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Workshop Report

Workshop and panel discussion: High-throughput cryopreservation of germplasm as an exchange currency for genetic resources

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A workshop and panel discussion to address the needs of the aquatic research community for germplasm conservation was held during the *5th Aquatic Animal Models for Human Disease* Conference in Corvallis, Oregon (September 20–22, 2010), entitled *High-throughput Cryopreservation of Germplasm as an Exchange Currency for Genetic Resources.* Researchers and representatives of medaka, *Xiphophorus*, and zebrafish Stock Centers were present and participated in the discussion. The workshop aimed at exploring the transfer of successful germplasm banking approaches (e.g. in livestock) to aquatic species, by evaluating linkages across current needs and opportunities in facilities, equipment, and protocols.

Biomedical research using fish as model organisms has produced thousands of mutants and transgenic animals — a genetic resource that represents an enormous scientific and informational value. Additional value, utility value, is added when the information can also be tested, manipulated, and studied in living organisms. In recent years medaka, zebrafish, and *Xiphophorus* have been used with great success as laboratory model organisms and as tools to study biomedical questions. Inbred *Xiphophorus* lines for example, have been instrumental to study the causes and course of melanomas. Medaka and zebrafish have been used in genetic screens and thousands of mutants and transgenic strains have been produced and studied. As such, research in biomedical fishes generates genetic resources at an unprecedented rate. The utility value of these resources is now increasingly limited by the constraints of maintaining the fish strains alive, and there is potential danger that many if not most of them could be lost. In this scenario, germplasm can be viewed as a third form of value, an exchange currency that allows creation, maintenance, transport, and exchange of the informational and utility values of genetic resources. Cryopreservation of germplasm also allows better planning of phases of large-scale research projects over the course of decades, such as genetic screens.

The workshop began with a review by Tiersch about current cryopreservation efforts in aquatic species compared to established livestock industries that have used germplasm banking for decades. Although high-throughput processing has been widely applied for sperm cryopreservation in dairy bulls for example, application in biomedical model fishes is still in the concept-development stage, because of the limited sample volumes and the biological characteristics of fish sperm. High-throughput processing in livestock was developed based on advances made in the laboratory and was scaled up for increased processing speed, capability for mass production, and uniformity and quality assurance. Cryopreserved germplasm combined with high-throughput processing currently constitutes an independent industry, encompassing animal breeding, preservation of genetic diversity, and medical research. At present however, there are no specifically engineered systems available for high-throughput cryopreservation of germplasm for aquatic species. Current research in fish cryopreservation should establish a central pathway that can accommodate various levels and methods of application. The overall throughput of this pathway needs to be scalable according to the particular needs of individual laboratories. At the same time, it should funnel research activities into a standardized approach that can utilize industrial methods supported by commercial vendors of specialized equipment, supplies and reagents, and industrial-level service providers for cryopreservation, storage, and quality control.

The Zebrafish International Resource Center reported that the research community produces novel fish strains at staggering rates. Even small laboratories can now produce hundreds of transgenic fish lines that include enhancer and gene traps, GFP reporter constructs, and viral insertion mutant lines. With the advent of the sequencing and mapping of the zebrafish genome, several consortia take advantage of the molecular information and now attempt to mutagenize all protein-producing genes in the genome by viral insertion mutagenesis, TILLING, or by targeted gene knock-out using Zinc-Finger Nucleases. Thus, it can be expected that several tens of thousands of mutant and transgenic lines will be generated in the coming decade. In fact, approximately 20,000 of these lines already exist across various research groups, and many are preserved in liquid nitrogen based on existing methods. The capacity to bank and exchange current and future resources critically depends on establishment of scalable, high-throughput cryopreservation methods.

For example, the current working model of the Resource Center might have to be adapted to deal efficiently with the increased numbers of prospective strains. Currently, approximately 350 frequently requested zebrafish lines are maintained alive at ZIRC to satisfy user requests with a short turn-around time. However even of these, only 50 lines are requested more than 5–6 times per year. In

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contrast, the less frequently requested strains (currently approximately 1000) are cryopreserved, thawed, and *in vitro* fertilized upon request, and freshly fertilized embryos are shipped to the researcher. For each type of line, the Resource Center currently stores only 10–20 frozen samples in its repository, limited mainly by the cost of cryopreservation with the available, labor-intensive techniques. This means that the Resource Center has to spend most of its funds on water, power, food, and personnel on 'popular' lines, which are requested only a few times per year. However, even with existing techniques, thawing of cryopreserved sperm and *in vitro* fertilization of eggs is a straightforward process and the cost of liquid nitrogen is far lower than live animal care. In the future, a high-throughput cryopreservation method, together with improvements that include optimization of male productivity and post-thaw fertility rates could allow the Resource Center to preserve *hundreds* or thousands of samples per line at reduced costs to save considerable resources including maintenance of popular lines. Thus, for each newly imported line, the Resource Center would shift investment to the beginning of the process to cryopreserve samples, but this would satisfy subsequent research requests for decades at very low cost.

Therefore, to fully access its value, germplasm banking must be performed on a scale of hundreds of thousands of samples. This can only be accomplished by development of high-throughput approaches that incorporate biological and cryobiological principles, equipment and facility development, process-control for sample handling, inventory and databasing, quality control and assessment, standardization and establishment of industrial practices, and institution of biosecurity systems — more than simply freezing a few sperm samples per line. A conceptual shift is also necessary from an informational bias in research to include utility value, particularly by recognizing the unexploited value of germplasm. As a practical example, the genetic improvement in global dairy herds has been accomplished through use of cryopreserved sperm to enable selective breeding of bulls to improve milk yields in their daughters. This has produced a multi-billion-dollar global market for germplasm because genetic information (milk production data) is converted into utility value (efficient dairy herds) through efficient use of an exchange currency (germplasm). Similarly, preservation of aquatic species germplasm can have enormous impact on preservation of biodiversity, food industries, and biomedical research.

As such, in the biomedical fish research community we are currently over-invested in informational value, constrained in utility value, and with only minimal investment in exploitation of an exchange currency. The establishment of large, interactive repositories that use scalable high-throughput cryopreservation pathways to bank genetic resources will create new opportunities to balance this equation.