

## CRYOPRESERVATION SERVICES

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The use of transgenics, knockouts, and other genetically-modified animal models has resulted in the increased production of valuable lines whose maintenance requires significant space and resources. Cryopreservation allows researchers the opportunity to preserve these unique models without having to maintain expensive nucleus colonies. Additionally, cryopreservation offers protection against the loss of valuable models due to microbial contamination, natural disasters such as fire, flood or earthquakes and most importantly, the cessation or alteration of genetic expression in later generations. Further, cryopreservation allows researchers the ability to transfer rodent models around the world with significantly reduced risk compared to live animal shipments. Charles River recommends cryopreserving all genetically-modified rodent strains as an insurance policy against catastrophic loss. Once embryos, sperm, or ovaries have been cryopreserved, researchers can store the cells inexpensively, but still have the ability to recover live animals in as little as 8-10 weeks. This ensures the briefest possible interruption to ongoing experiments in the event of an unrecoverable animal loss.

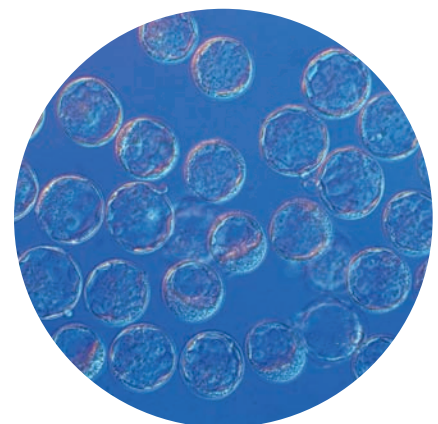
### Embryo Cryopreservation

Embryo cryopreservation remains the easiest and safest method of long-term cell storage. To successfully cryopreserve a genetically-modified 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 embryos transfer. The exact number of embryos that should be cryopreserved is also influenced by the genotype of the animals being used, the background strain of the model, and any special characteristics of a specific lineage. Charles River has decades of experience freezing rodent embryos and regularly assists 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, Charles River will collect embryos either via live matings or via *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-modified 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.

### 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 non-toxic. Equally important, the cells must be cooled gradually to assure 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 proficiency that result in minimal cryoinjury and high cryosurvival rates.





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### Sperm Cryopreservation

Comprehensive archiving of genetically-modified animals should include collecting samples throughout the entire course of model development. Since sperm cryopreservation is economical, sperm cells for a particular line can be stored at the beginning, middle, and end of model expansion. For sperm cryopreservation, we recommend cryopreserving sperm from at least 2 germline carriers. Sperm is collected from the caudal epididymis and vas deferens of the male reproductive tract, analyzed with a computerized sperm analyzer, treated with a cryoprotective agent, aliquotted into cryotubes, cooled, and stored in liquid nitrogen.

### Ovarian Cryopreservation

Ovarian cryopreservation permits haploid storage of genetic material from valuable female mice. It can also be used when the animal is aged or in poor health due to either an infection or adverse phenotype to rescue the line. Ovaries are collected from donor females, rinsed in medium with antibiotics, equilibrated with a cryoprotective agent, slow cooled to ultra-low temperatures, and plunged into liquid nitrogen. We recommend cryopreserving ovaries from at least 3 germline carriers, less than 6 months of age.

### Cell Storage

Clients can receive cryopreserved cells immediately, temporarily store them at Charles River, or use Charles River for long term storage. Inventories stored at Charles River are automatically divided in half and stored in two independent facilities with completely separate alarm and backup systems. As another alternative, cryopreserved cells can be divided between Charles River and the client's facility providing triple redundancy for valuable lines.

### Embryo Quality Control

Quality control is performed on every batch of cryopreserved embryos to verify the success of cryopreservation. Our quality assurance protocols require that 6-10% of every batch of embryos is thawed and cultured for 72 hours. In order to pass QA, at least 80% of the embryos must develop to blastocysts. Charles River's cryopreservation procedures regularly achieve greater than 95% survival of thawed embryos.

We offer scheduled quality control procedures to determine the viability of cryopreserved stocks throughout long-term storage as well as *in vivo* quality control to indicate the viability of embryos post-implantation. Under normal circumstances, colonies can be reconstituted at any time by surgically transferring thawed embryos into pseudopregnant recipient females. Typically, live birth rates following transfer of thawed embryos are equivalent to rates expected with freshly collected embryos. From over 25 years spent learning about metabolic reactions of cells at ultra low temperatures, Charles River is confident that cryopreserved cells, stored under proper conditions, last indefinitely.

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