

Transgenic Zebrafish Model Professor Peter Alestrom

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Norwegian School of Veterinary
Science



www.veths.no
aquamedicine.no (biochemistry)

The care and use of zebrafish in modern genomic research

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ABSTRACT. Zebrafish (*Danio rerio*) is **increasingly used in basic biology and biomedicine** and has a number of characters that makes it a good choice as lab research model. It's small

size, easy to breed, short generation interval, transparent embryo and well characterized early development (Streisinger et al. 1986; <http://zfin.org>) together with a fully sequenced genome (1,560,480,686 bp and ca 32000 genes) allows project design and success expectations which in many instances are much higher than would be expected using most other species of fish. As a manifest of the increasing use of the zebrafish model, a novel scientific journal named Zebrafish (www.liebertonline.com/loi/zeb) has been devoted to this niche of experimental biology.

In our laboratory the major projects aims at further refinement of the model (www.aquamedicine.no -biochemistry):

(1) Zebrafish ES cell cultures for targeted mutagenesis and **(2) development of targeting of vector DNA to improve DNA vaccine and other therapeutic use of gene constructs.** A third project area concerns **(3) monitoring in vivo effects of micro gravity conditions** and **(4) ecotoxins/toxicogenomics** in zebrafish.

As part of our research we are in the position of **upgrading our zebrafish laboratory to the standards set by AAALAC** and other international bodies for certification and accreditation of research animal units.



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Transgenic fish

By Peter Aleström, Professor PhD

Genetic modification of fish, using zebrafish as model, offers a unique research tool in several areas of aquamedicine or biomedicine in general. It allows characterization of gene expression patterns, functional genetics, methodological aspects of gene transfer and uptake, production of transgenic lines of fish with changed traits to be used as disease models etc. The value of the zebrafish model has recently increased with a fully sequenced genome and whole genome microarray technology available.

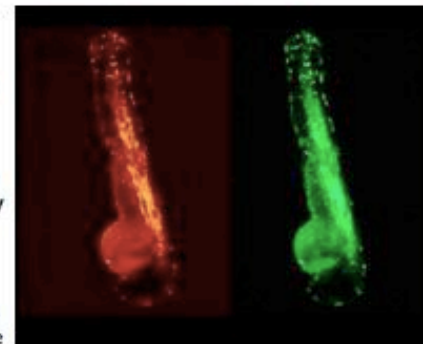
The Alestrom Zebrafish Laboratory at Norwegian School of Veterinary Science aims at being a regional center in an international network of transgenic fish laboratories. There are three major routes for genetic modification of fish in use in the lab:

- DNA transfer to somatic tissues for transient gene expression (DNA vaccines, gene therapy) [LINK to project](#)
- DNA transfer to one cell-stage embryos for production of stable transgenic lines of fish (expression of novel gene products, or over expression of already existing gene products)
- DNA transfer to ES-cells to achieve targeted mutagenesis through homologous recombination of gene constructs. Can be used for gene knock-out or gene replacement [LINK to project](#)

The Alestrom Zebrafish Lab has ongoing research projects in 3 areas:

- Reproductive biology with characterization of top level HPG-axis, the GnRH control of onset of sexual maturation and development of transgenic GnRH depleted sterile fish
- Bone metabolism, using transgenic fish to study osteoprotegerin-GFP reporter genes expression profiles in gravity on Earth as compared to microgravity in space [LINK to project](#)
- Fish prion protein as a model for Transmissible Spongiform Encephalopathy (TSE)

Link to [Biochemistry / Alestrom Zebrafish Lab home page](#)



Search

Transgenic fish

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Coming events

- Aquaculture Europe, Trondheim Norway August 5-9 2005
- International Marine Biotechnology Conference, June 7-12
- Harmonisation of the Care and Use of Fish in Research 23rd - 26th May 2005

News

- International meeting on the care & use of fish in...

Zebrafish (“fish–mouse”) & RRR

Reduction: Advantage with zebrafish is large numbers offspring and relative ease to use many groups – analysis at embryo stage reduce numbers of hatched fish

Cloned fish with same genetic background

Refinement: compared to other fish models (comp. mouse model). Genome sequence, established transgenic methods, functional genomics etc.

Replacement: in vivo vertebrate model: from mammal to fish



Old zebrafish system

New zebrafish system

1. Breed our fish - genetically defined
2. Pathogen free fish? Chlorine bleach embryos
3. Control water quality: reverse osmosis, UV sterilization, particle, bio and carbon filters; Control light, salinity and temperature; standardized (?) feeding
4. Monitoring pH & conductivity (salinity) and nitrogen

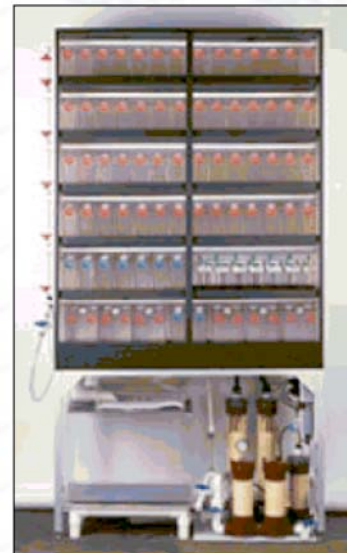
Marine Biotech, Inc.



AQUATIC RESEARCH SYSTEMS TECHNOLOGY



Marine Biotech Z-MOD® for Zebrafish (*Brachydanio rerio*)



The MBI **Z-MOD® System** is a partially enclosed cabinet with a full recirculating life support system that is used to house a variety of aquatic species. The system provides the muted light environment most aquatic species prefer. Also, the unit may be ordered with supplementary lighting and photo-period controls for the manipulation of day-night cycles.

It is the most space efficient, flexible, and cost effective research environment available. A fifty-four inch wide unit holds 1,008 adult zebrafish (6 adults per liter), or 176 frogs when configured for *Xenopus tropicalis* (1 adult per liter). The

OBLIGATORY WEEKLY DUTIES

Must be performed a least once per week on ALL water systems

(Complete fields as appropriate)

Biochemical/ Biological Analysis	Outer Room		Room 1				Room 2	Comments <i>(e.g. action taken)</i>
	System Water	Nursery	System A	System B	System C	System D	System E	
Temperature (°C)								
pH								
Nitrite Conc ⁿ (mg/ml)								
Nitrate Conc ⁿ (mg/ml)								
Ammonia Conc ⁿ (mg/ml)								
Water Hardness (dH)								

OTHER TASKS

Perform when necessary

Refilling of main system tank (% of tank refilled): % Other (Please specify; if necessary, continue on reverse):

--

A comprehensive manual



George Streisinger

This book is dedicated to the late George Streisinger who first began the study of zebrafish in Eugene and whose insight and gentle support got us all started.

<http://zfin.org>

http://agsci.oregonstate.edu/research/hand_zebrafish.html

Care and Use of Zerbrafish -- College of Agricultural Sciences

OSU Oregon State University

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College of Agricultural Sciences

Animal Handler Occupational Health and Safety Program

- OSU Policy on Animal Handler Occupational Health and Safety Program
- Allergies and Diseases Communicable from Animals to Humans
- rabbits
- rodents
- owls
- fish
- zebrafish
- frogs
- Animal Contact Review Questionnaire
- Health Questionnaire

Faculty Resources

- Plan of Work & Annual Report
- AES Projects
- Multi-State Projects
- Equipment Replacement
- Animal Handler Occupational Health and Safety Program
- Research Proposal
- Competitive Research Grants
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Cultivating excellence
in food, natural resource,
and biobased sciences

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Care and Use of Zerbrafish



Potential Zoonotic Diseases

Aside from food poisonings, the overall incidence of transmission of disease-producing agents from fish to humans is low. There are, however, a number of agents that are found in fish and aquarium water that have the potential to be transmitted to humans.



Norwegian School of Veterinary Science









Norwegian School of Veterinary Science





Norwegian School of Veterinary Science

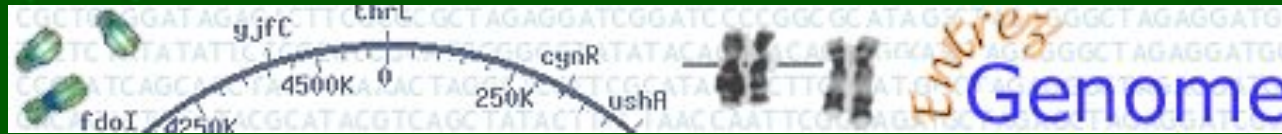




Artemia live food – good
but risk for parasites ?

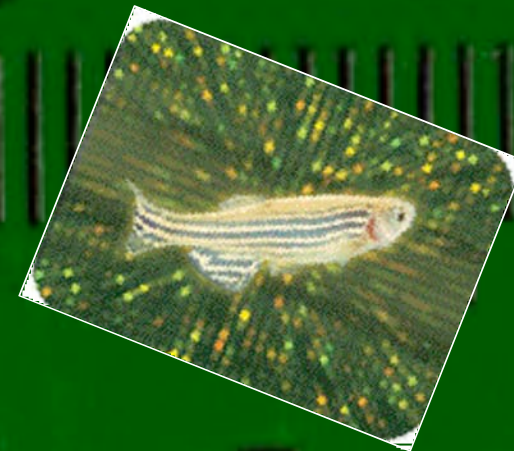






transgenics

somatic tissues – transient gene expression
 1-cell embryos – transgenic lines of zebrafish
 ES-cells for targeted mutagenesis (KO, gene replacement)



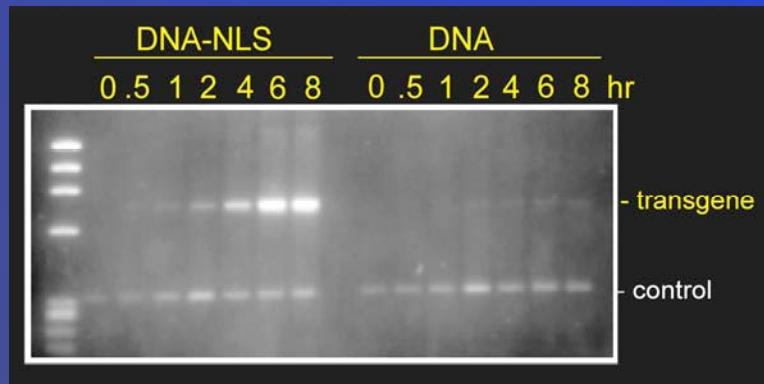
“GM light”

Intramuscular (i.m.)
injection or
Gene Gun (BioRad)
mediated biolistic
transfer

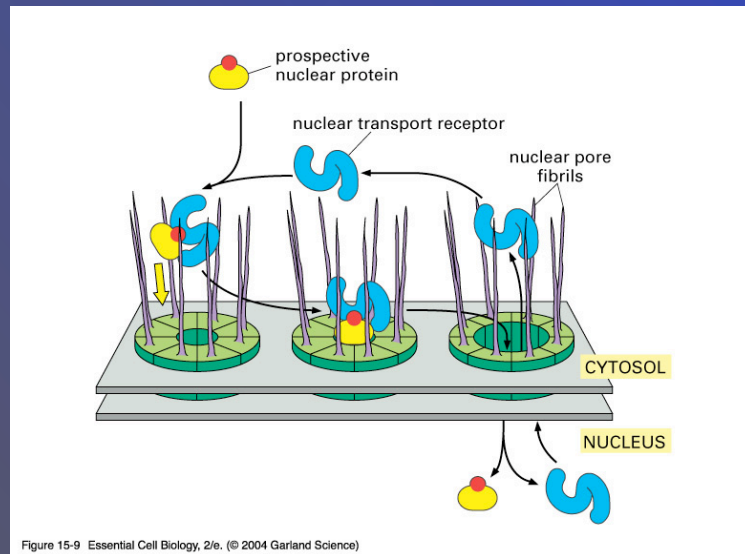
DNA vaccines

Gene therapy

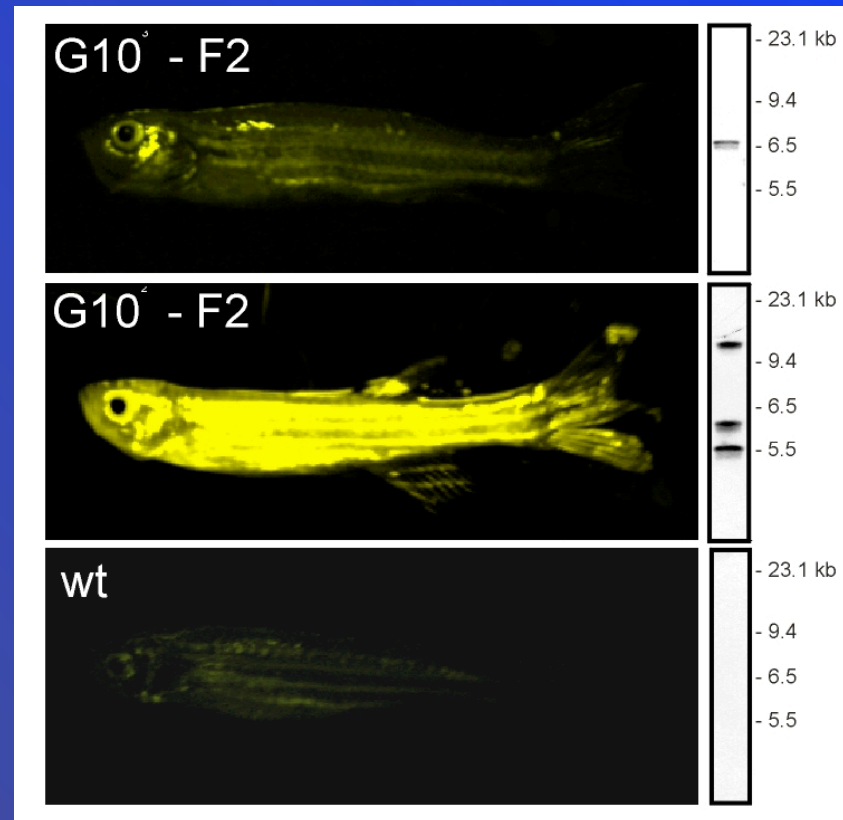




SV40 T-ag NLS peptide
CGGPKKKRKVG-NH2 mediated
 nuclear import of DNA



Glowing zebrafish expressing firefly luciferase reporter gene



Liang, M-r, Aleström, P. and Collas, P. 2000. Mol Reprod Dev 55:8-13

brain GnRH



pituitary
GTH

sex steroid hormones
& gonad development

Transgenic sterile fish

Inactivation of *gnrh*

Gene knock-down (anti-sense RNA)

Gene knock-out

Uzbekova S, Chyb J, Ferrière F, Bailhache T, Prunet P,
Aleström P, Breton B. 2000. *J Mol Endocrinol* 25:337-50

http://fugu.hgmp.mrc.ac.uk/ fugu genome

MRC HGMP-RC
The Fugu Genomics Project



The Wellcome Trust
Sanger Institute

ourGenome | Ensembl | Trace Server | Library
Infrastructure | HGP | CGP | **Projects** | Software | Teams | Search
Data Release Policy | Conditions of Use

The *Danio rerio* Sequencing Project

Fourth

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gene (fish) geno These betw evol navig

National Bio Resource Project - Medakafish Genome Project -

http://shigen.lab.nig.ac.jp/medaka/genome/top.jsp medaka.sci.hokudai.ac.jp/

NBRP Medakafish Genome Project



Salmon Genome Project - Netscape

http://www.salmongenome.no/cgi-bin/sgp.cgi

SGP Salmon Genome Project

Last Update
Aug. 25, 2004

Version 1.2.4

SGP Database

Database status :
Number of entries
Last update: 18-M

SGP Tools

Blast search

SGP Notes


Guest Bo

Tilapia

Introducti

Tilapia Genome Project

http://tilapia.unh.edu/WWWPages/TGP/TGP.html Zv4 assembly



Rainbow Trout Genome Project Update

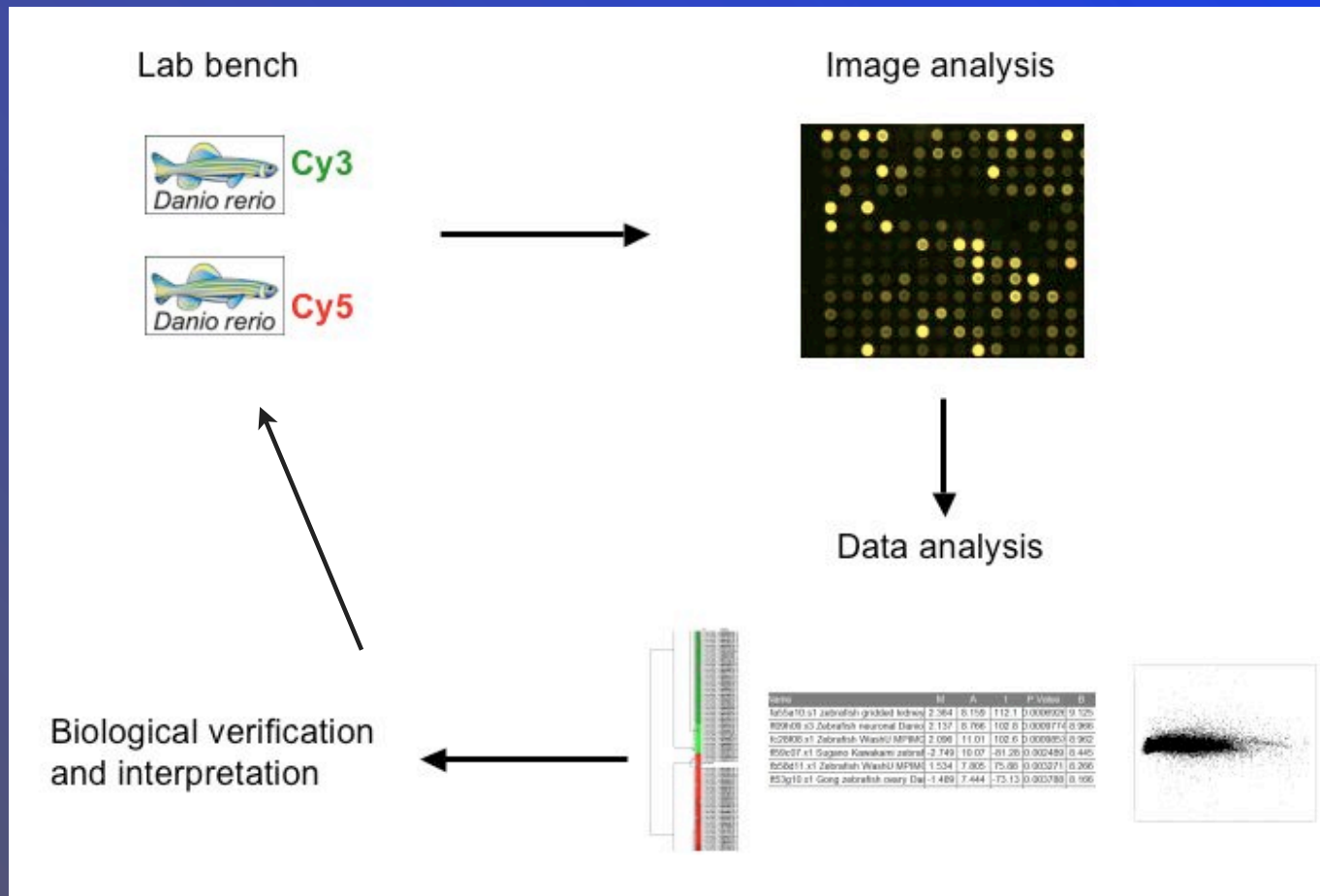
Michele C. McGuinness, PhD

global gene expression profiles

ES cell culture conditions
transgenics
environmental exposures



standardized system
nontreated fish constant



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ENFORM

European Network using Fish as Osteoporosis Research Models

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 AG [Alestrom](#), Oslo, Norway
 AG [Goerlich](#), Aachen, Germany


INDUSTRY
[Yeso](#), Oslo, Norway
[Lemnatec](#), Würselen, Germany
[OHB-System](#), Bremen, Germany



Oryzias latipes *Danio rerio*

Coordination by
 Prof. Dr. [Roland Goerlich](#), Institute of Biology, Molecular Biotechnology, RWTH Aachen, Worringerweg 1, 52074 Aachen, Germany
 Tel.: +(49) 241 – 8026521

ESA Microgravity Application Promotion Research Programme



PROBLEMS AND RATIONALE:

Bone loss affects astronauts at microG during space missions

Osteoporosis is a medical problem on Earth

Skeletal deformities is a problem for farmed fish

Osteoprotegerin (OPG) is a key factor in bone metabolism

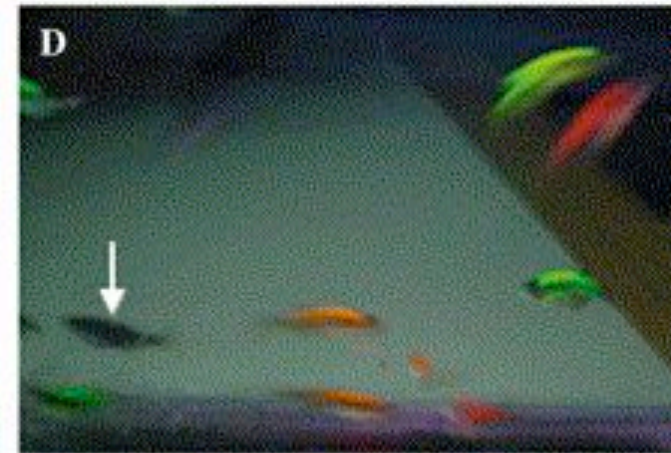
pet fish and toxicogenomics

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Research group:
[Biotechnology](#),
[Fish Biology](#)

Glofish-
World's first
genetically
engineered pet
made headlines
in 2003 >>[more](#)





Research

Articles

Published online: 22 July 2002, doi:10.1038/nbt721
August 2002 Volume 20 Number 8 pp 795 - 799

Cloned zebrafish by nuclear transfer from long-term-cultured cells

Ki-Young Lee^{1, 2}, Haigen Huang¹,
Bensheng Ju¹, Zhongan Yang¹ & Shuo Lin¹

1. Department of Molecular, Cellular, and Developmental Biology, University of California Los Angeles, Los Angeles, CA 90095-1606.

2. School of Agricultural Biotechnology, Seoul National University, Seoul, Korea, 151-742.

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FUGE 2004-2008 KNOCKOUT ZEBRAFISH (KO-ZFISH) MODEL

Alestrom Zebrafish Lab
Norwegian School of Veterinary Science
www.veths.no
aquamedicine.no



"KO-ZFISH" (FUGE 2004-2008)
Jethro L Holter
Rasoul Nourizadeh-Lillabadi
Inger-Marie Haaland, Jan Roger Torp

International
P. Collodi, L. Fan, Purdue University
ScanBalt Marine Biotech Network, ESMB
NoE Marine Genomics Europe

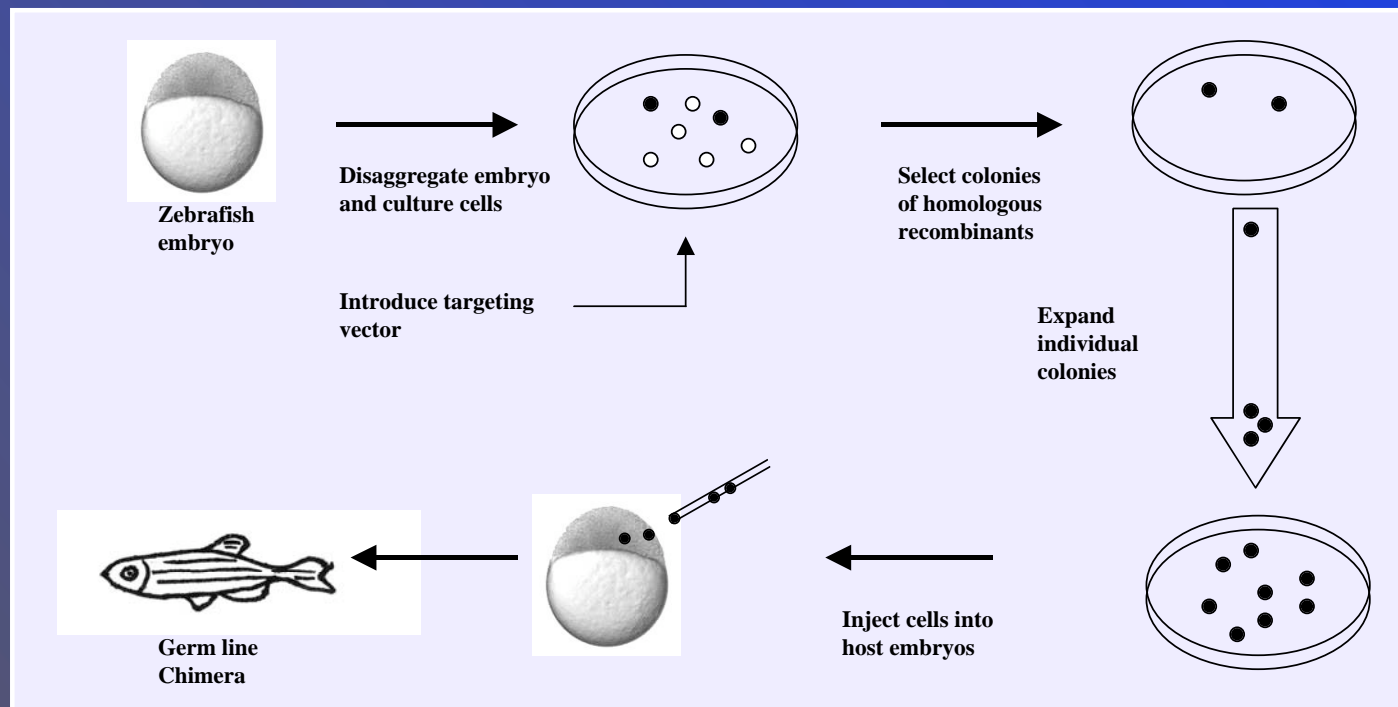
Other project areas
bone homeostasis in microgravity
toxicogenomics

WP1 KO-zfish
Zfish ES cells
Gene targeting

WP2 sterile zfish
gnrh KO zfish

WP3 TSE model (M Syed)
PrP transgenic zfish
PrP targeted mutagenese

FUGE Platforms
Norwegiam Microarray Consortium
Zebrafish platform, Sars Centre
Norwegian Transgenic Animal Forum



The new set-up in combination with our research area have motivated us to aim at:

The screenshot shows the AAALAC International website. At the top left is the AAALAC International logo, which includes a globe icon and the text "aaalac INTERNATIONAL". To the right of the logo is the full name "ASSOCIATION FOR ASSESSMENT AND ACCREDITATION OF LABORATORY ANIMAL CARE INTERNATIONAL". In the top right corner, there is a gold starburst graphic with the text "40 years of excellence in animal care 1965-2005". Below the logo and name is a navigation bar with "CONTACT" and "SEARCH:" followed by a search input field and a "GO" button. The main content area features a large background image of a person's hands holding a small white mouse. Overlaid on this image is the text "where science and responsible animal care connect". To the right of the mouse image is a text box that reads: "AAALAC International promotes the humane treatment of animals in science through accreditation and assessment programs. More than 670 institutions in 24 countries have earned AAALAC International accreditation." On the left side of the main content area is a vertical menu with the following items: "ABOUT AAALAC", "ACCREDITATION", "PROGRAM STATUS EVALUATION", "APPLY", "RESOURCES", "NEWS", and "PUBLICATIONS". Below the main content area is a "QUICK LINKS:" section with arrows pointing to "ACCREDITED ORGANIZATIONS", "MEMBERS ONLY", "REFERENCE RESOURCES", "PROGRAM DESCRIPTION", and "GLOBAL GATEWAY". At the bottom of the page, there is contact information for the USA office (11300 Rockville Pike, Suite 1211, Rockville, Maryland 20852 USA; t: 301-231-5353; f: 301-231-8282; email: accredit@aaalac.org) and the Europe office (Avenue de Tervuren 402, 1150 Brussels, Belgium; t: +32-2-761-66-78; f: +32-2-761-66-79; email: accredit_europe@aaalac.org). The footer also includes the copyright notice "© 2005 AAALAC International".

Alestrom Zebrafish Lab



Thank you for the attention