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

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Options for the rederivation of mouse lines maintained at Vanderbilt:

**In Vitro fertilization (IVF)**

*In vitro* fertilization requires fresh (non-frozen) 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 retrieval and transfer, the investigator notifies the VGER regarding how many singly housed males per line are planned for rederivation matings. The VGER orders wild-type females and superovulates for embryo production. The superovulated females are provided to the investigator 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.

Options for the rederivation of mouse lines from external sources:

**Frozen Embryo Cryorecovery**

This service can usually be scheduled within two weeks of receiving cryopreserved embryos and is the most efficient means of rederiving a line. Frozen embryos will be accepted at the 2- to 8-cell stage. The embryos will be thawed according to the protocol used by the lab that froze the embryos and washed numerous times before being transferred into a recipient female. Pups will be born approximately 19 days later. At 3 weeks of age, the PI will receive tail biopsies for genotyping, which must be completed before serological testing. Serological testing is routinely performed at 6 weeks of age. When these results have been obtained, usually by 7 weeks of age, the mice are moved to the investigator's room for housing.

**Fresh Embryo Rederivation**

This approach is similar to the frozen embryo rederivation above and is also an efficient means of rederiving a line. However, the performance of this service requires more planning and communication between the lab sending the embryos and the Vanderbilt Genome Editing Resource to prepare for the embryo transfer surgeries. Embryos can be shipped between the 2-cell stage to the morula/blastocyst stage for transfer. The pups will be born approximately 19 days after the embryo transfer. Again, at 3 weeks of age, the PI will receive tail biopsies for genotyping, which must be completed before serological testing. At 6 weeks of age, serological testing is done and by 7 weeks of age, the mice are moved to the investigator's room for housing.

**In Vitro Fertilization**

Another option for rederivation is in vitro fertilization (IVF) using frozen sperm. The performance of IVF and embryo transfer usually requires about a month of planning before the experiment. The day following the IVF procedure, the fertilized embryos will be washed and prepared for an embryo transfer into appropriate recipients. As above, once the embryos are transferred, pups will be born approximately 19 days later. At 3 weeks of age, the PI will receive tail biopsies for genotyping, which must be completed before serological testing. At 6 weeks of age, serological testing is done and by 7 weeks of age, the mice are moved to the investigator's room for housing.

**Incoming Shipments of Embryos or Sperm**

When an investigator would like to import either sperm or embryos to the Vanderbilt Genome Editing Resource (VGER) for cryorecovery, an order will be submitted in Topaz. Health reports will not be required for shipment approval as cryorecovery will produce clean animals.

Topaz order submission will enable DAC to ensure adequate animal numbers are available on the protocol, track shipments, and create cage cards once animals are created.

1. PI submits animal order in Topaz
   a. Select Non-approved eCatalog for vendor
   b. Select both “male” and “female” mice
   c. In Order Notes, document that either sperm or embryos will be received from XXXXX institution for cryorecovery
   d. Place delivery date of July 4th (or close to date if grayed out) for the following year as a “waitlisted” animal

For MTA's, please contact the Office of Technology Transfer at 615-936-7585.
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.

1. PI submits appropriate service form to VGER
2. Service forms are reviewed and approved by the Co-Directors of the resource
3. Rederivation is scheduled, mice are ordered (if required)
4. Rederivation is performed, embryos are transferred to pseudopregnant females
5. Pups born 17 to 20 days following the transfer
6. Pups weaned, tailed and ear punched by VGER staff at 3 weeks of age
7. PI identifies transgenic founders from tail DNA using PCR and Southern blotting
8. PI notifies VGER staff about transgenic founders
9. DAC staff review serology results, approve the movement of mice and transfer transgenic founders to PIs mouse housing room.

1. PI submits appropriate service form to VGER
2. Service forms are reviewed and approved by the Co-Directors of the resource
3. Ovary transplantation is scheduled
4. Ovary transplant is performed
5. Mice will be housed in the barrier facility for 4 weeks
6. Females will be serology tested after 4 weeks
7. DAC staff review serology results, approve the movement of mice and perform the transfer of mice to PI's mouse housing room.