Assisted Reproduction Technologies

When new transgenic lines need to be introduced into animal facilities, or when animals need to be rederived to remove specific pathogens, this Shared Resource is capable of performing a variety of techniques necessary to prevent the introduction of pathogens in the mouse facilities. This includes in vitro fertilization, rederivation, and ovarian transplantation. Specific Pathogen Free animals will be generated and delivered to your facility.

Request services through iLab: https://vanderbilt.corefacilities.org/service_center/show_external/5102

Keywords: rederivation, TMESCSR, service, reproduction, mouse, mice, IVF, embryo transfer, in vitro fertilization

**In vitro fertilization (IVF)**

*In vitro* fertilization requires fresh or frozen sperm from a transgenic male that is used to fertilize oocytes from superovulated female mice. IVF rederivations generally produce larger numbers of pups born when compared to the thawing of one or two straws of embryos for rederivation. After incubation overnight and fertilization, two-cell embryos are transferred into recipient animals. About 19 days later, pups are born and at 3 weeks of age the Vanderbilt Genome Editing Resource staff will wean, tail, and ear punch the pups. The investigator will then screen the tail DNA for transgenic founders and notify the resource of their results. This is also a valuable service for the quick expansion of a mouse colony, providing a large number of animals to establish a colony.

**Line Expansion by IVF**

Establishing a colony of experimental animals from a single founder can be very time-consuming. Sperm from N1 heterozygous males can be harvested and used to fertilize multiple isogenic wildtype embryos. Approximately half of the resulting N2 generation would be heterozygous. Aggressive breeding of heterozygous animals would provide a sufficient number for experimental analysis in the first F1 generation, potentially saving two generations of natural breeding time.

**Embryo Transfer Rederivation**

For rederivations, fresh or frozen 0.5 to 3.5-day old embryos are washed numerous times in sterile medium and are then transferred into recipient females. Seventeen to nineteen days later pups are born and at 3 weeks of age the Vanderbilt Genome Editing Resource staff will wean, tail, and ear punch the pups. The investigator will then screen the tail DNA for transgenic founders and notify the resource of their results. This service is recommended for homozygous lines or lines with multiple genetic mutations.

**Embryo Retrieval and Transfer Rederivation**

For embryo transfer, females are superovulated and bred to males. Embryos are collected the following day, washed to further reduce pathogen transfer and then surgically transferred into pseudopregnant females housed in the barrier facility. Pups will be born about 19 days later and weaned and tailed at 3 weeks of age. This service is recommended for homozygous lines or lines with multiple genetic mutations.

**Ovary Transplants**

For ovary transplantation, ovaries from a transgenic female are removed and transplanted into 1-2 recipient animals. This service, although not frequently utilized, is essential when a valuable line of animals ceases to reproduce and is at risk.

**In Vitro fertilization**

**Rederivation**

**Ovary transplantation**
1. PI submits appropriate service form to VGER
2. Service forms are reviewed and approved by the Co-Directors of the resource
3. IVF is scheduled, mice are ordered
4. IVF is performed
5. The following day, fertilized embryos are transferred to pseudopregnant females
6. Pups born 19 to 20 days post fertilization
7. Pups weaned, tailed and ear punched by VGER staff at 3 weeks of age
8. DAC staff review serology results, approve the movement of mice and transfer transgenic founders to PIs mouse housing room.
9. PI identifies transgenic founders from tail DNA using PCR and Southern blotting
10. PI notifies VGER staff about transgenic founders

Attachment

![IVF_Dishes.jpg](IVF_Dishes.jpg) - Added on March 18, 2016 at 2:48 PM by Jennifer Skelton

IVF dishes sitting in a modular incubator.