

# TRANSGENIC OUTLOOK



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## EMBRYOLOGY SERVICES

The creation of genetically engineered animal models is developing at a remarkable rate. Coinciding with this growth is the critical need to resourcefully archive germplasm and to safeguard the ever-increasing number of models with reproductive challenges. To complement the embryo cryopreservation program, Transgenic Services has expanded its Embryology Services to provide sperm and ovarian cryopreservation. In addition, comprehensive fertility evaluations and assisted reproductive technologies including *in vitro* fertilization, ovarian transplantation, and intracytoplasmic sperm injection are available.

Transgenic Services is pleased to take this opportunity to provide you with the latest information on our newly expanded Embryology Services. For further information please call 1-877-CRIVER-1, or email [askcrl@criver.com](mailto:askcrl@criver.com).

## SPERM CRYOPRESERVATION

Comprehensive archiving of genetically modified animals should include collecting samples throughout the entire course of model development. Since sperm cryopreservation is fairly economical, sperm cells for a particular line can be stored at the beginning, middle, and end of model expansion. Charles River recommends cryopreserving sperm from at least two germline carriers at less than 6 months of age. Post-thaw motility rates vary significantly from strain to strain.

Sperm is collected from the caudal epididymus and vas deferens of the male reproductive tract, treated with a cryoprotective agent, aliquotted into cryotubes, cooled, and stored in liquid nitrogen. We currently reconstitute lines from frozen sperm on a FVB or B6/F1 background. We also transfer frozen sperm to another institution that offers intracytoplasmic sperm injection (ICSI), the technique used for the generation of offspring from cryopreserved sperm. Charles River will provide ICSI services by the summer of 2004 (see back page).

## OVARIAN CRYOPRESERVATION

Ovarian cryopreservation permits haploid storage of genetic material from valuable female mice. It can also be used for cases in which the animal is aged or in poor condition due to either an infection or adverse phenotype. Ovaries are collected from donor females, rinsed in medium with antibiotics, equilibrated with



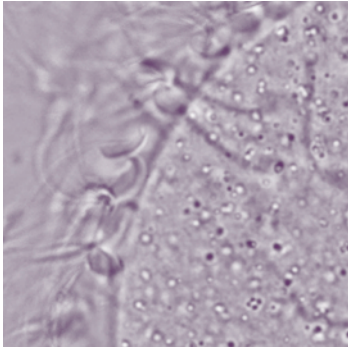
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a cryoprotective agent, slow cooled to ultra-low temperatures, and plunged in liquid nitrogen. Charles River recommends cryopreserving ovaries from at least three germline carriers at less than 6 months of age.

### **IN VITRO FERTILIZATION (IVF)**

*In vitro* fertilization is the process by which mature oocytes are fertilized in a Petri dish with capacitated sperm. IVF is commonly used to generate large numbers of embryos, which can be cryopreserved or transferred to recipient females. It is performed as a rescue effort for aged, ill, or unproductive males or to generate offspring from cryopreserved sperm. It is also used in the production of large numbers of embryos and/or offspring and to decrease the timeline for backcrossing. We are pleased to offer IVF to complement our standard cryopreservation and rederivation services. At Charles River, IVF is commonly performed on C57BL/6, FVB, and B6/F1 hybrid strains.



Sperm penetrating mouse oocyte during *in vitro* fertilization

### **FERTILITY ASSESSMENT**

The comprehensive fertility evaluation aids in identifying the cause of poor reproductive performance and the best route for overcoming infertility. Assessment includes computer assisted sperm analysis and the evaluation of both reproductive performance and genotyping results. Sperm analysis captures sperm parameters such as motility, concentration, and percent progressive.

Males and females can be scored separately or as mating pairs. The female fertility assessment includes estrus synchronization with exogenous hormones (PMSG and hCG), mating to a fertile male, and the ability to produce fertilized embryos.

### **OVARIAN TRANSPLANTATION**

Neonatal and adult ovaries can be transplanted to histocompatible or immunodeficient recipients as a means of overcoming obstacles (x-linked mutations, aged, ill or unproductive females) to rescue valuable female mice. The technique is similar with freshly collected or cryopreserved ovaries. Furthermore, donor ovaries can be split in half, or quartered, resulting in several potential germline carriers.

Ovaries of the recipient female are removed by making a small slit in the ovarian bursal membrane. The stalk between the ovary and oviduct is cut and the ovary is removed. The donor ovary is inserted into the opening in the bursa, where it is physically held in place. Within a few weeks, the blood supply to the ovary will be reestablished, and the ovary should function normally.

### **ARTIFICIAL INSEMINATION**

Artificial insemination (AI) is performed as a rescue effort for aged, ill, or unproductive males or to generate offspring from cryopreserved sperm. AI with fresh or frozen-thawed sperm can be performed surgically or nonsurgically. The sperm from one male is diluted in order to inseminate numerous females - the exact number contingent on sperm quality. The estrus cycle of the recipient female mouse must be synchronized with PMSG and hCG and/or by mating with a vasectomized male to produce a vaginal plug.

## RAPID REDERIVATION

For customers who wish to have a quicker turn-around time for rederived animals, we are now offering a rapid rederivation Service. Starting with a minimum of two proven breeder males at less than 6 months of age, and prepubescent females, 3-4 weeks of age, Charles River will perform up to four embryo transfers using isolator-reared CD-1 females as pseudo-pregnant recipients. Recipient mothers will be maintained in a sterile isolator for two weeks post-embryo transfer. For each line, at least two visibly pregnant females will be returned to the customer. The pre-rederived colony will be terminated seven days following shipment. Rapid rederivation offers a significant price decrease from standard rederivation and a turnaround time of approximately four weeks.

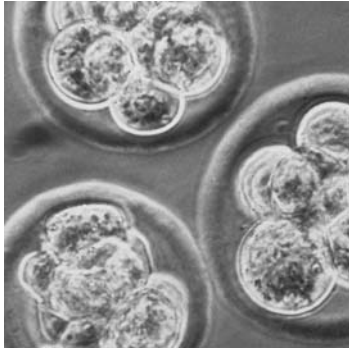


### Comparison Of Standard vs. Rapid Rederivation

#### Standard Rederivation

#### Rapid Rederivation

<p><b>Animal Requirements</b></p> <ul style="list-style-type: none"> <li>• 2 proven breeder males (less than 6 months of age)</li> <li>• At least 10-15 females (3-4 weeks of age)</li> </ul>	<p><b>Animal Requirements</b></p> <ul style="list-style-type: none"> <li>• 2 proven breeder males (less than 6 months of age)</li> <li>• 3-week-old females in quantities and at times requested by Charles River</li> </ul>
<p><b>Procedure</b></p> <ul style="list-style-type: none"> <li>• Upon arrival, all animals will be transferred into the biosecurity of a flexible film or semi-rigid isolator</li> <li>• Males will be bred with superovulated females for collection of preimplantation stage embryos</li> <li>• Embryos will be transferred to recipient females who then carry and deliver the rederived offspring</li> <li>• 4 embryo transfers will be performed</li> <li>• All recipient females used for rederivation efforts are reared within an isolator environment</li> </ul>	<p><b>Procedure</b></p> <ul style="list-style-type: none"> <li>• Upon arrival, all animals will be transferred into the biosecurity of a flexible film or semi-rigid isolator</li> <li>• Males will be bred with superovulated females for collection of preimplantation stage embryos</li> <li>• Recipient mothers will be maintained in a sterile isolator for 2 weeks post embryo transfer</li> <li>• 3-4 embryo transfers will be performed</li> <li>• All recipient females used for rederivation efforts are reared within an isolator environment</li> </ul>
<p><b>Health Monitoring</b></p> <ul style="list-style-type: none"> <li>• Comprehensive Health Monitoring (serology, bacteriology, pathology, and parasitology) and Helicobacter PCR will be performed on each cage housing recipient females</li> </ul>	<p><b>Health Monitoring</b></p> <ul style="list-style-type: none"> <li>• Health monitoring will not be performed on embryo transfer recipients</li> <li>• We cannot guarantee the health status of the rederived litters</li> </ul>
<p><b>Deliverable</b></p> <ul style="list-style-type: none"> <li>• Following rederivation, Charles River will return to you 3 rederived litters or a minimum of 3 rederived breeding pairs</li> <li>• Pre-rederived colony will be terminated when the health status of the rederived colony is confirmed</li> <li>• Charles River cannot guarantee the zygosity of the rederived offspring</li> </ul>	<p><b>Deliverable</b></p> <ul style="list-style-type: none"> <li>• Following rederivation, Charles River will return to you at least 2 visibly pregnant females</li> <li>• The rederivation is considered complete when the recipient females leave Charles River</li> <li>• Pre-rederived colony will be terminated 7 days following the shipment of pregnant females</li> <li>• Charles River cannot guarantee the zygosity of the rederived offspring</li> </ul>
<p><b>Timeline</b></p> <ul style="list-style-type: none"> <li>• 1 week to acclimate and set up matings</li> <li>• 2 weeks of embryo collections and transfers</li> <li>• 3 weeks gestation</li> <li>• 3 weeks weaning</li> <li>• 2 weeks to receive approved health reports</li> </ul> <p>Total time is approximately 11 weeks</p>	<p><b>Timeline</b></p> <ul style="list-style-type: none"> <li>• 1 week to acclimate and set up matings</li> <li>• 1 week embryo collections and transfers</li> <li>• 1 week post surgery recovery of embryo transfer recipients and to coordinate shipping arrangements</li> <li>• 1 week to ship visibly pregnant (10-14 days gestation) females</li> </ul> <p>Total time is approximately 4 weeks</p>



## **INTRACYTOPLASMIC SPERM INJECTION (ICSI): CURRENTLY IN R&D**

ICSI is the process by which a single spermatozoan is mechanically inserted into a donor oocyte in order to induce fertilization. Spermatozoa do not need to be motile, or "alive" in the conventional sense. In fact, with mice, only the head of the sperm cell is actually injected into the oocyte. ICSI is performed as a rescue effort for males with poor quality sperm and to generate offspring from cryopreserved or freeze-dried sperm.

## **CRYO RESERVE**

In conjunction with embryo transfer rederivation, we are pleased to offer Cryo Reserve. Charles River will cryopreserve an additional 100 embryos per line and store them in two separate locations at our facility. Should the need arise (catastrophe or repeat outbreak at your facility), Charles River can immediately perform embryo transfers in order to generate additional rederived offspring. Cryo Reserve is a convenient and cost-effective approach to providing further insurance for valuable research stocks and strains.