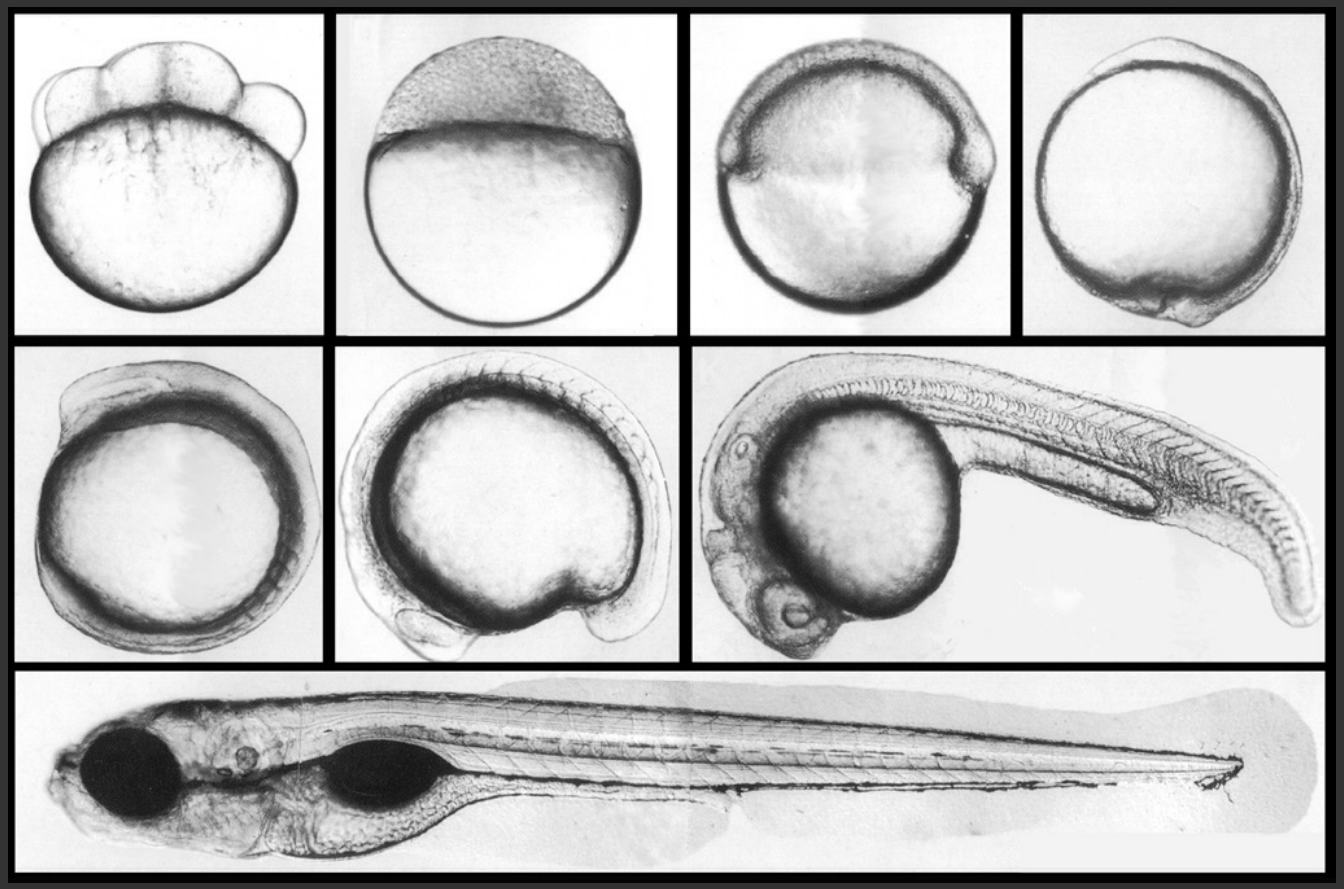
Zebrafish as a model organism

Susana Lopes
BDA
FCUL

Development is easy to follow



Haffter et al. *Development* (1996)

Zebrafish Biology

- Zebrafish, *Danio rerio*, is a teleost (bony fish)
- Adult size: 3-4 cm length
- Life cycle: 3-4 months
- Breed easily in the lab
- Lifespan: 2-3 years
- High fecundity and fertility (hundreds of eggs per clutch)
- External fertilization and external development
- Development is fast and transparent (tiny fish @24hpf)

Zebrafish Ecology
Life in the wild

- Origin: Asia (India, Pakistan, Nepal)

- Inhabits streams, canals, ditches, ponds, and slow-moving to stagnant water bodies, including rice fields

- Omnivorous

Zebrafish Research
early days

George Streisinger
University of Oregon

- Pioneered the study of zebrafish in the 70s.

- Zebrafish can be genetically modified easily, and researchers can modify them to mimic the traits of certain human diseases.

Zebrafish Research becomes popular in the 90s

- Speedy expansion due to two large scale forward genetic screens initiated in 1992-93
- Boston and Tubingen Screens

Christiane Nüsslein-Volhard

Wolfgang Driever

Danio rerio genome sequencing project

Started in 2001 @ Sanger Institute

LETTER

OPEN

doi:10.1038/nature12111

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

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

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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A genetic screen for mutations affecting embryogenesis in zebrafish

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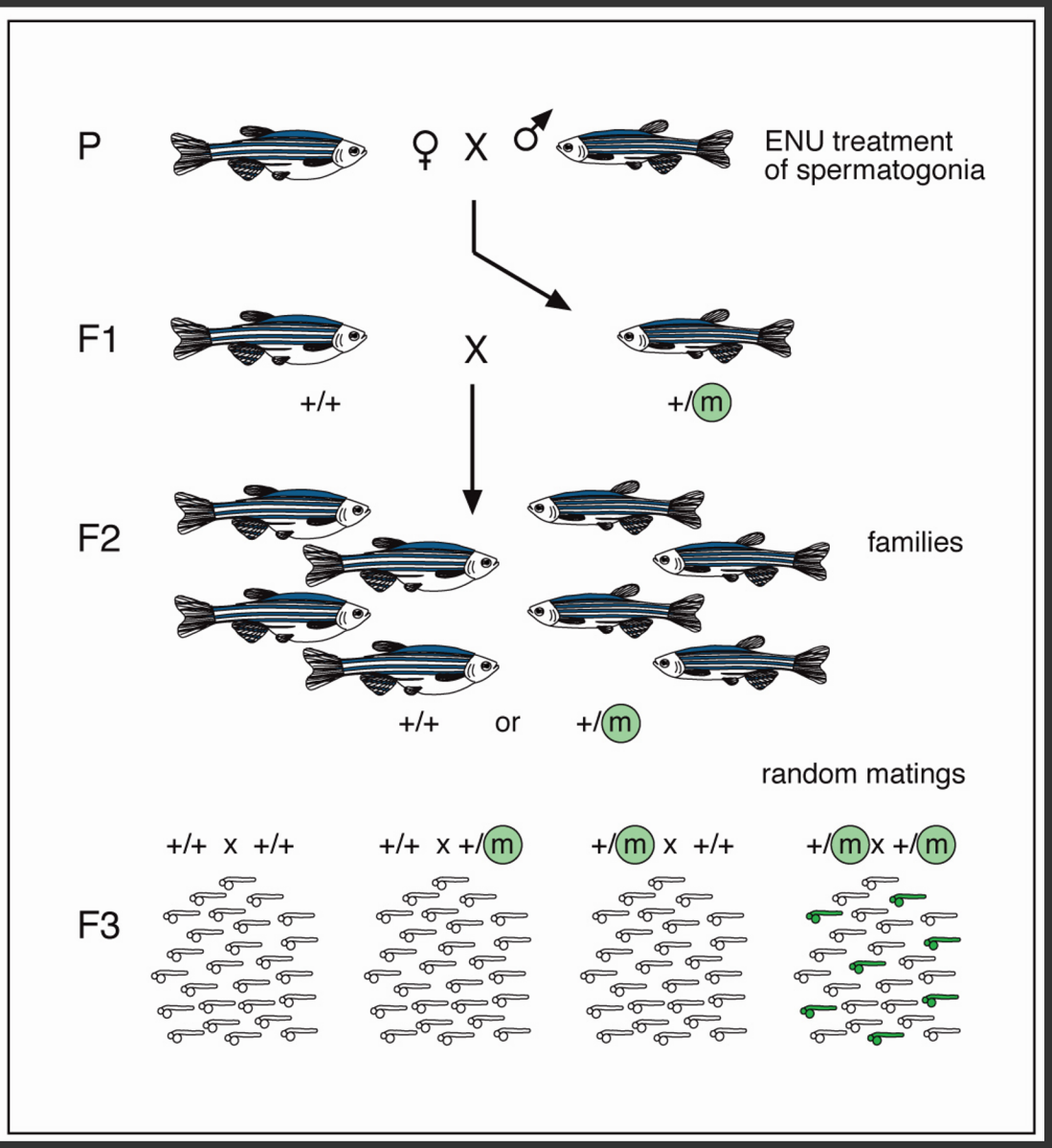
large-scale genetic screens



300 ENU
founder males

5.000 F2
families

2.000 mutated
developmental
genes

Haffter et al. *Development* (1996)

Forward genetics

From mutant to gene

Positional cloning

↓

Find the chromosome

↓

Find the closest z markers

↓

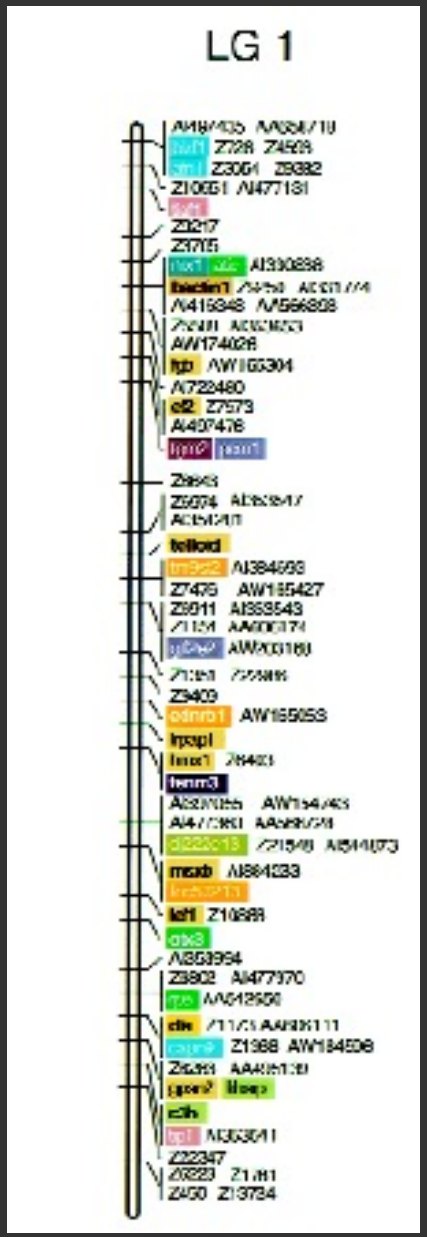
Genomic walk

↓

Narrow the Genomic interval

↓

Identify gene of interest



Reverse Genetics

From gene to mutant

- Morpholinos
- Tilling
- Zinc-Finger Nucleases
- TALENs
- CRISPRs

Screens using zebrafish

DRUG DISCOVERY

Zebrafish – an *in vivo* model for drug screening

Various characterisics of the zebrafish make it an ideal tool for drug screening.

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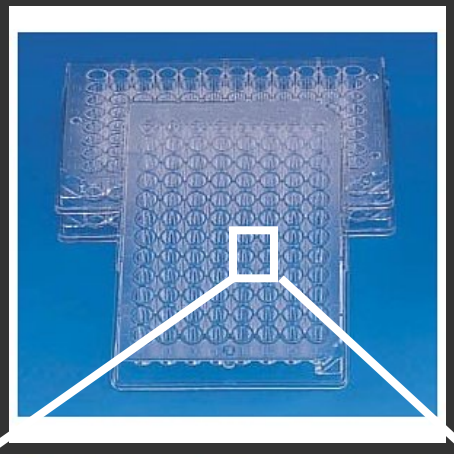


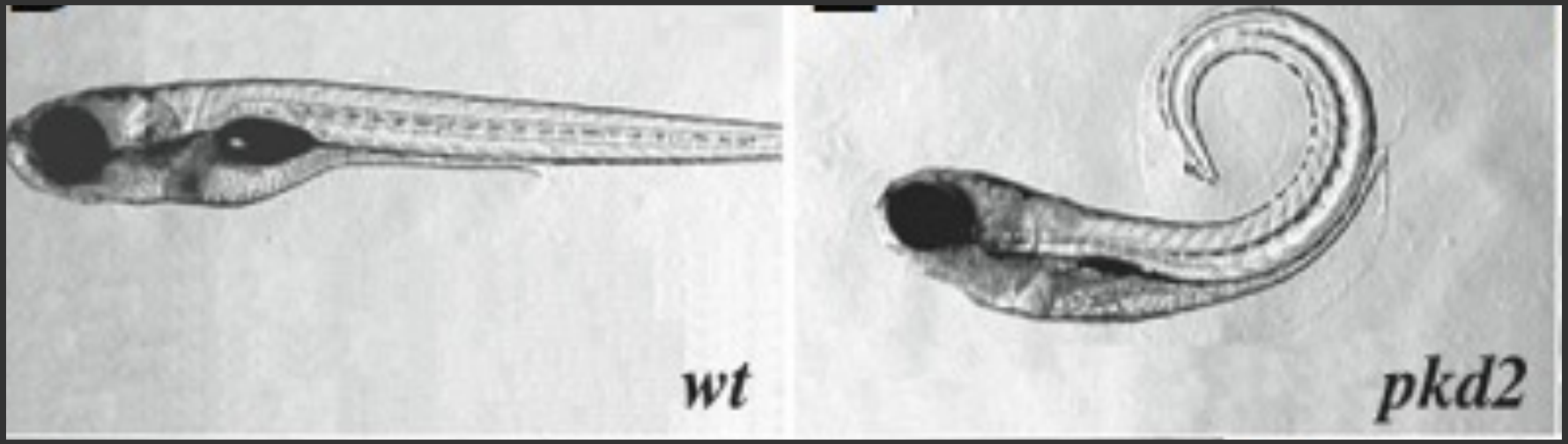
Above: Zebrafish hatchlings in 96-well plate for 'high throughput' drug testing

High throughput chemical genetic screens for compounds with specific biological activity in a whole organism are feasible using zebrafish.

Polycystic kidney disease

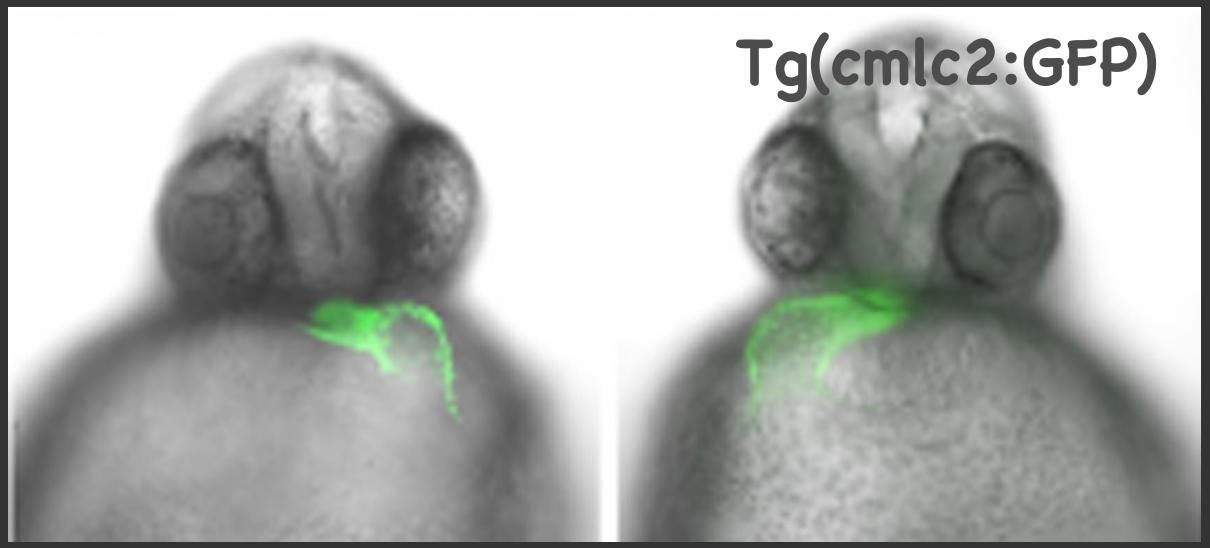
Rescue screens are potent to find potential therapies

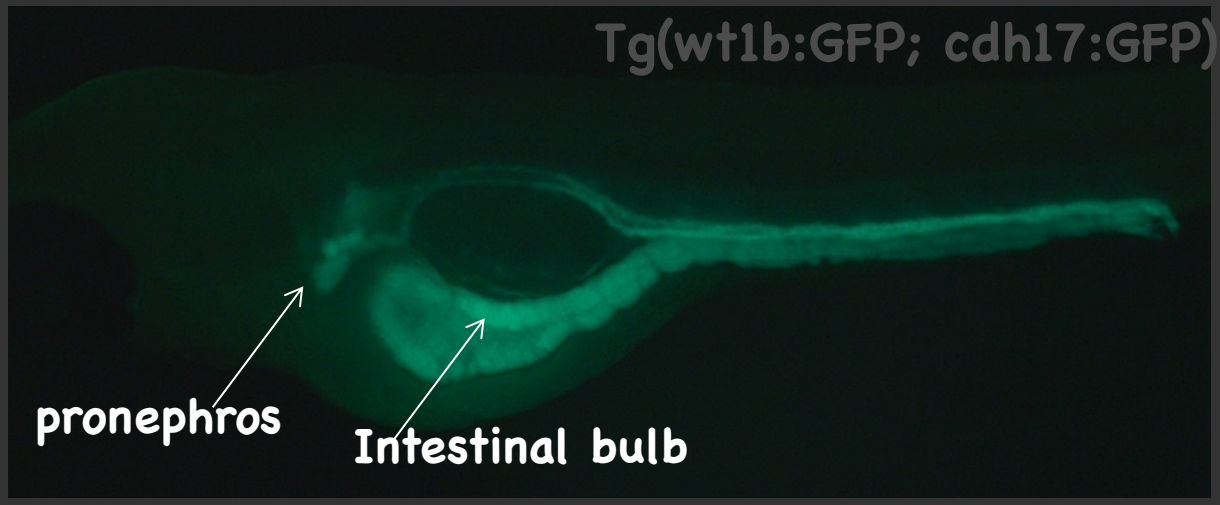
Polycystic kidney disease (1:1000)



Cao et al. PNAS (2009)

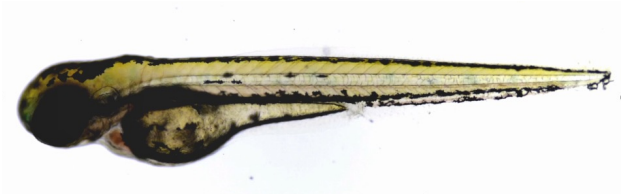
Reporter lines are useful tools for drug screens





Zebrafish screens can involve large numbers

Zebrafish larvae

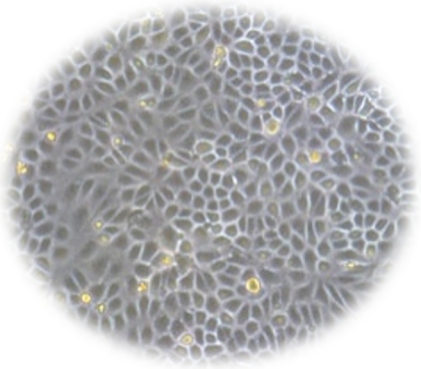


According to *Directive 2010/63/EU* *larvae* do not count for animal statistical reports up to 5 days old

Complementarity

Cell Culture

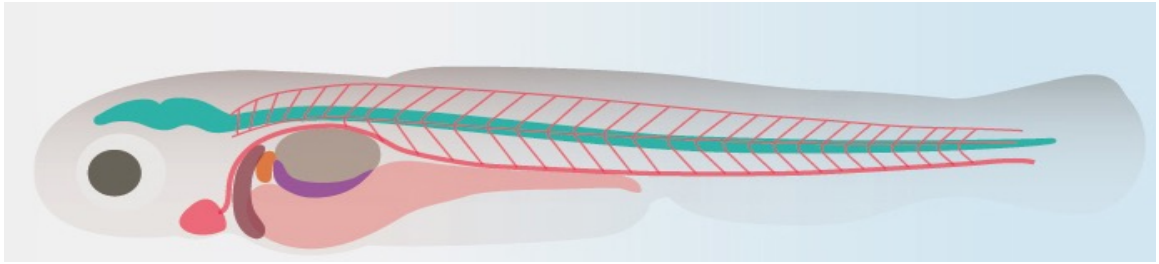
Very limited to assess organ physiology



Zebrafish larvae

live vertebrates with organs

Vertebrate biomedical model



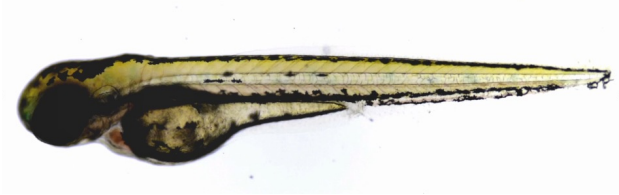
- LIVER
- PANCREAS
- GALL BLADDER
- CIRCULATORY SYSTEM
- DIGESTIVE SYSTEM
- LUNGS/ SWIM BLADDER
- CENTRAL NERVOUS SYSTEM

> 70% shared genes

From: *https://animal.research.utah.edu/faqs.php*

Summary

Living test tubes



Similar to humans

Fast results on hundreds of larvae

Why Zebrafish?

Husbandry

Needs recirculating systems

- Mechanical filtration, biological filtration, UV sterilization and carbon filtration.
- Easy maintenance
- Stable water quality
- Controlled conditions

Advantages and disadvantages of zebrafish

+

- Everyday 100 eggs per couple (we cross 10 couples usually)
- Genetics as in Drosophila (high numbers of embryos)
- Generation period 3 months - as in mouse
- Easy to breed and cheaper maintenance than mouse cages
- Transparent embryogenesis out of the mothers body (better than medaka)
- Transplantations are possible - as in chicken (but no genetics in chicken)
- Screens can be both by forward or reverse genetics
- Gene knockdown easy and under control
- Gene editing blooming (CRISPR/cas make it easier
- Live imaging - unbeatable model
- Transgenics can be induced by temperature or light
- Beautiful

−

- Duplicated genome sometimes leads to some genetic redundancy
- Not a mammal

Breeding box