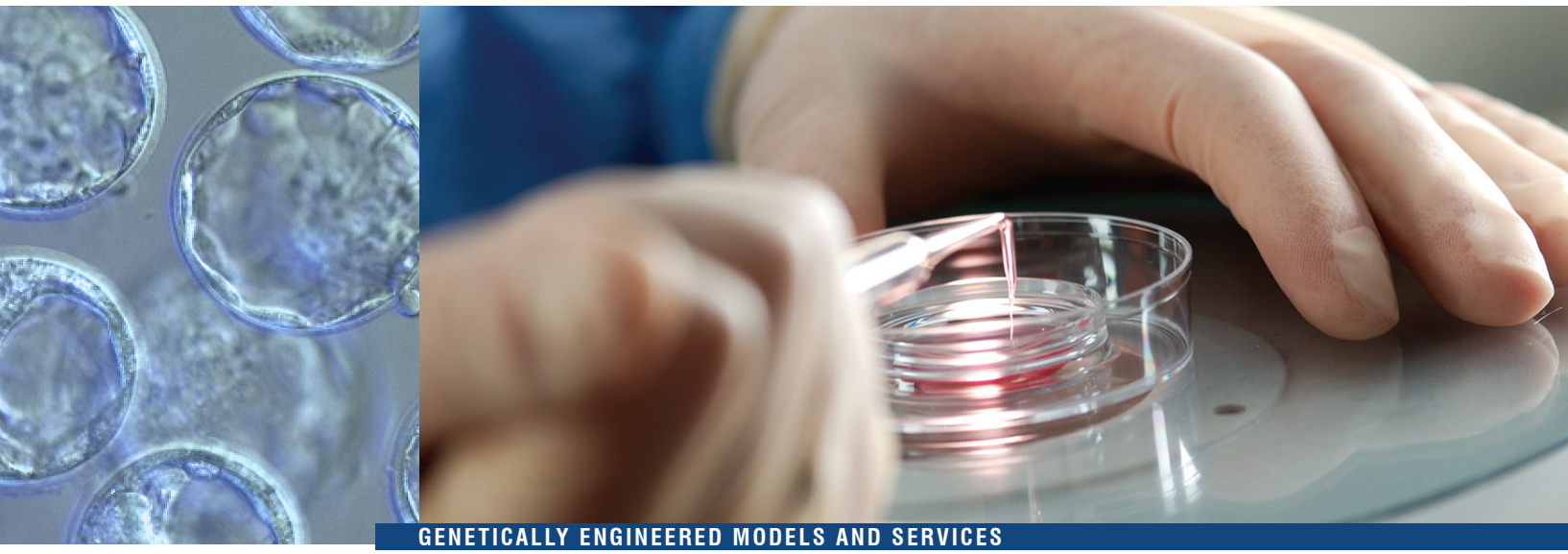
charles river



GENETICALLY ENGINEERED MODELS AND SERVICES

Cryopreservation

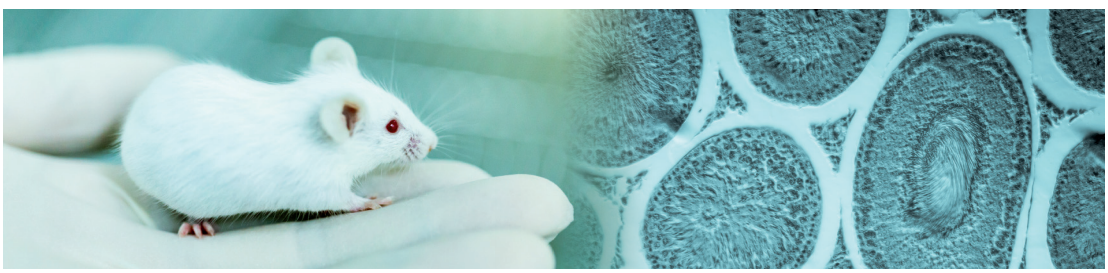
Charles River strengths:

- Over 25 years of experience in the cryopreservation of transgenic lines.
- A dedicated Project Manager and the support of experts to help you define the best strategy to protect the integrity of your line and to prevent genetic drift.
- A partnership with The Jackson Laboratory to provide local supplies of JAX™ Mice to biomedical researchers in Europe. Our breeding facilities also serve as The Jackson Laboratory's local provider of certain research services using JAX™ Mice, including cryopreservation of C57BL/6J or BALB/cByJ.

The Jackson Laboratory

Cryopreservation of rodent strains is vital to maintaining research integrity. It creates a cost-effective backup to live animal colonies in the event of a microbial contamination, catastrophic accident or natural disaster, and/or the cessation or alteration of genetic expression in later generations. Cryopreservation also provides an alternative to maintaining live animals for strains that are not currently being used, but may serve a purpose in the future. This backup can save significant space and animal care resources, allowing better management of the colonies being actively used for research.

Once a cryopreserved stock is created, animal lines can be recovered in as few as 13 weeks with a SOPF - VAF/Elite® health status. This quick recovery time ensures the briefest possible interruption to ongoing experiments in the event of total animal loss.



Cryopreservation European Services

<table>
<tr><th>Service</th><th>Description</th><th>Requirements</th><th>Deliverables (*)</th><th>Quality Control</th></tr>
<tr><td rowspan="4">Embryo Cryopreservation (Mice and Rats)</td><td rowspan="2">Transgenic males x Charles River wildtype females</td><td rowspan="2">Mice:
2 males†

Rats:
6 males†</td><td>HE x WT:
300 embryos cryopreserved</td><td rowspan="4">Included in vitro quality control:
Thawing of embryos and monitoring of in vitro development from 2-cell to 4-8-cell stage.

Optional in vivo quality control:
Thawing of embryos followed by reimplantation into two recipient females for birth check.</td></tr>
<tr><td>HO x WT:
150 embryos cryopreserved</td></tr>
<tr><td rowspan="2">Transgenic males x transgenic females</td><td rowspan="2">Mice:
5 males† + 10 females‡

Rats:
6 males† + 6 females‡</td><td>HE x HE:
200 embryos cryopreserved</td></tr>
<tr><td>All others matings:
150 embryos cryopreserved</td></tr>
<tr><td>Sperm Cryopreservation (Mice Only)</td><td>Freezing of sperm collected from two males</td><td>2 males†</td><td>~15 straws of cryopreserved sperm</td><td>Included in vitro quality control:
Thawing of one straw, IVF, and monitoring of generated embryos development to 2-cell stage.

Optional in vivo quality control:
Thawing of one straw, IVF, and transfer of generated embryos into two recipient females for birth check.</td></tr>
</table>

WT = wildtype; HO = homozygous; HE = heterozygous.
* This assumes the phenotype is not lethal or toxic and does not severely impair reproduction capacity.
† Mice or rat males: <16 weeks old for naïve males or <24 weeks old for proven male breeders.
‡ Mice and rat females: 7-16 weeks old.

Embryo Cryopreservation

Embryo cryopreservation remains the easiest and safest method of long-term cell storage. To successfully cryopreserve a genetically engineered rodent strain, it is important to consider several factors. These factors include: the percentage of thawed embryos that will carry the mutation, the percentage of thawed embryos that will be viable, and the anticipated live birth/weaning rates following embryo transfer.

The exact number of embryos that should be cryopreserved is influenced by the genotype of the animals being used, the background strain of the model, and any special characteristics of a specific lineage. We have decades of experience in freezing rodent embryos and regularly assist clients in determining the appropriate quantity to cryopreserve for each model. Depending on the number of males provided, the background strain of the model, and the specifics of the genetic mutation, we will collect embryos either via live matings or *in vitro* fertilization (IVF). Embryo cryopreservation may also be combined with embryo transfer rederivation to achieve pathogen-free strains of animals.

Animals required for embryo cryopreservation can be bred at Charles River or supplied at regular intervals from the client's facility. While here, all genetically engineered animal lines are housed within flexible film or semi-rigid isolators. The isolator not only guards against microbiological contamination, but also against genetic contamination by physically separating individual lines. Procedures for cryopreservation include collection of preimplantation-stage embryos, treating suitable embryos with a cryoprotective agent, loading the selected embryos into Cryotech™ straws, and freezing the embryos at a controlled rate. Each cryopreservation session is verified via thawing of one straw (20 embryos on average) and *in vitro* development monitoring. This ensures that the model will be safely recovered in the future.

Embryo cryostocks allow for a production of about 25-50 pups on average.

Sperm Cryopreservation

Sperm cryopreservation is becoming a fast, safe, and cost-effective way to protect genetically engineered mouse colonies. With sperm cryopreservation, it is feasible to cryopreserve stocks at multiple stages of model development (e.g., after creation or backcrossing), which provides an opportunity to recover a model from an earlier state if it ever becomes necessary. Charles River offers sperm cryopreservation for all genetically engineered mouse strains, providing a permanent backup for valuable lines.

For some mouse models, sperm cryopreservation may not be the best way to protect a colony due to specific genetics, phenotype, or composition of the model. Our dedicated team of project initiation specialists works with each client to determine if their line is a good candidate for sperm cryopreservation and ensure the success of those that are.

When animals are submitted for sperm cryopreservation, sperm is collected from the caudal epididymis and vas deferens of the male reproductive tract, treated with a cryoprotective agent, aliquoted into straws, cooled, and stored in liquid nitrogen. We recommend cryopreserving sperm from at least two gene-carrier males. The cryopreserved stock is verified using IVF, which is the optimal functional test to ensure the model can be recovered in the future.

Sperm cryostocks allow for a production of about 80-1,500 pups on average.

Embryo and Sperm Storage

Once a model's backup has been successfully created through cryopreservation, it is important to maintain the frozen stock in a tightly controlled facility. At Charles River, we maintain two independent facilities that are equipped with bulk liquid nitrogen storage tanks and are monitored and alarmed 24 hours a day for tank temperature and LN^2 level. All cryopreserved stocks are automatically split between the two storage facilities, providing redundant backup. In addition to storing material cryopreserved by Charles River, we also routinely accept cryopreserved material frozen by our clients for storage. This service provides an offsite backup in the event that a client's facility is compromised.

Shipping

Cryopreserved stocks can easily be transferred from our facilities to anywhere in the world utilizing our dry LN_2 shippers. This allows models to be shared with collaborators without having to ship live colony animals. To help guarantee the integrity of the shipment, the LN_2 shippers can be equipped with a data logger to ensure the temperature of the shipper has been maintained throughout transit. To further protect the cryopreserved models, Charles River recommends splitting the shipped material into two separate transfers, providing a redundancy in the event of a shipping delay.

Genotyping Services

Our full-service, molecular-based genetic characterization laboratory is equipped to run multiple types of allele-specific assays including PCR, qPCR, SNP, and TaqMan® assays within the frame of your cryopreservation process or once your animals are recovered.

Genetic and Health Status Refresh Pack

To ensure the genetic stability of transgenic and mutant mouse lines and increase the reproducibility and validity of *in vivo* studies, Charles River offers the Genetic and Health Status Refresh Pack. This integrated solution provides a simple and economical plan for backcrossing and cryopreserving your lines to eliminate unwanted mutations and refresh your mouse colonies.

Cryorecovery Services

Service	Deliverables
Embryo Reconstitution	Three breeding pairs minimum or 10 pups. Stock recovered from outside institutions will be evaluated for quality, and the number of animals recovered may vary.
Sperm Reconstitution	Three breeding pairs minimum or 10 pups. Stock recovered from outside institutions will be evaluated for quality, and the number of animals recovered may vary.
IVF Rapid Expansion	Recovery numbers are variable, based on available stock.