Workshop Training Series

BORC Services Can Leverage Your Research

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Director of Biomedical and Obesity Research Core

Nebraska Center for the Prevention of Obesity Diseases through Dietary Molecules

Introduction

- The Biomedical and Obesity Research Core (BORC) is a one-stop shop core facility in the Nebraska Center for Prevention of Obesity Diseases (NPOD).
- BORC provides cutting-edge biomedical research services for investigators in the University of Nebraska system and also external users.
- BORC Provides training for researchers on services provided in the BORC.
- BORC supports research tool development programs that benefit research community at large and contributes to their research discovery and external funding.

Services Provided by BORC

BORC provides biomedical research services in three ways:

1. **BORC is equipped with a variety of instruments that can be utilized to perform a range of biomedical experiments from molecular biology to in vivo study.**
2. **BORC provides monthly workshops to users on instrument operation and scientific technique developments. BORC also invites manufacturers to train users for new equipment.**
3. **BORC can carry out biomedical assay for clients.**

Introduction

The BORC provides research services in two locations at Leverton Hall and Life Science Annex on the east campus of University of Nebraska-Lincoln. The services are managed online through iLab operation software. First-time users need to register an account on iLAB using their emails (https://my.ilabsolutions.com/service_center/show_external/3591).

https://cehs.unl.edu/borc/

How Does BORC Charge?

- BORC provided subsidies to NPOD and University of Nebraska System (NU) investigators. The NPOD directors and project leaders are offered 90% subsidy for service cost occurred in BORC, while pilot project and seed grant investigators are offered 80%, other NPOD members like mentors and previous project leaders are offered 50%. The rest of NU investigators get 20% subsidy.
- The external users pay full price and industry users pay 140%.
- BORC bills clients quarterly. The users receive bills in January, April, July and October.

Major Equipment at BORC

- Molecular and Cell Biology
- Metabolic Study
- Animal Behavior Research
- Small Animal Imaging

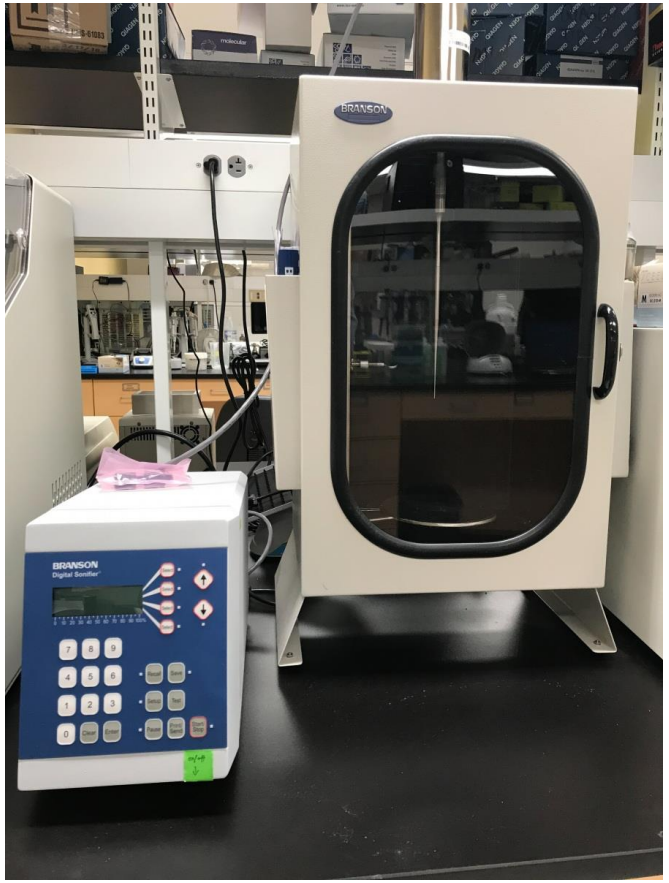
Molecular and Cell Biology

- DIGITAL Sonifier® UNITS Models S-450D
- FreeZone® 4.5 Liter Freeze Dry Systems
- Malvern NanoSight NS300
- BioTek Synergy™ H1m
- LICOR ODYSSEY® CLx
- Bio-Rad QX200 ddPCR system
- CFX Connect™ Real-Time PCR

Digital Sonifier® Unit Models S-450D

The Branson 450 is a ideal sniffier for:

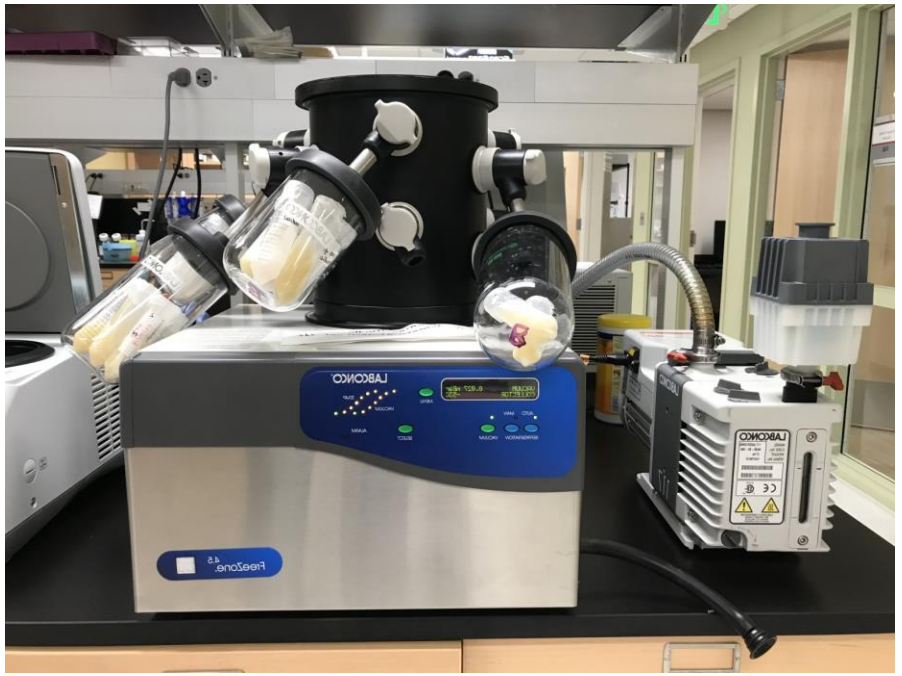
- Cell disruption and lysing
- Nano particles production
- Chip assay,
- Emulsification
- Homogenization
- Processing DNA and proteins



FreeZone® 4.5 Liter Freeze Dry Systems

Freeze drying is an important process in sample preparation and for the preservation and storage of biologicals, pharmaceuticals and foods.

- Milk
- Antibiotics
- Proteins,
- Plasma,
- cell lines
- Viruses
- Microorganisms.



NanoSight NS300 Nanoparticle Tracking Analysis System

Capable of visualizing and measuring particle size and distribution in suspension in the size range of 10-2000nm.

- **Protein aggregation studies**
- **Exosomes and microvesicles**
- **Pharmaceutical nanoparticles-liposomes**
- **Fluorescently labeled particles (not available)**

BioTek Synergy™ H1m

Synergy™ H1m is a flexible monochromator-based multi-mode microplate reader.

Measuring signals:

- UV-Vis absorbance
- Fluorescence intensity
- Luminescence
- Fluorescence polarization
- Time-resolved fluorescence

Application:

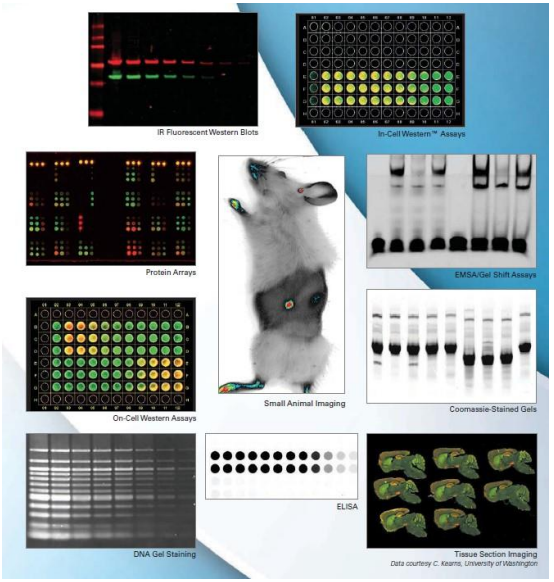
- DNA/RNA， protein quantification.
- ELISA
- Reporter gene expression (luciferase assay)
- Other biochemical or cell based assay using absorbance, fluorescence or luminescence as readout signals.

LICOR ODYSSEY® CLx

The Odyssey CLx is the next generation multifunctional imaging platform that can provide a wide range of applications.

Odyssey CLx Applications:

- Protein Detection: Coomassie-Stained Gels, Membrane and Slide Arrays
- Western blots: Two-Color Infrared and In-Gel
- Nucleic Acid Detection: Mobility Shift Assays,
- DNA Gel Staining (Syto®60), and Arrays
- Cell-Based Assays: In-Cell Western™ and On-Cell Western
- Microwell Assays: ELISA/FLISA, Protein Arrays, and rnai Analysis.
- Small Animal Imaging



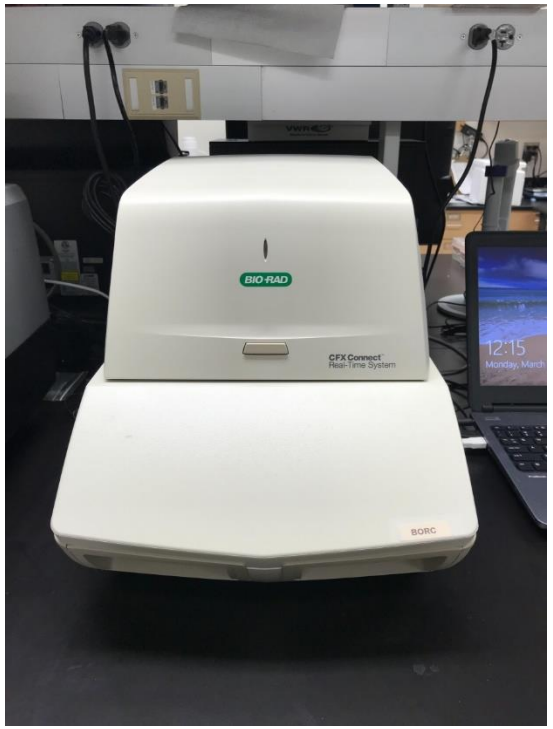
Droplet Digital PCR and Real-Time PCR

Bio-Rad QX200 ddPCR system

CFX Connect™ Real-Time PCR Detection System

CFX Connect™ Real-Time PCR

- **Easy startup** – obtain great results right away with factory calibration, quick setup, and intuitive software
- **Effortless optimization** – save time and reduce costs optimizing assays in a single run using the thermal gradient
- **Powerful data analysis tools** – quickly and accurately validate and analyze your data with the advance analysis modules of CFX Manager software
- **Accelerate publication submission** – include MIQE annotations and generate RDML files using Biogazelle's qbase PLUS software
- **Run high resolution melt (HRM) experiments** on the CFX Connect and perform data analysis using seamless data import to Precision Melt Analysis software



Benefits of Droplet Digital PCR

- **Absolute quantification — provide absolute quantification of nucleic acid target with wide-ranging applications for both research and clinical diagnostic applications.**
- **Unparalleled precision — the massive sample partitioning afforded by ddPCR enables small fold differences in target DNA sequence between samples to be reliably measured.**
- **Increased signal-to-noise — enrich for rare targets by reducing competition that comes from high-copy templates.**
- **Removal of PCR efficiency bias — error rates are reduced by removing the amplification efficiency reliance of PCR, enabling accurate quantification of targets.**
- **Simplified quantification — a standard curve is not required for absolute quantification.**

Metabolic Study

Metabolic cages (TSE Systems)

Treadmill

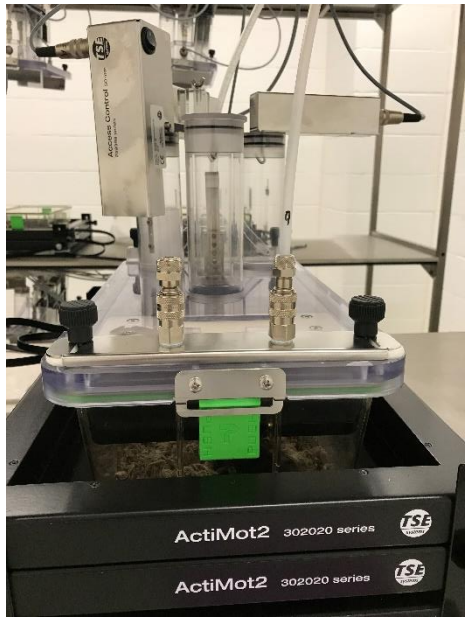
XFe-24 Extracellular Flux Analyzer

Vitros 250 Chemistry Analyzer

Agilent GC-MSD

TSE PhenoMaster Metabolic Cages

A multi-modular platform that allows researchers to carry out metabolic, behavioral and physiological analysis of mice in an automated and synchronized manner. Currently our system has 12 cages with modules to measure metabolic performance, activity, as well as feeding and drinking behavior.



- **Calorimetry:**
- **Activity**
- **Drinking and feeding behavior**

Parameters affecting energy expenditure

Parameter	Description	Unit	Remarks
Flow	Flow	l/min	
Temp	Temperature	°C	Measurement in the box.
O2	Concentration	%	Reference and per box.
CO2	Concentration	%	Reference and per box.
dO2	Difference	%	Reference O2 - Box O2.
dCO2	Difference	%	Reference CO2 - Box CO2.
VO2	O2 consumption	ml/(kg x h) or ml/h	
VCO2	CO2 production	ml/(kg x h) or ml/h	
RER	Respiratory Exchange Rate		VCO2/VO2
H	Heat	kcal/(kg*h) or Kcal/h	Also possible in W/kg

Calorimetry Results Parameter

Parameter	Description
XT, YT	Breaks X-beam total (is equivalent to XA + XF) Breaks Y-beam total (is equivalent to YA + YF)
XF, YF	Breaks X-beam, fine movements Breaks Y-beam, fine movements
XA, YA	Breaks X-beam, ambulatory movements Breaks Y-beam, ambulatory movements
Z	Breaks Z-beam, rearing
Z2	Breaks Z2-beam, rearing
CenT, PerT	Sum central and peripheral ambulatory and fine movement
CenA, CenF	Central ambulatory and fine movement
PerA, PerF	Peripheral ambulatory and fine movement

RER is A Marker of Energy Source

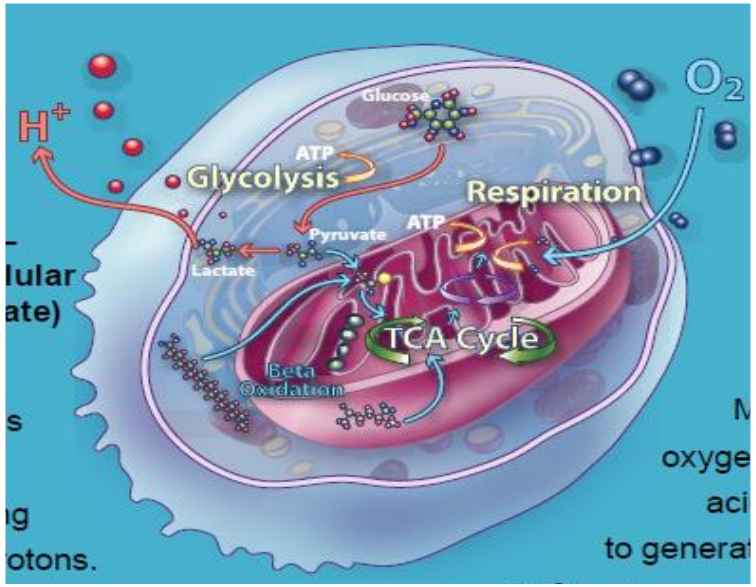
- RQ = 1 for pure carbohydrates
- RQ = 0.7 for pure lipids

% Dietary Macronutrients Carbohydrates / Lipids	RQ (RER) mol CO_2 / mol O_2
100 / 0	1.00
80 / 20	0.88
60 / 40	0.80
40 / 60	0.76
20 / 80	0.73
0 / 100	0.71

Treadmill

- The treadmill is a simple tool to evaluate physical health, cognitive and mental health of mice.
- The apparatus can also be used to investigate physical exhaustion that can often be a symptom of diseases and disorders.
- The treadmill can be used to assess post-recovery of motor and locomotion function from injuries.

Seahorse XFe Extracellular Flux Analyzer



MITOCHONDRIAL RESPIRATION- Mitochondria consume oxygen when oxidizing fatty acids or other substrates to generate ATP. The Seahorse XFe Analyzer measures mitochondrial respiration by measuring the **oxygen consumption rate** (OCR) of cells.

GLYCOLYSIS-ECAR-Cells generate ATP via glycolysis independent of oxygen, producing lactic acid and protons. The Seahorse XFe Analyzer measures glycolysis by measuring the **extracellular acidification rate (ECAR)** of cells.

The application of Seahorse XFe24

Assay

- Mitochondrial stress test
- Glycolysis stress test
- Fatty acid oxidation assay
- Cell energy phenotype test

Application

- Obesity/Diabetes
- Cancer Biology
- Immunology
- Neurodegeneration
- Other pathways involve changes of glycolysis and mitochondria respiration

Vitros 250 Dry Chemistry Analyzer

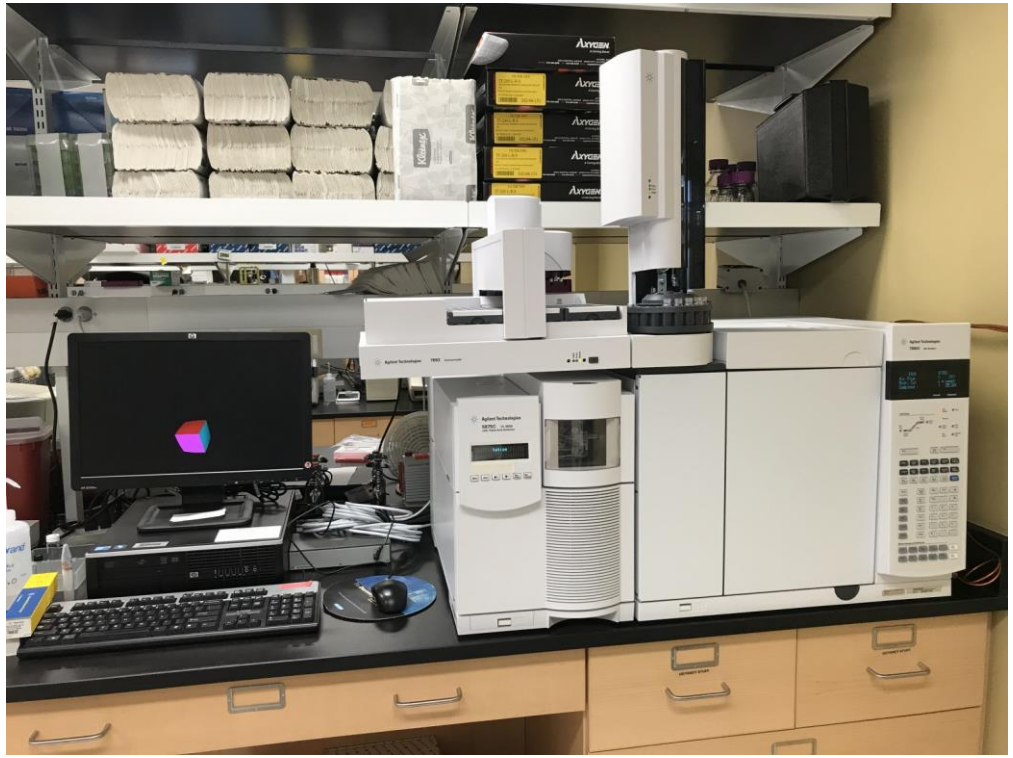
The Ortho Clinical Vitros 250 Chemistry System is chemical analyzer for Serum, Plasma, Urine, Cerebrospinal fluid, and cell culture medium (no color)

- **The VITROS 250 uses dry slides that have multilayered analytical elements coated on polyester supports.**
- **The analyte in the sample catalyzes the reaction sequence to yield products which absorb light at wavelengths in various regions (340 – 680nm).**
- **Depending on the analytes, the test types can be colorimetric, enzymatic end point, two-point or multi-point rate, or potentiometric.**

Chemistry Panels

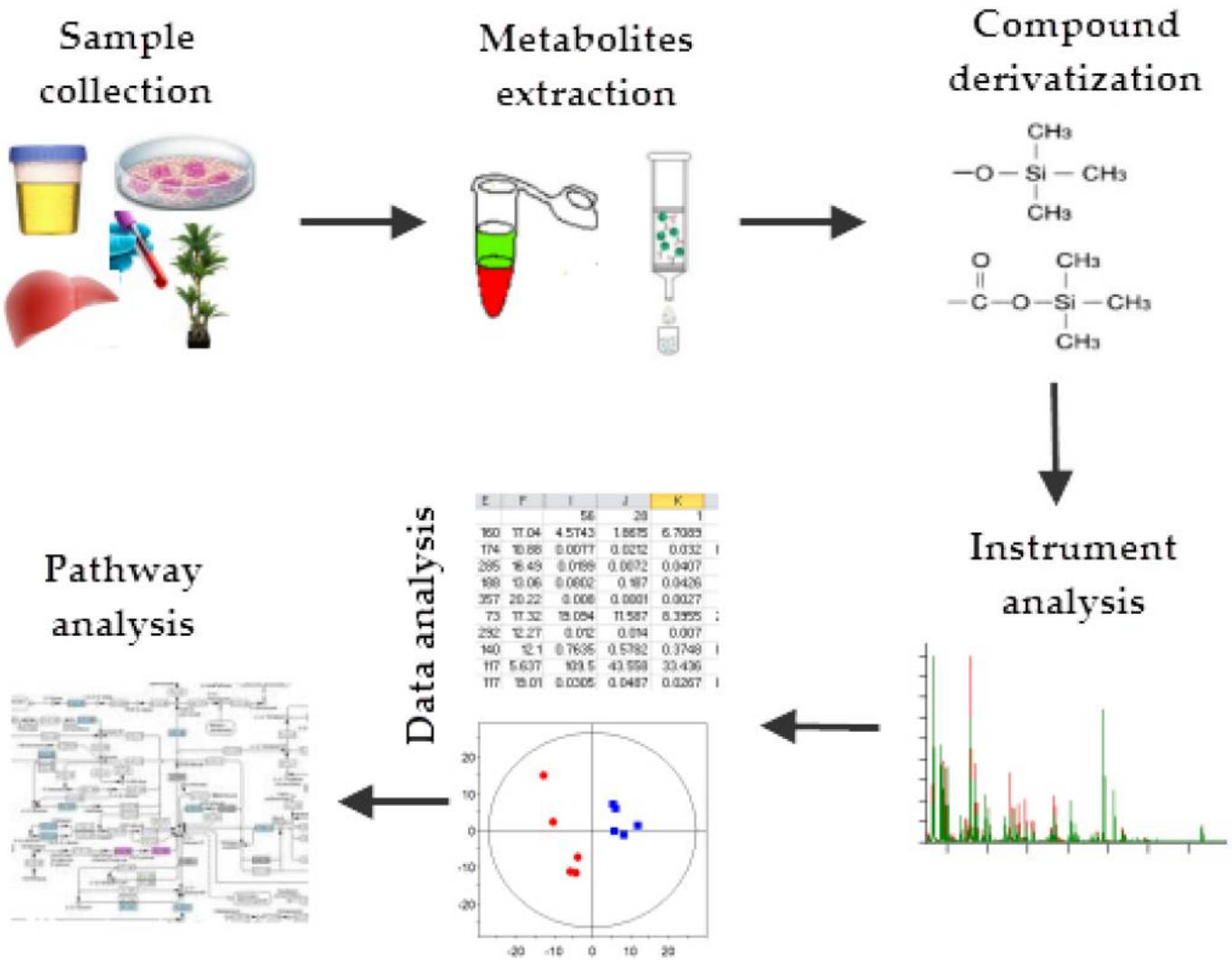
Basic Metabolic Panel	Comprehensive Metabolic Panel	Lipid Panel
Glucose	Glucose	Triglycerides
Calcium	Total Protein	Total Cholesterol
BUN	Albumin	HDL Cholesterol
Creatinine	Globulin	CHOL/HDLC
BUN/Creatinine ratio	A/G Ratio	LDL
Sodium	Total Bilirubin	VLDL
Potassium	ALT (SGOT)	
Chloride	AST (SGPT)	
Bicarbonate (ECO_2)	Alkaline Phosphatase	Hepatic Function Panel
	Calcium	
Electrolyte Panel	BUN	
	Creatinine	Albumin
	BUN/Creatinine	Total Bilirubin
Sodium	Sodium	Direct Bilirubin
Potassium	Potassium	Alkaline Phosphatase
Chloride	Chloride	AST (SGPT)
Bicarbonate (ECO_2)	Bicarbonate (ECO_2)	ALT (SGPT)

Agilent GC/MS



- Gas chromatography–mass spectrometry (GC-MS) has many advantages to analyze small and volatile molecules such as steroids, fatty acids, and hormones.
- It can separate complex samples, quantify analytes, and determine trace levels of organic contamination.
- The technique can be applied towards the study of liquid, gaseous and solid samples.
- GC-MS becomes one of the key technologies for metabolite profiling and increasingly contributes to our understanding of the metabolic pathway.

The procedure for GC-MS-based metabolomics

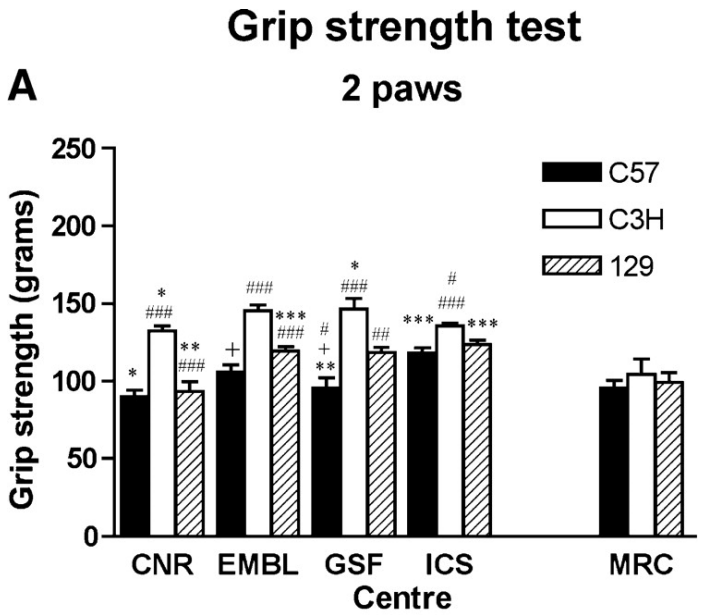


Animal Behavior Research

- Grip Strength System
- ROTOR-ROD™ System
- SR-LABTM Startle Response System
- Place Conditioning Preference
- Barnes Maze
- Morris Water Maze
- The Radial Arm Maze

Animal Grip Strength System

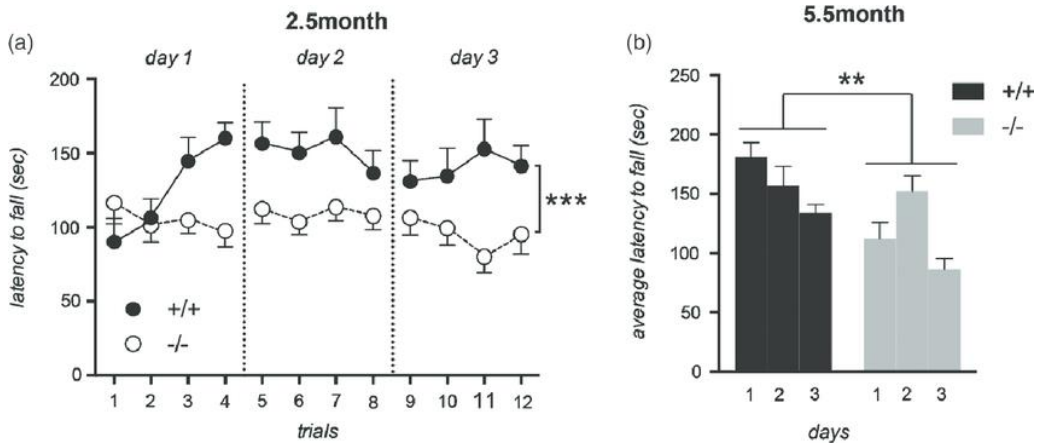
The Grip Strength test is used to evaluate motor function and deficit in rodent models of CNS disorders.



A cross-laboratory study of grip strength test.
Physiological Genomics Published Vol. 34 no. 3, 243-255

ROTOR-ROD™ System

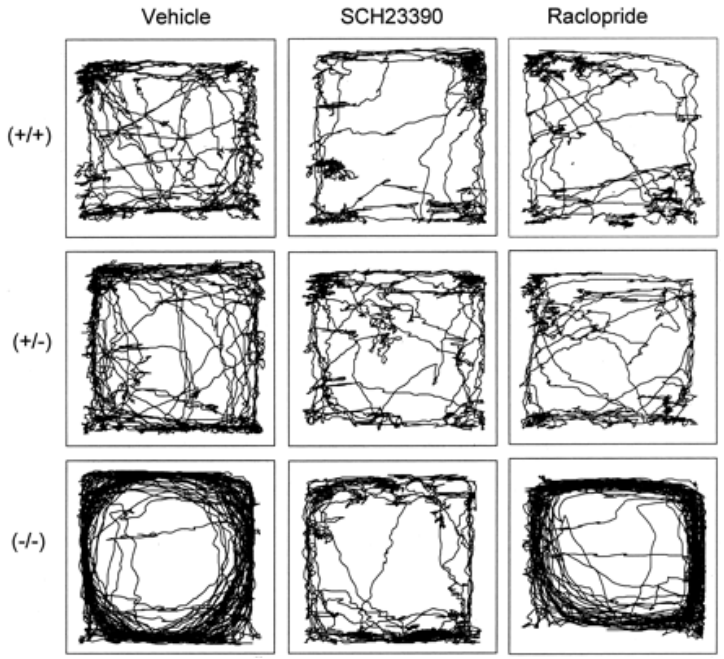
The ROTOR-ROD™ System measures motor function, motor learning, coordination, and equilibrium in both rats and mice.

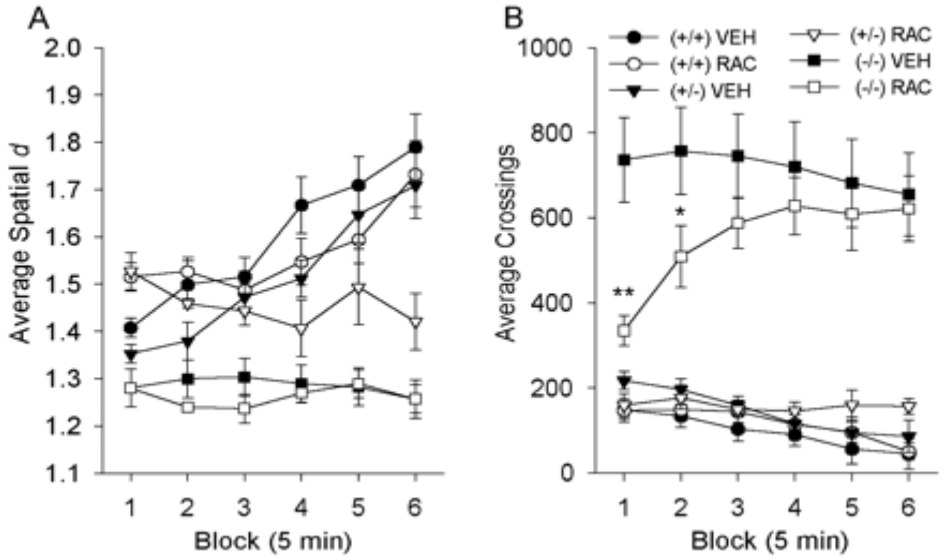


CDKL5 mutated animals fell down earlier compared with the respective WT
Genes Brain and Behavior 15(5) · March 2016

The SR-LAB Startle Response System

The world's most widely used system for startle reflex measurement and by far the most successful for fear potentiated startle and pre-pulse inhibition testing.

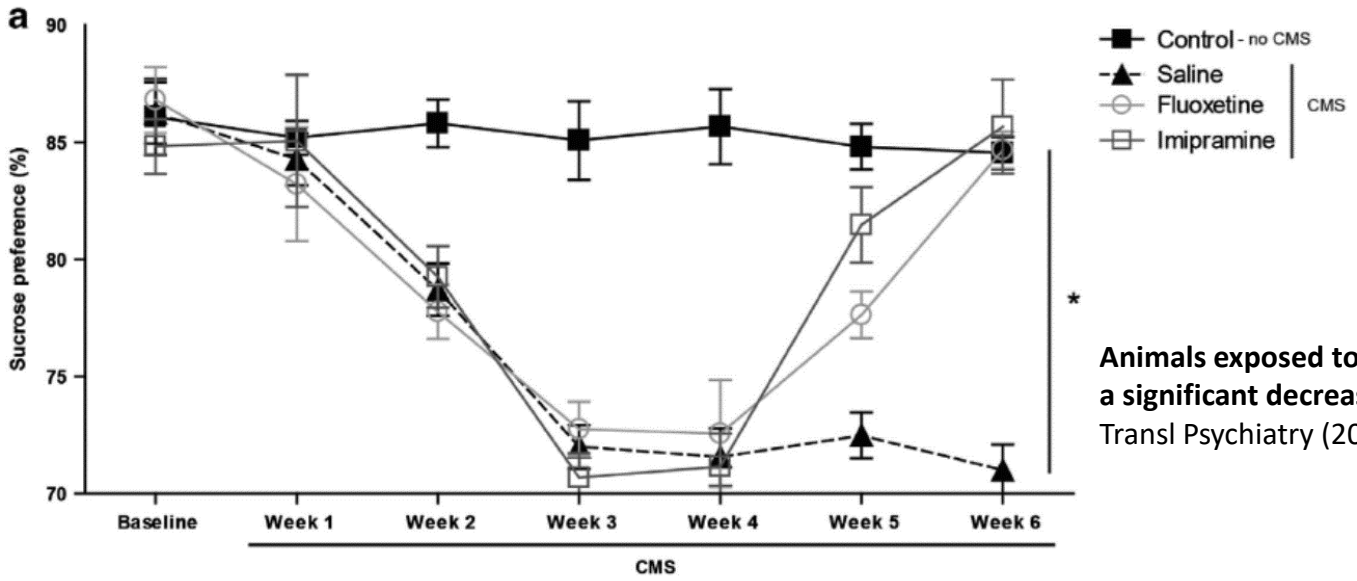




Prepulse Inhibition Deficits and Perseverative Motor Patterns in Dopamine Transporter Knock-Out Mice: Differential Effects of D1 and D2 Receptor Antagonists. Journal of Neuroscience 1 January 2001, 21 (1) 305-313

Place Preference Enclosure Equipment

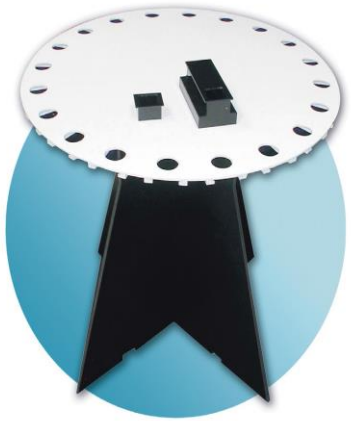
Place conditioning is most often used with rodents (rats, mice) to study the positive (rewarding) or negative (aversive) motivational effects of objects (e.g., food pellets, novel toys) or experiences (e.g., brain stimulation, drug intoxication, drug withdrawal, footshock, illness, wheel running and copulation).

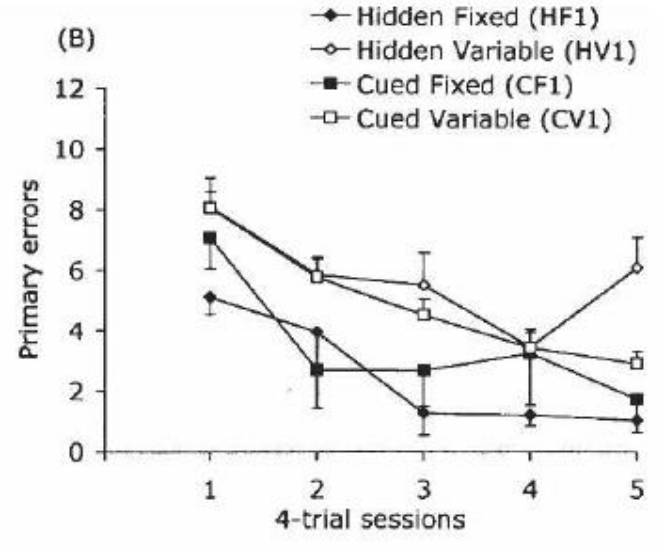


Animals exposed to chronical mild stress revealed a significant decrease in sucrose preference.
Transl Psychiatry (2013) 3, e266

Equipment for spatial learning and memory -Barnes Maze

The Barnes maze is a spatial memory task that requires subjects to learn the position of a hole that can be used to escape the brightly lit, open surface of the maze.



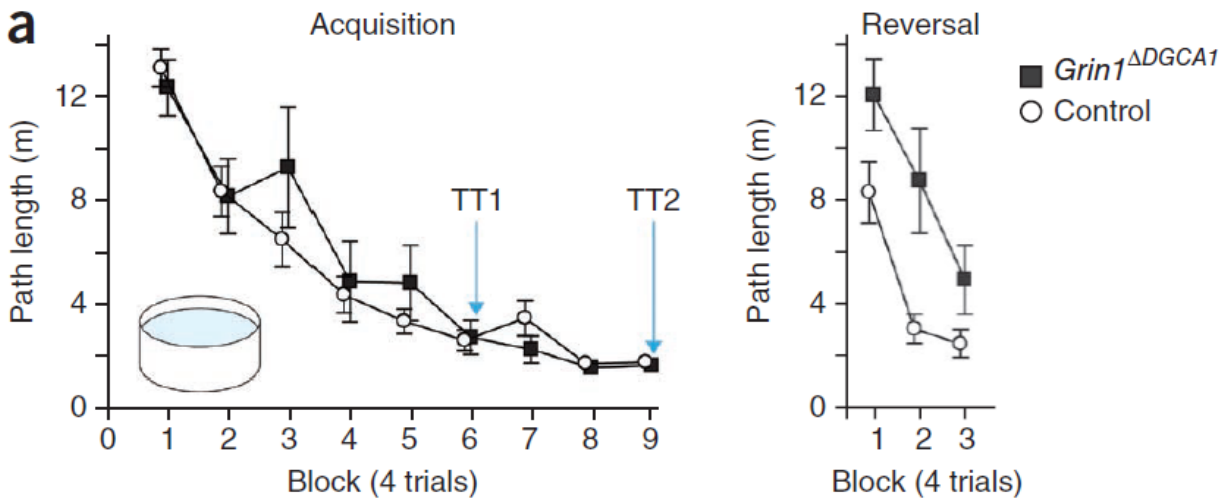


Mice trained with a fixed target location (HF1 and CF1 groups) made significantly fewer primary errors.
Cold Spring Harbor Laboratory Press ISSN 1072-0502/06; www.learnmem.org

Equipment for spatial learning and memory -Morris Water Maze

The Morris water maze (MWM) is a test of spatial learning for rodents that relies on distal cues to navigate from start locations around the perimeter of an open swimming arena to locate a submerged escape platform. Spatial learning is assessed across repeated trials and reference memory is determined by preference for the platform area when the platform is absent. Reversal and shift trials enhance the detection of spatial impairments.

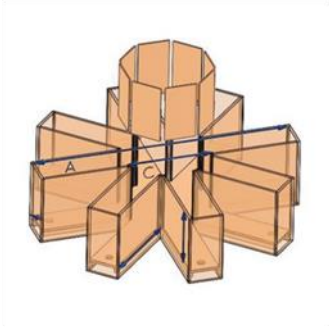
Morris water maze

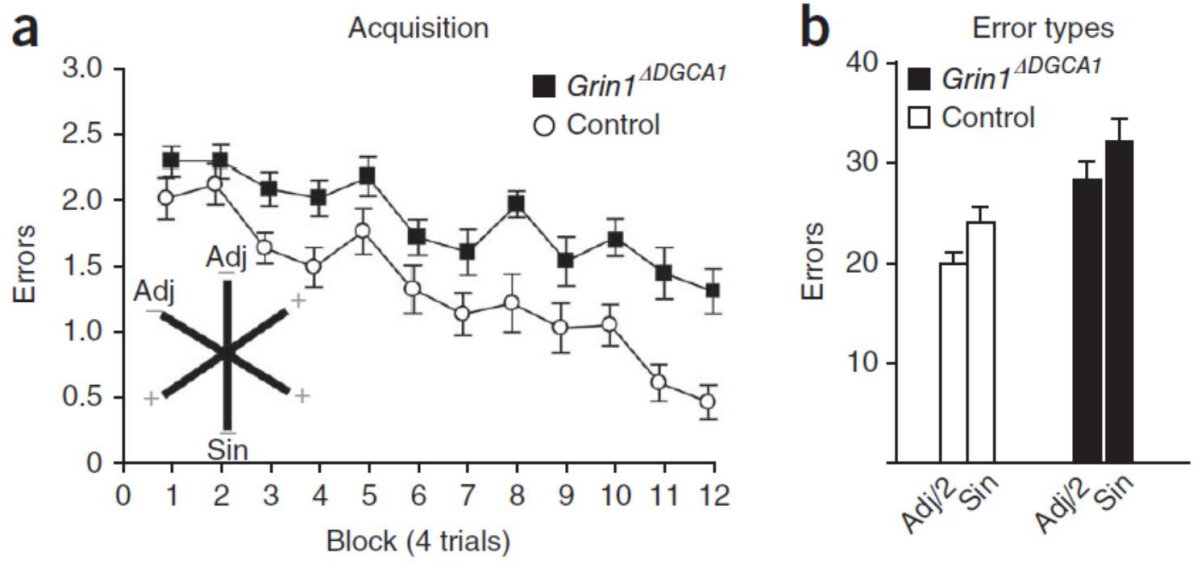


The path lengths of Grin1ΔDGCA1 mice were significantly longer than those of controls
Nature Neuroscienen volume 15, number 8

Equipment for spatial learning and memory -Radial Arm Maze

The system provides an evaluation of locomotor activity patterns. Experimentally induced changes in locomotor activity may show as an increase or reduced level of activity or an uneven distribution of locomotion, i.e. restriction to specific sectors of the maze or preference for distinct arms.





Grin1ΔDGCA1 mice were impaired in learning a radial maze task
Nature Neuroscienen volume 15, number 8

Small Animal Imaging System

- iBox® Scientia™ Small Animal Imaging System
- Pearl® Impulse Small Animal Imaging System
- LI-COR Odyssey® CLx
- UltraFocus DXA

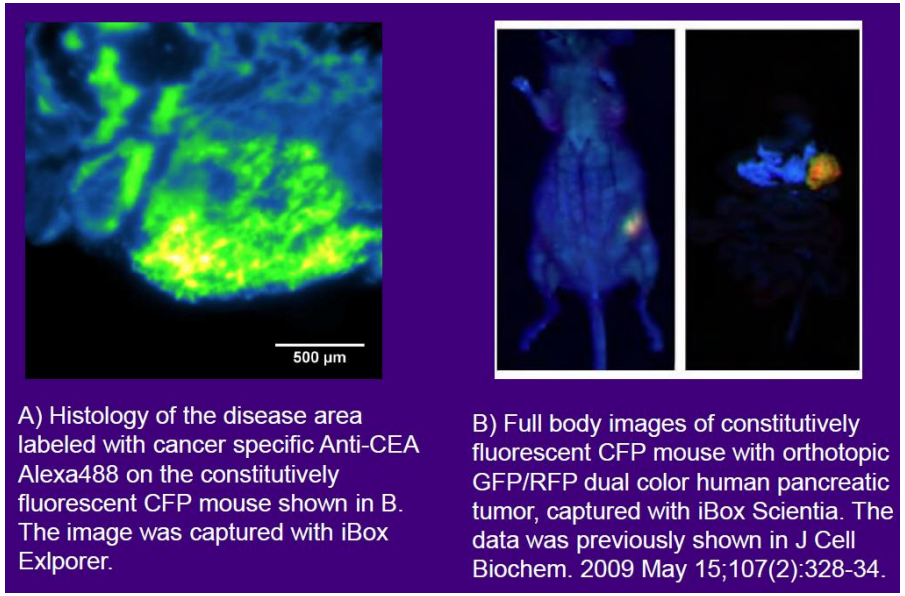
Small Animal Imaging System

-iBox Scientia

Non-invasive, visible and NIR in vivo imaging for detection of fluorophores/fluorescent protein markers in small animals

Application

- Tumor studies
- Cancer research
- Heart disease
- Gene therapy
- Other research using fluorescent protein



A) Histology of the disease area labeled with cancer specific Anti-CEA Alexa488 on the constitutively fluorescent CFP mouse shown in B. The image was captured with iBox Exlporer.

B) Full body images of constitutively fluorescent CFP mouse with orthotopic GFP/RFP dual color human pancreatic tumor, captured with iBox Scientia. The data was previously shown in J Cell Biochem. 2009 May 15;107(2):328-34.

Small Animal Imaging System

-iBox Scientia

- The Pearl Impulse Animal Imaging System is a small animal in vivo imaging system that offers dual-channel near-infrared fluorescent detection.

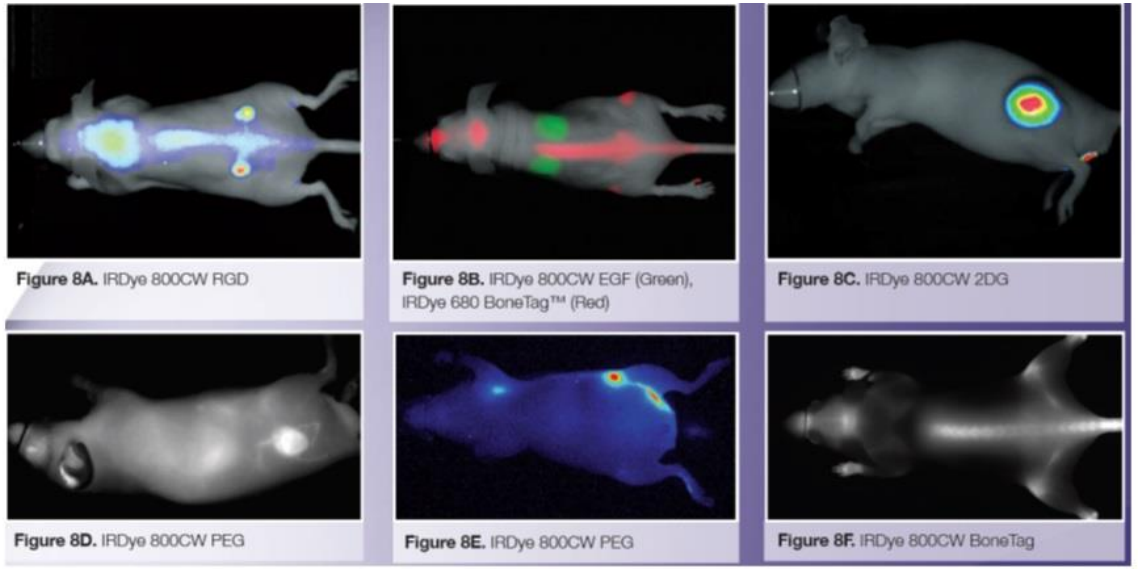


Figure 8A. IRDye 800CW RGD

Figure 8B. IRDye 800CW EGF (Green), IRDye 680 BoneTag™ (Red)

Figure 8C. IRDye 800CW 2DG

Figure 8D. IRDye 800CW PEG

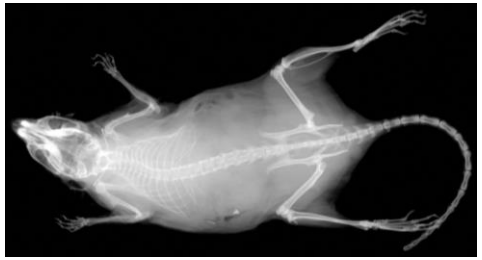
Figure 8E. IRDye 800CW PEG

Figure 8F. IRDye 800CW BoneTag

Small Animal Imaging System

-UltraFocus DXA

- Fully shielded x-ray cabinet designed for ultra high-resolution imaging and dual-energy x-ray absorptiometry (DXA) analysis .
- Pre-clinical imaging and body composition data



Services Provided at BORC

- Cell Based Assays and Biochemical Assays
- Chemistry Panel Analysis
- DNA & RNA extraction
- Gene Cloning and Subcloning
- Genotyping
- Real Time PCR and Digital Droplet PCR

Workshop List

- Sept BORC service can leverage your research.
- Oct Metabolic study services provided by BORC.
- Nov. Metabolic cages: What data can we get and how to explain them.
- Dec. Metabolomics research using GC-MS
- Jan. Molecular and Biochemical research services at BORC.
- Feb. Gene cloning methods and services at BORC.
- Mar. Real time PCR and ddPCR.
- Apr Introduction to animal cell culture.
- May CRISPR: Theory and technique.
- Jun Animal behavior services at BORC.
- Jul Nanosight 300 and its application in biomedical research.
- Aug The application of LiCOR CLx and iBox® Scientia™.

Research Tool Development Program

The BORC will support two Research Tool Development annually. The eligible applicants may be either NPOD internal investigators or other UNL investigators. The proposed projects are expected to benefit research for multiple labs. Applications are accepted on a rolling basis. The applications must include the following information in three pages (maximum, word or PDF format)

- **Application Title**
- **PI Contact Information**
- **Rationale and Objectives of the Proposed Project**
- **Experimental Plan**
- **A justification for Funding Request**

Core Facility Grant Program for New Users

This grant program is aimed at encouraging new users to employ NU core facilities in their research. The program is open to all faculty from any of the NU campuses. Awards are intended to be used to gather new or additional data for strengthening a grant proposal (new or resubmission) or for addressing reviewer concerns associated with a peer-reviewed manuscript.
For details, see
https://research.unl.edu/core-facility-grant-program-for-new-users/.

Rules and Precautions

- Reserve before you use the services. Provide the cost object number when reserve the services.
- Don’t let anybody else enter BORC lab without permission.
- Don’t take anything belonging to BORC out of BORC lab without permission.
- First-time users must get trained before using any instruments.
- Observe the protocol strictly when operate the equipment
- Inform BORC staff right away when you find the equipment doesn’t work properly.

Thank you