



Cryopreservation

Permanently archiving your genetically engineered rodent strains through cryopreservation is vital to maintaining the integrity of your research. Cryopreservation of sperm and/or embryos allows you to maintain a cost-effective backup to your live animal colony in the event of a microbial contamination, a 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 in your facility, allowing you to better manage the colonies you are actively using for research.

At Charles River, our project initiation specialists and embryologists will work closely with you to determine the cryopreservation program that best fits your needs based on factors such as importance of the line, number of genes involved, and purpose of cryopreservation. Once a cryopreserved stock is created, you can recover live animals 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.

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 also 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 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 your 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.

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.

Visit www.criver.com/info/quotes
for project estimates



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 (i.e. after creation, after backcrossing, etc.), which provides an opportunity to recover a model from an earlier state if it ever becomes necessary. At Charles River, we offer sperm cryopreservation for all genetically engineered mouse strains, providing a permanent backup to your valuable lines. However, there are some mouse models where sperm cryopreservation may not be the best way to protect your colony due to specific genetics, phenotype, or composition of the model. Our dedicated team of project initiation specialists will work with you to ensure that your line is a good candidate for sperm cryopreservation. 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, aliquotted 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 your model can be recovered in the future.



Embryo & Sperm Storage

Once you have successfully created a backup for your model 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 LN² level. All cryopreserved stocks are automatically split between the two storage facilities, providing redundant backup for your valuable lines. 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 to you in the event that your facility is compromised.

Shipping

Cryopreserved stocks can easily be transferred from our facilities to anywhere in the world utilizing our dry LN² shippers. This offering allows you to share your model with collaborators without having to ship live animals from your colony. To help guarantee the integrity of the shipment, the LN² shippers can be equipped with a data logger to ensure the temperature of the shipper has been maintained throughout transit. To further protect your valuable cryopreserved model, Charles River recommends splitting the shipped material into two separate transfers, providing a redundancy in the event of a shipping delay.