



**National Center for
Research Resources**

NATIONAL INSTITUTES OF HEALTH

Achieving High-Throughput Repositories for Biomedical Germplasm Preservation

Natcher Conference Center

April 10-11, 2007

Final Workshop Report

June 4, 2007

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1. Executive Summary

The workshop entitled, *Achieving High-Throughput Repositories for Biomedical Germplasm Preservation*, sponsored by National Center for Research Resources (NCRR), National Institutes of Health (NIH), and co-sponsored with the National Institute of Child Health and Human Development (NICHD), took place at the NIH Natcher Conference Center on April 10-11, 2007.

The need and purpose for convening the workshop were related to the large numbers of genetically-engineered animals (i.e., laboratory mouse, laboratory rat, pig, nonhuman primate, and aquarium fish) from multiple species that have been created, or will be created, as a result of NIH Roadmap and Trans-NIH initiatives, such as the Knockout Mouse Project, Target-Selected Mutagenesis in Zebrafish, and Neuromouse Project. Novel preservation technologies, improved cryopreservation methods, and high-throughput processes are urgently needed to ensure that living animals, cryopreserved germplasm, and related biological materials are readily available to biomedical investigators at low cost.

The workshop was divided into four sessions. Each session was moderated, consisted of invited presenters, and allowed ample time for discussion by the participants. The first session focused on the current state-of-the-art for the cryopreservation of sperm, oocytes, embryos, ovarian tissue, testicular tissue, and embryonic stem cells for each of the target species listed above.

The second session focused on examples of equipment and processes for achieving high-throughput germplasm preservation. Two presentations by commercial companies described current equipment, processes, and new prototypes. A third presentation described the National Animal Germplasm Program, a component of the United States Department of Agriculture's Agricultural Research Service. That presentation described experiences in developing and applying high-throughput processes for the cryopreservation of germplasm from domestic livestock species, including cattle, sheep, pigs, goats, chicken, aquatic species, and agriculturally important insects.

The third session consisted of six presentations on subjects concerning cryopreservation methods and optimization of preservation protocols. Topics included mechanistic and empirical approaches for developing improved cryopreservation methods, an update on mechanisms of chilling injury and cold shock, and presentations on promising and emerging technologies, such as evaporative drying of sperm, ultra-rapid cooling procedures using nanofluids and microfabrication techniques, and a new optimized cryopreservation procedure for mouse sperm.

The fourth session consisted of five presentations on important physiological, reproduction, biosecurity, disease transmission, genetic and epigenetic factors that are not inherently "cryobiological" but play increasingly important roles in ensuring the success of germplasm cryopreservation, and development of state-of-the art animal model repositories.

Overall, there were 24 presenters, including moderators of sessions. There were a total of 70 participants, including NIH-supported extramural and intramural researchers, NIH program and review staff, USDA intramural scientists, and commercial suppliers of high-throughput equipment for germplasm cryopreservation.

At the end of each session and following the last session, wide-ranging discussions were moderated by members of the workshop advisory committee and workshop organizers. Participants were asked to provide comments and suggestions on the opportunities and needs to address the current roadblocks to the efficient collection, cryopreservation, distribution, and use of animal germplasm. The major [Recommendations](#) are listed below.

2. Purposes and Objectives of the Workshop

The purposes and objectives of the workshop were to:

- assess the status of germplasm cryopreservation for the following animal models for translational research: laboratory mouse, laboratory rat, pig, nonhuman primate, and aquarium fish;
- summarize current problem areas, especially the availability of germplasm preservation technologies, and special challenges encountered by some of the target species;
- identify gaps in current scientific knowledge, such as the mechanisms of injury during cold shock, chilling injury, and other limiting factors;
- evaluate novel and emerging technologies that may increase the efficacy and efficiency of germplasm preservation, such as high-throughput and scalable germplasm preservation capabilities; and
- identify the most promising areas for potential research support.

3. Summary Of Presentations And Discussion

Session 1. Summaries of Germplasm Preservation for Each Target Species

Introduction and Overview: A select group of workshop participants constructed a template to develop qualitative measures of the current status of germplasm cryopreservation for each target species (mouse, rat, swine, rhesus macaque, aquarium fish), and “germplasm format” (sperm, embryo, oocyte, ovarian tissue, testicular tissue, embryonic stem cells). The overall assessment was based on several considerations. First, the extent to which each germplasm format could be used to establish a bank of cryopreserved germplasm. Second, the availability of appropriate assisted reproduction technologies, such as semen collection, artificial insemination, and intracytoplasmic sperm injection. Third, advantages and disadvantages of genetic factors such as haploid gametes inbred and outbred strains, and pluripotent embryonic stem cell lines. Fourth, the need for high-throughput and scaleable technologies for germplasm collection, evaluation, processing, and cryopreservation.

The “template” analysis indicated extensive variation in the efficiency, efficacy, and front-end/back-end costs of germplasm collection, cryopreservation, and re-establishment of living animals for each of the target species. Generally speaking, sperm cryopreservation is the most successfully used method and has been successfully applied to the mouse, swine, and zebrafish, but rat sperm cryopreservation is much less effective. Embryo cryopreservation has been

demonstrated to be effective for the cryopreservation of mice and rats. Progress has been made in the cryopreservation of swine embryos, but litter sizes are reduced, presumably due to chilling injury. Cryopreserving germplasm from the rhesus macaque is especially challenging, due to the difficulties of collecting high-quality sperm.

Session 2. Examples of Equipment and Processes for High-Throughput Repositories

Introduction and Overview: High-throughput and scalable technologies are required to address the large numbers of genetically-engineered animals from multiple species that have been created, or will be created, as a result of NIH Roadmap and Trans-NIH initiatives, such as the Knockout Mouse Project, Target-Selected Mutagenesis in Zebrafish, and Neuromouse Projects.

Technical directors from Minitube of America, Inc., and Cryo Bio Systems presented their respective product lines of equipment, consumables, and germplasm storage systems for collection, processing, testing, and cryopreservation of animal germplasm. The focus of the presentations was on current and planned equipment, supplies and protocols for high-throughput and scaleable germplasm preservation technologies. Discussions covered high security containers that reduce cross-contamination and biosecurity issues, equipment for semen processing and quality control, manual and automated filling, sealing and barcode printing on semen containers, programmable freezing and vitrification equipment, sperm sexing technology, microfluidic processing of embryos and sperm, livestock cloning, and laser-assisted hatching technologies.

A presentation by a representative from the National Animal Germplasm Program, a component of the USDA's Agricultural Research Service, described experiences in developing and applying high-throughput processes for the cryopreservation of germplasm from domestic livestock species, including cattle, sheep, pigs, goats, chicken, aquatic species, and insects of economic importance.

Session 3. Cryopreservation Methods and Optimization of Protocols

Introduction And Overview: The design and optimization of cryopreservation and thawing protocols continues to be a central topic in cryobiology research since the first report of successful cryopreservation of semen and hatching of chicks following insemination in 1949, and the birth of the first mammal (bull) following thawing and artificial insemination of a heifer in 1951.

Two presentations discussed the current status of strategies to design and optimize cryopreservation protocols. In the past three decades, two schools of thought have emerged. The first employs empirical methods to optimize the rate of cooling and concentration of cryoprotectant. The second approach is to measure a limited number of "fundamental" parameters (esp., water and cryoprotectant permeability coefficients) and use computer modeling to predict the appropriate cooling and warming conditions. Both approaches have been successful, and workshop participants agreed that both approaches have merit.

A presentation updating the current status of cold shock and chilling injury indicated that little progress has been made in addressing these bottlenecks. The general consensus was that additional research is needed to understand the mechanisms leading to chilling injury.

Two presentations described promising new emerging technologies for the preservation of animal germplasm. One report described progress towards the long-term preservation of mouse sperm following evaporative drying in a trehalose-EGTA solution and storage at temperatures as low as -80°C . Although the partially dried sperm did not retain motility, in some cases oocytes could be fertilized by intracytoplasmic sperm injection and the embryos can develop into normal mice following transfer to recipient females. Another report described progress on ultra-rapid cooling, using novel technologies such as micro-fabrication techniques, oscillating heat pipes, and nanofluid that might attain cooling rates one thousand fold higher than current technologies.

The final presentation reported an optimized protocol for the cryopreservation of mouse spermatozoa for C57BL/6J, C3H/HeJ, BALB/cByJ, FVB/NJ, 129S1/ScImJ and other standard strains of mice. The principle elements contributing to the improved viability of thawed mouse sperm include the addition of antioxidants to the cryopreservation medium, and uniform cooling and warming of the sperm containers during the cryopreservation and warming.

Session 4. Physiological, Reproduction, Disease and Epigenetic Considerations

Introduction and Overview: The successful cryopreservation of germplasm depends on practical and biological factors that not directly related to cryobiology. Considerations include the choice of germplasm format, the roles of physiological and reproduction, issues relate to the quality of germplasm, the detection and elimination of transmissible diseases, and the interplay between genetics and epigenetics. Five presentations discussed the biological factors that underpin the successful cryopreservation of animal germplasm.

The first presentation focused on the choice of germplasm format. The available choices are determined by species characteristics and biological limitations. Factors to consider include the availability of suitable cryopreservation protocols, costs of cryopreservation, storage, and rederivation of living animals. Whenever circumstances permit, the development of a high-throughput pipeline for germplasm preservation should be established. In other cases, opportunities may allow scalable preservation processes to be applied.

Limited opportunities exist to improve the quality of animal germplasm, either by adhering to proper management conditions, manipulating reproductive cycles, or acting proactively to address known damage mechanisms.

Biosecurity was identified as an essential component for germplasm repository activities. Although vertical transmission of disease is well understood, attention must also focus on broader issues, such as “horizontal transmission” that may result in the release of pathogens or exotic species into the environment. Germplasm repositories must be cognizant of the responsibility to ensure that risks be identified and minimized. Health testing programs can be

used to detect the vertical and horizontal transmission of disease. Once disease is identified, “best practice” analyses should be applied to decide what actions are needed.

The final presentation of the workshop updated participants on the status of epigenetic factors that may result in different phenotype outcomes that are not due to environmental or genetic factors. Examples of epigenetic effects include gametic imprint, X-chromosome inactivation, differentiation imprint, and cell migration. Subtle genetic and epigenetic effects are difficult to study and may or may not be important. The epigenetic effects on cryopreservation are largely unstudied, and most studies indicate no problems. However, researchers should be aware of the potential problems related to epigenetics.

4. Recommendations

The participants of the workshop provided comments and suggestions on the most important topics and priorities during the discussion sessions following each presentation, session, and the final discussion session of the workshop. Additional comments and suggestions were received via e-mail shortly after the workshop.

The major recommendations include:

- encourage the development of high-throughput and scalable technologies for germplasm collection, evaluation, processing and cryopreservation;
- establish multi-disciplinary teams to establish new approaches to the collection; cryopreservation and distribution of germplasm for high-priority translational species;
- support research on biosecurity of cryopreserved animal germplasm, and the detection and elimination of laboratory animal pathogens that might compromise research findings;
- support research to address long-standing bottlenecks to cryopreservation of animal germplasm, such as cold shock, chilling injury, protocol optimization, male-to-male variation; and
- support novel “high-risk/high-return” preservation technologies that are not dependent on freezing or cryopreservation and break new ground.

5. Conclusions

The workshop represented a unique opportunity to assemble the majority of animal germplasm experts in the USA to interact with experts in the fields of cryobiology, physiology, reproduction, biosecurity, animal health, animal husbandry, genetics, and epigenetics.

6. Contact Information

For more information about this workshop, please contact:

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For more information about the National Center for Research Resources, please visit:
www.ncrr.nih.gov.

Achieving High Throughput Repositories for Biomedical Germplasm Preservation Workshop

Natcher Conference Center—National Institutes of Health

April 10–11, 2007

Tuesday, April 10, 2007

- 8:00 am – 8:30 am **Registration and Continental Breakfast**
- 8:30 am – 8:40 am **Welcome**
Franziska Grieder, D.V.M., Ph.D., National Center for Research Resources (NCRR)
- 8:40 am – 8:50 am **Charge to the Workshop Participants**
Louise Ramm, Ph.D., NCRR
- 8:50 am – 9:00 am **Definition of High Throughput Repositories**
Moderator: William F. Rall, Ph.D., NCRR
- 9:00 am – 10:30 am **Status of Germplasm Cryopreservation by Species and Germplasm Format**
Moderator: Kent Lloyd, D.V.M., Ph.D., University of California, Davis
- Current Status of the Achieving High Throughput Repositories for Mouse and Rat Germplasm Preservation**
Presenter: John Critser, Ph.D., University of Missouri, Columbia
- High Throughput Preservation of Swine Germplasm**
Presenter: Phillip Purdy, Ph.D., U.S. Department of Agriculture (USDA)
- Preservation of Germplasm of Nonhuman Primates**
Presenter: Catherine VandeVoort, Ph.D., University of California, Davis
- Current Status of Cryopreservation in Aquatic Species**
*Presenter: Terrence Tiersch, Ph.D., Louisiana State University
Agricultural Center*
- 10:30 am – 10:50 am **Break**
- 10:50 am – 12:00 pm **Examples of Equipment and Processes for High Throughput Repositories**
Moderator: Terrence Tiersch, Ph.D.
- Cryo Bio System**
Presenter: Brad Belstra, Ph.D., IMV International Corporation

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State-of-the-Art Research and Development for Maximizing Germplasm Preservation

Presenter: John Dobrinsky, Ph.D., Minitube International Center for Biotechnology

The National Animal Germplasm Program’s Development and Use of High Throughput Processes

Presenter: Harvey Blackburn, Ph.D., USDA

12:00 pm – 1:00 pm

Lunch (on your own)

1:00 pm – 2:30 pm

Panel Discussion: Cryopreservation Methods and Optimization of Protocols

Moderator: George E. Seidel, Ph.D., Colorado State University

Fundamental (Mechanistic) and Empirical Methods: Fundamental Approaches

Mechanistic

Presenter: John Critser, Ph.D.

Empirical

Presenter: Stanley Leibo, Ph.D., University of New Orleans

Chilling Injury and Cold Shock

Presenter: Stanley Leibo, Ph.D.

2:30 pm – 2:50 pm

Afternoon Break

2:50 pm – 5:00 pm

Summary of Evaporative Drying of Mouse Sperm

Presenters: Mehmet Toner, Ph.D., Massachusetts General Hospital; and Kent Lloyd, D.V.M., Ph.D.

Ultra-Rapid Cooling Using Oscillating Heat Pipe, Nanofluid, and Microfabrication Techniques

Presenter: John Critser, Ph.D.

Improving Mouse Sperm Cryopreservation

Presenter: Robert Taft, Ph.D., The Jackson Laboratory

5:00 pm

Meeting Adjourns

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Wednesday, April 11, 2007

- 8:00 am – 8:30 am **Continental Breakfast**
- 8:30 am – 10:30 am **Panel Discussion: Physiological, Reproduction, and Disease Considerations**
Moderator: Thomas Kuehl, Ph.D., Scott and White Memorial Hospital
- Choice of Germplasm Formats**
Presenters: Kent Lloyd, D.V.M., Ph.D., and Phillip Purdy, Ph.D.
- Improving Gamete and Embryo Quality**
Presenters: James Graham, Ph.D., Colorado State University; and George E. Seidel, Ph.D.
- 10:30 am – 11:00 am **Break**
- 11:00 am – 12:00 pm **Biosecurity for High Throughput Cryopreservation**
Presenter: Terrence Tiersch, Ph.D.
- 12:00 pm – 1:00 pm **Lunch (on your own)**
- 1:00 pm – 2:50 pm **Guidelines for Health Testing**
Presenter: Lela Riley, Ph.D., University of Missouri
- Epigenetics**
Presenter: George Seidel, Ph.D.
- 2:50 pm – 3:00 pm **Break**
- 3:00 pm – 4:00 pm **Group Discussion: Formation of a Consensus**
Moderators: Advisory Committee Members
- 4:00 pm **Meeting Adjourns**

Workshop Agenda



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