



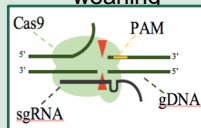
Transgenic & Genetically Engineered Models (TGEMs) Institutional Core

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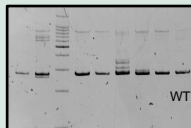
CRISPR/Cas9

Complete CRISPR model design and creation

- Guide design and preparation
- Blastocyst assay to assess activity of guides
- Genotyping design/strategy
- Repair template design
- Compatibility with most efficient guide(s)
- Unique restriction site for downstream screening
- Pronuclear microinjection of 150 embryos
- Transfer of embryos to pseudopregnant recipients
- Optional founder screening
- Transfer of founder litters at weaning



DNA Cleavage by Cas9 endonuclease.



Genotyping of blastocysts by PAGE.

Rodent Model Generation

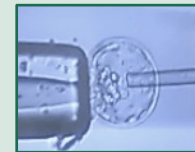
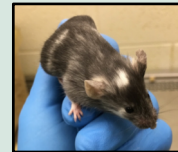
Pronuclear Microinjection / Electroporation

CRISPR/Cas9

- ❖ Loss of function alleles
 - Knockout / indels
 - Large genomic deletions
 - Hypomorphic
- ❖ Targeted insertions
 - Patient mutations
 - SNPs
 - Conditional alleles
 - ROSA26 insertions
 - Reporter tags

Conventional Transgenics

- Reporters
- Overexpression vectors



ES Cell Microinjection

ES Cell Targeting

- Conventional
- CRISPR induced recombination
- CRISPR guides and compatible ROSA26 targeting vector

Preparation of commercially available ES cells (EUCOMM)

Assisted Reproductive Techniques

Cryopreservation

- | | |
|-----------|-----------|
| Mouse | Rat |
| • Sperm | • Sperm |
| • Embryos | • Embryos |
| • Oocytes | |
| • Ovaries | |

Rederivation

- | | |
|--------------------------------------|--------------------------------------|
| Mouse | Rat |
| • In vitro fertilization | • Transfer of cryo-preserved embryos |
| • Transfer of cryo-preserved embryos | |
| • Pathogen-free | |
| • Ovarian transplant | |

Services

Consultation

- Design of transgenic constructs
- Screening strategies
- Breeding schemes

Maintenance of Stock Lines

- Cre strains (ex. Ella)
- Reporters (ex. TdTomato, GFP)
- Male/female breeders and controls (ex. C57B6J)

NIH P30 Support

- Comprehensive Cancer Center (CCC)
- Hepatorenal Fibrocystic Diseases Core (HRFDCC)
- Nutrition and Obesity Research Core (NORC)
- Diabetes Research and Training Center (DRTC)
- The Office of the Vice President for Research (OVPR)



Contact

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