U.S. Department of Health and Human Services
National Institutes of Health
Division of Program Coordination, Planning, and Strategic Initiatives
Office of Research Infrastructure Programs

Cryopreservation and Other Preservation Approaches for Animal Models Workshop

Session III. Cryoresearch: Supporting Technology and Resources

September 30, 2024
Virtual Meeting

Final Report

Table of Contents

Executive Summary

The Cryopreservation and Other Preservation Approaches for Animal Models Workshop was held in six sessions to address topics related to cryopreservation and other preservation methods, including but not limited to: (1) the needs and scientific status of cryopreservation and other preservation of gametes (sperm, oocytes, and embryos), reproductive tissues, larvae, and whole animals and their production of live offspring after revival; (2) emerging cryopreservation and other preservation methods and technologies, as well as how to optimize and implement them; (3) methods, technologies, and infrastructure to assess the impact of intrinsic and extrinsic factors on the quality, efficiency, and success of cryopreservation and other preservation protocols and revival, including scalability and reproducibility; (4) the sharing of technologies, including hands-on training for cryopreservation best practices and training of next-generation scientists; and (5) the preservation and management of samples, from collection to utilization.

Cryoresearch: Supporting Technology and Resources, the third of the six sessions of the workshop series, addressed topics related to the technology and resource needs of the cryopreservation research community. The goals of the workshop were to improve the understanding of challenges and technologies associated with sustaining the viability, physiological and functional integrity, maintenance, and sharing of animal models and other biological samples via cryopreservation. These technologies and resources are relevant to promoting the rigor, reproducibility, and translatability of animal research to develop interventions and therapeutics for human health. One session focused on recent scientific advances and physical infrastructure in cryobiology, and a second session focused on enabling technologies and resource management. The workshop panelists and participants identified challenges related to understanding the biophysics of cryopreservation, discovering new cryoprotective agents, physical and logistic infrastructure required for biorepositories, and the need to develop a workforce trained in cross-disciplinary sciences at the convergence of engineering and medicine.

Session Chair
Robyn Tanguay, Ph.D., Oregon State University

Presenters
Wah Chiu, Ph.D., Stanford University
Xu Han, Ph.D., CryoCrate LLC and Wake Forest University
Xiaoming (Shawn) He, Ph.D., University of Maryland
Adam Higgins, Ph.D., Oregon State University
Yoed Rabin, D.Sc., Carnegie Mellon University
Michael Sheldon, Ph.D., Sampled
Mehmet Toner, Ph.D., Harvard Medical School

Session III Organizing Committee

Robyn Tanguay, Ph.D., Session Chair, Oregon State University
Wah Chiu, Ph.D., Stanford University
Xu Han, Ph.D., CryoCrate LLC and Wake Forest University
Adam Higgins, Ph.D., Oregon State University
Yoed Rabin, D.Sc., Carnegie Mellon University
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Workshop Report

Opening Remarks

Monika Aggarwal, Ph.D., Office of Research Infrastructure Programs (ORIP), Division of Program Coordination, Planning, and Strategic Initiatives (DPCPSI), Office of the Director (OD), National Institutes of Health (NIH)
Yong Chen, Ph.D., ORIP, DPCPSI, OD, NIH
Franziska Grieder, D.V.M., Ph.D., ORIP, DPCPSI, OD, NIH
Robyn Tanguay, Ph.D., Oregon State University

Dr. Monika Aggarwal welcomed the participants to the meeting and introduced Dr. Franziska Grieder, who provided an overview of ORIP's mission—"Infrastructure for Innovation"—and structure. Dr. Grieder shared that ORIP comprises two divisions: the Division of Comparative Medicine (which supports research centers and resources, research project grants, and training programs for veterinary scientists) and the Division of Construction and Instruments (which sustains physical research infrastructure through construction and instrumentation awards and an equipment program). ORIP also supports small businesses through Small Business Innovation Research and Small Business Technology Transfer programs. Dr. Grieder reviewed the Division of Construction and Instruments' physical infrastructure programs, including the C06 Construction Program for supporting modern research laboratories, the S10 Shared Instrumentation Programs for state-of-the-art instruments, and the S15 Equipment Program for modern research-supporting equipment to enhance the operations in biomedical research facilities.

Dr. Grieder highlighted challenges encountered in supporting animal resources. Diverse animal models—which include worms, flies, aquatic animals, rodents, pigs, and nonhuman primates—require different strategies for maintenance and distribution. These cryopreserved animal models must be maintained constantly and efficiently to ensure experimental rigor and reproducibility, while also upholding reliable animal welfare standards. The rapid growth of animal resource development due to genome editing breakthroughs has increased costs for physical infrastructure and personnel at animal resource centers, but the NIH budget has remained relatively flat. Dr. Grieder pointed out that reliable cryopreservation was one way to address many of these challenges. She explained that the remaining three sessions in the series of six workshops will cover rodents, nonhuman primates, and swine.

Dr. Aggarwal emphasized that Session III will review the status of the current scientific state of cryopreservation and other preservation approaches, as well as gaps in the field and emerging technologies. She encouraged attendees to participate actively in discussions to identify enabling technologies, best practices, and resource management strategies for advancing cryoresearch. The goals of the workshop are to improve the maintenance and sharing of animal models and other biological samples and to promote rigor, reproducibility, and translatability of animal research to develop interventions and therapeutics for human health. Dr. Aggarwal expressed appreciation to the members of the workshop's organizing committee and introduced Dr. Robyn Tanguay, who reviewed the agenda and noted that input from the meeting would be compiled into a report.

Session 1: Technological Advances and Physical Infrastructure in Cryobiology
Moderator: Rosaly Correa-de-Araujo, M.D., Ph.D., M.Sc., National Institute on Aging (NIA), NIH

Keynote Presentation: Technological Advances in Cryobiology
Mehmet Toner, Ph.D., Harvard Medical School

Dr. Mehmet Toner shared an overview of the history of cryobiology and recent technological advances in the field, highlighting the dual drivers of biodiversity preservation and increasing use of gene-editing techniques in model organisms. Dr. Toner displayed a phase diagram demonstrating where various approaches to biopreservation (e.g., freeze–thaw, vitrification, desiccation) fall along the axes of temperature and cryoprotective agent (CPA) concentration. He noted challenges associated with navigating the biopreservation landscape, including controlling when, where, and whether ice is formed. Slow freezing requires low CPA concentration and involves ice formation outside the sample. Cryomicroscopy studies have provided insight into the physicochemical basis of cryobiology. Cryomicroscopy analyses have shown that during slow cooling, extracellular ice formation causes cellular dehydration via the rapid exit of water from cells. Cellular damage arises from increases in intracellular ionic concentration. During rapid cooling, cells are damaged by intracellular ice formation. Thermodynamic and theoretical analyses have advanced the ability to predict cellular responses to freezing, enhancing process optimization.

Vitrification involves rapid cooling in the presence of high CPA concentrations and/or CPA cocktails to avoid ice formation altogether. During vitrification, the cooling rate and temperature can be manipulated to almost completely inhibit the formation of ice. Reagents like glycerol increase the viscosity of a solution and reduce the cooling rate needed to achieve vitrification. The presence of CPAs can alter the vitrification temperature of a sample. Molecular approaches are being used to engineer the production of CPA-like sugars within cells genetically, and biomimetic agents are being used to inhibit ice crystallization and deliver CPAs into samples. Cellular metabolism can be regulated to increase resilience during cryopreservation. Organ vitrification has been achieved using 7- to 8-molar CPA cocktail solutions. Dr. Toner noted that warming rates and cooling rates are equally critical for avoiding recrystallization and devitrification, both of which are deleterious to biological samples. Novel techniques involving nanobeads, lasers, or radiofrequency coils currently are being developed for rapid and uniform warming that is enabling vitrification of organisms and tissues with unique needs and structures (e.g., pancreatic islets, zebrafish embryos, marine invertebrate games, coral larvae).

Explorations into alternative cryobiology strategies also are underway. Metabolic suppression—which certain animals use to achieve "suspended animation" and survive supercooling at high subzero temperatures with partial or no ice formation—is one avenue being investigated. For example, rat and human livers have been stabilized in supercooled states for short-term storage. Isochoric (constant pressure) cryopreservation prevents damage due to increased intracellular ionic concentration during freezing and has several applications in cryopreservation, organ transplantation, and food storage.

Challenges in Cryogenic Preservation of Macromolecules, Cells, and Tissues for Cryogenic Electron Microscopy (Cryo-EM) and Cryogenic Electron Tomography (Cryo-ET) Structural Investigations
Wah Chiu, Ph.D., Stanford University

Dr. Wah Chiu reminded the participants that multiscale imaging, spanning fluorescence microscopy (~200-nanometer [nm] to 500-nm resolution) to crystallography (0.1-nm to 0.3-nm resolution), is essential for understanding the complexity of biological processes. He described single-particle cryo-EM, which involves imaging proteins, nucleic acids, or molecular machines suspended in vitreous ice that are preserved by rapid plunge freezing into liquid ethane. The 2D particle images, which are oriented randomly within the vitreous ice, are classified and combined into a computed 3D structure. Vitrification for cryo-EM must be achieved through the rapid cooling of samples with small mass. A 1-micron (µm)-

thick layer of pure water cooled at 1 million degrees Celsius per second (°C/sec) can be vitrified in the range of microseconds to milliseconds, depending on exact cooling and thermal gradients. Dr. Chiu reviewed the basic requirements for a vitrification apparatus and shared images of an instrument built by members of his laboratory and more recently developed commercial models. He shared a 1.27-angstrom (Å) cryo-EM density map of apoferritin—a benchmark sample protein, which showed high-resolution structure details, including polypeptide side-chain atoms, water molecules, and ions. The formation of "bad ice" (i.e., crystalline ice) remains a challenging problem with current vitrification methods of many samples. Another concern is that vitrified samples might exhibit preferential orientation, aggregation, or denaturation patterns, leading to reconstruction of inaccurate structures; however, this issue often can be overcome by adding detergent to the sample. Dr. Chiu listed promising avenues for cryo-EM research, which include further understanding the chemistry of vitrifying biomolecules of various sizes in different chemical environments, optimizing individual samples, improving various freezing mechanisms, developing alternative support films to minimize macromolecule denaturation and optimize image contrast, and improving the sample transfer mechanism.

Dr. Chiu reviewed principles of cryo-ET, a technique in which samples are flash or high-pressure frozen to preserve their native state and then are incrementally tilted along an axis while multiple 2D images are captured. The images are merged using computational techniques to reconstruct a 3D tomogram. Dr. Chiu shared cryo-ET images visualizing SARS-CoV-2-infected liver cells and Chikungunya virus budding from a cell membrane to infect other cells. He noted that larger tissues can be frozen whole, milled, and imaged sequentially in a process known as serial cryogenic focused ion beam (cryo-FIB) scanning EM (SEM) imaging. Cryo-FIB SEM 3D-volume imaging of mouse brain tissue shows detailed subcellular structures, like mitochondria and other organelles. The remaining challenges in vitrifying cells and tissues for cryo-ET include developing a deeper understanding of the vitrification process in different cell environments, preserving the native structures of tissues after extraction from the organisms and before vitrification, developing high-pressure freezing instruments that do not require antifreeze chemicals, and improving the mechanism of transferring vitrified samples between imaging instruments. Dr. Chiu highlighted the almost exponential growth of EM structures during the past decade. He recommended the formation of interdisciplinary teams of physicians, engineers, computational scientists, and structural biologists to explore this new research opportunity to characterize ultrastructural organization in healthy and diseased tissues and cells.

Discovery of New Cryoprotective Agents for Improved Cryopreservation
Adam Higgins, Ph.D., Oregon State University

Dr. Adam Higgins discussed his efforts to develop more efficient methods for discovering new CPAs. Commonly used CPAs include propylene glycol, dimethyl sulfoxide (DMSO), and formamide, which share similar properties (e.g., small molecular size, organic composition, hydrophilicity) that can be used to identify potential CPAs. Dr. Higgins noted that more CPAs are used in mixtures because they appear to exhibit synergies. For example, despite increasing a sample's CPA concentration, the addition of DMSO can protect against the damaging effects of formamide. The potential for discovering new synergistic mixtures has not yet been widely explored.

Dr. Higgins reviewed approaches developed by his group for high-throughput screening of CPA candidates, which will be enhanced with a machine learning (ML) algorithm integrated into an active learning loop for CPA discovery. He described screens for characteristics like glass formation (by chemical concentration), membrane permeability, and toxicity at low and high concentrations. Preliminary screens identified 22 of 27 assessed chemicals with appropriate permeability and toxicity characteristics to serve as CPAs. Of the 22 candidates, 14 chemicals passed an additional toxicity screen at higher concentrations that utilized automated multistep methods and temperature control. Approximately 100 combinations of these chemicals were tested at even higher concentrations. At

6 molal concentrations, 20% of CPA mixtures had significantly higher viability than both single-CPA solutions, and at 12 molal concentrations, 15% of mixtures had significantly higher viability than both single-CPA solutions. This screening approach presents a significant opportunity to identify improved CPAs and CPA combinations at an accelerated rate.

Life in Nano Ice®: Achieving Biocompatible Cell and Tissue Cryopreservation Through Nanoscale Cubic Ice Formation and Molecular Assembly Technologies
Xu Han, Ph.D., CryoCrate LLC and Wake Forest University

Dr. Xu Han highlighted numerous challenges in the field of cryopreservation, including the storage and distribution needs of fast-advancing "living drug" industries. He explained that traditional cryopreservation necessitates dependence on liquid nitrogen facilities and that the use of cell-permeating CPAs combined with spontaneous expansion and recrystallization of hexagonal ice in solutions often leads to cryopreservation failures. Dr. Han described his theory of biocompatible cryopreservation, which would achieve efficient cryoprotection without using cell-permeating CPAs. In his theory, biocompatible cryopreservation could be achieved by producing a layer of thermally stable nanoscale ice crystals that coats the cell's surface. In practice, his team's development of the "Hexagonal-ice Ablation on Nanoscale" (HAN) mechanism—which uses a biocompatible polymer-based molecular assembly on the cell surface to generate stable cubic ice (i.e., an ice structure distinct from hexagonal ice)—successfully fulfills his theoretical prediction.

The HAN mechanism for biocompatible cryopreservation enables long-term storage in standard −80°C freezers and eliminates the need for CPAs and liquid nitrogen. This method was developed into a commercial product called OdinSol®, which is being finalized to significantly improve cryopreservation of induced pluripotent stem cell (iPSC)–derived tissues, corneal tissues, human skin and blood, and immune cells for immunotherapy. OdinSol has been modified for cryopreservation of organoids and derived into DionySol® (a biocompatible tissue decellularization and ultrastructural preservation medium), and the polymer-based molecular assembly alone can be used to engineer advanced 3D organoid models. With support from ORIP and the National Eye Institute, Dr. Han's group has developed biocompatible cryopreservation devices like InstaVitria® (to quench liquid nitrogen evaporation on very large surfaces) and IcyEye® (a cryopreservation device for corneal tissue that filters out damaging ice). They also are involved in efforts to develop a "Life in Nano Ice®" cryo-inventory network, in which industry partners and academic collaborators synchronize tissue and blood donation and transplantation with the demands of the "living drug" industry. His group also has developed DoriSol® for cryopreservation of samples from marine invertebrates (e.g., sponges) without permeating CPAs at −20°C to facilitate biodiversity restoration.

Discussion

- A participant asked how researchers should choose between slow freezing or vitrification for cryopreserving model organisms. Dr. Toner explained that vitrification is difficult to implement and should be avoided where possible. However, certain cold-sensitive organisms (e.g., pig embryos) are best preserved via vitrification.

- Dr. Michael Sheldon asked whether organoids exhibit normal developmental progression after biocompatible cryopreservation. Dr. Han responded that the organoids that were cryopreserved by his team at the 2-week stage could develop for an additional 2 weeks after thawing. He noted that in this experiment, organoids could not be cultured for longer than 2 weeks after thawing using current organoid-culturing technology.

Session 2: Enabling Technologies, Best Practices, and Resource Management
Moderator: Xu Han, Ph.D., CryoCrate LLC and Wake Forest University

Keynote Presentation: Enabling Technology for Low-Temperature Preservation—From Underlying Principles to Unmet Needs
Yoed Rabin, D.Sc., Carnegie Mellon University

Dr. Yoed Rabin presented an engineering perspective on cryopreservation science and technological advancements in the field. He reviewed temperatures traditionally accessed for clinical organ preservation (0°C to 10°C for hours), high subzero tissue preservation (−25°C to 0°C for hours to days), vitrification (indefinite storage below −135°C), and long-term storage of certain cell and tissue specimens in liquid nitrogen (−196°C, indefinitely). Dr. Rabin noted that although temperature values are most frequently used for reference, the complexity of the multiphysics involved in cryopreservation success is largely underappreciated. Cryopreservation success is affected by a combination of the thermal history, pressure history, fluid mechanics, solid mechanics, crystallization kinetics, toxicity, environmental factors, and protocols used for specimen storage and recovery. Enabling technologies have been developed to minimize the harmful effects associated with those effects while controlling ice formation in small specimens. However, scaling up by five orders of magnitude to cryopreservation of bulky tissues and large organs (e.g., whole liver) remains an outstanding challenge. Computer modeling can help bridge that gap while adopting a mechanistic approach to cryopreservation research and applying the underlying scientific principles. Dr. Rabin presented several examples in which engineering modeling has improved cryopreservation outcomes, such as geometric modeling of the liver vasculature to study CPA loading, thermal modeling of the heart to study organ recovery from cryogenic storage using nanowarming, and ultrasound-based geometric modeling of the ovary to assess its potential preservation for women undergoing cancer treatment. These approaches addressed thermal aspects of cryopreservation while also preserving structural integrity and preventing fractures. The same modeling approaches and computational tools are translational to other organs and to a variety of cryopreservation approaches.

Concepts from thermodynamics, crystallization kinetics, and heat transfer have traditionally been applied to address challenges related to controlling ice formation and reducing toxicity. Unfortunately, expertise from the traditional disciplines of fluid mechanics, solid mechanics, electromagnetism, medical imaging, and geometric modeling has been underutilized for tackling questions of material behavior, tailoring and optimizing preservation protocols, and designing new instrumentation. Dr. Rabin has developed several devices to measure material behavior during cryopreservation. One such device is the cryomacroscope, which is used as an add-on with a commercial, controlled-rate cooler. This device enables visualization of large-size specimens during the cryopreservation process to correlate the quality of the cryopreserved product with fractures, ice nucleation and crystal growth, and other physical events. Dr. Rabin commented that developing the mechanistic approach to cryopreservation research will require advanced computational tools, biomedical data integration using artificial intelligence (AI) and machine learning (ML), and expanding the workforce involved in preservation research. He added that target applications, logistics, and cryopreservation needs must be sufficiently mapped to prioritize knowledge acquisition and instrumentation development.

The Importance of Keeping Your Cool—State-of-the-Art Biorepositories Play an Essential Role in Safeguarding and Disseminating Cryopreserved Biomaterials
Michael Sheldon, Ph.D., Sampled

Dr. Sheldon introduced Sampled, a company that offers biomedical storage, project management, multi-omic analysis, research, and transport services. Sampled originated as the Rutgers University Cell and DNA Repository (RUCDR) in 1999. RUCDR was established to provide secure and centralized processing and storage of biomaterials with enhanced quality control. In 2020, RUCDR became an

independent commercial organization called Infinity BiologiX, and in 2021, Infinity BiologiX acquired a company called Roylance Pharma and gave the combined entities a new name. Sampled's state-of-the-art biorepository facilities in New Jersey include a 100,000-square-foot dedicated biorepository and a multi-omics, sample processing, and cell services laboratory. A Sampled storage facility is located in the United Kingdom. Sampled stores approximately 11 million cells and tissue samples at ambient, low, high subzero, and low subzero temperatures. Dr. Sheldon noted that modern biorepositories require critical infrastructure, including bulk liquid nitrogen tanks, super-insulated vacuumed lines, automated tank filling, constant monitoring, and emergency and disaster backup plans. Sample acquisition requires a full audit trail documenting all sample movement and custody until storage. Sampled utilizes a laboratory information monitoring system (LIMS) that is fully compliant with College of American Pathologists and Clinical Laboratory Improvement Amendments regulatory requirements and has developed a customized user-forward interface to maximize sample visibility and data management.

Dr. Sheldon emphasized the need for centralized biorepositories that archive valuable resources, such as genetically engineered mice, current good manufacturing practice (cGMP)–grade cells for translational and clinical studies, and phenotyped samples from subjects affected by a particular disease. Centralization ensures the quality and integrity of these resources and offers the widest access to investigators worldwide. Integrated analytical biobanks can offer additional services, such as quality control, biosample processing, and in-house multi-omics analysis. Technological innovations in cryopreservation methods, reagents, monitoring systems, and automated storage are bringing the field forward and are particularly vital for critical materials, such as those stored in reproductive clinics.

An Automatic Ice-Seeding Cryovial (aiCryovial) for Enhanced and Convenient Cell and Tissue Cryopreservation
Xiaoming (Shawn) He, Ph.D., University of Maryland

Dr. Xiaoming (Shawn) He explained that cryopreservation is an enabling technology that can provide ready availability and wide distribution for cell-based medicine. However, reagents used to seed extracellular ice during slow freezing (i.e., ice nucleators) often are not biocompatible and hinder compliance with cGMP regulations. Sand is biocompatible and a natural mediator of ice seeding. Dr. He's group fabricated polydimethylsiloxane (PDMS) films embedded with sand and incorporated them into conventional cryovials to generate automated ice-seeding cryovials (aiCryovials). The sand was pre-sifted to ensure homogeneous particle sizes, and X-ray spectroscopy was used to confirm the presence of sands on the PDMS film. Samples frozen in aiCryovials experienced consistent ice seeding at higher subzero temperatures than those frozen in standard cryovials (with or without PDMS film). Human iPSCs frozen in aiCryovials exhibit increased immediate viability upon thawing, increased attachment efficiency (i.e., long-term viability), and improved functional survival (based on pluripotency marker analysis and guided differentiation) compared to iPSCs frozen in conventional cryovials. Similar improvements were observed when mouse ovarian follicles were frozen and thawed in aiCryovials. Dr. He noted that the aiCryovial technology is being commercialized by HOHCells, LLC.

Discussion

- Dr. Han asked about potential regulatory requirements for organ cryopreservation media and cryopreservation devices. Dr. Rabin responded that these requirements should be tailored to the particular application or protocol. For example, nanoparticles are notoriously difficult to remove from tissue samples, and assessments should be made about how to remove them and their long-term effects if they remain in the tissue.

- A participant asked about the rate-limiting steps of biorepository logistics that most urgently require attention. Dr. Sheldon noted the need for more precise and sophisticated devices to monitor sample temperature during transportation.

- In response to a question about whether aiCryovial technology is equally effective for single-cell iPSC suspensions and iPSC clumps, Dr. He remarked that single cells were not separated from cell clumps during the aiCryovial experiments. He noted that iPSCs were grown in the presence of a Rho kinase inhibitor, which enables the survival of dissociated iPSCs.

- Another participant asked whether particles with rounder shapes might be less harmful to cells than sharp sand particles. Dr. He speculated that the sharpness of the sand might be damaging to particularly large or delicate samples; however, no damage was observed in samples of relatively large ovarian follicles.

Group Discussion
Moderator: Robyn Tanguay, Ph.D., Oregon State University

Dr. Tanguay opened the discussion with a question-and-answer session involving the workshop's committee members, moderators, and speakers.

- A participant asked Dr. Chiu about the process for freezing his samples, which involves plunging them into ethane instead of liquid nitrogen. Dr. Chiu explained that Dr. Jacques Dubochet proposed plunging samples in ethane to induce a faster cooling rate than liquid nitrogen; vitrification is determined by cooling rate. Dr. Dubochet won the 2017 Nobel Prize in Chemistry for this contribution to the development of cryo-EM for the high-resolution structure determination of biomolecules.

- When asked whether cryo-EM can be used to determine different ice structures, Dr. Chiu affirmed that ice structures can be visualized in cryo-EM images or diffraction patterns.

- A participant asked whether additives are used to promote vitrification. Dr. Chiu noted that, in general, no additives are used for macromolecule studies. CPAs are sometimes added when larger tissue samples are analyzed. Dr. Chiu expressed interest in testing CPAs for toxic effects and investigating the use of antifreeze proteins.

- In response to a question about the accuracy of using sample transparency to assess vitrification in his high-throughput assay, Dr. Higgins agreed that the method is crude but added that it is a reasonable approach for estimating the chemical concentrations required to prevent visible ice formation. Dr. Higgins emphasized that larger ice crystals are more damaging during cryopreservation. Dr. Tanguay asked Dr. Higgins a follow-up question about the need for an additional level of viability assessment. He agreed on the potential need to include additional assays (and possibly systems) in the screening pipeline.

- Dr. Chiu noted that common CPAs often are used as reagent solvents and might exert biological effects. He shared an example of a drug targeting coronaviruses that must be dissolved in DMSO; treating coronaviruses with DMSO alone resulted in notable changes to the viral structure. Dr. Sheldon remarked that these results indicate that Dr. Higgins should include an assessment of viral toxicity in his screening pipeline.

- Dr. Chiu stated that his group evaluated the structure of COVID-19 vaccines after leaving them at room temperature for several hours before freezing. Changes were observed, but they have not been correlated with altered efficacy.

- In response to a participant question, Dr. Higgins noted that his group has not yet evaluated potential CPAs during slow-cooling processes. He added that different chemical characteristics will be required of CPAs used during slow cooling.

- When asked about Sampled's LIMS, Dr. Sheldon explained that LabVantage software, which supports regulatory compliance, has been customized for use by the company. Sampled previously used a customized version of STARLIMS but switched to accommodate the needs of pharmaceutical clients. Both systems are very capable.

- When asked about Sampled's backup system, Dr. Sheldon explained that both dynamic (cryostorage maintained at temperature and activated upon emergency) and static (storage in a remote facility) systems are in use.

- A participant asked about supply chain risks related to liquid nitrogen delivery. Dr. Sheldon responded that a distributor has access to Sampled's tank monitor and delivers liquid nitrogen via tanker truck when needed. More concern has been expressed about interruptions to fuel supply for the backup generators; Sampled acquired both diesel and natural gas generators to mitigate these concerns.

- In response to a question about storage in liquid nitrogen versus storage in a freezer, Dr. Sheldon noted that storage in liquid nitrogen is preferred because freezers are cost prohibitive.

- Dr. Tanguay asked the presenters to share more information about their experiences with AI/ML approaches. Dr. Rabin highlighted the complexity of cryopreservation and the difficulty of determining how AI frameworks make decisions. He noted the significant amount of human error experienced when developing cryopreservation techniques. Dr. Higgins expressed interest in developing an AI model that can be trained on molecular parameters to predict such characteristics as membrane permeability or concentration required for vitrification.

- A participant asked about monitoring sample temperature during shipping or other processes. Dr. Sheldon explained that this level of monitoring is required for certain samples (e.g., samples for clinical trials).

- When asked about the long-term stability of aiCryovials, Dr. He noted that the sand-embedded PDMS film is stable enough for long-term storage at low temperatures. In response to a follow-up question from Dr. Tanguay, Dr. He confirmed that no equipment in addition to that used for conventional cryovials is required for freezing samples in aiCryovials. He added that seeding ice at higher temperatures than the aiCryovial is undesirable because large ice crystals that can damage cells form under these conditions during the thawing step of a typical slow-freezing cryopreservation protocol.

- Dr. Tanguay asked the panelists about the challenge of managing the integrity and quality of samples stored in biorepositories. Drs. Toner and Rabin discussed the need to assess more physicochemical properties of cryopreserved samples stored under different conditions. Dr. Sheldon commented that sample misidentification is an important source of errors that affect

sample integrity. Repositories should develop fingerprinting systems to confirm sample identities.

- Dr. Toner noted the need to develop desiccation techniques to enable storage of mammalian samples at ambient temperature.

- Dr. Tanguay commented on the difficulty of developing broad best practices in a field that is so dynamic. Dr. Rabin suggested that improved computer models would advance the state of cryopreservation science while saving time and money and reducing human error. Dr. Toner noted that biotechnology companies also will expand the field and develop processes for quality control.

- Drs. Tanguay and Toner emphasized the need for cryopreservation workforce development. Dr. Tanguay remarked that funding for cross-disciplinary training would be very beneficial.

- Drs. Toner and Rabin noted that adapting current cryopreservation techniques to large animal models likely will require novel CPAs and tailored protocols.

Concluding Remarks
Xiang-Ning Li, M.D., Ph.D., ORIP, DPCPSI, OD, NIH

Dr. Aggarwal introduced Dr. Xiang-Ning Li, who presented a brief summary of the workshop. He highlighted challenges and opportunities identified during the workshop, including the need to develop a deeper understanding of the cellular responses to cryopreservation; the need for novel, synergistic CPAs; and the potential presented by computational models of ice formation. Dr. Li noted that ORIP can support needs related to buildings, instrumentation, and equipment that were identified during the meeting. He thanked the session chair, workshop organizing committee, speakers, and attendees for their participation and adjourned the workshop.

Appendix A: Meeting Agenda

Session III. Cryoresearch: Supporting Technology and Resources

Virtual Meeting
September 30, 2024

11:00 a.m.–11:15 a.m. **Opening Remarks**
Monika Aggarwal, Ph.D., Office of Research Infrastructure Programs (ORIP), Division of Program Coordination, Planning, and Strategic Initiatives (DPCPSI), Office of the Director (OD), National Institutes of Health (NIH)
Yong Chen, Ph.D., ORIP, DPCPSI, OD, NIH
Franziska Grieder, D.V.M., Ph.D., ORIP, DPCPSI, OD, NIH
Robyn Tanguay, Ph.D., Oregon State University

11:15 a.m.–12:40 p.m. **Session 1: Technological Advancements and Physical Infrastructure in Cryobiology**
Moderator: Rosaly Correa-de-Araujo, M.D., Ph.D., M.Sc., National Institute on Aging, NIH

Keynote Presentation: Technological Advances in Cryobiology
Mehmet Toner, Ph.D., Harvard Medical School

Challenges in Cryogenic Preservation of Macromolecules, Cells, and Tissues for Cryogenic Electron Microscopy (Cryo-EM) and Cryogenic Electron Tomography (Cryo-ET) Structural Investigations
Wah Chiu, Ph.D., Stanford University

Discovery of New Cryoprotective Agents for Improved Cryopreservation
Adam Higgins, Ph.D., Oregon State University

Life in Nano Ice®—Achieving Biocompatible Cell and Tissue Cryopreservation Through Nanoscale Cubic Ice Formation and Molecular Assembly Technologies
Xu Han, Ph.D., CryoCrate LLC and Wake Forest University

12:40–1:00 p.m. **Lunch Break**

1:00–2:05 p.m. **Session 2: Enabling Technologies, Best Practices, and Resource Management**
Moderator: Xu Han, Ph.D., CryoCrate LLC and Wake Forest University

Keynote Presentation: Enabling Technology for Low-Temperature Preservation—From Underlying Principles to Unmet Needs
Yoed Rabin, D.Sc., Carnegie Mellon University

The Importance of Keeping Your Cool—State-of-the-Art Biorepositories Play an Essential Role in Safeguarding and Disseminating Cryopreserved Biomaterials
Michael Sheldon, Ph.D., Sampled

An Automatic Ice-Seeding Cryovial (aiCryovial) for Enhanced and Convenient Cell and Tissue Cryopreservation
Xiaoming (Shawn) He, Ph.D., University of Maryland

2:10–2:55 p.m.	**Group Discussion** *Moderator: Robyn Tanguay, Ph.D., Oregon State University*
2:55–3:00 p.m.	**Concluding Remarks** *Xiang-Ning Li, M.D., Ph.D., ORIP, DPCPSI, OD, NIH*
3:00 p.m.	**Adjournment**

Appendix B: Biosketches

Session III. Cryoresearch: Supporting Technology and Resources

Virtual Meeting
September 30, 2024

Dr. Robyn Leigh Tanguay holds the title of University Distinguished Professor within the Department of Environmental and Molecular Toxicology at Oregon State University. Additionally, she serves as the Director of the Superfund Research Center and the Sinnhuber Aquatic Research Laboratory at the same institution. Her educational background includes a Bachelor of Arts in biology from California State University, San Bernardino, a doctorate in biochemistry from the University of California, Riverside, and postdoctoral training at the University of Wisconsin–Madison. Dr. Tanguay has published more than 300 peer-reviewed journal articles, firmly establishing her as a leading authority in her field. Her work has not only received widespread recognition but also has played a pivotal role in shaping policies related to chemical safety and environmental protection.

Dr. Rosaly Correa-de-Araujo is a Senior Scientific Advisor in the National Institute on Aging's Division of Geriatrics and Clinical Gerontology. She oversees the scientific and business operations of the AgingResearchBiobank, including compliance with the National Institutes of Health (NIH) data sharing policy, and facilitates access to the Biobank's study collections by the national and international scientific communities. She is a cardiovascular pathologist trained at the National Heart, Lung, and Blood Institute. Her degrees are from the Federal University of Bahia School of Medicine and the University of São Paulo School of Medicine, both in Brazil. She has special training on Evidence-Based Clinical Practice from McMaster University, Canada, and numerous years of academic experience as Adjunct Associate Professor, George Washington University School of Medicine and Health Sciences; Clinical Assistant Professor (Geriatrics Pharmacotherapy), University of Maryland School of Pharmacy Experiential Learning Program; Assistant Professor, University of São Paulo School of Medicine, Brazil; and Chairman and Associate Professor of the Department of Pathology and Forensic Medicine and Chief of the Autopsy Section at the University Hospital, Federal University of Uberaba School of Medicine, Brazil. She has served in diverse positions in several bodies of the U.S. Department of Health and Human Services, including the Office of the Secretary, where she led a major Secretarial/Presidential initiative on global health specifically targeting health diplomacy in the Western Hemisphere. She also served as the Secretary's Delegate to the U.S.–Mexico Border Health Commission and as Deputy Director of the former Office on Disability. As former Director of Women's Health and Gender-Based Research in the Agency for Healthcare Research and Quality (AHRQ), she received numerous awards, including the AHRQ Director's Award of Excellence for revitalizing the women's health program and introducing the gender-based medicine concept to women's health and health services research. She has numerous scientific publications, including chapters in medical books on various topics. Areas of expertise and interest include skeletal muscle function deficit/loss of muscle mass, strength and function with aging, myosteatosis, multimorbidity, medication management, ethnogeriatrics, and older women's health. She has served as an expert on scientific panels of the United Nations Aging Agenda and the World Health Organization (WHO) initiative on Integrated Care for Older People and the WHO Clinical Consortium on Health Aging.

Dr. Mehmet Toner holds a Bachelor of Science degree from Istanbul Technical University and a Master of Science degree from Massachusetts Institute of Technology (MIT), both in mechanical engineering. He earned his doctorate in medical engineering from the Harvard–MIT Division of Health Sciences and Technology (HST). Currently, he is an Assistant Professor of Biomedical Engineering at Massachusetts General Hospital and Harvard Medical School, with a joint appointment as a Professor of Health Sciences and Technology at HST. Dr. Toner is a member of the senior scientific staff at Shriners Hospital for

Children and co-founder of the Center for Engineering in Medicine and Surgery. He founded the NIH BioMicroElectroMechanical Systems (BioMEMS) Resource Center at Massachusetts General Hospital and directs the Biomedical Engineering Research and Education Program for physicians there. He actively participates in numerous national and international professional committees and serves on the editorial boards of various scientific journals. In 1998, Dr. Toner was named a Fellow of the American Institute of Medical and Biological Engineering. He also serves on the scientific advisory boards of multiple biotechnology and medical device companies and has played a key role as a scientific founder of several startup companies.

Dr. Wah Chiu earned his Bachelor of Arts in physics and his doctorate in biophysics from the University of California, Berkeley. He is a recognized pioneer in cryogenic electron microscopy (cryo-EM), known for his transformative contributions to the development of single-particle cryo-EM for determining the structures of molecular machines at atomic resolution. Collaborating with scientists worldwide, his laboratory has successfully solved numerous cryo-EM structures, including those of viruses, chaperonins, membrane proteins, ion channels, antigen-antibody complexes, and RNAs. Dr. Chiu continues to establish high standards for testing and characterizing cryo-EM instrumentation while innovating new image processing and modeling algorithms for structure determination. His current research focuses on advancing cryogenic electron tomography (cryo-ET) to achieve near–atomic resolution structures of molecular complexes *in situ*. In addition to his academic roles, Dr. Chiu serves as the Director of the Division of CryoEM and Bioimaging at the Stanford Synchrotron Radiation Lightsource (SSRL) at SLAC National Accelerator Laboratory, a position he has held since 2018. His accolades include the M.J. Buerger Award from the American Crystallographic Association (2021), the inaugural Wallenberg-Bienenstock Professorship at Stanford University (2020), and election to the National Academy of Sciences (2012). Dr. Chiu is actively involved in the scientific community, serving on various boards and advisory committees. He is also a member of the Advisory Committee for the Worldwide Protein Data Bank and has been on the Scientific Advisory Board for the Research Collaboratory for Structural Bioinformatics Protein Data Bank since 2005.

Dr. Adam Higgins is a Professor of Bioengineering at Oregon State University. His primary research focus is cell, tissue, and organ preservation. Dr. Higgins has more than 20 years of experience in the field, and his work has resulted in more than 40 publications, including an article that was highlighted on the cover of *Biophysical Journal* and an article that was selected as the 2018 best paper in the journal *Cryobiology*. He has held various leadership positions, including service as the President of the Society for Cryobiology (2020–2021).

As a cryobiologist, **Dr. Xu Han** has been recognized as a Top Reviewer for *Cryobiology*, the official journal of the Society for Cryobiology, and has contributed as a reviewer for 27 NIH study section panels. In his role as an Assistant Professor at the Wake Forest Institute for Regenerative Medicine, his research focuses on advancing tissue engineering. As an Associate Professor of Clinical Medicine at the University of Missouri, his work is dedicated to promoting innovations in tissue transplantation. He is also the founder and Chief Technology Officer of CryoCrate LLC, an NIH Innovation Showcase Company. The CryoCrate team, along with their co-developers or collaborators, has received support from the Office of Research Infrastructure Programs, National Cancer Institute, National Eye Institute, National Institute on Aging, National Institute of Allergy and Infectious Diseases, and various other funding agencies and foundations for their pioneering work in developing an efficient biocompatible cell and tissue cryopreservation technology platform. This recognition highlights his contributions to both scientific innovation and entrepreneurship.

Dr. Yoed Rabin is a Professor of Mechanical Engineering at Carnegie Mellon University. He received his Bachelor of Science (1989) and Master of Science (1991) from Ben Gurion University in Israel and his Doctor of Science (1994) from the Technion–Israel Institute of Technology (IIT). Previously, Dr. Rabin held primary academic positions at the Division of Surgical Oncology (1994–1996) and the

Department of Human Oncology (1996–1998) at Allegheny University of the Health Sciences in affiliation with Hahnemann University Hospital, and with the Department of Mechanical Engineering at the Technion IIT (1997–2000). Dr. Rabin has been affiliated with 16 industrial companies over the years, working on a wide range of thermal engineering and biothermal technology applications. He has a broad range of research interests in areas of energy modalities in biology and medicine, including cryopreservation, cryosurgery, thermal ablation, thermal regulation in biological systems, blood perfusion, medical imaging, nitrogen-based and helium-based enabling cryogenic technologies, and the underlying principles of thermal sciences.

Dr. Michael Sheldon received his Bachelor of Arts from Cornell University in 1983 and a doctorate from the State University of New York, Stony Brook in 1993. He served on the faculties of Rutgers, The State University of New Jersey and Baylor College of Medicine with a background as a bench scientist in genetics and neurodevelopment. During his nearly 30 years of experience, he has served in several roles in the biobanking and integrated analytics fields. Dr. Sheldon founded the Sampled Scientific Affairs department, with the mission of providing expert technical resources to clients, discovering and adopting new technologies, and coordinating outreach initiatives designed to enhance the profile of the company. Previously, he served as Senior Director of Sample Processing Services at RUCDR Infinite Biologics (now Sampled), with oversight of all sample processing services relating to blood fractionation, cell and stem cell culture, and nucleic acid extraction. He was Director of the team that established the Sampled SARS-CoV-2 testing laboratory, serving the states of New Jersey and Minnesota, as well as many other sites across the United States. To date, Sampled has tested more than 12 million samples. He is also Director of the Sampled College of American Pathologists (CAP) Biorepository and is a certified CAP Inspection Team Leader.

Dr. Xiaoming (Shawn) He is a Professor of Bioengineering at the University of Maryland, College Park. He obtained his doctoral degree in mechanical engineering in 2004 from the University of Minnesota Twin Cities and conducted postdoctoral training from 2004 to 2007 at Harvard Medical School–Massachusetts General Hospital. He was an Assistant Professor at the University of South Carolina and Associate Professor and Full Professor at The Ohio State University from 2011 to 2017. His current research is focused on developing microscale and nanoscale biomaterials and devices to engineer, bank, and deliver stem and immune cells for the treatment and early detection of various diseases and disorders, including, but not limited to, cancer, cardiovascular diseases, diabetes, neurological disorders, and infertility. His research has been funded by grants with him as the principal investigator from various private foundations (e.g., the American Cancer Society) and government agencies (e.g., NIH, U.S. National Science Foundation). He has published 150 peer-reviewed articles in high-ranking journals, including *Nature Biomedical Engineering*, *Nature Nanotechnology*, and *Nature Communications*, as well as one book and four book chapters. He is an Editor-in-Chief of the *Journal of Medical Devices*. He is a fellow of the American Society of Mechanical Engineers and the American Institute of Medical and Biological Engineering and a member of the European Academy of Sciences and Arts.

Appendix C: Attendees

Session III. Cryoresearch: Supporting Technology and Resources

Virtual Meeting
September 30, 2024

Gloria Abizanda Sarasa, D.V.M., Universidad de Navarra
Jason Acker, Ph.D., University of Alberta
Yuksel Agca, Ph.D., D.V.M., University of Missouri
Monika Aggarwal, Ph.D., Office of Research Infrastructure Programs (ORIP), Division of Program Coordination, Planning, and Strategic Initiatives (DPCPSI), Office of the Director (OD), National Institutes of Health (NIH)
Alptekin Aksan, Ph.D., U.S. National Science Foundation
Simin Aliabadi, M.S., University of Nevada, Reno
Julio Aurelio Sarabia Alonso, Ph.D., University of California, Riverside
Michelle Altemara, M.S., The University of North Carolina at Chapel Hill
James Amos-Landgraf, Ph.D., University of Missouri
Carl Anderson, National *Xenopus* Resource, Marine Biological Laboratory, The University of Chicago
Katelyn Anderson, University of California, Irving
Guilherme Antunes, Champalimaud Foundation
Renee Araiza, University of California, Davis
Gediendson Araujo, Federal University of Mato Grosso do Sul
Shinya Ayabe, D.V.M., Ph.D., RIKEN BioResource Research Center
Lisa Baker, University of California, Davis
Ravi Balijepalli, Ph.D., National Heart, Lung, and Blood Institute, NIH
Amy Banes-Berceli, Ph.D., Oakland University
John Baust, Ph.D., CPSI Biotech
Karen Beeri, M.S., Vanderbilt University Medical Center
Jessica Bell, Stowers Institute for Medical Research
Thomas Bell, Ph.D., National Disease Research Interchange
Jhony Lisboa Benato, M.S., Universidade Federal do Rio Grande do Sul
Abby Bernardini, D.V.M., The University of Oklahoma Health Sciences Center
Maria Bewley, Ph.D., Penn State University College of Medicine
Laura Francesca Bianchi, Centre for Genomic Regulation
John Bischof, Ph.D., University of Minnesota
Haddy Bittaye, M.S., B.S., Max Planck Institute for Immunobiology and Epigenetics
Ana Borges, Ph.D., Instituto Gulbenkian de Ciência
Jeri Brandom, Ph.D., The University of Alabama at Birmingham
Tobias Braun, M.S., University of Veterinary Medicine Hannover
Jackie Brooks, Mutant Mouse Resource and Research Centers, The University of North Carolina
Elizabeth Bryda, Ph.D., University of Missouri
Bettina Buhring, Ph.D., ORIP, DPCPSI, OD, NIH
Kathy Burge, Ph.D., The University of Oklahoma Health Sciences Center
Melanie Burns, Ph.D., University of Minnesota
Nate Buzzell, M.S., BioMedical Research
Kevin Esteban Piñeros Cano, D.V.M., Universidad de los Llanos
Taylor Carlson, The University of Oklahoma Health Sciences Center
Alison Cash, D.V.M., Ph.D., Cleveland Clinic Lerner Research Institute
Joana Castro, M.S., Champalimaud Foundation
Raissa Cecil, M.S., University of Kentucky

Jasper Chan, M.S., The University of Hong Kong
Susan Chandran, M.S., ORIP, DPCPSI, OD, NIH
Brooke Chang, M.S., University of California, Berkeley
Rajan Chaudhary, M.S., Agroforestry Polytechnic Institute
Kallayanee Chawengsaksophak, Ph.D., Institute of Cancer Research
Ken Chen, M.D., Albert Einstein College of Medicine
Yong Chen, Ph.D., ORIP, DPCPSI, OD, NIH
Yong Cheng, Ph.D., Wistar Institute
Wah Chiu, Ph.D., Stanford University
Heonhwa Choi, Ph.D., Rutgers, The State University of New Jersey
Chi Kei Chow, The University of Hong Kong
Jonathan Clayton, D.V.M., Ph.D., University of Nebraska
Autumn Cole, University of California, Davis
Tony Consiglio, Ph.D., University of California, Berkeley
Miguel Contreras, Ph.D., ORIP, DPCPSI, OD, NIH
Rosaly Correa-de-Araujo, M.D., Ph.D., M.Sc., National Institute on Aging
Jennifer Corrigan, M.S., The Jackson Laboratory
Mitra Cowan, M.S., McGill University
Rachel Cox, Ph.D., Uniformed Services University of the Health Sciences
Carme Cucarella, Ph.D., Instituto de Biomedicina de Valencia
Anza Darehshouri, Ph.D., University of Colorado Anschutz Medical Campus
Aric Daul, Ph.D., University of Minnesota
Hanan Davidowitz, Ph.D., BioTillion
Tarek Deeb, M.S., Leibniz University of Hanover
Isabel Clara Rollan Delgado, Ph.D., European Molecular Biology Laboratory
Victoria Denham, Louisiana State University
Daina Domahidi, University of Toronto
Veronica Dominguez, Centro de Biología Molecular Severo Ochoa
Sheri Dorsam, Ph.D., U.S. Department of Agriculture (USDA)
Erin Ducharme, M.S., University of New England
Eden Dulka, Ph.D., University of Michigan
Michael Durnin, Stowers Institute for Medical Research
Melinda Dwinell, Ph.D., Medical College of Wisconsin
Charles Elder, M.S., University of Louisville
Bradley Ellis, Ph.D., Massachusetts General Hospital
Al Feinberg, D.V.M., Rutgers, The State University of New Jersey
Madison Floden, Ph.D., North Dakota State University
Thales Franca, D.Sc., Ph.D., M.S., Universitat Politecnica de Valencia
Martin Fray, Ph.D., The Mary Lyon Centre at MRC Harwell
Stephen Frederickson, National Human Genome Research Institute, NIH
Susanne Freedrich, Eidgenössische Technische Hochschule Zurich
Sabrina Gacem, Ph.D., Valencia University
Lakshya Gangwar, Ph.D., University of Minnesota
Victoria Gilbert, University of California, Davis
Birgit Glasmacher, Ph.D., Leibniz University Hannover
Inês Gonçalves, M.S., Champalimaud Foundation
Nitzan Gonen, Ph.D., Bar-Ilan University
Magdalena Góra, M.S., International Institute of Molecular and Cell Biology
Vijay Kanth Govindharajan, D.V.M., Ph.D., Qatar University
Linda Gower, Vanderbilt University
Ryan Gray, Ph.D., The University of Texas at Austin

Franziska Grieder, D.V.M., Ph.D., ORIP, DPCPSI, OD, NIH
Audra Guikema, Van Andel Research Institute
Maria Teresa Gutierrez-Wing, Ph.D., Louisiana State University
Jenna Hakkesteeg, University College London
Richard Hall, Ph.D., Stowers Institute for Medical Research
Xu Han, Ph.D., CryoCrate LLC and Wake Forest University
Zonghu Han, Ph.D., University of Minnesota
Dorit Hanein, Ph.D., University of California, Santa Barbara
Xiaoming (Shawn) He, Ph.D., University of Maryland
Mike Heinrich, University of Michigan
Kris Helke, D.V.M., Ph.D., Medical University of South Carolina
Brian Hermann, Ph.D., The University of Texas at San Antonio
Nikki Hernandez, D.V.M., Baylor College of Medicine
Adam Higgins, Ph.D., Oregon State University
David Hike, Ph.D., Massachusetts General Hospital
Kristy Hood, M.S., Novartis
Juliette Horwood, The Francis Crick Institute
Allison Hubel, Ph.D., University of Minnesota
Oleksandra Hubenia, Leibniz University Hannover
Antonio Icaro, Ph.D., Maranhao State University
Abdul-Rashid Iddi, M.S., Leibniz University Hannover
Aasma Iqbal, M.S., The University of Lahore Sargodha Campus
Khursheed Iqbal, Ph.D., Oklahoma State University
Mayumi Isaka, Regeneron Pharmaceuticals
Nicholas Jean, University of California, Berkeley
Frank Jenkins, Ph.D., University of Pittsburgh
Katie Johnson, D.V.M., Boise State University
Larry Johnson, M.S., The University of Arizona
Sarah Johnson, M.S., Harvard University
Purva Joshi, Ph.D., Massachusetts General Hospital and Harvard Medical School
Swapnil Kamble, D.V.M., Biological E Limited
Thomas Kaufman, Ph.D., Indiana University
Kanav Khosla, Ph.D., Sana Biotechnology
Natasha Kiel, Stowers Institute for Medical Research
Yongdeok Kim, Ph.D., University of California, Berkeley
Jack Koch, Ph.D., Aquatic Germplasm and Genetic Resources Center, Louisiana State University
Thomas Kolbe, Ph.D., University of Veterinary Medicine Vienna
Suman Komjeti, The University of Texas Southwestern Medical Center
Xiangbo Kong, M.S., University of Michigan
John Koomen, Ph.D., Moffitt Cancer Center
Malgorzata Korzeniowska, Ph.D., Instytut Medycyny Doświadczalnej i Klinicznej Polskiej Akademii Nauk
Elizabeth Kotus, University of Michigan
Peter Koulen, Ph.D., University of Missouri–Kansas City
Kathy Krentz, M.S., University of Wisconsin
Melissa Larson, Ph.D., University of Kansas Medical Center
P.S. Li, M.S., The University of Hong Kong
Wenping Li, Ph.D., University of Illinois Chicago
Xiang-Ning Li, M.D., Ph.D., ORIP, DPCPSI, OD, NIH
DoYoung Lim, Ph.D., Mayo Clinic
Advait Limaye, National Institute of Dental and Craniofacial Research (NIDCR), NIH

Shu-Wha Lin, Ph.D., National Taiwan University
Dejia Liu, University of Veterinary Medicine Hannover
Kent Lloyd, Ph.D., University of California, Davis
Glenn Longenecker, NIDCR, NIH
Ryan Lopez, M.S., Oregon State University
Leo Lou, University of California, Berkeley
Evyn Loucks, M.S., University of Oregon
Maor Lubman, M.S., Broward International University
Andreia Joana Miguel Madalena, Institute of Science and Technology Austria
Chethan Magnan, Michigan Technological University
Jenna Mangiarelli, M.S., Novartis Institutes for Biomedical Research
Carl Manner, Ph.D., Duke University
Elizabeth Marden, M.S., Penn State College of Medicine
Josep M. Marimon, D.V.M., University of Barcelona
Sara Leal Marin, M.S., Leibniz University Hannover
Saumya Mathew, Ph.D., Baylor College of Medicine
Stephanie Mauthner, Ph.D., Indiana University Bloomington
Rachel McAdoo, University of California, Davis
Elise McBurney, Novartis Institute for Biomedical Research
Maura McGrail, Ph.D., Iowa State University
Maria Noel Meikle, M.S., Institut Pasteur de Montevideo
Maria de Grecia Cauti Mendoza, M.S., Wistar Institute
Rebecca Mercier, Ph.D., PanTHERA
Leanne Miceli, The Jackson Laboratory
Reginald Miller, D.V.M., Icahn School of Medicine at Mount Sinai
Bradley Mills, Ph.D., University of Rochester
Oleg Mirochnitchenko, Ph.D., ORIP, DPCPSI, OD, NIH
Natalia Moncaut, Ph.D., Cancer Research UK Manchester Institute
Kathleen Moosbrugger, University of Pennsylvania School of Medicine
Anna Morgunowicz, Łukasiewicz Research Network–PORT Polish Centre for Technology Development
Arun Murahari, M.S., Centre for DNA Fingerprinting and Diagnostics
Stephanie Murphy, D.V.M., Ph.D., ORIP, DPCPSI, OD, NIH
Tara Murray, Boise State University
Allison Neely, D.V.M., University of Kansas Medical Center
Henrike Nelson, ORIP, DPCPSI, OD, NIH
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Harriette Oldenhof, Ph.D., University of Veterinary Medicine Hannover
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Tuncer Onay, Ph.D., Northwestern University
Armedia O'Neill-Blair, University of Missouri
Felipe Ongaratto, D.V.M., Ph.D., University of Wisconsin
Mariette Ouellet, Le Centre de Recherche du Centre Hospitalier de l'Université de Montréal
Nathalie Oulhen, Ph.D., Brown University
Yu Ouyag, University of California, Berkeley

Ken Overturf, Ph.D., USDA
Marta Batet Palau, Centre for Genomic Regulation
Theofilos Papadopoulos, Ph.D., Max Planck Institute for Multidisciplinary Sciences
Annette Parks, Ph.D., Bloomington *Drosophila* Stock Center, Indiana University
David Pasnik, D.V.M., USDA
Susan Penrose, Washington University Medical School
Leon Peshkin, Ph.D., Harvard University
Cornelia Peterson, D.V.M., Ph.D., Tufts University
Michael Pettigrew, Archive Sciences, Inc.
Dominick Pierre-Jacques, University of Illinois Chicago
Corinne Piotter, Purdue University
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Sukumal Prukudom, D.V.M., Ph.D., M.S., Kasetsart University
Anna Pujol, Ph.D., Universitat Autònoma de Barcelona
Sumanth Kumar Putta, D.V.M., Ph.D., Genentech
Paulina Pyrek, D.V.M., Norwegian University of Life Sciences
Yoed Rabin, D.Sc., Carnegie Mellon University
Laura Rangel, M.S., Cicese
Prateek Rauthan, M.S., Swami Rama Himalayan University
Gerardo Reyes, Brown University
Joe Rinehart, Ph.D., USDA
Víctor MauricioMedina Robles, Ph.D., Universidad de los Llanos
Samson Rokkarukala, Ph.D., National Institute of Oceanography, Council of Scientific and Industrial Research
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Ann Rougvie, Ph.D., University of Minnesota
René Rudat, Medical Faculty Magdeburg
Miheer Sabale, M.S., Macquarie University
Niloofar Sadeghi, Ph.D., Texas Biomedical Research Institute
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Manfred Schartl, Ph.D., *Xiphophorus* Genetic Stock Center, Texas State University
Jacqueline Schlamp, Northwestern State University
Michael Schmale, Ph.D., University of Miami
Jean-Francois Schmouth, Ph.D., Le Centre de Recherche du Centre Hospitalier de l'Université de Montréal
Christine Schnitzler, Ph.D., University of Florida
Nikko-Ideen Shaidani, M.S., Marine Biological Laboratory
Soaleha Shams, Ph.D., Mayo Clinic
Nataliia Shapovalova, M.S., University of Zurich
Michael Sheldon, Ph.D., Sampled
Karen Siu, The University of Hong Kong
Hannah Skaggs, University of Louisville
Stephanie Slater, Seattle Children's Research Institute
Jennifer Sloppy, Ph.D., Penn State College of Medicine
Dionísio Sousa, M.S., Champalimaud Foundation
Tina St Laurent, Van Andel Institute

Stephanie Sterling, University of Pennsylvania
Barbara Stone, Ph.D., ParaTechs Corporation
Joyce Stuckey, D.V.M., M.S., Rutgers University, The State University of New Jersey
Daniel Stutts, The University of North Carolina
Lucia Suárez López, Ph.D., Cicese
Yongjun Sui, Ph.D., National Cancer Institute, NIH
Tsung-Chang Sung, Ph.D., The Salk Institute
Sonya Swing, D.V.M., Ph.D., The University of Alabama at Birmingham
Roman Szabo, Ph.D., NIDCR, NIH
Robert Taft, Ph.D., The Jackson Laboratory
Toru Takeo, Ph.D., Kumamoto University
Akiko Takizawa, Ph.D., Medical College of Wisconsin
Ritesh Tandon, Ph.D., ORIP, DPCPSI, OD, NIH
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Nathalia Teixeira, M.S., Universidade Federal do Rio Grande do Sul
Amand Thayer, M.S.P.H., Genentech
Riley Thompson, D.V.M., Ph.D., Colorado State University
Biao Tian, Ph.D., ORIP, DPCPSI, OD, NIH
Simon Tinman, D.V.M., Bar Ilan University
Irene Tirado-Gonzalez, Ph.D., Georg-Speyer-Haus
Carmen De Sena Tomas, Ph.D., Instituto de Investigaciones Biomédicas August Pi i Sunyer
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Simon Tröder, Ph.D., University of Cologne
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John Vandeberg, Ph.D., The University of Texas Rio Grande Valley
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Ziran Wei, Ph.D., Louisiana State University
Cale Whitworth, Ph.D., Indiana University
Brian Will, University of New England
Evan Williams, University of Oregon Institute of Neuroscience
Wendy Williams. D.V.M., M.S., The University of Oklahoma Health Sciences Center
Michelle Winter, University of Kansas Medical Center
Michelle Wodzak, University of Toronto
Wim Wolkers, Ph.D., University of Veterinary Medicine Hannover
Stephanie Womack, M.S., University of Oregon
HeatherWood, Cleveland Clinic
Mark Woodward, Ph.D., Wake Forest University
Lin Wu, Ph.D., Harvard University
Xiaoli Wu, M.S., University of Manitoba
Xiaojun Xing, M.S., Yale University

Ping Xu, Ph.D., UMass Chan Medical School
Fatima Nnaminin Yahaya, M.S., Kwara State University
Huanghe Yang, Ph.D., Duke University
Alora Yarbrough, M.S., University of California, San Diego
Yun You, Ph.D., University of Minnesota
Zhe Yuan, Ph.D., Wistar Institute
Taisiia Yurchuk, Ph.D., Institute of Animal Reproduction and Food Research
Ioana Maria Zah, University of Copenhagen
Mary Zelinski, Ph.D., M.S., Oregon National Primate Research Center
Li Zhan, Ph.D., Massachusetts General Hospital
Shumei Zhao, Ph.D., Howard Hughes Medical Institute
Sige Zou, Ph.D., ORIP, DPCPSI, OD, NIH
Nikolas Zuchowicz, M.S., University of Minnesota