Alternative models (part II): zebrafish

- Interdisciplinary Technical Journal Club: special series on Laboratory Animal Science -

Silvia Sorce

4th October 2016

“After completing a training course, heads of animal facilities and experimenters must periodically take part in continuing education courses.
At least 4 training days are required per 4 years.”
https://www.blv.admin.ch/blv/en/home/tiere/tierversuche/aus-weiterbildung-tv.html

University of Zurich[UZH] | UniversitätsSpital Zürich | Institute of Neuropathology

Education and Training of Persons Conducting Animal Experiments

Interdisciplinary Technical Journal Club:
special series on Laboratory Animal Science recognized by the Veterinary Office of the Canton of Zurich

CONFIRMATION OF ATTENDANCE

Name: XXXX Surname: XXXXX

Date	Title	Speaker	Duration	Organizer signature
05.01.2016	Germ-free mice and the effect of microbiome on animal experiments	Dr. Silvia Sorce	1h	
02.02.2016	Successful replacement of animal models: cell-based assays	Dr. Asvin Lakkaraju	1h	
01.03.2016	Successful translation of basic research into clinical practice	Dr. Renier Myburgh	1h	
05.04.2016	Pitfalls in the use of experimental mice	Dr. Mario Nuvolone	1h	
03.05.2016	Successful replacement of animal models: production of antibodies	Dr. Assunta Senatore	1h	
07.06.2016	New methods for gene engineering in animals	Dr. Caihong Zhu	1h	

Organizers: Prof. A. Aguzzi, Dr. Silvia Sorce, Dr. Mario Nuvolone

6 hours
1 day

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CONFIRMATION OF ATTENDANCE

Name: XXXX Surname: XXXXX

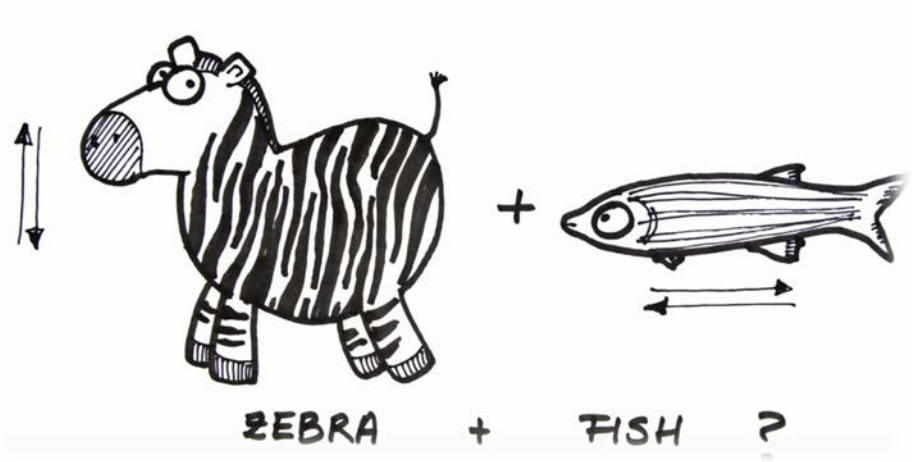
Date	Title	Speaker	Duration	Organizer signature
05.07.2016	Successful replacement of animal models: cell-based assays (part II)	Dr. Vijay Chandrasekar	1h	
02.08.2016	Longitudinal imaging in small animals	Dr. Regina Reimann	1h	
06.09.2016	Alternative models (part I): drosophila	Dr. Daniel Kirschenbaum	1h	
04.10.2016	Alternative models (part II): zebrafish	Dr. Silvia Sorce	1h	
01.11.2016	Alternative models (part III): yeast	Dr. Asvin Lakkaraju	1h	
06.12.2016	Alternative models (part IV): nematodes	Dr. Claudia Scheckel	1h	

Organizers: Prof. A. Aguzzi, Dr. Silvia Sorce, Dr. Mario Nuvolone

3 hours
1/2 day

Outline

- Introduction
- Zebrafish models of genetic diseases
- Zebrafish models of acquired disorders
- Zebrafish as tools for drug discovery



http://www.roche.com/research_and_development/drawn_to_science/zebrafish.htm

General information

The zebrafish (*Danio rerio*) is a tropical freshwater fish

Small fish (3–4 cm long as an adult), lifespan in captivity is around 2 to 3 years

Native to the Himalayan region: typical habitat of zebrafish in the wild shallow waters, e.g. rice fields

Zebrafish are available at pet stores throughout the world

Zebrafish International Resource Center (ZIRC):
http://www.zebrafish.org/home/guide.php
8 inbred strains, numerous mutants and outbred

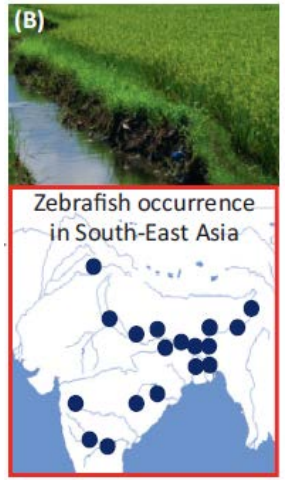
Easily maintained in 45 liter aquaria heated to 28.5C with 25 fish per tank

Adults should be fed 1-2 times per day with a variety of food

Although zebrafish reach sexual maturity in 10-12 weeks, the breeding fish should be between 7 and 18 months of age for maximum embryo production

Zebrafish are photoperiodic in their breeding, and produce embryos every morning, shortly after sunrise → Control the day-night cycle with an automatic timer (14 hr light/10 hr dark)

https://devbiootago.wordpress.com



Kalueff et al., 2014

https://www.nc3rs.org.uk/

Some historical notes

1960s : George Streisinger, Founding Father of Zebrafish Developmental and Genetic Research
As a fish hobbyist who knew how easy it was to raise and maintain zebrafish, he began using it as a model system.
→ Idea of applying mutational analysis to study zebrafish
→ aspired to unravel the genetic logic of neural development in a vertebrate

!! Little appreciation of the degree to which
vastly divergent species would share regulatory pathways

1980s : establishment of a research community focused on developmental and genetic studies with the zebrafish → especially neurobiologists interested in observing neurite outgrowth

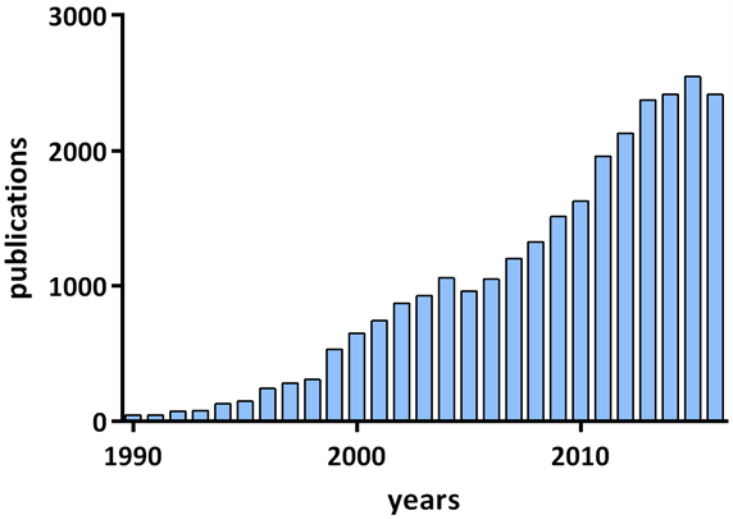
1990s : «Big Screen» for embryonic mutants is performed in parallel in Tübingen and Boston

Christiane Nüsslein-Volhard

Marc Fishman

Wolfgang Driever

Zebrafish in Pubmed



L'ordonnance sur la protection des animaux OPAn

Tierschutzverordnung TschV

Définit le champ d'application de la loi sur l'expérimentation animale :

Anwendungsbereiche des Gesetze:

- ✓ aux vertébrés;
- ✓ aux décapodes marcheurs et aux céphalopodes;
- ✓ aux mammifères, aux oiseaux et aux reptiles dès le dernier tiers de leur gestation ou de leur développement avant éclosion;
- ✓ aux stades larvaires des poissons et des amphibiens qui se nourrissent par eux-mêmes.

- ✓ Wirbeltiere;
- ✓ Panzerkrebse und Kopffüsser;
- ✓ Säugetiere, Vögel und Reptilien im letzten Drittel der Entwicklungszeit vor der Geburt oder dem Schlüpfen;
- ✓ Larvenstadien von Fischen und Amphibiens, die frei Futter aufnehmen.

https://www.admin.ch/opc/fr/classified-compilation/20080796/index.html

Anzahl Tiere nach Tierart und Verwendungszweck

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

	Grundlagenforschung	Entdeckung, Entwicklung und Qualitätskontrolle	Krankheitsdiagnostik	Bildung und Ausbildung	Schutz von Mensch, Tier und Umwelt	Anderer Zusammenhang	Total 2015	Veränderung 2014 – 2015
Mäuse	324'433	77'798	303	3'576	4'575	1'856	412'541	+ 5.7 %
Ratten	24'692	48'252	505	1'717	2'962	6	78'134	-5.9 %
Hamster		101	38				139	-44.2 %
Meerschweinchen	38	492	185	16	44	18	793	-18.5 %
Andere Nager	154	2'248	116				2'518	+ 3.2 %
Kaninchen	445	143	4	29		36	657	-30.9 %
Hunde	1'222	756	162	258	34	527	2'959	-10 %
Katzen	164	261	73	42		81	621	-21.2 %
Primaten	76	103		11	8		198	-21.1 %
Rindvieh	749	327	208	1'341	36	798	3'459	-32.6 %
Schafe, Ziegen	162	162	402	17		504	1'247	-16.6 %
Schweine	2'104	224	319	113	26	1'028	3'814	-17.6 %
Pferde, Esel	418	55	173	200		491	1'337	-6.5 %
Diverse Säuger	2'977					109	3'086	+ 131.9 %
Vögel (inkl. Geflügel)	17'041	94	4	64		57'214	74'417	+ 19.1 %
Amphibien, Reptilien	30'073		126	52		2'102	32'353	+ 337.2 %
Fische	46'035		1'133	710	4'094	11'716	63'688	+ 59.7 %
Wirbellose	372						372	-40.4 %
Total	**451'155**	**131'016**	**3'751**	**8'146**	**11'779**	**76'486**	**682'333**	**+ 12.5 %**
2014	361'463	134'836	6'728	9'426	21'754	72'298	606'505	
Differenz in %	+ 24.8 %	-2.8 %	-44.2 %	-13.6 %	-45.9 %	+ 5.8 %		

http://tv-statistik.ch/fr/statistique-simples/index.php#a2

Anzahl Tiere nach Tierart und Schweregrad

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

	Schweregrad 0		Schweregrad 1		Schweregrad 2		Schweregrad 3		Total	Veränderung 2015 – 2014
Mäuse	116'938	28.3 %	163'108	39.5 %	121'934	29.6 %	10'561	2.6 %	412'541	+ 5.7 %
Ratten	40'742	52.1 %	19'144	24.5 %	16'458	21.1 %	1'790	2.3 %	78'134	-5.9 %
Hamster	56	40.3 %	35	25.2 %	44	31.7 %	4	2.9 %	139	-44.2 %
Meerschweinchen	214	27 %	512	64.6 %	63	7.9 %	4	0.5 %	793	-18.5 %
Andere Nager	16	0.6 %	1'837	73 %	506	20.1 %	159	6.3 %	2'518	+ 3.2 %
Kaninchen	138	21 %	217	33 %	294	44.7 %	8	1.2 %	657	-30.9 %
Hunde	2'149	72.6 %	546	18.5 %	262	8.9 %	2	0.1 %	2'959	-10 %
Katzen	323	52 %	222	35.7 %	75	12.1 %	1	0.2 %	621	-21.2 %
Primaten	98	49.5 %	77	38.9 %	23	11.6 %			198	-21.1 %
Rindvieh	1'660	48 %	1'605	46.4 %	194	5.6 %			3'459	-32.6 %
Schafe, Ziegen	911	73.1 %	163	13.1 %	158	12.7 %	15	1.2 %	1'247	-16.6 %
Schweine	2'752	72.2 %	925	24.3 %	137	3.6 %			3'814	-17.6 %
Pferde, Esel	1'007	75.3 %	330	24.7 %					1'337	-6.5 %
Diverse Säuger	2'121	68.7 %	938	30.4 %			27	0.9 %	3'086	+ 131.9 %
Vögel (inkl. Geflügel)	68'313	91.8 %	5'714	7.7 %	385	0.5 %	5	0 %	74'417	+ 19.1 %
Amphibien, Reptilien	23'349	72.2 %	8'985	27.8 %	19	0.1 %			32'353	+ 337.2 %
Fische	31'494	49.5 %	27'770	43.6 %	2'765	4.3 %	1'659	2.6 %	63'688	+ 59.7 %
Wirbellose	372	100 %							372	-40.4 %
Total	**292'653**	**42.9 %**	**232'128**	**34 %**	**143'317**	**21 %**	**14'235**	**2.1 %**	**682'333**	**+ 12.5 %**
2014	253'714		215'521		124'985		12'285			
Differenz in %		+ 15.3 %		+ 7.7 %		+ 14.7 %		+ 15.9 %		

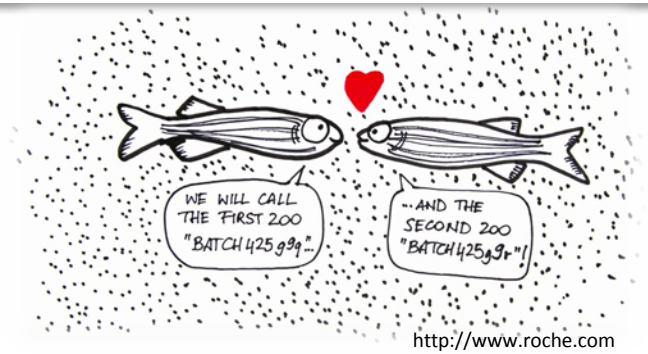
http://tv-statistik.ch/fr/statistique-simples/index.php#a2

Positives aspects of using zebrafish

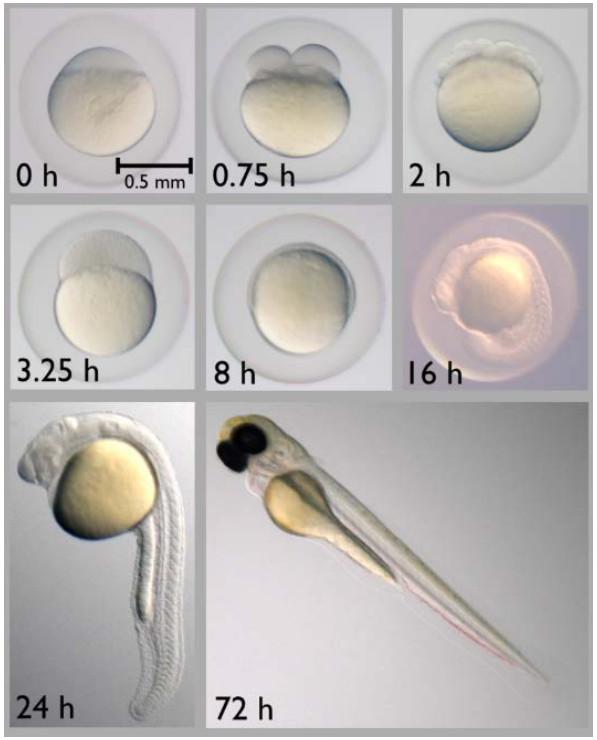
High fecundity: A single adult mating pair can produce 200 embryos or more per week

Transparent embryos that develop outside the mother: Embryonic phenotypes are strongly predictive of adult phenotypes in most organs, allowing for the screening of relevant adult phenotypes using space-efficient embryos

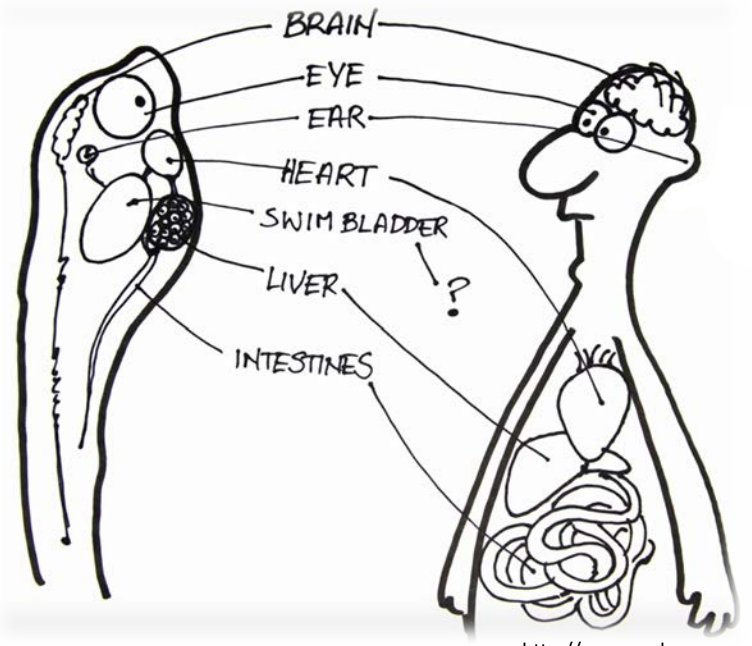
Conservation of vertebrate organs: zebrafish share nearly all organs with mammals, including the brain, eyes, heart, intestines and liver



http://www.roche.com



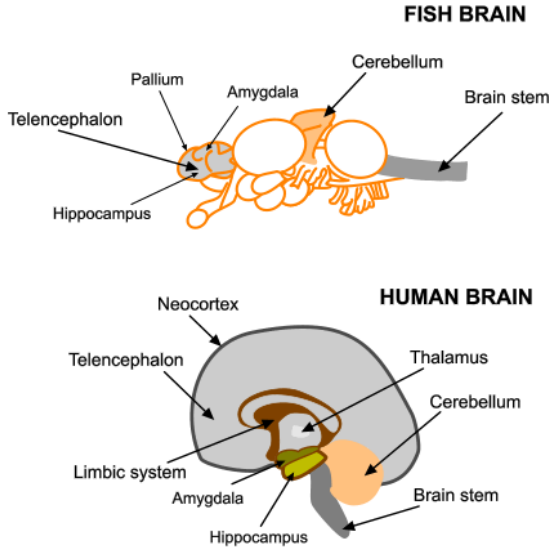
https://en.wikipedia.org/wiki/Zebrafish



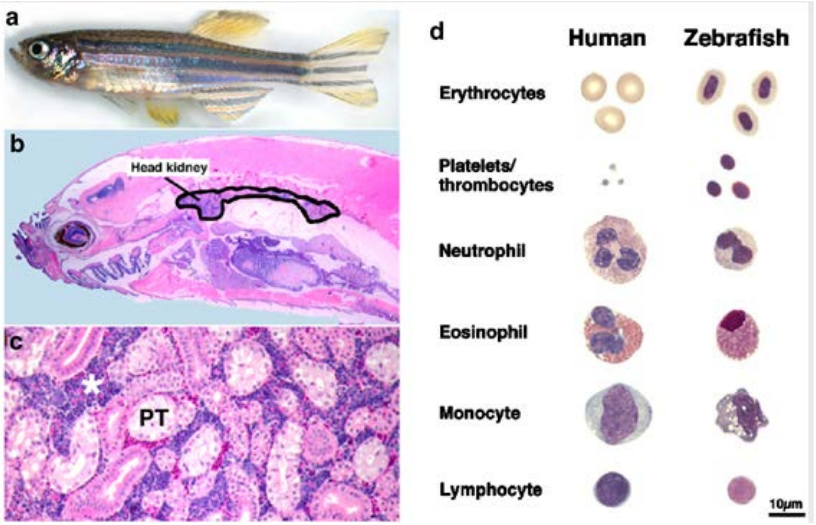
http://www.roche.com

Table 2 | **Comparative zebrafish biology for modelling human disease**

Characteristics	Key similarities to humans	Key differences and unknowns
Nervous system and behaviour	Representative anatomy: fore-, mid- and hind-brain, including diencephalon, telencephalon and cerebellum; peripheral nervous system with motor and sensory components; enteric and autonomic nervous systems; specialized sensory organs: eye, olfactory system and ear; exhibit 'higher' behaviours and integrated neural function: memory, conditioned responses and social behaviours (for example, schooling)	Telencephalon has only a rudimentary cortex; fish-specific sensory organs, such as the lateral line; fish behaviours and cognitive function are abstracted or simplified compared with human behaviour
Haematopoietic and lymphoid/ immune systems	Multiple haematopoietic cell types: erythrocytes, myeloid cells (neutrophils, eosinophils, monocytes and macrophages), T- and B-lymphocytes; coagulation cascade for haemostasis; innate and adaptive humoral and cellular immunity	Erythrocytes are nucleated; possess thrombocytes rather than platelets; kidney interstitium is the haematopoietic site; details of humoral regulation of haematopoiesis are largely unknown; could have evolved fish-specific immune system components (for example, a family of immune receptors)



http://www.fishpain.com/fish-and-pain-brain-structures.htm



Davidson and Zon, 2004

Lieschke and Currie, *Nat Rev Genet* 2007

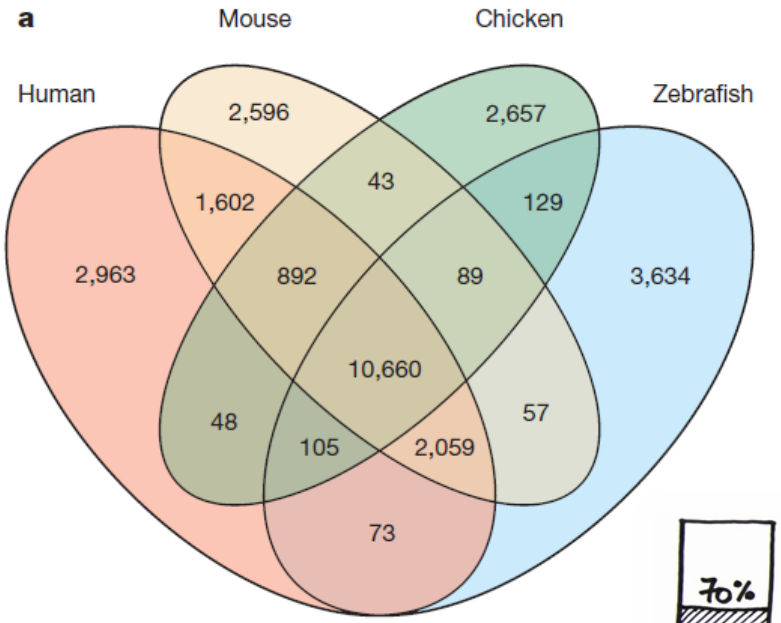
LETTER

OPEN

doi:10.1038/nature12111

The zebrafish reference genome sequence and its relationship to the human genome

498 | NATURE | VOL 496 | 25 APRIL 2013



http://www.roche.com

→ 70% of human genes have at least one obvious zebrafish orthologue

→ 82% of human morbid genes can be related to at least one zebrafish orthologue

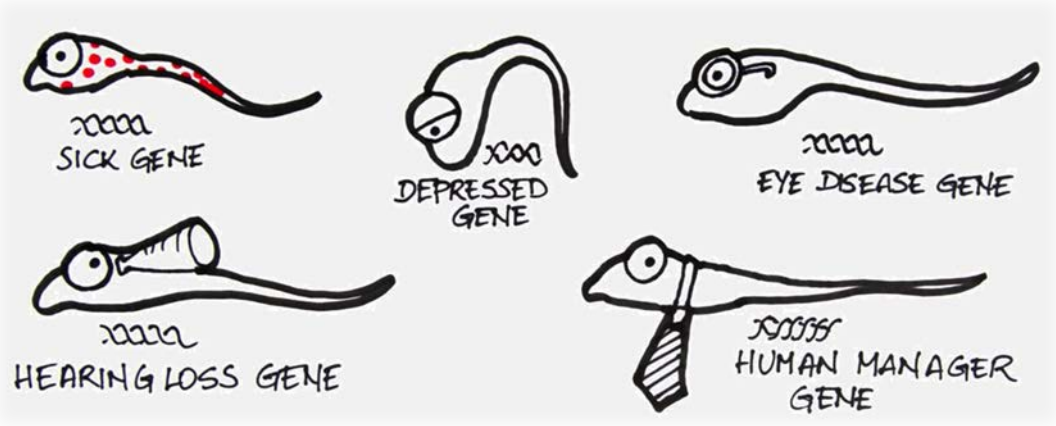
Table 1 | **Attributes of some key animals used to model human disease**

Attribute of disease model	Model organism			
	Fly	*Zebrafish*	*Mouse*	*Rat*
Practical issues				
Husbandry infrastructure	$	$	$$$	$$$
Cost per animal per year	$	$	$$$	$$$
Characterized inbred strains	+	-	++++	+++
Outbred laboratory strains	+	+++	++	++
Anatomical similarity	-	+	++	++
Molecular or genetic similarity	+	++	+++	+++
Pathological similarity	-	++	+++	+++
Storage; for example, freezing sperm	No	Yes	Yes	Yes
Molecular biology tools				
Transgenesis*	++	++	++	++
Targeted gene modification*	+	-	++++	+
Transient *in vivo* assays*	++	++++	+	+
Allelic series from TILLING*	+++	++++	++	+
Feasibility of large-scale screens‡	++++	+++	++	+
Affordability of large-scale screens‡	++++	+++	+	-
Sequencing progress§	+++	++	+++	++
Annotation progress§	++	++	++++	++
Cell-biology tools				
Cell lines and tissue culture	++	+	++++	+
Antibody reagents	++	+	++++	++

*Reverse-genetics approach; ‡forward-genetics approach; §genome sequence; -, not relevant, or not a strength; $, $$, $$$ and +, ++, +++, relative cost ($) and strength (+) of the model in each category; ++++, outstanding strength of the model; TILLING, targeting induced local lesions in genomes.

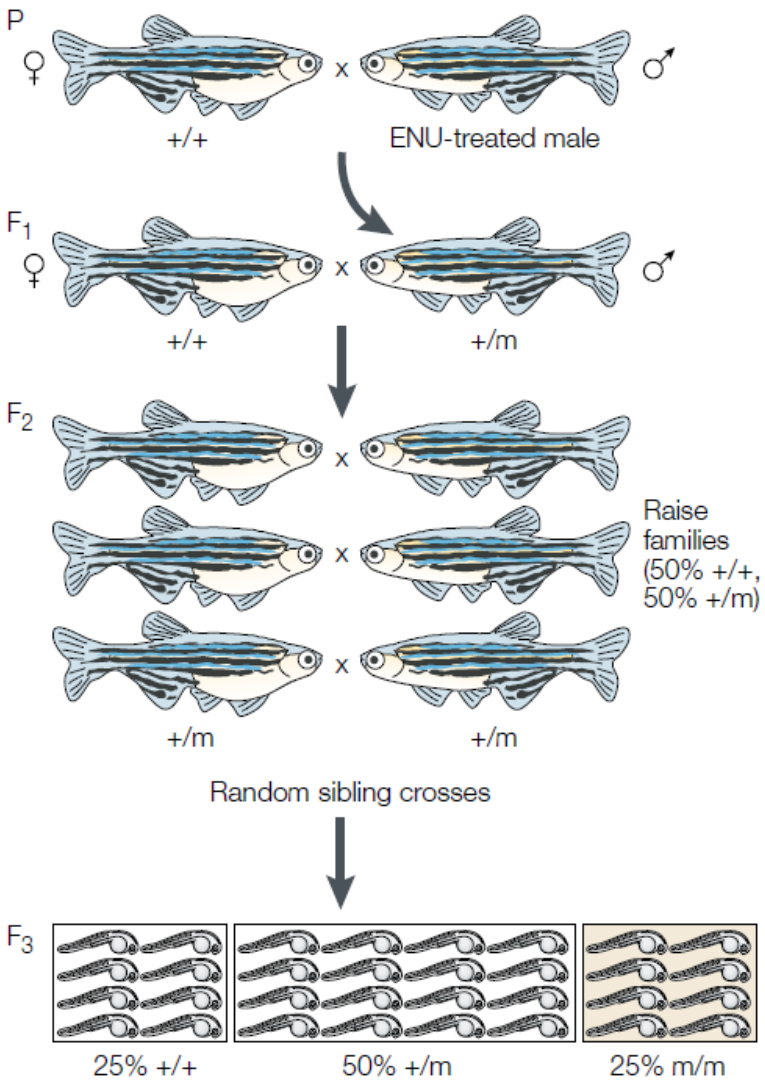
Lieschke and Currie, *Nat Rev Genet* 2007

Zebrafish models of genetic disorders



http://www.roche.com

Forward-genetic screens



1. ethylnitrosourea (ENU), is used to generate hundreds of point mutations in the male premeiotic germ cells (spermatogonia)
ENU-treated males are crossed to wild-type females to produce the F1 heterozygous progeny

2. F1 fish are then crossed to create F2 families, half of which are genotypically heterozygous for a specific mutation (m), whereas the other half are wild type

3. F2 siblings are crossed, and the resulting F3 progeny are 25% wild type (+/+), 50% heterozygous (+/m) and 25% homozygous (m/m) for a recessive mutation

Together, the Boston and Tübingen screens, starting from about 300 ENU founder males, involved raising more than 5,000 F2 families, analysing more than 6,000 mutagenized genomes and selecting more than 2,000 new developmental mutants for characterization

Patton and Zon, *Nat Rev Genet* 2001

Development 123, 1-36
Printed in Great Britain © The Company of Biologists Limited 1996
DEV3343

The identification of genes with unique and essential functions in the development of the zebrafish, *Danio rerio*

Pascal Haffter, Michael Granato‡, Michael Brand†, Mary C. Mullins‡, Matthias Hammerschmidt§, Donald A. Kane¶, Jörg Odenthal, Fredericus J. M. van Eeden, Yun-Jin Jiang, Carl-Philipp Heisenberg, Robert N. Kelsh¶, Makoto Furutani-Seiki, Elisabeth Vogelsang, Dirk Beuchle††, Ursula Schach, Cosima Fabian and Christiane Nüsslein-Volhard***

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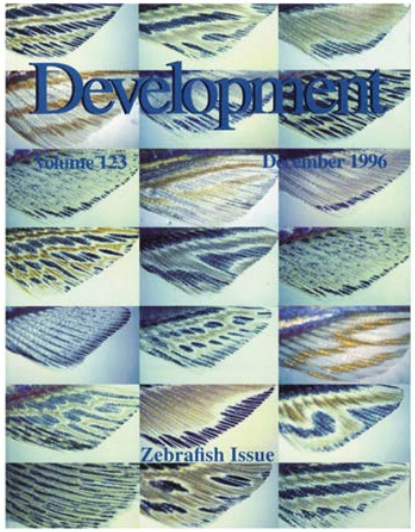

Development 123, 37-46
Printed in Great Britain © The Company of Biologists Limited 1996
DEV3351

A genetic screen for mutations affecting embryogenesis in zebrafish

W. Driever*, L. Solnica-Krezel, A. F. Schier, S. C. F. Neuhauss, J. Malicki, D. L. Stemple, D. Y. R. Stainier†, F. Zwartkruis‡, S. Abdelilah, Z. Rangini§, J. Belak and C. Boggs

Cardiovascular Research Center, Massachusetts General Hospital and Harvard Medical School, 149 13th Street, Charlestown, MA 02129, USA

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§Present address: Department of Oncology, Sharett Institute, Hadassah Hospital, Jerusalem 91120, Israel

Large-scale froward-genetic screens uncovered numerous zebrafish mutations in genes orthologous to those causing human congenital disease, and result in clear phenotypic similarities

Dystrophin is required for the formation of stable muscle attachments in the zebrafish embryo

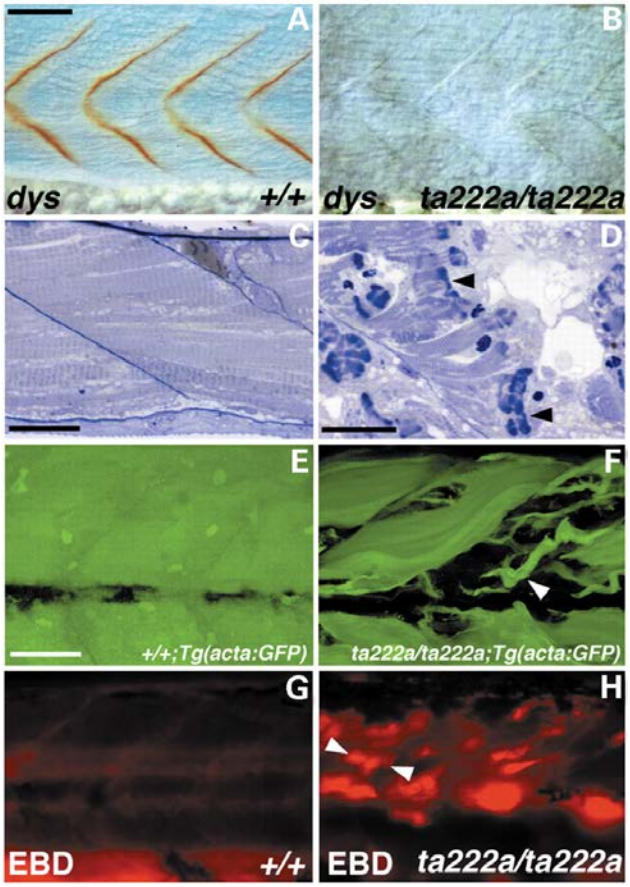
David I. Bassett[1,2,*], Robert J. Bryson-Richardson[3], David F. Daggett[4], Philippe Gautier[1], David G. Keenan[3] and Peter D. Currie[3]

[1]Comparative and Developmental Genetics Section, MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK
[2]Institute of Human Genetics, University of Newcastle upon Tyne, International Centre for Life, Central Parkway, Newcastle upon Tyne NE1 3BZ, UK
[3]Victor Chang Cardiac Research Institute, 384 Victoria Street, Darlinghurst, Sydney 2010, Australia
[4]Department of Molecular and Cell Biology, University of California Berkeley, 555 Life Sciences Addition #3200, Berkeley, CA 94720-3200, USA
*Author for correspondence (e-mail: dbassett@hgu.mrc.ac.uk)

Accepted 12 August 2003

Development 130, 5851-5860

doi:10.1242/dev.00799



sapje is a mutation of zebrafish that causes fibre detachment. Dystrophin C-terminal immunoreactivity is localized to somite boundaries in wild-type (in **A**, 27 h post-fertilisation, lateral views) but lacking in *sapje* mutant embryos (**B**).

Toluidene blue histology reveals detached, retracted fibres in association with lesions in *sapje* (arrowhead in **D**; parasagittal sections, 72 h post-fertilisation) but not in wild-type (**C**) embryos.

Surface reconstructions of somites made using confocal microscopy to image fluorescence from a transgene expressing EGFP in skeletal muscle under the control of an alpha-actin promoter reveals extensive fibre loss in *sapje* mutant (arrowhead in **F**), but not in wild-type embryos (**E**), and in particular the apparently random nature of the damage sites.

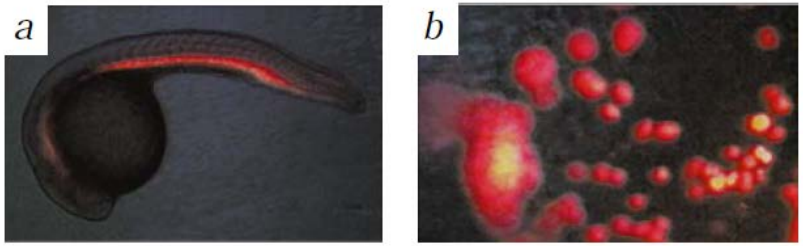
Evans blue is a vital dye (EBD, red fluorescence) that does not accumulate in wild-type somites (**G**), but labels fibres with compromised sarcolemmal membranes in *sapje* (**H**). Labelled fibres are visible that have both detached and retracted (arrowheads).

sapje, disrupts the zebrafish orthologue of the X-linked human Duchenne muscular dystrophy (DMD) gene

A zebrafish model for hepatoerythropoietic porphyria

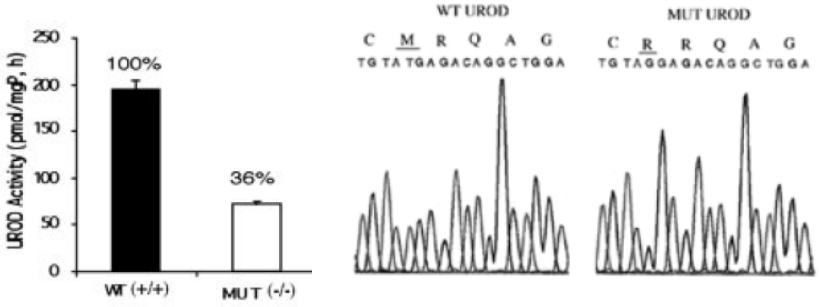
Han Wang[1], Qiaoming Long[1], Scott D. Marty[1], Shigeru Sassa[2] & Shuo Lin[1]

Defects in the enzymes involved in the haem biosynthetic pathway can lead to a group of human diseases known as the porphyrias. *yquem* (*yqe*^{tp61}) is a zebrafish mutant with a photosensitive porphyria syndrome. Here we show that the porphyric phenotype is due to an inherited homozygous mutation in the gene encoding uroporphyrinogen decarboxylase (UROD); a homozygous deficiency of this enzyme causes hepatoerythropoietic porphyria (HEP) in humans. The zebrafish mutant represents the first genetically 'accurate' animal model of HEP, and should be useful for studying the pathogenesis of UROD deficiency and evaluating gene therapy vectors. We rescued the mutant phenotype by transient and germline expression of the wild-type allele.



yquem (*yqe*^{tp61}) homozygous embryos die due to photo-ablation of their auto-fluorescent blood cells upon light exposure

The red auto-fluorescence of the blood cells can be observed in the haematopoietic tissue of the intermediate cell mass (ICM) as early as 30 hours postfertilization (hpf), and later in circulating blood cells (Fig. 1a,b), presumably due to the excessive accumulation of photosensitive porphyrins.



→ excessive amounts of uroporphyrinogens I and III and 7-carboxylate porphyrin in homozygous *yquem* embryos compared with wild-type embryos, suggesting a UROD deficiency in the mutant.

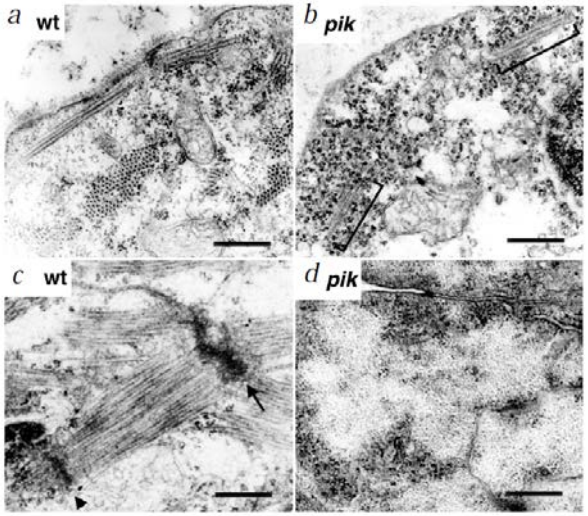
→ Similar to hepatoerythropoietic porphyria: clinically characterized by light-sensitive dermatitis, due to defects in UROD gene

Cardiomyopathy in zebrafish due to mutation in an alternatively spliced exon of *titin*

Xiaolei Xu[1]*, Steffen E. Meiler[1]*, Tao P. Zhong[1], Manzoor Mohideen[1], Dane A. Crossley[2], Warren W. Burggren[3] & Mark C. Fishman[1]

*These authors contributed equally to this work.

nature genetics • volume 30 • february 2002



pickwick mutant: normal heart, but poorly contractile
Transversal sections of ventricle cells analysed by transmisison electromicroscopy

(A-B) In wild-type hearts at 36hpf, there are nascent myofibrils. In pick mutant, the number is reduced

(C-D)In wild-type hearts at 48 hpf, myofibrils assemble into higher-order sarcomere structures. In pick mutant, no myofibrils are detectable

The causative mutation is an alternatively-spliced exon of the gene ttn, encoding Titin

Mutations of *TTN*, encoding the giant muscle filament titin, cause familial dilated cardiomyopathy

Brenda Gerull[1,2]*, Michael Gramlich[1]*, John Atherton[3], Mark McNabb[4], Karoly Trombitás[4], Sabine Sasse-Klaassen[1], J.G. Seidman[5], Christine Seidman[6], Henk Granzier[4], Siegfried Labeit[7], Michael Frenneaux[8] & Ludwig Thierfelder[1,2]

*These authors contributed equally to this work.

Published online: 14 January 2002, DOI: 10.1038/ng815

nature genetics • volume 30 • february 2002

Once a zebrafish disease model is generated, the ease of embryo manipulation enables further investigation of the molecular and cellular basis of the disease

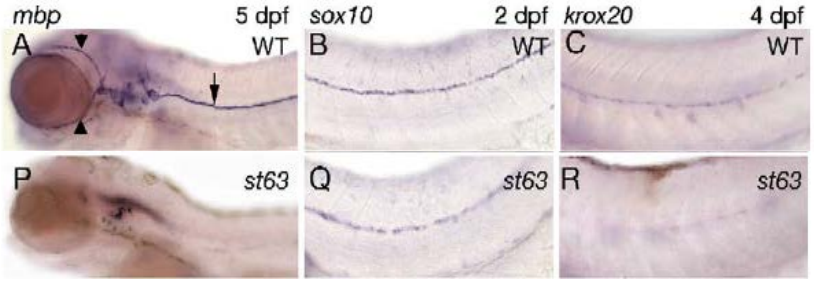
Developmental Biology 298 (2006) 118 – 131

A genetic screen identifies genes essential for development of myelinated axons in zebrafish

Hans-Martin Pogoda [a,1], Nitzan Sternheim [a,1], David A. Lyons [a], Brianne Diamond [a], Thomas A. Hawkins [a], Ian G. Woods [a], Dimple H. Bhatt [b], Clara Franzini-Armstrong [c], Claudia Dominguez [a], Naomi Arana [a], Jennifer Jacobs [a], Rebecca Nix [a], Joseph R. Fetcho [d], William S. Talbot [a,*]

[a] Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305, USA
[b] Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, NY 11794, USA
[c] Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA
[d] Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, USA

→ N-nitroso-N-ethylurea (ENU)-treated males
→ The screen identified 13 mutations in 10 zebrafish genes essential for the development of myelinated axons

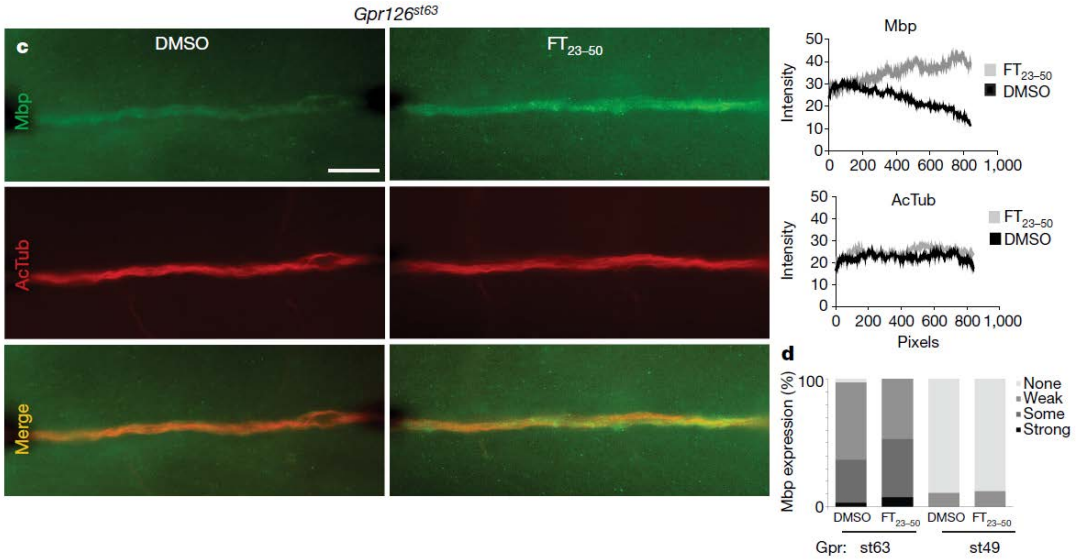


→ *gpr126*^{st63} zebrafish mutant has a point mutation that reduces Gpr126 signalling and shows decreased myelin basic protein (Mbp) expression by Schwann cells of the posterior lateral line nerve (PLLn)
Monk *et al.*, 2009, *Science*

The prion protein is an agonistic ligand of the G protein–coupled receptor Adgrg6

Alexander Küffer[1]*, Asvin K. K. Lakkaraju[1]*, Amit Mogha[2], Sarah C. Petersen[2], Kristina Airich[1], Cédric Doucerain[1], Rajlakshmi Marpakwar[1], Pamela Bakirci[1], Assunta Senatore[1], Arnaud Monnard[1], Carmen Schiavi[1], Mario Nuvolone[1], Bianka Grosshans[3], Simone Hornemann[1], Frederic Bassilana[3], Kelly R. Monk[2] & Adriano Aguzzi[1]

464 | NATURE | VOL 536 | 25 AUGUST 2016



→ When applied to *gpr126*^{st63} zebrafish larvae at 50–55 h post-fertilization (hpf), FT23–50 (20 µM) increased Mbp expression in the PLLn at 5 days post-fertilization (dpf) compared to DMSO-treated larvae
→ Corroborated by results in *gpr126*^{st49} larvae, which encode a truncated Gpr126 incapable of Gs signalling

Reverse genetic approach

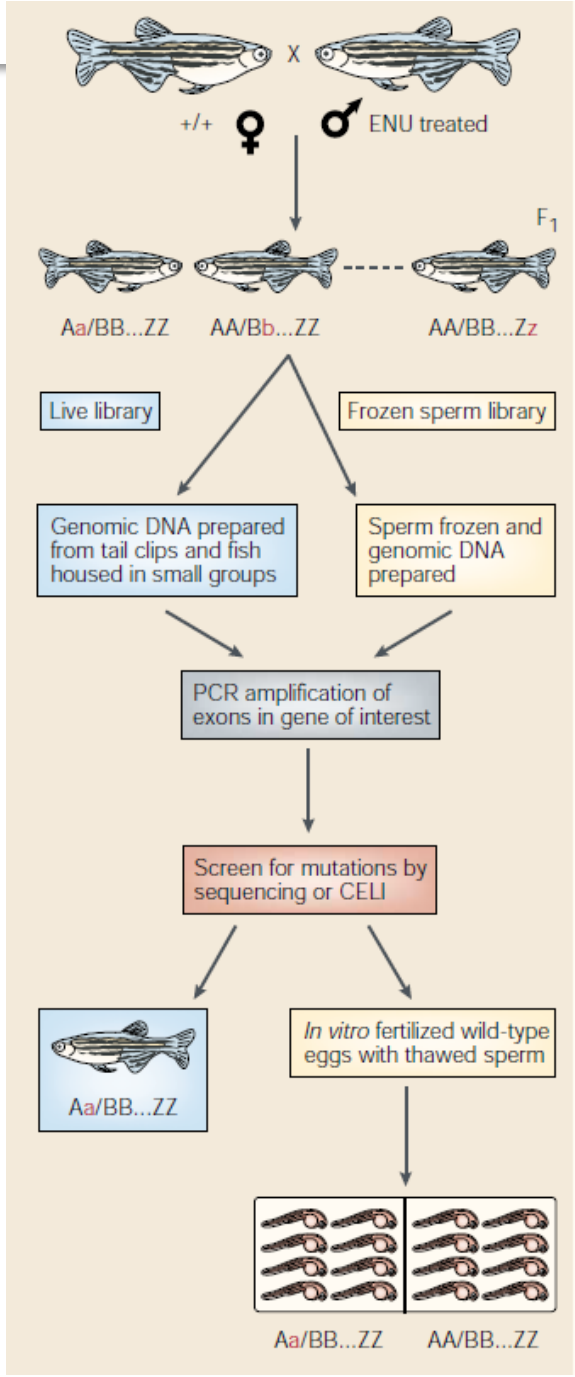
→ Studying the phenotypic consequences of perturbing the function of a gene of interest

→ TILLING: targeting induced local lesions in genomes

Target-Selected Inactivation of the Zebrafish *rag1* Gene

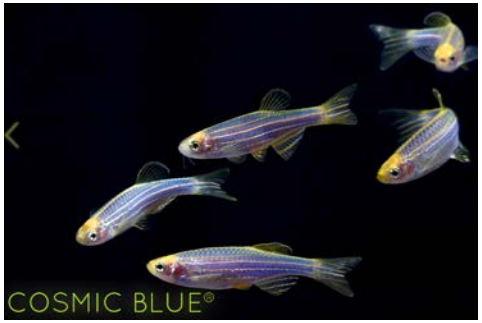
Erno Wienholds,[1] Stefan Schulte-Merker,[2] Brigitte Walderich,[2] Ronald H. A. Plasterk[1]*

The zebrafish has become a favorite organism for genetic analysis of vertebrate development, but methods for generating mutants by reverse genetic approaches have been lacking. We report a method to obtain stable mutants of a gene based on knowledge of the gene sequence only. Parental fish were mutagenized with *N*-ethyl-*N*-nitrosourea; in 2679 F_1 fish, the *rag1* gene was analyzed for heterozygous mutations by resequencing. In total, we found 15 mutations: 9 resulted in amino acid substitutions and 1 resulted in a premature stop codon. This truncation mutant was found to be homozygous viable and defective in V(D)J joining. Although presumably immune deficient, these homozygous *rag1* mutant fish are able to reach adulthood and are fertile. As sperm samples from all 2679 F_1 fish were collected and cryopreserved, we have in principle generated a mutant library from which mutants of most zebrafish genes can be isolated.



Transgenic zebrafish

- → Transgenic zebrafish are created by injecting a DNA construct into fertilized oocytes
- → 50–80% efficiency of germline transgenesis
- → Given the optical clarity of zebrafish embryos, such transgenes are frequently coupled to a fluorescent protein tag such as green fluorescent protein (GFP)
- → GloFish®: strains carry transgenes that cause them to express high levels of different fluorescent proteins. These fluorescent proteins cause the fish to be brightly colored under normal room light, and to fluoresce, or glow, when they absorb specific wavelengths of light



Lieschke and Currie, *Nat Rev Genet* 2007

Transparent Adult Zebrafish as a Tool for In Vivo Transplantation Analysis

Richard Mark White,[1,2,3] Anna Sessa,[2] Christopher Burke,[2] Teresa Bowman,[2] Jocelyn LeBlanc,[2] Craig Ceol,[2] Caitlin Bourque,[2] Michael Dovey,[2,3] Wolfram Goessling,[2,3] Caroline Erter Burns,[4] and Leonard I. Zon[1,2,3,*]
[1]Department of Medical Oncology, Dana Farber Cancer Institute, Boston, MA 02115, USA
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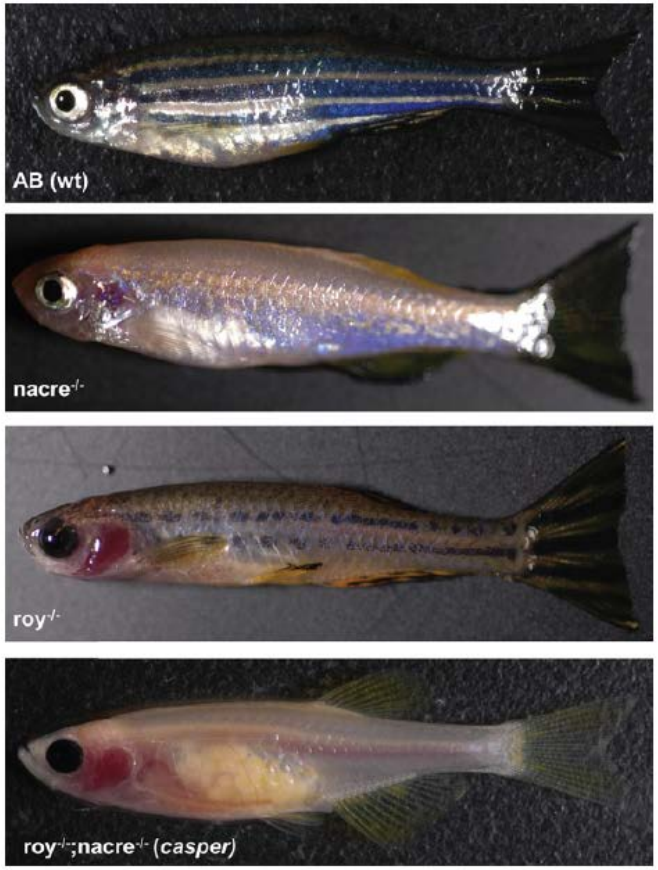
*Correspondence: zon@enders.tch.harvard.edu
DOI 10.1016/j.stem.2007.11.002
Cell Stem Cell 2, 183–189, February 2008



adult pigmentation pattern of the zebrafish consists of 3 distinct classes of pigment cells arranged in stripes: black melanophores, reflective iridophores, and yellow xanthophores

The *nacre* mutant has a complete lack of melanocytes due to a mutation in the gene encoding the *mitfa* gene. (Lister et al., 1999).

The *roy orbison* (roy) zebrafish is a spontaneous mutant and has a complete lack of iridophores, uniformly pigmented eyes, sparse melanocytes, and a translucency of the skin. The gene responsible is unkown.

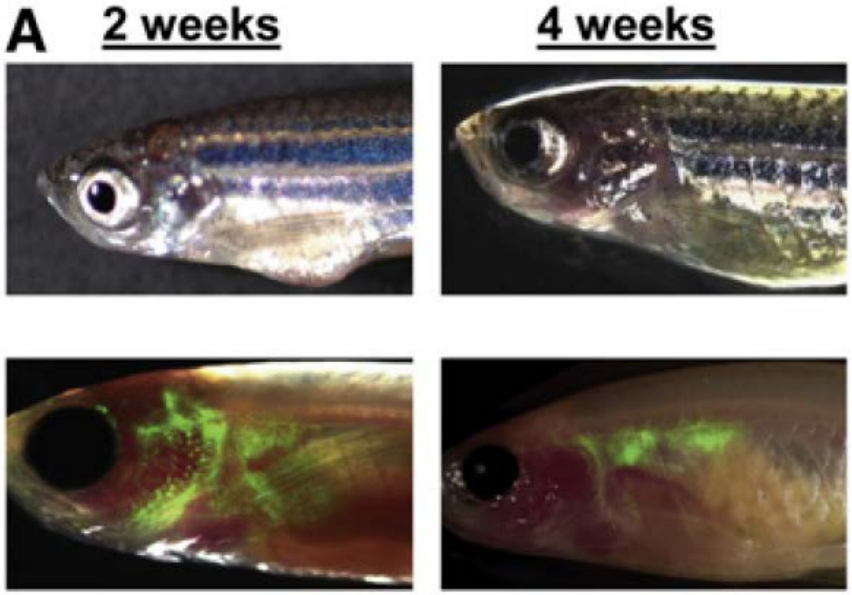
doubly mutant for *nacre* and *roy* fish demonstrates a complete lack of all melanocytes and iridophores in both embryogenesis and adulthood → named ***casper*** for its ghost like appearance

Figure 1. Combinatorial Pigmentation Mutants Yield Transparent Adult Zebrafish

Transparent zebrafish: *casper line*

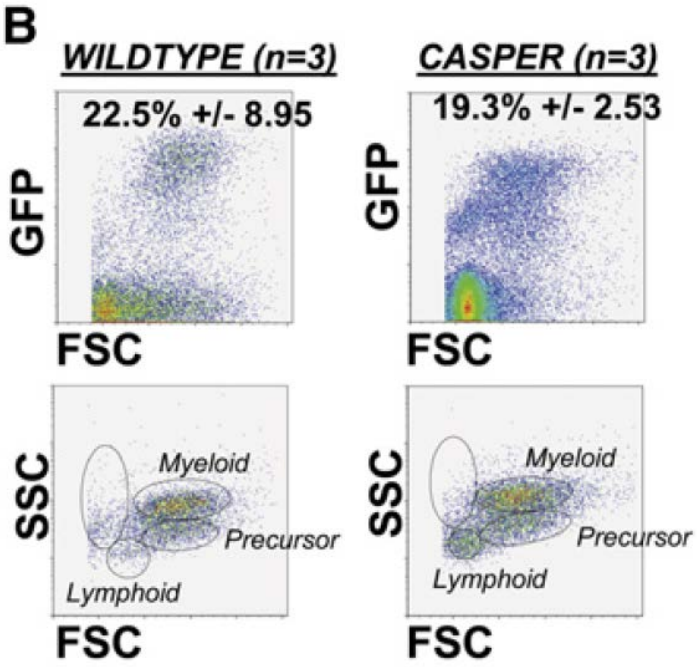
→ Transplantable hematopoietic stem/progenitor (HSPC) marrow population resides within the adult kidney (Traver et al., 2003)

→ whole kidney marrow from beta-actin:GFP transgenic fish (which labels all cell types except red blood cells) and performed intracardiac (ventricular) transplantation of 100,000 whole kidney marrow cells along with 200,000 carrier red blood cells into a recipient casper mutant that had previously been irradiated with 25 Gy



At 2 weeks , GFP-positive cells can be seen to circulate and home to the region near the gills and head kidney of the recipient only in the casper line.

By 4 weeks, a population of GFP-positive cells is tightly localized to the zebrafish kidney, where most adult hematopoietic tissue is known to reside



Both the wild-type and transparent mutant repopulated their kidney marrow with a full repertoire of hematopoietic lineages. In both types of recipients, a subset of the sorted cells was GFP positive (19%–22%), as is expected in a mosaic transplantation assay

White *et al., Cell Stem Cell* 2008

Efficient genome editing in zebrafish using a CRISPR-Cas system

Woong Y Hwang[1,7], Yanfang Fu[2,3,7], Deepak Reyon[2,3], Morgan L Maeder[2,4], Shengdar Q Tsai[2,3], Jeffry D Sander[2,3], Randall T Peterson[1,5,6], J-R Joanna Yeh[1,5] & J Keith Joung[2–4]

NATURE BIOTECHNOLOGY VOLUME 31 NUMBER 3 MARCH 2013

→ First CRISPR-Cas–based genome editing in whole organism

→ customizable sgRNAs can direct Cas9 endonuclease–mediated alteration of endogenous genes in zebrafish embryos :

- sgRNA and Cas9-encoding mRNA microinjected into one-cell-stage zebrafish embryos
- successfully targeted >80% of the sites tested in zebrafish

nature COMMUNICATIONS

ARTICLE

Received 11 Sep 2015 | Accepted 12 Apr 2016 | Published 27 May 2016

DOI: 10.1038/ncomms11601 OPEN

Mutations in *SLC39A14* disrupt manganese homeostasis and cause childhood-onset parkinsonism–dystonia

Karin Tuschl *et al.*#

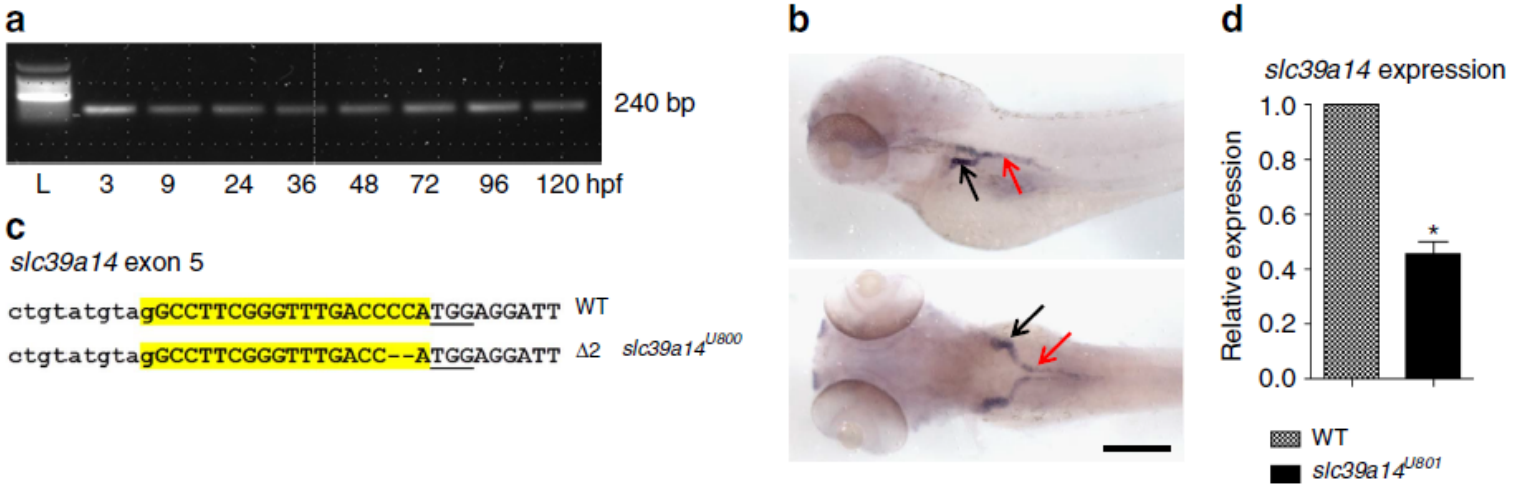
Identified cohort of patients with a novel autosomal recessive manganese transporter defect caused by mutations in *SLC39A14*

Excessive accumulation of manganese in these patients results in rapidly progressive childhood-onset parkinsonism–dystonia with distinctive brain magnetic resonance imaging appearances and neurodegenerative features on *post-mortem* examination

Table 2 | Whole-blood Mn levels are raised in SLC39A14 deficiency.

ng ml^{-1}	Control	F98V	E105X	Father	Mother	Reference
Mn	6.69–11.29	*159*	*445*	15.1	12.2	5–12.8 (ref. 40)
Fe	409–462	434	397	370	386	236–614 (ref. 41)
Cu	635–1,096	1,130	944	770	1,108	590–1,470 (ref. 41)
Zn	4,364–5,284	5,076	4,580	5,804	5,424	4,800–7,800 (ref. 41)
Cd	0.35	1.42	0.18	0	0.89	0.15–2.04 (ref. 40)

Table showing whole-blood metal levels for two affected individuals (F98V and E106X), three healthy control subjects, parents heterozygous for the E105X mutation and published reference ranges[40,41]. While Mn levels are significantly raised in both patients, other metal levels are within the normal range. Abnormal Mn levels are indicated in italics.

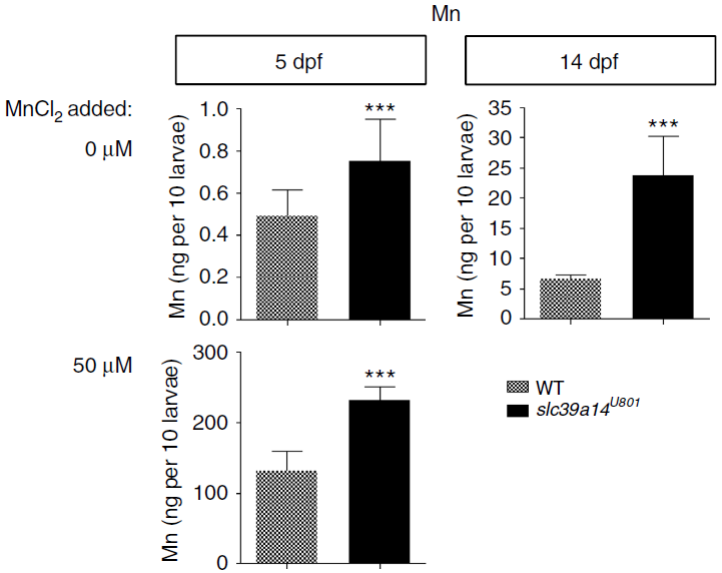


(a) RT–PCR showing *slc39a14* expression between 3 and 120 hpf in zebrafish.

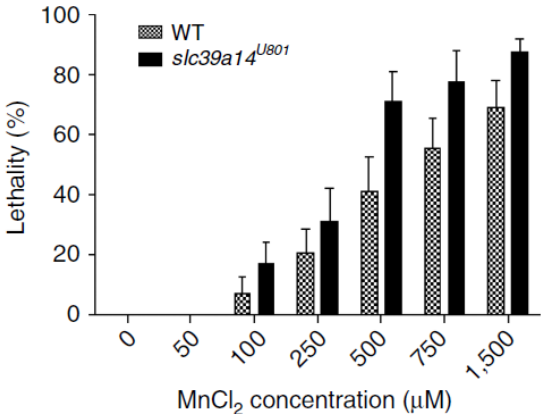
(b) Whole-mount in situ hybridization using a DIG-labelled antisense RNA probe showing *slc39a14* expression in the proximal convoluted (black arrows) and straight (red arrows) pronephric tubules in zebrafish larvae at 4 dpf. Top, lateral view; bottom, dorsal view. Scale bar, 200 mm.

(c) DNA sequence of the region within exon 5 of slc39a14 targeted by a CRISPR guide RNA is highlighted in yellow and the 2-bp deletion introduced in the *slc39a14*^{U801} mutant indicated by dashes. Pam sequence underlined.

(d) qRT–PCR demonstrates a 2.2-fold reduction in slc39a14 expression in homozygous *slc39a14*^{U801} mutants

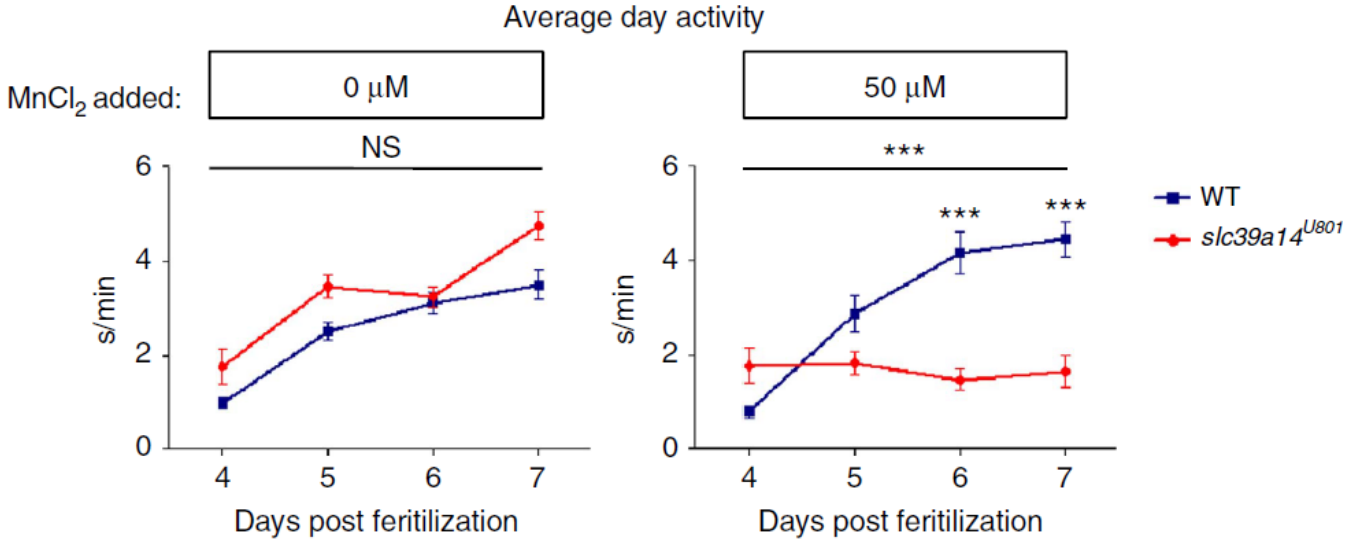


mutant larvae have significantly raised Mn levels at 5 dpf and 14 dpf, and Mn accumulation on $MnCl_2$ exposure is significantly higher in mutant compared with WT larvae at 5 dpf



	LC_{50} (µm)	95% CI
WT	661	548–808
slc39a14^{U801}	377	313–455

graph presenting the lethality in homozygous *slc39a14*^{U801} and WT larvae at 5 dpf on $MnCl_2$ exposure between 2 and 5 dpf. Median lethal concentration (LC_{50}) of $MnCl_2$



in unexposed conditions there is no significant difference in locomotor activity between *slc39a14*^{U801} and WT larvae on $MnCl_2$ exposure, locomotor activity is markedly reduced in mutant larvae compared with WT

Zebrafish models of acquired disorders

Zebrafish to study cancer

→ Genomic alterations of most malignancies have been identified
→ Tools are required to place these abnormalities into a biological context

The histone methyltransferase SETDB1 is recurrently amplified in melanoma and accelerates its onset

Craig J. Ceol[1]†*, Yariv Houvras[1,2]†*, Judit Jane-Valbuena[3,4], Steve Bilodeau[5], David A. Orlando[5], Valentine Battisti[6], Lauriane Fritsch[6], William M. Lin[3,4], Travis J. Hollmann[7], Fabrizio Ferré[8], Caitlin Bourque[1], Christopher J. Burke[1], Laura Turner[1], Audrey Uong[1], Laura A. Johnson[3,4], Rameen Beroukhim[3,4], Craig H. Mermel[3,4], Massimo Loda[7], Slimane Ait-Si-Ali[6], Levi A. Garraway[3,4], Richard A. Young[5] & Leonard I. Zon[1]

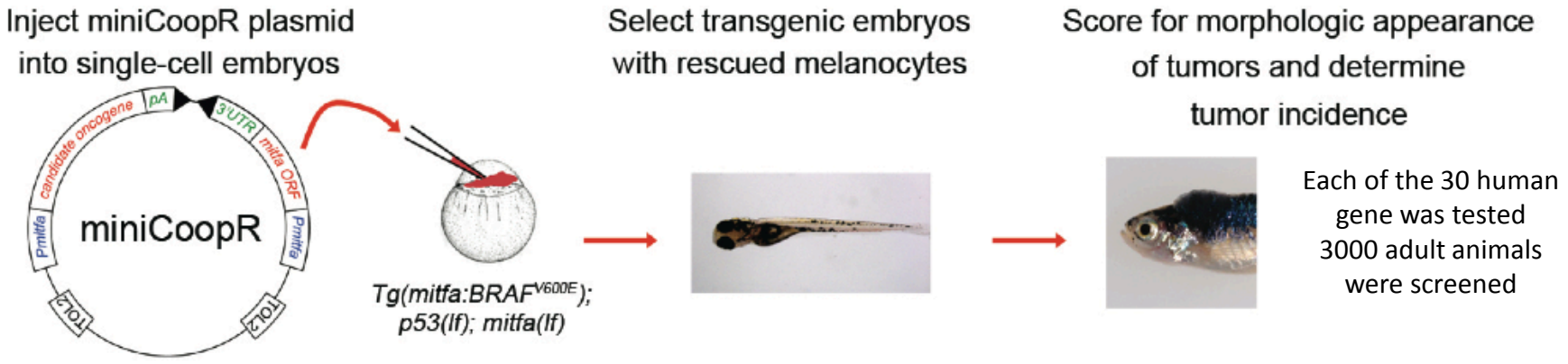
24 MARCH 2011 | VOL 471 | NATURE | 513

→ miniCoopR assay to identify key genes in region of chromosomal gain 1q21 comprising 30 genes and emerged from analysis of human melanoma samples

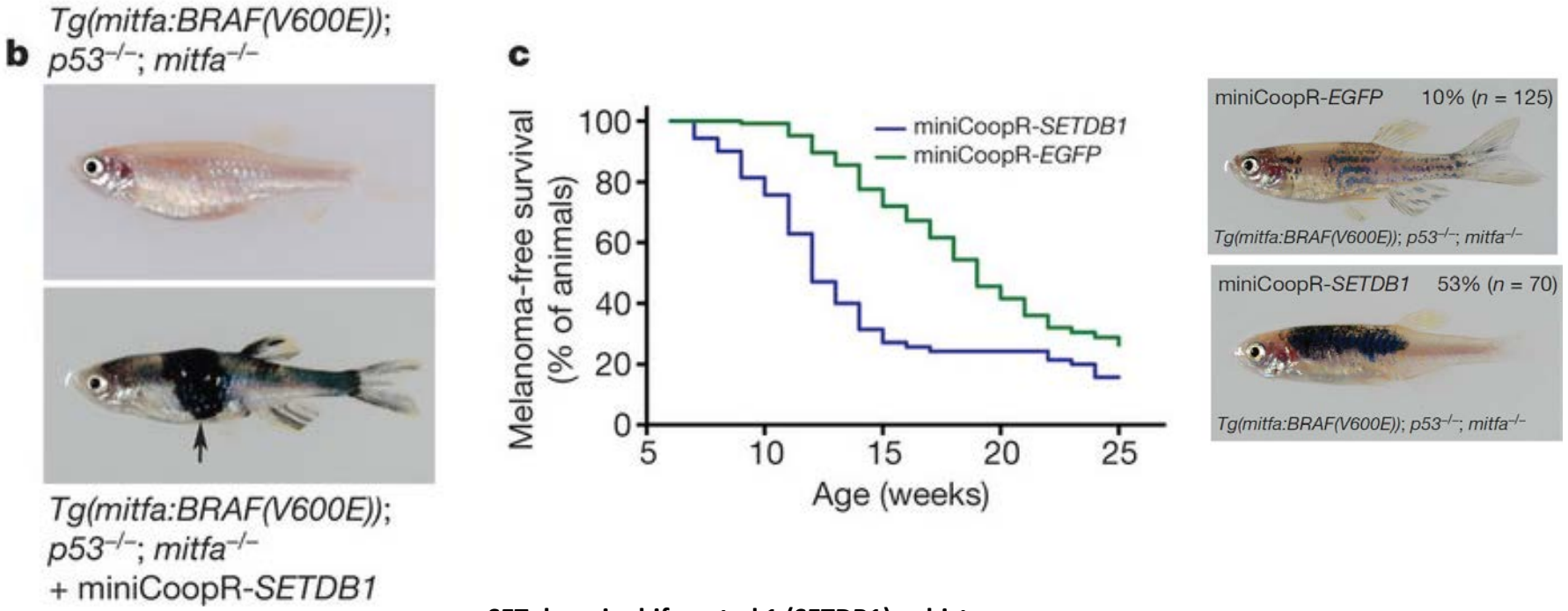
BRAF$^{(V600E)}$ is expressed under the control of a melanocyte-specific gene (*mitfa*) promoter on a p53 (also known as tp53) mutant background (*p53*$^{-/-}$)

melanomas and melanocytes that develop in Tg(mitfa:BRAF$^{(V600E)}$);p53-/- zebrafish are suppressed by a *mitfa*$^{-/-}$ mutation.

Tg(mitfa:BRAF(V600E)); p53$^{-/-}$*; mitfa*$^{-/-}$



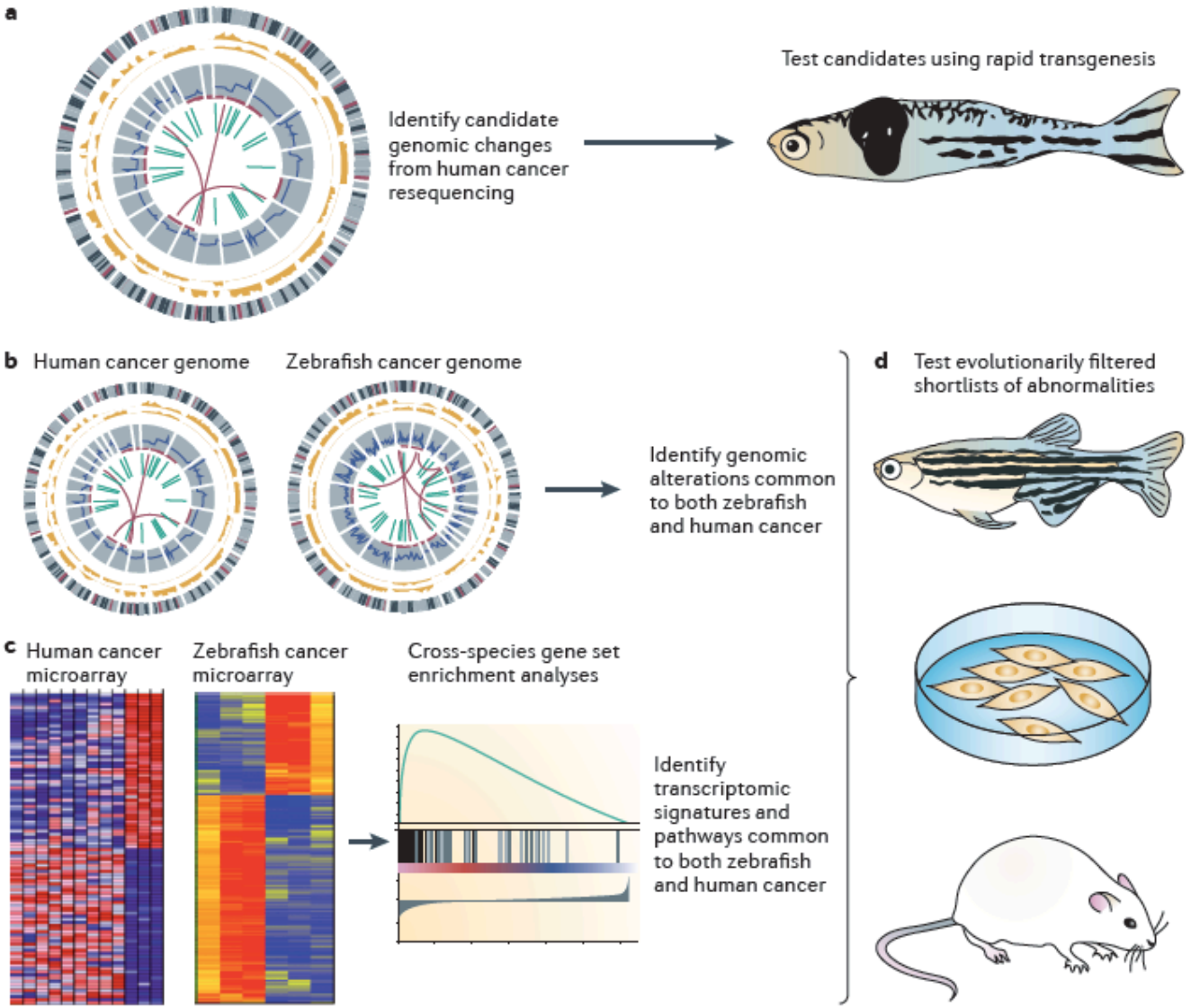
miniCoopR that rescues melanocytes and melanomas and drives the expression of a candidate gene in these rescued tissues



SET domain, bifurcated 1 (SETDB1), a histone methyltransferase, was found to cooperate with BRAF^{V600E} in mediating melanoma

Zebrafish to study cancer (ii)

→ Genomic alterations of most malignancies have been identified
→ Tools are required to place these abnormalities into a biological context



Conservation of gene expression signatures between zebrafish and human liver tumors and tumor progression

Siew Hong Lam[1], Yi Lian Wu[1], Vinsensius B Vega[2], Lance D Miller[2], Jan Spitsbergen[3], Yan Tong[1], Huiqing Zhan[1], Kunde R Govindarajan[2], Serene Lee[2], Sinnakarupan Mathavan[2], Karuturi R Krishna Murthy[2], Donald R Buhler[3], Edison T Liu[2] & Zhiyuan Gong[1]

NATURE BIOTECHNOLOGY VOLUME 24 NUMBER 1 JANUARY 2006

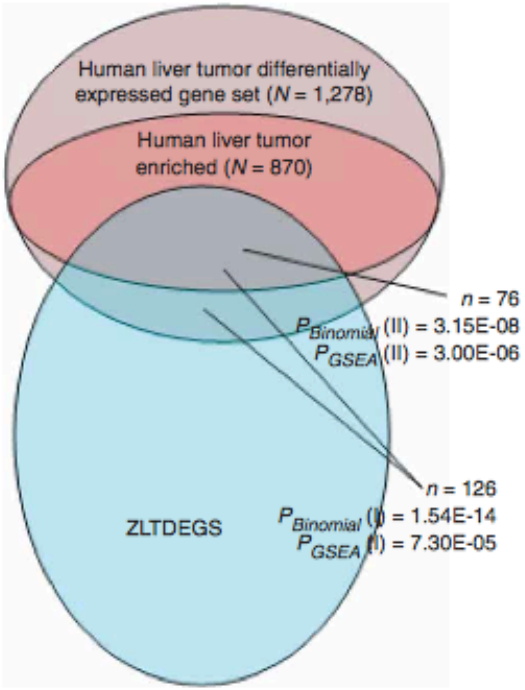
generated liver tumors in zebrafish, by treating them with carcinogens, for comparative gene-expression analysis

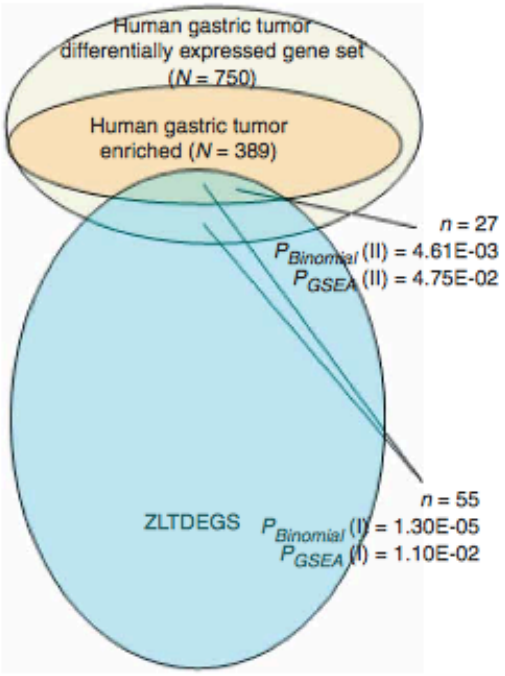
ZLTDEGS; Zebrafish Liver Tumor Differentially Expressed Gene Set), consisting of 2,315 gene features

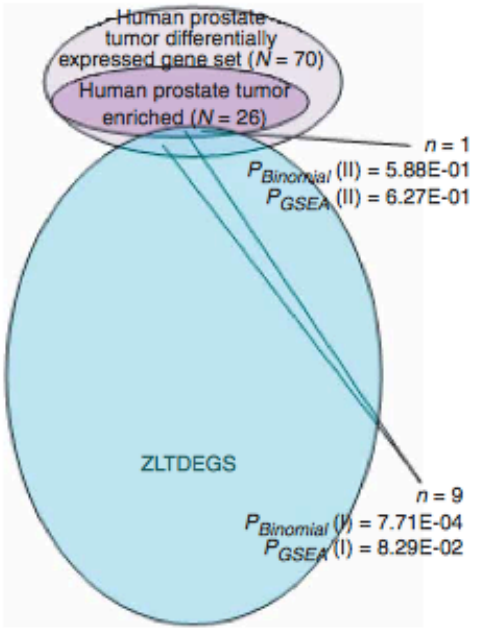
Intersection with human cancer microarray data from four cancer types: liver, gastric, prostate, lung

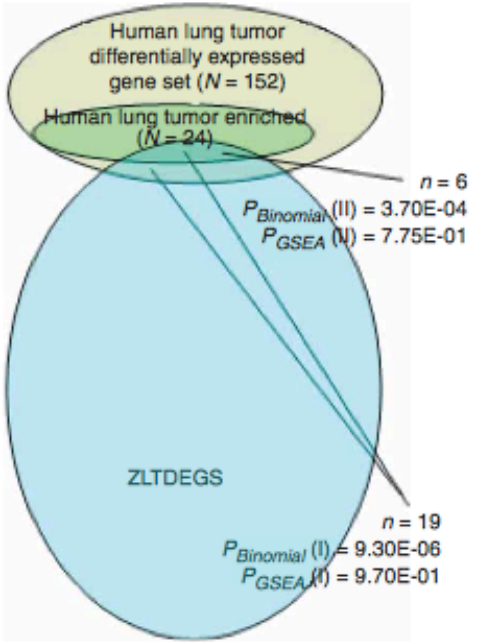
126 genes, represents a conserved expression signature that is correlated with genes that are highly significant in human liver tumors

the molecular conservation underscores their basic role in liver tumor.



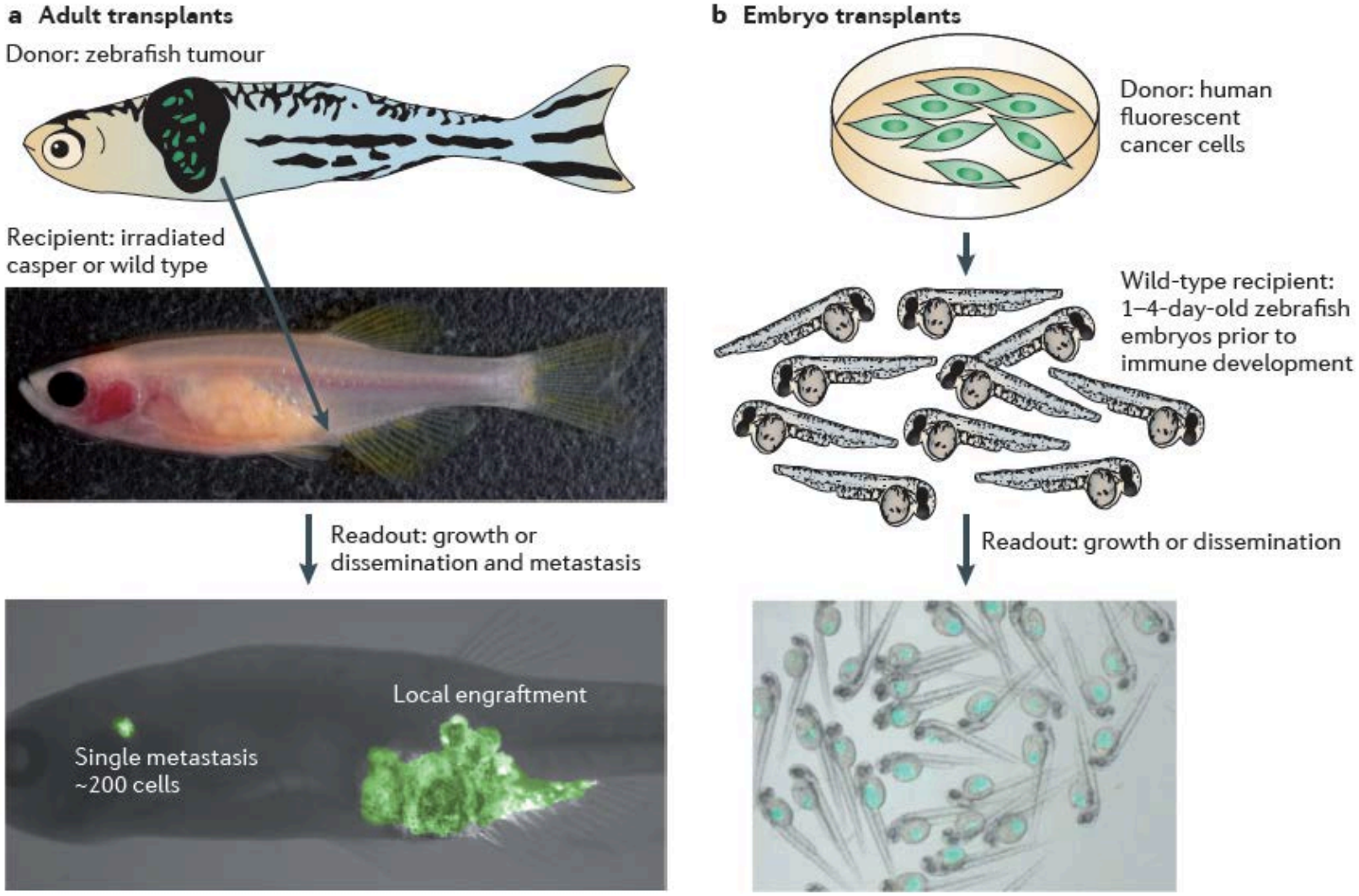






Zebrafish to study cancer (iii)

→ Transplantation of cancer cells can be performed in zebrafish to generate allograft or xenograft



study of engraftment and tumour cell subpopulations

study the effect of drugs on transplanted cells

Zebrafish as tools for drug discovery

Zebrafish screenings

- Tipically carried out in living zebrafish embryos or larvae in 96-well plates (3-5 embryos/well)

- Large numbers of fish can be mated to generate thousands of embryos

- Chemical libraries can be robotically aliquoted

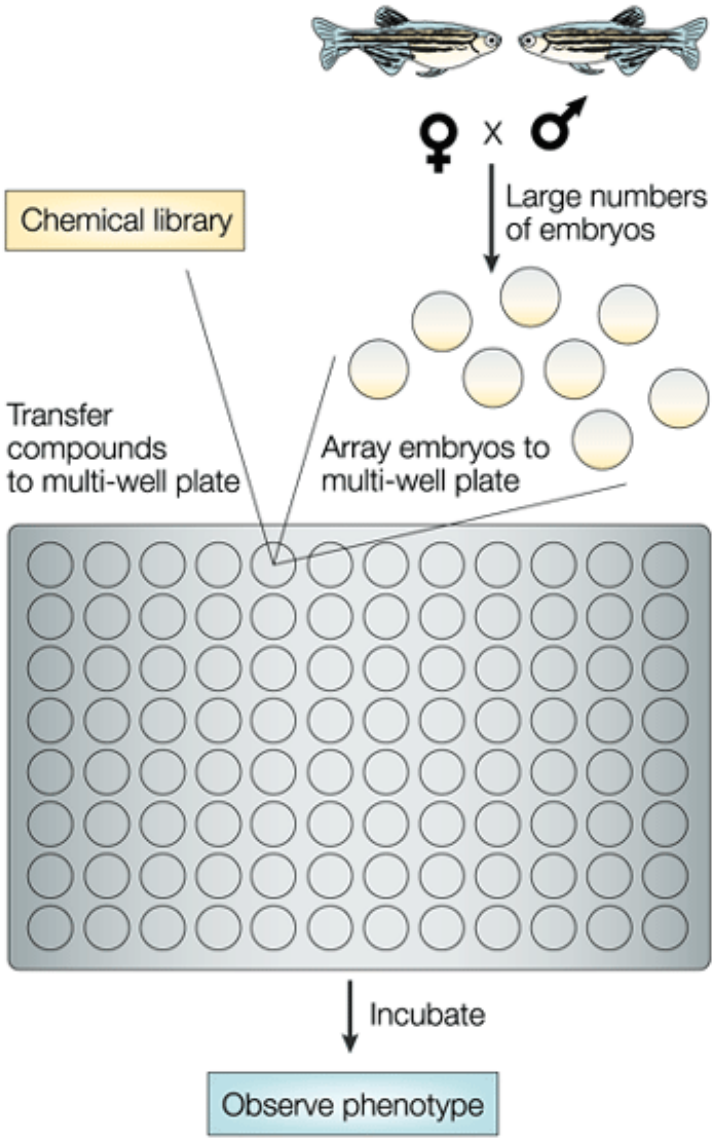
- Techniques have been established to perform whole-mount immunohistochemistry or in situ hybridization on large numbers of samples.

- Advantages *vs* cultured cells:

➢ possess fully integrated vertebrate organ system

➢ pain, sedation, tumour metastasis, vascular tone and gut motility are examples of observable disease-relevant phenotypes

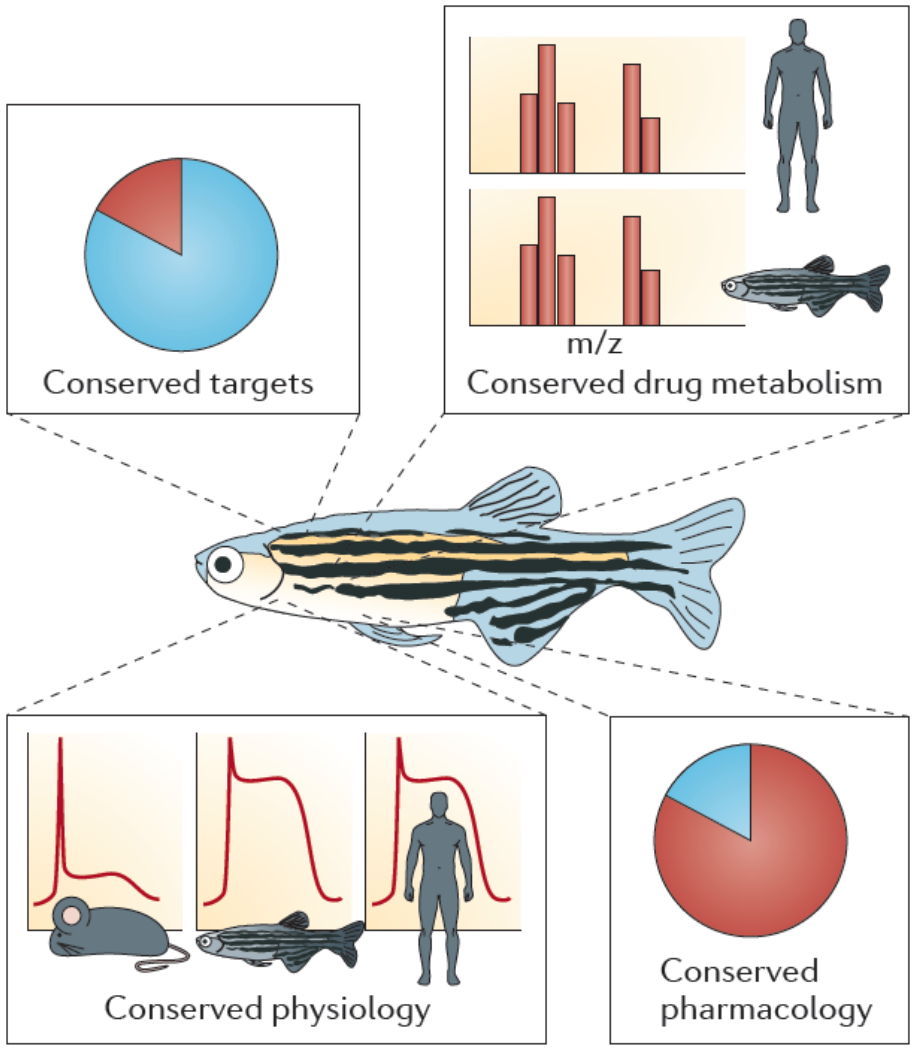
➢ Already offer insights about ADME-Tox characteristics → rapidly translated to *in vivo* mammalian models with minimal optimization



MacRae and Peterson, *Nat Rev Drug Disc* 2015

Zebrafish screenings: are the results relevant for human diseases?

functional domains of target proteins are very conserved between human and zebrafish
e.g. glucocorticoid receptor: overall 50% sequence identity, but 74% at ligand-binding domain



humans and zebrafish share a large number of drug metabolism pathways

zebrafish physiology is often well conserved: *e.g.* cardiac electrophysiology (ECG tracings)

compounds discovered in zebrafish screens have been shown to exhibit similar effects in rodent models and humans

→ More than 65 small-molecule screens in zebrafish have been published

MacRae and Peterson, *Nat Rev Drug Disc* 2015

Vol 447|21 June 2007|doi:10.1038/nature05883 nature

LETTERS

Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis

Trista E. North[1,2], Wolfram Goessling[1,2], Carl R. Walkley[1,3], Claudia Lengerke[1], Kamden R. Kopani[1,2], Allegra M. Lord[1,2], Gerhard J. Weber[1,2], Teresa V. Bowman[1,2], Il-Ho Jang[1], Tilo Grosser[4], Garret A. FitzGerald[4], George Q. Daley[1], Stuart H. Orkin[1,2,3] & Leonard I. Zon[1,2]

- A chemical genetic screen was conducted to identify new pathways modulating definitive HSC formation during zebrafish embryogenesis

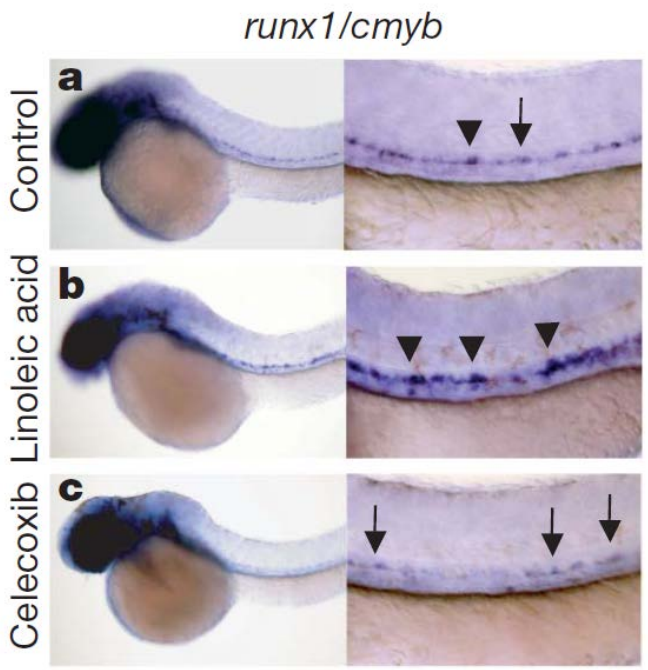
- *runx1* and *cmyb*, required for mammalian HSC development, are expressed in the ventral wall of the dorsal aorta in a region analogous to the mammalian aorta–gonad–mesonephros (AGM) at 36 h post fertilization (h.p.f.)

- Wild-type embryos, incubated with individual chemicals, were examined for alterations in runx11/cmyb1 expression in HSCs by automated *in situ* hybridization at 36 h.p.f.

- 91.7% of these compounds (2,275 of 2,357) failed to alter HSC expression
- whereas 35 (1.4%) and 47 (1.9%) led to increased or decreased numbers of HSCs, respectively

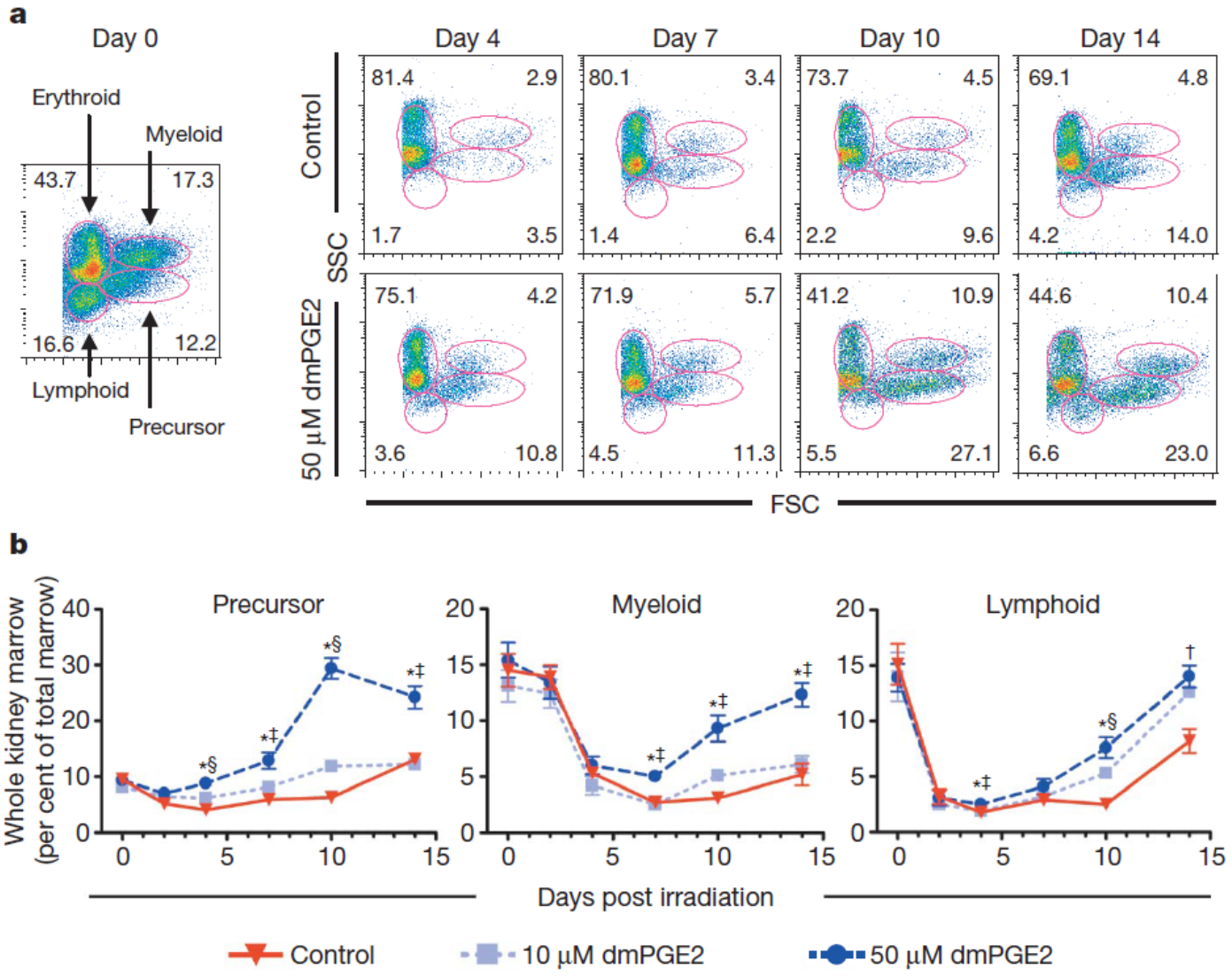
- Among these substances, 10 affected the prostaglandin pathway

Compounds	# of Times Identified	Effect on HSCs
Celecoxib	2	Decrease (26/31)
Febufen	1	Decrease (20/26)
Prostaglandin J2	1	Decrease (12/22)
Suxibuzone	1	Decrease (16/30)
Sulindac	1	Decrease (18/31)
Mead Acid	2	Increase (24/32)
Linoelic Acid	1	Increase (22/30)
13(S)-HODE	1	Increase (15/25)
Ly-171883	1	Increase (17/26)
Epoxyeicosatrienoic Acid	1	Increase (17/25)



prostaglandins come from the omega-6 fatty acids, with linoleic acid serving as the starting point

celecoxib acts by inhibiting prostaglandin synthesis via inhibition of COX2



sublethal irradiation of wild-type fish → kidney marrow irradiation-recovery assay
rate of kidney marrow repopulation was significantly enhanced after exposure to 50 µM dmPGE2
dmPGE2: a long-acting derivative of PGE2, 16,16-dimethyl-PGE2

TRANSPLANTATION

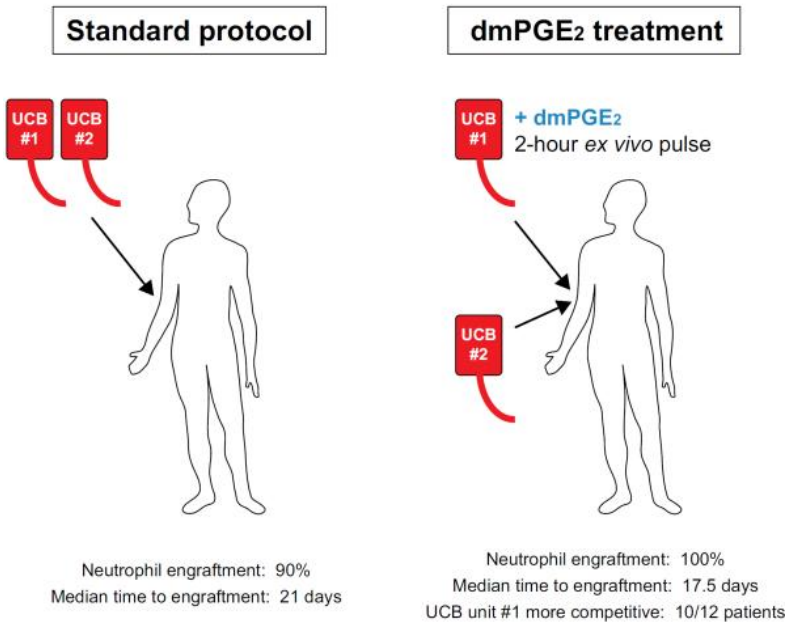
Prostaglandin-modulated umbilical cord blood hematopoietic stem cell transplantation

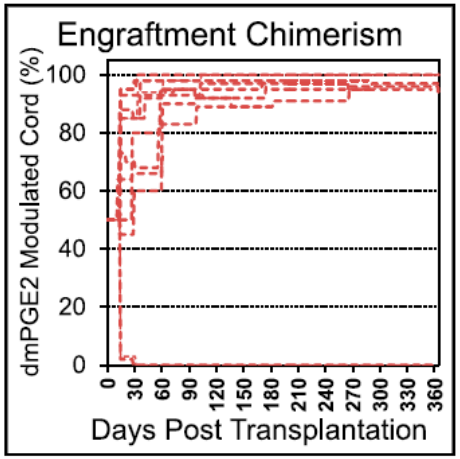
Corey Cutler,[1,2] Pratik Multani,[3] David Robbins,[3] Haesook T. Kim,[1] Thuy Le,[3] Jonathan Hoggatt,[2,4] Louis M. Pelus,[5] Caroline Desponts,[3] Yi-Bin Chen,[4] Betsy Rezner,[3] Philippe Armand,[1] John Koreth,[1] Brett Glotzbecker,[1] Vincent T. Ho,[1] Edwin Alyea,[1] Marlisa Isom,[1] Grace Kao,[1] Myriam Armant,[6] Leslie Silberstein,[2,6] Peirong Hu,[5] Robert J. Soiffer,[1] David T. Scadden,[2,4] Jerome Ritz,[1] Wolfram Goessling,[1,2,7] Trista E. North,[2,8,1] John Mendlein,[3] Karen Ballen,[4] Leonard I. Zon,[1,2,6,9] Joseph H. Antin,[1] and Daniel D. Shoemaker[3]

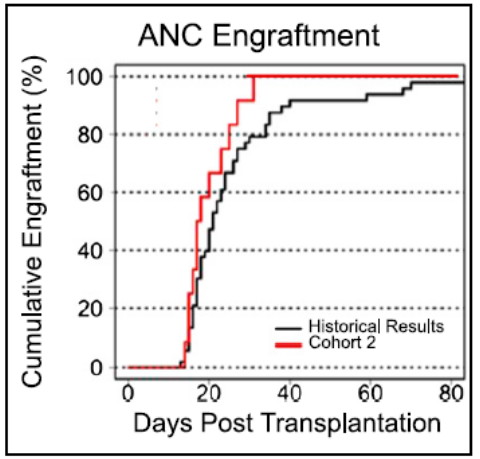
BLOOD, 24 OCTOBER 2013 • VOLUME 122, NUMBER 17

- hematopoietic stem cells (HSCs) from umbilical cord blood (UCB) can be used for allogeneic transplantation when a suitable adult donor is unavailable
- low HSC content in UCB : brief pulse treatment with a small molecule modulator to enhance the homing and engraftment potential of HSCs? → stable prostaglandin E2 (PGE2) derivative 16,16-dimethyl PGE2 (dmPGE2)

- phase 1 trial to evaluate the safety of dmPGE2-treated UCB (dmPGE2-UCB) cotransplantation with an unmanipulated UCB unit in patients with hematologic malignancies







Exp Cell Res. 2014 Dec 10

→ Phase II trial ongoing

Discovering chemical modifiers of oncogene-regulated hematopoietic differentiation

Jing-Ruey J Yeh[1–3], Kathleen M Munson[1–3], Kamaleldin E Elagib[4], Adam N Goldfarb[4], David A Sweetser[2,5] & Randall T Peterson[1–3]

VOLUME 5 NUMBER 4 APRIL 2009 NATURE CHEMICAL BIOLOGY

- → transgenic zebrafish line Tg(*hsp*:*AML1-ETO*), which expresses the leukemic oncogene AML1-ETO (AE) under the control of the zebrafish *hsp70* heat shock promoter
- → converts erythropoiesis to granulopoiesis and blocks the maturation of the granulocytes in the posterior blood island of the embryonic zebrafish: changes are evident from the downregulation of *gata1 (*erythroid transcription factor*)*

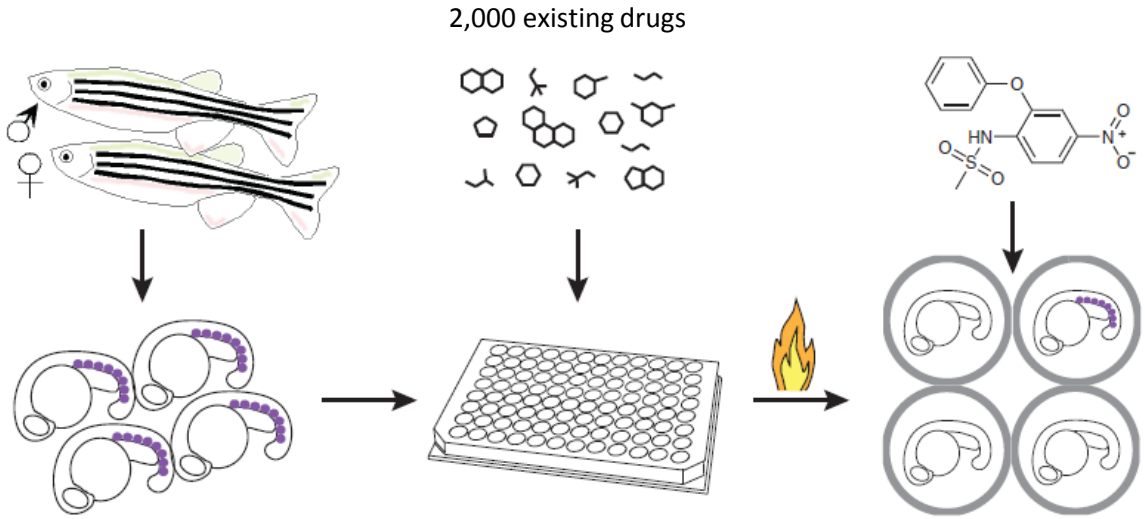


Figure 1 Screening for chemical suppressors of AE. Homozygous Tg(*hsp*:*AML1-ETO*) fish were crossed with wild-type fish to generate thousands of heterozygous Tg(*hsp*:*AML1-ETO*) embryos. These embryos were raised for 12–16 hpf, at which point five embryos were arrayed into each well of the 96-well plates. The compounds from the library were added to the plates. An hour later, the plates were heat-shocked at 40 °C for 1 h to induce AE expression. At 90 min after the heat shock, the embryos were processed for *in situ* hybridization of *gata1*. Induced expression of AE resulted in lost of *gata1*$^+$ hematopoietic cells (indicated as purple dots) in the posterior blood islands of the zebrafish embryos. However, the chemical suppressors of AE, such as nimesulide (top right), antagonized AE's effect, restoring *gata1* expression in Tg(*hsp*:*AML1-ETO*) embryos.

cyclooxygenase-2 inhibitor

selective COX-2 inhibitor NS-398

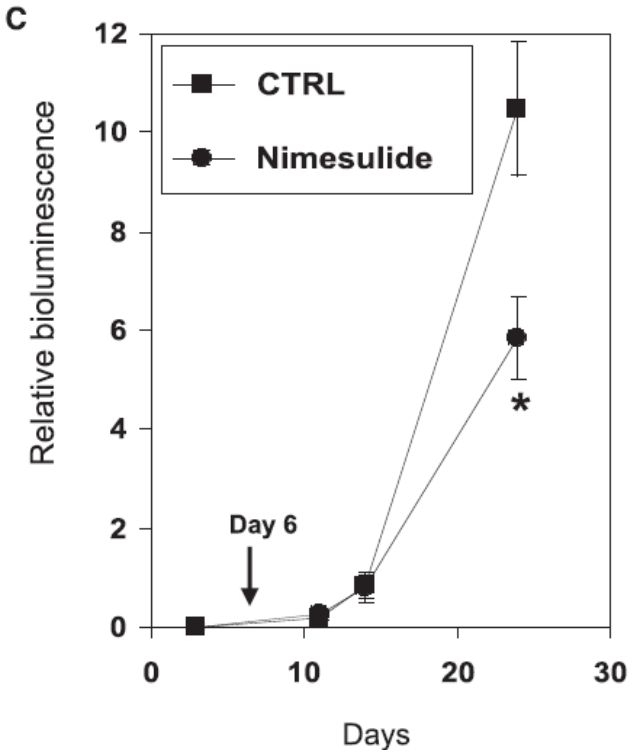
nonselective COX-2 inhibitor indomethacin

AML1-ETO mediates hematopoietic self-renewal and leukemogenesis through a COX/β-catenin signaling pathway

Yiyun Zhang,[1,2] Jianfeng Wang,[2,3] Justin Wheat,[3] Xi Chen,[3] Shan Jin,[1,2] Hossein Sadrzadeh,[4] Amir T. Fathi,[2,4] Randall T. Peterson,[1,2,5] Andrew L. Kung,[5,6] David A. Sweetser,[2,3] and Jing-Ruey Joanna Yeh[1,2]

[1]Cardiovascular Research Center, Massachusetts General Hospital, Charlestown, MA; [2]Department of Medicine, Harvard Medical School, Boston, MA; [3]Department of Pediatrics, Divisions of Pediatric Hematology/Oncology and Medical Genetics, Massachusetts General Hospital, Boston, MA; [4]Massachusetts General Hospital Cancer Center, Boston, MA; [5]The Broad Institute, Cambridge, MA; and [6]Department of Pediatrics, Columbia University Medical Center, New York, NY

BLOOD, 13 JUNE 2013 • VOLUME 121, NUMBER 24



SKNO-1 cells, a human myelogenous leukemia cell line established from an AML patient

NSG mice were inoculated via tail vein injections with SKNO-1 cells that have been engineered to express luciferase.

Injected mice were fed with normal powder food (square) (n = 5) or normal powder food mixed with nimesulide (dot) (n = 6) from day 6 (black arrow).

Progression of SKNO-1 cells was monitored by in vivo imaging of bioluminesce

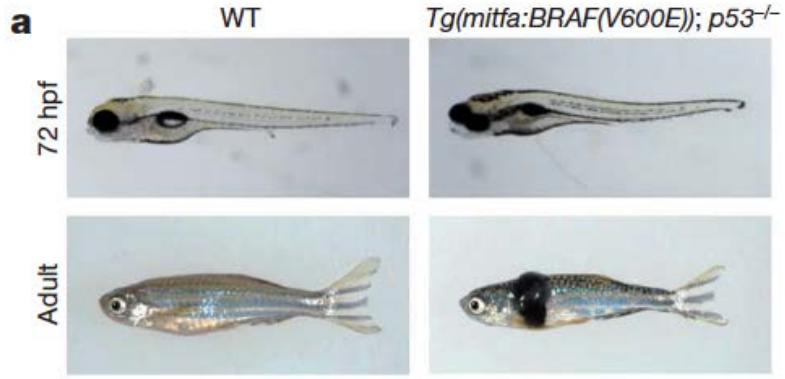
→ Phase I trial ongoing

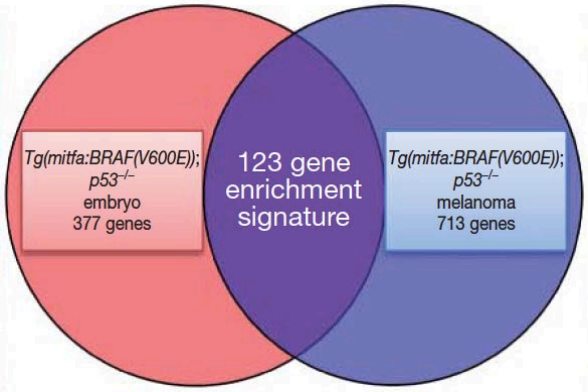
LETTER

doi:10.1038/nature09882

DHODH modulates transcriptional elongation in the neural crest and melanoma

Richard Mark White[1,2], Jennifer Cech[1], Sutheera Ratanasirintrawoot[1], Charles Y. Lin[3,4], Peter B. Rahl[3], Christopher J. Burke[1], Erin Langdon[1], Matthew L. Tomlinson[5], Jack Mosher[6], Charles Kaufman[1,2], Frank Chen[7], Hannah K. Long[8], Martin Kramer[9], Sumon Datta[1], Donna Neuberg[10], Scott Granter[11], Richard A. Young[3,4], Sean Morrison[6], Grant N. Wheeler[5] & Leonard I. Zon[1]



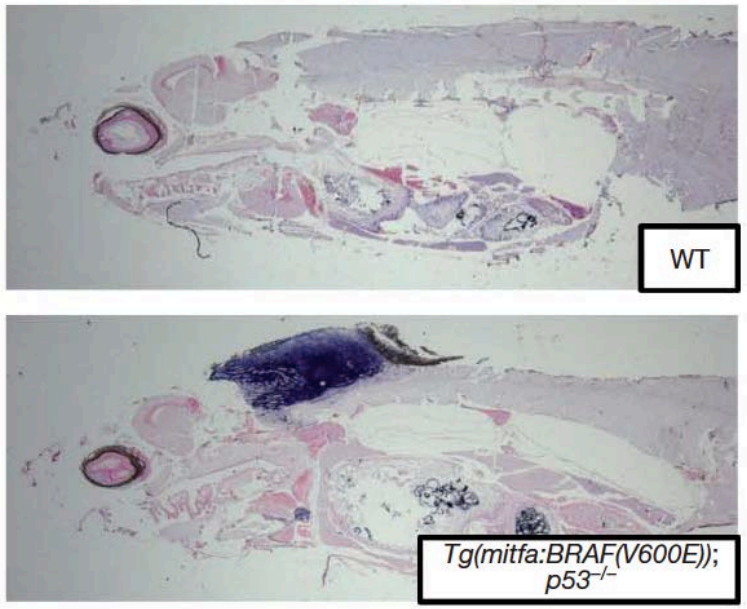


enriched for markers of embryonic neural crest progenitors → e.g. crestin

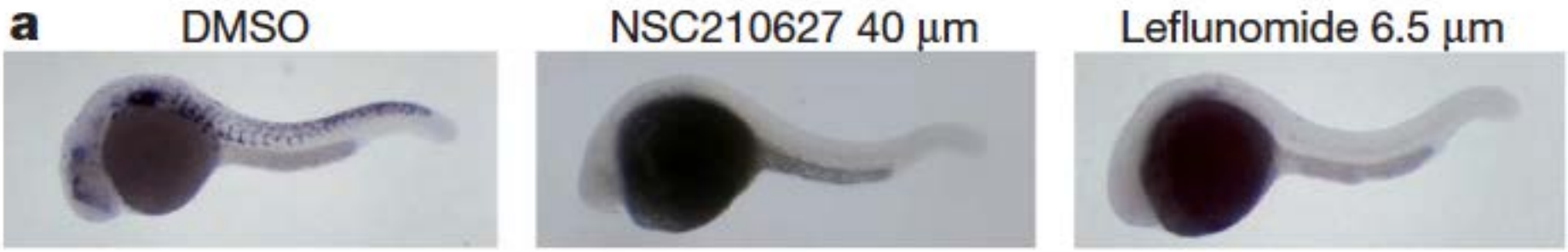
crestin is zebrafish specific and is normally downregulated after the terminal differentiation of neural crest progenitors

activated BRAF$^{(V600E)}$ promotes the maintenance of multipotency in neural crest progenitors, which become expanded during tumorigenesis

in adult Tg(mitfa:BRAF(V600E)); p53$^{-/-}$ melanomas, almost all tumour cells, but no normal cells, were positive for crestin



→ screened 2,000 chemicals to identify compounds that inhibit crestin expression during embryogenesis
→ most chemicals (90%) had a minimal effect or were toxic
→ NSC210627 and Leflunomide, inhibitors of dihydroorotate dehydrogenase (DHODH) abrogated the expression of crestin

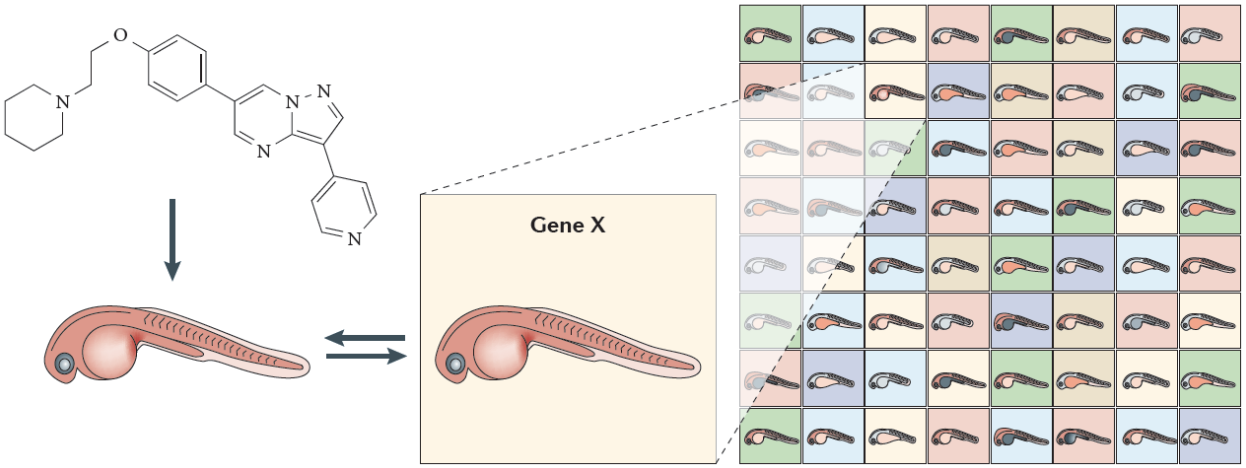


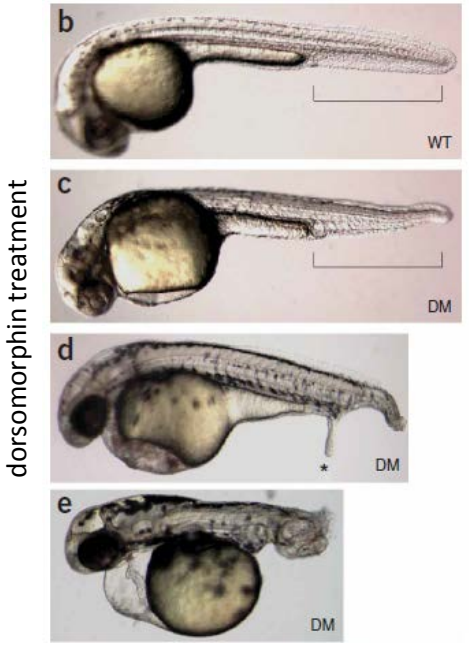
→ Leflunomide exerts these effects by inhibiting the transcriptional elongation of genes that are required for neural crest development and melanoma growth
→ Leflunomide is a well-tolerated anti-arthritis drug in humans

→ To be completed in July 2017

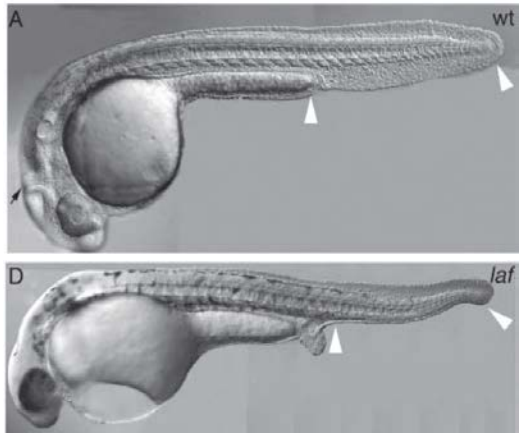
Zebrafish phenotypes to identify drug mechanism of action

→ similarity between a drug-induced phenotype and a genetic phenotype can often provide clues as to the principal drug target or targets





Yu *et al.,* Nat Chem Biol. 2008 Jan;4(1):33-41



Mullins *et al.,* Development 123, 81-93 (1995)

lost-a-fin phenotype due to *Alk8* mutation (in human ALK2 activin receptor-like kinase-2)

ALK2 is important in the bone morphogenic protein (BMP) pathway which is responsible for the development and repair of the skeletal system

MacRae and Peterson, *Nat Rev Drug Disc* 2015

Zebrafish for toxicology study

- zebrafish toxicology is much less expensive and can be performed rapidly on large numbers of compounds in parallel

- testing can be performed on hundreds or thousands of hits from a primary high-throughput screen to eliminate toxic compounds at an early stage

→ enabling toxic compounds to 'fail fast', before substantial resources have been wasted

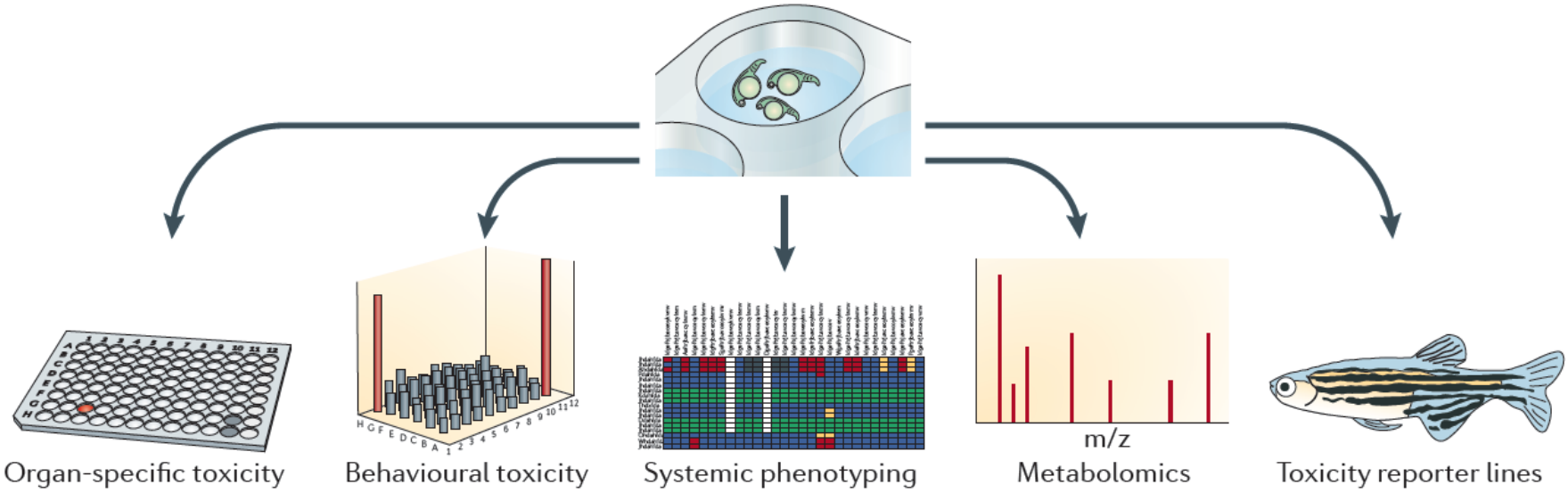


Figure 4 | **Use of zebrafish in toxicology.** Zebrafish have been validated for high-throughput screening focused on specific organ toxicity (liver, kidney and heart) or behavioural toxicity. Alternatively, they have been used to profile compounds systematically for toxicity across multiple systems simultaneously. New technologies, including metabolomic profiling and toxicity reporter lines, promise to extend the utility of zebrafish further. m/z, mass to charge ratio.

MacRae and Peterson, *Nat Rev Drug Disc* 2015

Challenges of zebrafish screenings

- Controlling and quantifying drug exposures:

  difficult to predict how much drug is absorbed

  false negative in screening due to poor uptake

  especially problematic for toxicity test

- Natural variations in *in vivo* phenotypes, *e.g.* behaviours

- Standardization of conditions applied: well volume, timing and mode of exposure, temperature, lighting, pH …

- Producing enough zebrafish for truly large-scale screens : !! Especially for fragile or inbred transgenic lines

OPEN ACCESS Freely available online

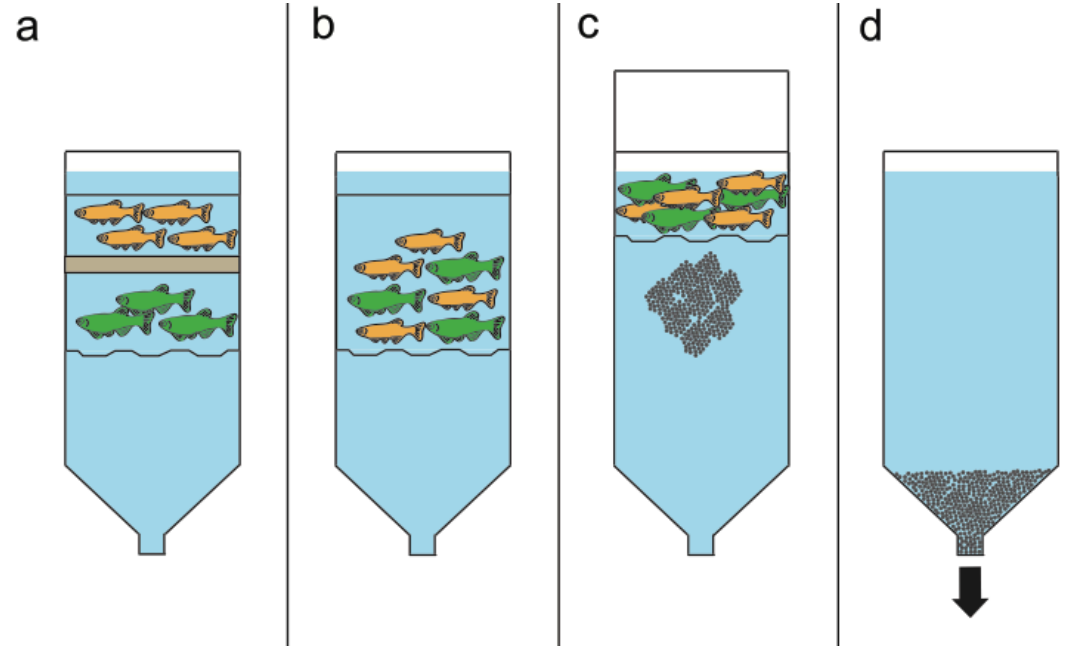
PLOS one

A New System for the Rapid Collection of Large Numbers of Developmentally Staged Zebrafish Embryos

Isaac Adatto[1,2], Christian Lawrence[5], Michael Thompson[6], Leonard I. Zon[1,2,3,4]*

1 Stem Cell Program and Division of Hematology/Oncology, Children's Hospital and Dana Farber Cancer Institute, Boston, Massachusetts, United States of America, **2** Harvard Stem Cell Institute, Boston, Massachusetts, United States of America, **3** Harvard Medical School, Boston, Massachusetts, United States of America, **4** Howard Hughes Medical Institute, Boston, Massachusetts, United States of America, **5** Aquatic Resources Program, Children's Hospital Boston, Boston, Massachusetts, United States of America, **6** Plastics Concepts, Inc., Billerica, Massachusetts, United States of America

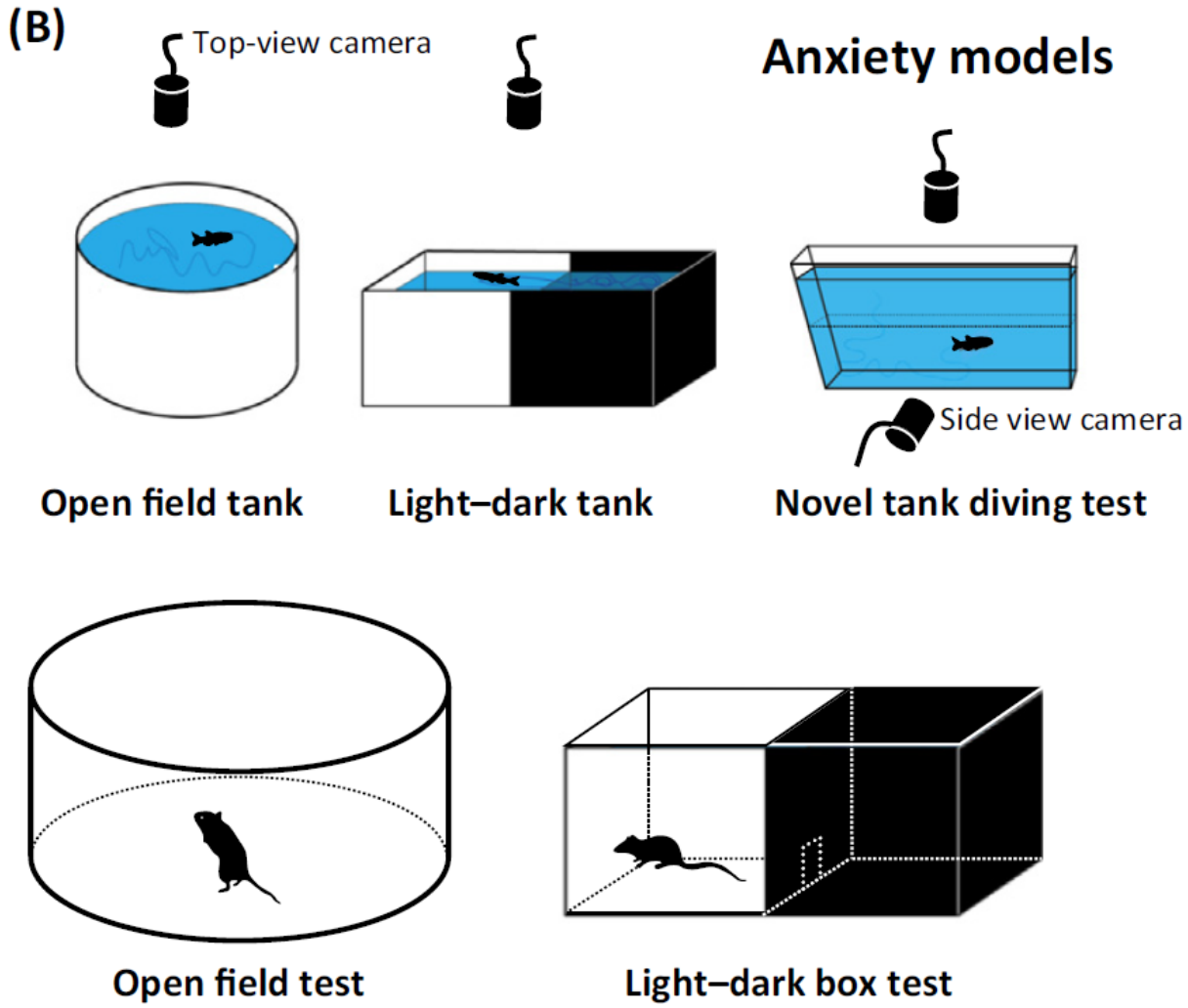
http://www.tecniplast.it/us/product/ispawn.html



(A)The breeding vessel is filled with conditioned water, and fish are added to it so that female fish and male fish are contained within the spawning platform, below and above the separator, respectively. (B) The separator is removed and the male and female fish swim together in deep water. (C) The platform is raised within the outer chamber so that the male and female fish swim together and spawn in shallow water. The fertilized embryos fall through the floor of the spawning platform. (D) After the fish are removed from the breeding vessel, the fertilized embryos that have settled at the bottom of the outer chamber are collected.

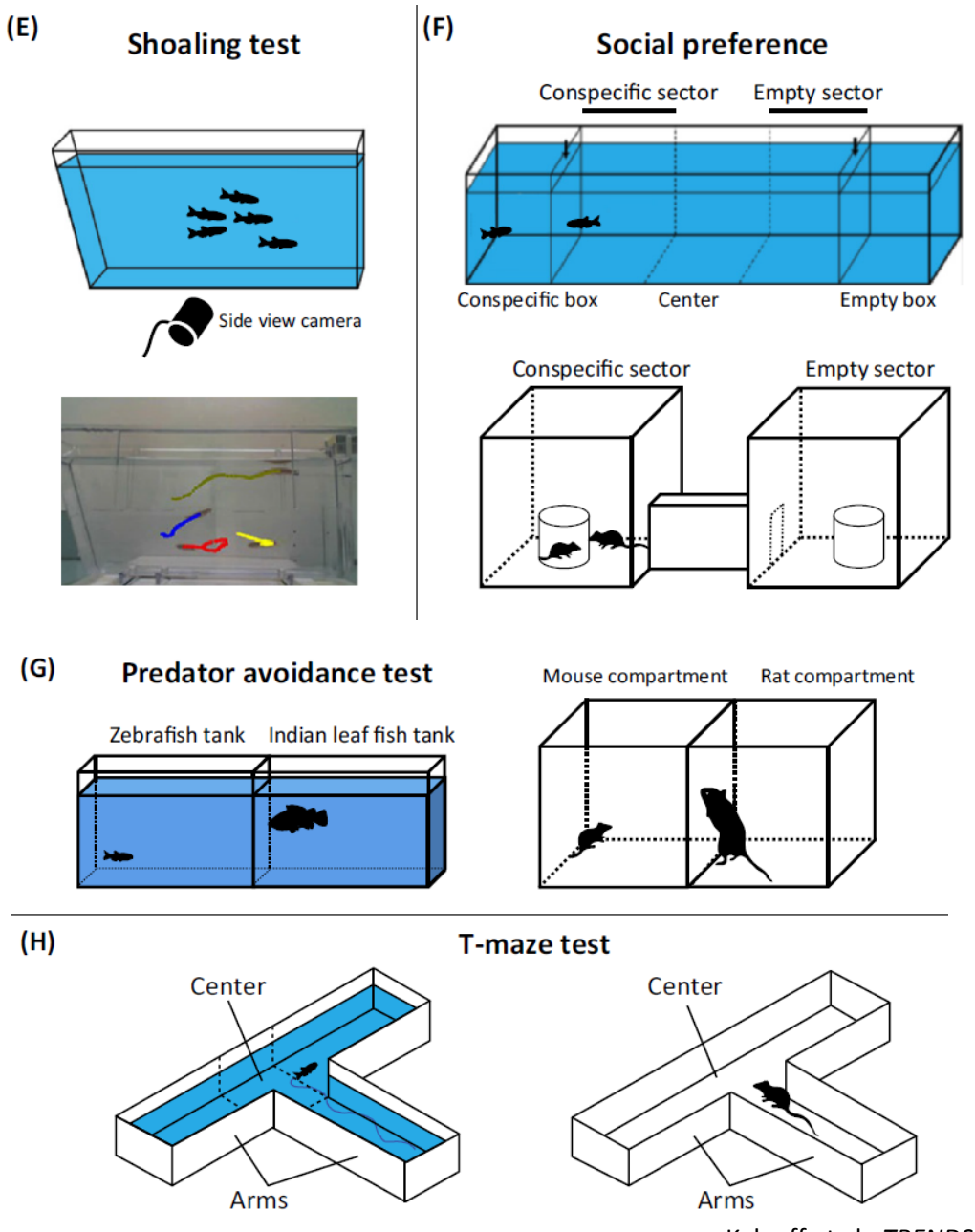
Zebrafish to study complex neurological functions that affect behaviour

Zebrafish neurobehavioral tests of exploration, anxiety, and locomotion



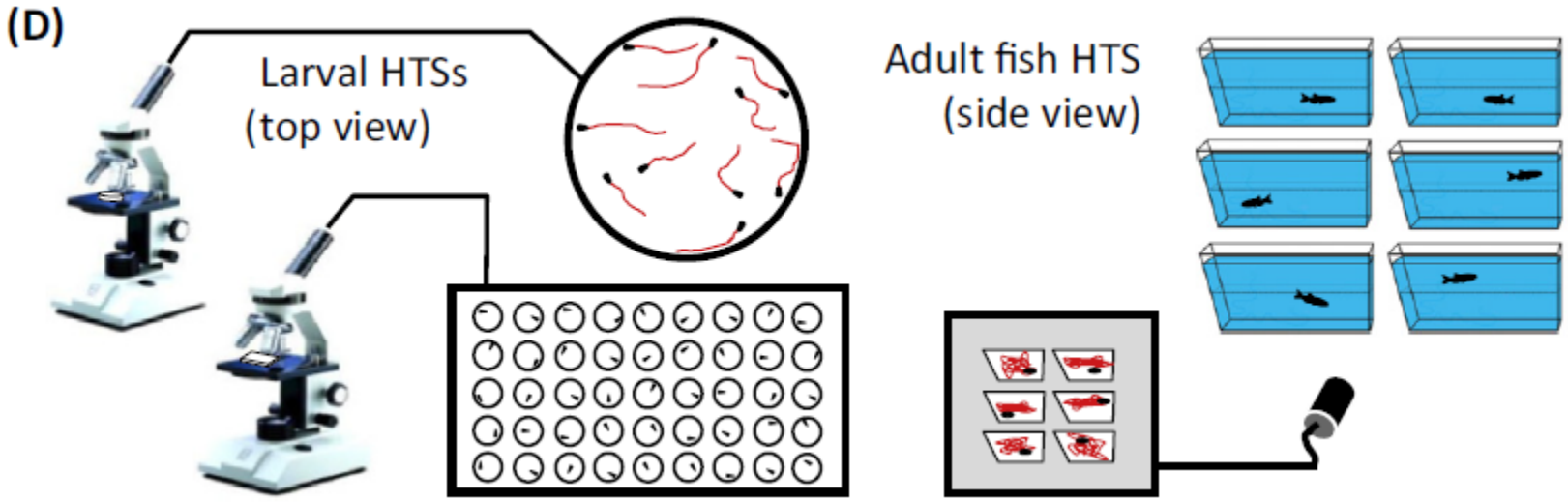
Kalueff et al., *TRENDS in Pharmacological Sciences*, 2014

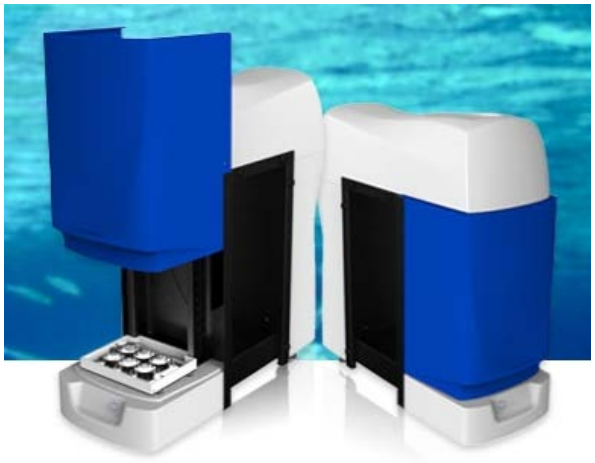
Examples of zebrafish social and cognitive behavior tests



Kalueff et al., *TRENDS in Pharmacological Sciences*, 2014

high-throughput screens (HTSs)using embryos and adult





http://www.viewpoint.fr/en/p/equipment/zebrabox

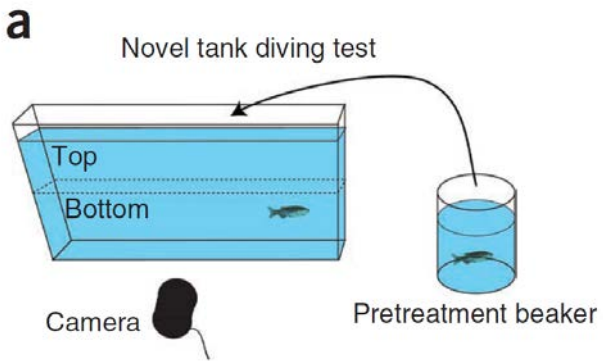
Kalueff et al., *TRENDS in Pharmacological Sciences*, 2014

Measuring behavioral and endocrine responses to novelty stress in adult zebrafish

Jonathan Cachat[1,2], Adam Stewart[1,2], Leah Grossman[1], Siddharth Gaikwad[1], Ferdous Kadri[1], Kyung Min Chung[1], Nadine Wu[1], Keith Wong[1], Sudipta Roy[1], Christopher Suciu[1], Jason Goodspeed[1], Marco Elegante[1], Brett Bartels[1], Salem Elkhayat[1], David Tien[1], Julia Tan[1], Ashley Denmark[1], Thomas Gilder[1], Evan Kyzar[1], John DiLeo[1], Kevin Frank[1], Katie Chang[1], Eli Utterback[1], Peter Hart[1] & Allan V Kalueff[1]

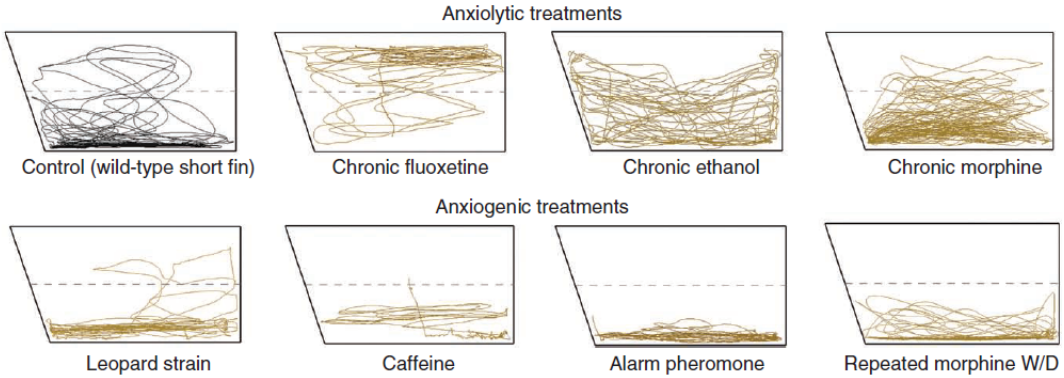
[1]Department of Pharmacology and Neuroscience Program, Zebrafish Neuroscience Research Consortium, Tulane University Medical School, New Orleans, Louisiana, USA. [2]These authors contributed equally to this work. Correspondence should be addressed to A.V.K. (avkalueff@gmail.com).

Published online 14 October 2010; doi:10.1038/nprot.2010.140



→ animal's natural instinct to seek protection in an unfamiliar environment by diving, freezing and reducing exploration.

→ As the fish gradually acclimates to the new environment, an increase in exploration (e.g., increased locomotion, decreased freezing and more entries to the top half of the tank) usually occurs



→ drug administration is performed by immersion
→ In some cases (e.g., high drug toxicity, fast hydrolysis, poor solubility in water), intraperitoneal (i.p.) injections of small (5–10 µl) volumes may also be used in adult anesthetized fish

Conclusions

- ✧ No animal model is perfect: greater degree of abstraction than for mammalian models
  ! Gene function can be divergent between zebrafish and humans
  ! Aspects of zebrafish physiology can complicate the model

- ✧ Zebrafish can be used to study and model a wide range of human diseases

- ✧ Growing community - ongoing efforts to improve methods and develop new technologies

- ✧ Growing interaction with researchers using mouse and human systems

…Thank you!