

Approval Date _____
1st Renewal Due Date _____
2nd Renewal Due Date _____
Expiration Date _____

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(For office use only)

**ANIMAL USE APPLICATION TO  
THE INSTITUTIONAL ANIMAL CARE & USE COMMITTEE (IACUC)  
UNIVERSITY OF OREGON  
ZEBRAFISH FORM**

*(Must be typewritten - Diskette available upon request in IACUC, OVSAC and some departmental offices)*

Date Submitted March 17, 2006

**I. TITLE, DATES, AND PERSONNEL**

Title of Research Project Zebrafish International Resource Center

Project Dates 05/01/98 (new funding period begins 05/01/03) to 04/30/08

Principal Investigator Monte Westerfield Title/Rank Professor / PI

Dept/Institute Institute Of Neuroscience Ext. 6-4607 Emergency Phone 343-5637

Co-Investigator Michael Kent Title/Rank Co-PI

Dept/Institute Microbiology, OSU Ext. 7-8652 Emergency Phone (541) 738-9499

Co-Investigator Zoltan Varga Title/Rank Res. Assoc.

Dept/Institute ION, ZIRC Ext. 6-6099 Emergency Phone 689-2347

Other Personnel: Jen Matthews, Andrzej Nasiadka, April Mazanec, Austin Bailey, David Lains, Carrie Cleeton, Carrie Jones, Jennifer Ramsay, Tim Mason, Ron Holland, Beth Murrill, Erin Quinn, Terra Hiebert, Renee Clark, Amelia Westerfield, Konstantine Buirlin, Jen-Jen Hwang-Shum

**II. PURPOSE (Check if applicable):**

Research Project     Pilot Project     Teaching     Student Special Project

**III. FUNDING (POTENTIAL AND AWARDED)**

A. **PROTOCOL STATUS:**  New     Amendment     Annual Renewal

B. **FUNDING PROPOSAL TYPE:**  New     Continuation     Renewal     Revision

Was this project originally funded or reviewed without the use of animals, or are there new significant changes involving animals which were not previously outlined in the grant proposal? **YES** \_\_\_ **NO** **X**.  
If yes, you will need to send a letter addressed to the program officer of the granting agency detailing the proposed significant changes involving the use of animals. The letter requires a counter-signature by the institution. The Office of Research Services & Administration (ORSA) will sign on behalf of Richard Linton, Vice Provost for Research. You may attach Section IX of the protocol application to satisfy funding agency requirements and IACUC policies and procedures. If you have any questions regarding the requirements for the letter, please contact ORSA, 346-5131, or OVSAC, 346-4957. Please attach a copy of the letter with the appropriate signatures to this application.

C. **EXTRAMURAL FUNDING:** (When more than one funding source is solicited, a single IACUC animal use request may be submitted provided the species, number, and procedures are the same for each grant proposal application.)

Agency National Institutes of Health Grant # 1 P40 RR012546

Grant Title National Resource of Zebrafish

Proposed Dates: 05/01/98 to 04/30/03

Agency National Institutes of Health Grant # 1 P40 RR012546 Supplement

Grant Title National Resource of Zebrafish Supplement to 1 P40RR HD12546

Proposed Dates: 07/01/00 to 04/30/04

Agency National Institutes of Health Grant # 2 P40 RR012546-06

Grant Title Zebrafish International Resource Center

Proposed Dates: 05/01/03 to 04/30/08

D. **INTRAMURAL/NON-COMPETITIVE FUNDING** (See page 2 of instructions)

Funding Source N/A

Proposed Dates \_\_\_\_\_ to \_\_\_\_\_

E. **COOPERATIVE RESEARCH**

Is this a cooperative research project (are there principal investigators from more than one institution involved)? X Yes \_\_\_ No **If yes**, please see the UO's Policy to Address Cooperative Research which states that all Principal Investigators engaged in cooperative research with another institution must have approval by the UO's IACUC for all those projects which utilize vertebrate animals. Additionally, if the animals are housed at a cooperating facility, then there must be approval by the cooperating institution's IACUC.

F. **PEER REVIEW OF UNSPONSORED RESEARCH** (1. For teaching applications. 2. Other)

**1. Departmental Curriculum Committee Review:**

N/A \_\_\_\_\_  
(Department) (Committee Chair) (Date Approved)

**2. Other Peer Review:**

I have reviewed the attached animal use application and find it to be scientifically valid and consistent with University of Oregon policy.

\_\_\_\_\_  
Signature (Authorized reviewer) Name & Title (typed)

\_\_\_\_\_  
Signature (Authorized reviewer) Name & Title (typed)

#### IV. ANIMAL REQUIREMENTS AND FACILITIES

Common Name Zebrafish Source ZIRC and various world wide labs

Strain Various # of embryos per year  $\sim 1.47 \times 10^7$  # of adults per year  $\sim 145,000$

Housing Location Zebrafish International Resource Center and OSU (see details below)

Lab Room# ZIRC rms.103 and 118

Will special housing be needed? No If yes, explain: \_\_\_\_\_

Will animals be held more than 12 hours outside of OVSAC? Yes If yes, explain: All housing locations are outside of OVSAC; adults and larvae are kept within these locations.

Will facilities outside of University of Oregon campus be used? Yes If yes, explain: Fish will be kept by Dr. Michael Kent for fish disease studies at OSU in Nash Hall Room 542 and at the Center for Fish Disease Research.

Are animals wild or laboratory-bred? Laboratory-bred

Is live feed required? Yes If yes, explain: Larval fish are fed live paramecium (protozoan). Fish of all life stages (larval-adult) are fed live *Artemia* nauplii (newly hatched brine shrimp).

#### V. WHAT IS THE OBJECTIVE OF THIS STUDY? HOW IS THIS STUDY RELEVANT TO HUMAN OR ANIMAL HEALTH, THE ADVANCEMENT OF KNOWLEDGE, OR THE GOOD OF SOCIETY?

The last several years have witnessed an explosion in our understanding of vertebrate development, largely based on work from a few model genetic organisms. The embryonic zebrafish is the newest of these model organisms. Because the basic principles of body patterning appear similar during embryogenesis of 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.

Genetic research on zebrafish was begun at the University of Oregon and for many years Eugene was the only place it was performed. Recently, however, international interest in this organism has grown tremendously (Balter, 1995); studies of the embryology and genetics of zebrafish and the recent identification of over 7,000 genetic mutations, have lead to a dramatic increase in the number of laboratories using this organism to study the basic mechanisms of vertebrate development. Currently these genetic stocks are distributed among more than 300 laboratories in 28 countries. To make room for new mutants, laboratories must discontinue some of their current stocks. Although mutations can be preserved in frozen sperm, not all laboratories are proficient with this technique. Thus, some discontinued stocks are permanently lost. There is no central site to preserve and keep track of these stocks.

This center will maintain wild-type and mutant stocks of zebrafish and make these stocks widely available to the international research community. Specifically we are:

**1. 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 from the research community and breed them to produce new generations. We will freeze and store sperm from all these lines. We will acquire the most widely used wild-type lines and maintain them in a manner that preserves their genetic diversity. We will receive and store antibodies, gene probes and markers 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 on-line about stocks, materials, ordering procedures and methods through ZFIN, the zebrafish model organism database.

**2. To provide consultation and pathology services.** We will provide diagnostic services and health status testing for laboratory zebrafish. We will use histopathology, bacteriology and virology to analyze specific or suspected disease problems. We will 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 will continue to develop methods to detect and control disease in laboratory colonies. We will maintain and update our on-line manual for the prevention, diagnosis and treatment of diseases affecting zebrafish available through ZFIN.

**3. To develop improved zebrafish husbandry methods.** We will establish standards and procedures for generating healthier and more vigorous colonies. We will study the influence of stress, diet, water conditions, housing and husbandry on larval and juvenile growth, adult fecundity, reproductive longevity and disease. We will test aquaria products and algal/cyanobacterium flora for toxicity to larval and adult fish. We will develop and improve standardized methods for cryopreservation and reconstitution of genetic lines. We will continue to investigate the transmission and pathology of the microsporidian parasite, *Pseudoloma neurophilia*.

VI. **DOES THIS STUDY UNNECESSARILY DUPLICATE PREVIOUS EXPERIMENTS? IF SO, EXPLAIN.**

We confidently assert that the studies that the Resource Center will support do not unnecessarily duplicate previous work.

**CHECKLIST.** (Please give details in Section IX).

**TYPE OF PROJECT** (If necessary, please consult OVSAC for further information concerning pain categories. This section is only a checklist.)

**PAIN CATEGORY** (Indicate species and number of animals in each pain category):

**C** 100,000 adult zebrafish and 1.47 X 10<sup>7</sup> embryos

Procedures that are considered to produce minimal, transient, or no pain or distress when performed by competent individuals (e.g. all zebrafish embryos\*\*)

**D** 45,000 adult zebrafish

Procedures or tests involving the administration of appropriate anesthetic, analgesic, or tranquilizer drugs to avoid pain or distress (e.g., fin clips, in vitro fertilization)

**E\*** \_\_\_\_\_

Procedures or tests that, for scientific validity, are performed involving pain or distress without administration of appropriate anesthetic, analgesic, or tranquilizer drugs (e.g., chemical mutagenesis of adults, ENU)

\* Please note that when a protocol falls into the "E" category, the investigator must attach a written justification for the procedure and may be requested to attend an IACUC meeting to discuss the proposed research.

\*\* In practice, tricaine anesthesia is sometimes used to facilitate capture and handling of the fish at any stage after the embryos become motile even though the procedures produce no or minimal discomfort. Even invasive procedures done with embryos could not produce discomfort because the neural centers mediating pain sensation are still undeveloped.

**PROCEDURE**

\_\_\_ Blood Collection    X Surgical    \_\_\_ Non-Surgical    \_\_\_ Behavioral

Field Study     Other (Describe): Care and maintenance of adults; breeding and obtaining gametes and embryos (including parthenogenetic embryos); raising larvae, cryogenic preservation of sperm; strain record-keeping; fin clips, quarantine and other procedures relating to disease control and euthanasia

**TYPE OF STUDY**

Terminal (Acute): Animal never awakens from initial procedure.  
 Survival (Chronic): Animal awakens and survives for hours/days after initial procedure.

**SPECIAL CONSIDERATIONS:** (Check if applicable)

Multiple surgeries            (If yes, explain in Section IX)  
 Restraint device(s)            (If yes, explain in Section IX)  
 Neuromuscular blocking agents (If yes, explain in Section IX)  
 Complete Freund's Adjuvant    (If yes, include signed copy of the U of O Adjuvant Policy)  
 Breeding Colony (If yes, include the standard operating procedure for care and breeding)  
 Food or Water Deprivation      (If yes, explain in Section IX)

**VIII. ANIMAL EXPERIMENTATION INVOLVING HAZARDOUS AGENTS**

Are any hazardous agents including infectious agents, biohazards, carcinogens (ENU for mutagenizing), toxic chemicals, or radioisotopes, gamma rays for mutagenizing used on live animals for this study?

\* Yes     No    \* Michael Kent's infectious disease studies at OSU

**If hazardous agents are being used, attach a use authorization from the appropriate committee or office.**

Authorized by:

Biosafety Committee (Infectious agents and biohazards)?             Yes     No \*OSU Biosafety  
Environmental Health & Safety Office (Carcinogens and toxic chemicals)?     Yes     No  
(EHS has reviewed and acknowledges the use of ENU for zebrafish mutagenesis.)  
Radiation Committee (Radioisotopes)?             Yes     No

**NOTE:** Since the use of animals in experimentation involving hazardous agents requires special consideration, the procedures and the facilities to be used must be reviewed by both the Office of Environmental Health and Safety and the IACUC. Formal safety programs should be established to assess the hazards, to determine the safeguards needed for their control, and to ensure that the staff is competent, and that the facilities are adequate for the safe conduct of the research (PHS *Guide*).

**IX. PLEASE PROVIDE DETAILED INFORMATION FOR THIS SECTION ON A SEPARATE SHEET**

This application form has been reformatted in order to accommodate the Vertebrate Animal Section of the Research Plan of the Public Health Service Grant Application form, PHS 398. Items 1-5 in the bold print are quoted directly from the PHS Application Packet. The light print is to serve as a guide (check sheet) in preparing your response to meet funding agency and IACUC requirements. This format is applicable for all animal use protocols, even when the funding source is other than PHS.

**If PHS is the funding source, please answer the following questions and attach a copy of the Vertebrate Animal Section of the Research Plan. For funding other than PHS, please answer the**

**following questions and attach a copy of all relevant portions of the grant application pertaining to animal care and use.**

1. **Provide a detailed description of the proposed use of animals in the work previously outlined in the experimental design and methods section. Identify the species, strains, ages, sex, and numbers of animals to be used in the proposed work.**

Experimental/Non Surgical Study: Identify procedure and duration of study.

Behavioral Study: Describe any conditioning, deprivation, or stimulation that might be involved.

For Surgical, Blood & Tissue Collection, Address:

- Drugs and/or antigens used                      Quantity
- Route of administration                      Frequency
- Injection sites                      Pain associated with procedures

For Surgical Procedure, Address:

- Pre-operative care                      < Methods to prevent dehydration/hypothermia
- Surgical procedure                      < Anticipated duration of surgery
- Multiple surgeries                      < Anticipated duration of surgeries/type
- Use of paralyzing drugs                      < Anticipated duration of study/endpoint/pain
- Post-operative care                      < Anticipated nursing care medication & duration

Field Study: For capture or any invasive procedure

2. **Justify the use of animals, the choice of species, and the numbers used. If the animals are in short supply, costly, or to be used in large numbers, provide additional rationale for their selection and their numbers.**
3. **Provide information on the veterinary care of the animals involved.** (Note: It is not necessary to complete this section for the IACUC. It is only necessary to state that veterinary service is being provided by the Office of Veterinary Services & Animal Care as described in routine facility standard operating procedures or PHS-approved assurance statements.)
4. **Describe procedures of ensuring that discomfort, distress, pain and injury will be limited to that which is unavoidable in the conduct of scientifically sound research. Describe the use of analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices where appropriate to minimize discomfort, distress, pain and injury.**

Address:

- Analgesic/anesthetic/tranquilizing drugs
  - Dose
  - Frequency
  - Route of administration
  - Criteria to assess pain/discomfort
- Describe use of comfortable restraining devices
  - Dimensions and/or type
  - Duration of confinement (continual observation required)
- Describe any other animal manipulations that may produce pain, discomfort, or anxiety not mentioned previously

- Describe any physical or psychological impairment of the animal resulting from experimental manipulation (e.g. blindness, loss of motor abilities)
  - Describe the methods used to assess adequate levels of anesthesia
  - Describe indices used to help assess possible signs of pain, distress or discomfort
5. **Describe any euthanasia method to be used and the reasons for its selection. State whether this method is consistent with the recommendations on the panel of euthanasia of the American Veterinary Medical Association available in the OVSAC library and the *Researcher's Handbook*. If not, present a justification for not following the recommendations.**
- A. For chemical or gas euthanasia, please include the agent, dose and route.
- B. For physical euthanasia, please indicate the specific method:

## IX. Detailed Information

**If PHS is the funding source, please answer the following questions and attach a copy of the Vertebrate Animal Section of the Research Plan. For funding other than PHS, please answer the following questions and attach a copy of all relevant portions of the grant application pertaining to animal care and use.**

### INTRODUCTION: Standard Description of the Proposed Use of Animals

**Species:** Zebrafish, *Danio rerio*  
**Sex:** Both sexes  
**Ages:** All ages  
**Number:** Approximately 145,000 adults and  $1.47 \times 10^7$  embryos

The zebrafish group has prepared its own detailed user manual that describes these standard procedures. This was done because of the special requirements of the zebrafish, and how they were used, as contrasted with use of other vertebrates at Oregon (particularly birds and mammals), that are covered by OVSAC's Standard Operating Procedures Document. The zebrafish standard operating procedure manual is *The Zebrafish Book* (ed. 4, 2000). The manual has been approved by the IACUC and is currently included within the IACUC packet.

The Zebrafish Standard Operating Procedures covered by this application does cover all procedures, including invasive ones, carried out with embryos, either done within the facility (e.g. DNA injection into early embryonic cells) or in the user laboratories (e.g. cell labeling, microsurgery, laser microablation, cell transplantation, donors for cell & tissue culture). In addition, all usual facility operations are included: care and maintenance of adults, breeding and obtaining gametes and embryos (including parthenogenetic embryos), raising larvae, cryogenic preservation of sperm, fin clips, mutagenesis, strain record keeping, sending fish to and receiving fish from other laboratories, quarantine and other procedures relating to disease control, and euthanasia.

**1a. Briefly summarize the methods to be used in achieving the objectives of your proposal. Please emphasize any procedures not covered by the *The Zebrafish Book* (please give a brief description in the space provided below).**

In addition to the procedures in the zebrafish book, fish may be exposed to *Pseudoloma neurophilia* (microsporidia) or potential aquatic environmental toxins (e.g. algae/cyanobacteria toxins, aquaria

parts). Studies involving pathogen exposures will primarily occur at OSU (please see attached OSU Animal Care & Use Proposals and Approval Letters)

**2a. Standard justification for the use of animals and choice of species:**

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.

**2b. Are there any other justifications for this project not outlined above? No If yes, please list.**

**2c. Justify the number of animals proposed:**

Our goal is to house 600 genetically distinct zebrafish lines. We will need an average of 150 fish to maintain each line. This requires about  $600 \times 150 = 90,000$  adults. The mutant lines are maintained by crossing mutant carriers and growing up the babies. Only 1 out of 4 (or 1 out of 3, depending upon the type of cross) of these offspring will inherit the mutant chromosome. To identify these carriers, offspring are crossed back to the previous generation and on average 4 crosses will need to be done to identify a carrier. On average, about 200 embryos are produced by each cross even though we need many fewer to recognize the mutation. This process will be repeated 3 times a year to have young, healthy breeders available at all times. To identify 10 carriers for each line, we will generate:  $10 \text{ carriers} \times 4 \text{ crosses each} \times 200 \text{ embryos per cross} \times 600 \text{ lines} \times 3 \text{ times per year} = 1.44 \times 10^7$  embryos. Currently we have 372 distinct zebrafish lines.

The Resource Center provides adult zebrafish and zebrafish embryos to research laboratories throughout the world. In 2005, the Resource Center supplied 8,500 adult zebrafish and 85,000 embryos to the research community. We expect the customer demand for zebrafish embryos and adults to increase over the next three years. The estimated number of adult zebrafish that will be shipped over the follow three years is 52,000 fish. The estimated number of embryos that will be shipped over the follow three years is 310,000 embryos.

Experiments on the transmission and pathology of *Pseudoloma neurophilia* are expected to utilize 500 adult fish per year. [1500 total fish]

Toxicity and stress studies will utilize an estimated 500 larval or adult fish per year. [1500 total fish]

Experiments on optimizing husbandry conditions will be performed on wild-type (AB) shipping stock and should not require additional fish.

### 3. Veterinary Care:

Veterinary care is provided by the Resource Center staff; Dr. Jen Matthews, ZIRC veterinarian; Dr. Michael Kent, fish pathologist and co-PI; and Dr. Jan Spitsbergen, board certified veterinary pathologist and co-PI, as described in routine standard operating procedures. They consult with OVSAC as required.

#### 4a. Standard Procedures for Alleviation of Pain, Discomfort, Distress, and Injury:

Most of the procedures on embryos will be done at very early developmental stages before the nervous system has matured. Indeed, the neural crest cells that we study are the *source* of sensory neurons that ultimately develop in these organisms. We feel, therefore, that without the structures necessary to detect pain, embryos at this stage are unlikely to be susceptible to painful stimuli. On the other hand, the developing muscle cells in the embryos twitch spontaneously causing the embryos to move. To prevent such movements, which make observations of cells more difficult, embryos older than 17 hours will be anesthetized in Tricaine, also called MS 222, added to the water. Tricaine is the best anesthesia available for lower (aquatic "cold-blooded") vertebrates. The dosage is age dependent. Anesthesia is administered by immersing the animal in the anesthetic to facilitate handling of the fish, e.g. during procedures to obtain gametes from adults which involves handling of the fish but produces minimal discomfort even if the fish were alert. There is no permanent impairment.

#### 4b. Which standard procedures outlined above or any others not mentioned will be utilized to ensure minimization of pain, discomfort, distress and injury?

The only non-standard situation at the Resource Center will involve disease, toxicity or neoplasia studies. Fish may be exposed to agents in one of three ways, by immersion (category C), by feeding (category C) or by injection of anesthetized fish in order to make the fish easier to handle (category D). Cohorts of potentially infected fish will maintained through the study period until they are sacrificed in order to assess the results of the experiment. Individual fish that are displaying obvious signs of disease prior to that time will be sacrificed in order to minimize their experience of pain and to preserve the usefulness to the study (category C).

#### 5a. Standard method of euthanasia:

Standard methods of euthanasia include:

- 1) Immobilization by submersion in ice water immediately followed by cranial concussion and decapitation via an in-sink garbage disposal.
- 2) Overdose of tricaine methanesulfonate (MS-222, 200-300mg/l) by prolonged immersion. Fish should be left in the solution for at least 10 minutes following cessation of opercular movement.
- 3) Anesthesia with tricaine methanesulfonate (MS-222, 168mg/l) followed by rapid freezing in liquid nitrogen.

5b. Which standard method or other will be utilized? All methods listed above will be used.

**X. PERSONNEL<sup>2</sup>** Please fill out **section A** with the P.I.'s information; complete **section B** by listing each research assistant, student, postdoc, and **primary** lab employee who will be involved in this study.

**A. P.I. QUALIFICATIONS AND TRAINING**

NAME Monte Westerfield Campus Phone 6-4607  
 Position Professor of Biology Institute/Department Institute of Neuroscience Work Location 337 Huestis  
 Credentials/Experience 25 years experience with zebrafish, trained by George Sreisinger  
 Signature \_\_\_\_\_ Emergency Phone 343-5637

In accordance with federal regulations, please provide the following information. **Be advised that the P.I. must assure that all persons participating have demonstrated competence for those techniques that they will be performing as part of this study.**

**B. LAB PERSONNEL QUALIFICATIONS AND TRAINING**

**I have read the protocol and understand my responsibilities outlined therein. I have also read the University of Oregon's Animal Care & Use Training Handbook.**

Name	Credentials/Experience	Personnel Signature	Trained By	Training Required (Y/N)	OHP Review Date
Zoltan Varga, Ph.D	4 years post-doc in Monte Westerfield lab, 4.5 years experience with zebrafish as Assistant Professor in Freiburg Germany, 2 years as director of ZIRC		Monte Westerfield, ZIRC Staff	No	10/2004
Mike Kent	Director for Salmon Disease Research, Microbiology, 23 years experience conducting in vitro experiments with fish		Jen Matthews, Tim Mason	No	
Jen Matthews, DVM, Ph.D.	3 years small animal clinical vet experience, 6 years experience as a ZIRC veterinarian		Zebrafish Facility Staff	No	6/2000
April Mazanec	4 years experience with UO Zebrafish Facility, 6 years experience with the ZIRC, 6 years assisting ZF course in Woods Hole		Zebrafish Facility Staff	No	3/2001
Erin Quinn	2 years experience with ZIRC		ZIRC Staff	No	

<sup>2</sup> . NOTE: Federal regulations require that all personnel involved with animal care and use (including antigen preparation for antibody production) be qualified to perform their duties. Those personnel with any live animal contact must also be a part of the University of Oregon's Occupational Health Program. **In order for this animal use application to be approved, the IACUC must have on file training information and qualifications for each individual and documentation of participation in the Occupational Health Program for those individuals with any live animal contact.**

Andrzej Nasiadka	3 years experience at the University of Freiburg, 2 years experience at ZIRC		Driever Lab, ZIRC Staff	No	10/2004
Austin Bailey	5 years experience with UO Zebrafish Facility, 6 years experience with ZIRC		Zebrafish Facility Staff, ZIRC staff	No	5/2000
David Lains	5 years experience with ZIRC, attended aquaculture conferences		Zebrafish Facility Staff, ZIRC staff	No	1/2001
Tim Mason	Over 14 years experience with the UO Zebrafish Facility		Zebrafish Facility Staff, Pat Lambert, Ed Sullivan	No	9/2002
Carrie Cleeton	4 years experience with UO Zebrafish Facility, 5 years experience with ZIRC		Zebrafish Facility Staff, ZIRC staff	No	3/2002
Carrie Jones	2 years experience with UO Zebrafish Facility, 4 years experience with ZIRC		Zebrafish Facility Staff, ZIRC staff	No	1/2002
Beth Murrill	1.5 years experience with the ZIRC		ZIRC staff	No	12/2004
Terra Hiebert	11 months experience with the ZIRC		ZIRC staff	No	6/2005
Amelia Westerfield	3.5 years experience with UO Zebrafish Facility, 9 months experience with ZIRC		Zebrafish Facility Staff, Westerfield Laboratory Staff	No	10/2005
Kostia Birulin	9 months experience with ZIRC		ZIRC Staff	No	10/2005
Jen-Jen Hwang-Shum, Ph.D	8 months experience with ZIRC		ZIRC Staff	No	10/2005
Renee Clark	10 months experience with ZIRC		ZIRC Staff	No	4/2005
Ron Holland	3 years experience with ZIRC		Tim Mason, ZIRC Staff	No	3/2004
Jennifer Ramsey, MS	Masters degree in Fish Pathology, 9 years experience working in fish research, 4 years working with zebrafish at OSU and ZIRC		Michael Kent and ZIRC Staff	No	3/2006
Aurora Evans	1.5 years experience at ZIRC as a student employee		ZIRC Staff	No	7/2004
Sarah Hendrickson	1.5 years experience at ZIRC as a student employee		ZIRC Staff	No	12/2004
Sarah Terpin	2 years experience at ZIRC as a student employee		ZIRC Staff	No	4/2004
Andrew Van Gordon	2.5 years experience at ZIRC as a student employee		ZIRC Staff	No	8/2003
Evan Williams	1 year experience at ZIRC as a student employee		ZIRC Staff	No	10/2005
Kellie Masuda	1 year experience at ZIRC as a student employee		ZIRC Staff	No	10/2005
Noah Clarke	6 months experience at ZIRC as a student employee		ZIRC Staff	No	
Ben Clark	2 years experience at ZIRC as a student employee		ZIRC Staff	No	1/2004

## XI. ASSURANCE STATEMENTS

A. **ALTERNATIVES.** The following alternatives must be addressed prior to the use of animals in accordance with Federal policy:

1. Replacement:

I have considered the use of alternatives to the present species, i.e. the use of other species and/or the use of non-animal models and have found them to be unacceptable. X Yes \_\_\_ No

2. Reduction:

I have designed my experimental protocol with careful attention to using the appropriate number of animals and have considered appropriate statistical methods used to reduce the number of animals in this study. X Yes \_\_\_ No

3. Refinement:

I have planned this project to assure that animals are subjected to the minimum amount of pain and distress by the adequate administration of anesthetics, tranquilizers; humane euthanasia; that they receive careful scrutiny of behavioral indices of pain or distress; and that noninvasive imaging technologies are used when appropriate. X Yes \_\_\_ No

4. Alternative Methods: (The following is from USDA Policy #12, June 21, 2000)

*Alternatives or alternative methods* are generally regarded as those that incorporate some aspect of replacement, reduction, or refinement of animal use in pursuit of the minimization of animal pain and distress consistent with the goals of the research. These include methods that use non-animal systems or less sentient animal species to partially or fully *replace* animals (for example, the use of an *in vitro* or insect model to replace a mammalian model), methods that *reduce* the number of animals to the minimum required to obtain scientifically valid data, and methods that *refine* animal use by lessening or eliminating pain or distress and, thereby, enhancing animal well-being. Potential alternatives that do not allow the attainment of the goals of the research are not, by definition, alternatives.

The USDA believes that the performance of a database search remains the most effective and efficient method for demonstrating compliance with the requirement to consider alternatives to painful/distressful procedures. However, in some circumstances (as in highly specialized fields of study), conferences, colloquia, subject expert consultants, or other sources may provide relevant and up-to-date information regarding alternatives in lieu of, or in addition to, a database search. When other sources are the primary means of considering alternatives, the Institutional Animal Care and Use Committee (IACUC) and the inspecting Veterinary Medical Officer should closely scrutinize the results. Sufficient documentation, such as the consultant's name and qualifications and the date and content of the consult, should be provided to the IACUC to demonstrate the expert's knowledge of the availability of alternatives in the specific field of study. For example, an immunologist cited as a subject expert may or may not possess expertise concerning alternatives to *in vivo* antibody production.

When a database search is the primary means of meeting this requirement, the narrative must, at a minimum, include:

1. The names of the databases search;
2. The date the search was performed;
3. The period covered by the search; and
4. The key words and/or the search strategy used.

The Animal Welfare Information Center (AWIC) is an information service of the National Agricultural Library specifically established to provide information about alternatives. AWIC offers expertise in formulation of the search strategy and selection of key words and databases, access to unique databases, on- and off-site training of institute personnel in conducting effective alternative searches, and is able to perform no-cost or low-cost electronic database searches. AWIC can be contacted at (301) 504-6212,

via e-mail at [awic@nal.usda.gov](mailto:awic@nal.usda.gov), or via its web site at <http://www.nal.usda.gov/awic>. Other excellent resources for assistance with alternative searches are available and may be equally acceptable.

Regardless of the alternative source(s) used, the written narrative should include adequate information for the IACUC to assess that a reasonable and good faith effort was made to determine the availability of alternatives or alternative methods. If a database search or other source identifies a *bona fide* alternative method (one that could be used to accomplish the goals of the animal use proposal), the written narrative should justify why this alternative was not used.

**In accordance with the information provided on the preceding page from USDA Policy #12, please provide in the space below a written narrative description of the methods and sources used to determine that alternatives were not available or appropriate for this study.**

The Zebrafish International Resource Center seeks to support studies involving the use of zebrafish by providing fish, materials, information, expertise, health services, and disease and husbandry research to the zebrafish research community. Supplying zebrafish cannot be done without zebrafish. Zebrafish health and husbandry studies require the use of zebrafish to assess the actual effects of proposed experiments. Mutant animals are provided for use in a wide variety of studies (developmental or other) as well as being possibly used in studies of the genetic effects upon disease susceptibility. For experiments requiring experimental tractable genetics, zebrafish is the only vertebrate except mouse where successful genetic analyses are possible and well-developed genetic tools are available.

To some extent, various studies including disease mechanisms can be studied in zebrafish cells growing in cell culture. However, primary cell cultures must be obtained from developing embryos, and ultimately, the predictions of hypotheses about the behavior of embryonic cells in their normal environments must be tested directly in intact systems.

On 3/14/06, the databases of ASFA (Aquatic Sciences and Fisheries Abstracts), Toxline, and the Zoological Record Plus and Google Scholar were searched between the years 1996 and the present for the keywords: zebrafish and disease, husbandry, microsporidia, cryopreservation, neoplasia, and toxicity. There were numerous hits on the searches but the articles were not relevant to alternatives to the proposed studies or services.

**B. ASSURANCE FOR THE HUMANE CARE AND USE OF ANIMALS USED FOR TEACHING AND RESEARCH**

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 and understand the assurance statements.

_____	<u>Monte Westerfield</u>
P.I. Signature	Name and Title (typed)
_____	<u>Michael Kent</u>
Co-P.I. Signature	Name and Title (typed)
_____	<u>Zoltan Varga</u>
Co-P.I. Signature	Name and Title (typed)

**NOTE:** Person applying for an animal use approval must be eligible for Principal Investigator status.