ORIP

OFFICE OF RESEARCH INFRASTRUCTURE PROGRAMS

CRYOPRESERVATION 2022

Research and Resources

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ORIP'S MISSION

ORIP advances the NIH mission by supporting infrastructure for innovation. This support is focused on research resources, including animal models for human diseases, cutting-edge scientific instrumentation, construction and modernization of research facilities, and research training opportunities for veterinary scientists. Through continued engagement with NIH Institutes, Centers, and Offices and the biomedical research community, ORIP empowers and expands existing programs and develops new initiatives to support NIH research at the forefront of scientific progress.

NIH National Institutes of Health
Office of Research Infrastructure Programs

OVERVIEW

Animal models are essential to understanding diseases and maintaining health of humans through development of diagnostic approaches and therapeutic interventions. These disease models are being generated at an unprecedented rate due to rapidly evolving technological advancements, such as gene editing. This rapid increase in animal models, however, is creating challenges in how to maintain these critical resources in reliable and cost-effective ways. Long-term preservation of the genetic stock of such models is needed to ensure efficiency, rigor, reproducibility, and transparency in biomedical research.

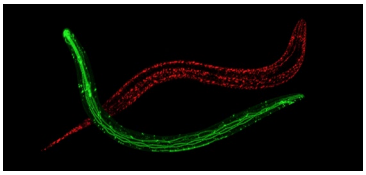
One solution to these challenges is to use long-term germplasm preservation methods. ORIP supports preserving model organisms using established practices, including invertebrate, aquatic, mouse, rat, pig, and nonhuman primate (NHP) models. ORIP also supports research projects on developing efficient germplasm preservation methods, including cryopreservation, for diverse animal models. These projects are improving existing methods to preserve embryos (including NHP embryos), eggs, or sperm for a variety of animal model species or are developing new preservation methods, such as for zebrafish or *Drosophila melanogaster* embryos. Development of effective germplasm preservation technologies will serve the entire biomedical research community by preserving haploid (half) or diploid (full) genetic material and will further promote and conserve valuable animal models for human diseases.

Cryopreservation tank with vials. Photo courtesy of the U.S. Department of Agriculture.

LONG-TERM PRESERVATION OR CRYOPRESERVATION PRACTICES IN ORIP-SUPPORTED CENTERS

Accidents, natural disasters, and other incidents—such as pathogen contamination—can impact ORIP-supported resource centers, resulting in loss of valuable genetic stocks. To address this concern, ORIP partnered with the U.S. Department of Agriculture to store backup collections of *Caenorhabditis elegans*, zebrafish, other aquatic models, and rodents at the National Laboratory for Genetic Resources Preservation (NLGRP) in Fort Collins, Colorado. The NLGRP is the world's largest agricultural gene bank, storing more than 1 million samples from more than 55,000 animals, including livestock and aquatic species used for food consumption. The NLGRP's storage vault, designed to resist earthquakes and floods, offers automated liquid nitrogen tanks for cryopreservation that are self-sufficient for up to 4 weeks.

Long-Term Preservation of *Caenorhabditis elegans* Genetic Stock Collections



Two C. elegans *worms. Images courtesy of Dr. Ann Rougvie, University of Minnesota.*

The *Caenorhabditis* Genetics Center (CGC) supported by ORIP currently maintains more than 22,000 high-quality *C. elegans* genetic strains for distribution to the research community. The CGC routinely uses well-established cryopreservation methods to effectively collect and store long term tens of thousands of *C. elegans* stocks. The CGC makes at least three frozen copies of every stock collected: two copies are cryopreserved onsite but in different buildings at the University of Minnesota, where the CGC is located, and a third copy is transferred to the NLGRP to protect the CGC collection against any local catastrophic event. The latter approach requires safe and reliable long-distance shipment of stock and the maintenance of a separate database for efficient strain retrieval when needed.

Long-Term Preservation of Aquatic Genetic Stock Collections

The Zebrafish International Resource Center (ZIRC) is the largest repository of living and cryopreserved zebrafish genetic stocks (more than 11,000 lines) in the United States. An area of special interest is zebrafish sperm cryopreservation, which has become essential for preserving large numbers of relevant lines for the biomedical research community. ZIRC is capable of storing only the haploid genetic material using this approach and lacks the technology to preserve embryos. The Aquatic Germplasm and Genetic Resources Center (AGGRC) at Louisiana State University is developing reliable cryopreservation methodologies to apply to the NIH-funded *Xenopus*, *Ambystoma*, and *Aplysia* stock centers for the establishment of their own germplasm repositories (repository development).

ORIP also supports development of new technologies, devices, and methods to improve cryopreservation of aquatic species germplasm/embryos. Technologies under development include the following:

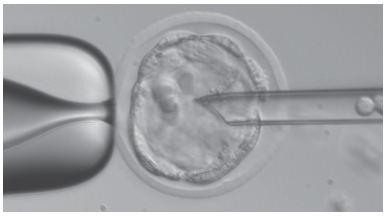
- A system for tracking the precise location of biological samples in freezers or liquid nitrogen containers that includes special vials carrying radio-frequency identification technology and companion hardware and software.
- Construction of an ultrafast cooling device for vitrification (the process of rapid cooling of liquid

medium in the absence of ice crystal formation, an alternative to slow freezing for the cryopreservation of biological material) of cells and tissues.

Long-Term Preservation of Mouse Genetic Stock Collections

As one of the largest biorepositories and genome resources in the world, the Mutant Mouse Resource and Research Centers (MMRRC) creates, cryobanks, cryorecovers, and distributes scientifically valuable genetically modified mouse models to investigators. After extensive quality control assessments, mouse lines deposited by research scientists into the MMRRC system are cryopreserved in one or more formats, such as sperm, embryos, or embryonic stem (ES) cells. The MMRRC develops and applies a variety of high-fidelity cryopreservation protocols (e.g., slow freezing, rapid freezing, vitrification) defined by multiple critical factors (e.g., cryoprotectant type and concentration, cell type and developmental stage, cooling rate, warming rate, cryocontainer type) to prevent cells and tissues from cryodamage caused by cold or osmotic shock (e.g., ice crystal formation, solution effect, oxidative stress) and ensure cryorecovery. The MMRRC also provides consultation on cryopreservation protocols, practices, and procedures to the research community, including emergency services.

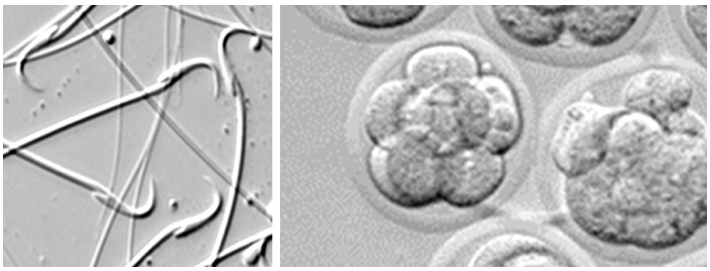
Long-Term Preservation of Rat Genetic Stock Collections



A rat blastocyst injection. Image courtesy of Dr. Elizabeth Bryda, University of Missouri.

Gamete cryopreservation is the optimal method for archiving rat strains and stocks used in research. Cryobanking is highly recommended to preserve valuable rat models; protect against model loss due to environmental disaster, human error, or the need to temporarily reduce animal colonies, as was experienced during the COVID-19 pandemic; and enable genetic refreshment of live colonies to protect against genetic drift. Methods that allow efficient embryo freezing are best for preserving rat models. It also is possible to cryopreserve rat spermatozoa, although current approaches are not reliable or routine and require further development. As one of the few rat repositories in the world, the Rat Resource & Research Center (RRRC) maintains a biobank of cryopreserved rat strains and stocks and has extensive expertise with cryopreservation methods and cryorecovery techniques, including intracytoplasmic sperm injection, that are needed to re-animate cryopreserved strains and stocks.

The goal of the Hybrid Rat Diversity Panel (HRDP) is to establish a panel of 96 genetically and phenotypically diverse inbred rat strains as a resource for complex trait mapping and systems genetic approaches. The HRDP includes strain rederivation, whole-genome sequencing, phenotypic characterization, tissue biobanking, and cryopreservation to ensure long-term stability and availability. After establishing living colonies for each HRDP strain, re-cryopreservation begins with embryo collection and storage to provide stock maintained at the Medical College of Wisconsin and the RRRC for archiving, distribution, and preparation for disaster recovery.



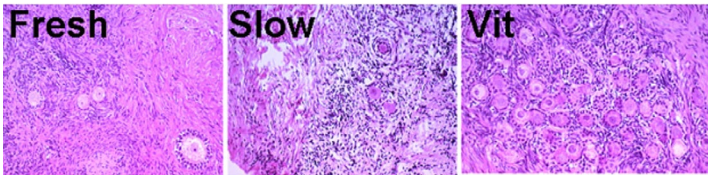
Sperm and embryos from a biobank of cryopreserved rat strains. Images courtesy of Dr. Elizabeth Bryda, University of Missouri.

Long-Term Preservation of Pig Genetic Stock Collections

Pluripotent stem (PS) cell biobanking and cryopreservation are important for regenerative medicine, species preservation, and animal biotechnology. To date, however, lack of stable and bona fide PS cells from pigs and other mammalian species greatly hampers such efforts. Investigators from The University of Texas Southwestern Medical Center have successfully derived nine ES cell lines from pig blastocysts using a novel ES cell culture condition developed in the laboratory. These pig ES cells can be recovered efficiently after multiple freeze/thaw cycles and maintain genetic and epigenetic stability after long-term culture, thereby offering invaluable material for animal biotechnology.

Long-Term Preservation of Nonhuman Primate Genetic Gametes and Embryos

The Oregon National Primate Research Center (ONPRC) includes support for an Assisted Reproductively Technology (ART) Core, which encompasses macaque sperm and embryo cryopreservation. Sperm cryopreservation represents the gold standard for macaques but is cumbersome and time consuming. Hence, the ART Core is exploring and optimizing vitrification and post-thaw retrieval methods. Although female gamete (oocyte) preservation has not been established in NHPs, safeguarding of macaque ovarian cortex using vitrification followed by



Sections of macaque ovarian cortical tissue before (Fresh) and after slow-freezing (Slow) and vitrification (Vit). Primordial and primary follicles are preserved after slow-freezing, but secondary follicles and stroma show cryodamage. All classes of follicles are preserved, and stromal damage is minimal after vitrification. Images courtesy of Dr. Mary Zelinski, ONPRC.

future transplantation is under development. Methods of trophectoderm biopsy of blastocysts for genotyping followed by macaque embryo cryopreservation allow fertility preservation and creation of gene-edited disease models.

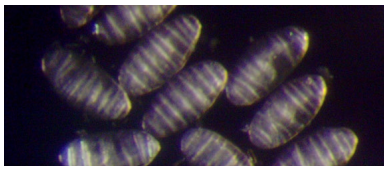
Recently developed genome-editing technologies, such as the CRISPR/Cas system, have opened the door for generating genetically modified NHP models for a variety of biomedical fields. These technologies also allow generation of reporter animals for testing genome-editing approaches for development of new somatic cell therapies. The Oregon Health & Science University and Massachusetts Institute of Technology are working on generating reporter rhesus macaques and marmosets, respectively. To generate gene-edited NHP embryos following reporter integration requires an efficient blastocyst cryopreservation and post-thaw recovery. Improving these procedures will affect the overall ability of the biomedical community to generate, preserve, and share new NHP models.

IMPROVEMENT OR DEVELOPMENT OF LONG-TERM PRESERVATION AND CRYOPRESERVATION METHODS

Drying, Storing, and Reanimating Egg Germinal Vesicles to Preserve Fertility

Using the domestic cat, ORIP-supported investigators are examining the impact of compaction, desiccation, and storage at room temperature on an egg's DNA and its ability to reconstitute an oocyte that can undergo meiosis, fertilization, and embryo development to term. Epigenetic studies that explored the protein content of the germinal vesicle revealed that desiccation in a controlled environment leads to less damage than cryopreservation. Researchers collaborated with bioengineers and developed optimized microwave-assisted drying and storage of trehalose buffer. This drying method resulted in less structural damage to germinal vesicles of feline oocytes compared to cryopreservation. Follow-up research has focused on desiccation of early oocyte stages in the ovarian cortex. Findings could have widespread practical application to more effective and economical germplasm preservation, propagation, and management.

Drosophila Cryopreservation and Rewarming for Long-Term Storage



Dehydrated Drosophila *embryos. Image courtesy of Dr. John Bischof, University of Minnesota.*

Drosophila stock preservation currently relies on the labor-intensive and costly method of passing live cultures to fresh food, which imposes a huge burden on fly laboratories and stock centers to maintain *Drosophila* stocks. ORIP supports development of reliable, manageable, and cost-effective cryopreservation methods for long-term storage and effective revival of *Drosophila* embryos. Investigators are augmenting pre-cryopreservation procedures by determining optimal conditions for permeabilization solvent concentration, embryo immersion time, cryoprotective agent (CPA) concentration and treatment time, and embryo stage for permeabilization and CPA treatment. Optimization of vitrification and warming procedures by determining cooling and warming conditions of embryos is another area of need, as is enhancement of post-warming procedures by developing effective post-warming culture methods. Researchers supported by ORIP recently published a robust and efficient method for cryopreservation of *Drosophila melanogaster* embryos by optimizing key steps described above. The optimized method resulted in more than 10% of embryos developing into fertile adults after cryopreservation for 25 distinct strains from different sources. The further optimization and wide adoption of this cryopreservation protocol will have a significant impact on long-term preservation of *Drosophila* stocks for biomedical research.