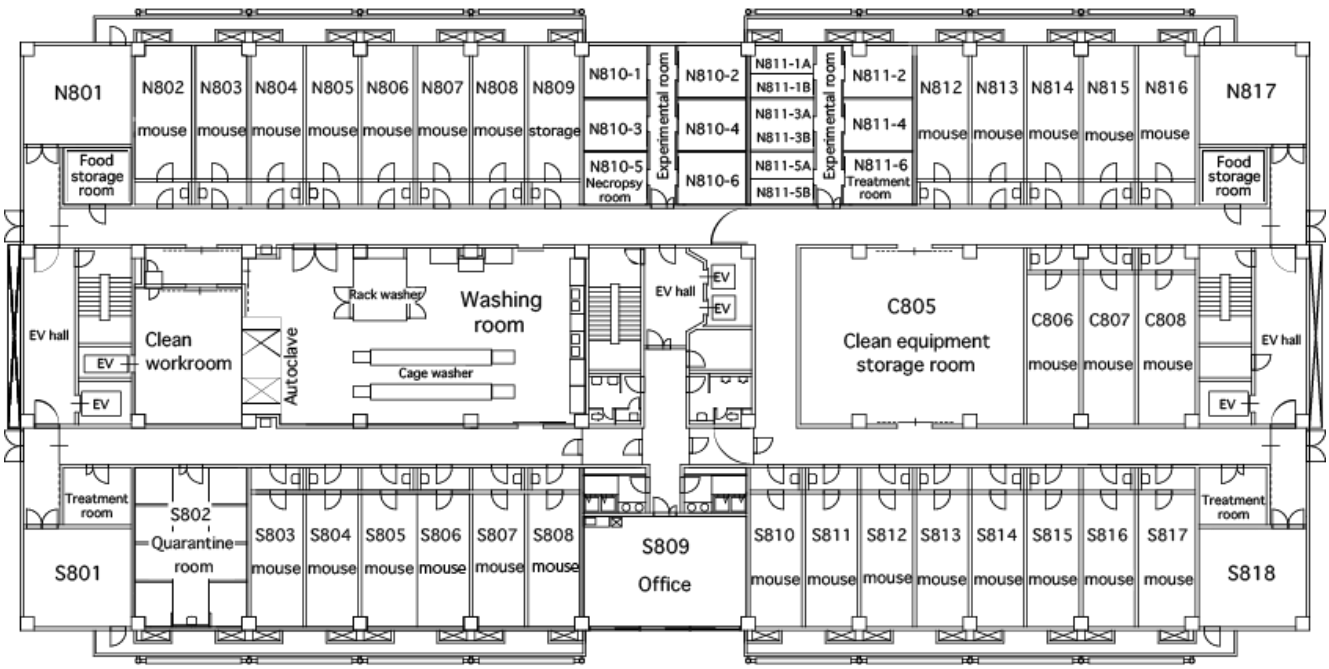
Appendix

Location of Common Facilities

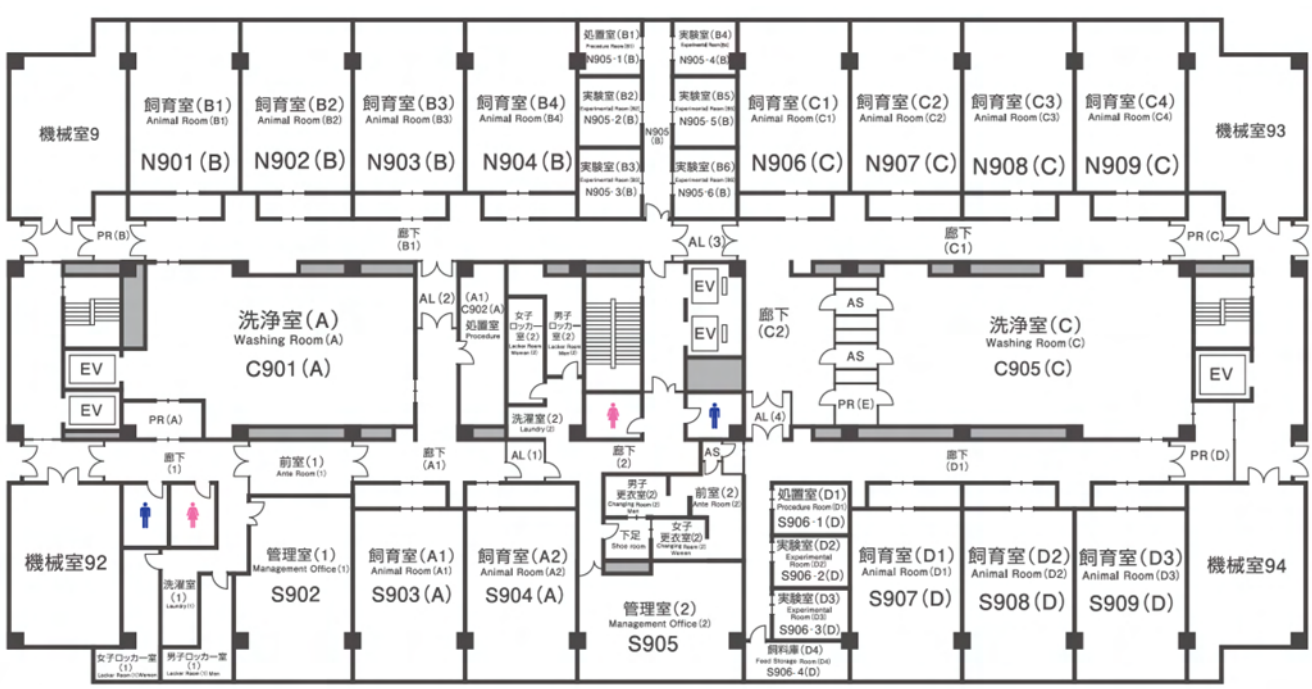
CBS NCGR Bldg. 3F :
Animal facility [mice / rats / rabbits / manipulation room / behavior testing rooms]

CBS NCGR Bldg. 2F :
Animal facility [mice / rats / behavior testing rooms / cage washing room]

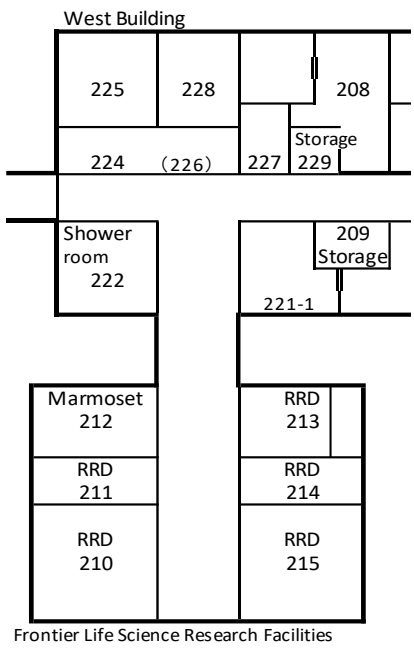
CBS Cent. Bldg. 8F : Animal facility [mice / mouse behavior analysis rooms / quarantine room]



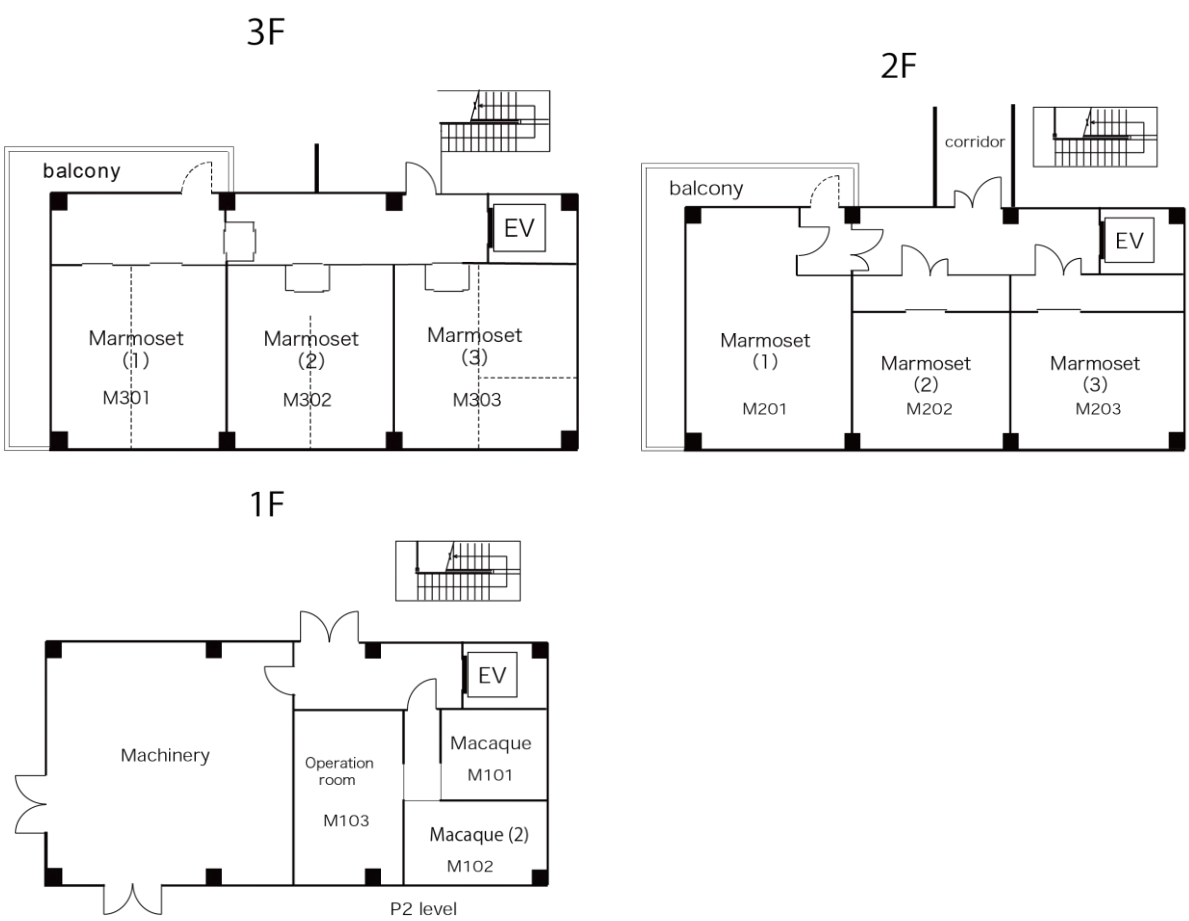
CBS Cent. Bldg. 9F : Animal facility [marmosets]



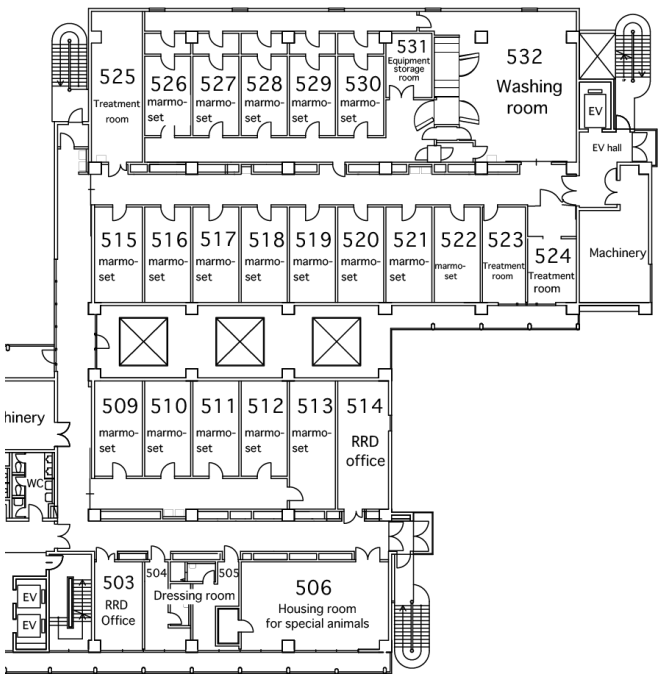
Frontier Life Science Research Facilities 2F: Animal facility [marmosets]



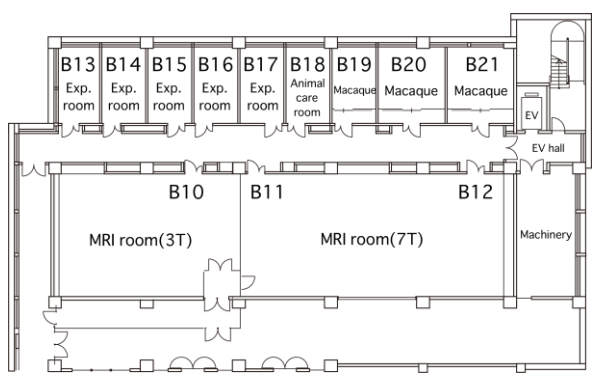
CBS West Bldg. Annex : Animal facility [macaques, marmosets]



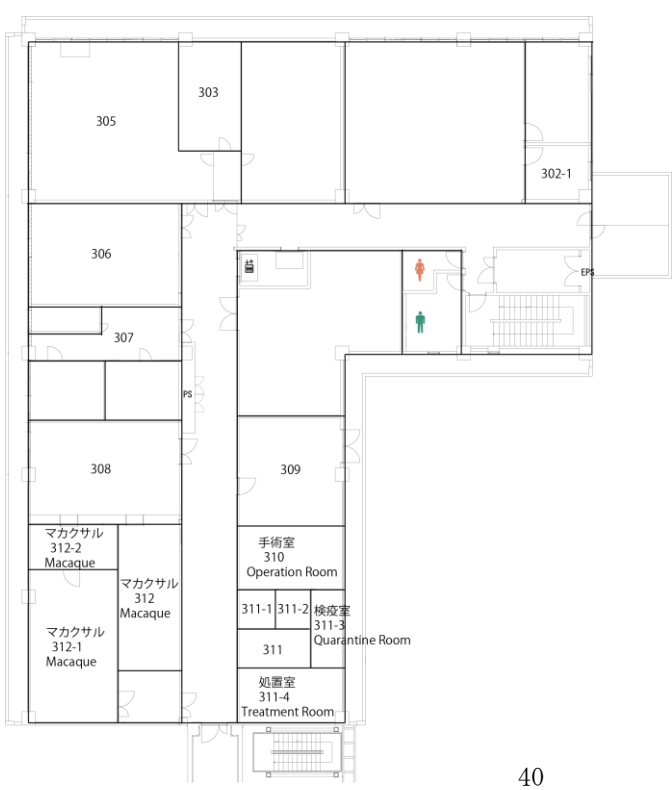
CBS East Bldg. 5F : Animal facility [marmosets / special animals]



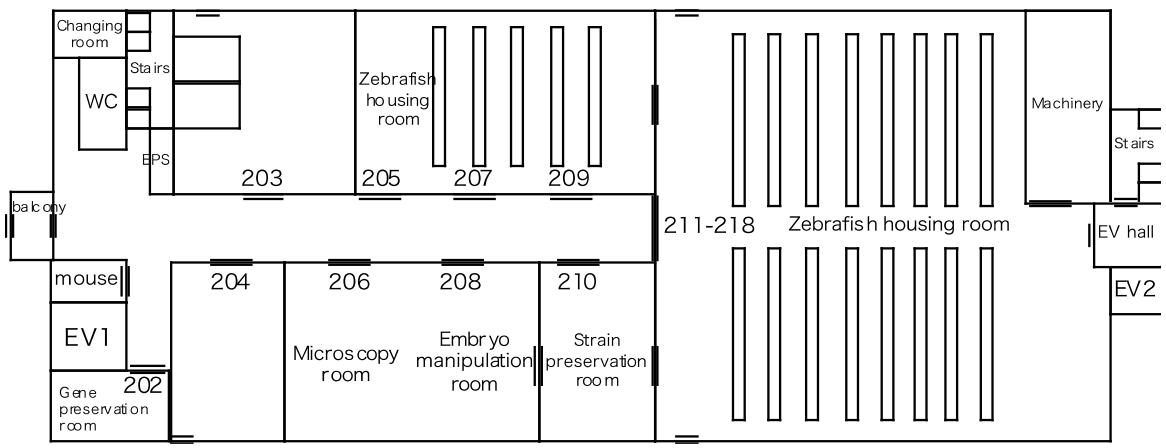
CBS East Bldg. B1F : Animal facility [macaques], MRI room



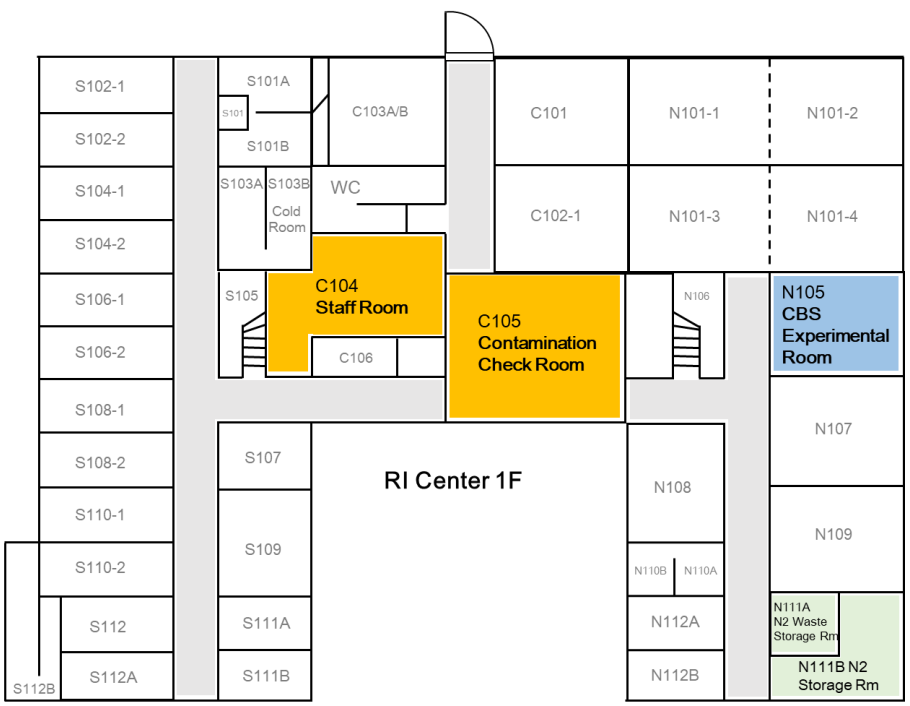
CBS West Bldg. 3F : Animal facility [macaques]



CBS Ikenohata Bldg. 2F : Zebrafish facility

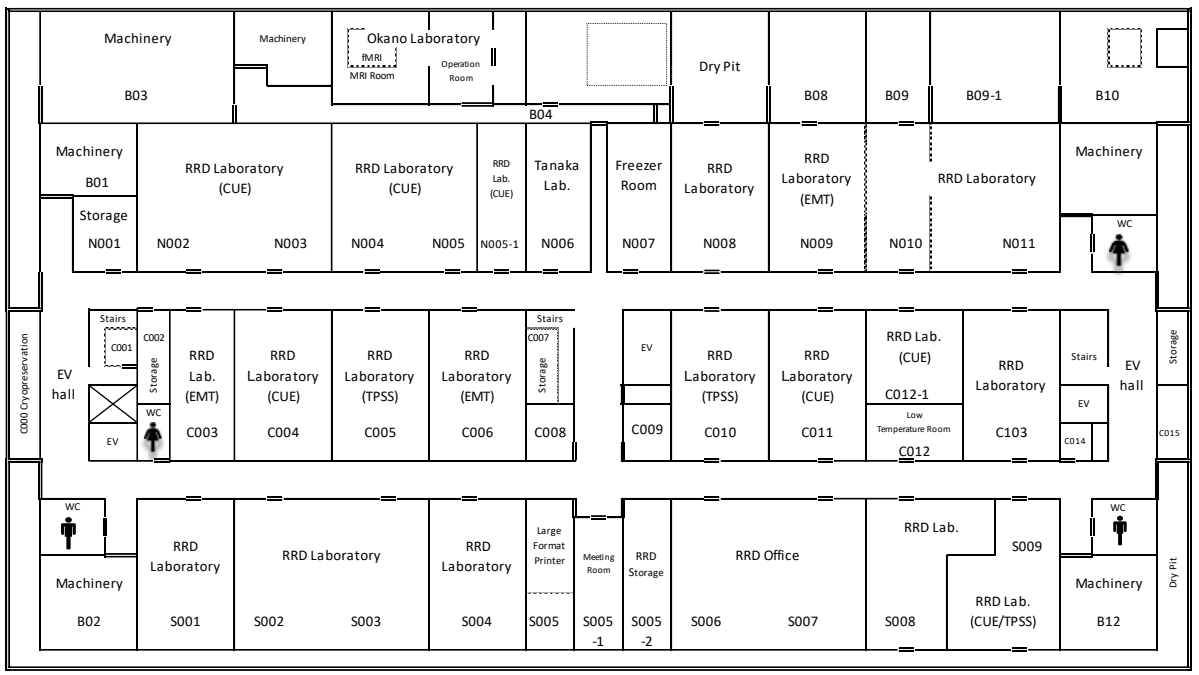


RI Center : RI facility



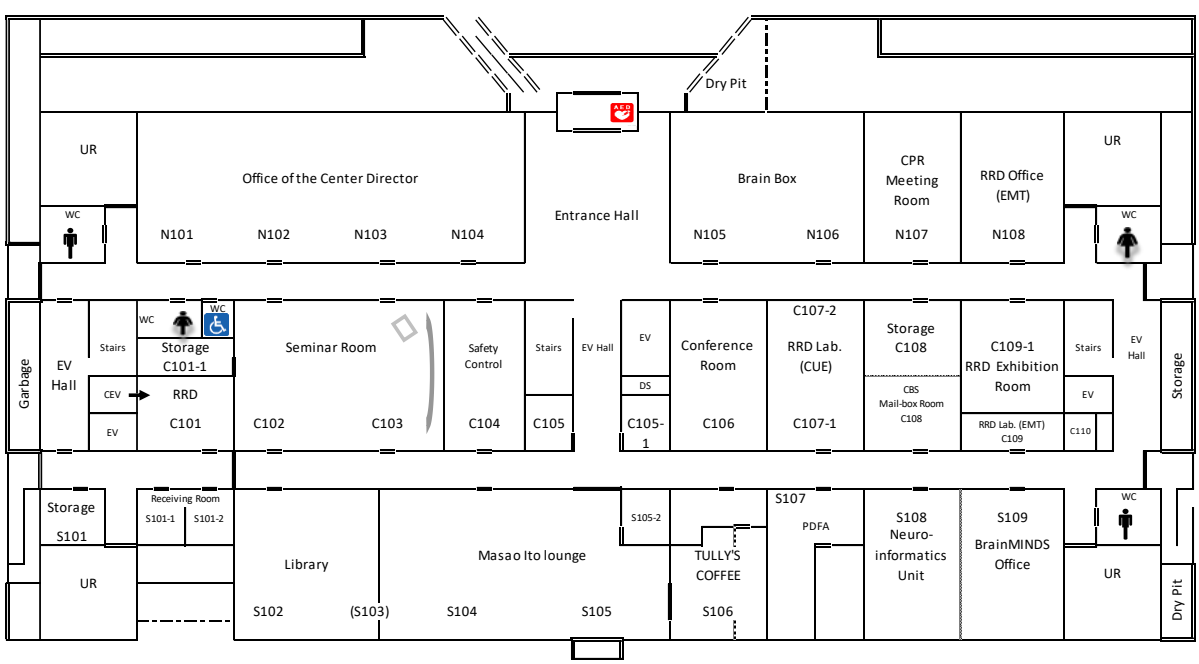
CBS Cent. Bldg. B1F :

Bio-material analysis room [staff-run/TPSS/ common use equipment (CUE)], Electron microscopy room, Cryopreservation room



CBS Cent. Bldg. 1F:

Bio-material analysis room [common use equipment (CUE)], Electron microscopy room, Office of the Center Director




RIKEN CBS
Center for Brain Science