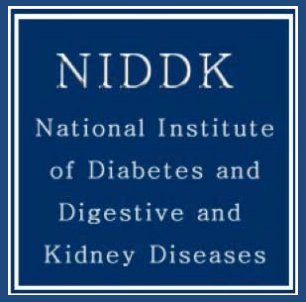
AMDCC Mouse Generation and Husbandry Core at The Jackson Laboratory



T1D-MGHC Project Highlights

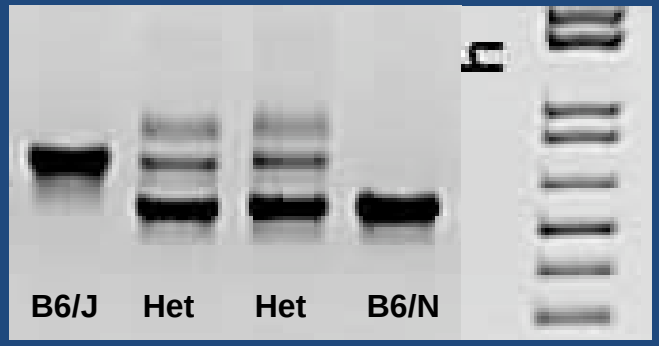
Ed Leiter, T1DR Principal Investigator
Cathleen Lutz, T1DR Co-Principal Investigator
Racheal Wallace, T1DR Operations Manager
Peter Reifsnyder, Senior Research Assistant
Pam Stanley, Research Assistant

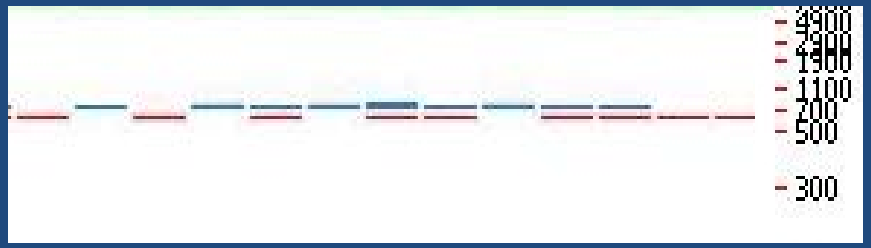
Drs Oliver Smithies/Masao Kakoki

Project: Produce Bradykinin B1 and B2 receptor double knockout with the $Ins2^{Akita}$ mutation on a C57BL/6 background to explore the impact of defective bradykinin signaling.

1. Imported and rederived B6/N Bradykinin B1 and B2 receptor double knockout mice and mated with B6/J-Akita.
2. Bred B6 Bradykinin B1 and B2 receptor mutation to homozygosity with Akita segregating.
3. Distributed B6 Bradykinin B1 and B2 receptor knockout, Akita/+ and breeders
4. Fix segregating population for B6/J *Nnt* mutation

Nnt mutation in B6/J





Segregating *Nnt* in Phenotyped *B6.Bdkrb1/2-/-, Akita*

Dr. Firouz Daneshgari

Project: **To combine the floxed *Sod2* allele with the transgene transgelin-Cre/Esr (TagIn-creER) on a C57BL/6 background to explore the impact of increased ROS production in smooth muscle, specifically diabetic bladder dysfunction**

1. Imported/rederived B6. *SOD2^{floxed}* and B6-TagIn-cre/Esr stocks
2. Bred the 2 alleles together.
3. Distributed heterozygous and homozygous B6. *SOD2^{floxed}* mice also heterozygous for TagIn-cre/Esr
4. Discovered that the imported *SOD2^{floxed}* allele had lost its *neo* cassette.

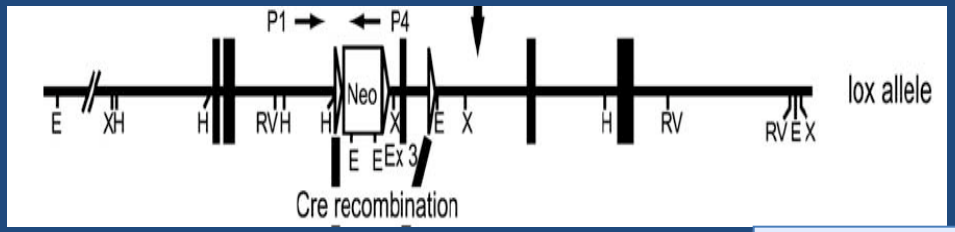
Dr. Eva Feldman

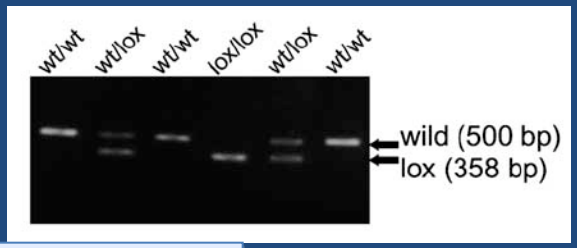
Project: Transfer the B6.*SOD2*^{floxed} and B6.Nestin-cre alleles onto the BKS.*Lepr*^{db} background to evaluate the role of ROS production in diabetic neuropathy.

SNP Marker	Animal ID						
sample	BH377	BH381	BH382	C57BL/6J	C57BLKS/J	DBA/2J	SJL
04-006370062-M	G	G	G	A	G	G	A
04-032923355-M	T	T	T	C	T	T	T
04-085096243-M	T	T	T	C	T	T	C
04-097015585-M	G	G	G	A	G	G	G
04-122948823-M	G	G	G	A	G	G	G
12-017285410-N	G	G	G	A	G	G	A
12-031510473-N	A	A	A	G	A	A	G
12-048364436-M	C	C	C	C	C	C	T
12-049281625-M	T	T	T	C	T	T	T
12-059204373-M	T	T	T	T	T	T	G
12-063406537-M	A	A	A	A	A	A	G
12-064212930-N	G	G	G	A		G	G
12-067428336-M	A	A	A	A	A	A	C
12-083253104-M	het	het	het	A	A	A	G
12-092440423-M	G	G	G	G	G	G	C
12-099526845-G	G	G	G	G	G	T	T
12-104882822-M	T	T	T	T	T	T	C
12-113315003-M	C	C	C	C	C	G	G

1. Imported/rederived B6.*SOD2*^{floxed}
2. Transferred *SOD2*^{floxed} and Tg(Nes-cre) alleles to BKS.*Lepr*^{db} .
3. Developed SNP panel to distinguish donor strain from targeted background strain.
4. Bred *SOD2*^{floxed} in the BKS.*Lepr*^{db} background to homozygosity
5. Distribute BKS. *Lepr*^{db} *SOD2*^{floxed} and heterozygous BKS-*Lepr*^{db} *SOD2*^{floxed} Tg(Nes-cre) breeders to produce experimental animals.

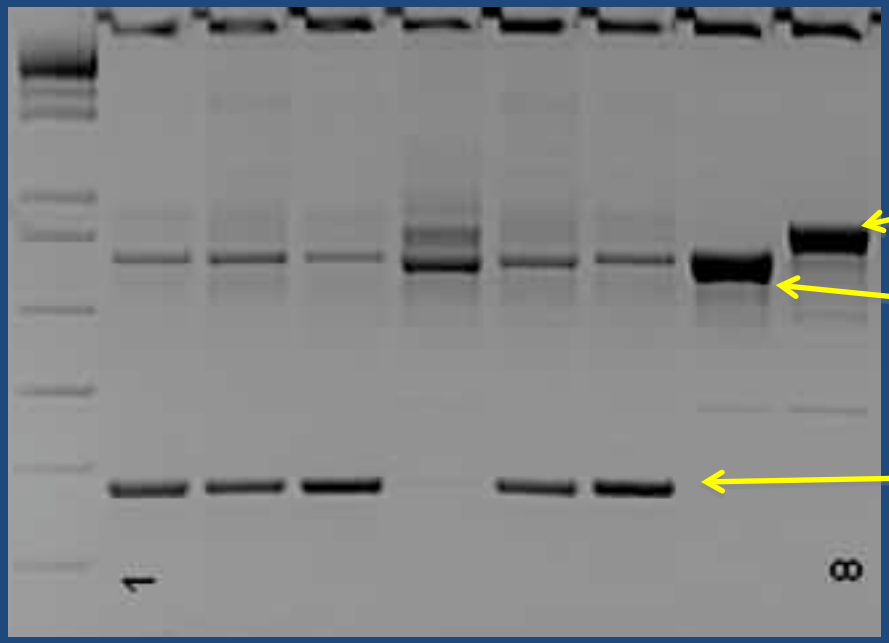
Sod2^{flox} allele





Ikegama, BBRC 296, 729-736

Sod2^{flox} cut by Tg(CMV-Cre)



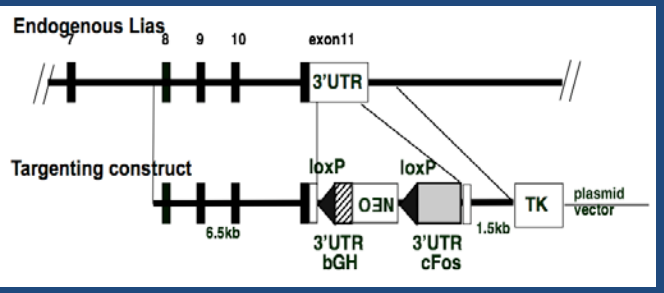
Sod2^{Flox}/*Sod2*^{Flox}, uncut by Cre

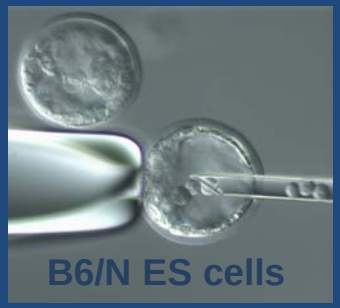
WT allele (CMV-cre)

Sod2^{Flox} allele cut by CMV-Cre

Dr. Nobuyo Maeda

Project: Produce a high/low set of B6 lines for Lipoic Acid Synthetase (Lias) expression

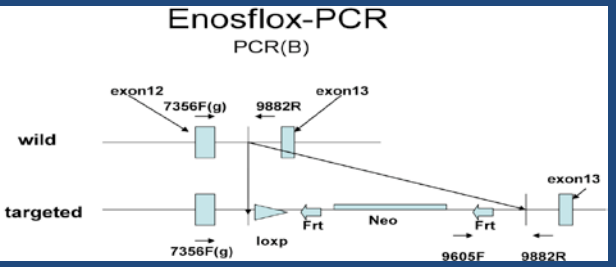




1. Identified chimeras and tested for for germline transmission
2. Mated founder's progeny to C57BL/6J (Tyr+ and Nnt-) and replaced Tyr^{c} and Nnt^{+} with the B6/J alleles.
3. Shipped heterozygous and homozygous mice to PI for $Lias^{high}$ activity determination
4. Mated to Tg(EIIA-cre) to create $Lias^{low}$
5. Ship heterozygous and homozygous mice to PI for $Lias^{low}$ activity determination
6. Mate $Lias^{low}$ to B6-$Ins2^{Akita}$
7. Cryopreserve all lines
8. Mate $Lias^{low}$ to B6-Ldlr-KO

Dr. Ray Harris

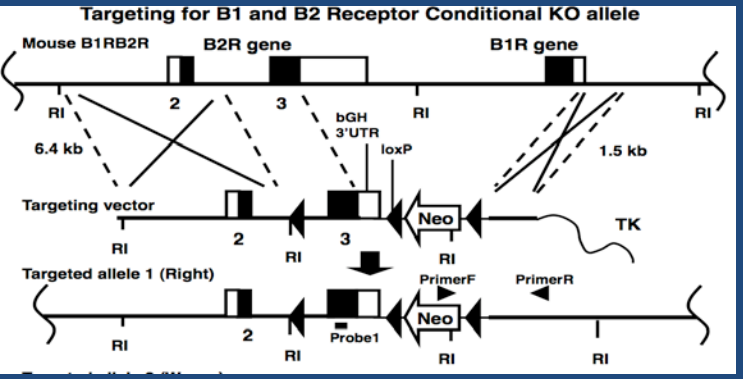
Project: **Produce 129 targeted lines for a conditional nitric oxide synthetase (Nos3) to explore tissue specific pathogenic endothelial changes.**

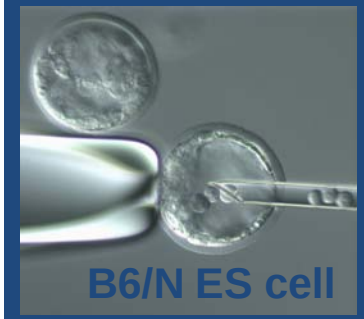


1. Tested 12 chimeric males for germline transmission containing correct construct
2. Mated chimeric founders to 129S4-Flpe
3. Shipped mice Nos3^{floxed}, Flpe heterozygous mice from 4 chimeric founder lines to PI for further breeding and to test Nos3 expression
4. Maintaining each founder line until expression data are available
5. Transfer conditional Nos3 mutation onto the DBA/2J background.

Drs Oliver Smithies / Masao Kakoki

Project: Produce a conditional bradykinin 2 receptor knockout in a C57BL/6 background to explore tissue specific impact of impaired bradykinin signaling, when combined with the *Ins2*^{Akita} mutation.

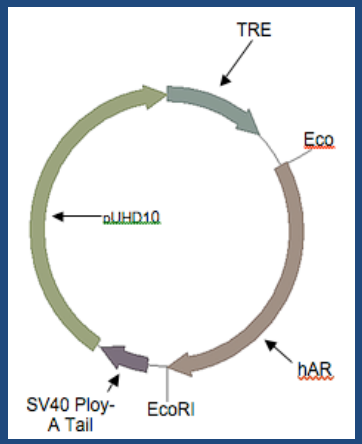


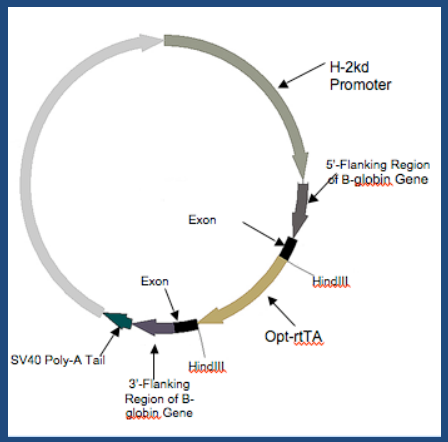


1. Test for germline transmission.
2. 8 medium – low chimeric males
   -No germline expression.
3. Re-inject ES cell clone using laser assisted technology
   -No chimeric pups born
4. Restart project using B6/J-albino ES cells for targeting.

Dr Ed Fisher

Project: **Produce a tetracycline inducible (tet-On), bi-transgenic tetO-human aldose reductase and H2-K^{d} -rtTA in the B6/J background.**

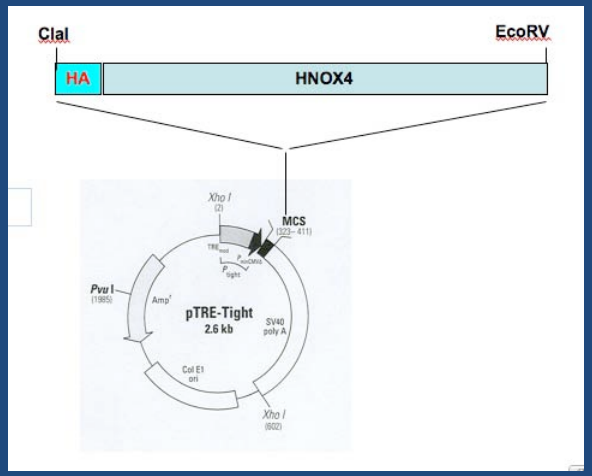


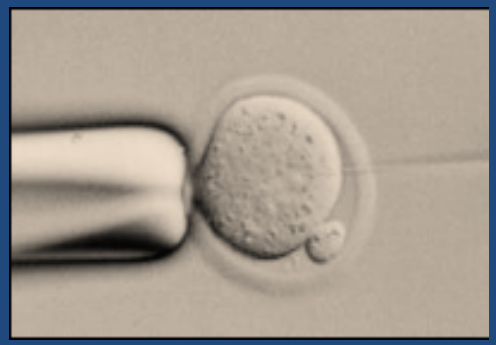


1. Established germline transmission of 14 founders.
2. Shipped double transgenic mice from each founder line.
2. Maintained each founder line until expression studies complete
3. Mating 3 high expressing founder lines to B6-Ldlr KO
4. Ship double transgenic, Ldlr KO mice

Dr. Kumar Sharma

Project: Produce a tetracycline inducible (tet-On), Nox4 transgenic stock on FVB/N and DBA/2 backgrounds. The objective is to assess the effects of hyperglycemia on nephropathy after tissue specific over-expression of human NOX4.





1. Established germline transmission of 10 FVB/N Tet0-Nox4 founder lines
2. Shipped transgenic offspring from each Tet0-Nox4 transgenic founder
3. Maintaining each founder line until expression data are available
4. Transfer Teto-Nox4 and NPHS2-rtTA transgenes to DBA/2J background

Dr. Moshe Levi

Project: Transfer the Farnesoid X receptor (FXR) knockout allele onto DBA/2J and FVB/NJ with Ins2^{Akita} to evaluate the consequences of FXR deficiency in diabetic renal and cardiovascular complications.

SNP	FVB-FXR, Akita Mouse ID					
	FX351	FX352	FX353	129	FVB	C57BL/6
10-077371167-G	A	A	A	G	A	G
10-086567143-M	T	T	T	T	C	T
10-086817590-G	T	T	T	T	A	A
M-05799_1	G	G	G	G	C	G
FXR (Nr1h4 by PCR)	**-/-**	**-/-**	**-/-**			
10-099683776-N	C	C	C	C	G	C
10-100345477-M	T	T	T	T	C	T
10-107333522-M	T	T	T	T	C	C
10-107789430-G	G	G	G	A	A	G
10-120289167-M	G	G	G	A	G	A

1. Using speed congenic technology, transferred FXR KO from B6 to FVB/NJ and DBA/2J.
2. Developed SNP panel to distinguish donor strain from target background strain.
3. Bred to Ins2^{Akita} mutation of same background strain.
4. Bred FXR mutation to homozygosity.
5. Distributing mice

Dr. Dale Abel

Project: Produce congenic C57BL/6-*Ins2*^{Akita} Netrin^{floxed} Tg(CAG-cre/Esr1) for evaluation of selective over-expression of Netrin

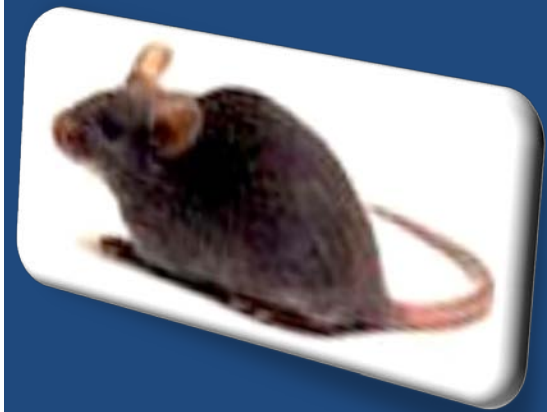
generation = N5	B6-Ntn1<floxed>						
SNP Marker	Mouse#						
	BS183	BS301	BS309	BS313	C57BL/6J	129S1	B6+129
05-149044358-M	C	C	C		C	A	het
06-003167392-M	C	C	C	C	C	T	het
06-030516093-G	A	A	A	A	A	C	het
06-060887613-G	A	A	A	A	A	G	het
06-090142535-M	C	C	C	C	C	A	het
06-095139289-M	het	het	het	het	T	A	het
Gt(Rosa)26 - Ntn1	heterozygous by PCR						
06-112199886-M	het	het	het	het	G	T	het
06-119728826-G	het	het	het	het	T	A	het
06-122941044-M	het	het	het	het	T	A	het
06-128926170-M	C	C	C	C	C	T	het
06-135955068-M	C	C	C	C	C	A	het
06-140532504-G	C	C	C	C	C	G	het
06-146079591-M	A	A	A	A	A	C	het
06-149052281-M	C	C	C	C	C	G	het
07-022997618-M	C	C	C	C	C	T	het
08-123340602-N	C	C	C	C	C	T	het
09-102158958-M	C	C	C	C	C	T	het

1. Imported /rederived Ntn1^{floxed} mutation on a mixed background
2. Developed a C57BL/6/129 SNP marker panel
3. Transferred the Ntn1 mutation onto a congenic C57BL/6J background.
4. Combine the B6-Ntn^{floxed} with B6.Tg(CAG-cre/ESR1) and B6.Ins2^{Akita} to eliminate the 129 derived Y-chromosome
5. Distribute mice

Dr. Frank Brosius

PROJECT: Produce congenic 129S6-*Ins2*^{Akita} Jak2^{floxed} Tg(Nphs2-cre) for evaluation of selective over-expression of Janus kinase.

SNP Marker	129S6.B6-Akita	129S6.B6-Akita	129S6.Cg-NPHS2-cre	129S6.Cg-NPHS2-cre	129S6-Gt(ROSA)-Jak2	129S6	129S1	C57BL/6J	129S1 XB6	SJL
01-184035421-M	C	C	C	C	C	C	T	C	het	
03-096257069-G	C	C	C	C	C	C	T	C	het	
04-003163167-M	T	T	het	het	T	T	T	G	het	
04-009404597-G	NA	NA	het	het	NA	NA	G	A	het	
04-010285677-G	NA	NA	het	het	NA	NA	G	A	het	A
04-013376218-G	NA	NA	het	het	NA	NA	T	C	het	
04-020201305-G	NA	NA	het	het	NA	NA	A	T	het	
04-030080285-M	NA	NA	het	het	NA	NA	G	A	het	
04-032923355-M	T	T	het	het	T	T	T	C	het	
04-039199957-M	NA	NA	het	het	NA	NA	NA	NA	NA	
04-058850394-M	T	T	T	T	T	T	T	C	het	
04-092052171-M	G	G	G	G	G	G	G	A	het	
06-003167392-M	T	T	T	T	T	T	T	C	het	
06-029636236-G	T	T	T	T	T	T	T	G	het	
06-060887613-G	G	G	G	G	G	G	G	A	het	
06-090142535-M	A	A	A	A	A	A	A	C	het	
Gt(ROSA)26										
06-122941044-M	A	A	A	A	A	A	A	T	het	
06-149052281-M	G	G	G	G	G	G	G	C	het	
11-094206790-M	C	C	C	C	C	C	T	C	het	
11-118804416-N	A	A	A		A	A	A	G	het	



1. Imported/rederived stop-floxed-*Jak2*, NPHS2-cre mutations, 129-*Ins2* ^{Akita} and 129S6-Tac
2. Developed 129S6 / B6 /FVB SNP panel
3. Completed 129S6 congenic backcross of the NPHS2-cre
4. Shipped 129S6-stop-floxed-*Jak2* and 129S6-NPHS2-cre breeders
5. Mating *Jak2* mutation to *Ins2* ^{Akita} and NPHS2-cre

MGHC stocks available only to AMDCC members

Stock# 006861 - C57BL/6-*Ins2*^{Akita}*Bdkrb1/Bdkrb2*^{tm2Mki}

Stock# 007709 - B6.129-Tg(Tagln-cre)1Feil/Fdmd

Stock# 007023 - B6.Cg-*Sod2*^{tm1Shs}/Elf

Stock# 007688 - 129S6.B6-*Ins2*^{Akita}

Stock# 008523 - 129S6.Cg-Tg(NPHS2-cre)295Lbh/Bro

***The following strains will need approval from Dr. Nobuyuki Takahashi at U. of North Carolina:**

- **-Stock# 008286 - 129.Cg-Nos3^{tm1Unc}/J**
  - **(Nos3 homozygous KO)**
- **-Stock# 008693 - B6.Cg – Ins2^{Akita} Nos3^{tm1Unc}/J**
  - **(Nos3 heterozygous, Akita heterozygous)**

Other stocks should be available shortly, but will most likely be distributed for AMDCC collaborations

Next 6-8 Months

1. We will complete 11 of 15 approved projects maintaining small quantities for AMDCC distribution or transferring to public distribution (Akita stocks).
   - We have used or created 43 inbred or mutant stocks to accomplish these goals.
     - This does not account for multiple founder lines.

2. Cryopreservation of newly established stocks with multiple or single mutations.

3. Phenotyping studies of multigenic stocks.

4. Continue/start speed congenic projects for newly developed mutations – Drs. Sharma and Harris.

5. Continue efforts to develop conditional B1RB2R KO.

6. Lias-L in combination with other mutations.

TJL Scientific Resources Utilized

Assisted Reproductive Services

- Importation
- Re-derivation
- Cryopreservation
- Cell Biology
- Microinjection

Phenotyping Services

- Necropsy Service
- Histotechnical Service
- Physiology Service
- Pathology/Veterinary Science

Genome Services

- Molecular Biology Service
- Transgenic Genotyping Service
- SNP Genotyping Service
- Fine Mapping Service

Vivarium Management

- Research Animal Facility
  - Basic Animal Husbandry
- Laboratory Animal Health
  - Microbiologic screening of barrier colonies

Operations

- TJL Customer Service
  - Mouse orders/shipping
- TJL Marketing
  - information/catalog
- Nomenclature
- Information Technology
- Multimedia Services

Phenotyping Data