



UNIVERSITY OF OREGON

June 8, 2021

To Whom It May Concern:

The following application, # AUP-21-15 v.1, was reviewed and approved by the University of Oregon Animal Care and Use Committee.

**Title of Protocol Application:** Zebrafish International Resource Center

**Name of Applicant:** Monte Westerfield

**Name of Institution:** ION

Sponsoring Agency	Title of Grant Application	Sponsored Grant #	UO Grant #	EPCS #
NIH	Competing continuation of Zebrafish International	P40 OD011021-16	217090	24999
NIH	Development of Germplasm Resources for Preservation of Aquatic Models	1 R24 RR023998-01A1	2416S0	25538
NIH	Development of Community-driven Reproducibility for Preservation of Germplasm Resources of Aquatic Models	TBA	TBA	30176

**Protocol Review Type:** Continuation of Expiring Protocol 18-05

**Approval Date:** 06-08-2021

**Original Approval Date:** 06-08-2021

**Protocol Expiration Date:** 06-08-2024

This institution has an Animal Welfare Assurance on file with the NIH Office of Laboratory Animal Welfare (OLAW). The UO PHS Assurance number is D16-00004.

IACUC Member Designated  
to Verify Acceptance

**INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE**

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# Animal Use Protocol

## Administrative Information

Title: Zebrafish International Resource Center

## Protocol Purpose

### Protocol Status

*Check One*

**Continuation of Expiring Protocol**

*18-05*

*Original Approval and Expiration Dates*

**Original Approval Date**

*12 June, 2018*

**Protocol Expiration Date**

*12 June, 2021*

## Protocol Status

### Protocol Type

*Please check all that apply.*

**Research Project**

**Colony Health Surveillance Project**

## Funding Information

Funding Source Grant Title	Funding Status ("New", "Continuation", "Renewal", "Revision")	Funding Status ("New", "Continuation", "Renewal", "Revision")	Grant #	Index #	EPCS #
NIH/Zebrafish International Resource Center	Continuation		P40 OD11021-16	217090	24999
NIH/Development of Germplasm Resources for Preservation of Aquatic Models	Continuation/Expiring		1 R24 RR023998-01A1	2416S0	25538
NIH/Development of Community-driven Reproducibility for Preservation of Germplasm Resources of Aquatic Models	New		TBA	TBA	TBA

## Funding Information

*Was this project originally funded or previously reviewed without the use of animals, or are there new significant changes in scope that now involve animals which were not previously outlined in the grant proposal?*

**No**

*What is your Funding Type?*

**Extramural**

**I have attached a copy of my grant(s).**

*Please attach a copy of your entire grant(s), including research plan, materials and methods, personnel, budget, etc. by clicking the paperclip "Attachments" icon at the top.*

**Important Reminder:**

*You must affirm both items.*

**I understand by signing this application I am affirming protocol and grant congruency.**

*Although federal regulations don't require institutions to perform a side by side comparison of the protocol with the grant application or proposal, institutions are responsible for assuring all animal care and use procedures that the IACUC reviews and approves are congruent with all of the animal care and use information contained in the grant application.*

**Important Reminder:**

*You must affirm both items.*

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## Cooperative Research

### Cooperative Research

*Is this a cooperative research project (are there principal investigators from more than one institution involved)?*

**Yes**

*Is any of the animal work and animal housing being conducted at the cooperating institution?*

**No, all live animal work will be conducted here on UO campus or UO field work.**

**Please describe the nature of the collaboration.**

**For additional guidance, please see UO [Cooperative Research](#) policy.**

NIH/Development of Germplasm Resources for Preservation of Aquatic Models: Software and 3-D printed accessories/tools for zebrafish sperm cryopreservation will be developed, engineered, and produced by our collaborators at LSU. These tools will be shipped to ZIRC and evaluated for their utility in standardizing, optimizing, and refining cryopreservation. Fish will be anesthetized and sperm or oocytes harvested as outlined in the protocol section of this application. After 2 4 year periods this program is expiring. A new grant application has been submitted instead with the title: Development of Community-driven Reproducibility for Preservation of Germplasm Resources of Aquatic Models.

## Peer Review of Un-sponsored Research

### Peer Review of Un-sponsored Research

*Is this a teaching protocol or a protocol involving un-sponsored research, such as departmental or start up funds?*

*The IACUC requires that all proposals to use animals in research projects receive scientific peer review before being submitted to the IACUC. Most sponsored proposals receive this review during the funding submission process.*

**No**

## Personnel

### Principal investigator

Westerfield, Monte

### Co-Investigators

Varga, Zoltan

## **Research personnel**

Clark, Renee  
Core, Keely  
Freeman, April  
Holland, Ronald "Ron"  
Hwang-Shum, Jen-Jen  
Lains, David  
Loucks, Evyn  
Matthews, Jen  
Murphy, Joy  
Murray, Katy  
Nasiadka, Andrzej  
Quinn, Erin  
Schumann, Alex  
Smith, Calvin  
Varga, Zoltan  
Westerfield, Monte  
Williams, Evan

## **Student Research Personnel**

Not applicable

## **Teaching Personnel**

Not applicable

## **Animal Care and Veterinary Support Staff**

Murray, Katy

## **Pre-Reviewers**

Not applicable

## **Signers**

Not applicable

## Request addition of a person to the database

### Collaborative Research Personnel

*Provide Names and Job Titles for Collaborative Research Personnel*  
**Provide Information:**

**1) Dr. Terrence R. Tiersch**

*Professor & Director*

*Aquatic Germplasm and Genetic Resources Center (AGGRC)*

*School of Renewable Natural Resources*

*Louisiana State University,*

*Agricultural Center,*

*2288 Gourrier Avenue*

*Baton Rouge, Louisiana, 70820*

**2) Michael Kent, MS, PhD,**

*Professor - Microbiology, Parasitology*

*Oregon State University*

*Nash Hall 220*

*2820 SW Campus Way*

*Corvallis, OR 97331*

### Collaborative Research Personnel

Not applicable

### Emergency Contact

Varga, Zoltan

### Rationale and Purpose

#### Rationale and Purpose

*What are the study objectives or aims of the study?*

*Although there are no specific requirements to list separately all of the benefits and harms of the proposal, there is an expectation that the IACUC*

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*"will weigh the objectives of the study against potential animal welfare concerns" (NRC 2011, 27). The information provided below should be written in lay terms that a non-scientist would understand, and should not be a copy and paste of the aims from the grant. The information provided in this section could be used for possible press release.*

**Provide study objectives or aims of the study:**

**To serve as a central repository for zebrafish genetic stocks and research materials.** We will maintain healthy stocks of fish and frozen sperm of identified genotypes and make them widely available to the research community. We will continue to expand our collection by obtaining carriers of mutations and transgenes from the research community and breeding them to produce new generations. We will freeze and store sperm from all these lines. We will develop genotyping protocols where applicable and make them available online through the ZIRC website (<http://zebrafish.org/zirc/home/guide.php>). We will acquire the most widely used wild-type lines and maintain them in a manner that preserves their genetic purpose. We will receive and store antibodies and gene probes that are used to identify and analyze wild-type and mutant stocks. Upon request, we will ship fish and materials to research laboratories throughout the world. We will provide information online about stocks, materials, ordering procedures, and protocols, and we will provide links to corresponding pages in ZFIN, the zebrafish model organism database (<http://zfin.org>). By providing these services in an efficient manner, ZIRC saves laboratories time and expense that can be better used for their research goals.

**To provide pathology and consultation services.** We will provide diagnostic services and health status testing for laboratory zebrafish. We will use histopathology, bacteriology, and necropsy to analyze specific or suspected disease problems (<http://zebrafish.org/health/index.php>). We will provide routine sentinel and quality control testing of zebrafish from healthy laboratory colonies for early detection of problems and to fulfill institutional animal care and use committee (IACUC) health monitoring requirements. We will provide consultations to aid fish facilities in maintaining healthy fish populations and to deal effectively with disease. We will continue to characterize the significant diseases of zebrafish and develop methods to detect and control disease in laboratory colonies. We will maintain and update our online manual for the prevention, diagnosis, and treatment of diseases affecting zebrafish. <http://zebrafish.org/zirc/health/diseaseManual.php>

**We will develop a platform, based on a panel of PCR assays, to screen for the most prevalent pathogens of laboratory zebrafish.** We will use genomic sequence information from known zebrafish pathogens to design and test PCR primers for species-specific detection. We will validate the sensitivity of these molecular assays with standard health screening by histopathology. We will use these PCR assays and other diagnostic tools to establish and implement screening procedures for in-house detection and monitoring of zebrafish pathogens. We will establish a platform, through the ZIRC website, for sharing the ZIRC pathogen monitoring program and tools for diagnostic testing with the research community, and we will provide consultation and diagnostic services, using this new platform, to identify pathogens in research laboratories, through our Pathology Service (Resource Aim 2).

*How are the procedures and design of the study relevant and important for human or animal health, the advancement of knowledge, or the good of society?*

*See US Government Principle II online at*

*Remember, our Animal Welfare Program is required to follow the US Government Principles as outlined in the Public Health Service Assurance: "This Institution is guided by the 'U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training'."*

**Provide relevance and importance for human or animal health, the advancement of knowledge, or the good of society:**

*A major goal of biomedical research is to understand how genes regulate developmental and physiological processes. By studying genes, including the regulation of their expression and the functions of their products, we can obtain a deeper and richer understanding of biological processes and gain important insights into vertebrate, including human, health and disease. In recent years, we have witnessed an explosion in our understanding of how genes regulate biological processes, largely based on work from a few model genetic organisms including mouse, fruit fly, nematode worm, yeast, and zebrafish. Each system has specific and complementary strengths. Our understanding of human development, hereditary medical conditions and disease has been tremendously augmented by the research performed with these genetic model organisms. The zebrafish is the newest of them. Because the basic genetic principles of embryonic development are very similar for vertebrates, insights gained from the research with zebrafish have implications for human health and disease. Moreover, research on this organism meets the intent of the Animal Welfare Act and the "3 R"s because higher vertebrate models can now be replaced with this 'lower' vertebrate.*

*In the past decades, there was a dramatic increase in the number of laboratories using this organism to study the basic mechanisms of vertebrate development. Laboratories have generated several thousand transgenic fish lines, identified over 20,000 genetic mutations, and due to the completion of the zebrafish genome sequencing project, plans are underway to produce a mutation in every gene of the zebrafish genome (ca. 25,000 genes). Even more recently, genome editing methods have become available that enable scientists to target previously not mutagenized genes by conventional forward genetic methods. Most of the genetic stocks are distributed among more than 1000 laboratories in more than 40 countries. To make room for*

*new mutants, laboratories must discontinue some of their current stocks. Although mutations can be preserved as frozen sperm, not all laboratories are proficient with this technique. Thus, discontinued stocks may be permanently lost unless a central site serves as a repository to preserve and redistribute them for future research.*

*The Zebrafish International Resource Center acquires and maintains wild-type, transgenic, and mutant zebrafish stocks and makes them available to the international biomedical research community.*

## Animal Subjects Information

fish: Zebrafish

### Total number of animals

Species	Min	Max
fish: Zebrafish	70330	70330

### USDA Pain Category

USDA Pain Category	Species	Procedure or Experimental Group	Procedure or Experimental Group
B	fish: Zebrafish	Shipping and Breeding Colony	0
D	fish: Zebrafish	Food/Diet Study	0
B	fish: Zebrafish	15 alpha-methyltestosterone	0
D	fish: Zebrafish	PCR Panel Development	0
D	fish: Zebrafish	Cryopreservation Equipment testing	0



## Animal Numbers Justification

*For all animals in all Aims provide the scientific justification for the number of animals in each experimental group.*

**Provide justification narrative.**

The requested animal numbers for ZIRC are based on actual shipping and breeding database entries (animal census) for the years 2018-2020, inflated by 10% (as buffer). In contrast to regular laboratories, colony breeding and distribution is *the* key part of ZIRC's mission, whereas "research activities" have somewhat lower priority. Hence, we list breeding colony and distribution animal numbers also in the research section.

*Provide common basis for animal numbers, if applicable. Check all that apply.*

**Numbers are based upon a statistical analysis or analyses.**

On average, ZIRC breeds a new generation once a year for each of its stocks and maintains the current animal census in its database. Annual averages of the census data show that in 2018; 2019; and 2020, ZIRC housed on average 56,176; 48,958; and 39,674 animals in its facility. Because animal numbers were particularly low in 2020 due to the impact of the Covid-19 pandemic, and a return to normal operations is expected for the year 2021/22, animal use was projected from the averages of 2018/2019 equaling 53,000 adults/year plus 10% = 58,000/per year. Fish for masculinization, and antioxidant supplement testing are included. We will breed an extra 8,880 fish for PCR Panel/Droplet DNA testing and 3,450 fish for Cryopreservation research each year. Thus a total of 70,330 fish per year.

**Lacking statistical data, this is the estimated minimum number necessary to provide valid results.**

## fish: Zebrafish

### Animal Characteristics

*For the current species (see highlighted subtab above), provide animal gender information. Check all that apply.*

**Female**

**Male**

*For the current species (see highlighted subtab above), provide animal age/developmental stage information. Check all that apply.*

**Embryonic**

**Larval (4 dpf through 7 dpf)**

**Larval (8 dpf through 30 dpf)**

**Juvenile**

**Adult**

*STRAIN and BREEDING LINE Information*

*Are there strains or breeding lines you will use that are not available via the checkboxes below in this section?*

**Yes**

<http://zebrafish.org/fish/lineAll.php/> and attached IBC registration of ZIRC lines (attached in Procedures)

## Animal Source

*For the current species (see highlighted subtab above), what is the source of the animals you will use? Check all that apply.*

### Approved Vendor(s)

#### Aquatic

Aquatic Animal Care Services Huestis Facility (AqACS)

Sinnhuber Aquatic Research Laboratory(SARL)

The European Zebrafish Resource Center (EZRC)

Zebrafish International Resource Center (ZIRC)

#### In-house Breeding Colony

#### Transfer from other UO PI(s)

ZIRC imports novel zebrafish lines from any submitting zebrafish laboratory at the UO if the line has scientific value for the research community. Specific PIs to be determined.

#### Other Source

ZIRC imports novel zebrafish lines from any submitting zebrafish laboratory from other institutions domestic and international, if the line has scientific value for the research community. Specific PIs to be determined.

## Animal Health Status

*For the current species (see highlighted subtab above), describe the health status of the animals. Check all that apply.*

### Conventional

#### Other Health Status

*The ZIRC conducts quarterly diagnostic testing of its colony to provide up-to-date health status information to the public. All recent and archived health reports can be obtained here:*

[https://zebrafish.org/wiki/health/health\\_reports/start](https://zebrafish.org/wiki/health/health_reports/start)

#### **Diagnostic testing includes:**

*The majority of moribund fish are submitted for histopathology.*

*A subset of all 8-month wild-type stocks is submitted for histopathology or PCR for *P. neurophilia*.*

*A subset of retired stocks is submitted for histopathology or PCR for *P. neurophilia*.*

*A subset of fish from the sentinel source tank is screened for *P. neurophilia* by histology or PCR.*

*Pre- and post-filtration sentinel fish are submitted quarterly for histopathology. Sentinel samples represent 6 months and 1 year of exposure to system parameters. One-year-exposure sentinels are sampled every 6 months.*

*Mycobacterium species are identified by qPCR on frozen fish.*

#### **The following pathogens are detected in the ZIRC colony:**

*Mycobacterium spp.: A single species of Mycobacterium, *M. chelonae*, has been identified from zebrafish and biofilms sampled from the ZIRC aquaculture facility (Whipps et al., 2008). We continue to periodically test fish by qPCR for Mycobacterium. *M. chelonae* is the only species that has been identified in fish reared in the ZIRC main fish room.*

#### Pathogens detected (all fish sampled):

*In last 3 months: Mycobacterium chelonae, Pseudoloma neurophilia*

*In last 12 months: Mycobacterium chelonae, Pseudoloma neurophilia*

*In last 36 months: Mycobacterium chelonae, Pseudoloma neurophilia*

## Acclimation Period

*Acclimation upon arrival at University of Oregon:*

**Policies on acclimation and quarantine will be followed for this species.**

Not applicable

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## Breeding and Genetic Information

*For the current species (see highlighted subtab above), will animals be bred?*

**Yes**

*Are the animals bred under this protocol available from a commercial supplier?*

**No**

*Provide source(s) for animals that will be bred. Check all that apply.*

**Colony at Other Institution Managed by non-UO Investigator**

Lines submitted to the ZIRC are bred by domestic and international laboratories of the zebrafish research community before being submitted to ZIRC as frozen sperm sample or live males (quarantined) for cryopreservation.

*What is the final disposition of unwanted adult breeders and unneeded offspring? Check all that apply.*

**Euthanized using Approved Procedure(s)**

**List euthanasia methods under the "Animal Procedures/Experimental Design" tab.**

*What is the breeding scheme?*

**Fish**

*Will the IACUC-approved species-specific fish SOP for breeding be used?*

**Yes**

*Will offspring require special care or special monitoring?*

**No**

*Will genotyping be required?*

**Yes**

**Fish**

*Select genotyping method(s):*

**Fin Clip**

**Visual Phenotype in Experimental Animals**

We carry >12,600 distinct lines that harbor 46,191 alleles among them. Embryonic, larval, or adult phenotypes (or gene expression) will be assessed at appropriate stages, according to published, or submitter-provided information to determine the genotype of parents (recessive traits) or individual fish (dominant). In addition, ZFIN records will be looked up and used to identify genetic carriers, as needed (or as available) for each individual fish line. Molecular genotyping is more efficient, therefore morphology/phenotype will only be assessed if molecular characterization protocols do not exist, or if additional confirmation is needed (e.g. for transgenes that have been detected by PCR, but require additional functional expression of the gene).

*When is the phenotype visible?*

**0 dpf through 7 dpf**

**8 dpf or later**

**Visual Phenotype in Offspring Used to ID Parent(s)**

As provided by ZFIN, submitting laboratory, or publication.

*When is the phenotype visible?*

**0 dpf through 7 dpf**

**8 dpf or later**

*For the current species (see highlighted subtab above), will genetically engineered animals be used?*

*Genetic Engineering is directed modification of the gene complement of a living organism by such techniques as altering the DNA, substituting genetic material by means of a virus, transplanting whole nuclei, transplanting cell hybrids, etc.*

**Yes**

*Important Reminder: Complete the "Use of Genetically Engineered Animals" questions under the "Safety, Hazards, and IBC" tab.*

*For the current species (see highlighted subtab above), what type of animal welfare documentation will be used?*

**Standard IACUC-Approved Checklist(s)**

## Multiple Protocol Animal Use

*Will the individual animal(s) in this subtab be used on other protocols?*

**No**

AB

AB/TU

CB Cooch Behar

Nadia

NHGRI-1

SAT Sanger AB Tübingen

TL

TU

WIK

# Animal Care, Housing and Transportation

## Husbandry

### Husbandry

*Will animals need care at UO?*

**Yes**

*For the all species on this protocol, select the animal type. Check all that apply.*

**Fish**

*Will UO standard species-specific water quality parameters be used?*

**Yes**

*Will UO standard species-specific feed types and feeding frequency be used?*

**Yes**

*Will UO standard species-specific illumination and photoperiod be used?*

**Yes**

*Will UO standard species-specific noise/sound ranges be used?*

**Yes**

*Will UO standard species-specific temperature ranges be used?*

**Yes**

*Will UO standard species-specific tank/cage change schedule be used?*

**Yes**

*Will the animals need any other type of non-standard care?*

**No**

## Housing

### Housing

*Will animals be housed at UO?*

**Yes**

*For the all species on this protocol, select the animal type. Check all that apply.*

**Fish**

*Will UO standard species-specific tank/cage type(s) be used?*

**Yes**

*Will UO standard species-specific environmental enrichment be used?*

**Yes**

*Will it be necessary to house animals individually other than for fin clips?*

**Yes**

*Why must animals be housed individually?*

*If it is necessary to house animals singly—for example, when justified for experimental purposes, for provision of veterinary care, or for incompatible animals—this arrangement should be for the shortest duration possible. If single animals are housed in small enclosures, an opportunity for periodic release into larger enclosures with additional enrichment items should be considered, particularly for animals housed singly for extended periods of time. (Guide, p. 60)*

**No Reliable Method for Tagging or Labeling Individual Animal Exists**

**Other**

We isolate individual fish mainly for fin-clipping and house them in crossing cages until PCR results have been obtained. Typically 2 animals are stored in a crossing cage with a central divider. Animals remain up to 2x2 days in static water conditions. They receive food on the second day, followed by a fresh water change. They are returned to the recirculating water system as soon as possible, after 4 days maximally, and are housed in groups of up to 20 fish.

For some lines, for which we do not have molecular identification protocols, we identify the carriers of genetic modifications by breeding them, and observing the embryonic or larval phenotypes of their offspring. This identifies the genotype of the parents and these adults are also housed individually until the genetic identification based on embryo/larval phenotypes is possible. However, fish stay never longer than 4 days in static conditions, and they receive food and water change on the second day in static water tank isolation.

*Will the fish be outside the main vivarium for more than 24 hours?*

*For aquatic animals "main vivarium" includes the Aquatic Animal Care Zebrafish Facility, Stickleback Facility, Zebrafish International Resource Center and Oregon Institute of Marine Biology.*

**No**

## Location Assignments

Site	Building	Floor	Section	Room	Room Type
Main Campus	ZIRC	First (Ground)	Conventional	118 Housing & Procedure	Housing and Procedure
Main Campus	ZIRC	First (Ground)	Quarantine	103 Quarantine	Housing and Procedure

### Location Assignments

*HOUSING: Will you need to house animals in rooms not listed in the table above?*

**No**

*Will any of the Procedure Rooms contain or house animals for longer than 12 hours?*

**No**

*EXPERIMENTS: Will you need to perform experiments on animals in rooms not listed in the table above?*

**No**

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## Transportation

### Transportation

*Will you need to transport animals outside the main vivarium? (Not Field Studies)*

**Yes**

*Movement of animals within or between sites or institutions should be planned and coordinated by responsible and well-trained persons at the sending and receiving sites to minimize animal transit time or delays in receipt. Shipping should be coordinated to ensure that animals arrive during normal business hours or, if delivery occurs outside of this time, that someone is available to receive them. Defining and delegating responsibility to the appropriate persons, who are knowledgeable about the needs of the species being shipped, will help ensure effective communication and planning of animal transport (AVMA 2002). (Guide pp. 107-108)*

*What type of animal transport will be used? Check all that apply.*

**Intrainstitutional**

*Careful planning for all types of transportation should occur to ensure animal safety and well-being. The process of transportation should provide an appropriate level of animal biosecurity while minimizing zoonotic risks, protecting against environmental extremes, avoiding overcrowding, providing for the animals' physical, physiologic, or behavioral needs and comfort, and protecting the animals and personnel from physical trauma. (Guide, p. 107)*

**Transport Between Main Campus Locations**

**Interinstitutional**

*Institutions should contact appropriate authorities to ensure compliance with any relevant statutes and other animal transportation requirements that must be met for animals to cross international boundaries, including those not of the country of final destination. The NRC publication *Guidelines for the humane Transportation of Research Animals* provides a comprehensive review of this topic (NRC 2006). (Guide, p. 107)*

**Commercial Transport Between Vendor(s) and Laboratories**

*Why will animals be transported outside the main vivarium? Check all that apply.*

**Collaboration(s) Between Laboratories**

**Acting as Vendor or Supplier to other Labs**

*Will animals return to the main vivarium?*

**No**

*Will the animals pass through public spaces or patient areas while in transit?*

**No**

*What method(s) will be used to transport the animals? Check all that apply.*

*REMINDER: Vehicles are Animal Facilities according to PHS Policy and, as such, are subject to IACUC inspection.*

*B. Animal Facility - Any and all buildings, rooms, areas, enclosures, or vehicles, including satellite facilities, used for animal confinement, transport, maintenance, breeding, or experiments inclusive of surgical manipulation. (PHS Policy, III. Definitions)*

**Non-Vehicle Transport**

**Cart**

**Insulated Container ("Cooler")**

**Tray**

**Vehicle Transport**

**Vendor Vehicle Transport**

*Will you follow the UO species-specific SOP on animal transportation?*

**Yes**

*Who will be responsible for the animal transport? Check all that apply.*

**Principal Investigator**

**Laboratory Personnel**

approved ZIRC personnel listed in personnel tab.

**Main Vivarium Personnel**

approved ZIRC personnel listed in personnel tab.

*Where will the animals be transported?*

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**Provide departure and arrival locations:**

various locations, depending on request.

*How long will the animals be in transit?*

**Provide travel duration estimate:**

travel duration will vary based on the location. Transit time will always be attempted to be minimized.

International Shipments <4 days transit (Monday - Friday arrival)

Domestic Shipments: Over night, (Wednesday - Thursday)

## Animal Procedures/Experimental Design

Experimental Design / Animal Procedures	Animal Subjects Details
Zebrafish International Resource Center	fish: Zebrafish

## Zebrafish International Resource Center

### Animal Species Use

fish: Zebrafish

### Procedures Summary

*For the current aim (see highlighted subtab above), explain the experimental design and provide a clear, concise and sequential description of the procedures involving the use of animals.*

*This narrative should explain the overall design and include things like experimental animal groups, group sizes, and group use.*

**IMPORTANT:** Provide a clear and concise sequential description of the procedures (especially those that are listed in the procedures tab) involving the use of animals that is easily understood by all members of the IACUC. Details of any of the procedures should be described in the "Procedures" tab. This description should allow the IACUC to understand the experimental use of an animal from its entry into the experiment to the endpoint of the study.

*NOTE: You may attach diagrams, flowcharts, etc., by using the paperclip icon at the top or bottom of this page.*

**Provide Experimental Design (see IMPORTANT help text above):**

#### **1: Genetic Repository and Distribution**

##### **1. To serve as a central repository for zebrafish genetic stocks and research materials.**

1. ZIRC acquires live males or cryopreserved sperm samples from submitting laboratories, after the health status of the submitting fish facility has been obtained. If not available all materials are treated as contagious. Live fish undergo stringent quarantine and additional health monitoring procedures in the quarantine room. Healthy males are bred and bleach surface sanitized embryos are introduced into the main fish facility nursery. Testes are dissected or sperm samples are collected and cryopreserved and stored in designated vapor phase liquid nitrogen freezers. We continue to expand our collection by obtaining carriers of mutations and transgenes from the research community and breed them to produce new generations. We freeze and store sperm from all imported lines. We also acquire the most widely used wild-type lines and maintain them in a manner that preserves their genetic diversity. We receive and store antibodies, gene probes and markers used to identify and analyze wild-type and mutant stocks.

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## 2. ZIRC propagates healthy stocks of fish (2.1) and maintains frozen sperm (2.2) of identified genotypes.

*2.1 Live fish are maintained, identified and propagated in a manner that preserves their genetic characteristics and makes these available to requesting researchers. Live fish are also generated and maintained to amplify cryopreserved stocks or to be distributed directly.*

*2.2 Cryopreserved stocks are thawed if the sample count reaches a low threshold, and fish are raised, identified, and gametes are harvested to amplify the resource. Cryopreserved samples are also thawed upon request for shipment. After in vitro fertilization, embryos are shipped to clients, and/or introduced to the main fish facility nursery to raise a new generation and reamplify frozen stocks. Popular lines that are requested frequently or needed for teaching are maintained alive as long as needed and are retired to the sperm bank when requests reach a low annual threshold.*

3. We make frozen and live stocks widely available to the research community. Upon request, we ship fish and materials to domestic and international research laboratories using commercial courier services. Animal shipments are packaged according to IATA regulations plus a heat pack if destinations or seasons require it. Live fish are bred and their offspring is sorted, cleaned, packaged and shipped as larvae, juveniles or young adults. Cryopreserved stocks are thawed, used for *in vitro* fertilization, and embryos, juveniles or young adults are packaged and shipped to requesting laboratories. We also provide online information about the stocks, materials, ordering procedures and methods (<http://zebrafish.org/zirc/home/guide.php>) in collaboration with ZFIN, the zebrafish model organism database (<http://zfin.org>).

Please see attached SOP collection of ZIRC's routine operations for import, maintenance, preservation, and distribution of fish lines.

## **2: Diagnostic Health Service**

***ZIRC provides diagnostic pathology and consultation services.***

*ZIRC provides diagnostic services and health status testing for laboratory zebrafish, including ZIRC's own colony. We use histopathology, bacteriology, necropsy, and virology to analyze specific or suspected disease problems. We provide routine sentinel or quality control testing of zebrafish from healthy laboratory colonies for early detection of problems and to fulfill institutional animal care and use committee, IACUC, health status monitoring requirements. We continue to develop methods to detect and control disease in laboratory colonies. We continue to investigate the transmission and pathology of the microsporidian parasite, *Pseudoloma neurophilia*, and *Mycobacterium chelonae* and we continue to discover novel pathogens through the diagnostic service.*

*The ZIRC veterinarian monitors the health status of the ZIRC fish colony and maintains biosafety during line exchanges. The ZIRC veterinarian assesses the presence and prevalence of pathogens in the facility by monitoring sentinel fish populations, or strategically and randomly selected fish, and proposes strategies to control or eliminate any pathogens identified in the colony. The ZIRC veterinarian is also in charge to put in place policies to prevent novel pathogens from entering the facility during import and provides health statements to clients upon fish exports. A quarterly colony health statement is prepared and made available online to the research community.*

Please see attached SOP collection of ZIRC's routine operations for the colony health monitoring procedures and Diagnostic Health Services.

***Research Aim. We will develop a platform, based on a panel of PCR assays, to screen for the most prevalent pathogens of laboratory zebrafish.*** We will use genomic sequence information from known zebrafish pathogens to design and test PCR primers for species-specific detection. We will validate the sensitivity of these molecular assays with standard health screening by histopathology. We will use these PCR assays and other diagnostic tools to establish and implement screening procedures for in-house detection and monitoring of zebrafish pathogens. We will establish a platform, through the ZIRC website, for sharing the ZIRC pathogen monitoring program and tools for diagnostic testing with the research community, and we will provide consultation and diagnostic services, using this new platform, to identify pathogens in research laboratories, through our Pathology Service (Resource Aim 2).

## **3: Cryopreservation Research**

All fish lines acquired by the ZIRC are preserved as cryogenically frozen sperm samples. Currently, ZIRC preserves 98,625 samples of 46,161 distinct alleles (~2.1 samples/allele) in approximately 12,600 fish lines (~7.8 samples/line).

Typically, live males are imported into the quarantine room, acclimated, observed for health status, and either bred with AB females and cryopreserved, or cryopreserved only. When cryopreserved samples are imported in liquid



nitrogen, samples are transferred into a vapor phase cryogenic freezer. When fish lines are rederived from frozen sperm to fulfill a request, to reamplify a stock, or to genetically identify carriers, the health status of the submitting laboratory will dictate what biosafety measures are taken with the live embryos and larvae. Frequently requested fish lines are maintained live in the fish facility, however once the number of requests drops below 9 per year, lines are cryopreserved and live propagation is discontinued. Similarly, frequent requests trigger the reactivation of a frozen line to live maintenance. In addition, when regular requests for frozen samples deplete a frozen resource, lines are reactivated to freeze more sperm samples and reamplify the resource. Hence ZIRC's genetic line management revolves around the constant freezing and thawing of fish lines based on user request frequencies and resource availability, minimizing the number of live lines as much as possible to manage the genetic resources as efficiently as possible.

An emergency back-up site exists in Fort Collins, Colorado at the National Center for Genetic Resource Preservation (ARS/USDA) for cryopreserved ZIRC samples that are not backed up reliably in other laboratories or Resource Centers.

Please see attached SOP collection of ZIRC's *routine cryopreservation procedures*.

### **Cryopreservation Research Component**

The zebrafish research community has produced several tens of thousand mutations, transgenes, and continues to produce more novel modifications of the zebrafish genome. However, the success of zebrafish comes with a price: there are simply too many lines to maintain as live populations within laboratories. Sperm cryopreservation has become an extraordinarily efficient storage and management tool that keeps expenses in check for the Zebrafish International Resource Center (ZIRC) and, hence, the biomedical research community. However, cryopreservation harbors risks for the integrity of germplasm, including cellular and DNA damage from Reactive Oxygen Species (ROS) that are generated during short-term storage of freshly collected milt, and subsequent freezing and thawing. Because the community lacks standards for sample quality, ZIRC wastes significant effort trying to reactivate fish lines from cryopreserved samples imported from laboratories with varying freezing protocols. We will assay ROS and cell damage using fluorescent vital dyes and fluorescence cytometry together with quantitative sample quality assessment, including computer assisted cell analysis (CASA) of sperm motility, cell morphology, and fertilization success with males *in vivo* and after thawing samples *in vitro*. We will then test antioxidants and other compounds to develop a new protocol that minimizes cellular damage incurred before and during cryopreservation and thawing. We will test these as additives in fish food and in dilution (extender) and cryoprotective media. Our goal is to improve the viability and quality of fresh milt during short- (hours) and intermediate-term (days) storage before freezing and after thawing. This will lead to novel and safer means of transport and experimentation with sperm cells. By adding new cryopreservation-based services to ZIRC's portfolio, centered around sperm sample quality assessment, quality data tracking, and a highly reliable cryopreservation protocol with do-it yourself cryo-kits, we will also achieve better standardization of cryopreservation methods for research laboratories, and for sample acquisition by ZIRC from the community. Specifically, we will analyze and improve sperm extender and cryoprotective media to minimize oxidative stress and physical damage to cells during refrigerated storage, cryopreservation, and thawing. In addition, we will extend refrigerated storage time and enable shipping of refrigerated, rather than frozen sperm samples. We will also develop new cryopreservation services and database tools to support community standards for quality assessment, storage, and exchange of sperm samples.

### **Animal Biosafety Level**

*For the current aim (see highlighted subtab above), will the risks associated with the experiments exceed Animal Biosafety Level 1 (ABSL-1)?*

*Where biologic agents are used, the Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH) publication biosafety in Microbiological and biomedical Laboratories (BMBL; DHHS 2009) and the USDA standards (USDA 2002) should be consulted for appropriate facility design and safety procedures. These design and safety features are based on the level of risk posed by the agents used. Special facilities and safety equipment may be needed to protect the animal care and investigative staff, other occupants of the facility, the public, animals, and the environment from exposure to hazardous biologic, chemical, and physical agents used in animal experimentation. (Guide, p. 19)*

**No, ABSL-1 is the risk level associated with the animal use in these experiments.**

## Humane Endpoints

*For the current aim (see highlighted subtab above), what are the humane endpoints?*

*Humane Endpoints adapted from information [online](#).*

**Select Animal Type(s) Used in this Aim. Check all that apply.**

### **Fish**

*Types of Humane Endpoints (Check all that apply.)*

**Abnormal Color Change**

**Abscess**

**Distended Abdomen**

**Emaciation**

**Eye Bulging (Exophthalmos)**

**Fungal Growth on Body or Fins**

**Gas Bubbles**

**Hemorrhage or Redness**

**Masses or Swellings**

**Skeletal Deformity**

**Tumor Formation**

**Twirling**

**Ulcer**

**Wound**

**Placeholder**

## Final Disposition and Euthanasia

Not applicable

### Euthanasia / Final Disposition

*For this aim, what will be the final disposition of the animals? Check all that apply.*

#### Euthanasia

*METHODS of EUTHANASIA and CONFIRMATION of DEATH*

**Select Animal Type(s) Used in this Aim. Check all that apply.**

##### Fish

*What method(s) of euthanasia will be used? Check all that apply.*

##### IACUC-Approved Methods

(zebrafish, adult) Immersion in solution of buffered tricaine methanesulfonate (MS-222), 0.03% (w/v)

*Finfish should be left in the anesthetic solution for a minimum of 10 minutes after cessation of opercular movement. (AVMA Guidelines for the Euthanasia of Animals (2013), p. 72)*

(zebrafish, all developmental stages) Rapid Chilling (Hypothermic Shock)

*Transfer from acclimatized temperatures to water associated with a 2° to 4°C ice slurry must occur rapidly with as little transfer of warmer water as possible. (AVMA Guidelines for the Euthanasia of Animals (2013), p. 73)*

##### Other Method(s)

Accidental death by electrosedation (infrequent, usually males)

*How will you confirm animal death? Check all that apply.*

##### Cessation of Vital Signs

#### Other

Fish can be euthanized or maintained alive for future electrosedation testing, but are never returned to the breeding colony or transferred to other protocols/tests. I.e. the same animal may be electrosedated 2-3 times over a period of 6-9 months. A minimal resting period between electrosedation tests of 2 weeks is observed.

### Antibody Production

*Will you produce antibodies as part of this Aim?*

No

### Surgery

*Will you be performing surgery as part of this Aim?*

Yes

*What type of surgery will be performed? Check all that apply.*

#### MAJOR SURGERY

*As a general guideline, major survival surgery penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection (Brown et al. 1993).*

#### MINOR SURGERY

*Minor survival surgery does not expose a body cavity and causes little or no physical impairment; this category includes wound suturing, peripheral vessel cannulation, percutaneous biopsy, routine agricultural animal procedures such as castration, and most procedures routinely done on an "outpatient" basis in veterinary clinical practice.*

#### Minor Surgery

*What surgery category is part of this Aim? Check all that apply.*

*Surgical procedures are categorized as major or minor and, in the laboratory setting, can be further divided into survival and nonsurvival. As a general guideline, major survival surgery (e.g., laparotomy, thoracotomy, joint replacement, and limb amputation) penetrates and exposes a*

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body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection (Brown et al. 1993). Minor survival surgery does not expose a body cavity and causes little or no physical impairment; this category includes wound suturing, peripheral vessel cannulation, percutaneous biopsy, routine agricultural animal procedures such as castration, and most procedures routinely done on an “outpatient” basis in veterinary clinical practice. Animals recovering from these minor procedures typically do not show significant signs of postoperative pain, have minimal complications, and return to normal function in a relatively short time. When attempting to categorize a particular surgical procedure, the following should be considered: the potential for pain and other postoperative complications; the nature of the procedure as well as the size and location of the incision(s); the duration of the procedure; and the species, health status, and age of the animal. Laparoscopic surgeries and some procedures associated with neuroscience research (e.g., craniotomy, neurectomy) may be classified as major or minor surgery depending on their impact on the animal (Devitt et al. 2005; Hancock et al. 2005; NRC 2003; Perret-Gentil et al. 1999, 2000). For example, laparoscopic techniques with minimal associated trauma and sequelae (e.g., avian sexing and oocyte collection) could be considered minor, whereas others (e.g., hepatic lobectomy and cholecystectomy) should be considered major. Although minor laparoscopic procedures are often performed on an “outpatient” basis, appropriate aseptic technique, instruments, anesthesia, and analgesia are necessary. Whether a laparoscopic procedure is deemed major or minor should be evaluated on a case-by-case basis by the veterinarian and IACUC. (Guide, p. 117-118)

### **Survival Surgery**

### **Nonsurvival Surgery**

*Was the veterinarian involved in the presurgical planning?*

Presurgical planning should include input from all members of the surgical team (e.g., the surgeon, anesthetist, veterinarian, surgical technicians, animal care staff, and investigator). The surgical plan should identify personnel, their roles and training needs, and equipment and supplies required for the procedures planned (Cunliffe-Beamer 1993); the location and nature of the facilities in which the procedures will be conducted; and perioperative animal health assessment and care (Brown and Schofield 1994). A veterinarian should be involved in discussions of the selection of anesthetic agents and doses as well as the plan for perioperative analgesic use. If a nonsterile part of an animal, such as the gastrointestinal tract, is to be surgically exposed or if a procedure is likely to cause immunosuppression, preoperative antibiotics may be appropriate (Klement et al. 1987); however, the routine use of antibiotics should never be considered a replacement for proper aseptic surgical techniques. Presurgical planning should specify the requirements for postsurgical monitoring, care, and recordkeeping, including the personnel who will perform these duties. The investigator and veterinarian share responsibility for ensuring that postsurgical care is appropriate. (Guide, p. 116)

**Yes**

*Will surgery be performed outside of the central animal facility?*

Unless an exception is specifically justified as an essential component of the research protocol and approved by the IACUC, aseptic surgery should be conducted in dedicated facilities or spaces. When determining the appropriate location for a surgical procedure (either a dedicated operating room/suite or an area that provides separation from other activities), the choice may depend on the species, the nature of the procedure (major, minor, or emergency), and the potential for physical impairment or post-operative complications, such as infection. Most bacteria are carried on airborne particles or fomites, so surgical facilities should be maintained and operated in a manner that ensures cleanliness and minimizes unnecessary traffic (AORN 2006; Bartley 1993). If it is necessary to use an operating room for other purposes, it is imperative that the room be returned to an appropriate level of hygiene before its use for major survival surgery. (Guide, p. 116-117)

**No**

*Which personnel will be involved?*

### **Which personnel will perform non-survival surgery?**

Keely Core (Testes dissection) Jen Matthews (Testes dissection) Joy Murphy (Testes dissection) Eryn Loucks (Testes dissection) Dagmara Marston (Testes dissection) Evan Williams (Testes dissection) Zoltan Varga (Testes dissection)

### **Which personnel will perform survival surgery?**

Jen-Jen Hwang Shum (Fin Clips) Keely Core (Fin Clips) Eryn Loucks (Fin Clips) Dagmara Marston (Fin Clips) Evan Williams (Fin Clips)

### **Which personnel will provide training of lab-specific surgical procedures to new lab members?**

Jen-Jen Hwang Shum (Fin Clips); Jen Matthews (VMD; testes dissections)

*Other than veterinary health reasons, have animals been surgically manipulated in other studies prior to your use?*

**No**

*Surgical Procedure Considerations (Check All)*

### **Preoperative Considerations**

*Which preoperative steps (not including anesthesia) will be used to prepare animals for surgery? Check all that apply.*

#### **Physical Exam**

*For nonsurvival surgeries, will the surgery be performed using aseptic technique, clean technique or a combination of both?*

*In nonsurvival surgery, an animal is euthanized before recovery from anesthesia. It may not be necessary to follow all the techniques outlined in this section if nonsurvival surgery is performed but, at a minimum, the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean (Slattum et al. 1991). For nonsurvival procedures of extended duration, attention to aseptic technique may be more important in order to ensure stability of the model and a successful outcome.*

(Guide, p. 118)

## Clean Technique

*For survival surgeries, will the surgery be performed using aseptic technique and in accordance with the Guide?*

*Aseptic technique is used to reduce microbial contamination to the lowest possible practical level (Mangram et al. 1999). No procedure, piece of equipment, or germicide alone can achieve that objective (Schonholtz 1976): aseptic technique requires the input and cooperation of everyone who enters the surgery area (Belkin 1992; McWilliams 1976). The contribution and importance of each practice varies with the procedure. Regardless of the species, aseptic technique includes preparation of the patient, such as hair or feather removal and disinfection of the operative site (Hofmann 1979); preparation of the surgeon, such as the provision of appropriate surgical attire, face masks, and sterile surgical gloves (Chamberlain and Houang 1984; Pereira et al. 1990; Schonholtz 1976); sterilization of instruments, supplies, and implanted materials (Bernal et al. 2009; Kagan 1992b); and the use of operative techniques to reduce the likelihood of infection (Ayliffe 1991; Kagan 1992a; Lovaglio and Lawson 1995; Ritter and Marmion 1987; Schofield 1994; Whyte 1988). While the species of animal may influence the manner in which principles of aseptic technique are achieved (Brown 1994; Cunliffe-Beamer 1983; Gentry and French 1994), inadequate or improper technique may lead to subclinical infections that can cause adverse physiologic and behavioral responses (Beamer 1972; Bradfield et al. 1992; Cunliffe-Beamer 1990; Waynforth 1980, 1987) affecting surgical success, animal well-being, and research results (Cooper et al. 2000). General principles of aseptic technique should be followed for all survival surgical procedures (ACLAM 2001). (Guide, p. 118)*

No

### Provide Explanation

Fin clips are performed under MS-222 anesthesia with a sterile, disposable blade on a clean piece of parafilm to avoid genetic cross contamination between specimen. Personnel is wearing sterile gloves. Infections have not been observed at the amputation site with this technique.

*What types of surgeon preparation will be used and what equipment will the surgeon employ? Check all that apply.*

### Sterile Gloves

*What types of animal patient preparation will be used and what equipment will the animal patient be given? Check all that apply.*

### Other

### Parafilm

*What method(s) will be used to sterilize the instruments? Check all that apply.*

*Specific sterilization methods should be selected on the basis of the physical characteristics of the materials to be sterilized (Callahan et al. 1995; Schofield 1994) and sterilization indicators should be used to validate that materials have been properly sterilized (Berg 1993). Autoclaving and plasma and gas sterilization are effective methods most commonly used to sterilize instruments and materials. Alternative methods, used primarily for rodent surgery, include liquid chemical sterilants and dry heat sterilization. Liquid chemical sterilants should be used with appropriate contact times and instruments should be rinsed with sterile water or saline before use. Bead or dry heat sterilizers are an effective and convenient means of rapidly sterilizing the working surfaces of surgical instruments but care should be taken to ensure that the instrument surfaces have cooled sufficiently before touching animal tissues to minimize the risk of burns. Alcohol is neither a sterilant nor a high-level disinfectant (Rutala 1990) but may be acceptable for some procedures if prolonged contact times are used (Huerkamp 2002). (Guide p. 119)*

### Autoclave

*Will preoperative anesthesia, sedation, or tranquilization be provided to the animals?*

**Yes. Preoperative drugs will be used to calm the animals.**

### MS-222

## Postoperative Considerations

*An important component of postsurgical care is observation of the animal and intervention as necessary during recovery from anesthesia and surgery (Haskins and Eisele 1997). The intensity of monitoring will vary with the species and the procedure and may be greater during the immediate anesthetic recovery period. During this period, animals should be in a clean, dry, and comfortable area where they can be observed frequently by trained personnel. Particular attention should be given to thermoregulation, cardiovascular and respiratory function, electrolyte and fluid balance, and management of postoperative pain or discomfort. Additional care may be warranted, including long-term administration of parenteral fluids, analgesics, and other drugs, as well as care of surgical incisions. Appropriate medical records should also be maintained. After recovery from anesthesia, monitoring is often less intense but should include attention to basic biologic functions of intake and elimination and to behavioral signs of postoperative pain, monitoring for postsurgical infections, monitoring of the surgical incision site for dehiscence, bandaging as appropriate, and timely removal of skin sutures, clips, or staples (UFAW 1989).*

(Guide, p. 119-120)

**Describe immediate postoperative recovery monitoring procedures, including duration and frequency.**

*What criteria will be used for postoperative monitoring of animal condition during recovery from anesthesia? Check all that apply.*

**Other**

Fish are placed in transparent 1-gallon static water tanks, and are observed for successful and full recovery from MS-222 anesthesia immediately after fin clipping. Fin clipped fish are placed in a dedicated area of the facility and are observed for any signs of pain, distress, or discomfort (or other humane endpoints listed previously) at least once a day until genotyping results are obtained and identified fish can be placed into recirculating water tanks (2-4 days).

*What postoperative method(s) of animal support will be used during immediate recovery? Check all that apply.*

**Mechanical Ventilation**

**Describe postoperative care support activities following surgery.**

*The selection of appropriate analgesics and anesthetics should reflect professional veterinary judgment as to which best meets clinical and humane requirements as well as the needs of the research protocol. The selection depends on many factors, such as the species, age, and strain or stock of the animal, the type and degree of pain, the likely effects of particular agents on specific organ systems, the nature and length of the surgical or pain-inducing procedure, and the safety of the agent, particularly if a physiologic deficit is induced by a surgical or other experimental procedure (Kona-Boun et al. 2005). Preemptive analgesia (the administration of preoperative and intraoperative analgesia) enhances intraoperative patient stability and optimizes postoperative care and well-being by reducing postoperative pain (Coderre et al. 1993; Hedenqvist et al. 2000). Analgesia may be achieved through timely enteral or parenteral administration of analgesic agents as well as by blocking nociceptive signaling via local anesthetics (e.g., bupivacaine). (Guide, p. 121-122)*

*Will analgesia be provided for relief of postoperative pain?*

**No**

Wound healing is extremely fast and typically, fish never show signs of discomfort pain or distress

*What is the estimated period of time to full recovery for the animal?*

**Provide Recovery Period**

2-3 minutes

*Where will the animal be located during postoperative recovery?*

**Provide Recovery Location**

Main fish room, transparent static tank; Water is changed every other day after a feeding with flake food. If genotyping exceeds 4 days, animals are placed individually in 1-gallon tanks on the recirculating water system (over the weekend and until results are available).

*Which personnel will be monitoring the animal during postoperative recovery?*

**Provide Names of Personnel**

Jen-Jen Hwang Shum, April Freeman, Zoltan Varga, Keely Core, Evyn Loucks, Dagmara Marston, Evan Williams

*What is the frequency of postoperative monitoring?*

**Provide Frequency of Postoperative Monitoring**

Immediately after MS-222 anesthesia, then at least once daily, at feeding, and at water changes

*What, if any, complications could reasonably be expected?*

*What parameters will be used to detect signs of pain, distress or discomfort? Check all that apply.*

**Wound Healing**

**IMPORTANT:** This parameter requires checking the wound at least daily until suture removal.

extremely rare

**Other**

All humane endpoints described earlier for zebrafish

*When observations indicate pain, discomfort, or distress, will the UO Emergency Veterinary Care SOP be followed?*

No

A decision will be made by the ZIRC veterinarian whether or not to euthanize the fish.

*What are the provisions for after hours, weekend, and holiday care for animals in recovery?*

**Provide Description of After Hours, Weekend, and Holiday Care for Animals in Recovery**

If genotyping exceeds 4 days, animals are placed individually in 1-gallon tanks on the recirculating water system (over the weekend and until results are available). They are fed and monitored along with all other animals in the facility.

## Unexpected Outcomes

*Will this Aim introduce novel experimental variables that may affect animal health and welfare?*

Yes

*What is the plan for monitoring the health and welfare of animals exposed to novel experimental variables?*

**Provide Plan for Monitoring Animal Health and Welfare**

Animals will be observed during the recovery period as outlined above and their recovery steps are timed and statistically evaluated with their body condition factor. Animals will be monitored as outlined above for pain distress and discomfort or humane endpoints. Animals will be monitored during recovery, after experimentation, at the end of the day of experimentation and then daily once for 3-4 days. If humane endpoints or unexpected outcomes are observed fish will be euthanized by IACUC approved methods (rapid chilling).

*What method of communication will you use to report animal welfare concerns to the IACUC when the initial characterization of a genetically modified animal (GMA) reveals a condition that negatively affects animal well-being? (Check all that apply.)*

Email

In-Person Office Visit

Phone

## Animal number calculation for experimental part Zebrafish International Resource Center

fish: Zebrafish			
	Min	Max	Procedure or Experimental Group
	0	0	<b>Animals</b>
	58000	58000	Routine Stock Center Operations (17-AMT, Diet)
+	8880	8880	PCR Panel/Droplet DNA Test development
+	3450	3450	Cryopreservation Equipment Testing
	70330	70330	<b>fish: Zebrafish</b>

## Procedures, Pre-Defined and Manually Entered

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Description	Species
ZIRC-L3: Masculinization of fish stocks with 17 alpha-Methyltestosterone	fish: Zebrafish
ZIRC-L3: Fish Food Testing	fish: Zebrafish
ZIRC-L3: Open-Platform PCR Screening Panel Development	fish: Zebrafish
ZIRC-L3: Pseudoloma Droplet DNA testing	fish: Zebrafish
ZIRC-L3: Cryopreservation Research: testing of micro fabricated and 3-D printed cryopreservation tools	fish: Zebrafish

## Description of Procedures

### 1. Genetic Repository and Distribution

Please see attached the updated collection of ZIRC SOPs for routine operations for import, maintenance, preservation, and distribution of fish lines.

Estimated number of animals for fish line propagation, identification and distribution: ca. **58,000 adult/year** (based on distribution and husbandry data of the past 3 years).

### 2. Diagnostic Health Service

Please see attached SOP collection of ZIRC's *routine operations for the colony health monitoring procedures and Diagnostic Health Services*.

### ZIRC-L3: Masculinization of fish stocks with 17 alpha-Methyltestosterone

ZIRC fish populations are sometimes skewed to females. However, ZIRC depends particularly on males and the cryopreservation of sperm to turn over and manage its resources efficiently. Therefore, a high ratio of females poses a problem especially in small stocks, when a certain minimal number (20) of cryopreserved samples is required. Therefore, we are adding 17-alpha-methyltestosterone (17-AMT) to larval flakes to induce male-skewed in a specific set of stocks that consistently produces more females than males. To this end, a batch of freshly prepared Juvenile flake mix is added to 17-AMT dissolved in Ethanol (99,9%) to yield 15mg 17-AMT/kg food. The flakes are vacuum dried and stored in a falcon tube wrapped in Aluminum foil in the food room fridge. Larvae receive standard portions of the 17-AMT diet.

Estimated number of animals, ca. 5,000 animals/year (a subpopulation of the 58,000 above)

### ZIRC-L3: Fish Food Testing

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Sperm cryopreservation has become an extraordinarily efficient storage and management tool that keeps expenses in check for the Zebrafish International Resource Center (ZIRC) and, hence, the biomedical research community. However, cryopreservation harbors risks for the integrity of germplasm, including cellular and DNA damage from Reactive Oxygen Species (ROS) that are generated during short-term storage of freshly collected milt, and subsequent freezing and thawing. Because the community lacks standards for sample quality, ZIRC wastes significant effort trying to reactivate fish lines from cryopreserved samples imported from laboratories with varying freezing protocols. We will assay ROS and cell damage using fluorescent vital dyes and fluorescence cytometry together with quantitative sample quality assessment, including computer assisted cell analysis (CASA) of sperm motility, cell morphology, and fertilization success with males *in vivo* and after thawing samples *in vitro*. We will then test antioxidants and other compounds to develop a new protocol that minimizes cellular damage incurred before and during cryopreservation and thawing. We will test these as additives in fish food and in dilution (extender) and cryoprotective media. Our goal is to improve the viability and quality of fresh milt during short- (hours) and intermediate-term (days) storage before freezing and after thawing. This will lead to novel and safer means of transport and experimentation with sperm cells. By adding new cryopreservation-based services to ZIRC's portfolio, centered around sperm sample quality assessment, quality data tracking, and a highly reliable cryopreservation protocol with do-it yourself cryo-kits, we will also achieve better standardization of cryopreservation methods for research laboratories, and for sample acquisition by ZIRC from the community. To these ends we will analyze gonads and gametes of fish that have been euthanized by standard UO/IACUC approved protocol (see Anesthesia Analgesia tabs). There are three key goals we want to achieve:

a) We will analyze and improve sperm extender and cryoprotective media to minimize oxidative stress and physical damage to cells during refrigerated storage, cryopreservation, and thawing. This will include A) visualization and quantification of ROS and DNA- and plasma-membrane damage in sperm cells. Specifically, we will determine at which steps in the current protocol ROS, plasma membrane-, or DNA-damage is generated. B) We will test whether antioxidants and cholesterol protect sperm cells from oxidative stress and plasma membrane damage before and during cryopreservation, or after thawing. To this end we will either add these substances to fish feed (as in Food diet testing) or expose sperm cells to these compounds in solution C) We will determine optimal dosage of lipid- and water soluble antioxidants and cholesterol-loaded cyclodextrin in extender and cryoprotective solutions or as food additives. D) We will evaluate whether cryopreservation, storage, shipping, and male fertility are improved by these additives, and we will adapt accordingly the formulation of fish diet, cryopreservation media, or both.

b) We will extend refrigerated storage time and enable shipping of refrigerated, rather than frozen sperm samples. A) We will evaluate the viability, motility, and fertilization rates of sperm samples in our current extender and in synthetic seminal fluid over a period of several days under room-temperature and refrigerated storage conditions. We will vary ionic composition and strength, availability of metabolites, antioxidants, and cell culture serum supplements to maximize storage time. B) We will test whether cultured testis tissue is a better alternative to refrigerated storage, shipping, and recovery of spermatozoa. To this end we will dissect testes or collect milt as described in previously IACUC approved protocols listed in our SOP collection. C) We will develop new storage and line exchange protocols depending on the results.

c) ZIRC will develop new cryopreservation services and database tools to support community standards for quality assessment, storage, and exchange of sperm samples. A) We will offer a new service to back up laboratory fish lines. Upon request, sperm samples will be collected from live males in the ZIRC quarantine room, cryopreserved, temporarily stored in a quarantine cryogenic freezer, and returned to clients for long-term storage. B) In coordination with the AGGRC (our scientific collaborators at LSU) we will provide basic and more advanced do-it-yourself cryo-kits for users to cryopreserve fish lines or to submit standardized-quality samples to ZIRC. C) We will add a tab on our home page and offer annual cryopreservation workshops, visits, and other training opportunities for researchers to learn ZIRC's cryopreservation platform. We will provide user interfaces to collect quality assessment data and develop analytical tools for sample quality control. Cryopreservation data will be cross-linked to ZFIN, aGRIN (ZIRC's emergency off-site repository), and AGGRC to support future analysis and research of sperm sample quality.

### **Animal breeding and space**

Space requirements: 3,000 larvae/nursery (a subpopulation of the 58,000 above)

### **ZIRC-L3: Open-Platform PCR Screening Panel Development**

In recent years, a number of zebrafish pathogens have been identified, many by our own studies. These pathogens have negative direct and indirect effects on research. Impacts include altered behavior, repressed fecundity, massive mortalities, and human infection with zoonotic pathogens. The research community currently lacks a platform for systematic health screening. Some commercial services are offered, but because they are for-profit, critical information about detection assays including sensitivity and validation are proprietary and not made available to researchers who use the service. If negative results are incorrectly reported to the user, this can

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provide a false sense of pathogen-free status that can lead to severe consequences. Similarly, false positives can result in unnecessary anxiety and culling of animals. ZIRC has already established successful health-monitoring programs. However, current monitoring is predominantly based on histopathology. We outsource molecular screening for pathogens to the Oregon Veterinary Diagnostic Lab (OVDL), and this testing is currently limited to only a few species. Our recent work in-house and our studies of cases submitted to our Pathology and Health Services have identified and characterized an increasing number of pathogens present in zebrafish research facilities (see list below). Thus, it is critically important for us to develop a reliable, standardized PCR platform for systematic pathogen detection within the ZIRC facility.

Ultimately, ZIRC will provide a complementary diagnostic service to the research community, by empowering individual laboratories and fish facilities to carry out their own pathogen monitoring, tailored specifically to their research programs. In doing so researchers will no longer need to rely on one-size-fits-all screening panels with uncertain detection sensitivities and unknown protocol validation, as are currently offered by commercial entities. To this end, we will develop a PCR panel for the most prevalent pathogens found in zebrafish research facilities. We will provide the information on our web site, along with general health monitoring methods and the most typical screening strategies that can be applied in fish facilities of various sizes, from small to very large. To help set up user-specific health monitoring programs, we will also advise laboratories how to apply the screening panels to their specific needs. We will charge a consultation fee for this service.

We will design PCR assays to detect *Pseudoloma neurophilia*, *Mycobacterium* species (i.e. *M. marinum*, *M. haemophilum*, *M. chelonae*, and *M. fortuitum*), *Mycoplasma* spp. (Karen Guillemin, personal communication), *Pleistophora hyphessobryconis*, *Edwardsiella ictaluri*, *Myxidium streisingeri*, and *Pseudocapillaria tomentosa*. We will use DNA sequences from these pathogens for heat-shock protein and ribosomal RNA (rRNA) genes. Sequence information is available. As we identify new pathogens through our ongoing Pathology and Health Services, we will use the same strategy to add them to the platform.

Unlike ZIRC, most laboratories do not have Kompetitive Allele Specific PCR (KASP; LGC Genomics) genotyping capability. Therefore, we will design two types of assays in parallel. In the first, the PCR products will be analyzed by conventional gel electrophoresis and visualized with ethidium bromide and UV illumination. In the second, KASP technology will be used, which relies on fluorescent probes. This will alleviate the need to use ethidium bromide gels and will make pathogen detection and monitoring faster, more efficient, and safer. Because KASP amplicons are typically 50 base pairs or shorter, we will use commercially synthesized DNA oligonucleotides of pathogen-specific SNPs for assay testing.

**Validation.** We will first test our KASP assays with DNA oligonucleotides alone and then with added wild-type genomic DNA. We will also use synthetic DNA oligonucleotides as positive and negative controls for routine pathogen molecular screening. In addition to single-PCR product assays (utilized for general pathogen detection), we will also design RFLP, dCAPS, and KASP assays to identify *Mycobacterium* substrains. We will validate tested molecular assays and determine their sensitivity by performing parallel histopathology analyses. We will test a number of preparations including samples prepared from different tissues, and samples prepared from water, biofilms, and aquarium debris.

We will determine the sensitivity of tested molecular assays by performing them in parallel with our routine in-house histopathology analyses that we have developed previously as part of our Pathology and Health Services. By using newly developed molecular and already established histopathology assays side-by-side for systematic in-house pathogen screening, we will validate results in the context of our own health program. Our molecular assays will also undergo independent testing and validation in external facilities, the OVDL and other laboratories. We will use the most optimal and reliable molecular assays for systematic in-house pathogen screening. No extra animals will be bred or used for this research, we will use cloned DNA provided by other laboratories, synthetic DNA, or DNA derived from tissue of routine in-house histopathology cases.

Animals obtained from other laboratories are usually submitted as fixed tissue. Whenever live animal are received, or whenever we will use live in-house fish for colony health monitoring to validate the PCR panel, we will euthanize fish according to IACUC approved euthanasia methods (MS-222 overdose or hypothermal shock, listed in the attached SOP collection). Freshly isolated tissue will be used for DNA isolation before the carcass is fixed for paraffin embedding, sectioning, and staining for histopathology analysis. The isolated fresh tissues will be enzyme-digested and DNA will be isolated using standard molecular laboratory protocols.

### **ZIRC-L3: Pseudoloma Droplet DNA testing**

**Background.** As part of their R24 grant aims, the Kent laboratory at OSU will be developing non-lethal PCR tests for prevalent zebrafish pathogens using digital droplet DNA testing of water. The laboratory has been developing such tests for salmon pathogens

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with excellent success, and now will be moving to adapt the technology for zebrafish pathogens.

The aim of the collaboration between the Kent laboratory and ZIRC is to validate a *Pseudoloma* Droplet DNA test, because 1) *Pseudoloma* occurs in our facility, 2) Our Veterinarian (Katy Murray) is already testing tanks with individual fish histology, and 3) such a test will be very valuable for the efficient diagnosis of live fish populations. We currently do not have a droplet DNA system, so the Kent Laboratory will provide replicate DNA samples for our in-house comparison with their own PCR test and the droplet test.

We routinely test all wild-type fish tanks when fish reach 8 months of age. On average, 20-gallon tanks house 150 to 250 fish. Presently, Katy is testing fish by histology with Luna stain and has recently started implementing in-house PCR in addition to histology (See Health Services Procedures). Tanks that are positive at 8 months for *Pseudoloma* are marked in the database and flagged for fish euthanasia and tank removal in the fish room. The fish are then euthanized according to approved hypothermal shock protocol as soon as possible. OSU will be provided euthanized fish from these tanks. We (Katy) will also freeze euthanized fish in Eppendorf tubes and save water following the Kent laboratory's filtering protocol (see below). Each month, approximately 10 tanks are routinely tested at ZIRC, and typically 2-3 are *Pseudoloma* positive.

## Procedures

### a) *Pseudoloma* samples from 8-month fish.

If a ZIRC tank with 8-month fish is tested positive for *Pseudoloma* during routine (histopathology) health monitoring, the Kent laboratory will test water from that tank by DNA droplet analysis. All fish from these tanks will be euthanized, frozen, stored in zip-lock bags (for incineration); however, 30 carcasses will be transferred to the Kent laboratory for subsequent *Pseudoloma* testing by PCR.

**3 x 30 x 12 = 1080 fish/year**

If all of the 8-month tanks are negative by histology, the water test is performed on up to 3 these randomly chosen tanks and 30 fish from these tanks are saved for and PCR (Kent and ZIRC tests). If 3 "negative by histology" tanks are not available on a given month, we will randomly test 3 different 8-month tanks to conduct this task.

**3 x 30 x 12 = 1080 fish/year**

### b) *Pseudoloma* sampling of 3-month old offspring from positively identified stock populations

By 8 month of age, a new generation has already been bred and is growing up in our facility. These fish are typically 2-3-month old. A key advantage of the droplet DNA testing will be that we will be able to test live fish (i.e. their water) for pathogens much earlier, before we breed them. Because we will test specific populations directly, this method will significantly reduce maintenance time of fish that would have tested positive at 8 months. They are eliminated earlier, which reduces the potential of pathogen transmission by our husbandry procedures.

Each month, 2 tanks with 3-month old fish derived from *Pseudoloma*-positive (8-month) fish will be used for water testing. In addition, 30 3-month old fish will also be selected from these tanks for euthanasia and subsequent PCR testing.

**2 x 30 x 12 = 720 fish/year**

Each of these tanks is tested once at 3 months, and then later again at 8 months. If the 3-month old fish test *Pseudoloma*-negative, they continue to be used for the ZIRC program. If a 3-month old fish tank tests positive, all fish (up to 250) will be transferred to OSU, maintained in the Kent fish facility, and tested again at 8-10 months to follow increases in prevalence within a tank/stock. This may happen up to 12 times per year (depending on fish availability at ZIRC, and/or space availability in the Kent fish facility)

**2x250x12 = 6000 fish/year (maximally).** These fish will be transported to OSU, maintained, and euthanized according to Kent laboratory, OSU-IACUC approved procedures.

**Total Fish used:** 1080 + 1080 + 720 + 6000 = **8880 Fish** (excluded from the 58,000 above)

## Pain Category: D

The 8880 fish used in this study are all part of ZIRC's routine breeding concept and health monitoring and are included in the animal numbers of the main protocol (Repository); Fish will be euthanized according to protocol before histopathology and PCR testing. Because fish may have other uses and may be anesthetized e.g. to collect gametes or for fin-clipping prior to diagnostic testing, we will list them in Pain Category D.

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### **Fish Data for Collection:**

1. Strain,
2. Age,
3. Number of Fish,
4. Time in Tank,
5. Tank Volume,
6. ZIRC tank location

### **Water Sampling Method**

- 1) Turn water off for 6 h, provide aeration
- 2) At the end of the static period, water in the tank is stirred and 4 liters of tank water are filtered using a vacuum pump. 4 filters are exposed per aquarium, 2 for droplet- and 2 for ZIRC PCR analysis. Katy Murray will oversee filtering at ZIRC. Filters are folded and saved frozen for future processing.
- 3) Prevalence of infection in fish: At least 30 fish will be screened by qPCR at OSU. Fish will be preserved in groups of five and frozen. The Kent laboratory will thaw, dissect brain and spinal cord, and process in pools of 5 as described in the Sanders et al. publication. Prevalence will be estimated using the algorithm of Williams and Moffitt (2005).

### **Justification for 30 fish/tank sample**

Dhand, N. K., & Khatkar, M. S. (2014) provides an online program for calculating the precision of prevalence results with a known subsample from a finite population <http://statulator.com/SampleSize/ss1P.html>

A low sample number of individuals will produce larger margins of error (standard deviation from the mean). However, this is acceptable to correlate water droplet scores (quantification of DNA by ddPCR) with a prevalence estimate in the fish population from the same tank, with a large number of tanks (over time) that show a range of prevalence of infection. Thus, although results have a broad range, with many tanks tested we will see how water testing and histopathology correlate

E.g. If 31% of the fish test positive in a population (based on histopathology of individual fish), and the average population size in 20-gallon ZIRC tanks is 200 fish, the study would require a sample size of 30 for estimating the expected proportion with 50% precision relative to the expected proportion (i.e.  $0.5 \times 0.31 = 15.5\%$  absolute precision) and 95% confidence. This means, if 30 fish are sampled randomly from a tank, and if we determine that 31% of subjects test positive for *Pseudoloma*, there will be 95% confidence that between (31% +/-) 15.5% and 46.5% of subjects in the population have the factor of interest. This is sufficient to correlate histopathology and DNA droplet water testing

### **References**

- Dhand, N. K., & Khatkar, M. S. (2014). Statulator: An online statistical calculator. Sample Size Calculator for Estimating a Single Proportion. Accessed 22 March 2018 at <http://statulator.com/SampleSize/ss1P.html>
- Sanders JL, Kent ML 2011. Development of a sensitive assay for the detection of *Pseudoloma neurophilia* in laboratory populations of the zebrafish *Danio rerio*. Dis Aquat Org. 96:145-156
- Williams and Moffitt. 2005. Estimation of pathogen prevalence in pooled samples using maximum likelihood methods and open-source software J. Aquat Animal Health 17: 386-391)

### **Personnel**

Katy Murray and Evyn Loucks will perform all animal work at ZIRC. Kent laboratory staff will instruct ZIRC in the water sampling and Droplet DNA testing methods. No personnel need to be added.

### **ZIRC-L3: Cryopreservation Research: testing of micro fabricated and 3-D printed cryopreservation tools**

Please see attached SOP collection of ZIRC's routine cryopreservation procedures.

Gamete collection: Males will be isolated from females at least two weeks before experimentation (up to 4 weeks), and maintained in 1-gallon tanks on the recirculating water system in room 118 at a density of 8 fish per tank (8 fish per gallon; ~2 fish / L). On the day of

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experimentation, fish will be transferred from their racks to workspace in 118 or to the laboratory in 109. Fish will be sedated in 50 mg/L MS-222, then shortly before milt collection transferred for ca. 1-2 minutes into 168 mg/L MS-222 for full anesthesia, transferred onto a slit sponge in dorsal recumbency, and sperm will be harvested with a 20  $\mu$ l glass capillary and gentle abdominal massage with rubber padded forceps close to the urogenital opening. Once sperm is obtained, fish are immediately transferred into a recovery chamber with fresh system water and their recovery is observed throughout the next 30-60 minutes. Fish are monitored again when they are placed back on the recirculating water system in room 118, and then daily, every evening when all fish are routinely monitored for their well-being and health status.

When sperm is collected, it is captured in 20  $\mu$ l of E400 extender, which blocks premature cell movement and activation. Cell density is determined and adjusted to an experimental standard, or a dilution series is prepared. Sperm samples are then tested for motility using CASA as indicated for each of the devices below, before freezing and/or after thawing. Samples will also be used for in-vitro fertilization to compare motility and fertilization capacity.

Zebrafish sperm will be tested using the following devices:

**3.1 Microfabricated Enumeration Grid Chamber (MEGC);** this microfabricated counting chamber will be compared to cell counting methods already used at ZIRC such as measuring the optical absorption of cells in extender at 400 nm with a nanodrop spectrophotometer. Cell densities are calculated from the A400 absorption value according to a predetermined second order binomial absorption curve for zebrafish sperm. Sperm density is also determined with a hemocytometer or a mackuler counting chamber, which are similar to the MEGC, but come at considerably higher cost. Analyses will address material toxicity, functionality, practicality, and reproducibility. **300 Males (includes assumed 3 rounds of feedback and device refinements): 300 Animals**

**3.2 Sequential Logarithmic Mixing Apparatus (SeLMA)/Microfabricated Activation and Motility Chamber (MAMC).** Previously, CASA measurements required the mixing of activating solution and sperm on the slide, cover slipping the slide and placing it under the microscope for recording of short, 60-frame video sequences. The SeLMA/MAMC provides controlled, hands-free mixing of water and sperm sample under the microscope for the timed activation and immediate observation of sperm cells for CASA measurements. Activation of sperm and observation of motility will become a straightforward 1-step process and will help laboratories to implement computer assisted sperm analysis (CASA) for reproducible quality control measurements. **300 Males (includes assumed 3 rounds of feedback and device refinements): 300 Animals**

**3.3 A belt-driven device and a cryopreservation float, named Positional Cooling Platform Device (PCPD),** for the standardized freezing of single straws and vials will be tested ("standard" = slow-cooling rates of 1-10 °C/min). Zebrafish sperm will be harvested as described above, and cell density and quality will be determined by Nanodrop optical absorption and CASA analysis. Cell density will be adjusted for optimal cryopreservation density and the devices will be tested for the controlled cooling of the samples. A thermocouple in control cryovials and straws (immersed in extender) will provide reference temperature readings to control the belt-driven device's speed before it drops samples into liquid nitrogen. The height of samples floating above liquid nitrogen in the PCPD will also be adjusted based on these controls. Once samples have been frozen in liquid nitrogen they will be stored in a liquid nitrogen freezer over night, and thawed for analysis the next morning or within a few following days. Material toxicity, functionality, practicality, and reproducibility will be assessed by thawing samples and analyzing them by CASA and in vitro fertilization. **Two devices x 300 Males (includes assumed 3 rounds of feedback and device refinements): 600 Animals**

**3.4** We will test 3-D printed loops (VSID), and optical control devices for standardized vitrification (ultra-rapid cooling 100 - 1000 °C/min cooling rates per minute). This includes a 3-D printed vitrification device (VSID) with a sample loop and a protective sleeve that can be used for the labeling (identification) of the vitrified sample. Loops with fresh samples are immersed at high speed into liquid nitrogen to achieve vitrification, the amorphous form of water crystallization. The vitrification of samples will be verified visually/optically before long term storage, by placing loops on a 3-D printed holder that permits the assessment of vitrification success with stripes imprinted under the sample. Perfectly vitrified samples will not obstruct the pattern/lines in any way, but will essentially behave like "glass". Opaque or non-transparent samples indicate crystal formation or lack of successful sample vitrification. Most test will be performed without sperm samples. We will first test material integrity and stability when 3-D printed materials transition from room-temperature to liquid nitrogen (brittleness), which exerts considerable material stresses in the used plastics. Next we will test extender, and extender-cryoprotectant mixes for their capability to form a film in the loop, to maintain the film during vitrification, and the level/quality of vitrification of these solutions when injected into liquid nitrogen. The composition of solutions will be adapted for maximal vitrification. Once materials and solutions have been tested, we will test survival of cells in the cryoprotectant by collecting and pooling samples from 5 males and incubating/equilibrating them in cryoprotectant for 5, 10, 20, 30, 40, 50, and 60 minutes on ice. We will also analyze the optimal dilution of cells in the cryoprotectant solution. Cells will be frozen with slow-cooling rate methods and thawed for analysis. Cell survival and functionality will be measured after each period with Nanodrop cell density, CASA, and in vitro fertilization assays. Because pooling of samples and adjustment of cell densities yields several samples per male animal numbers will not exceed: 10 variations of cryoprotective solution x 5 cell densities x 7 incubation time frames x 5 males = 1750 males). Once the optimal incubation time, cryoprotectant composition, and cell concentration have been determined, we will attempt

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vitirifying cells with the optimized procedure and 3-D printed loops. We will analyze the transparency (vitrification) of samples and the integrity of the vitrified sample film in the loop immediately after freezing. Cells will be thawed and analyzed with Nanodrop, CASA and IVF to determine vitrification/survival success. We anticipate that the thawing procedure will have to be adjusted to the time frame of the vitrification procedure (accelerated). We may have to try several thawing methods. We anticipate using 50 animals x 3 thawing methods x 3 vitrification methods ~ 500 animals for successful vitrification and thawing of samples. **2250 Animals** (Note, this is an absolute maximum, because males can be re-used for harvesting sperm samples after a resting period of a few weeks; thus the same male can be used 2-4 times before euthanasia due to age, or accidental death (rare) during anesthesia).

In combination with the MEGC and SeLMA/MAMC we hope to provide laboratories with low cost, efficient, standardized, and reliable sample quality control capabilities, before they use the PCPC for cryopreservation of samples in their laboratory. Streamlined QC capability at a fraction of the cost (compared to obtaining professional grade cryopreservation equipment) will empower laboratories to bank their own fish lines more effectively and efficiently, thereby reducing maintenance costs and the number of animals that need to be maintained in their facilities to propagate precious research lines. At the same time, it will also allow for standardization of cryopreservation methods across the zebrafish and other aquatic research communities, which will aid to improve genetic line preservation in aquatic biomedical laboratories and fisheries.

**Total: 3450 Animals (excluded from the 58,000 animal above)**

## Additional Procedure Details

### Prolonged Restraint

*Will any of the procedures listed above involve prolonged animal restraint?*

**No**



## Blood or Fluid or Tissue Collection

*Will any of the procedures listed above involve the collection of fluid from a live animal or the collection of tissues from a live animal?*

*Fluid can be blood, bile, spinal fluid, etc.*

*Do not include information about animal identification methods.*

**Yes**

### DETAILS

#### Provide Specimen Name

Gamete collection

Collection by abdominal massage (during standard anesthesia with MS-222) for *in vitro* fertilization

Cryopreservation: Zebrafish *Danio rerio* sperm cells/milt

Diagnostic health service and colony health monitoring, whole fish:

DNA Digest of Nervous tissue for PCR

Bacteriological Diagnostics: Aseptic blood-agar tissue culture

Embedding of fixed carcass in paraffin and sectioning for H&E stain for histopathology

#### Provide Collection Site

Gamete collection: *Urogenital opening*

Cryopreservation: *Urogenital opening, testes dissection*

Diagnostic health service, Colony Health monitoring: *Whole fish or organ dissection*

#### Provide Specimen Volume or Weight

Gamete collection: *Sperm <5 µl & Eggs ~ 500 µl*

Cryopreservation: *1-4 µl; testes weight ca. 4-6 mg*

Diagnostic health service: *Whole fish, 200-1000 mg, organ weight varies.*

#### Provide Number of Samples

Gamete collection: *Circa 25,000 fish*

Cryopreservation: *circa 10,000 fish, ~ 20,000 samples per year*

Diagnostic health service and health monitoring: *10,000 fish/specimen*

#### How will specimen be discarded?

Gamete collection: *Not discarded. Sperm samples are added to cryogenic repository and/or used for *in vitro* fertilization immediately or after thawing; eggs are used for *in vitro* fertilization.*

Sperm Sample Cryopreservation: *Not discarded. Cryopreservation storage and subsequent thawing for *in vitro* fertilization of eggs*

Diagnostic health service: *Not discarded - specimen are paraffin embedded and will be stored for re-sectioning in the ZIRC veterinary office or with Dr. Michael Kent at OSU. Culture plates are discarded via Biohazard collection, through appropriate UO/EHS procedures.*

## Substance Administration

*Will you be administering any substances to animals?*

*"Substances" here does not include agents used for anesthesia, analgesia, or euthanasia. Nor does it include hazardous agents. Questions about these are asked in other areas of the eAPM system.*

**Yes, please check all that apply**

**Will be using anesthetics, analgesics or euthanizing agents**

***Questions about these are asked in the Anesthesia/Analgesia tab***

*Is the substance a neuromuscular blocking agent?*

*Does the substance use cause animal welfare issues?*

## Use of Controlled Substances

*Will controlled substances be used for anesthesia, analgesia, restraint, animal management, testing, or euthanasia?*

**Yes**

*Controlled Substance Details*

**Provide Controlled Substance(s) Name(s)**

17- $\alpha$ -Methyltestosterone (Schedule IIIN Controlled Substance)

Used to masculinize stocks from fish lines with known female skew from which sperm samples need to be cryopreserved. The lines are bred and progeny are exposed to 17- $\alpha$ -Methyltestosterone during the first month of nursery, by receiving dry flakes supplemented with 17- $\alpha$ -MT. See procedures for detail.

**Provide your DEA license number and expiration date**

**IMPORTANT: Attach a copy of your DEA registration and provide your DEA license number and expiration date.**

DEA Registration #: RV0493104; valid through 05/31/2021 (previously RU0231578)

Oregon Exception Request to Conduct Research - OAR 855-080-0095; valid through May 31, 2021

Continuation of DEA registration has been requested (see attached PDF); Oregon Exception request will be submitted as soon as DEA registration is completed. Completed registrations and Oregon Exception will be added as soon as available.

**Provide Names of Authorized Individuals**

Zoltan Varga

Jen Matthews

Joy Murphy

**Provide Storage Details**

17 $\alpha$ -Methyltestosterone is maintained at room temperature in a locked, unmarked drawer in Room 109, which serves as an auxiliary laboratory space. Access to this space requires the building access code (keypad and card). A camera monitors the outside entrance to the room. The drawer is equipped with a Schlage safety lock, to which the keys are stored in a wall mounted key-safe with combination code access. Only the DEA registrant and the registered experimenters have access to the code (memorized, not captured in writing). An annual inventory is conducted according to federal law, and use of the CS is tracked and logged every time.

## Food or Fluid Regulation

*Will food or fluid regulation be used in this study?*

**Yes**

*What is the justification for the use of food and/or water regulation?*

**Provide Justification**

**Gamete Collection:** During the identification of individual carriers of a genetic modification, animals are held for up to 4 days in isolated, transparent, static water tanks (1.5 Liter). During this time they do not receive food for two days. Approximately 30 minutes after feeding (2nd day) animals receive a fresh water change and are held for another 2 days in static, isolated conditions without food. If the time to obtain genotyping results exceeds 4 days individual fish are placed in 1-gallon tanks on the recirculating water system, where they receive regular feeding (twice daily) and health monitoring every day. Once genotyping results are obtained, carriers of genetic modifications are pooled into 1-gallon tanks and placed on the recirculating water system (max. density 20 fish/gallon) with regular feeding and monitoring schedules.

**Cryopreservation:** Animals will be conditioned by being isolated from females at least two weeks before experimentation (up to 4 weeks), and maintained in 1-gallon tanks on the recirculating water system in rm 118 at a density of 9 fish per tank (9 fish per gallon; 2.5 fish / L). During isolation, fish will receive an extra feeding of Adult Master Mix flake feed at mid-day each day. The total number of feedings will be 3 per day for these fish. The conditioning improves male performance, reduces male variability between samples and males (cell density and function). Thereby, 25-50% fewer males have to be conditioned, anesthetized and squeezed for sperm samples.



*What alternative methods were considered, including positive reinforcement?*

*What is the nature, extent, and frequency of food and/or water regulation and how was this determination made?*

**Provide Details**

Gamete Collection: During the identification of individual carriers of a genetic modification, animals are held for up to 4 days in isolated, transparent, static water tanks (1.5 Liter). During this time they do not receive food for two days. Approximately 30 minutes after feeding (2nd day) animals receive a fresh water change and are held for another 2 days in static, isolated conditions without food.

Cryo: 2-4 weeks, one additional feeding per day, Males isolated from females at 2.5 fish/L

*What are the potential adverse consequences of the regulation on the animal's health and well-being?*

**Provide Animal Health and Welfare Details**

Same as all other fish at ZIRC

*What methods and signs will be used to assess the animal's health and well-being and how frequently will it be assessed?*

*Body weights should be recorded at least weekly and more often for animals requiring greater restrictions.*

*(Guide, p. 31)*

**Provide Details about Assessment of Health and Well-Being**

Animals are monitored visually at least once a day for humane endpoints or any signs of discomfort, pain or distress. If the latter is observed, water is changed and subsequent regular monitoring continues to ascertain that symptoms have been alleviated. If humane endpoints are observed, the veterinarian is consulted and a determination is made whether or not to euthanize the animal(s). Note: Humane endpoints as listed in this protocol include several/most signs of discomfort, pain or distress.

*Where will written records be located?*

*Written records should be maintained for each animal to document daily food and fluid consumption, hydration status, and any behavioral and clinical changes used as criteria for temporary or permanent removal of an animal from a protocol.*

*(Guide, p.31)*

**Provide Location of Records**

ZIRC breeding log (Database and excel spreadsheet).

Hard copy is maintained with animals during the 2x2 days of food and water restriction. Then data is transferred to the fish inventory database for permanent archiving.

*What criteria will be used to remove the animal from the study?*

**Provide Criteria Used to Remove Animals from Study**

Fish that do not carry the genetic modification that are characteristic for the strain are euthanized according to IACUC approved methods, carcasses are disposed of according to IACUC approved method.

**Imaging Studies**

**Post-Procedural Monitoring**

**Procedures Training Verification**

**fish: Zebrafish**

**PROCEDURES TRAINING VERIFICATION TOOL MATRIX**

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	Clark, Renee		Core, Keely		Freeman, April		Holland, Ronald "Ron"		Hwang-Shum, Jen- Jen	
ZIRC-L3: Cryopreservation Research: testing of micro fabricated and 3-D printed cryopreservation tools	<input type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input type="checkbox"/>	⚠
ZIRC-L3: Fish Food Testing	<input checked="" type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠
ZIRC-L3: Masculinization of fish stocks with 17 alpha- Methyltestosterone	<input checked="" type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠
ZIRC-L3: Open- Platform PCR Screening Panel Development	<input type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠
ZIRC-L3: Pseudoloma Droplet DNA testing	<input type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠

  

	Lains, David		Loucks, Evyn		Matthews, Jen		Murphy, Joy		Murray, Katy	
ZIRC-L3: Cryopreservation Research: testing of micro fabricated and 3-D printed cryopreservation tools	<input type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠	<input type="checkbox"/>	⚠
ZIRC-L3: Fish Food Testing	<input checked="" type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠
ZIRC-L3: Masculinization of fish stocks with 17 alpha- Methyltestosterone	<input checked="" type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠
ZIRC-L3: Open- Platform PCR Screening Panel Development	<input type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠
ZIRC-L3: Pseudoloma Droplet DNA testing	<input type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠

	Nasiadka, Andrzej		Quinn, Erin		Schumann, Alex		Smith, Calvin		Varga, Zoltan	
ZIRC-L3: Cryopreservation Research: testing of micro fabricated and 3-D printed cryopreservation tools	<input type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠
ZIRC-L3: Fish Food Testing	<input type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠
ZIRC-L3: Masculinization of fish stocks with 17 alpha-Methyltestosterone	<input checked="" type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠
ZIRC-L3: Open-Platform PCR Screening Panel Development	<input checked="" type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠
ZIRC-L3: Pseudoloma Droplet DNA testing	<input checked="" type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠

	Westerfield, Monte		Williams, Evan	
ZIRC-L3: Cryopreservation Research: testing of micro fabricated and 3-D printed cryopreservation tools	<input type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠
ZIRC-L3: Fish Food Testing	<input type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠
ZIRC-L3: Masculinization of fish stocks with 17 alpha-Methyltestosterone	<input type="checkbox"/>	⚠	<input checked="" type="checkbox"/>	⚠
ZIRC-L3: Open-Platform PCR Screening Panel Development	<input checked="" type="checkbox"/>	⚠	<input type="checkbox"/>	⚠
ZIRC-L3: Pseudoloma Droplet DNA testing	<input checked="" type="checkbox"/>	⚠	<input type="checkbox"/>	⚠

## Additional Procedure Training Details

### Additional General Training Details

Are there any additional General Training details for the IACUC to consider?

No

## Anesthesia/Analgesia

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## Anesthesia

Not applicable

## Analgesia

Not applicable

## General Training

### Principal Investigator Qualifications

#### General Training Assurance

*Will you as principal investigator assure that personnel listed in this project will be allowed adequate time to attend training sessions?*

**Yes**

### Required General AUP Training

ALL: Animal Occupational Health Awareness ( $\geq 85\%$ )  
 L1-A-2 Organizational Structure  
 L1-A-3 Nature of Research, Testing and Teaching Programs  
 L1-A-4 Key Institutional Representatives  
 L1-A-5 Accreditation History  
 L1-B-1 Animal Welfare Act  
 L1-B-2 U.S. Government Principles  
 L1-B-3 U.S. Public Health Service (NIH) Policy  
 L1-B-4 Other Regulations and Regulatory Agencies  
 L1-C-01 Institutional Animal Care and Use Committee (IACUC)  
 L1-C-02 Animal Care Services (ACS)  
 L1-C-05 PHS Assurance of Compliance  
 L1-C-06 Concern Policy  
 L1-C-07 Reportable Events Policy  
 L1-C-08 Occupational Health and Safety Program  
 L1-C-09 Transportation of Live Vertebrate Animals  
 L1-C-10 Euthanasia Policy  
 L1-C-11 Anesthesia Policy  
 L1-D-1 Animal Use Protocol  
 L1-D-2 Protocol Amendment Policy  
 L1-D-3 Peer Review of Research Services  
 L1-D-4 Cooperative Research Policy  
 L1-D-5 Designated Member Review  
 L1-D-6 Guidelines for Pain, Distress and Discomfort in Zebrafish  
 L2-ZIRC Aquaculture System Shut Down and Start Up  
 L2-ZIRC Aquarium and Plastic Tank Cleaning Procedure  
 L2-ZIRC Aretmia Decapsulation

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L2-ZIRC Bacterial Plating for UV Sterilizer Monitoring  
 L2-ZIRC Brine Shrimp (Artemia) Hatching Procedure and Feeding  
 L2-ZIRC Daily Monitoring of Fish Morbidity and Mortality  
 L2-ZIRC Embryo Media  
 L2-ZIRC Embryo Surface Sterilization  
 L2-ZIRC Euthanasia by Hypothermal Shock  
 L2-ZIRC Euthanasia of Fish for PCR or Fixation  
 L2-ZIRC Feeding Procedures  
 L2-ZIRC Fin Clipping and Fin Clip Processing  
 L2-ZIRC Fish Acquisition/Receiving  
 L2-ZIRC Fish Maintenance (strain identification and propagation)  
 L2-ZIRC Fixing Zebrafish for Histopathology  
 L2-ZIRC General Breeding  
 L2-ZIRC Molecular Genotyping of Mutant and Transgenic Lines  
 L2-ZIRC Nursery  
 L2-ZIRC Operation in Time of Crisis  
 L2-ZIRC Paramecia Feeding  
 L2-ZIRC Paramecia Maintenance  
 L2-ZIRC Powdered Food Preparation  
 L2-ZIRC Prospore Autoclave Sterilization Monitoring  
 L2-ZIRC Providing Fish & Materials  
 L2-ZIRC Quarantine Room and General Procedures  
 L2-ZIRC RODAC Procedure  
 L2-ZIRC Sentinel Fish Program  
 L2-ZIRC Sperm Cryopreservation  
 L2-ZIRC Sperm Thawing Procedure  
 L2-ZIRC Washroom Procedure  
 Physician's Clearance

## Required General AUP Training for Study Personnel

	Clark, Renee	Core, Keely	Freeman, April	Holland, Ronald "Ron"	Hwang-Shum, Jen- Jen
ALL: Animal Occupational Health Awareness (>=85%)	YES	YES	YES	YES	YES
Physician's Clearance	YES	YES	YES	YES	YES
L1-A-2 Organizational Structure	YES	YES	YES	YES	YES
L1-A-3 Nature of Research, Testing and Teaching Programs	YES	YES	YES	YES	YES
L1-A-4 Key Institutional Representatives	YES	YES	YES	YES	YES
L1-A-5 Accreditation History	YES	YES	YES	YES	YES
L1-B-1 Animal Welfare Act	YES	YES	YES	YES	YES
L1-B-2 U.S. Government	YES	YES	YES	YES	YES

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Principles					
L1-B-3 U.S. Public Health Service (NIH) Policy	YES	YES	YES	YES	YES
L1-B-4 Other Regulations and Regulatory Agencies	YES	YES	YES	YES	YES
L1-C-01 Institutional Animal Care and Use Committee (IACUC)	YES	YES	YES	YES	YES
L1-C-02 Animal Care Services (ACS)	YES	YES	YES	YES	YES
L1-C-05 PHS Assurance of Compliance	YES	YES	YES	YES	YES
L1-C-06 Concern Policy	YES	YES	YES	YES	YES
L1-C-07 Reportable Events Policy	YES	YES	YES	YES	YES
L1-C-08 Occupational Health and Safety Program	YES	YES	YES	YES	YES
L1-C-09 Transportation of Live Vertebrate Animals	YES	YES	YES	YES	YES
L1-C-10 Euthanasia Policy	YES	YES	YES	YES	YES
L1-C-11 Anesthesia Policy	YES	YES	YES	YES	YES
L1-D-1 Animal Use Protocol	YES	YES	YES	YES	YES
L1-D-2 Protocol Amendment Policy	YES	YES	YES	YES	YES
L1-D-3 Peer Review of Research Services	YES	YES	YES	YES	YES
L1-D-4 Cooperative Research Policy	YES	YES	YES	YES	YES
L1-D-5 Designated Member Review	YES	YES	YES	YES	YES
L1-D-6 Guidelines for Pain, Distress and Discomfort in Zebrafish	YES	YES	YES	YES	YES
L2-ZIRC Aquaculture System Shut Down and Start Up	NO	NO	YES	NO	NO
L2-ZIRC Aquarium and Plastic Tank Cleaning Procedure	YES	YES	YES	NO	YES
L2-ZIRC Aretmia Decapsulation	NO	NO	NO	NO	NO
L2-ZIRC Bacterial Plating for UV Sterilizer Monitoring	NO	NO	YES	NO	NO
L2-ZIRC Brine	YES	NO	YES	NO	NO

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Shrimp (Artemia) Hatching Procedure and Feeding					
L2-ZIRC Daily Monitoring of Fish Morbidity and Mortality	YES	YES	YES	NO	NO
L2-ZIRC Embryo Media	YES	NO	YES	NO	YES
L2-ZIRC Embryo Surface Sterilization	NO	YES	YES	NO	NO
L2-ZIRC Euthanasia by Hypothermal Shock	YES	YES	YES	NO	NO
L2-ZIRC Euthanasia of Fish for PCR or Fixation	YES	YES	NO	NO	NO
L2-ZIRC Feeding Procedures	YES	YES	YES	YES	YES
L2-ZIRC Fin Clipping and Fin Clip Processing	NO	NO	NO	NO	YES
L2-ZIRC Fish Acquisition/Receiving	NO	NO	YES	NO	NO
L2-ZIRC Fish Maintenance (strain identification and propagation)	YES	YES	NO	NO	YES
L2-ZIRC Fixing Zebrafish for Histopathology	YES	NO	YES	NO	NO
L2-ZIRC General Breeding	YES	YES	YES	NO	YES
L2-ZIRC Molecular Genotyping of Mutant and Transgenic Lines	NO	NO	NO	NO	YES
L2-ZIRC Nursery	YES	NO	YES	NO	NO
L2-ZIRC Operation in Time of Crisis	NO	YES	YES	YES	NO
L2-ZIRC Paramecia Feeding	NO	YES	YES	NO	NO
L2-ZIRC Paramecia Maintenance	YES	NO	YES	NO	NO
L2-ZIRC Powdered Food Preparation	NO	NO	YES	NO	NO
L2-ZIRC Prospore Autoclave Sterilization Monitoring	NO	NO	YES	NO	NO
L2-ZIRC Providing Fish & Materials	NO	YES	YES	NO	NO
L2-ZIRC Quarantine Room and General Procedures	NO	YES	YES	NO	NO
L2-ZIRC RODAC Procedure	YES	NO	YES	NO	NO
L2-ZIRC Sentinel Fish Program	NO	NO	YES	NO	NO

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L2-ZIRC Sperm Cryopreservation	YES	NO	NO	NO	NO
L2-ZIRC Sperm Thawing Procedure	YES	NO	NO	NO	NO
L2-ZIRC Washroom Procedure	YES	YES	YES	NO	NO

	Lains, David	Loucks, Evyn	Matthews, Jen	Murphy, Joy	Murray, Katy
ALL: Animal Occupational Health Awareness (>=85%)	YES	YES	YES	YES	YES
Physician's Clearance	YES	YES	YES	YES	YES
L1-A-2 Organizational Structure	YES	YES	YES	YES	YES
L1-A-3 Nature of Research, Testing and Teaching Programs	YES	YES	YES	YES	YES
L1-A-4 Key Institutional Representatives	YES	YES	YES	YES	YES
L1-A-5 Accreditation History	YES	YES	YES	YES	YES
L1-B-1 Animal Welfare Act	YES	YES	YES	YES	YES
L1-B-2 U.S. Government Principles	YES	YES	YES	YES	YES
L1-B-3 U.S. Public Health Service (NIH) Policy	YES	YES	YES	YES	YES
L1-B-4 Other Regulations and Regulatory Agencies	YES	YES	YES	YES	YES
L1-C-01 Institutional Animal Care and Use Committee (IACUC)	YES	YES	YES	YES	YES
L1-C-02 Animal Care Services (ACS)	YES	YES	YES	YES	YES
L1-C-05 PHS Assurance of Compliance	YES	YES	YES	YES	YES
L1-C-06 Concern Policy	YES	YES	YES	YES	YES
L1-C-07 Reportable Events Policy	YES	YES	YES	YES	YES
L1-C-08 Occupational Health and Safety Program	YES	YES	YES	YES	YES
L1-C-09 Transportation of Live Vertebrate Animals	YES	YES	YES	YES	YES
L1-C-10 Euthanasia Policy	YES	YES	YES	YES	YES
L1-C-11 Anesthesia	YES	YES	YES	YES	YES

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Policy					
L1-D-1 Animal Use Protocol	YES	YES	YES	YES	YES
L1-D-2 Protocol Amendment Policy	YES	YES	YES	YES	YES
L1-D-3 Peer Review of Research Services	YES	YES	YES	YES	YES
L1-D-4 Cooperative Research Policy	YES	YES	YES	YES	YES
L1-D-5 Designated Member Review	YES	YES	YES	YES	YES
L1-D-6 Guidelines for Pain, Distress and Discomfort in Zebrafish	YES	YES	YES	YES	YES
L2-ZIRC Aquaculture System Shut Down and Start Up	NO	NO	NO	NO	NO
L2-ZIRC Aquarium and Plastic Tank Cleaning Procedure	YES	YES	YES	YES	YES
L2-ZIRC Aretmia Decapsulation	NO	NO	NO	NO	NO
L2-ZIRC Bacterial Plating for UV Sterilizer Monitoring	NO	NO	NO	NO	NO
L2-ZIRC Brine Shrimp (Artemia) Hatching Procedure and Feeding	YES	YES	YES	YES	NO
L2-ZIRC Daily Monitoring of Fish Morbidity and Mortality	NO	YES	NO	YES	YES
L2-ZIRC Embryo Media	NO	YES	YES	YES	NO
L2-ZIRC Embryo Surface Sterilization	NO	YES	NO	YES	YES
L2-ZIRC Euthanasia by Hypothermal Shock	NO	YES	YES	YES	YES
L2-ZIRC Euthanasia of Fish for PCR or Fixation	NO	YES	NO	YES	YES
L2-ZIRC Feeding Procedures	NO	YES	YES	YES	NO
L2-ZIRC Fin Clipping and Fin Clip Processing	NO	YES	YES	YES	NO
L2-ZIRC Fish Acquisition/Receiving	NO	NO	YES	YES	NO
L2-ZIRC Fish Maintenance (strain identification and propagation)	NO	YES	YES	YES	NO
L2-ZIRC Fixing Zebrafish for Histopathology	NO	YES	NO	YES	YES

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L2-ZIRC General Breeding	YES	YES	YES	YES	NO
L2-ZIRC Molecular Genotyping of Mutant and Transgenic Lines	NO	YES	NO	NO	NO
L2-ZIRC Nursery	NO	YES	YES	YES	NO
L2-ZIRC Operation in Time of Crisis	YES	YES	YES	YES	YES
L2-ZIRC Paramecia Feeding	NO	YES	YES	YES	NO
L2-ZIRC Paramecia Maintenance	NO	NO	NO	YES	NO
L2-ZIRC Powdered Food Preparation	NO	NO	YES	NO	NO
L2-ZIRC Prospore Autoclave Sterilization Monitoring	NO	NO	YES	NO	NO
L2-ZIRC Providing Fish & Materials	NO	NO	NO	NO	NO
L2-ZIRC Quarantine Room and General Procedures	YES	YES	YES	YES	YES
L2-ZIRC RODAC Procedure	NO	NO	YES	NO	NO
L2-ZIRC Sentinel Fish Program	NO	NO	NO	YES	YES
L2-ZIRC Sperm Cryopreservation	NO	YES	YES	YES	NO
L2-ZIRC Sperm Thawing Procedure	NO	YES	YES	YES	NO
L2-ZIRC Washroom Procedure	NO	YES	YES	YES	NO
	Nasiadka, Andrzej	Quinn, Erin	Schumann, Alex	Smith, Calvin	Varga, Zoltan
ALL: Animal Occupational Health Awareness (>=85%)	YES	YES	YES	YES	YES
Physician's Clearance	YES	YES	YES	YES	YES
L1-A-2 Organizational Structure	YES	YES	YES	YES	YES
L1-A-3 Nature of Research, Testing and Teaching Programs	YES	YES	YES	YES	YES
L1-A-4 Key Institutional Representatives	YES	YES	YES	YES	YES
L1-A-5 Accreditation History	YES	YES	YES	YES	YES
L1-B-1 Animal Welfare Act	YES	YES	YES	YES	YES
L1-B-2 U.S. Government Principles	YES	YES	YES	YES	YES
L1-B-3 U.S. Public	YES	YES	YES	YES	YES

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Health Service (NIH) Policy					
L1-B-4 Other Regulations and Regulatory Agencies	YES	YES	YES	YES	YES
L1-C-01 Institutional Animal Care and Use Committee (IACUC)	YES	YES	YES	YES	YES
L1-C-02 Animal Care Services (ACS)	YES	YES	YES	YES	YES
L1-C-05 PHS Assurance of Compliance	YES	YES	YES	YES	YES
L1-C-06 Concern Policy	YES	YES	YES	YES	YES
L1-C-07 Reportable Events Policy	YES	YES	YES	YES	YES
L1-C-08 Occupational Health and Safety Program	YES	YES	YES	YES	YES
L1-C-09 Transportation of Live Vertebrate Animals	YES	YES	YES	YES	YES
L1-C-10 Euthanasia Policy	YES	YES	YES	YES	YES
L1-C-11 Anesthesia Policy	YES	YES	YES	YES	YES
L1-D-1 Animal Use Protocol	YES	YES	YES	YES	YES
L1-D-2 Protocol Amendment Policy	YES	YES	YES	YES	YES
L1-D-3 Peer Review of Research Services	YES	YES	YES	YES	YES
L1-D-4 Cooperative Research Policy	YES	YES	YES	YES	YES
L1-D-5 Designated Member Review	YES	YES	YES	YES	YES
L1-D-6 Guidelines for Pain, Distress and Discomfort in Zebrafish	YES	YES	YES	YES	YES
L2-ZIRC Aquaculture System Shut Down and Start Up	NO	NO	YES	YES	YES
L2-ZIRC Aquarium and Plastic Tank Cleaning Procedure	NO	NO	YES	YES	YES
L2-ZIRC Artemia Decapsulation	NO	NO	NO	NO	YES
L2-ZIRC Bacterial Plating for UV Sterilizer Monitoring	NO	NO	NO	NO	YES
L2-ZIRC Brine Shrimp (Artemia) Hatching Procedure	NO	NO	NO	YES	YES

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and Feeding					
L2-ZIRC Daily Monitoring of Fish Morbidity and Mortality	YES	NO	NO	YES	YES
L2-ZIRC Embryo Media	YES	NO	NO	NO	YES
L2-ZIRC Embryo Surface Sterilization	YES	NO	NO	NO	YES
L2-ZIRC Euthanasia by Hypothermal Shock	YES	YES	NO	YES	YES
L2-ZIRC Euthanasia of Fish for PCR or Fixation	YES	NO	NO	NO	YES
L2-ZIRC Feeding Procedures	NO	YES	YES	YES	YES
L2-ZIRC Fin Clipping and Fin Clip Processing	YES	NO	NO	NO	YES
L2-ZIRC Fish Acquisition/Receiving	YES	NO	NO	NO	YES
L2-ZIRC Fish Maintenance (strain identification and propagation)	NO	NO	NO	YES	YES
L2-ZIRC Fixing Zebrafish for Histopathology	YES	NO	NO	NO	YES
L2-ZIRC General Breeding	YES	NO	NO	NO	YES
L2-ZIRC Molecular Genotyping of Mutant and Transgenic Lines	YES	NO	NO	NO	YES
L2-ZIRC Nursery	NO	NO	NO	YES	YES
L2-ZIRC Operation in Time of Crisis	NO	NO	YES	YES	YES
L2-ZIRC Paramecia Feeding	NO	NO	NO	YES	YES
L2-ZIRC Paramecia Maintenance	NO	NO	NO	YES	YES
L2-ZIRC Powdered Food Preparation	NO	NO	NO	YES	YES
L2-ZIRC Prospore Autoclave Sterilization Monitoring	NO	NO	NO	YES	YES
L2-ZIRC Providing Fish & Materials	NO	YES	NO	NO	YES
L2-ZIRC Quarantine Room and General Procedures	YES	YES	NO	YES	YES
L2-ZIRC RODAC Procedure	NO	NO	NO	YES	YES
L2-ZIRC Sentinel Fish Program	YES	NO	NO	YES	YES
L2-ZIRC Sperm Cryopreservation	NO	NO	NO	NO	YES

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L2-ZIRC Sperm Thawing Procedure	NO	NO	NO	NO	YES
L2-ZIRC Washroom Procedure	NO	NO	YES	YES	YES

	Westerfield, Monte	Williams, Evan
ALL: Animal Occupational Health Awareness (>=85%)	YES	YES
Physician's Clearance	YES	YES
L1-A-2 Organizational Structure	YES	YES
L1-A-3 Nature of Research, Testing and Teaching Programs	YES	YES
L1-A-4 Key Institutional Representatives	YES	YES
L1-A-5 Accreditation History	YES	YES
L1-B-1 Animal Welfare Act	YES	YES
L1-B-2 U.S. Government Principles	YES	YES
L1-B-3 U.S. Public Health Service (NIH) Policy	YES	YES
L1-B-4 Other Regulations and Regulatory Agencies	YES	YES
L1-C-01 Institutional Animal Care and Use Committee (IACUC)	YES	YES
L1-C-02 Animal Care Services (ACS)	YES	YES
L1-C-05 PHS Assurance of Compliance	YES	YES
L1-C-06 Concern Policy	YES	YES
L1-C-07 Reportable Events Policy	YES	YES
L1-C-08 Occupational Health and Safety Program	YES	YES
L1-C-09 Transportation of Live Vertebrate Animals	YES	YES
L1-C-10 Euthanasia Policy	YES	YES
L1-C-11 Anesthesia Policy	YES	YES
L1-D-1 Animal Use	YES	YES

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Protocol		
L1-D-2 Protocol Amendment Policy	YES	YES
L1-D-3 Peer Review of Research Services	YES	YES
L1-D-4 Cooperative Research Policy	YES	YES
L1-D-5 Designated Member Review	YES	YES
L1-D-6 Guidelines for Pain, Distress and Discomfort in Zebrafish	YES	YES
L2-ZIRC Aquaculture System Shut Down and Start Up	YES	NO
L2-ZIRC Aquarium and Plastic Tank Cleaning Procedure	YES	YES
L2-ZIRC Aretmia Decapsulation	YES	YES
L2-ZIRC Bacterial Plating for UV Sterilizer Monitoring	YES	NO
L2-ZIRC Brine Shrimp (Artemia) Hatching Procedure and Feeding	YES	YES
L2-ZIRC Daily Monitoring of Fish Morbidity and Mortality	YES	YES
L2-ZIRC Embryo Media	YES	YES
L2-ZIRC Embryo Surface Sterilization	YES	YES
L2-ZIRC Euthanasia by Hypothermal Shock	YES	YES
L2-ZIRC Euthanasia of Fish for PCR or Fixation	YES	YES
L2-ZIRC Feeding Procedures	YES	YES
L2-ZIRC Fin Clipping and Fin Clip Processing	YES	YES
L2-ZIRC Fish Acquisition/Receiving	YES	NO
L2-ZIRC Fish Maintenance (strain identification and propagation)	YES	YES
L2-ZIRC Fixing Zebrafish for Histopathology	YES	YES
L2-ZIRC General Breeding	YES	YES

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L2-ZIRC Molecular Genotyping of Mutant and Transgenic Lines	YES	YES
L2-ZIRC Nursery	YES	YES
L2-ZIRC Operation in Time of Crisis	YES	YES
L2-ZIRC Paramecia Feeding	YES	YES
L2-ZIRC Paramecia Maintenance	YES	YES
L2-ZIRC Powdered Food Preparation	YES	YES
L2-ZIRC Prospore Autoclave Sterilization Monitoring	YES	NO
L2-ZIRC Providing Fish & Materials	YES	NO
L2-ZIRC Quarantine Room and General Procedures	YES	YES
L2-ZIRC RODAC Procedure	YES	YES
L2-ZIRC Sentinel Fish Program	YES	NO
L2-ZIRC Sperm Cryopreservation	YES	YES
L2-ZIRC Sperm Thawing Procedure	YES	YES
L2-ZIRC Washroom Procedure	YES	YES

## Additional General Training Details

### Additional General Training Details

*Are there any additional General Training details for the IACUC to consider?*  
No

## Protocol/Forms Links

### Links to other Protocols or Forms

Not applicable

### Additional details for linked protocols and forms

Not applicable

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# Alternatives

## Rationale for Animal Use

Not applicable

## Search Data

Not applicable

## Search Data

*Should additional databases be added?*

**No**

*Rationale for Animal Use and Consideration of Alternatives*

*In accordance with the information provided above, please provide a written narrative description that gives the rationale for the use of animals, the choice of species, and the methods and sources used to determine that alternatives were not available or appropriate for this study. The narrative should also address each of the 3 R's defined above.*

### Provide Narrative:

ZIRC is supported by the Office of Research Infrastructure Programs (NIH) with the specific purpose to function as a genetic repository of zebrafish (*Danio rerio*) research lines and not any other organism. Thus, there are no reasonable alternatives to the use of zebrafish at ZIRC.

A database search was not conducted as alternatives do not actually exist. As a resource center, ZIRC serves a relatively unique (replicated only twice worldwide) and very specific function that supports the assurance statements of all zebrafish researchers in general. Resource Centers exist for other species, however, ZIRC is supported by NIH/ORIP to function as the genetic repository for zebrafish specifically. Two other zebrafish Resource Centers exist, with which ZIRC cooperates to support assurance statements:

*EZRC - the European Zebrafish Resource Center and  
CZRC, the China Zebrafish Resource Center.*

### ZIRC's Role for the Assurance Statements, the 3 R's.

The ZIRC serves other research groups to *replace* other vertebrate model organisms with zebrafish for some of their research goals. In addition, because many lines are maintained in a centralized, efficiently operating facility, ZIRC also helps to *reduce* the overall number of zebrafish maintained globally for research purposes. Cryopreservation research at ZIRC is also aimed at *reducing* the number of animals that have to be maintained as live stocks at ZIRC or elsewhere. Thus, ZIRC activities are aimed to support two of the "R"s in the Assurance Statement XI.A (Alternatives): Replace and Reduce. ZIRC's husbandry research activities and publications are in addition aimed to *refine* and optimize maintenance standards and therefore support the third "R" also. Several regional Stock Centers (Taiwan, China, Japan, Germany) support the operations of ZIRC, because we will be able to focus better on the specific research programs of NIH and US based researchers. Importantly, shipping live fish to countries with complicated local regulations will become less frequent. Because Stock Centers are better equipped to exchange and work with cryopreserved samples, we will exchange frozen samples between Stock Centers in bulk, facilitating the regional distribution of live fish strains and minimizing potential discomfort or waiting times of animals in transit. In the long run, we aim to mirror the (cryopreserved) genetic stock inventories between ZIRC, EZRC, and CZRC to further reduce the numbers of animals that need to be maintained alive worldwide. The cooperation between Stock Centers thus refines animal handling and husbandry (shipping) and reduces overall animal numbers.

In general, there are no reasonable alternatives to the use of vertebrate animals such as zebrafish (*Danio rerio*) for research supported by the Resource Center. Some studies, like cellular differentiation, for example, can be conducted in cell culture using established cell lines. However, cell functions and interactions in culture are not completely normal. It is impossible to know with much precision whether proper patterning of tissues occurs in vitro; the developmental context of the embryo, including surrounding cells and extracellular material, is lost; many physiological processes depend upon tissues or organs and their interactions. If we wish to understand the mechanisms that regulate these processes, rather than just the mechanics of cellular differentiation, we need to study this process in live animals where the effects of specific mutations can be assessed. By studying processes in the animal, we will learn how these processes are regulated in their normal physiological context. Such research can



be done only with live animals.

Zebrafish are simple, small vertebrates that are easily reared in the lab. Development is rapid and the embryos are optically transparent so that we can directly observe the growth of individual cells, tissues, and organs. Many mutant strains are available and more are being continuously created and identified in laboratories throughout the world.

Because the basic principles of body patterning and physiology appear similar in all vertebrates, insights gained from work on embryonic zebrafish will have implications for human health and disease. Moreover, research on this organism meets the intent of the Animal Welfare Act because use of many higher vertebrates can now be replaced by use of this lower vertebrate.

Another potential alternative is the use of invertebrates, and much progress has been made in our understanding of genetics and development using these organisms. However, it is thought that vertebrates have evolved at least a few new tricks for organizing and forming their organs and bodies (see for example Easter et al., 1985) and, thus, vertebrates need to be studied, too. The fish is especially well suited for the proposed research because it sits close to the evolutionary step between invertebrates and vertebrates and it teaches us about both kinds of animals. Finally, it is important to remember that, because humans are vertebrates too, the study of fish will teach us something about ourselves.

*Standard Justification for Animal Use and Choice of Species*

**I am using zebrafish on this protocol.**

*Standard Justification for Zebrafish*

**Zebrafish Justification**

*The zebrafish has become widely accepted throughout the world as a particularly useful preparation to analyze how vertebrate development is regulated at the cellular, genetic, and molecular levels. There are a number of reasons for this assessment: (1) the fish are easy to maintain in large numbers and readily reproduce under laboratory conditions; (2) adult fish can be subjected to mutagenesis and mutations can be screened in the first generation by analyzing haploid embryos; (3) the zebrafish embryo has few cells relative to other vertebrates, thus making it a "simple" model for more complex vertebrates such as ourselves; (4) the embryos are optically clear and develop very rapidly and externally (not inside the mother or an eggshell) so that the events involved in the differentiation of tissue, such as the nervous system, can be readily observed; (5) direct access to the developing embryos make it possible to introduce foreign genetic material and to perform cell labeling and other experimental manipulations; and (6) the zebrafish is a small animal so that large numbers, required for genetics, can be kept and studied.*

*Assurance Regarding Unnecessary Duplication of Previous Experiments*

**As principal investigator, I certify the experiments in this project do not unnecessarily duplicate previous experiments.**

## Replacement

Not applicable

## Reduction

Not applicable

## Refinement

Not applicable

## Safety & Hazards

### Use of Biologicals or Cell Lines

Not applicable

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## Use of Animal Biological Products or Animal Cell Lines

*Are animal derived biologicals or cell lines used on animals under on this protocol?*

No

## Use of Infectious Agents

Infectious Agent	IBC Approval #	IBC Approval Date	_UNUSED_	Animal Phenotype
		16-Feb-2021		

## Use of Infectious Agents

*Are infectious agents used on animals under this protocol?*

No

## Use of rDNA

Not applicable

## Use of Recombinant or Synthetic Nucleic Acid Molecules (rsNA)

*Is recombinant/synthetic DNA used on animals under this protocol?*

No

## Use of Genetically Engineered Animals

Modification or Construct	IBC Approval #	IBC Approval Date	_UNUSED_	Animal Phenotype
transgenic zebrafish	2021-04	16-Feb-2021		mostly harmless

## Use of Genetically Engineered Animals / Transgenic Animals

*Will genetically engineered animals be used or created under this protocol?*

Yes

*What activates or silences the genetic modification(s)? Check all that apply.*

**Virus**

ZIRC does not generate any transgenic organisms *per se*, however, they are bred in our facility for identification and propagation purposes, before they are redistributed to other research labs.

A portion of ZIRC transgenic lines are so called "Ia" alleles. They contain an inactivated retroviral vector which, once integrated in the zebrafish genome, cannot replicate. The inserted vector is used to disrupt genes and their normal function (i.e. it creates a mutant line). Most Ia lines/samples have multiple insertion loci (sometimes several dozens), which are all listed as distinct alleles on the ZIRC ordering pages.

All strains, including mutations induced by gamm-ray or chemichals (ENU), are propagated as heterozygotes on the AB wild-type background. A) Lines without molecular markers are identified by visual morphology (phenotype) screening of heterozygous sibling incrosses. B) Lines with molecular markers are identified by fin-clip of heterozygotes and subsequent PCR with primers for gDNA sequences that characterize the modified genomic sequence. In case of transgenic modifications with fluorescent or other gene product the molecular identification is followed up by visual confirmation of the transgene product, e.g. fluorescence microscopy for GFP and other fluorescent markers.

**Other**

ZIRC does not generate any mutants or transgenic organisms, however, they are bred in our facility for

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identification and propagation purposes, before they are redistributed to research labs.

Some transgenes have a dominant effect and can therefore be maintained by outcrossing to wild-type AB fish. For example, all heterozygous carriers of GFP encoding transgenes will express green fluorescence at appropriate times of their embryonic development or during adulthood under the control of a promoter DNA sequence. Conversely, there are transgenes that have no effect on their own, typically they need to be crossed to another transgenic line in order to effect expression. For example, the Gal4 protein is generated under the control of an endogenous zebrafish gene regulatory element. On its own it has no effect, because the yeast Gal4 protein has no function in vertebrates. However, when the line is crossed to a UAS-reporter transgene carrier, Gal4 proteins can bind to the UAS sequence and thereby activate reporter gene expression. Reporter genes can be enzymes that catalyze a color reaction in the fish/embryo, or fluorescent proteins that can be detected by fluorescence microscopy.

Another type of transgenes has been created with a tol2 transposon vector, which facilitates (site-specific) integration of cloned promoter and/or reporter elements. These transgenes are also inactive once they integrated in the zebrafish genome and can not excise/relocate (transpose) again.

There are 3 general types of transgenes in the collection: 1) enhancer traps (allele postfix "Et" - e.g. smb576Et) where the transgene inserted in the gene's enhancer/regulatory elements (246 records); 2) gene traps, (allele postfix "Gt" - e.g. j1098cGt), where the transgene inserted in the gene's coding sequence or introns/exons (532 records); and "regular" transgenic lines (allele postfix "Tg" - e.g. hi1640Tg) where the transgene inserted in randomly in the genome (5521 records); this last type can contain endogenous regulatory sequences with reporters, or (the majority now) virus inserts that disrupt gene functions.

A database of transgenic lines handled at ZIRC can be found at <http://zebrafish.org/fish/lineAll.php>.

All strains, including mutations induced by gamm-ray or chemichals (ENU), are propagated as heterozygotes on the AB wild-type background. A) Lines without molecular markers are identified by visual morphology (phenotype) screening of heterozygous sibling incrosses. B) Lines with molecular markers are identified by fin-clip of heterozygotes and subsequent PCR with primers for gDNA sequences that characterize the modified genomic sequence. In case of transgenic modifications with fluorescent or other gene product the molecular identification is followed up by visual confirmation of the transgene product, e.g. fluorescence microscopy for GFP and other fluorescent markers.

*Provide the likely phenotypic result(s) of the modification(s):*

**No Problems for the Animal**

*Are there any special care or special monitoring requirements for the animals?*

**No**

## Use of Chemical Hazards

Not applicable

### Use of Chemical Hazards

*Will chemical hazards be used with animals named on this protocol?*

**No**

## Use of Radiological Hazards

Not applicable

### Use of Radiological Hazards

*Will radiological hazards be used with animals named on this protocol and are those radiological hazards listed in the table above?*

**No**

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## Other Hazards and Agents

Not applicable

### Other Hazards and Agents

*Are there any additional hazards and agents that apply to this protocol you have listed in the table above?*

No

## Human derived agents, tissues or cell lines

### Human or Non-Human Primate (NHP) Biological Products and Cell Lines

*Does this protocol use tissues, samples, or cell lines derived from human or non-human primate sources?*

No

## Waste and Animal Disposal Procedures

### Waste and Animal Disposal Procedures

*Describe disposal procedures for carcasses, recombinant/synthetic materials, contaminated items, and other hazardous materials.*

*IMPORTANT: Describe disposal procedures for items related to radiological work under the "Use of Radiological Hazards" group.*

#### Provide Description:

All adult animal carcasses (post-euthanasia) are collected in zip-lock bags and maintained in a dedicated freezer until collected by a commercial vendor for incineration. Embryos are dissolved in bleach over night (500 mg/L), which also neutralizes pathogens and destroys DNA.

## Additional Safety Considerations

### Additional Safety Considerations

*Are there any additional safety considerations not listed in the previous sections?*

No

## Field Studies

### Field Studies

*Will any part of this project be conducted in the field?*

No

## Assurances

### Assurances

#### INVESTIGATOR AGREEMENTS and ASSURANCES

1. I agree to abide by the University of Oregon policies for the care and use of animals; the provisions of the NIH Guide to the Care and Use of Laboratory Animals; and all federal, state, and local laws and regulations governing the use of animals in research. I understand that emergency veterinary care will be administered to animals showing evidence of pain or illness, in addition to routine veterinary care as prescribed for individual species in the Standard Operating Procedures.

2. I declare that all experiments involving live animals will be performed under my supervision or that of another qualified biomedical scientist listed on this protocol.

3. I certify that all personnel having direct animal contact, including myself, have been trained in humane and scientifically acceptable procedures in animal handling, administration of anesthetics, analgesics, and euthanasia to be used in this project. I assure that personnel will be allowed adequate time to attend training sessions.

4. I understand that personnel with live animal contact are required to participate in the Occupational Health and Safety Program.

5. I further declare that the information provided in the accompanying protocol is accurate to the best of my knowledge. Any proposed revisions to the animal care and use data will be promptly forwarded in writing to the IACUC for approval, including changes in personnel and location.

6. I am aware that any deviation from an approved protocol or violations of pertinent policies, guidelines or laws could result in immediate suspension of this project.

**I have read these assurance statements and I affirm all these statements are true.**

*If you agree with the statement displayed by the button:*

1. Select the Button
2. Use the "Action" menu in this tab and choose "Save."

## Workflow History

	File Version	Document Version	Status	Performed by	Effective date	Comment
	0.1.0	1.0	Draft	Callihan, Carissa	15-Apr-2021	
	0.2.0	2.0	ICCO Administrative Review	Callihan, Carissa	12-May-2021	
	0.3.0	3.0	IACUC eReview	Callihan, Carissa	12-May-2021	
	0.4.0	4.0	ICCO Administrative Review	Callihan, Carissa	19-May-2021	
	0.5.0	5.0	For Revision (ICCO)	Callihan, Carissa	19-May-2021	
	0.6.0	6.0	ICCO Administrative Review	Callihan, Carissa	07-Jun-2021	
	0.7.0	7.0	Full Committee Review	Callihan, Carissa	07-Jun-2021	Westerfield/Varga 21-15 ready to be reviewed at the June 8, 2021 IACUC Meeting
	1.0.0	8.0	Approved	Callihan, Carissa	08-Jun-2021	Approved at the June IACUC Meeting 6/8/21