The role of fish embryos in molecular toxicology studies - challenges and solutions -

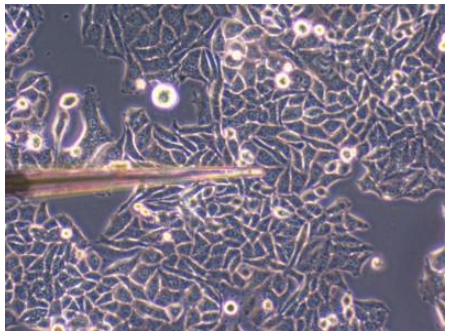
Zsolt Csenki-Bakos

Why fish?

- Mammalian model „first class"
- 3R strategy
- Atrict Animal Protection Acts
- High-throughput strategy
- Alternative models needed

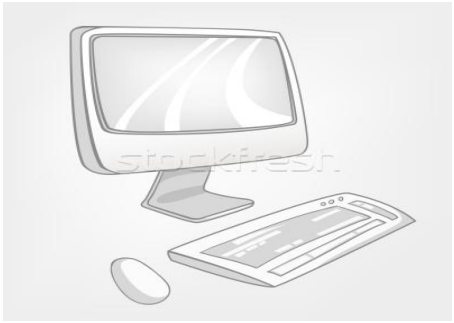
Alternative models

Cell and tissue cultures

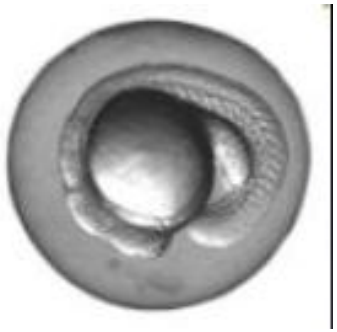


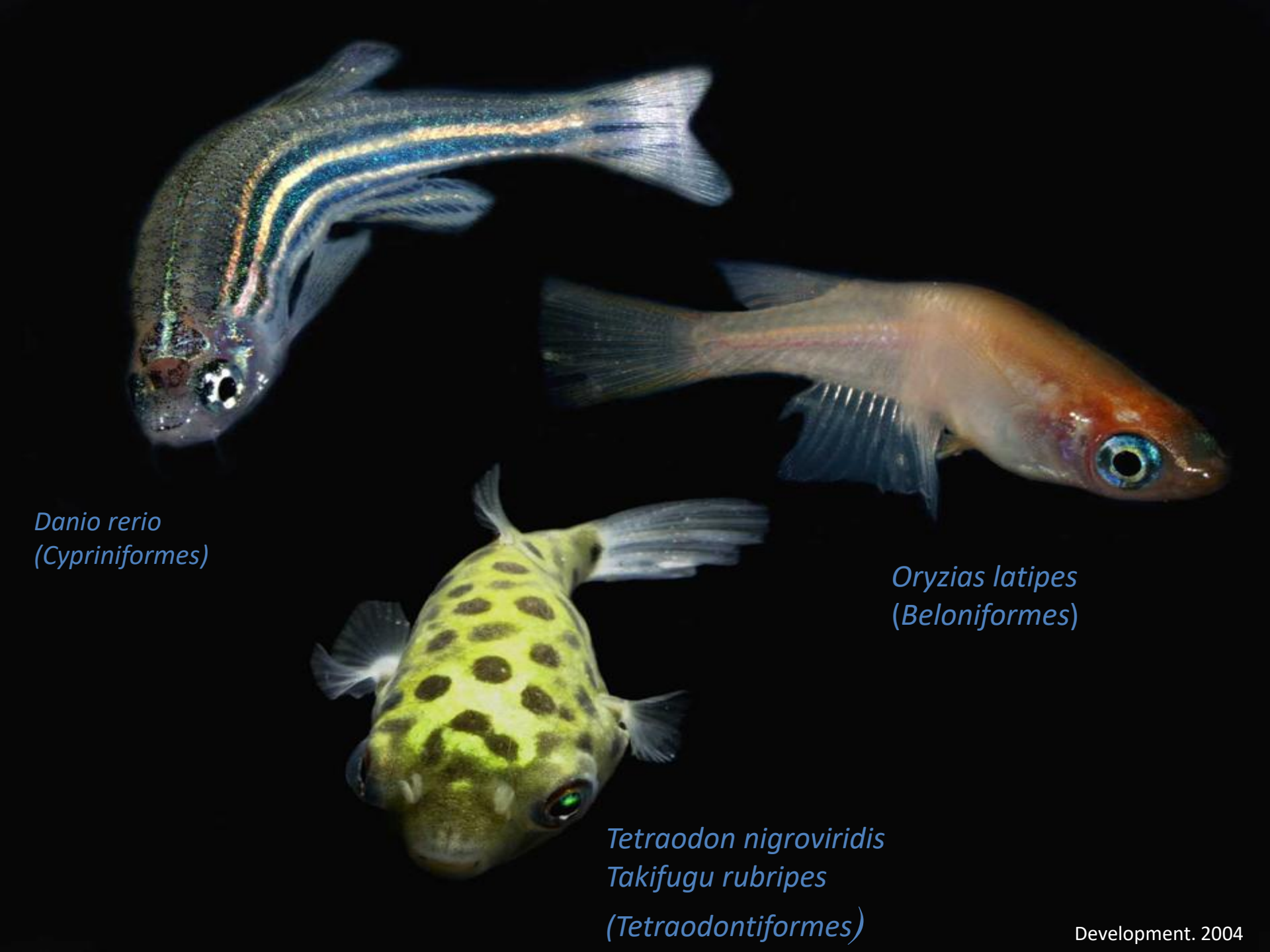
C. elegans

In silico model



Fish (embryos)





Development. 2004

Zebrafish

-Popular aquarium fish
- Peaceful nature
-Easy to keep and propagate
-Sexual dimorphism


http://diszhal.info

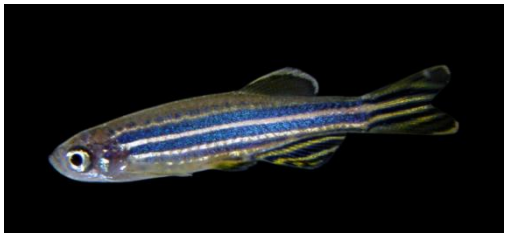

http://akvarista.gportal.hu

http://hac-aquascaping-contest.com/galeria_21.html

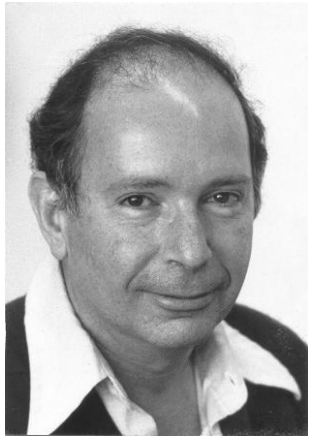

http://www.origo.hu

Why zebrafish?

- small
- short generation interval
- large number of gametes
- ex utero developing transparent embryo
- genes, receptors, molecular and physiological processes → humans
- simple and cheap experimental techniques
- full genome information
- whole life cycle in water → aquatic toxicology
- not considered animals up to the free-feeding age



The father of the zebrafish research



George Streisinger
(Streisinger György)

Scientific „multitool"

Ecotoxicology

Repr

Molec

Other

gy

rch

esearch

‘s recommendation

Developmental biology

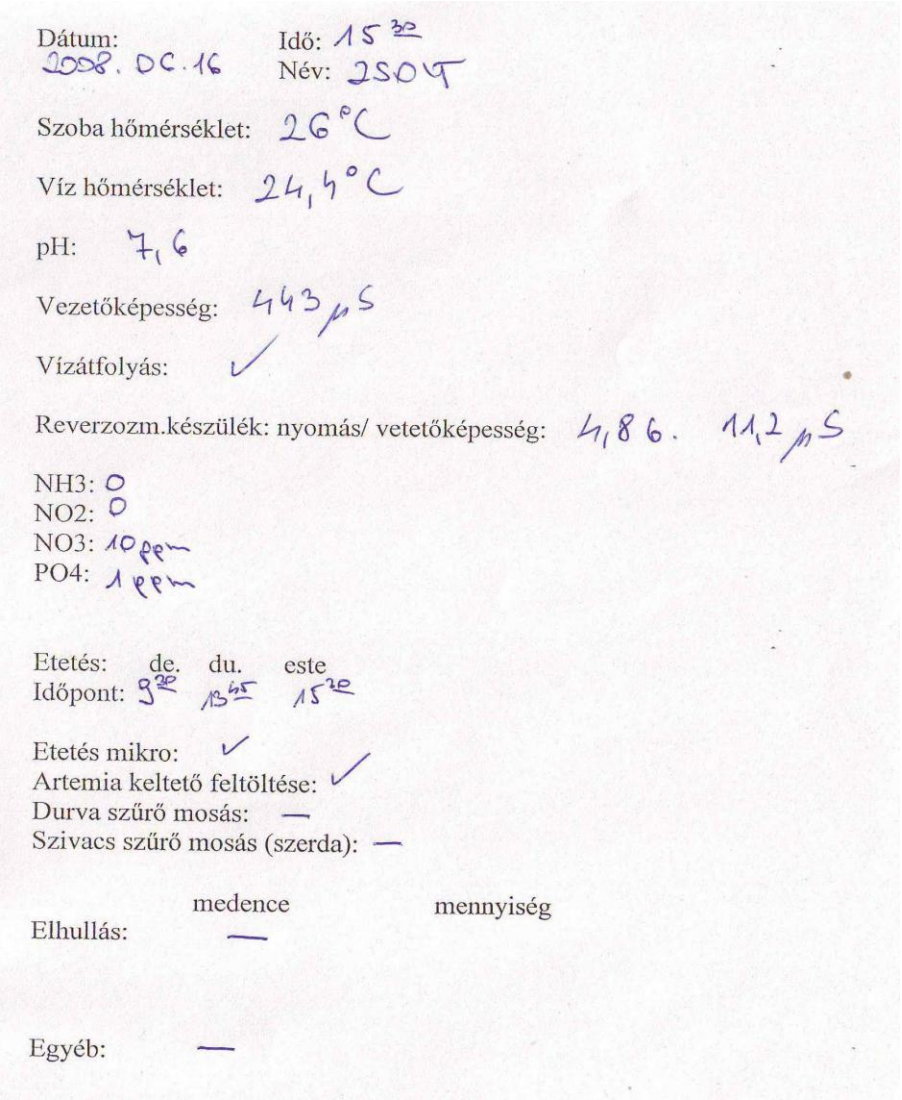
etc…

Comparison of laboratory models

			
Husbandry infrastructrure	$	$$$	$$$
Cost per animal per year	$	$$$	$$$
Characterized inbred strains	+	++++	+++
Outbred laboratory strains	+++	++	++
Storage (freezing sperm)	Yes	Yes	Yes
Transgenesis	++	++	++
Targeted gene modification	+	++++	+
Transient in vivo assays	++++	+	+
Affordability of large screens	+++	+	-
Cell lines and tissue cultures	+	++++	+

Lieschke et al., 2007

Housing conditions



Dátum: 2008. OC. 16 Idő: 15 30
Név: 2SOY

Szoba hőmérséklet: 26 °C

Víz hőmérséklet: 24,4 °C

pH: 7,6

Vezetőképesség: 443 µS

Vízátfolyás: ✓

Reverzozm.készülék: nyomás/ vetetőképesség: 4,86. 11,2 µS

NH3: 0
NO2: 0
NO3: 10 ppm
PO4: 1 ppm

Etetés: de. du. este
Időpont: 9 30 13 45 15 30

Etetés mikro: ✓
Artemia keltető feltöltése: ✓
Durva szűrő mosás: —
Szivacs szűrő mosás (szerda): —

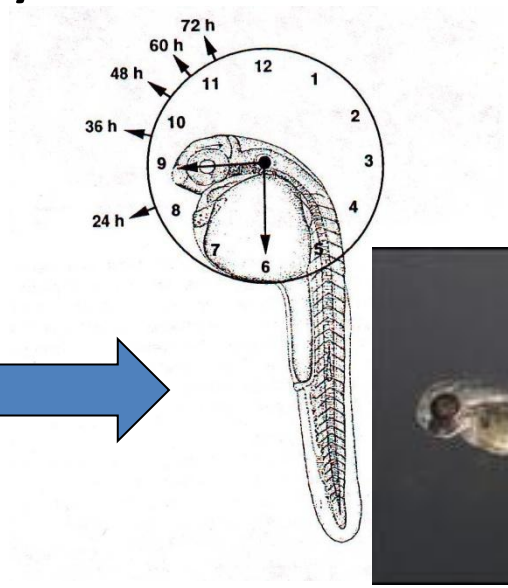
medence mennyiség
Elhullás: —

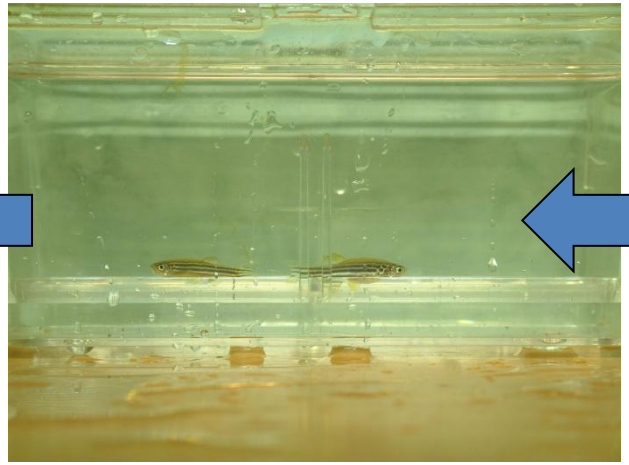
Egyéb: —

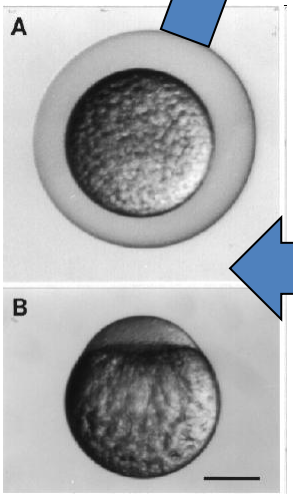
-Temperature: 25,5-27,5 C
-Conductivity: 500-600 mikroS
-pH 7,5-7,8
-Dark-light cycle (10/14 h)
(daily registered parameters)

24-hour supervision throughout the year

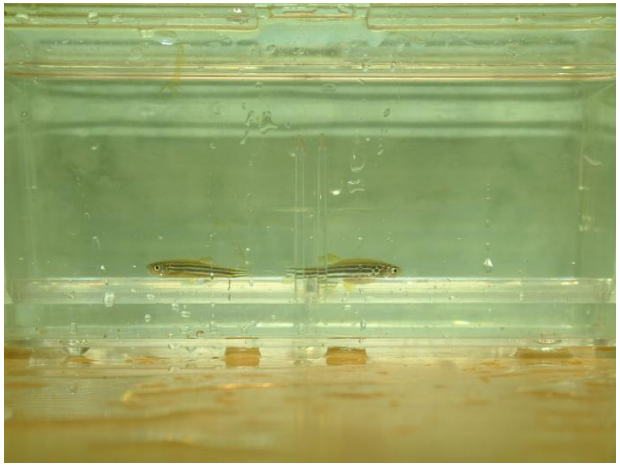
Zebrafish life-cycle in the lab



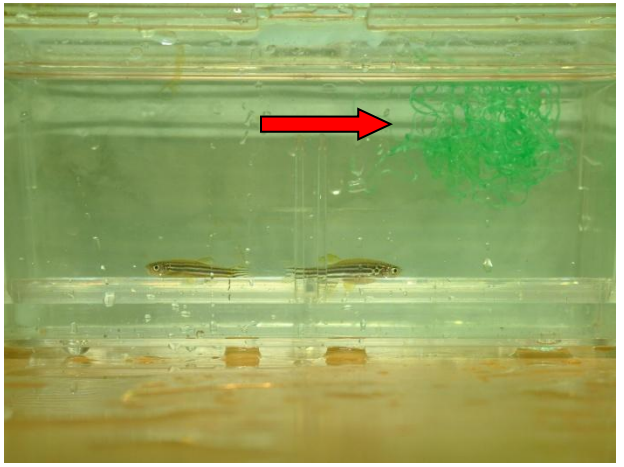




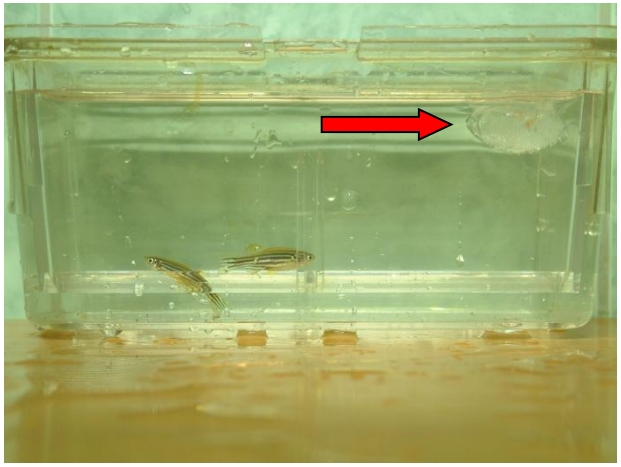
Propagation of zebrafish



Pair crossing



With plastics plant



With „stimulations”

Group crossing

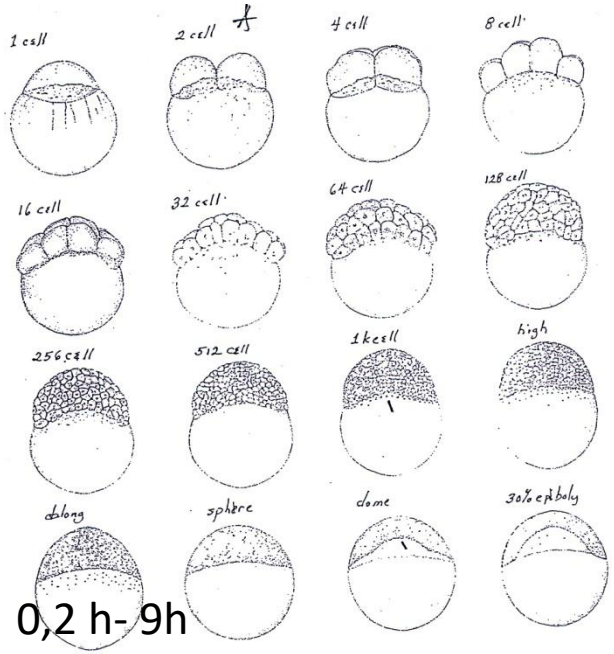
Feeding plan

Weekly feeding plan

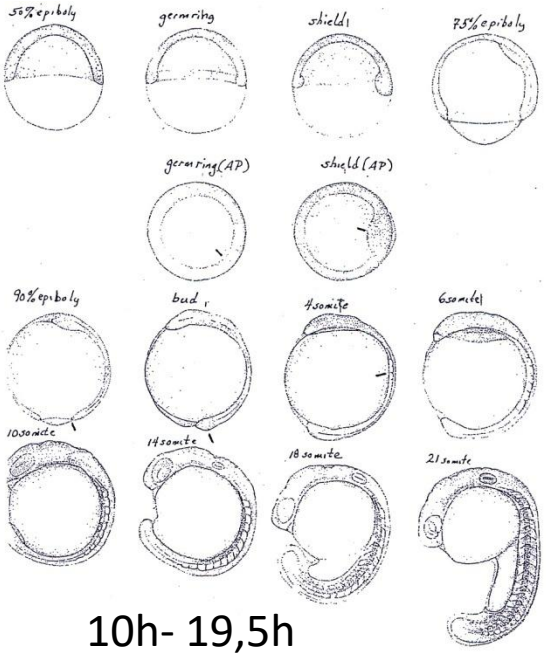
	Adult		Young			Baby*		
	morning	afternoon	morning	afternoon	evening	morning	afternoon	evening
Monday	SDS FOOD	SDS FOOD	ARTEMIA	SDS FOOD	SDS FOOD	SDS FOOD	SDS FOOD	SDS FOOD+ nematode
Tuesday	ARTEMIA	SDS FOOD	ARTEMIA	SDS FOOD	SDS FOOD	SDS FOOD	SDS FOOD	SDS FOOD+ nematode
Wednesday	SDS FOOD	SDS FOOD	ARTEMIA	SDS FOOD	SDS FOOD	SDS FOOD	SDS FOOD	SDS FOOD+ nematode
Thursday	SDS FOOD	SDS FOOD	ARTEMIA	SDS FOOD	SDS FOOD	SDS FOOD	SDS FOOD	SDS FOOD+ nematode
Friday	ARTEMIA	SDS FOOD	ARTEMIA	SDS FOOD	SDS FOOD	SDS FOOD	SDS FOOD	SDS FOOD+ nematode
Saturday	SDS FOOD	SDS FOOD	ARTEMIA	SDS FOOD		SDS FOOD	SDS FOOD+ nematode	
Sunday	SDS FOOD	SDS FOOD	ARTEMIA	SDS FOOD		SDS FOOD	SDS FOOD+ nematode	

* after the 10th day feed with artemia

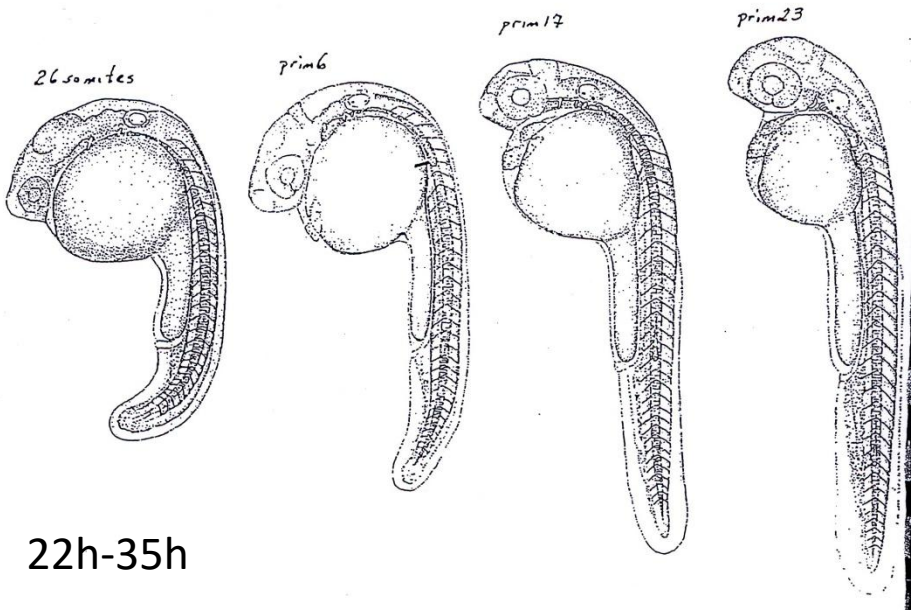
Development of zebrafish embryos (videos)



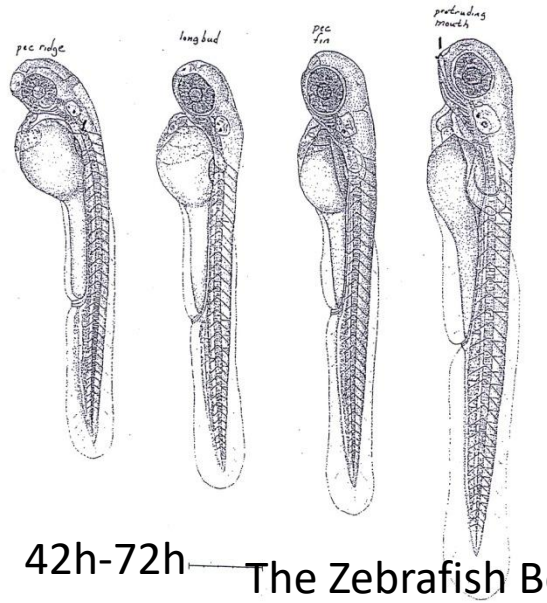
0,2 h- 9h



10h- 19,5h



22h-35h



42h-72h

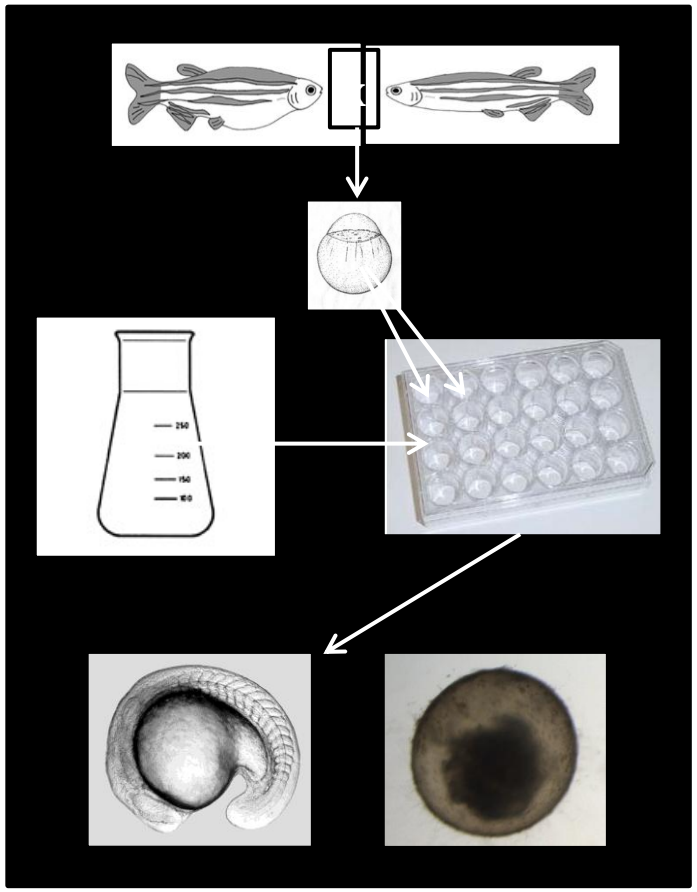
The Zebrafish Book 1995

Classical toxicity testing

- Acute (short term) tests

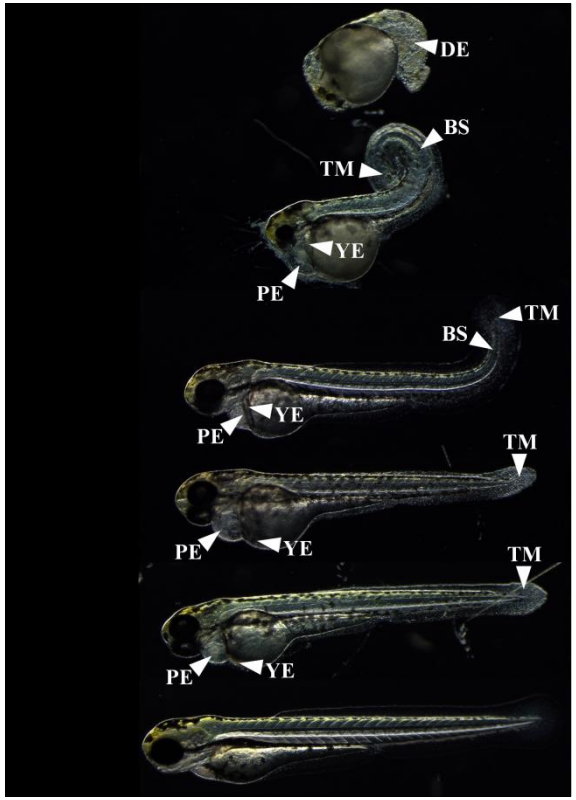
guideline	name	duration	stage	endpoints
OECD TG 236	Fish embryo toxicity test (FET)	48 h (-120 h)	embryo	number of coagulated eggs, formation of somites, tail detachment, heart beat
OECD TG 212	Fish, short-term toxicity test on embryo and sac-fry stages	before yolk sac is absorbed	embryo	hatching, mortality, appearance, behaviour, body length and weight
OECD TG 203	Fish acute toxicity test	96 h	adult	mortality

Fish Embryo Toxicity Test (FET)



- Embryos are individually exposed in 24-well plates within 1 hour
- After 24 and 48 hours the number of coagulated eggs is determined (LC50, NOEC, LOEC)

Fish Embryo Toxicity Test (FET)



- Additional endpoints: morphology, gene expression
- Duration can be expanded to 120 hours

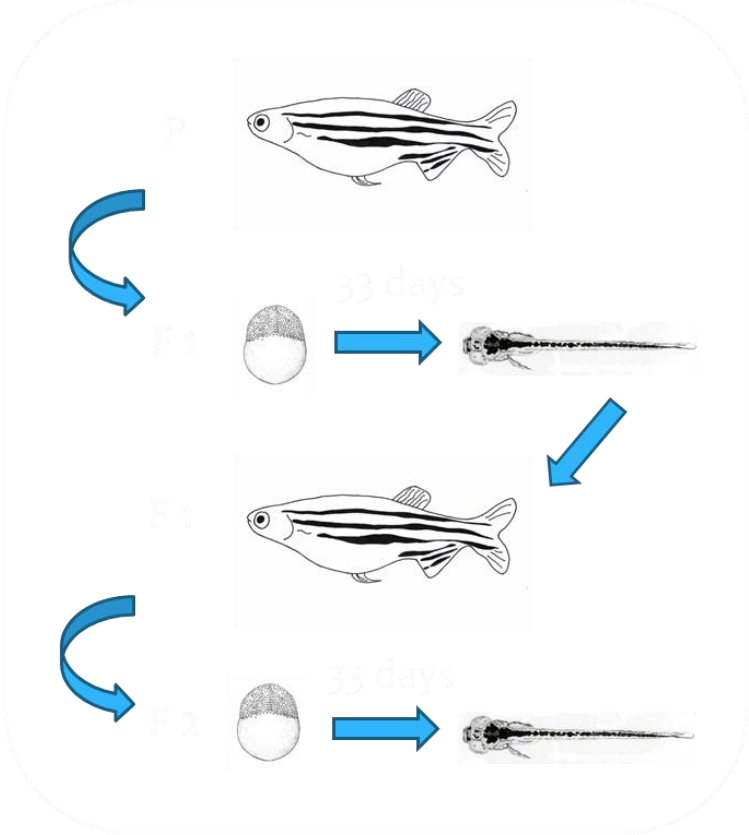
Long-term tests

- Low concentration, longer exposure >> chronic toxic effects

guideline	name	duration	stage	endpoints
OECD TG 210	Fish early life-stage test	30 days after hatching (app. 33 days)	embryo	cumulative mortality, hatching, body length and weight
OECD TG 215	Fish juvenile growth test	≥ 28 days	adult	mortality, external abnormalities, growth rate, weight
OECD TG 204	Fish prolonged toxicity test	14 days (+7-14 days)	adult	cumulative mortality, behaviour

Multi-generation test

- No exact protocol (EPA, 2002, Diekmann et al., 2004)



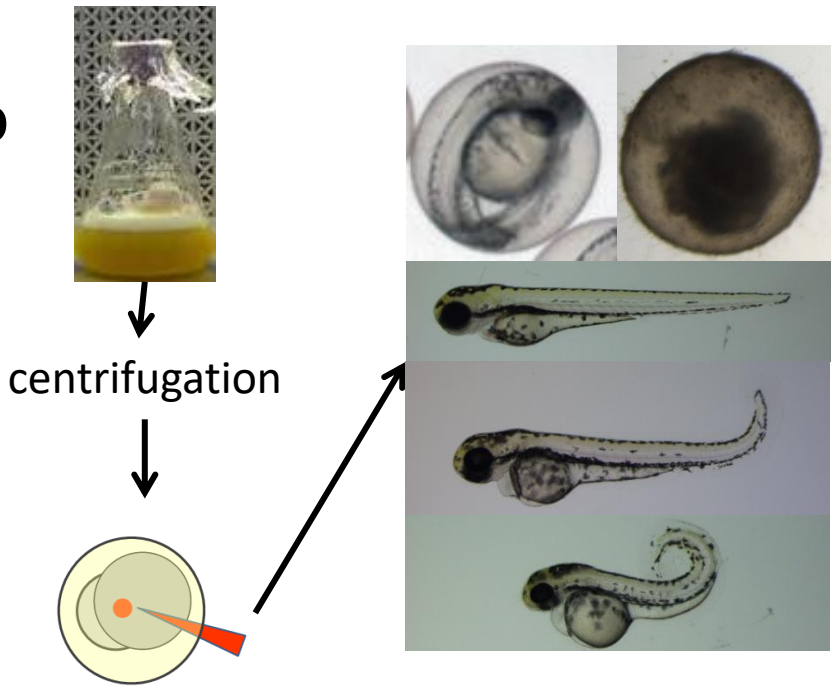
Timeline	Test day	Endpoints
Pre-test	4-7 days	secondary sex char., reproductive behaviour, spawning activity, fecundity, fertilization success
P	0-14 days	
F1	day 10-14	hatching, normal/abnormal
P	P adult termination	weight, length, sex, GSI, gonad histology, vtg, steroids
F1	day 21-week 12-14 (post hatch)	survival, length, time to maturity, sex ratio, secondary sex char., pre-spawn condition
F1	14-16 weeks	survival, secondary sex char., reroductive behaviour, spawning activity, fecundity, fertilization success
F2	14-16 weeks	hatching, normal/abnormal
F1	F1 adult termination	weight, length, sex, GSI, gonad histology, vtg, steroids
F2	16-20 weeks	survival, weight, length

Non classical alternative tests

- If organic matter content is too high
- Material can not be added to the water

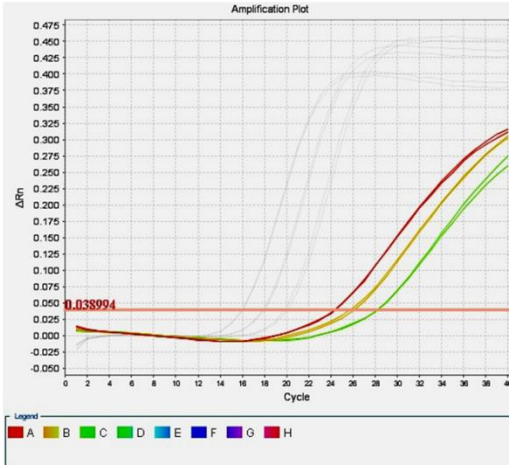
Solution: microinjection

- Used etc. for testing the degarding activity of microbes



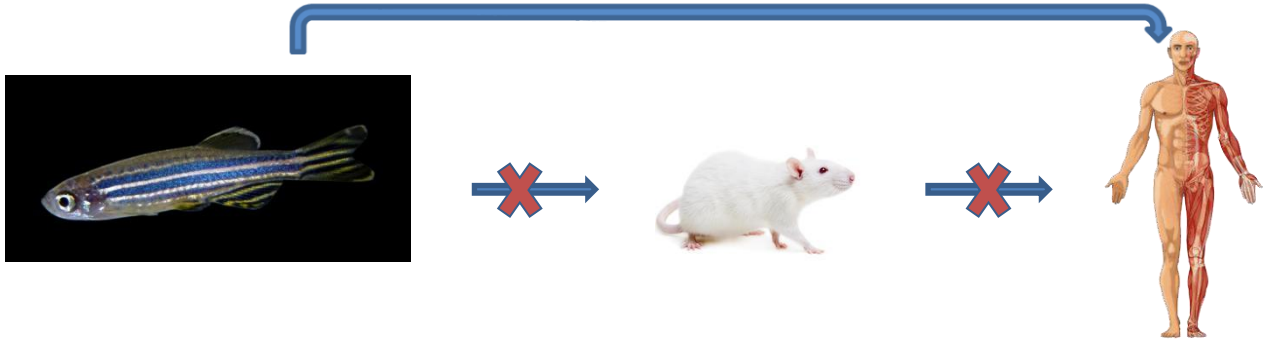
Gene expression analysis

- classical endpoints are mainly visible features (developmental disorders, lethality, etc.)
- some effects appear earlier
- can easily be deteced at the gene expression level, especially if genes specific for a certain effect (marker genes) are known.



Zebrafish to Human

direct use of test compounds in clinical testing



doi:10.1093/brain/aww342 BRAIN 2017: 140; 669–683 | 669

BRAIN
A JOURNAL OF NEUROLOGY

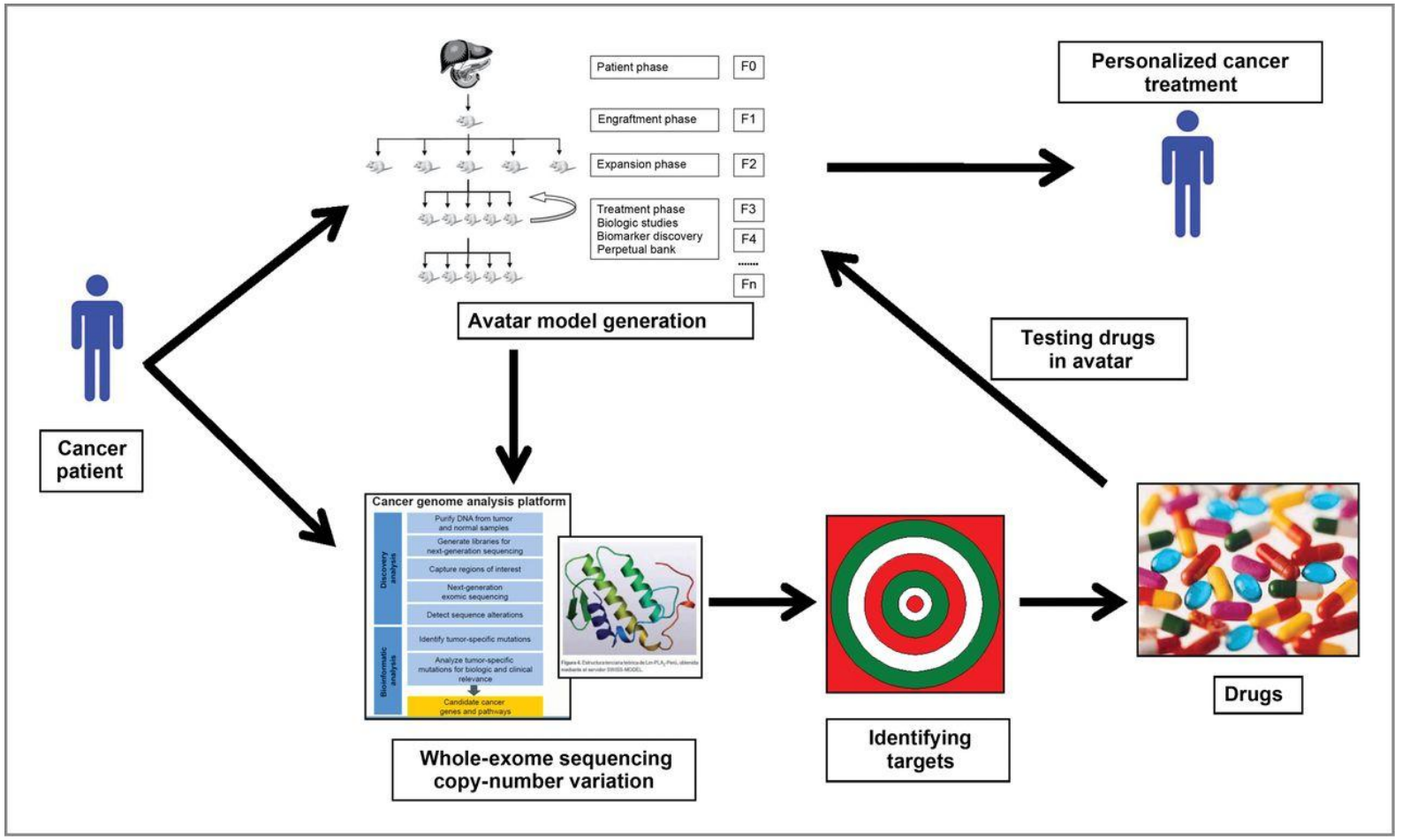
Clemizole and modulators of serotonin signalling suppress seizures in Dravet syndrome

Aliesha Griffin,[1] Kyla R. Hamling,[1] Kelly Knupp,[2] SoonGweon Hong,[3] Luke P. Lee[3] and Scott C. Baraban[1]

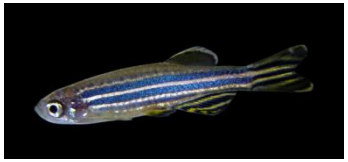
Dravet syndrome is a catastrophic childhood epilepsy with early-onset seizures, delayed language and motor development, sleep disturbances, anxiety-like behaviour, severe cognitive deficit and an increased risk of fatality. It is primarily caused by *de novo* mutations of the *SCN1A* gene encoding a neuronal voltage-activated sodium channel. Zebrafish with a mutation in the *SCN1A* homologue recapitulate spontaneous seizure activity and mimic the convulsive behavioural movements observed in Dravet syndrome. Here, we show that phenotypic screening of drug libraries in zebrafish *scn1* mutants rapidly and successfully identifies new therapeutics. We demonstrate that clemizole binds to serotonin receptors and its antiepileptic activity can be mimicked by drugs acting on serotonin signalling pathways e.g. trazodone and lorcaserin. Coincident with these zebrafish findings, we treated five medically intractable Dravet syndrome patients with a clinically-approved serotonin receptor agonist (lorcaserin, Belviq®) and observed some promising results in terms of reductions in seizure frequency and/or severity. Our findings demonstrate a rapid path from preclinical discovery in zebrafish, through target identification, to potential clinical treatments for Dravet syndrome.

Personalized medicine

-Avatar models-

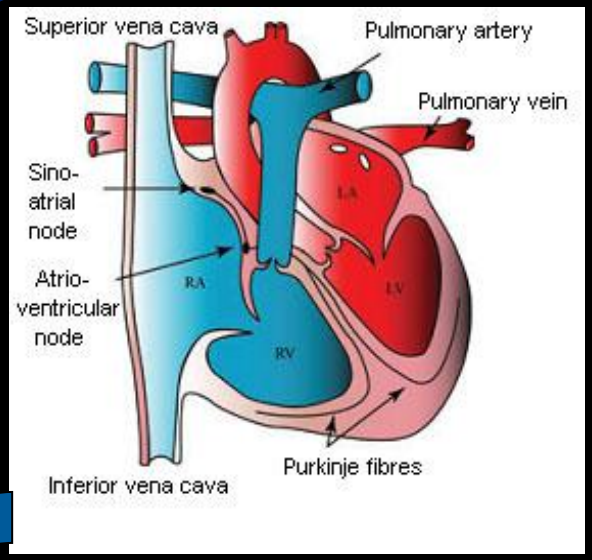


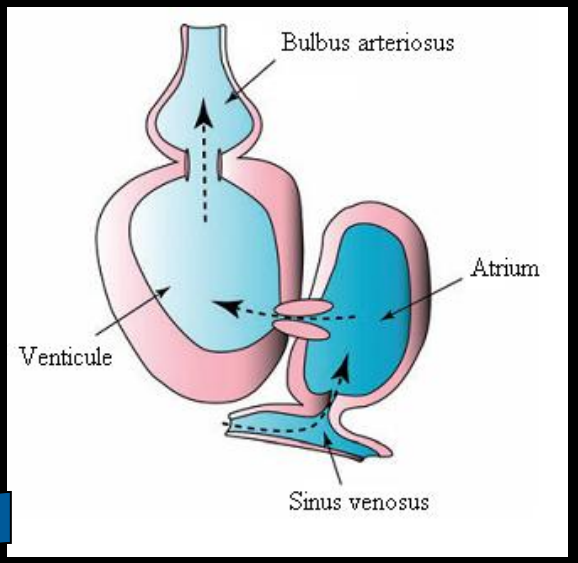
Fish - avatar

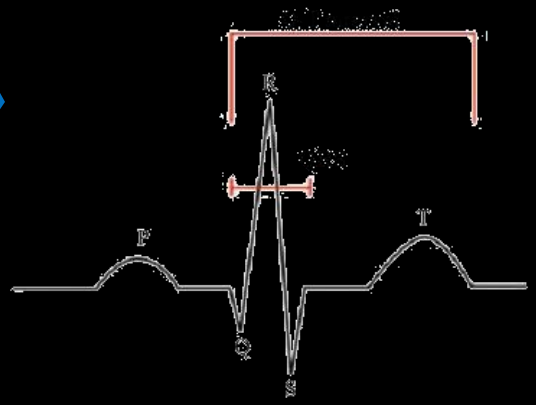


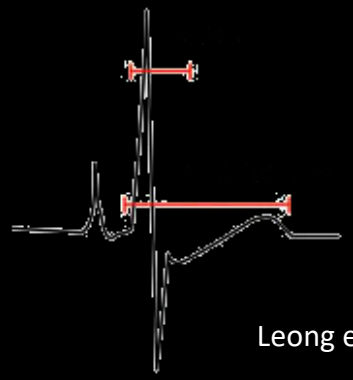
- Faster
  Cheaper
  More testing options
  Before 5 days, there is partically no immune system for fish
  Animal protection laws do not regulate
- Human cells --- Zebrafishn cells have a different temperature requirement - this problem is still waiting to be solved

ECG in zebrafish





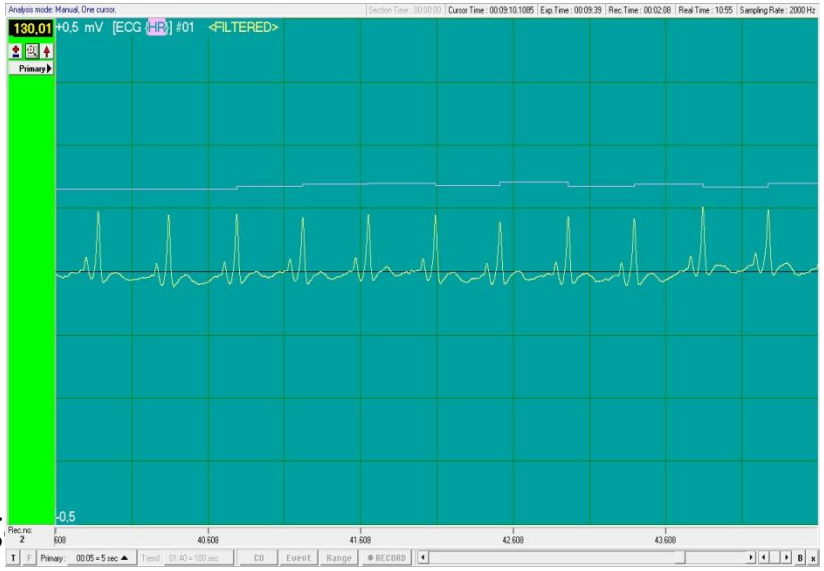




Leong et al. (2010)

ECG in zebrafish

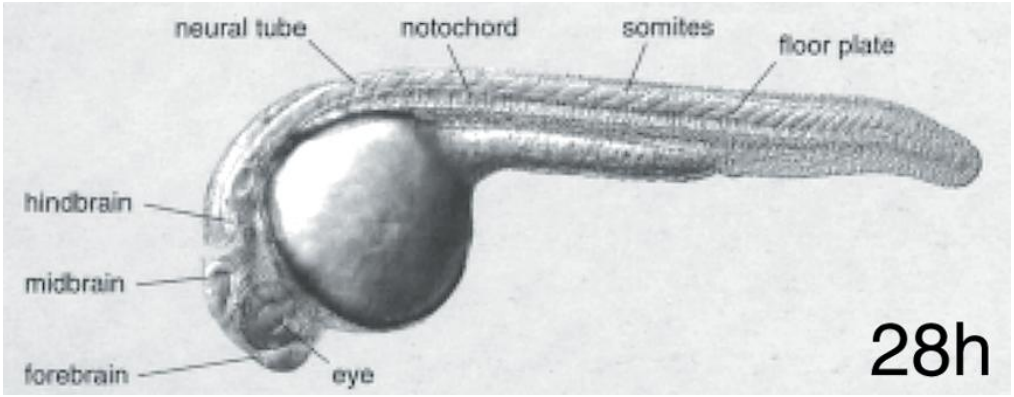
- Cardiotoxicity, safety pharmacology
- May be performed in adults and embryos
- In adults:

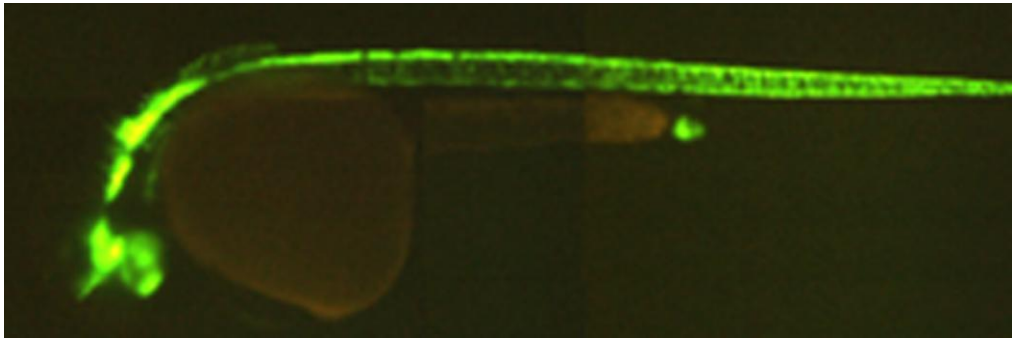


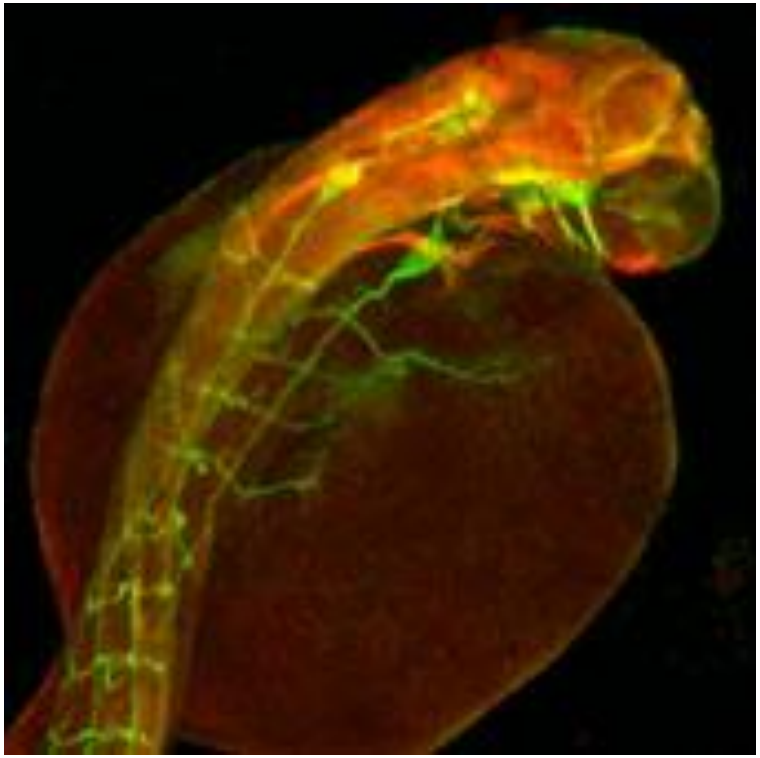
In collaboration with Experimetra Ltd. (Hung

Transgenic zebrafish

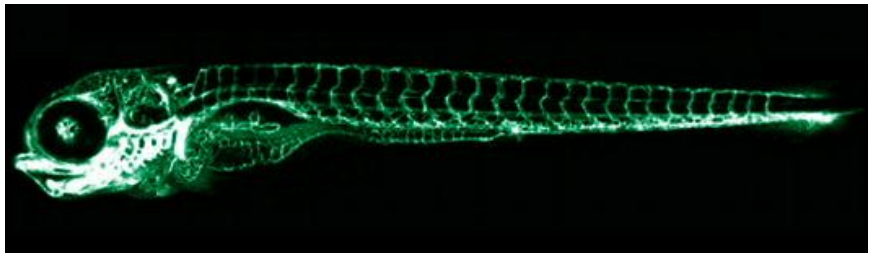
GFP (Green fluorescent protein)



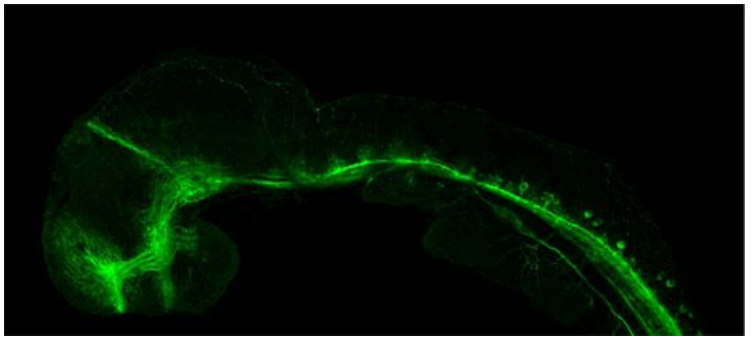




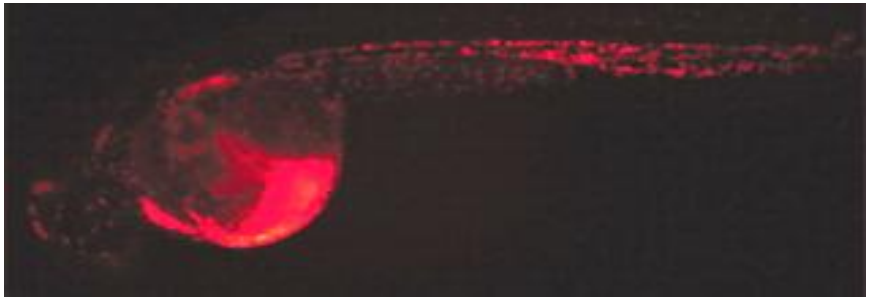
Transgenic zebrafish lines in toxicology



Fli-1 (Friend leukemia integration site-1)
(GFP: green)



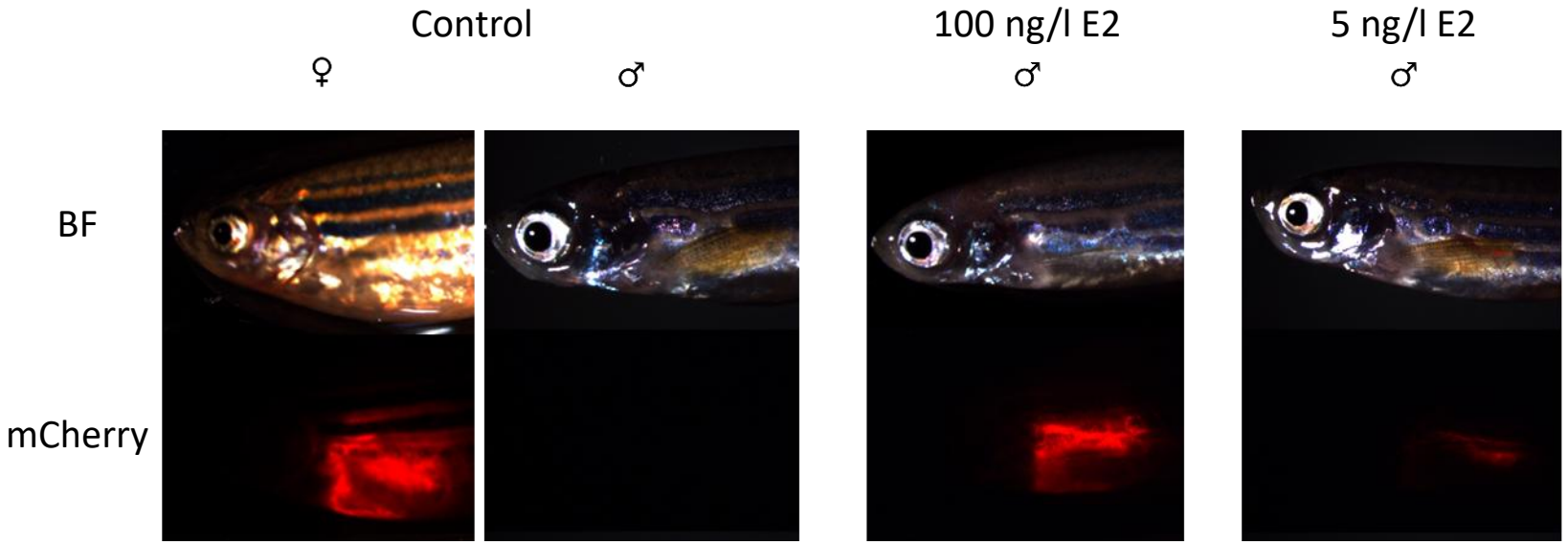
Neurogenin
(GFP=Green)



GATA-1 (globin transcription factor-1)
(DsRed, red)

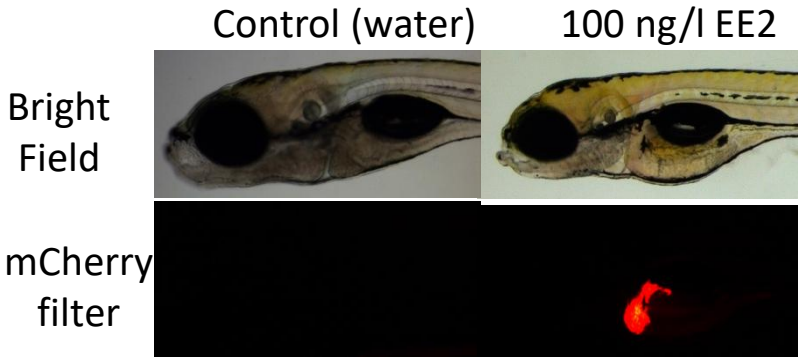
Detection of estrogenic substances: vtg1:mCherry

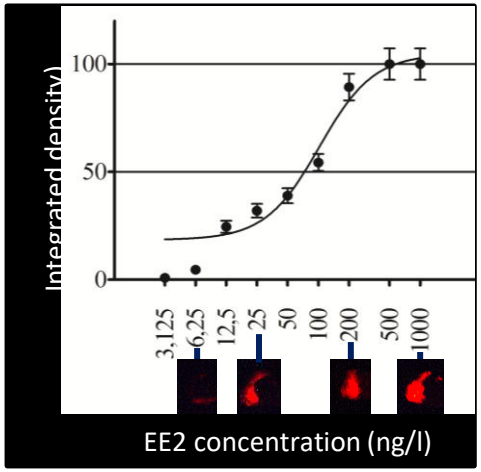
- **<u>Inducible promoters</u>**: detection of specific substances, concentration dependent response
- Estrogen sensitive, liver-specific promoter (vtg) + red fluorescent protein (mCherry)



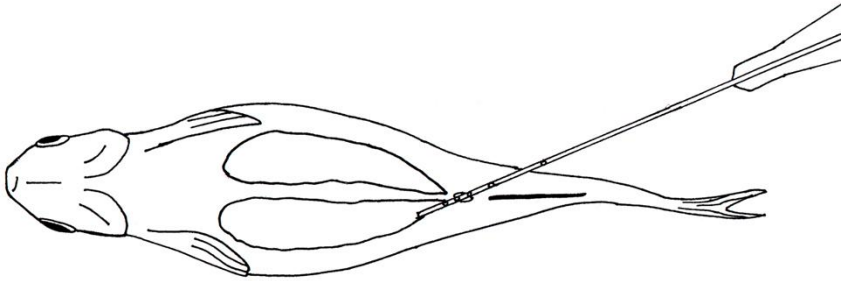
Development and validation of transgenic biomarker lines

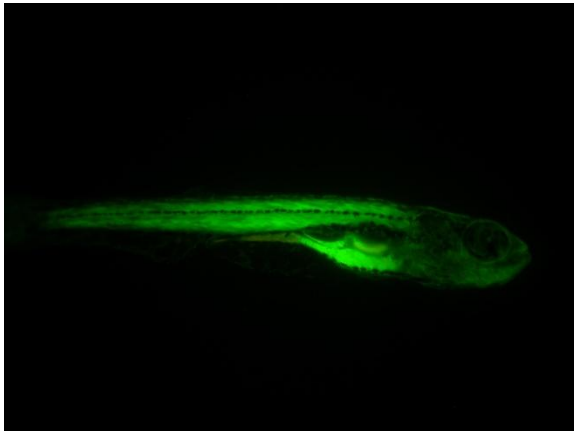
- Fluorescent proteins under the control of zebrafish endogenous promoters
- Toxicology model (vtg1:mCherry, slc5a5:mCherry)
- Pharmacology model (TetOn:GRß:Venus)

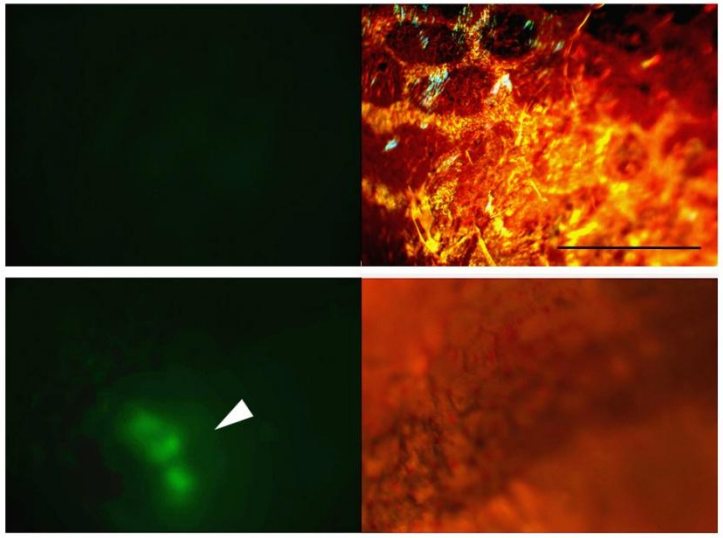


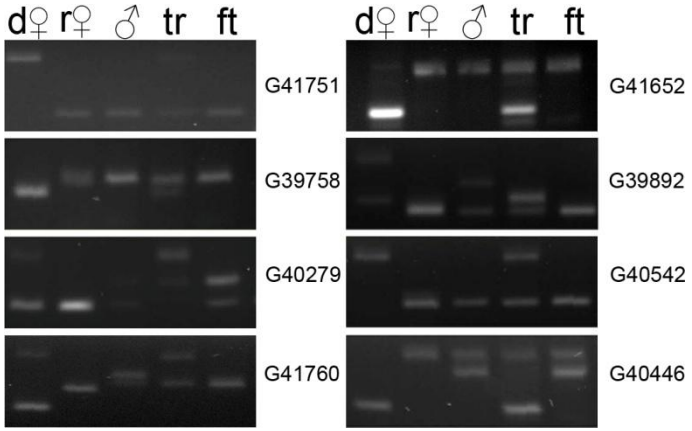


Follicle transplantation in zebrafish









Csenki Zs. és mtsai 2010

Atlanti tok (*Acipenser sturio*)

Európai angolna (*Anguilla anguilla*)