International Mouse Phenotyping Consortium Launch Meeting

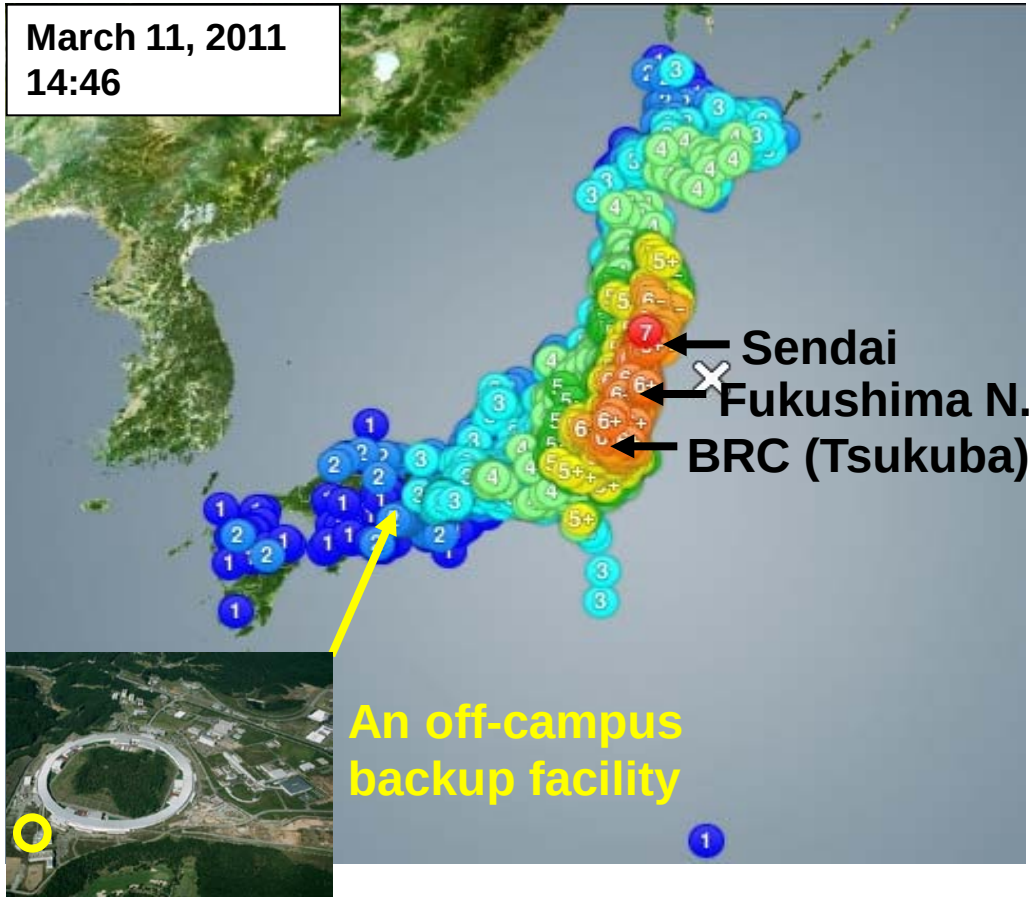
September 29, 2011
Washington DC, USA

RIKEN BioResource Center
Yuichi OBATA, Shigeharu WAKANA,
Atsushi YOSHIKI, Hiroshi MASUYA

Japan and RIKEN BRC Thank You for All Your Thoughts and Supports to Us after the Great Eastern Japan Disaster

March 11, 2011

14:46 Lower 6 (Magnitude 9) Coast of Sanriku (Miyagi, Sendai)
Multiple earthquakes
15:15 Lower 6 (Magnitude 7.4) Coast of Ibaraki (Tsukuba)
(Japanese earthquake scale; Level1: weak to Level 7: highest)



At RIKEN BRC

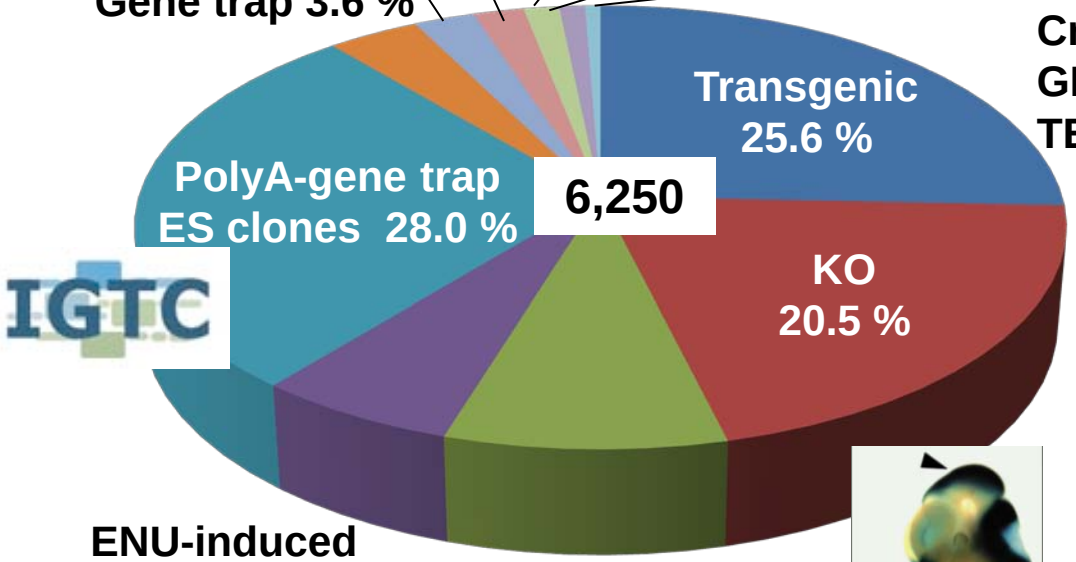
- No human casualties
- No losses or damages of resources
- Minimum damages to facilities and equipment: $120,000 to repair

Lifelines to preserve bioresources

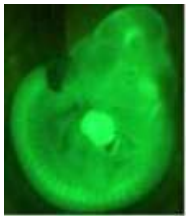
- Water supply: stopped for 4 days
- Electricity: only a flash blackout, but lacked fuel for emergency generators to last for two weeks
- Liquid nitrogen supply: stopped for 7 days
- An off-campus backup facility with N2 tanks

Mouse Resources at RIKEN BRC

Depositor	No. of Strains
Domestic academic	5,702
Domestic profit	285
Overseas academic	263
	6,250



Cre-driver (150)
GFP reporter (400)
TET mice (63)



Null KO
Conditional KO
Knockin
Knockin reporter

RIKEN BRC has collected strains mainly developed in Japan

Distribution of Mouse Resources
http://www.brc.riken.jp/lab/animal/en/

Distribution Items:

- Live mice
- Frozen embryos/ sperm
- Frozen strains/ES cells of FIMRe
- PolyA Gene Trap ES cells
- B6/N & MSM BAC clones
- Organs and tissues
- Genomic DNA

Cumulative no. since FY2001

Strains	No. of distribution (overseas)
Transgenic	7,703 (1,741)
KO	6,091 (2,526)
Inbred and mutant	3,330 (79)
Wild-derived	432 (26)
ENU-induced mutant	124 (31)
Total	17,680 (4,403)

International Distribution

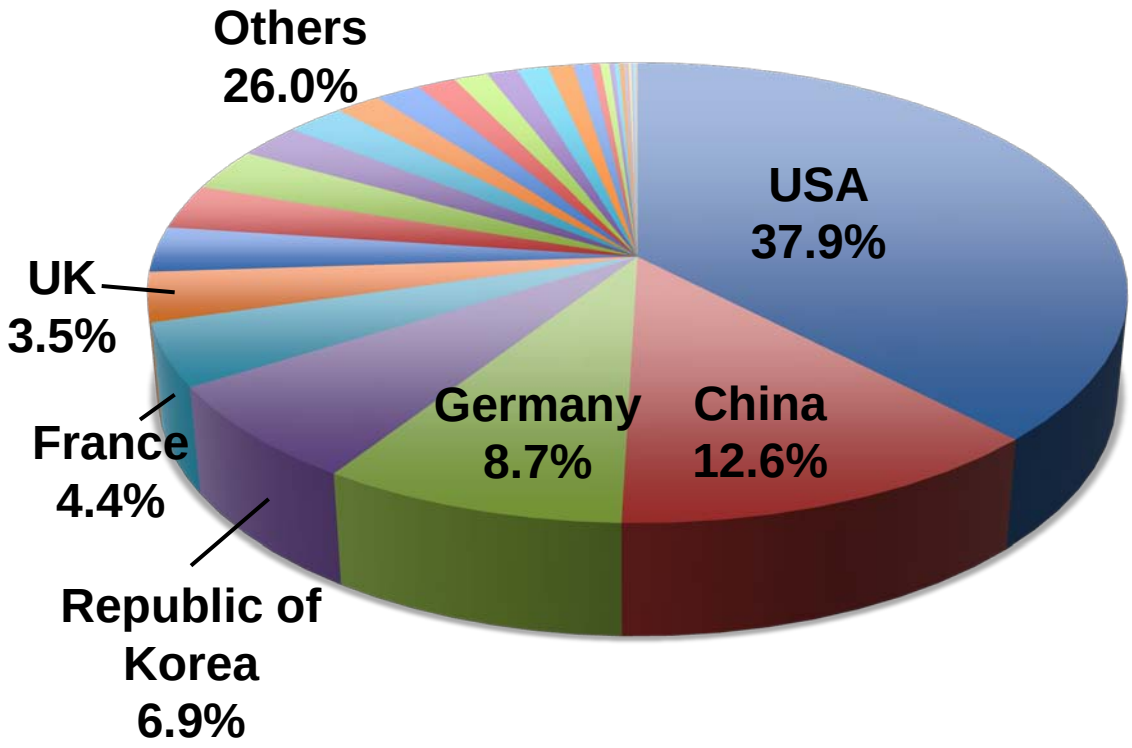
As of Sep 22, 2011

We have distributed mice to 478 organizations in 32 countries

Cumulative data since 2001

International Distribution

Organization	2011
Domestic Academic	50.2%
Domestic Profit	10.3%
Overseas Academic	38.7%
Overseas Profit	0.8%



<Others>

Belgium	Canada	Singapore	Italy
Australia	Spain	Switzerland	Netherlands
Taiwan	Austria	Sweden	Portugal
Israel	Denmark	Finland	Czech Republic
Thailand	Greece	South Africa	Philippines
Ireland	Croatia	Mexico	Brazil
Russia	Malaysia		

Cre-driver mice at RIKEN BRC

- Useful Cre mice collected from Japanese Scientists
- New Cre mice of C57BL/6N background generated by the BRC program
- 128 Cre and 4 Flp mice available

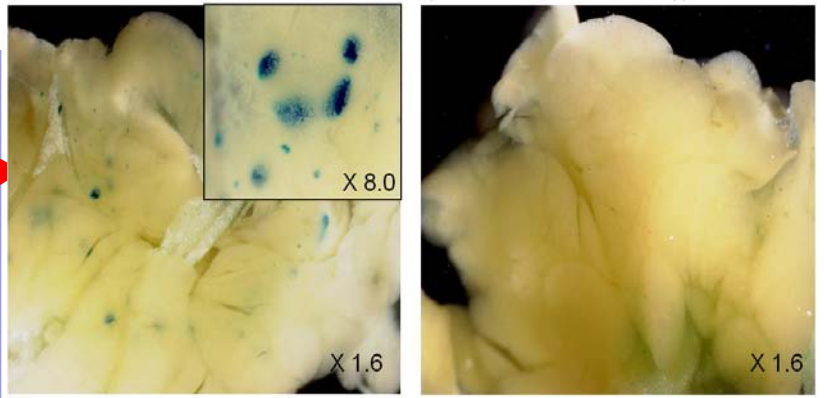
Search for Cre/Flp mice in BRC Web Catalog

RBRC04738	Aug 31, 2011	Transgene	B6.Cg-Tg(Lck-cre)1Jtak	Cre (Phage P1 Cre recombinase)	Cre/loxP system
RBRC03934	Aug 31, 2011	Transgene	C57BL/6N-Tg(Insl-cre)25Utr/Rbrc		
RBRC03970	Aug 31, 2011	Transgene	C57BL/6N-Tg(Krt14-cre)9Utr/Rbrc		
RBRC03702	Aug 31, 2011	Transgene	C57BL/6N-Tg(Nes-cre)1Utr/Rbrc	cre (Phage P1 Cre recombinase)	
RBRC03919	Aug 31, 2011	Transgene	C57BL/6N-Tg(Tagln-cre)707-1Utr/Rbrc		Cre/loxP system, FLP/frt system
RBRC03747	Aug 31, 2011	Transgene	C57BL/6N-Tg(Tek-cre)288-3Utr/Rbrc	cre (Phage P1 Cre recombinase)	
RBRC03809	Aug 31, 2011	Transgene	C57BL/6N-Tg(Vil1-cre/Esr1)2Utr/Rbrc	Esr1Esr1, Cre (Phage P1 Cre recombinase)	
RBRC03964	Aug 31, 2011	Transgene	C57BL/6N-Tg(Wap-cre)18Utr/Rbrc		
RBRC01345	Aug 31, 2011	Targeted Mutation Congenic	Emx1-Cre KI Δneo	Emx1Emx1, frt (yeast FRT (flippase recombination target) sites), nls (Simian virus 40 Large T antigen nuclear localization signal), cre (Phage P1 Cre recombinase)	Cre/loxP system

B6/N-Tg(Ins1-cre)

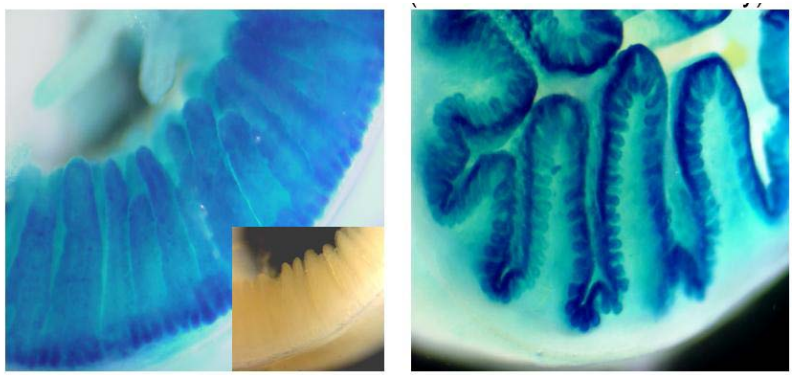
B6/N-Tg(Vil1-cre)

Specificity: Pancreas islet



Genotype: Cre/+, lacZ/+

Specificity: gut epithelium



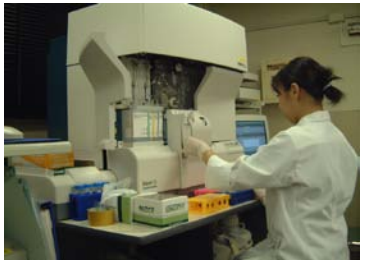
Small Intestine Large intestine

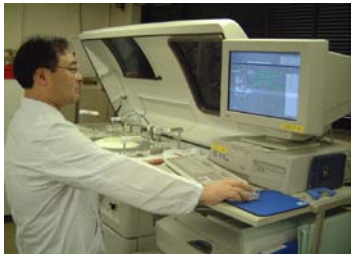
Genotype: Cre/+, lacZ/+, inset: control

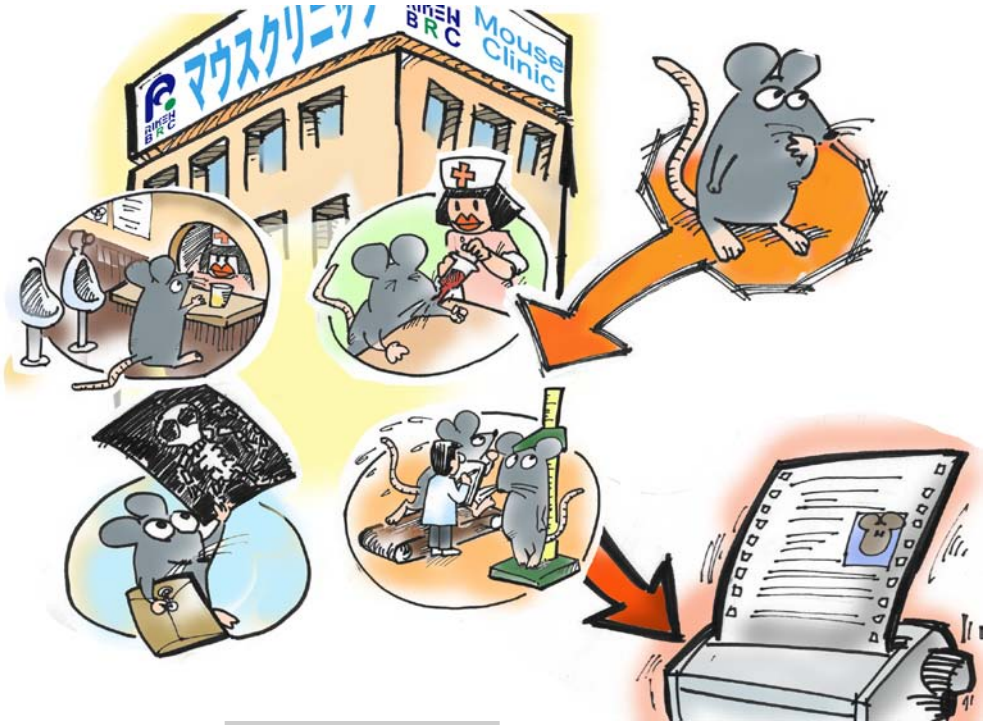
Japan Mouse Clinic

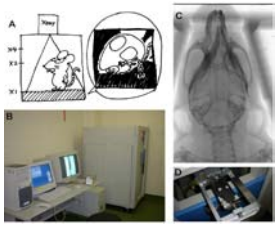
http://mouseclinic.brc.riken.jp/en

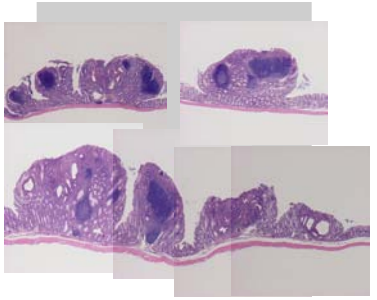
RIKEN BioResource Center

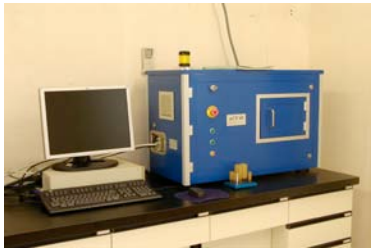


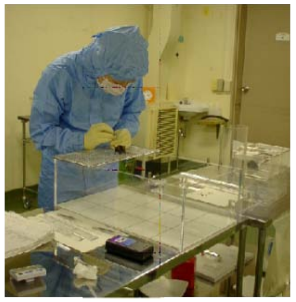


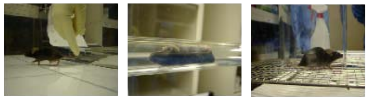


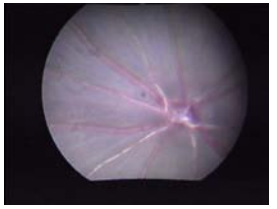


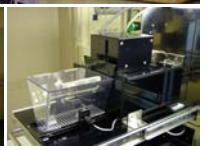












JMC: Started as Phenotyping of ENU Mutants

Mouse Resources in Japan and Abroad

Experimental Animal Division

Deposited Mutants in RIKEN BRC

Fundamental Phenotyping Pipeline (Broad Phenotyping)

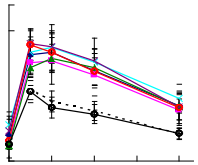
Dysmorphology | Haematology | Clinical biochemistry | Behavioural | Pathology

Detail Phenotyping Pipelines

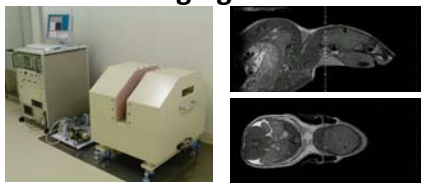
Sensory

Cardiovascular

Metabolic



Imaging



annotation

Disease model animals with standardized phenotype data

Pipeline 1 in JMC: Fundamental Screen

	No. of test	Screens	Methods	Age (weeks) 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
Fundamental screen	P1-01	Behavior	Open-field test	
	P1-02	Morphology/Behavioral /Sensory	Modified-SHIRPA	
	P1-03	Hematology/Clinical Chemistry	Hematological test	
	P1-04		Urinalysis	
	P1-05		Clinical biochemical test	
	P1-06	Pathology	Autopsy, Histology	
In depth screen	P1-07	Behavior	Rota-rod test	
	P1-08	Sensory	ABR (Auditory brainstem response)	
	P1-09	Metabolism	ITT (Insulin tolerance test)	
	P1-10		OGTT (Oral glucose tolerance test)	
	P1-11	Muscular	Lactate measurements in blood	
	P1-12	Metabolism	Adipocytokine and clinical biochemical test	
	P1-13	Sensory	Funduscopy	
	P1-14		ERG (Electroretinography)	
	P1-15	Cardiovascular	Blood pressure	
	P1-16	Metabolism	Body fat percentage and Bone Mineral Density (DEXA)	
	P1-17	Cardiovascular	Echocardiography	

Pipeline 2 in JMC: Behavioral Screen

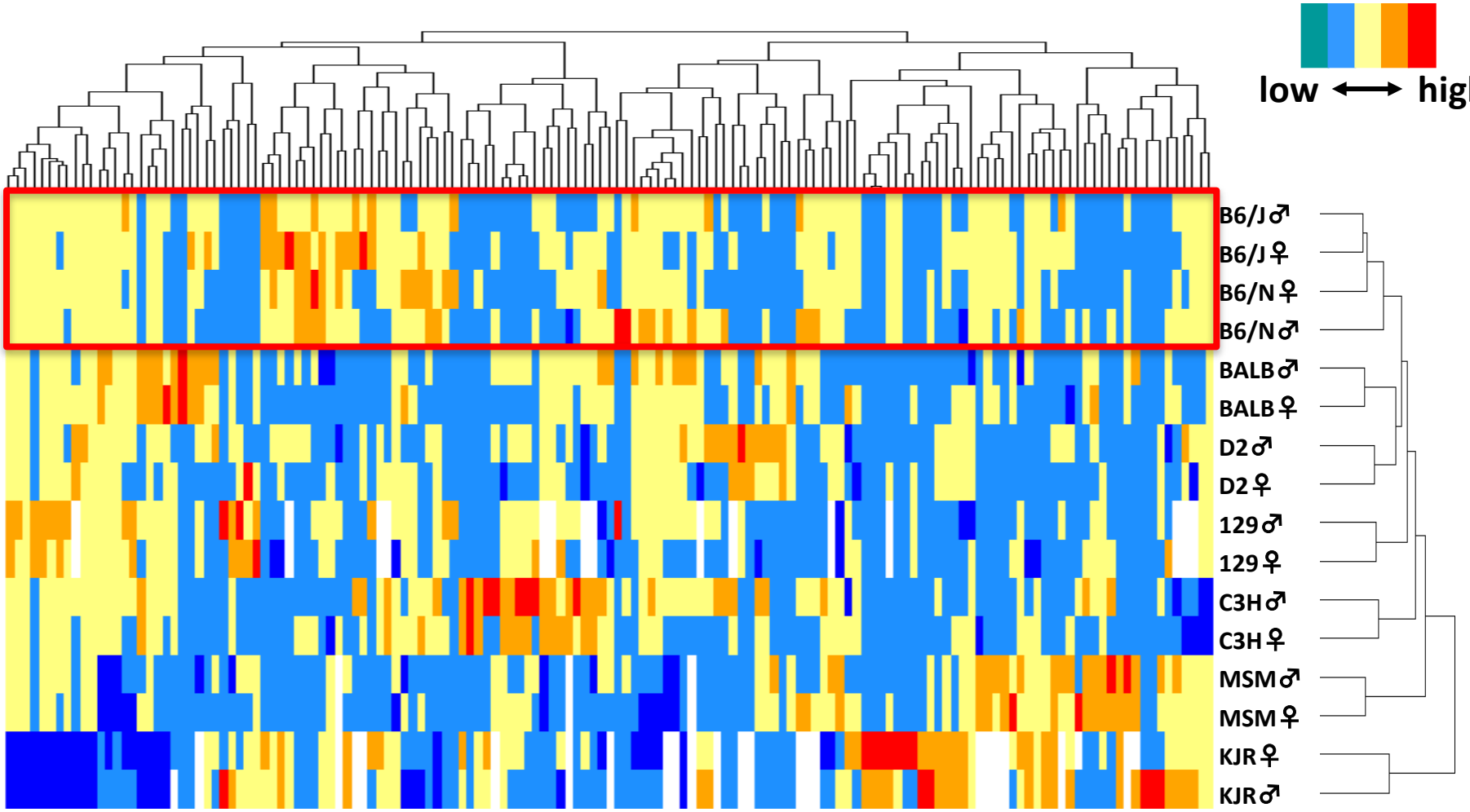
	No. of test	Screens	Methods	Age (weeks) 7	8	9	10	11	12	13	14	15	16	17
Behavior oriented screen A	P2-01	Behavior/ Neurology/ Psychiatry	Light/dark transition		■									
	P2-02		Open-field test			■	■							
	P2-03		Rota-rod test*1			■	■	■						
	P2-04		Home-cage activity test				■	■						
	P2-05		Passive avoidance test						■					
	P2-06		Tail suspension test								■			
	P2-07		Hot-plate test									■		
	P2-08		Tail-flick test											■

	No. of test	area	Methods	Age (weeks) 7	8	9	10	11	12
Behavior oriented screen B*2	P2-09	Behavior/ Neurology/ Psychiatry	Prepulse inhibition test				■		
	P2-10		Object exploration test					■	
	P2-11		Fear conditioning test						■






Heat map of the Parameters in JMC Phenotype Pipeline
- 8 inbred strains -

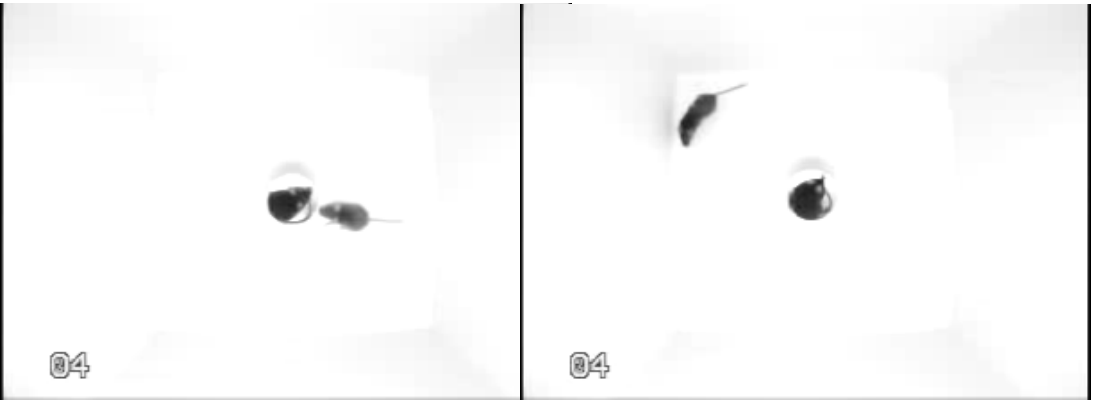


Attention Deficit Hyperactivity Disorder (ADHD) Model Mouse

BRC No. RBRC-GSC0036_Strain Name : M100174

Mutated gene: *Grin1*, Chr 2

Allele Symbol: *Grin1*^{Rgsc174}

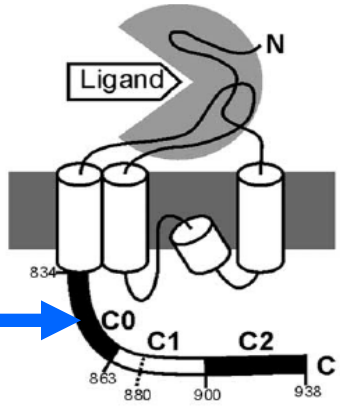


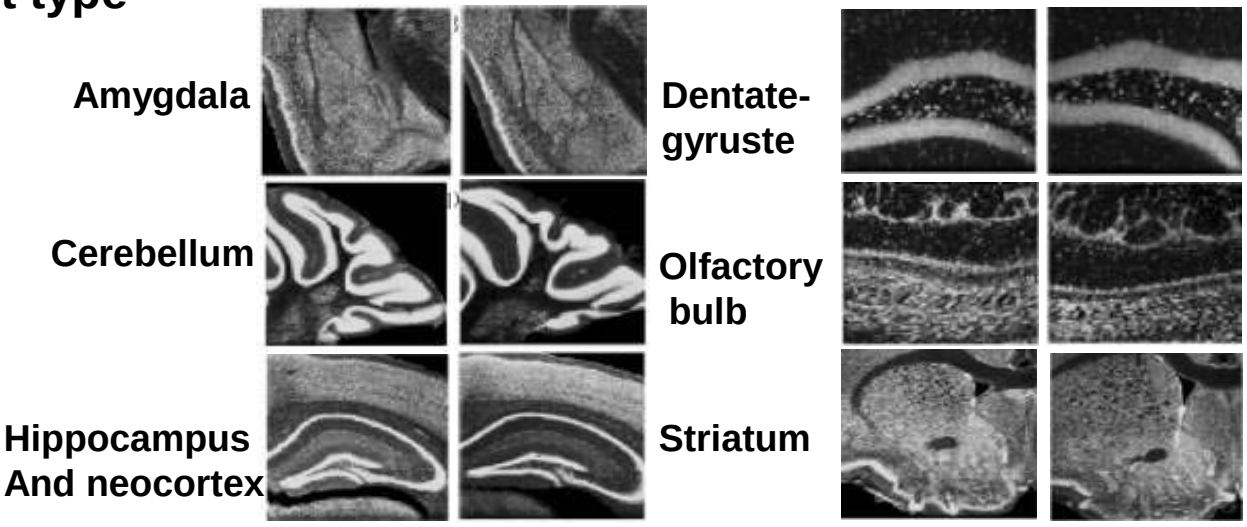
Wild type **Mutant type**

- Hyperactivity, shorter attention span to novel object.
- Suppression of hyperactivity by methylphenidate.
- No obvious abnormality in brain tissues.

Ser to Arg amino acid substitution (844th resic in intracellular C0 domain of GRIN1 proteir

Location of M-174 mutation (*Grin1*^{Rgsc174})





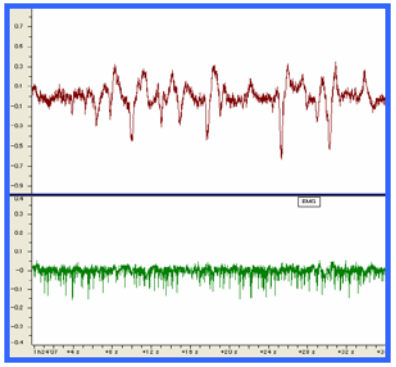
Furuse *et al.*, 2011

Glucose Transporter 1 (GLUT1) Deficit Syndrome Model Mouse

BRC No. RBRC-GSC0242_Strain Name : M100200

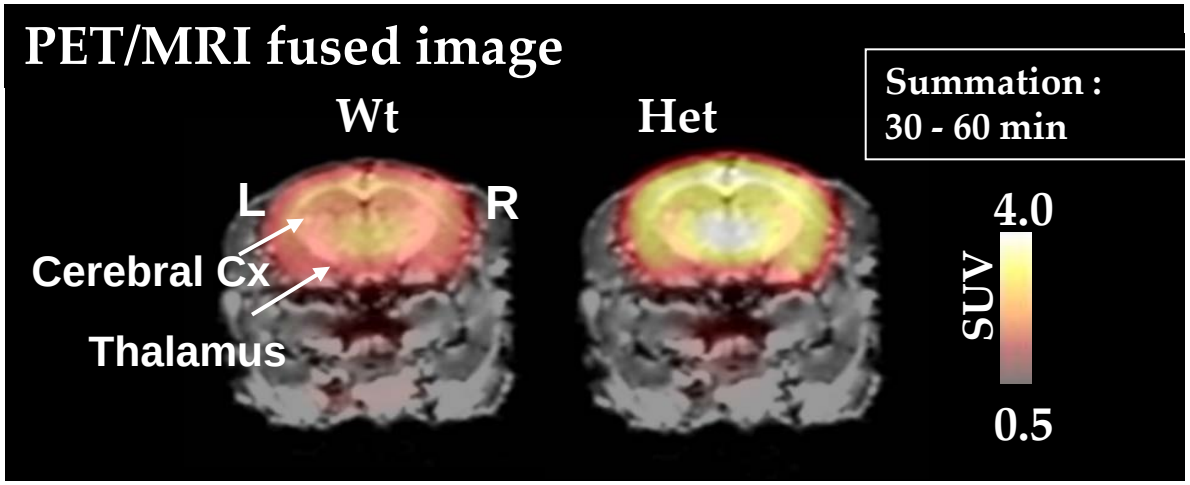
Mutated gene: *Glut1*, Chr. 4

Allele Symbol: $Glut1^{Rgsc174}$

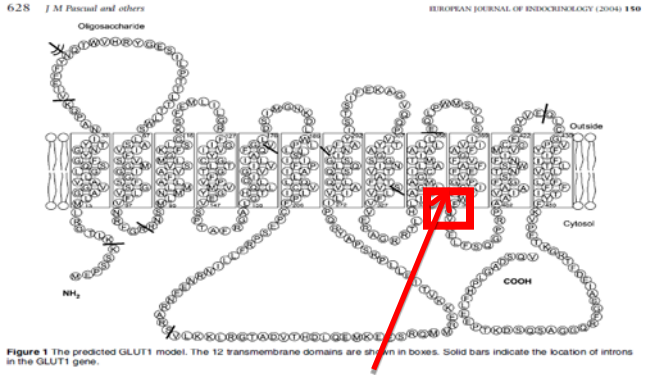


- Immobility
- Ataxia
- Epileptic seizure

Epileptic electroencephalogram



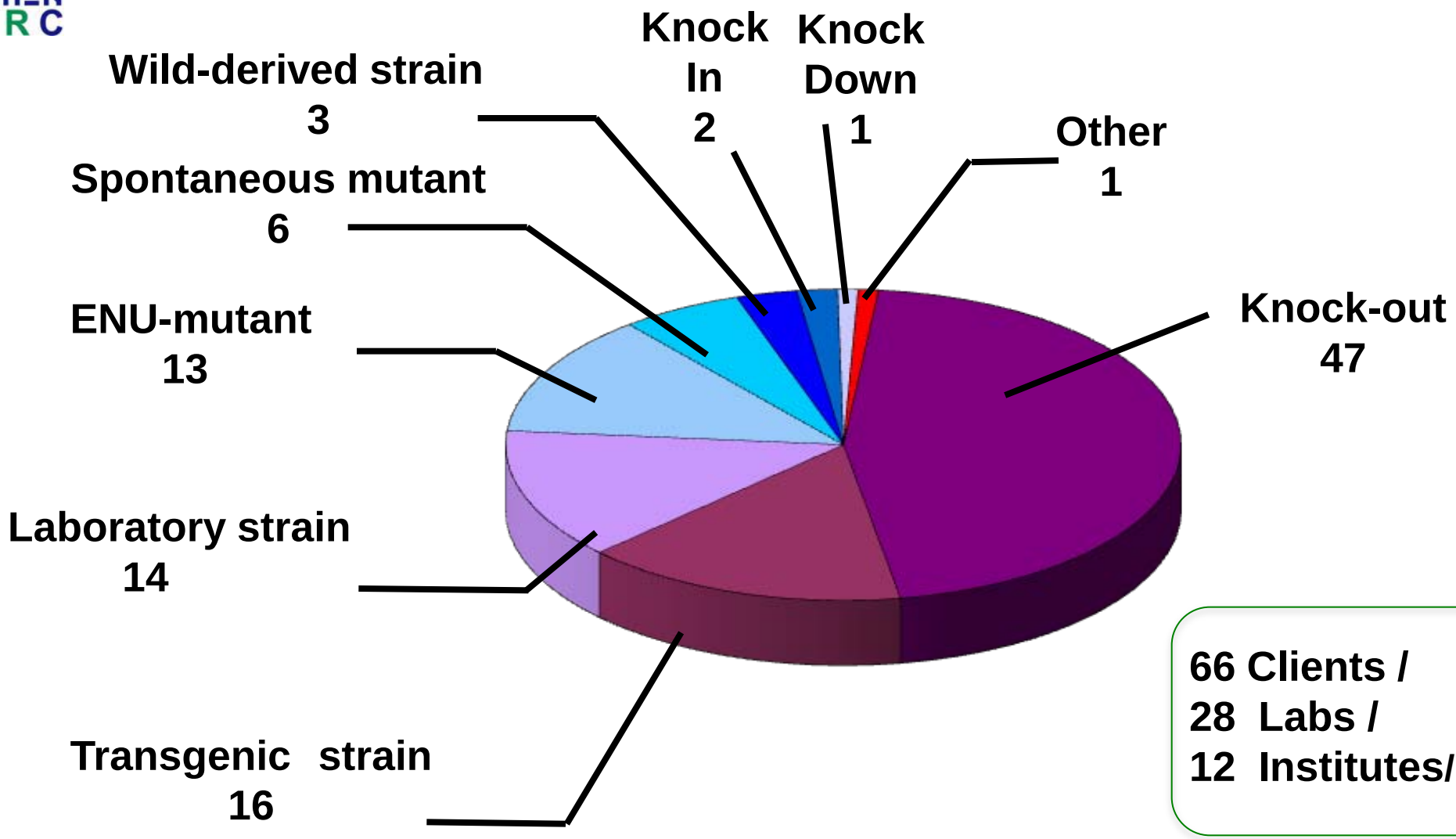
A kinetic analysis of Glucose using [18F]FDG PET imaging
Glucose transportation was decreased.



The missense mutation Ser324Pro in trans-membrane domain of GLUT1 protein

Mizuma *et al* in preparation

Progress of Phenotyping in JMC (from April 2008 to June 2011)



66 Clients /
28 Labs /
12 Institutes/

28 lines are going and 75 lines have been finished to screen.

Discovery Rate of Abnormal Phenotypes - Genetically Modified Mice -

item	Open Field	SHIRAPA	Hema-tology	Clinical Chemistry	Rota-Rod	ABR	Adipo-cytokines	Blood Pressure	DEXA	Behavior
wks	7w	8w	9w	11w	12w	13w	18w	21w	22w	7-17w
No. of Significant ／ No. of screened	16/35	31/53	33/39	32/39	4/15	2/23	22/32	4/33	9/21	11/19
Discovery rate	45.7	72.1	84.6	82.1	26.7	9.5	68.8	12.1	42.9	57.8

Pathology (26w)

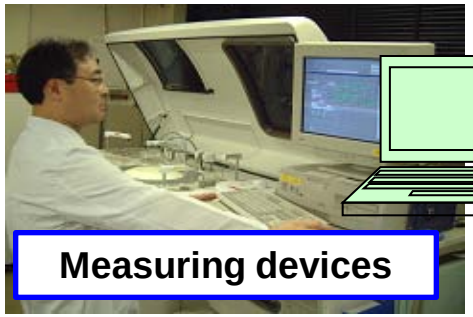
Item	decision	
	Macroscopic	Histology
No. of screened mice:	39	26
No. of abnormal phenotypes distinct from wild types:	37	15
discovery rate (%):	94.9	57.7

Informatics Infrastructure in RIKEN BRC

All of the workflows are semi-automated

Data capturing to central database → **Automated statistical pipeline** → **Posting statistical data on Web**

Direct input

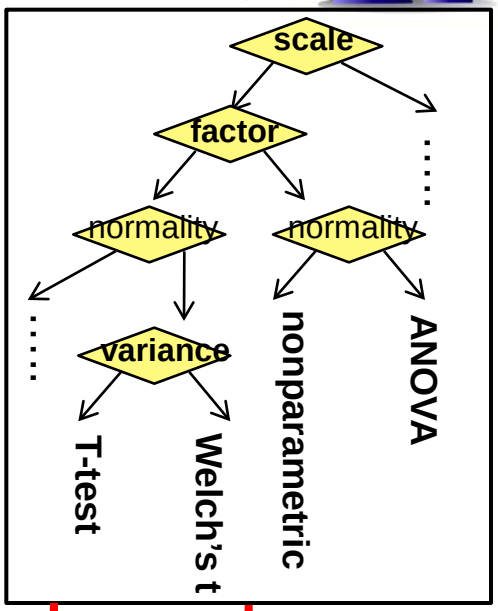


Measuring devices

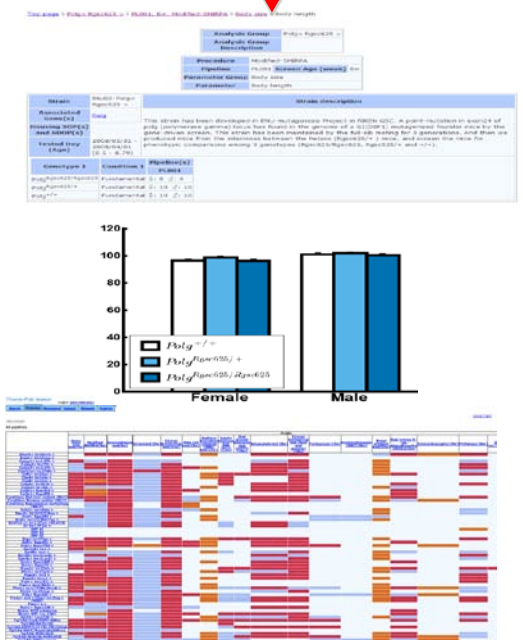
2 pipelines
28 analyses
>300 parameters

Raw experimental data

Central Database



Statistics, graphs



Now in development

Data transfer to IMPC

Dissemination of JMC Data: Pheno-Pub

http://phenopub.brc.riken.jp/

Browsing phenotype data from multiple view points

Search of strains

Overview of phenotype data:

Heat map of phenotypes

Detailed statistical report for mutant strains:

Statistics

Bartlett test result

Factor	df	Bartlett's K²	p
genotype1	2	1.832061	0.4001041
sex	1	4.256950	0.03909006

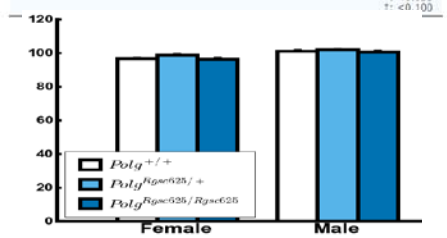
Summary statistics

Factor genotype1 - sex	mean	median	SD	SEM	N
genotype1 Rgsc625/Rgsc625 - F	97.57	97	1.81	0.69	7
genotype1 Rgsc625/Rgsc625 - M	98.73	101	3.72	1.12	11
genotype1 Rgsc625/+ - F	98.7	99	1.7	0.54	10
genotype1 Rgsc625/+ - M	101.8	102	1.69	0.53	10
genotype1 +/+ - F	96.44	97	1.88	0.63	9
genotype1 +/+ - M	100.45	100	2.46	0.74	11

ANOVA result

Factor	df	Sum Sq.	Mean Sq.	F value	Pr(>F)	Signif.
genotype1	2	42.658	21.329	3.7150	0.03104	*
sex	1	114.118	114.118	19.8768	4.433e-05	***
genotype1:sex	2	19.248	9.624	1.5763	0.19702	
Residuals	52	298.546	5.741			

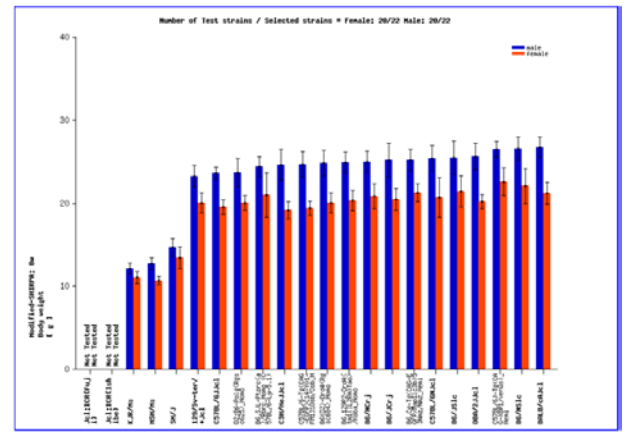
Signif. codes ***: <0.001 **: <0.010 *: <0.050



Images and comments

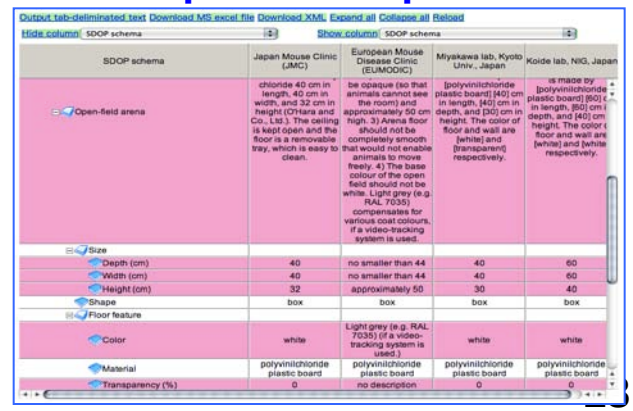
Strain differences

Bar graphs for phenotypes



Phenotyping procedure

Inter-comparison of procedures



International Cooperation Toward "Integrated Mouse Phenome"

2007-2010: Discussion of informatics issues with CASIMIR and InterPhenome consortiums

2009: International Phenome Integration Meeting
RIKEN/InterPhenome/CASIMIR
July 12 - 13, 2009, Kyoto, Japan

- Description phenotype with ontologies.
- Minimum requirement for phenotype data.
- IP issues on information.

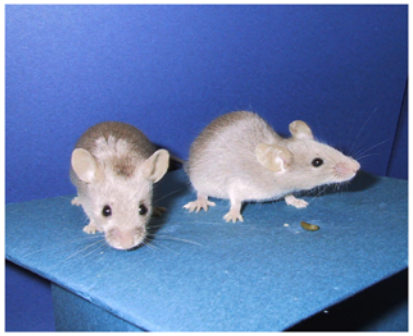
2010-: IT-working group member of IMPC

Investigation of technological issues for integration of mouse phenotype data.

Asian Efforts - Establishment of AMPC -

Top Consortium Phenotyping Databases/links Resources Meetings

AMPC Asian Mouse Phenotyping Consortium



1ST ASIAN MOUSE PHENOTYPING CONSORITUM WORKIG MEETING

20th July (Tue) 2010 – 22nd July (Thru) 2010

RIKEN BRC

China: Xiang Gao (Nanjing University)
Korea: Je Kyung Seong (Seoul National University)
Taiwan: Jeffrey Yen (Academia Sinica)
Si-Tse Jiang (NLAC)
Japan: Shigeharu Wakana (RIKEN BRC), Tomohiro Suzuki (RIKEN BRC)
Hiroshi Masuya (RIKEN BRC), Nobuhiko Tanaka (RIKEN BRC)
Atsushi Yoshiki (RIKEN BRC), Kazuo MORIWAKI (RIKEN BRC)

Phenotyping Consortium (AMPC).

The Modified SHIRPA Workshop in Taiwan

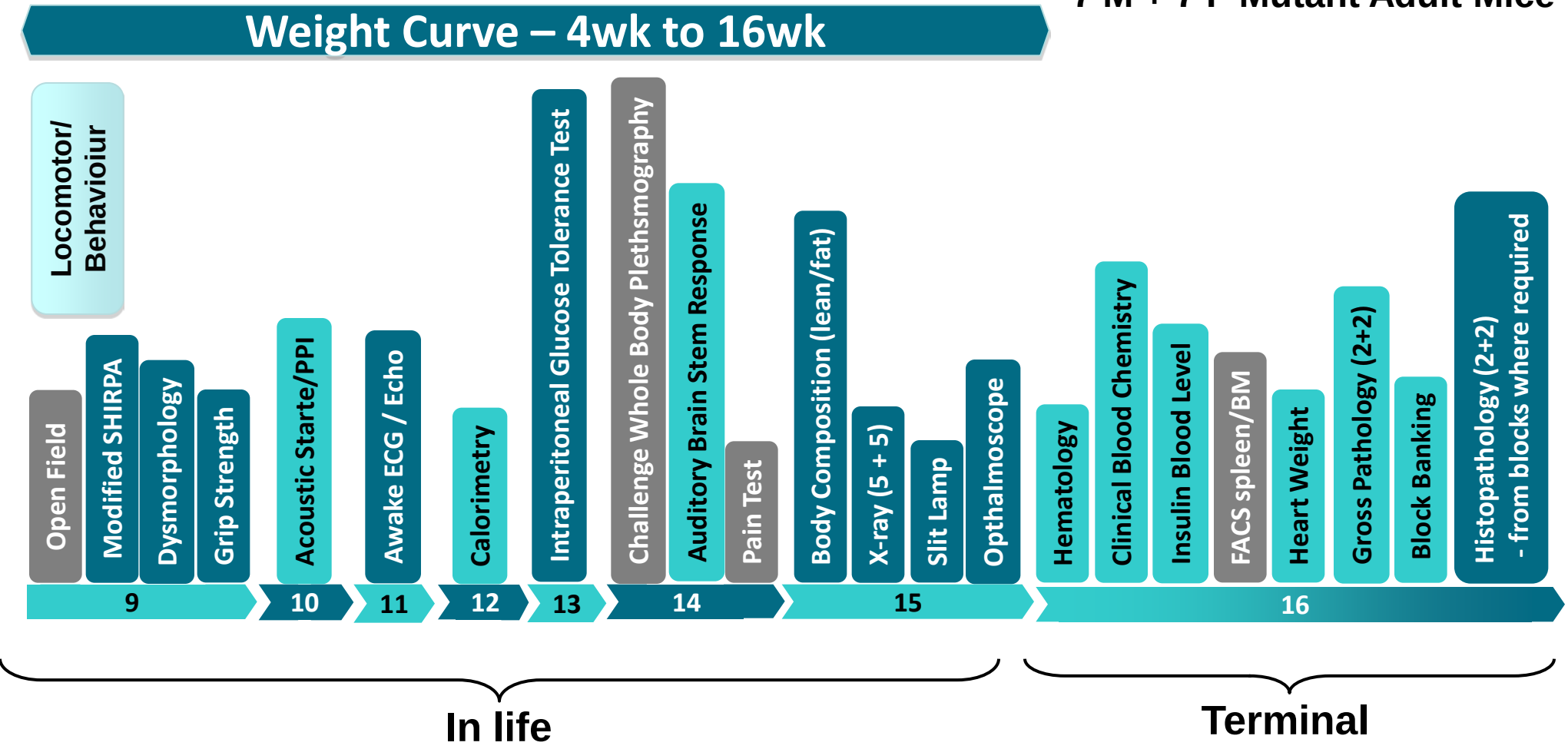
AMPC 1st Asian Mouse Phenotyping Consortium Council Meeting

June 12–14, 2011 Hoam Faculty House, Seoul Nat'l Univ., Seoul, Korea Supproted by KGEMC, NRF

IMPC Phenotyping Pipeline

International Mouse Phenotyping Consortium

7 M + 7 F Mutant Adult Mice



Japan Mouse Clinic has capacity of carrying out all of the tests in the IMPC pipeline.

RIKEN BRC

Steps for the future!

10th Anniversary

RIKEN BioResource Center