



GENETICALLY ENGINEERED MODELS AND SERVICES

## Cryopreservation

### Services

- Cryopreservation of rodent strains is vital to maintaining research integrity
- Charles River helps develop cryopreservation programs for our clients
- Programs are based on:
  - Importance of the line
  - Number of genes involved
  - Purpose of cryopreservation

Cryopreservation of sperm and/or embryos 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 eight to ten weeks. This quick recovery time ensures the briefest possible interruption to ongoing experiments in the event of total animal loss.

### How Does Cryopreservation Work?

The biological metabolism of living cells significantly decreases and eventually ceases at low temperatures, permitting the long-term preservation of living cells. There is an obvious contradiction between the concept of low temperature preservation and the fact that living cells can be damaged both by temperatures lower than the freezing point of water (0 °C) and by the cryoprotective agent itself. The key to successful cryopreservation is to minimize the creation of harmful ice crystals during the freezing process to ensure that little or no intracellular ice forms. Furthermore, the cryoprotectant, designed to prevent ice formation, must be relatively nontoxic. Equally important, the cells must be cooled gradually to ensure that they lose water slowly enough to dehydrate without freezing intracellularly, but quickly enough to avoid cell deterioration and death due to dehydration. To overcome this challenge, Charles River utilizes internally developed techniques backed by years of cryopreservation experience that result in minimal cryoinjury and high cryosurvival rates.

EVERY STEP OF THE WAY

## Cryopreservation Services

Service	Description	Requirements	Deliverables
Embryo cryopreservation	<ul style="list-style-type: none"> <li>For heterozygous X wild-type strain</li> </ul>	<ul style="list-style-type: none"> <li>8–10 breeder males between 12 weeks and 6 months old</li> <li>20 females 3–4 weeks old*</li> </ul>	<ul style="list-style-type: none"> <li>250–300 embryos (heterozygous lines)</li> <li>150–200 embryos (homozygous lines)</li> </ul>
Cryopreservation with HO breeding	<ul style="list-style-type: none"> <li>Homozygous strain undergoes preliminary expansion breeding</li> </ul>	<ul style="list-style-type: none"> <li>5 breeding pairs</li> </ul>	<ul style="list-style-type: none"> <li>150–200 embryos</li> <li>Homozygous expansion breeding</li> </ul>
Sperm cryopreservation	<ul style="list-style-type: none"> <li>Cryopreservation of sperm from 2 males</li> </ul>	<ul style="list-style-type: none"> <li>2 breeder males: between 12 weeks and 6 months old</li> </ul>	<ul style="list-style-type: none"> <li>Pre- and post-thaw QC</li> <li>15 straws preserved, most strains</li> </ul>

### 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: a) the percentage of thawed embryos that will carry the mutation; b) the percentage of thawed embryos that will be viable; and c) 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 at Charles River, 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.

---

## **Sperm Cryopreservation**

Sperm cryopreservation is increasingly 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. All samples receive a pre- and post-thaw computerized sperm analysis to provide definitive quality information to ensure the model can be recovered in the future.

## **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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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.

## Cryopreservation and Disaster Preparedness

Cryopreservation is an essential step in safeguarding valuable research strains against loss. Many fail to consider losing their research mice to natural disasters, disease outbreaks, breeding errors and genetic drift. Preserving sperm or embryos dramatically reduces costs associated with mouse colony maintenance, while protecting valuable research strains against disease, breeding cessation and disasters. It also limits genetic drift, prevents loss of phenotype, and enables an easy recovery of specific pathogen-free mice.

Cryopreservation provides an effective, low-cost way to back up live animal colonies or store lines that are not actively being used. At Charles River, embryos and sperm are divided between two independent locations, each with an automated liquid nitrogen filling system and an alarm network to help ensure the security of our clients' valuable animal models.

## Cryopreservation Services

Service	Deliverables
Embryo Reconstitution	10 offspring if recovered from stock cryopreserved and stored at Charles River. Stock recovered from outside institutions will be evaluated for quality, and the number of animals recovered may vary.
Sperm Reconstitution	10 offspring if recovered from stock cryopreserved and stored at Charles River. 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.