Analytical Genetics and Transgenics Core (P30-AR-048311; RDCC)

Director-
Dr. Bob Kesterson (kesterso@uab.edu)

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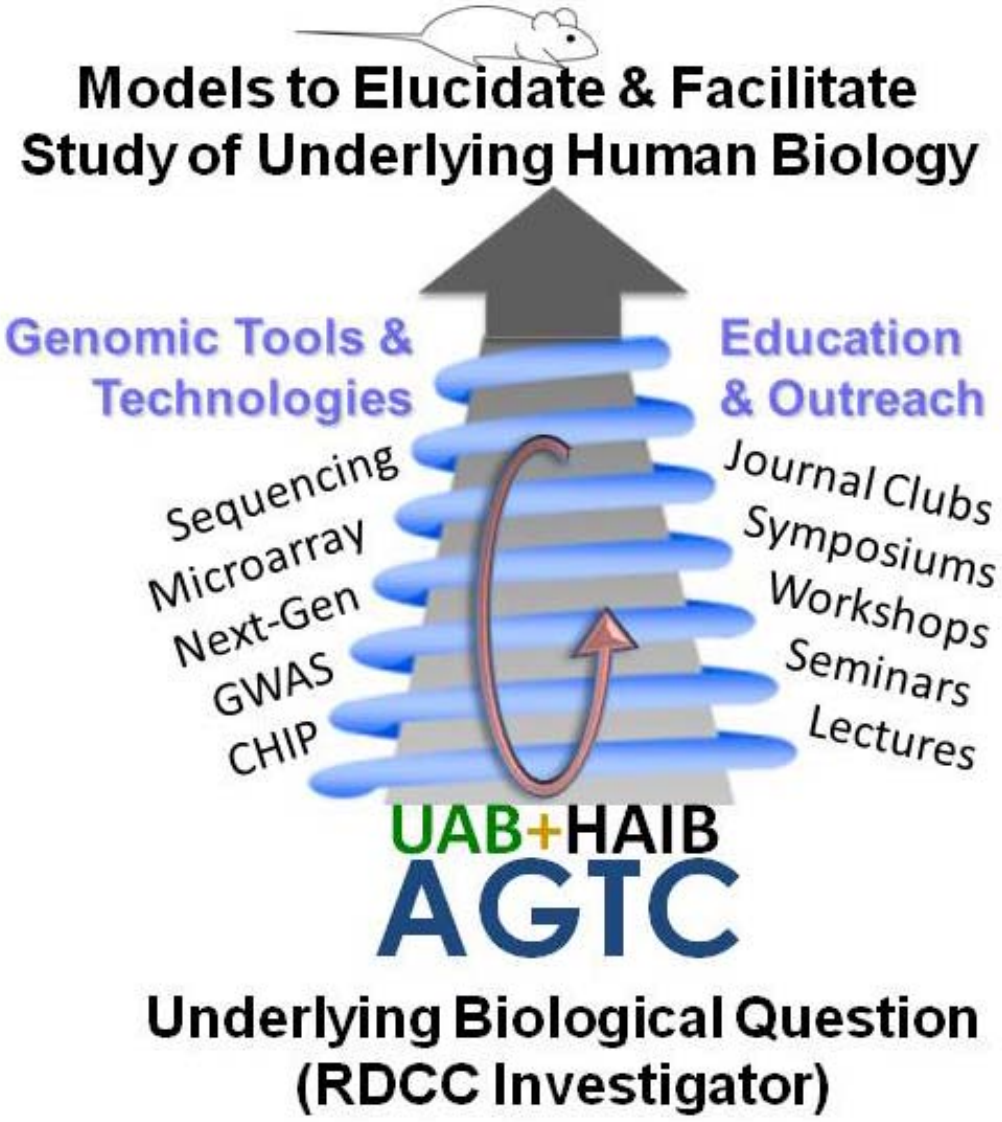
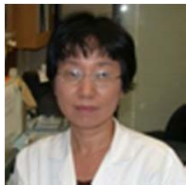


Core History

- 1991 - Creation of Transgenic Animal Core (CCC)
- 2002 - Creation of Gene Targeting Core Facility (GTCF)
- 2002 - New Lab Facilities in Kaul Human Genetics Building
- 2004 - Recruitment of Bob Kesterson from Vanderbilt
- 2005 - C57BL6 Embryonic Stem Cells Produced Germline Chimeras
- 2005 - HSF/GEF Grant for Cryopreservation Services & Equipment
- 2006 - Engineered Mouse Resource Established as Component of UAB Recessive Polycystic Kidney Disease Core Center
- 2008 – UAB Diabetes Research Training Center (DRTC) funded
- 2008 - Genetic Mouse Service Core (GMSC) Established as Component of CMBD
- 2010 – NIH SIGs Award to Purchase AutoGenprep 965

Transgenic Mouse Facility: Staff & Space




- **Staff**
  - **Larry Johnson -** microinjectionist and coordinator (1991)
  - **Jinju Zhang -** secondary microinjectionist, colony management (2000)
  - **Min Chen** – molecular biologist and tissue culture (2008)
  - **Judy Kesterson** – tissue culture and program administrator (2007)
  - **Daniel Kennedy** – microinjectionist and colony management (2012)
  - **Jennifer Zhang** – "cilia" colony management and genotyping (2011)

- **Hugh Kaul Human Genetics Building**
  - **606 Kaul -** molecular biology laboratory wet lab space (875 ft^2)
  - **611 Kaul –** common equipment room (200 ft^2)
  - **613 Kaul –** tissue culture laboratory (200 ft^2)
  - **Suite 128 Kaul –** Transgenic "Barrier" Facility includes: 4 animal rooms, 1 preparatory laboratory and 1 microinjection laboratory (1100 ft^2)
  - **Suite 602 Kaul –** Kesterson Office & Outer Office (180 ft^2)
  - **605 Kaul –** Coordinators Office (50 ft^2)

Larry Johnson
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Phone: 934-2998
KAUL 606

"www.uab.edu/transgenics"

MOUSER:
UAB mouse model database allows you to find mice that other labs have on campus!

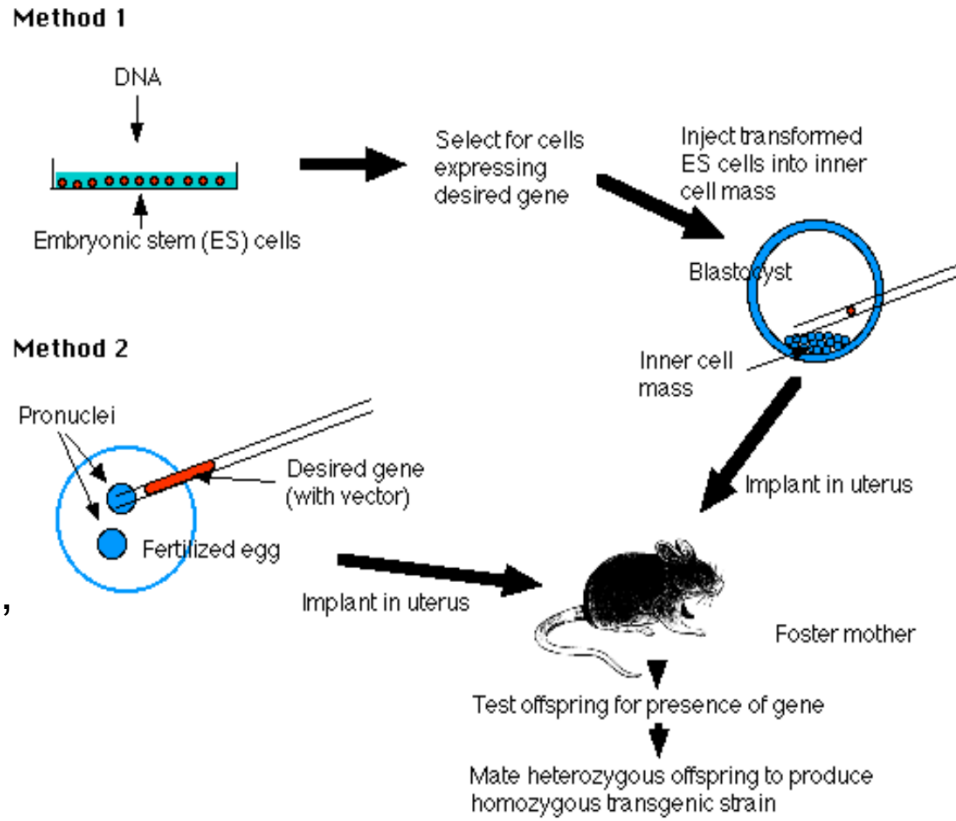
Services Provided by Transgenic Mouse Facility

Transgenic Animal Production:

- microinjection of gene constructs into fertilized eggs
- microinjection of targeted ES cells into blastocysts

Additional Services:

- cryopreservation of embryos
- embryo rederivation to produce pathogen-free mice
- assisted reproduction techniques (in vitro fertilization, superovulation, and embryo transfer).



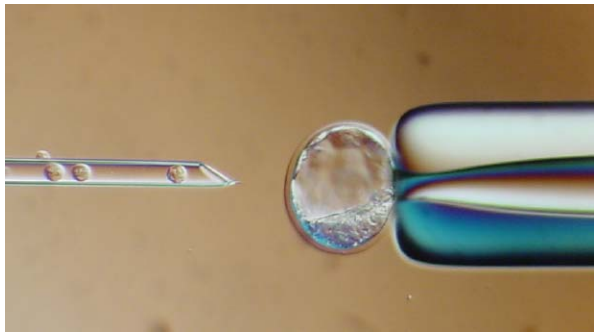
Consultation services:

- designing the most effective transgenic DNA constructs (promoter, reporter, knockout, etc.)
- strategies for molecular diagnosis of transgenic mice (PCR and Southern screens)
- database searches for available mouse reagents (ES clones)
- breeding schemes required for perpetuation of lines

Services Provided by Transgenic Mouse Facility

Commonly Used Services and Fees

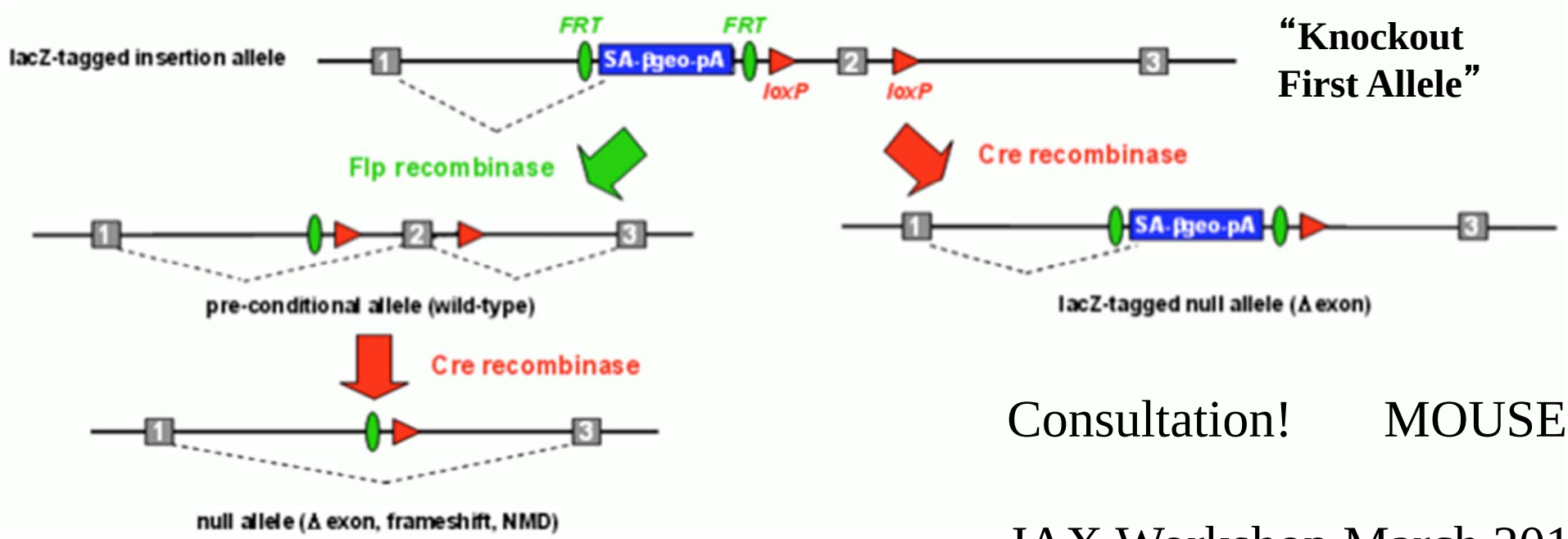
Project Type	Comments	Total fee
DNA Microinjection	150 fertilized oocytes injected	$2900
Gene Targeting	No screening (DNA plates to PI)	$4500
	With Southern blot screening	$6000
ES Cell Transfer	Inject ≥ 40 blastocysts	$2800
IVF	Includes transfer of two-cell embryos to up to six recipient females	$1800
IVF/Cryopreservation	Includes verification of pregnancy in recipient female using thawed embryos	$1800
Embryo Cryopreservation	Economy (viability not tested)	$900
	Standard (test for viable pregnancy)	$1000
	Premium (test pregnancy to term)	$1200
Transfer of cryopreserved embryos	Transfer of thawed embryos to up to six recipient females	$900
Rederivation	Transfer of fertilized oocytes to up to six recipient females	$1000
Sperm cryopreservation	1-2 males per line. Motility of thawed sperm tested	$250



www.uab.edu/transgenics

International Knockout Mouse Consortium

- **Knockout Mouse Project (KOMP) (USA)**
- **European Conditional Mouse Mutagenesis Program (EUCOMM) (Europe)**
- **North American Conditional Mouse Mutagenesis Project (NorCOMM) (Canada)**
- **Texas A&M Institute for Genomic Medicine (TIGM) (USA)**



"Knockout First Allele"

Consultation! MOUSER

JAX Workshop March 2012

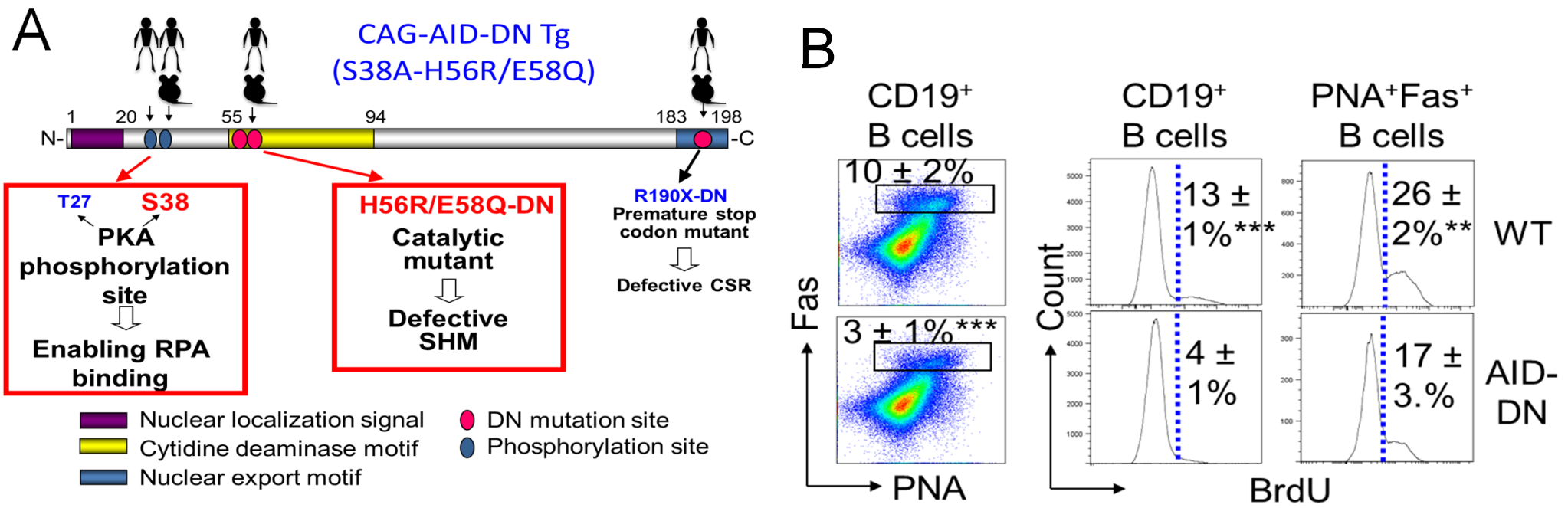
Analytical Genetics and Modeling Core (RDCC P30)

Gene	Knockout Phenotype	Status	Investigators Supported by Model
Birc3	Mice homozygous for disruptions in this gene have a reduced susceptibility to endotoxic shock. Mice homozygous for a knock-in allele exhibit increased B cell survival and proliferation, lymph node hyperplasia, lymphocytic infiltrates in the lungs, and enlarged gut-associated lympoid tissue.	Complete - conditional floxed allele mice	Schroeder, Zhou
Gsk3a	Mice homozygous for a null allele exhibit improved glucose tolerance, increased insulin sensitivity, decreased fat mass and increased lean mass.	Complete – conditional floxed allele mice	Raman
Gsk3b	Mice homozygous for disruptions in this gene may die embryonically around mid-gestation or neonatally. When mice die neonatally, cleft palate and sternum are present.	Complete - conditional floxed allele obtained from external source	Raman, Serra,
Il17ra	Homozygotes for a targeted null mutation exhibit reduced contact, delayed-type and airway hypersensitivity responses and impaired T-dependent antibody production.	Targeted allele germline – breeding with Flp mice	Elson, Harrington, Mountz, Raman, Weaver, Xu, Zajac, and Zhang
Il23r	Th17 T cells from homozygous null mice have less secretion of IL-9 upon secondary stimulation.	Chimeras have not produced germline offspring - ordered additional ES clones	Elson, Harrington, Mountz, Raman, Weaver, Xu, Zajac, and Zhang
Il20rb	Mice homozygous for a knock-out allele display enhanced antigen-specific T cell responses. Mice homozygous for a reporter allele fail to exhibit epidermal hyperplasia in an interleukin-23 (IL-23)-dependent psoriasis mouse model.	Complete – conditional floxed allele mice	Bullard, Weaver
Ptpn22	Homozygous null mice display antigen dependent increases in T cell proliferation and cytokine production, enlarged spleens and lymph nodes, increased spontaneous germinal center formation, increased B cell numbers, and increased serum IgG and IgE levels.	No chimeras from ES cells injected - ordered additional ES clones	Bridges, Edberg, Kimberly, Justement
Rgs16	Mice homozygous for a knock-out allele exhibit increased fatty acid oxidation and circulating ketone levels when fed a high-fat diet. Mice homozygous for a different knock-out allele exhibit impaired Th1 and Th2 chemotaxis and increased susceptibility to parasitic infection.	Complete – conditional floxed allele mice	Hsu, Justement, Mountz
Tgfbr3	Mice homozygous for disruptions in this gene usually die as embryos. The very few individuals that survive are poorly fertile with abnormalities of the spleen, liver, heart, and skeletal system.	Complete – conditional floxed allele mice	Feng, Raman, Yoder

Analytical Genetics and Modeling Core (RDCC P30)

Strain	Description	Source	Stock #
B6.Cg-TgN(Lck-Cre)548Jxm	This transgene expresses Cre recombinase under the control of the mouse lck promoter, active in the T cell lineage	Jackson Lab	003802
C.129P2-*Cd19*$^{tm1(cre)Cgn}$/J	Heterozygous mice are phenotypically normal and can be used for specific deletion of floxed targets in B-lymphocytes.	Jackson Lab	004126
B6.129P2-Lyzs$^{tm1(cre)Ifo}$/j	Cre recombinase is regulated by the *Lyzs* locus and can be used for deletion of targeted genes in the myeloid cell lineage.	Jackson Lab	004781
B6.FVB-TgN(EIIa-Cre)C5379Lmgd	Cre-mediated recombination occurs in a wide range of tissues, including the germ cells that transmit the genetic alteration to progeny. Used to remove LoxP flanked sequences, generally to create null allele.	Jackson Lab	***003724***
B6.Cg-Tg(ACTFLPe)9205Dym/Jor	This transgenic strain expresses a variant of the FLP1 recombinase gene under the direction of the human ACTB promoter. Used as deleter strain to remove FRT flanked sequences, generally selection cassettes.	Jackson Lab	005703

Suppression of the catalytic domain of activation-induced cytidine deaminase (AID) can suppress the hyper-reactive germinal center responses



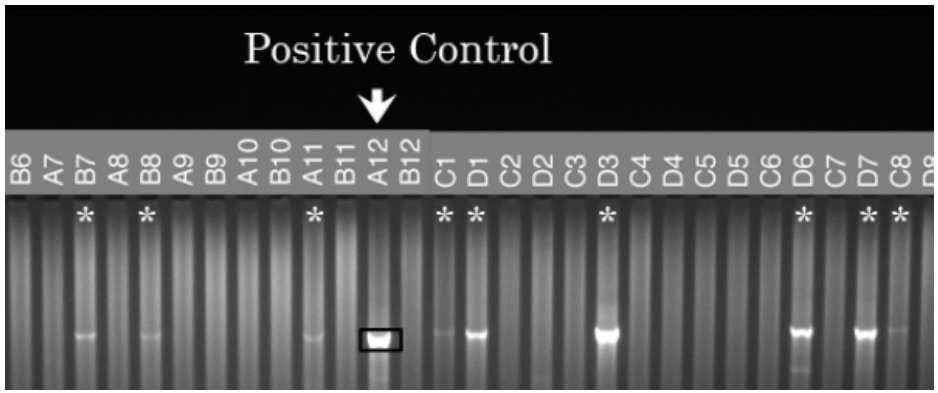
This study is to determine if functional suppression of the catalytic domain of activation-induced cytidine deaminase (AID) can suppress the hyper-reactive germinal center responses in autoimmune BXD2 mice.

(A) A transgenic (Tg) BXD2 mouse expressing a dominant negative (DN) form of AID(AID-DN) at the somatic hypermutation (SHM) site was generated by support provided by the AGTC. The H56R/E58Q AID-DN has been previously shown to function specifically in the post-cleavage step of the SHM process of AID. To prevent the excessive class-switch function of the AID-DN Tg, an additional Serine 38 (AGT) to Alanine 38 (GCT) mutation was introduced into the PKA phosphorylation site, leading to the generation of a S38A and H56R/E58Q-triple mutant of the AID-DN Tg. (**B**) The effects of AID-DN on *in vivo* germinal center (GCs) and B-cell proliferative responses were determined by flow cytometry at the RDCC – CFCC. Spleen cellular composition and GCs were characterized in WT BXD2 and BXD2-AID-DN Tg mice at 5-6-mo of age. Left: FACS analysis of percent of PNA+Fas+ B220+ GC B cells in the spleen (*** $P < 0.005$; $N \geq 6$ per group). Right: In vivo proliferation as determined by BrdU incorporation for CD19+ B cells and PNA+Fas+ CD19+GC B cells in WT BXD2 and BXD2-AID-DN Tg mice (*** $P < 0.005$; $N \geq 6$ per group). *Hsu et al, Arthritis Rheum. 2011 Jul;63(7):2038-48, PMID: 21305519*

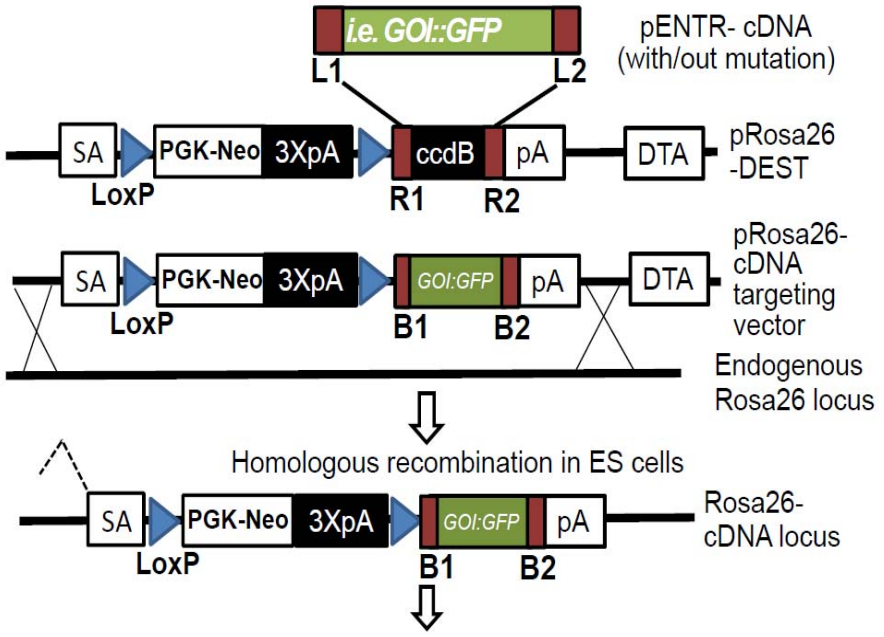
Core B: Cre-Inducible Transgene Expression

- Transgene targeted to ROSA26 locus for inducible “ubiquitous” expression for *in vivo* analysis of gene function.

Targeting in ES Cells

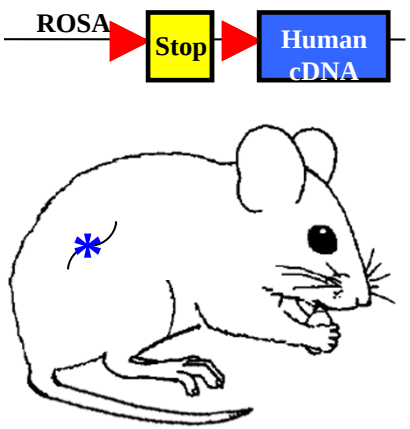


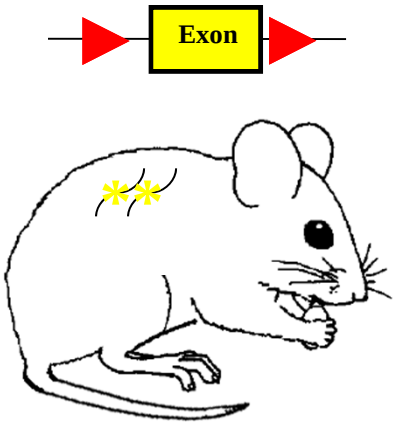
“Gateway cloning”

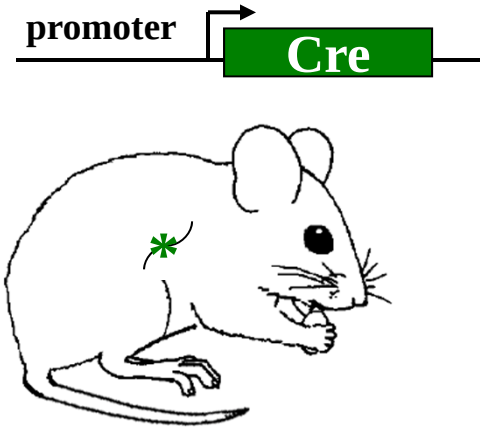


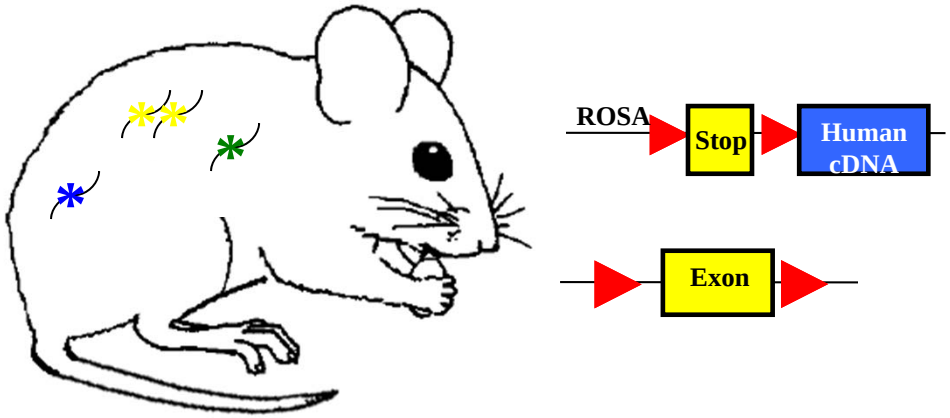
- Confirm Cre induced expression in targeted ES cells Make mouse line from targeted cells.
- Cross into conditional mutant background backgrounds (if desired) with congenital or tamoxifen induced Cre (i.e. KSPCreER, CaGG-CreER).
- Loss of endogenous loci and induction of the mutant form.

Conditional Expression of Human Allele in Knockout Context







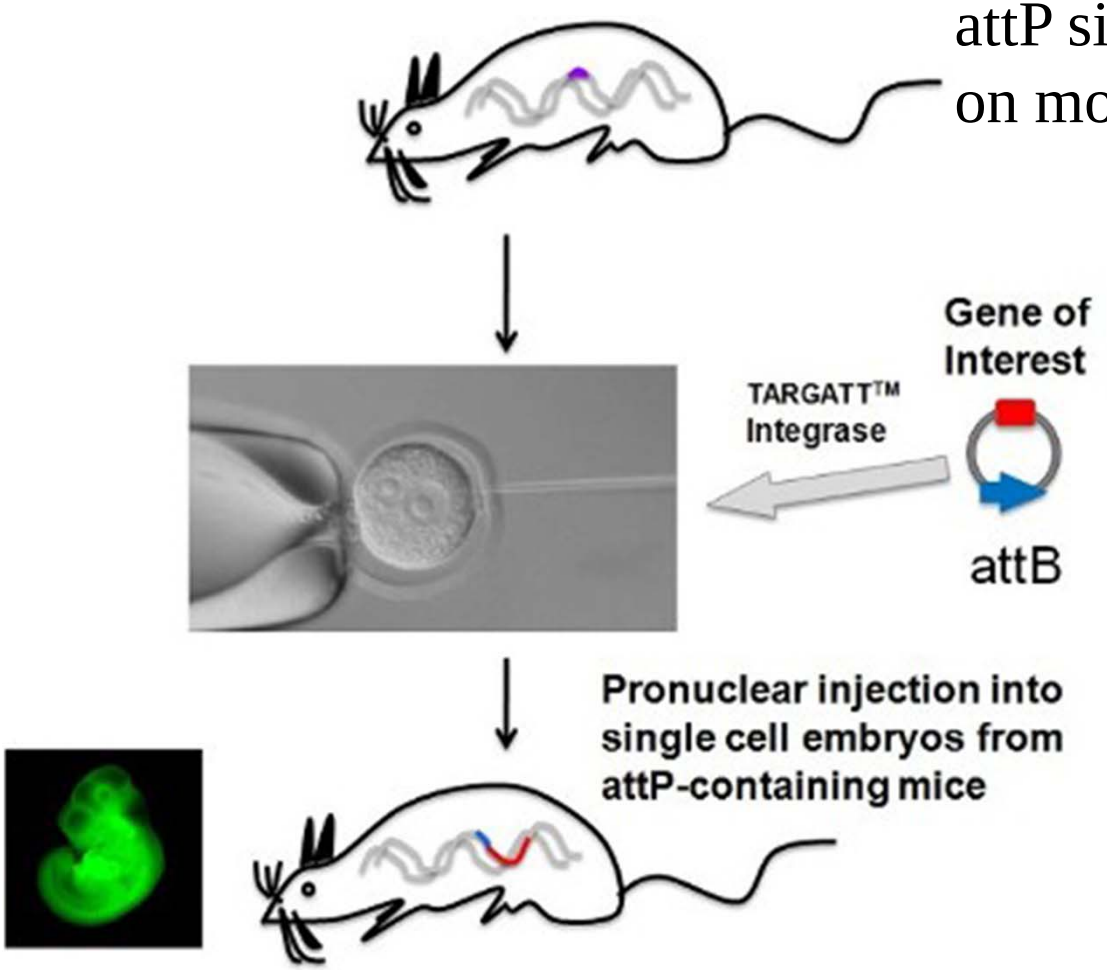


Inducible!

Future Plans

Site Specific Single Copy Transgenes

attP sites located in Hipp11 (H11) locus on mouse chromosome 11



\+ ϕC31 mRNA

ZFNs being tested

New Services

High Throughput DNA Isolation & Genotyping

AutoGenprep 965

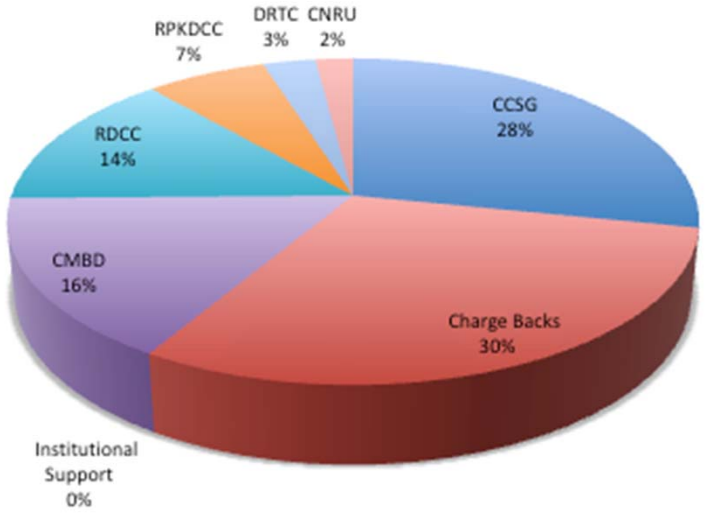
- fully automated
- isolate and purify genomic DNA from mouse tissues and ES cells (plasmids/BACs too).
- 384 samples in 4 hrs

Chromo4 Real-Time

ZFNs

Trangenic & Knockout/Knockin Rats!

Acknowledgements



NIH Funding

- **P30 AR48311 UAB Rheumatic Diseases Core Center (RDCC)**
- **P30 CA-13148 UAB Comprehensive Cancer Center (CCC)**
- **P30 DK074038 UAB Recessive Polycystic Kidney Disease Core Center (RPKDCC)**
- ***P30 AR046031-07 UAB Core Center for Basic Skeletal Research (CCBSR)***

- **P30 DK056336 UAB Clinical Nutrition Research Unit (CNRU)**
- **P60DK079626 UAB UAB Diabetes Research and Training Center (DRTC)**