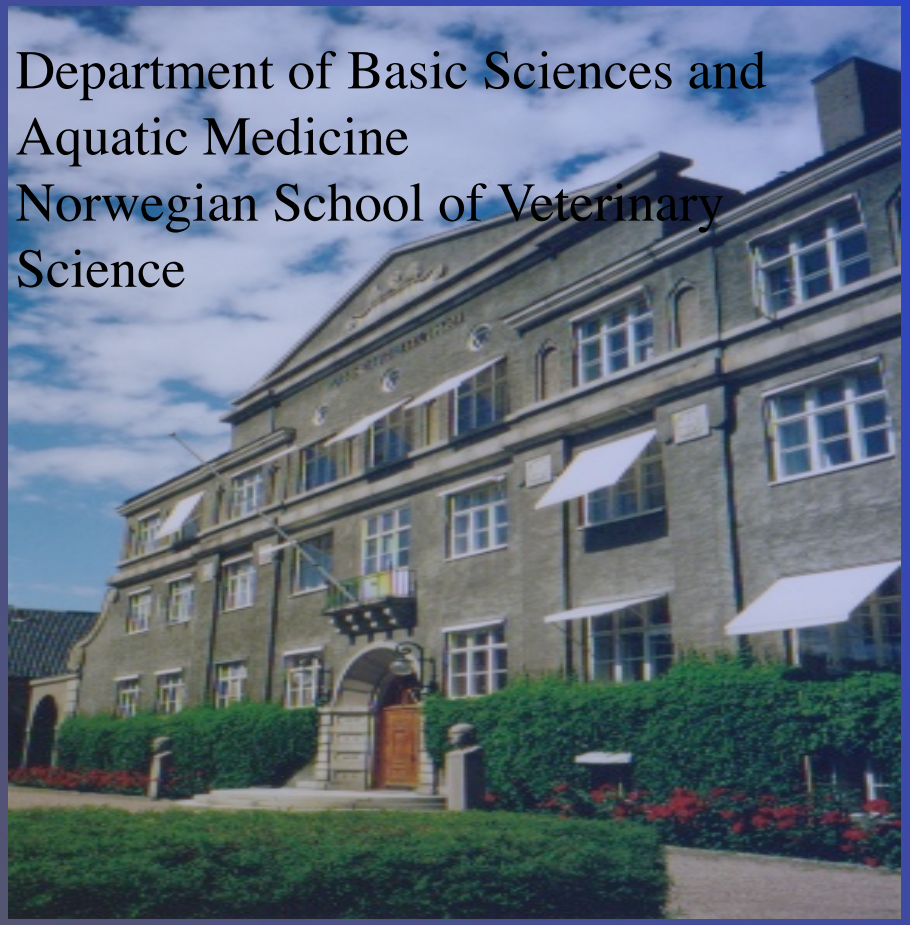
Transgenic Zebrafish Model
Professor Peter Alestrom



www.veths.no
aquamedicine.no (biochemistry)

The care and use of zebrafish in modern genomic research

Peter Alestrøm, Jethro Lee Holter, Rasoul Nourizadeh-Lillabadi,
Tom Ask, Inger Marie Haaland, Jan Roger Torp
Department of Basic Sciences and Aquatic Medicine
Norwegian School of Veterinary Science

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 then 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.

Aquamedicine.no

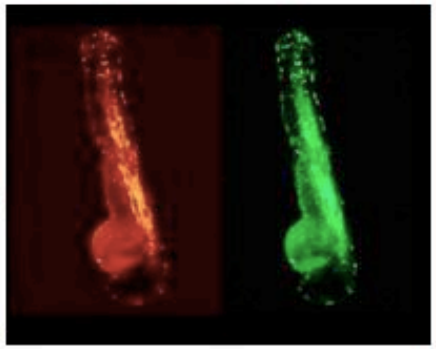
Norwegian School of Veterinary Science

Veterinærinstituttet
National Veterinary Institute

Transgenic fish

By Peter Alestrom, 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**

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 embyo 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

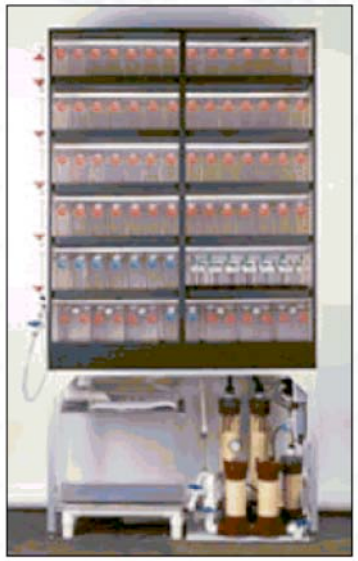
New zebrafish system

1. Breed our fish - genetically defined
2. Pathogen free fish? Chlorine bleech embyos
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

Systematic monitoring

ZEBRAFISH LABORATORY DAILY MONITORING FORM

Date:	Start Time:	Your Name:
Hands washed *(tick)*: ☐	**Time Artemia was prepared**	
	Morning:	Afternoon:

OBILGATORY DAILY DUTIES
Must be performed everyday on ALL water system
(Please tick boxes and complete fields when task is completed)

Feeding	Time	Outer room	Room 1				Room 2	Comments *(e.g. action taken/ treatment)*
		Nursery	System A	System B	System C	System D	System E	
Morning		☐	☐	☐	☐	☐	☐	
Noon		☐	☐	☐	☐	☐	☐	
Afternoon		☐	☐	☐	☐	☐	☐	
Water Quality Tasks/Checks								
10% Water Change		☐	☐	☐	☐	☐	☐	
Check UV-Lamp		N.A.	☐	☐	☐	☐	N.A.	
Check Pumps		☐	☐	☐	☐	☐	☐	
Check Carbon Filter		☐	☐	☐	☐	☐	☐	
Check Reservoir Filters		N.A.	☐	☐	☐	☐	N.A.	
Check Water Flow		☐	☐	☐	☐	☐	☐	
Check Fish Tank Filter Screens		N.A.	☐	☐	☐	☐	N.A.	
Check For Escaped Fish		☐	☐	☐	☐	☐	☐	
Cleaning/Disease Monitoring								
Check For Dead Fish (Log)		☐	☐	☐	☐	☐	☐	
Check For Signs Of Disease/Worms		☐	☐	☐	☐	☐	☐	
Check For Ill/Hurt Fish		☐	☐	☐	☐	☐	☐	
Check And Siphon Off Excess Debris		☐	☐	☐	☐	☐	☐	

OBILGATORY 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** ***(e.g. action taken)***
	System Water	**Nursery**	**System A**	**System B**	**System C**	**System D**	**System E**	
Temperature (°C)								
pH								
Nitrite Conc[0] (mg/ml)								
Nitrate Conc[1] (mg/ml)								
Ammonia Conc[2] (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):

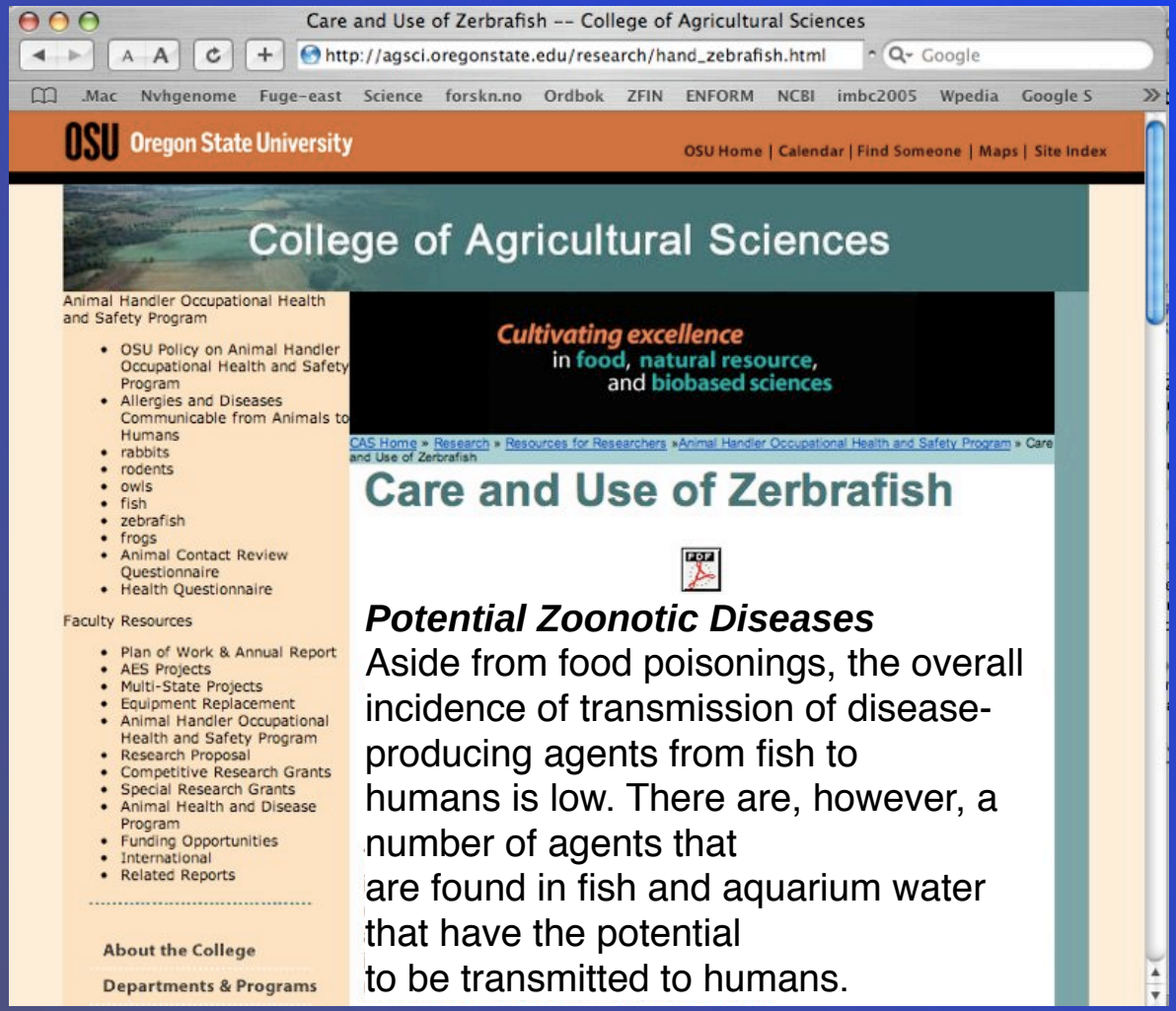
A comprehensive manual

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

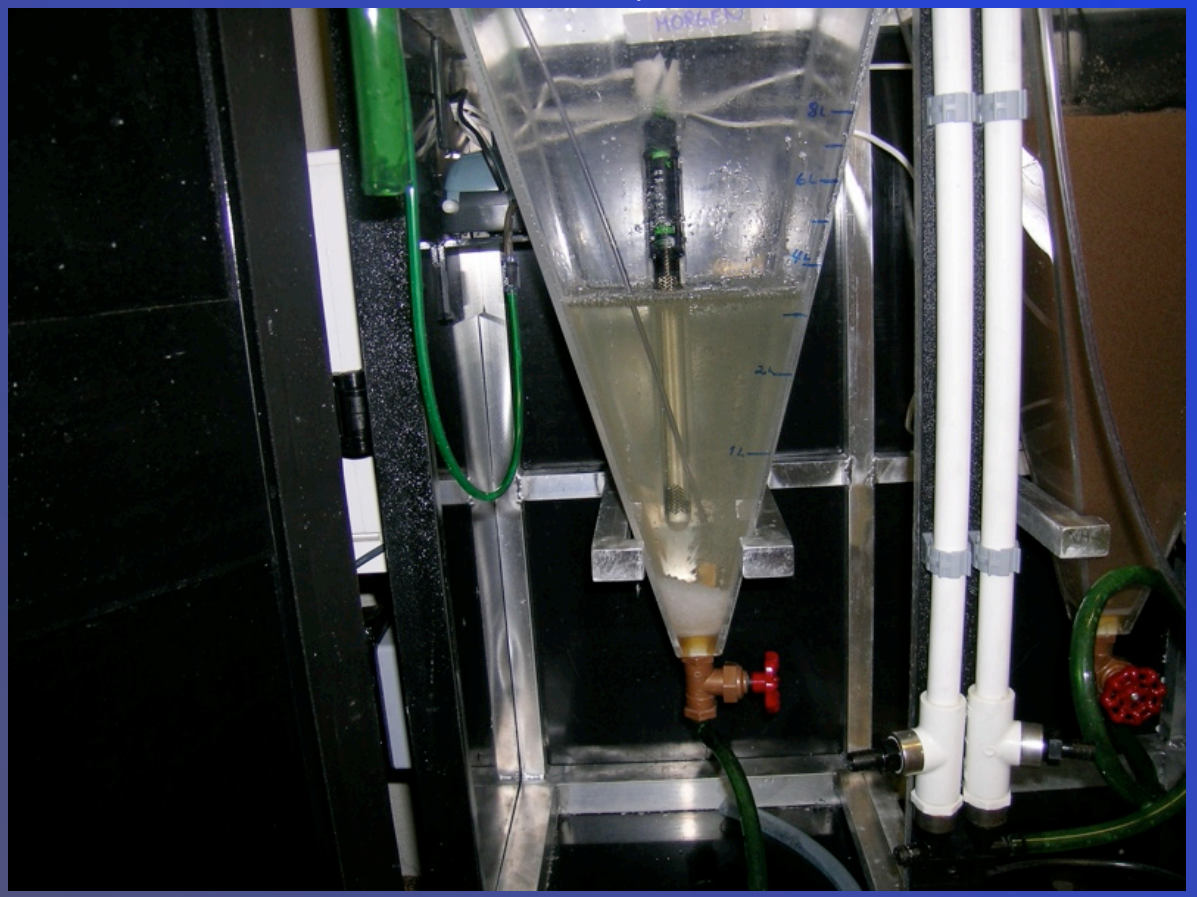



A4
LEVEL SWITCH


OPG
OPG
OPG
OPG
OPG
MARK
MARK
MARK


TVE

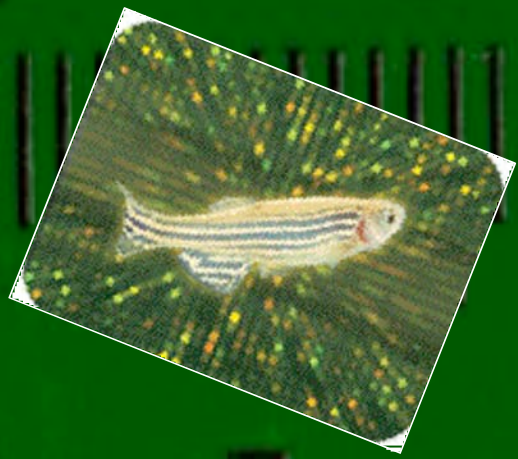
Arthemia live food - good but risk for parasites ?




TUE 7/12-04
CAMILLA
♂

transgenics

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

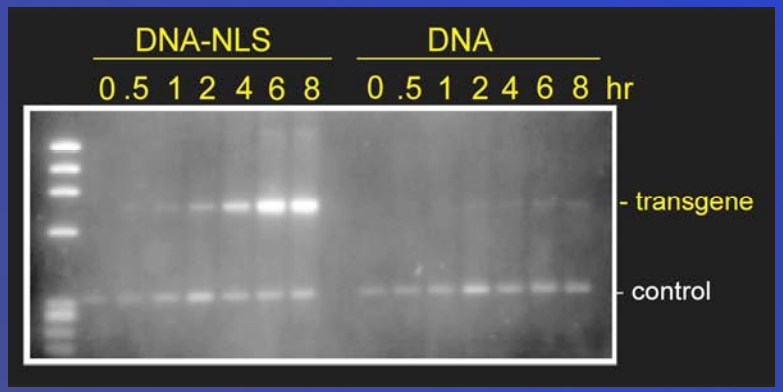


“GM light”

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

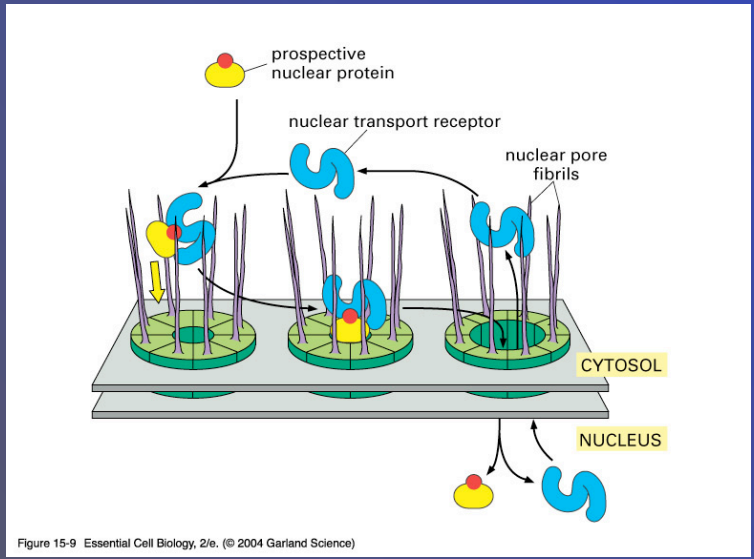
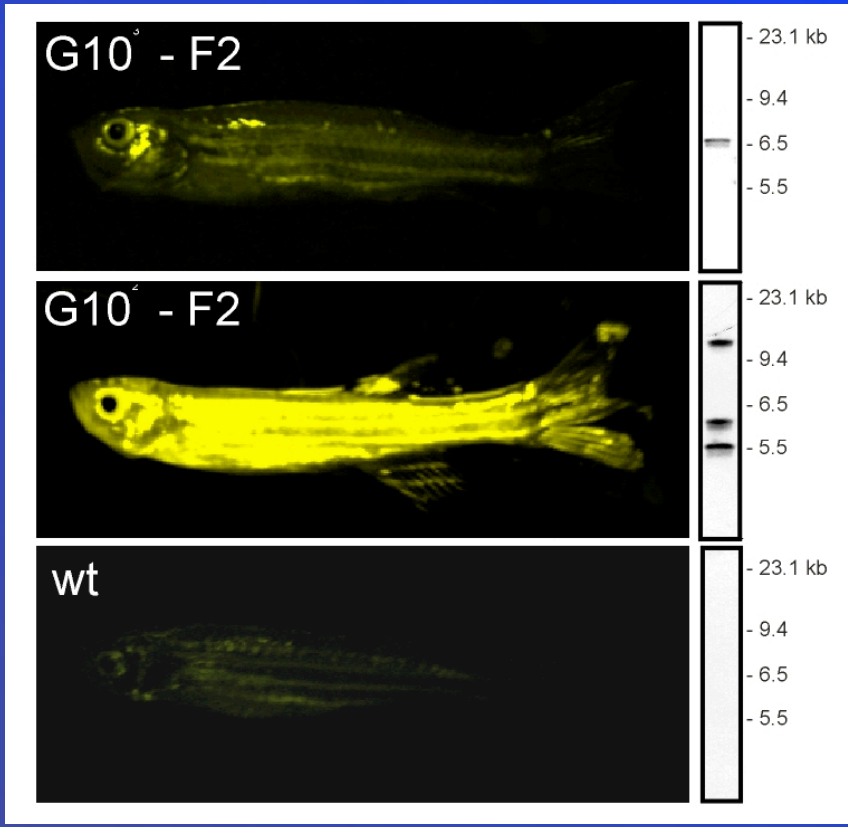
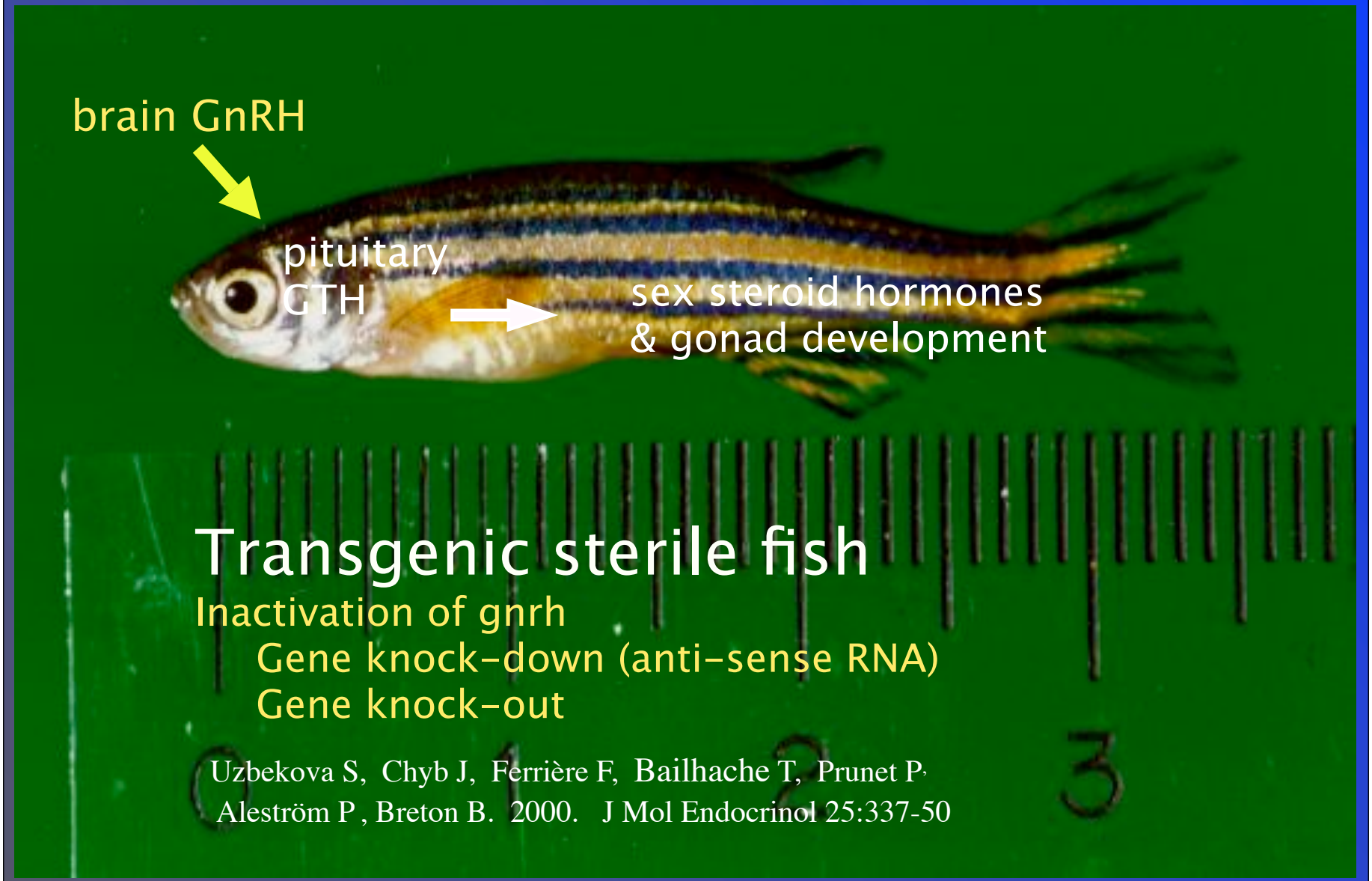


Figure 15-9 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Glowing zebrafish expressing firefly luciferase reporter gene



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

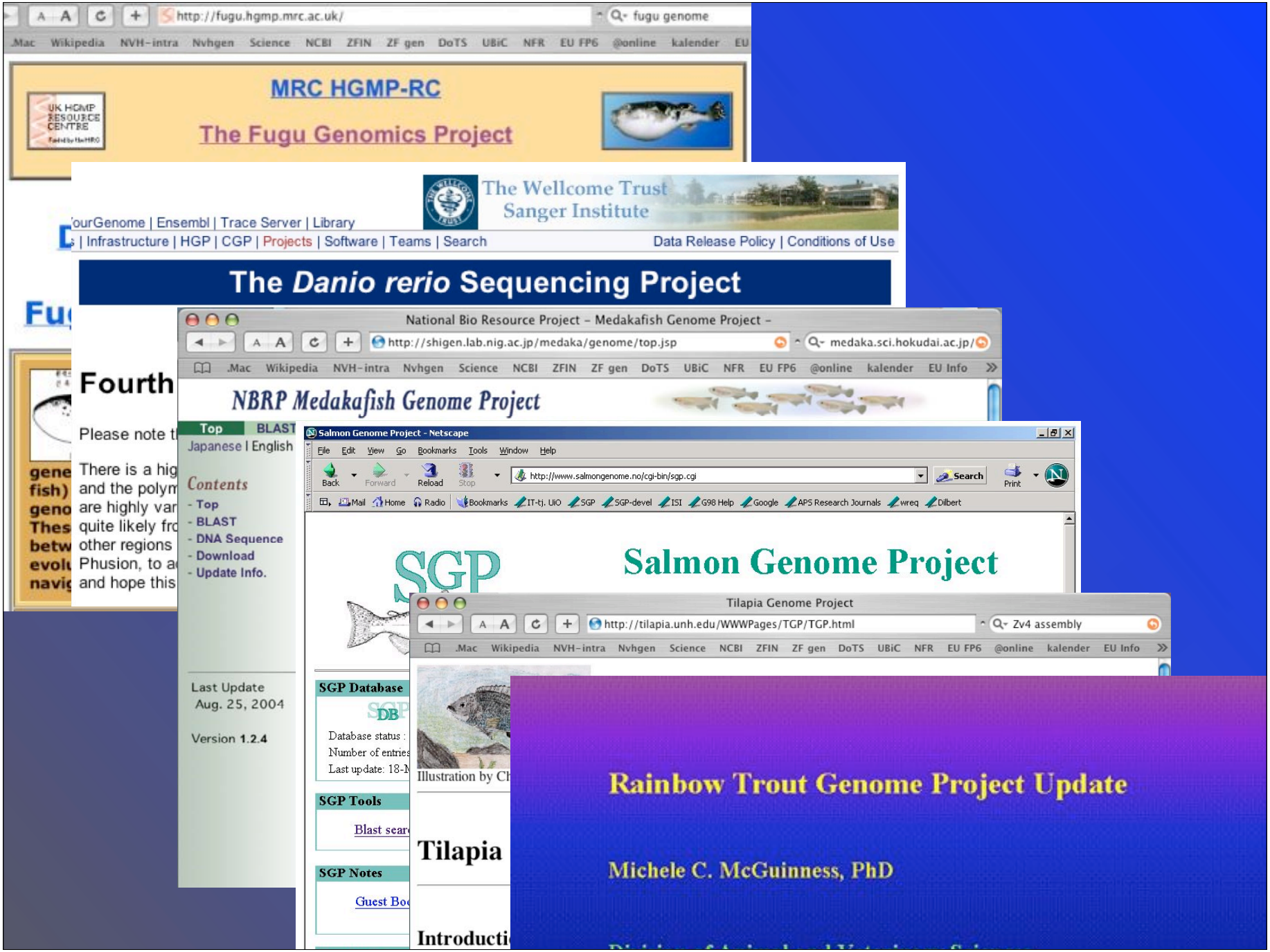


Transgenic sterile fish

Inactivation of gnrh

- Gene knock-down (anti-sense RNA)
- Gene knock-out

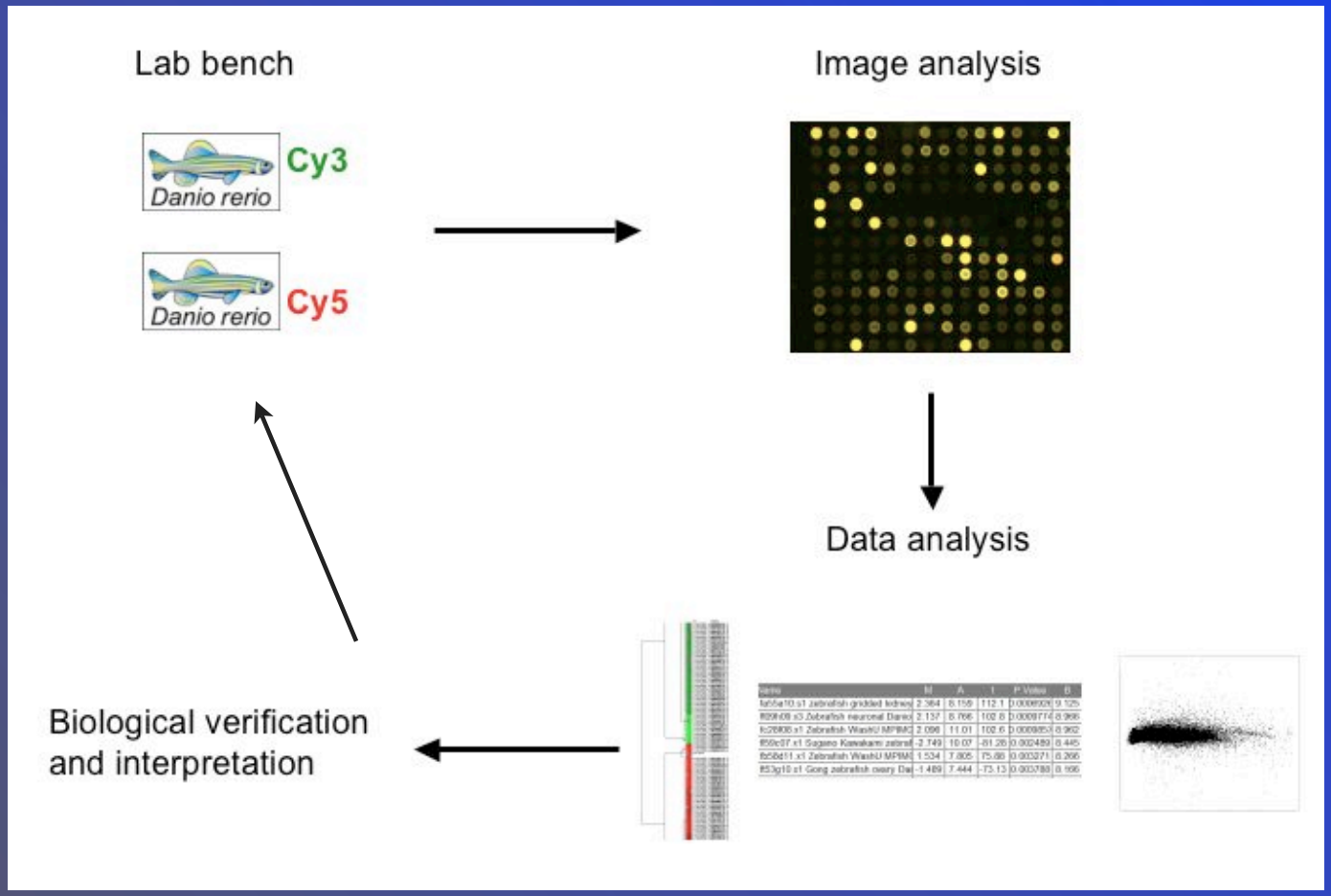
Uzbekova S, Chyb J, Ferrière F, Bailhache T, Prunet P, Alestrom P , Breton B. 2000. J Mol Endocrinol 25:337-50

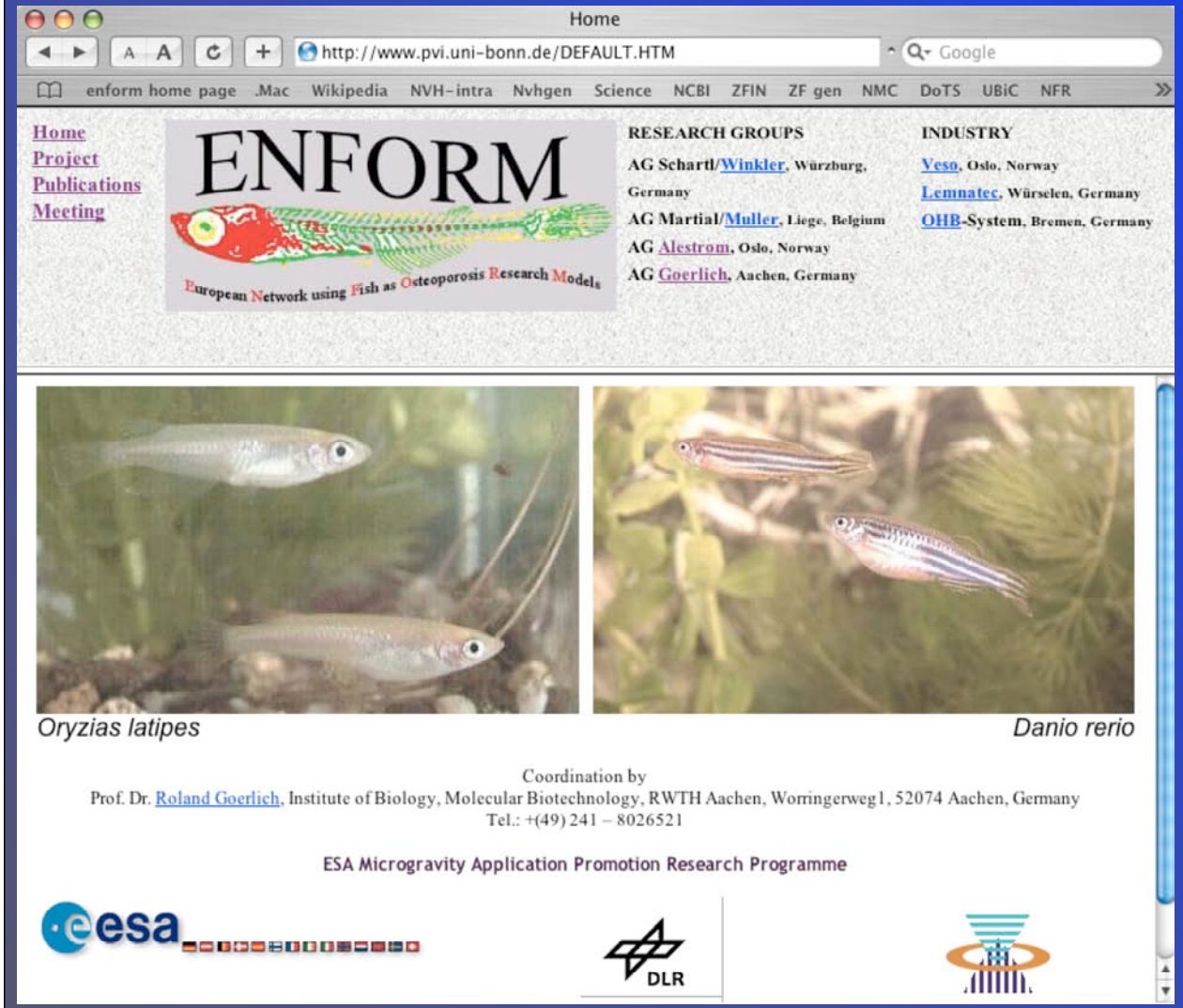

http://fugu.hgmp.mrc.ac.uk/
fugu genome
MRC HGMP-RC
The Fugu Genomics Project
The Wellcome Trust Sanger Institute
Data Release Policy | Conditions of Use
The Danio rerio Sequencing Project
National Bio Resource Project – Medakafish Genome Project –
http://shigen.lab.nig.ac.jp/medaka/genome/top.jsp
medaka.sci.hokudai.ac.jp/
NBRP Medakafish Genome Project
Salmon Genome Project - Netscape
http://www.salmongenome.no/cgi-bin/sgp.cgi
SGP
Salmon Genome Project
Tilapia Genome Project
http://tilapia.unh.edu/WWWPages/TGP/TGP.html
Zv4 assembly
Tilapia
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





PROBLEMS AND RATIONALE:

Bone loss affects astronauts at microG during space missions

Osteoporosis is a medical problem on Earth

Sceletal deformities is a problem for farmed fish

Osteoprotegerin (OPG) is a key factor in bone metabolism

pet fish and toxicogenomics

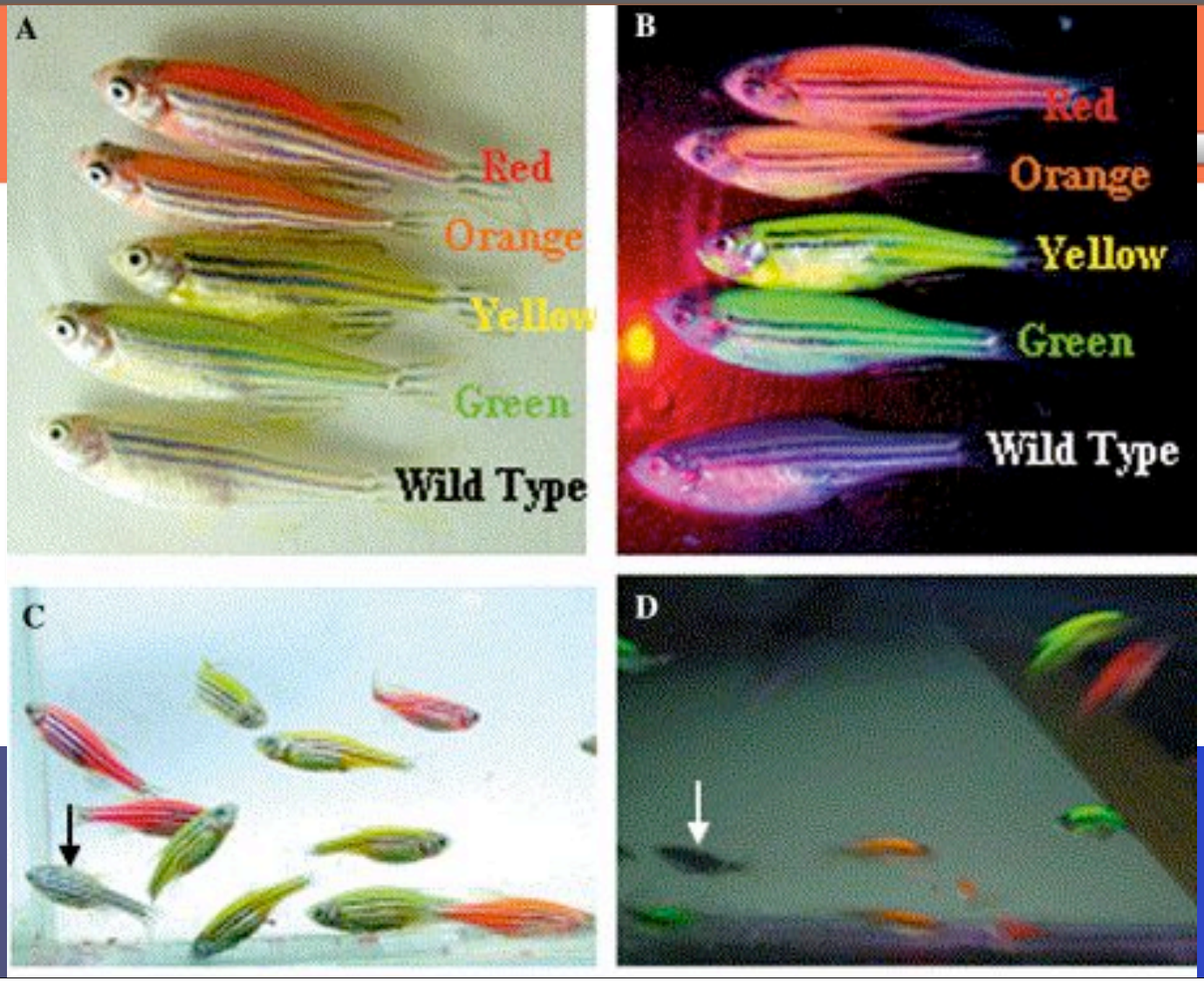
> Faculties & Courses > Sci

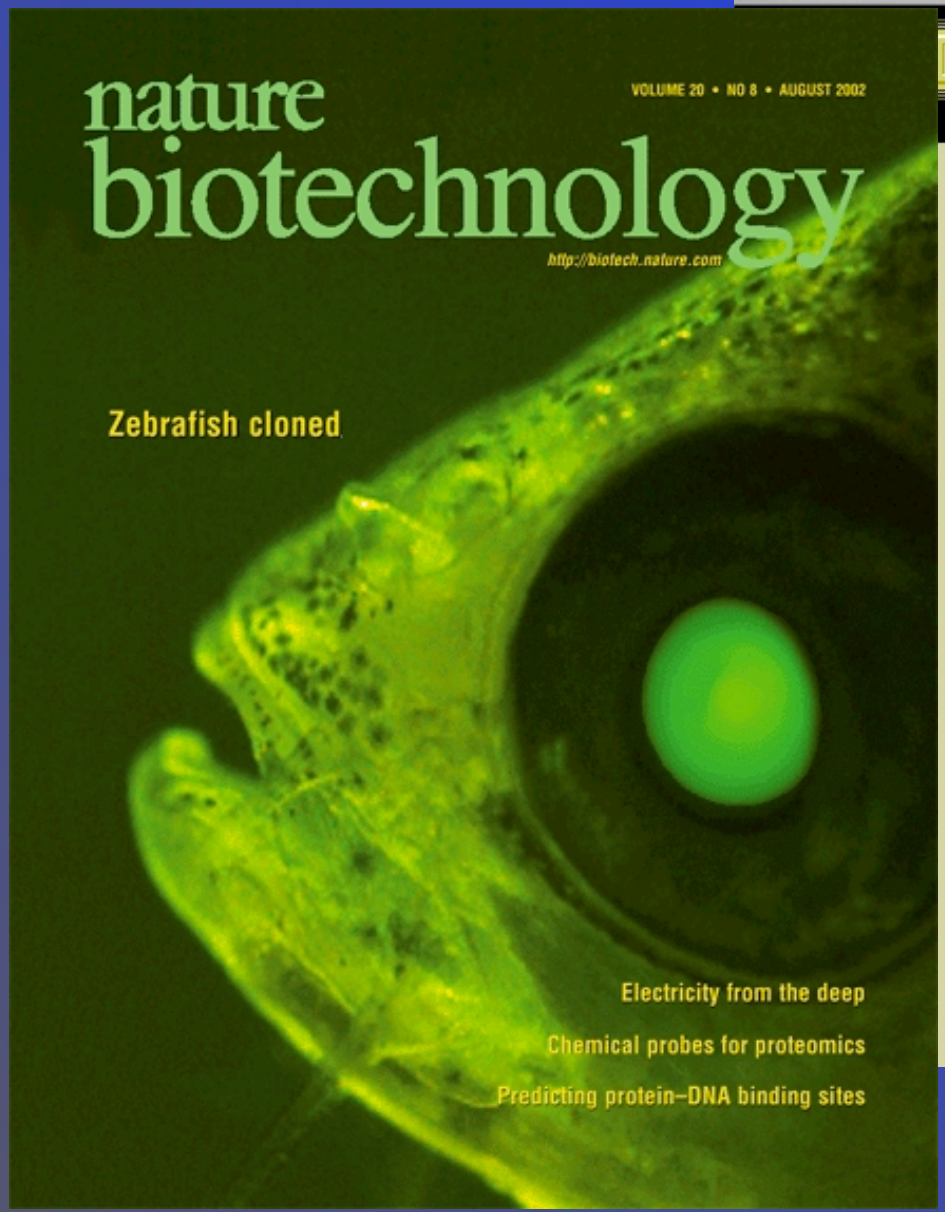
NUS National University of Singapore

Contact u

Research group: Biotechnology, Fish Biology

Glofish- World's first genetically engineered pet made headlines in 2003 >>more





http://www.nature.com/cgi-taf/DynaPage.taf?file=/nbt/

tlf.nr .Mac NVHmail NVH Science ZFIN ConSite NCBI EU Info EU FP6 NFR

ARCHIVE | SEARCH | INFORMATION | CLASSIFIED | SUBSCRIBE

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 Huang[1], Bensheng Ju[1], Zhongan Yang[1] & Shuo Lin[1]

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.
Correspondence should be addressed to S Lin. e-mail: shuolin@ucla.edu

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

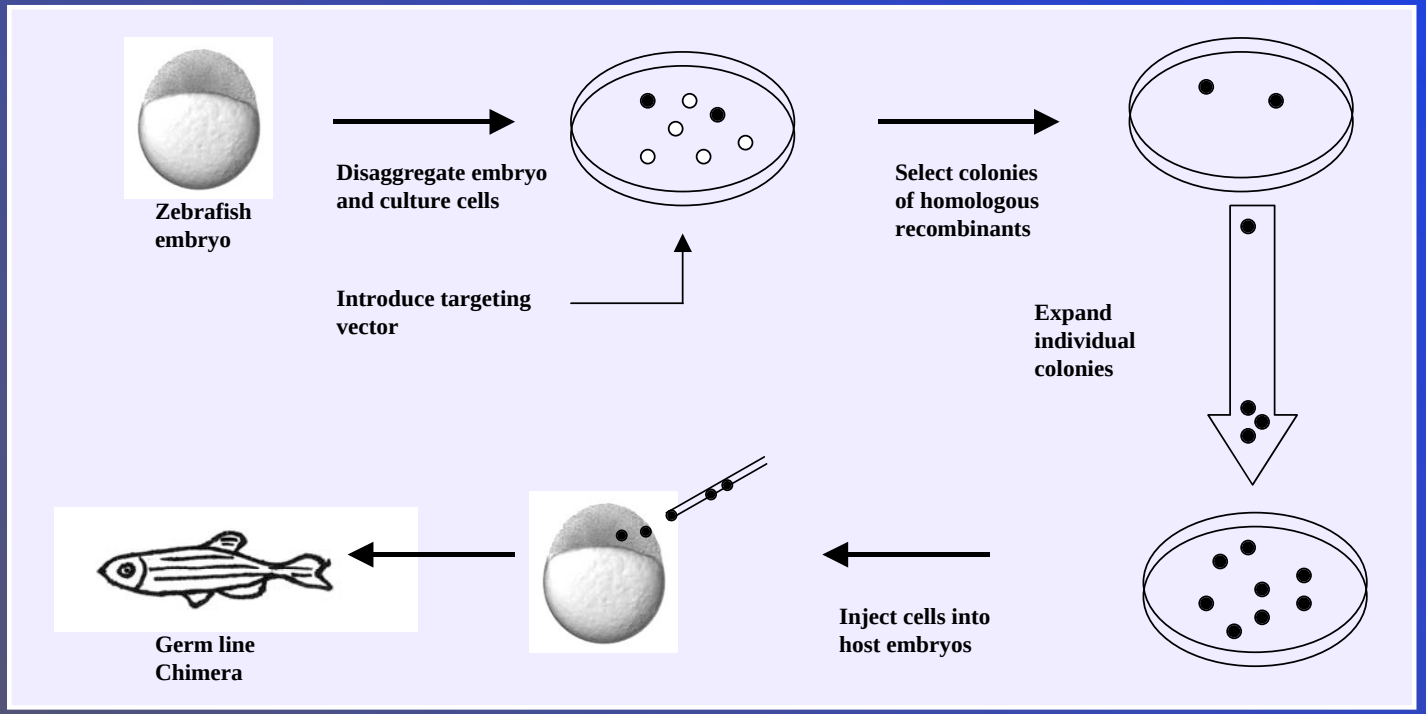
Other project areas
bone homeostasis in microgravity
toxicogenomics

WP1 KO-zfish
Zfish ES cells
Gene targeting

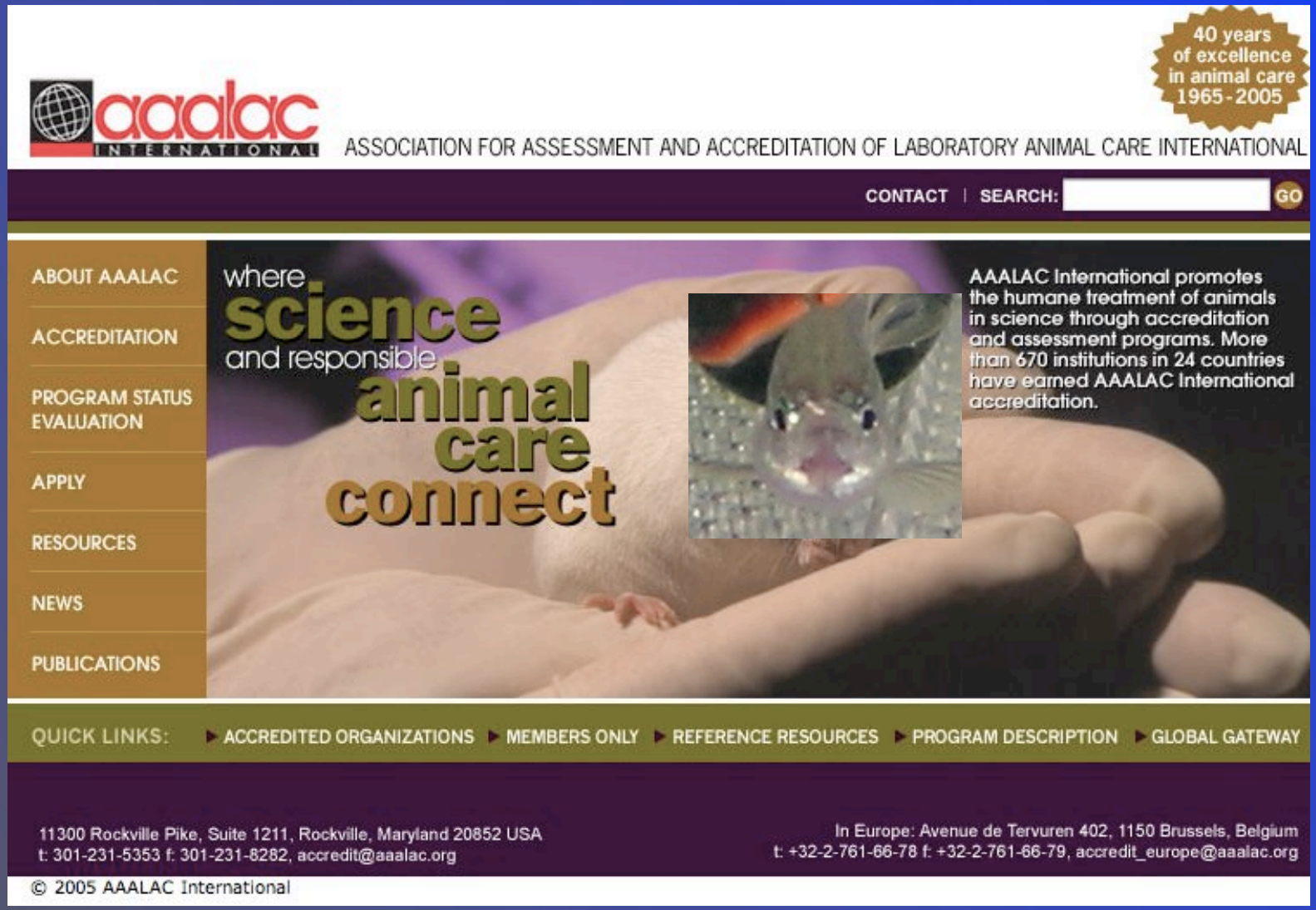
WP2 sterile zfish
gnrh KO zfish

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:



Alestrom Zebrafish Lab

Thank you for the attention

www.veths.no / aquamedicine.no / pabio.no

May 25, 2005