Progress of the
European Zebrafish Resource Center

EZRC
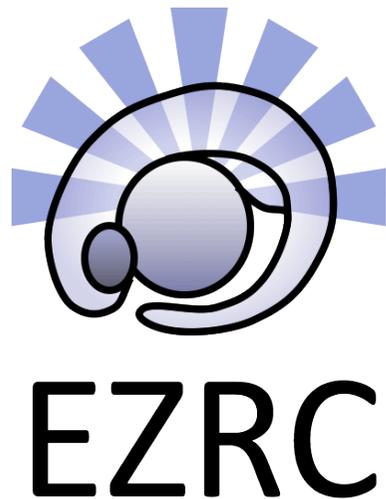
Aims

- Provide a **permanent repository** for zebrafish lines from European labs, including the Tübingen und Freiburg ENU screens, and the ongoing Sanger TILLING project (genome-wide knockout of protein-coding genes)

- Provide **easy and affordable access** to these lines for European labs

- **Mirror** popular lines from the U.S. resource center, ZIRC

- Provide **sequencing** services for SNP mapping and transcript counting

- Act as a **screening center**, hosting shelf screens and chemical screens

Status (I)

- **Funding** from the Helmholtz Society (mainly for equipment and construction), Tschira Foundation (for the first 3 years), European Commission (for work done as part of the ZF-HEALTH EU project)

- **Construction is finished**:

  - Main fish room and molecular biology lab in one building
  - Quarantine room and screening fish room in a separate building
  - Cryogenic freezers and sperm-freezing facitilies in both buildings

- **Approx. 3,500 tanks**, approx. 6,500 including experimental facility

- **Staff of 11**: Scientific director (part-time), managing director, business manager, secretary, husbandry manager, four animal keepers, two lab technicians, database programmer (part-time)

Status (II)

- Official opening was on **July 18, 2012**

- **Database** developed in house, now used for frozen stocks and being tested for fish stocks, will later be used for all experimental fish as well

- **SOPs** being developed for quality assurance

- Stock list will be available from <**itg.kit.edu/ezrc**>, pricing similar to ZIRC, webshop integrated with the model organism database, ZFIN is planned

What is available

- **Lines**

  - Initially a limited number of lines available (approx. 10 wildtype, 80 mutant and 130 transgenic)
  - All Sanger KO lines from summer 2013 (same as ZIRC, not outcrossed)
  - All Tübingen lines from summer 2013
  - MTA similar to ZIRC (non-commercial use, no redistribution, provider and EZRC must be acknowledged)
  - Shipment as bleached embryos (FedEx or GO Express within the EU, World Courier outside the EU)

- **Submissions**

  - Accepting **submissions**, same submission procedure as ZIRC
  - Submission as bleached embryos, will be grown up in quarantine for one generation, then frozen or transferred to the core fishroom

Sanger Mutants (TILLING project)

- Starting with the current library of mutagenized fish, EZRC will receive 2 sperm samples of each sequenced F1 fish, 3 will go to ZIRC and 3 will be retained by Sanger

- F1 fish contain approx. 15 putative KO mutations, outcrossing and genotyping will have to be carried out by the recipient (sequencing or KASPar assay)

- Same procedure as at ZIRC: After the first request fish will be raised, sperm of 10 – 20 F2 males + fin clips will be frozen without genotyping; if more requests are received, KASpar genotyping of the F2 males is carried out

- Cost will be the same as for any other IVF irrespective of effort

- Scientists may receive embryos immediately if IVF is sufficiently successful

- Not all fish may really contain the mutation, negative fish will be replaced if possible

- Genotyped fish may be offered at additional cost
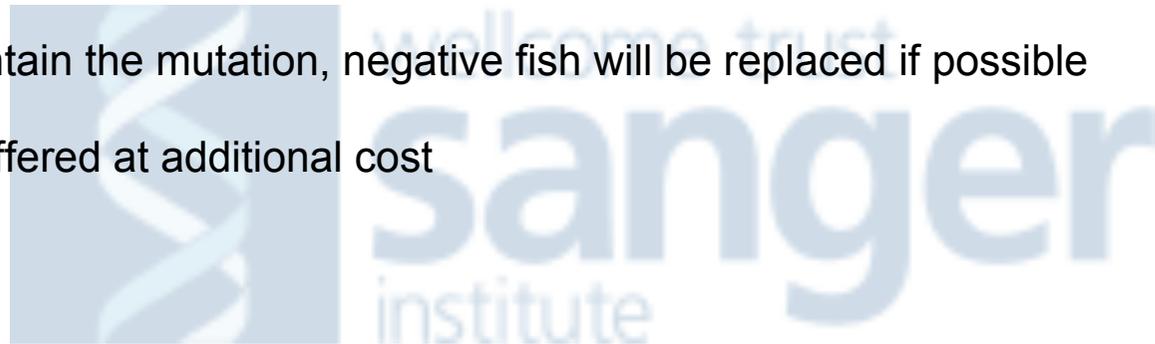
Tübingen Mutants

- All Tübingen mutants will be imported as frozen samples in summer of 2013

- 1,200 lines from Tübingen I screen, 6,000 from Artemis screen, 2,700 EU screen

- Entire containers with frozen sperm will be transferred to minimize risk of transport

- Most of the lines have been submitted to the ZFIN database, the remainder will be submitted by EZRC

- Lines will shipped from Tübingen sperm, no quality control by EZRC

- Tübingen MTAs will be passed on by EZRC, slightly different versions for the three screens

Database

- Challenge: develop a common database for EZRC and ITG fish groups
- Implemented by Wolf Thies using open-source tools (Linux, Apache, MySQL, PHP)
- Separate webshop for external requesters planned (possibly a commercial solution)

- **Stock** records refer to fish in a tank or sperm in a tube, and are never deleted
- Each stock has **events** associated with it which log its life history
- Each stock is **linked** to its parents recording the pedigree

- Utilities include to-do lists, graphical display of freezer boxes and fish racks
- In use for sperm, currently in beta testing for fish, release to ITG scientists by 2014

- **Access rights** can be set per stock, by owner / group / all users
- Features to be added: bar-code scanner / mobile interface, pedigrees
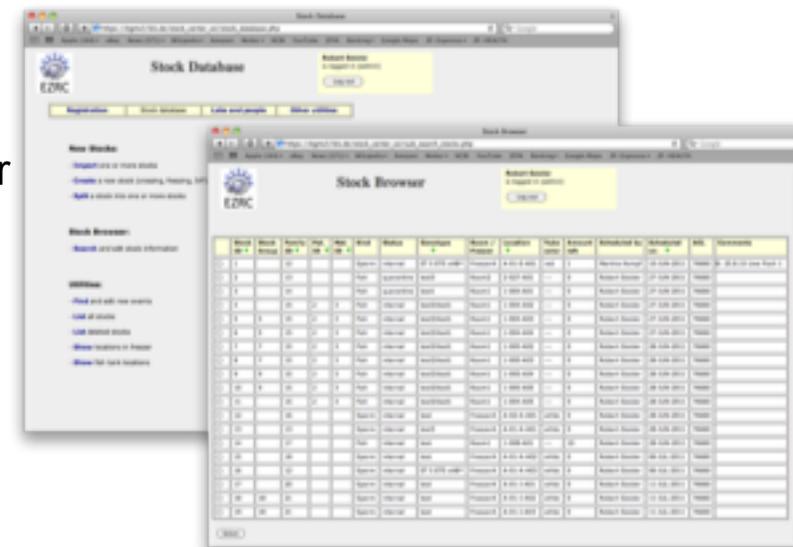
Health Monitoring

- **Incoming inspection:** Representative subset of larvae

- **Core Fishroom:** Sentinel tanks with AB fish, pre- and post filter; 10 fish each are examined every 6 months

- **Quarantine and Screening Fishroom:** 5 random live fish + 5 dead fish (preserved in formaldehyde) examined every 6 months

- **Parasitological examination** by an external veterinarian (Dr. Sandra Lechleiter)

- Squash preparations, examined for worms (nematodes, cestodes, trematodes), protozoa (Ichthyophthirius multifiliis, Piscinoodinium, Tetrahymena), fungi (Microsporidium), bacteria (Mycobacterium)

- If necessary the veterinarian will forward samples to a service company (LABOKLIN GmbH) for **histology** or to Leipzig University for **bacteriology**

- Histology and PCR (for Microsporidium and Mycobacterium) will be established in-house at the ITG
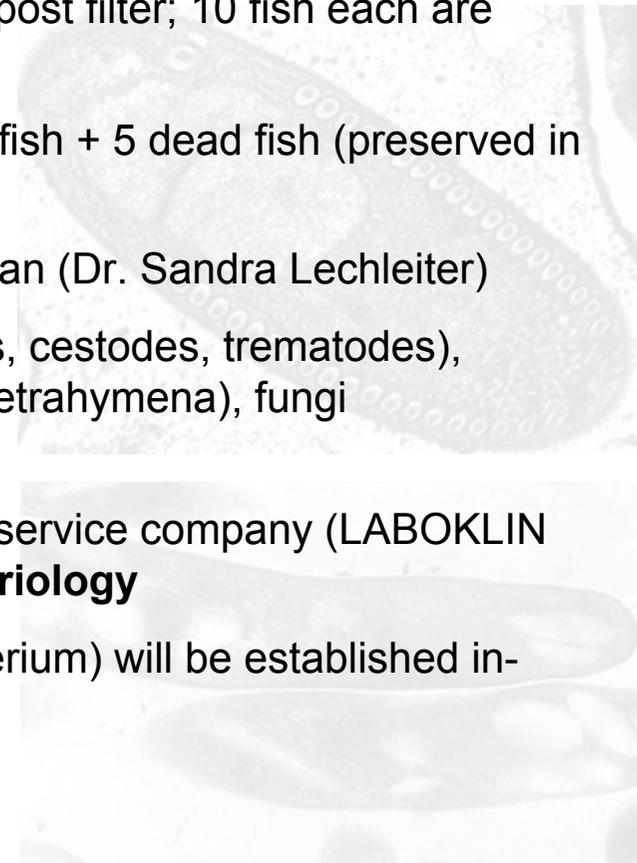
Sequencing service

An Illumina HiSeq 1000 is being operated by Olivier Armant (Strähle group), data analysis is done in collaboration with Wouter Coppieters and Bernard Peers, Liege

Sequencing services will be offered by the EZRC for zebrafish-related projects, mainly for:

- **Expression profiling** of zebrafish lines by transcript counting (RNAseq), with output provided to end users in an easy-to-use format

- **Genetic mapping** of mutations by genome-wide SNP analysis (multiplexed sequencing of exons)

Currently we are evaluating two different approaches for mapping, **exome sequencing** (gives 50x coverage for exons only in one Illumina lane), or **genome sequencing** (gives 6x coverage), and the potential for multiplexing to lower costs

ZF-HEALTH project
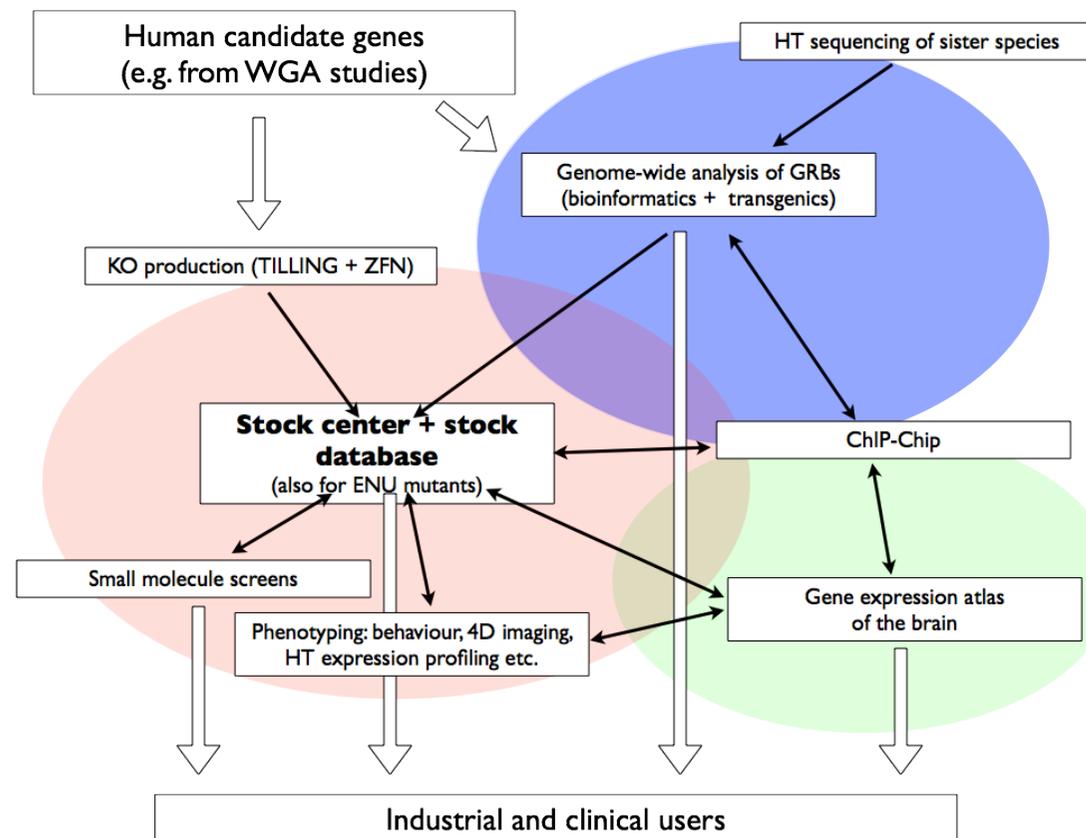
Three components:

- Analysis of genomic regulatory blocks

ZF-HEALTH project

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- Gene expression atlas of the brain
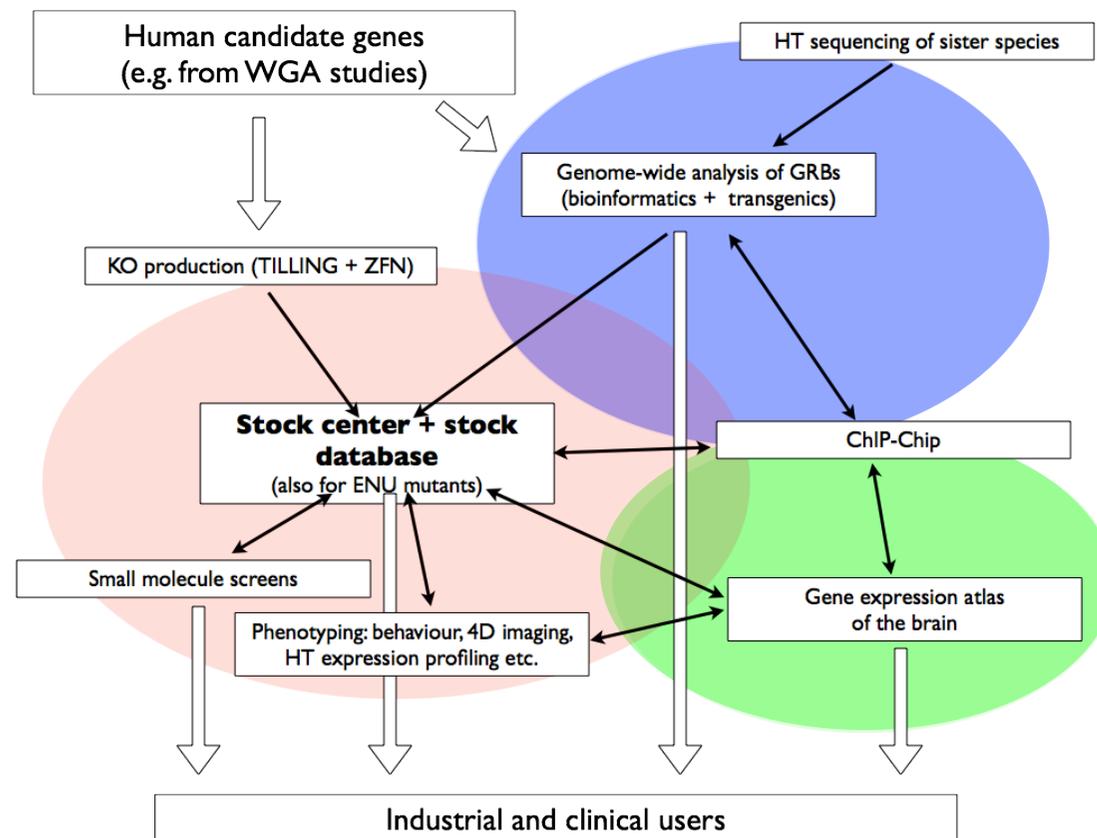
ZF-HEALTH project

Three components:

- Analysis of genomic regulatory blocks

- Gene expression atlas of the brain

- Production, phenotyping and chemical screening of KO mutants

Human candidate genes (e.g. from WGA studies)

HT sequencing of sister species

Genome-wide analysis of GRBs (bioinformatics + transgenics)

KO production (TILLING + ZFN)

Stock center + stock database (also for ENU mutants)

ChIP-Chip

Small molecule screens

Phenotyping: behaviour, 4D imaging, HT expression profiling etc.

Gene expression atlas of the brain

Industrial and clinical users

Screens

- **Collaborative screens** where KIT will provide the facilities, biological material, software and coordination, while guests bring their own assays

- Mainly KO mutants which will be continuously supplied by Sanger as part of the ZF-HEALTH project (approx. 24 F2 crosses per week, representing a total of 4 exomes or 60 KOs)

- High-throughput screening pipeline available (Screening Center, Ravindra Peravali; collaborations with Stefan Schulz and Ralf Mikut, Institute for Applied Informatics)

- At least 4 **shelf screens** envisioned in the course of ZF-HEALTH, with a total of at least 20 assays to be employed, as well as **chemical screens** on mutants of interest

| Maintain and breed fish | Separate, distribute and expose fertilised eggs | Acquire images and store | Find, crop and analyse embryos |
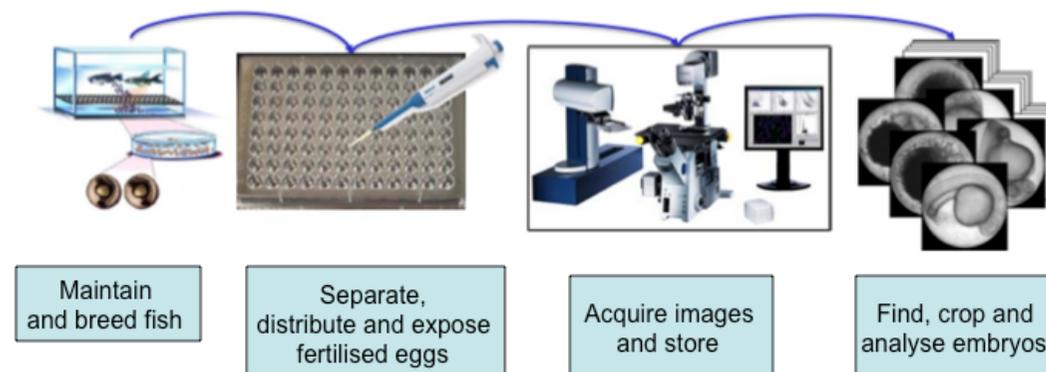
Illustration: U. Liebel

Behavioral barcoding

- Photomotor response (PMR) of embryos at 28 hpf
- Yields multiple numeric measurements that can be used for clustering of responses
- Peterson lab performed a large screen of small neuroactive molecules, could cluster known molecules and find some new candidates
- Will allow to annotate mutants by neural pathway affected
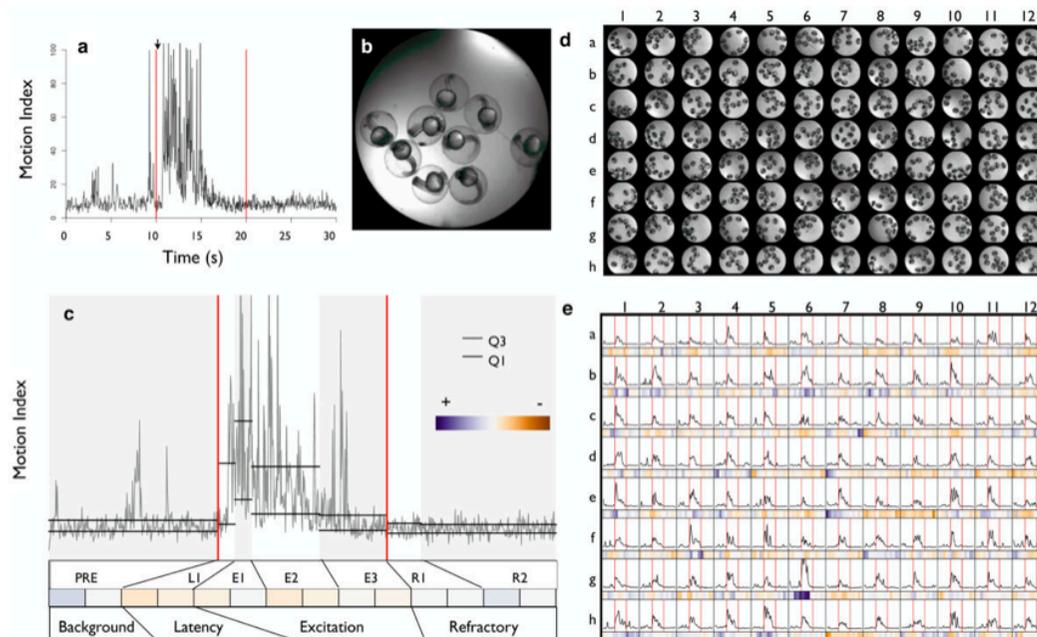
D. Kokel et al, 2010

Further screening assays

Assays currently being developed by Ravi Peravali to assess natural variation in medaka, but adaptable for zebrafish as well:

- larval morphometry (imaging not yet automated)
- larval "social" feeding
- adult feeding
- mating

- Screening for metabolic parameters, e.g. in blood? Possible collaboration with KIT-IOC

Synchrotron microCT

3D micron-scale imaging unaffected by pigmentation and tissue thickness

Proposed as the basis for a US zebrafish phenome project

Pilot project:

- **Optimization of X-ray imaging methods (KIT-ISS / ANKA)**
  Protocols, imaging software, tomography stage

- **Applications to the zebrafish model (ITG)**
  Wildtye zebrafish development, mutants and toxicology (e.g. vasculogenesis)

K. Cheng
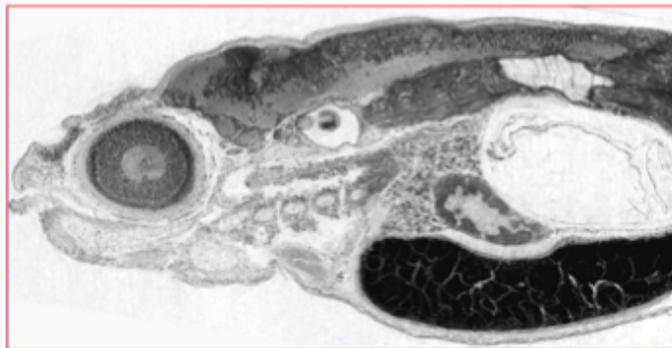
Thanks

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