Transgenic Mouse Facility

UCI Chao Family Comprehensive Cancer Center

Leadership

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Scientific Director

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Managing Director

Further information regarding all offered services can be found at the website:
https://tinyurl.com/yda7q9yy

Mission

Facilitate use of the mouse as a mammalian model genetic system to investigate mechanisms of oncogenesis and testing of cancer therapeutics

To fulfill this mission the **TMF:**

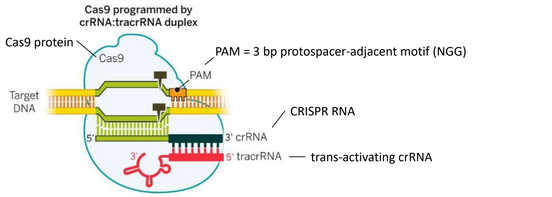
- Provides advice on experimental design and analysis to investigators on the use of genetically modified mice.
- Provides specialized expertise, technical support and obtains reagents required to manipulate the mouse genome and analyze the consequences thereof.
- Develops and communicates awareness of novel mouse-related resources, facilitates their acquisition, and provides practical assistance with their use.

Services

- Consult with, raise awareness and educate Cancer Center members about strategies to modify the mouse genome and to use genetically engineered mouse models in their research programs.
- Genome engineering in mice and ES cells via CRISPR /Cas9.
- Production of gene targeted mouse models using KOMP, EUCOMM ES cells as well as JM8 (C57BL/6N) ES cell-mediated targeting in-house.
- Targeted transgenesis at *Hipp11* (Ch 11) & *ROSA26* (Ch 6).
- Production of small transgene and BAC random-integration transgenic mice.
- Southern analysis of mouse genomic DNA, including probe design and testing.
- Cryopreservation, import, export of mouse embryos and sperm.
- IVF and embryo transfer to re-animate, re-derive, expand or develop large cohorts of identical aged mice for experimental studies.
- Provision of oocytes or pre-implantation embryos for *in vitro* investigation.
- Sourcing of existing mouse models for UCI investigators.
- Molecular genotyping and breeding services.
- Customized services to accommodate investigator.
- Provision of commonly used CRE and FLP expression mice.
- Provision of letters of support for grant applications.
- Provision of materials and methods for manuscripts.

Popular Services (Examples)

CRISPR-mediated Genome Engineering – background and common production pipeline



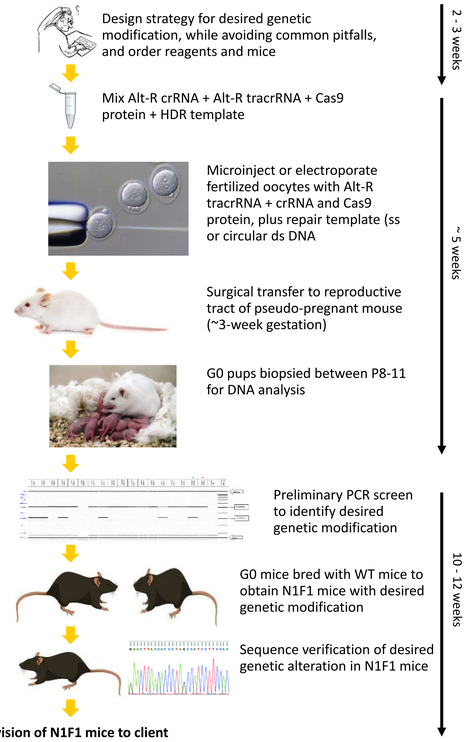
Production of null allele in first generation mice - useful if gene is previously uncharacterized

Repair Template

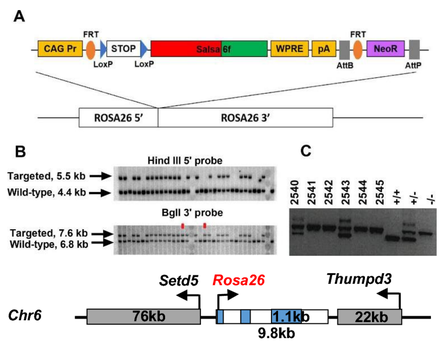
5′ 3′

Examples of HDR templates with commonly requested modifications

- Missense mutation(s)
- Splicing variant
- Enhancer variant
- Floxed allele
- 2A-reporter allele
- 2A-Cre allele
- Humanized exon(s)

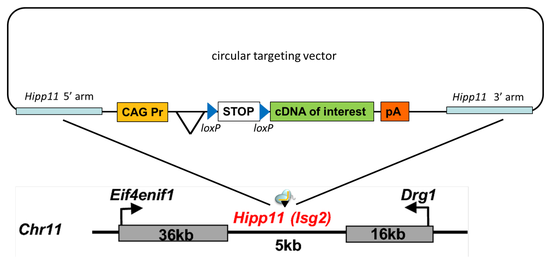


Targeted Transgenesis at *Rosa26* locus in mouse ES cells



Targeted transgenesis at *Hipp11* locus in mouse embryos.

Transgene expression at the *Hipp11* (intergenic) locus can be more consistent than that obtained at *Rosa26* locus. Plasmid transgenes can be targeted with high efficiency (~ 50%) via CRISPR/Cas9. Use of two independent loci enables combination of targeted transgenes.

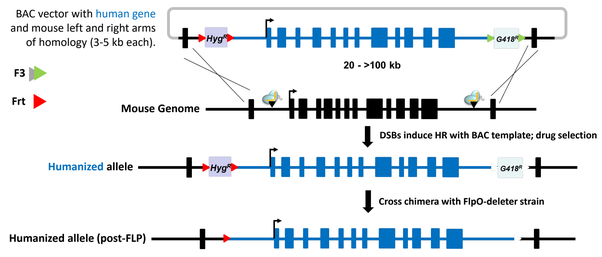


Assisted reproduction to develop large cohorts of mice for experimental studies

- Labs may find it challenging to produce cohorts of age- and sex-matched animals for experimental analysis.
- *In vitro* fertilization (IVF) and embryo transfer (ET) to pseudo-pregnant females can be used to produce mice efficiently for appropriately powered experimental analysis.
- Production of large cohorts of mice for experiments via IVF + ET is a generally under-utilized method to accelerate research using genetically modified mice.

Humanization of mouse loci.

Combining ES cell and CRISPR/CAS9 to substitute mouse genes with their human counterpart.



Key Equipment & Technologies

- Culture and LN_2 cryogenic storage of sperm, embryos, mES cell lines

- Microinjection and electroporation of zygotes / preimplantation embryos

- TaqMan, rhAMP based genotyping via Bio-Rad RT-PCR systems

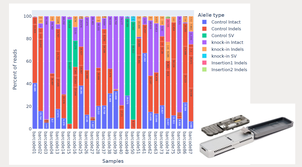
- IVF-based mouse production

- PFGE and Southern analysis using Bio-Rad CHEF Mapper

- High-throughput (3 x 96-well tray) analysis of standard PCR reactions using Agilent zcapillary array Fragment Analyzers

- Multiple animal holding rooms with ventilated cage racks and sterile caging.

- ONT MinION sequencing and Open-source sequence clustering program DAJIN2 analyzes detailed sequence and composition data of CRISPR edits make selection of right founder mice faster.



- Tissue culture suite with incubators, hoods and electroporation apparatus for ES cell culture (not shown)

Under Development

- **Improved production efficiency CRISPR-engineered CRE-conditional (floxed allele) mice via sequential electroporation of pre-implantation embryos.** Electroporation of ssODN containing 1st loxP site at zygote stage, followed by electroporation of 2nd loxP site at 2-cell stage prior to transfer into reproductive tract of pseudo-pregnant female.
- **Faster throughput DNA sequence analysis of targeted ES cells and founder (G0) generation mice generated by CRISPR via targeted sequencing using Oxford Nanopore MinION system.** Applications: Long-range PCR amplicon ligation sequence to identify animals/cells with 2 correct mutations in *cis* (on the same chromosome)