

Cryopreservation provides a safe path for preserving the quality and protection of the genome edited mouse line for any potential projected future experiments.

Cryopreservation benefits include:

1. Safeguards valuable mouse strains against catastrophe
2. Limits genetic drift
3. Protects lines from disease outbreak
4. Protects lines from breeding cessation
5. Eases distribution of mouse lines without the need of shipping live animals
6. Reduces housing and genotyping costs

Keywords: sperm cryopreservation mouse embryo

Expand

Sperm Cryopreservation:

The freezing of mature spermatozoa has become a viable alternative to embryo cryopreservation. Freezing sperm is more cost-effective and less labor-intensive than freezing embryos. Improved protocols developed by The Jackson Laboratories have enhanced previous methods of sperm cryopreservation and reconstitution. The improved protocols yield higher percent viability than when cryopreserving sperm in cryovials.

The investigator is responsible for providing a complete description of the mouse line to be cryopreserved (strain, genotype, etc.). Upon completing the appropriate form and online documentation of the mouse line to be cryopreserved, sperm is obtained from the epididymides and vas deferentia of a male transgenic or knock-out mouse and cryopreserved.

Straws of frozen sperm are stored in liquid nitrogen storage tanks. A yearly storage fee applies to each line stored. These tanks are monitored weekly by personnel of the resource, and cannot be accessed by other individuals.

Sperm cryopreservation requires two male mice that are proven breeders from your colony. This cryopreservation service includes the quality control check of thawing one or two straws of sperm and performing a small scale IVF to produce 2-cell stage embryos. Additional breeding steps may be required if more than one mutation is involved when rederiving a line from cryopreserved sperm.

Timeline:

1. PI submits a service request [iLab Organizer \(corefacilities.org\)](https://corefacilities.org)
2. Service requests are reviewed and approved by the Director and Manager of the facility
3. Experiments are scheduled
4. PI submits transfer request through DAC for males to be moved on the specified cryopreservation date to Vanderbilt Genome Editing Resource procedure room.
5. Cryopreservation is performed and 26 straws are placed into long term storage
6. A small scale IVF is scheduled
7. One to two straws of sperm is utilized for IVF
8. Confirmation of fertilized embryos at the 2-cell stage
9. PI notified about results of IVF

Embryo Cryopreservation:

Cryopreservation provides a safe path for preserving the quality and protection of the transgenic line for any potential projected future

experiments. The investigator is responsible for providing a complete description of the mouse line to be cryopreserved (strain, genotype, etc.) Wildtype (purchased) female mice are superovulated and mated with a minimum of 8 heterozygous or 7 homozygous male stud mice between the ages of 2-6 months that are provided by the investigator. Superovulation, mating, embryo recovery and cryopreservation will occur on a weekly basis until sufficient embryos are obtained. The goal is to cryopreserve a minimum of 250 heterozygous or 150 homozygous embryos. This service is recommended for homozygous lines or lines with multiple genetic mutations.

Embryos are cryopreserved at the 2-cell to blastocyst stage in straws. There is a higher percent viability cryopreserving in straws versus cryovials. Two cell embryos have a higher survival rate than 8-cells embryos because they are less susceptible to osmotic shock.

Embryos can also be cryopreserved after performing an *in vitro* fertilization. Fresh or frozen sperm is cultured with wildtype eggs donated from superovulated females. The resulting fertilized embryos can be transferred to produce live pups or can be cryopreserved at the two-cell stage.

Frozen embryos are stored in liquid nitrogen storage tanks. A yearly storage fee applies for each line. These tanks are monitored weekly by personnel of the resource, and cannot be accessed by other individuals.


Timeline:

1. PI submits a service request [iLab Organizer \(corefacilities.org\)](http://iLab Organizer (corefacilities.org))
2. Service requests are reviewed and approved by the Director and Manager of the facility
3. Experiments are scheduled
4. Cryopreservation is performed and straws are placed into long term storage
5. One to two straws of embryos will be thawed to check embryo viability
6. PI notified about results of viability check

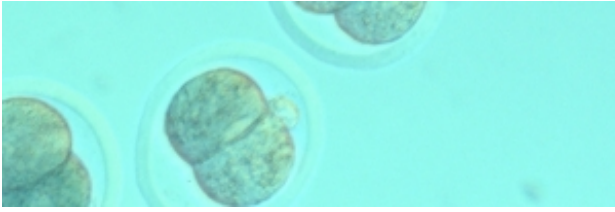
Factor	Embryo	Sperm
Cost to Freeze	Medium to High	Low
Cost to Recover	Low	Medium to High
Time to Freeze	Low to High	Low
Time to Recover	Low	Medium
Reliability	High	Variable
Ease of Use	High	Low to Medium
Genotype Produced	Heterozygous/Homozygous	Heterozygous
Advantages	Can be quickly rederived	Only requires 2 males
Limitations	Requires up to 10 males and females to maintain homozygous lines per cryopreservation	Pups from IVF rederivation will be heterozygous
Optimal Use	Strains with multiple mutations	Strains with single mutations

[Click here to contact the core](#)

Attachments

 [2_cell_embryos.jpg](#) - Added on June 13, 2017 at 1:56 PM by [Jennifer Skelton](#)

Embryos at the 2-cell stage.



 [Biocool.jpg](#) - Added on June 13, 2017 at 1:56 PM by [Jennifer Skelton](#)


Biocool freezer used for embryo cryopreservation.



 [Cassette_Freezer_Box.jpg](#) - Added on March 17, 2016 at 3:08 PM by [Jennifer Skelton](#)

Liquid nitrogen storage box containing cassettes and straws.



 [Cryo_Straws.jpg](#) - Added on March 17, 2016 at 3:08 PM by [Jennifer Skelton](#)

Cryopreservation straws

