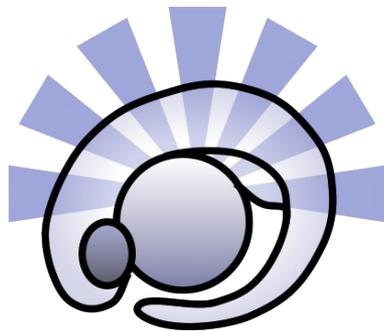


## Update on the European Zebrafish Resource Center

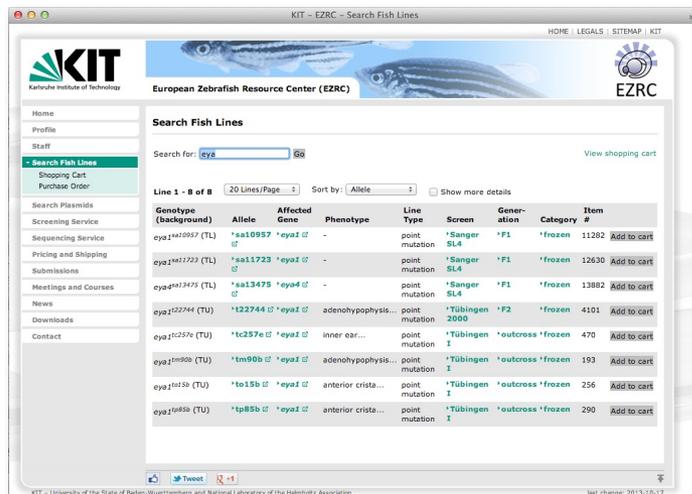


**EZRC**



# Aims

- Provide a **permanent repository** for zebrafish lines from European labs, including the Tübingen und Freiburg ENU screens, and the Sanger ZMP project (genome-wide knockout of protein-coding genes)
- Provide **easy and cost-effective access** to these lines for European labs
- **Mirror** popular lines from the U.S. resource center, ZIRC
- Provide **additional resources** e.g. plasmids, genome mapping, screening, training



KIT - EZRC - Search Fish Lines

European Zebrafish Resource Center (EZRC)

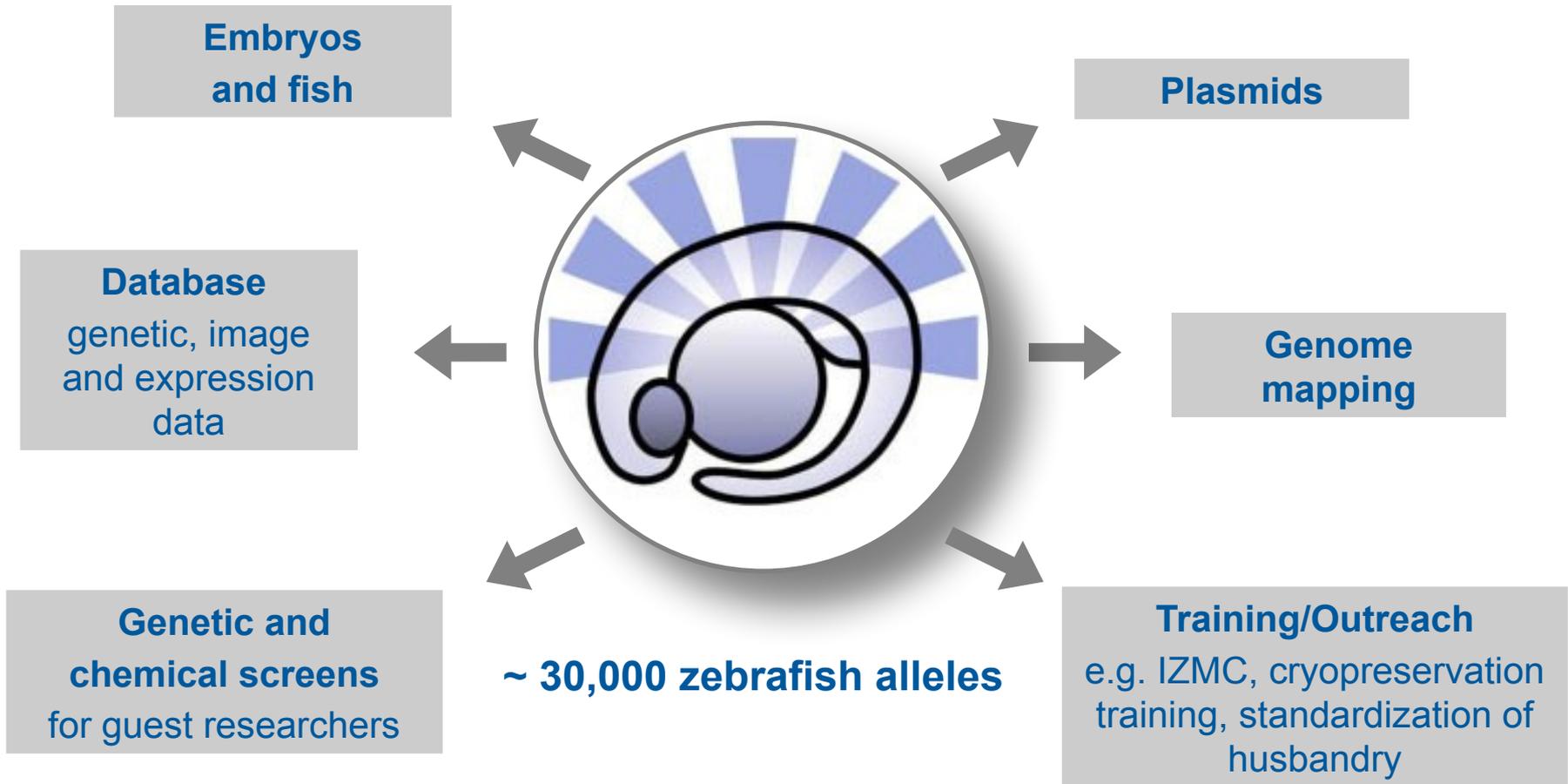
Search for:   [View shopping cart](#)

Line #	Genotype (background)	Allele	Affected Gene	Phenotype	Line Type	Screen	Generation	Category	Item #	
eya21470987 (TL)	*sa10957	*eya2	0	-	point mutation	*Sanger SL4	*F1	*frozen	11282	<a href="#">Add to cart</a>
eya21471723 (TL)	*sa11723	*eya2	0	-	point mutation	*Sanger SL4	*F1	*frozen	12630	<a href="#">Add to cart</a>
eya214717475 (TL)	*sa13475	*eya4	0	-	point mutation	*Sanger SL4	*F1	*frozen	13882	<a href="#">Add to cart</a>
eya2122744 (TU)	*t22744	*eya2	0	adenohypophysis...	point mutation	*Tübingen 2000	*F2	*frozen	4101	<a href="#">Add to cart</a>
eya2125776 (TU)	*t25776	*eya2	0	inner ear...	point mutation	*Tübingen I	*outcross	*frozen	470	<a href="#">Add to cart</a>
eya21m90b (TU)	*tm90b	*eya2	0	adenohypophysis...	point mutation	*Tübingen I	*outcross	*frozen	193	<a href="#">Add to cart</a>
eya21415b (TU)	*t1415b	*eya2	0	anterior crista...	point mutation	*Tübingen I	*outcross	*frozen	256	<a href="#">Add to cart</a>
eya21465b (TU)	*t465b	*eya2	0	anterior crista...	point mutation	*Tübingen I	*outcross	*frozen	290	<a href="#">Add to cart</a>



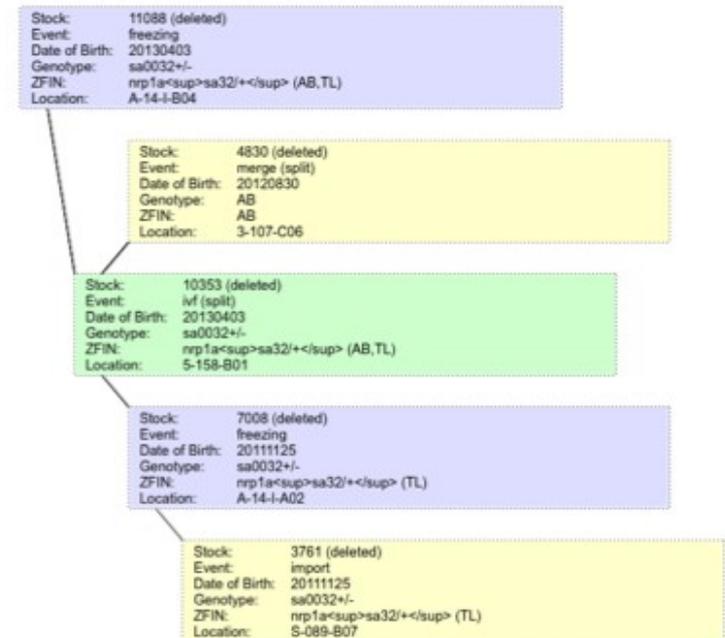
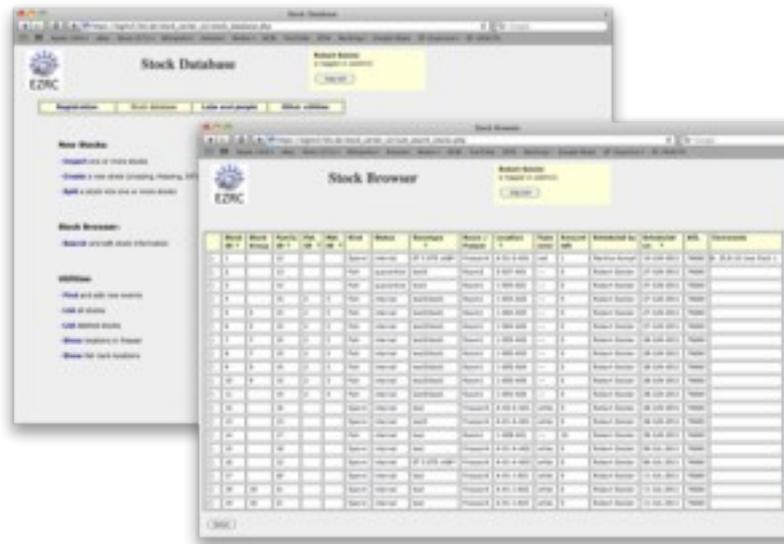
## EZRC in overview

Official opening July 2012, web store online November 2013



# Resource Center Database (RCDB)

- Built with open-source tools (Linux, Apache, MySQL, PHP)
- Supports EZRC and experimental fish facility
- Permanent record of each line and its life history
- Provides pedigrees and bar-code tank labels
- Also supports processing of orders



## EZRC – what is available

- Tübingen I screen (1996) 1,100 alleles
- Tübingen 2000 screen in collaboration with Artemis 100 alleles
- Tübingen ZF-MODELS screen (2004) 1,000 alleles
- Zebrafish Mutation Project (ZMP), Sanger Institute 27,100 alleles  
12,200 mutated genes
- FISHTRAP transgenics (Karuna Sampath) 200 lines
- ZETRAP (V. Korzh) and other transgenics 200 lines
- Wildtype lines (AB, TU, WIK, SJD, NHGRI-1, ABO, TL, LEO, Casper...) ~30,000 alleles / lines



- Planorbella spec.



# Archiving of CRISPR lines

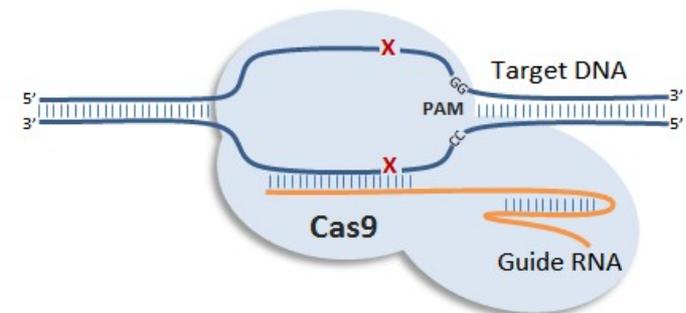
Genome editing by CRISPR can easily produce new mutant lines, however:

- Mutagenized sites must be **sequenced** to verify
- **Outcrossing** necessary to get rid of second site effects (though less than ENU mutants)
- Each CRISPR experiment requires a **permit** and evaluation of animals for harmful phenotypes

Conclusions:

- EZRC archives CRISPR lines as frozen sperm
- Library of CRISPR lines will complement Sanger ENU mutants
- Will save both money and animal experiments
- We archive published lines free of charge if we can distribute them

(Geisler et al., EMBO Reports 2016)



# Current challenges

## Brexit

- Several shipments to Britain since Brexit, but more paperwork (TRACES export certificate, veterinary inspection, recipient must supply CEFAS license number)
- Shipping only with World Courier or Barbican
- For shipment to Germany, recipient needs to apply for two import permits (animal diseases + animal welfare), original must be included with the shipment

## Covid

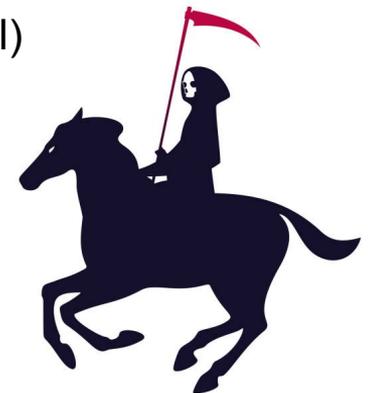
- Air embargoes in 2020 and 2021, now lifted
- Shipping costs and lead time increased (World Courier)
- But embryo shipping within the EU is fine (GO! Express, DHL Medical)

## Climate change

- Shipments paused when outside temperature is  $\gg 30\text{ }^{\circ}\text{C}$

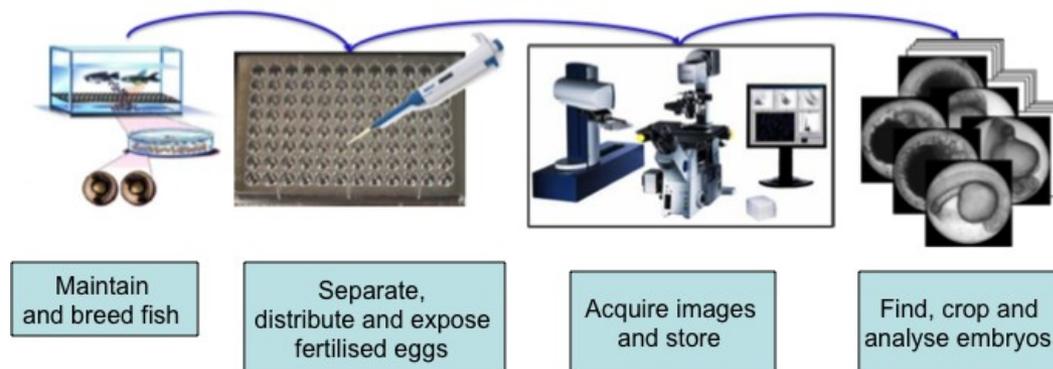
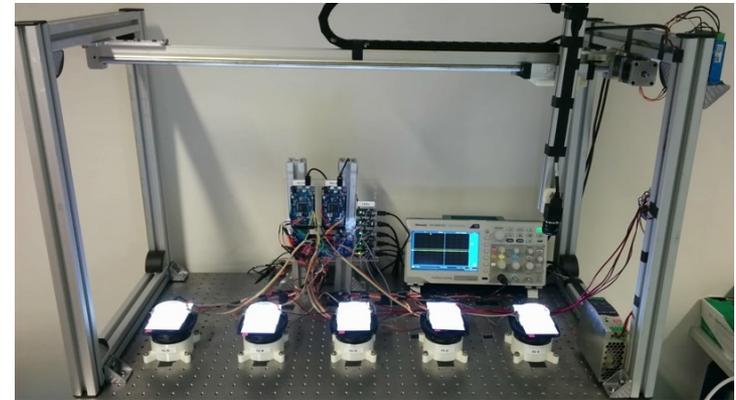
## Ukraine war

- So far no problems...



# Behavioral screening platform

- Automated, high-throughput assays developed at the IBCS screening center for:
  - Photomotor response
  - Touch response
  - Startle response (vibration)
- Automated embryo sorter
- Morphometry
- Image analysis in collaboration with KIT-IAI



Ravindra Peravali

# Behavioral screening

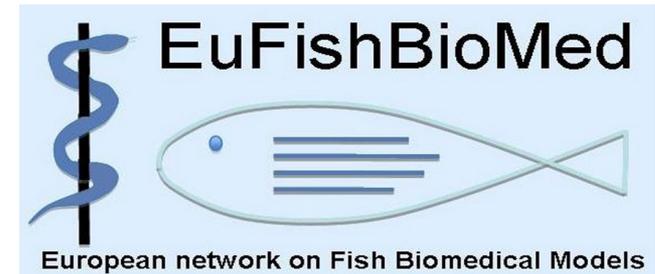
- Screen wildtype lines and inbred sublines established at the EZRC
- AB, WIK (Kolkata), SJD (Nepal) are derived from separate wild catches
- Useful as a baseline for future studies
- Potential to map trait differences

Behavior	Stage	Neurons involved	Disease model for
Photomotor response	Approx. 30 hpf	Photosensitive hindbrain neurons (Kokel et al., 2013)	Neurotoxicity, effects of many classes of psychotropic drugs (Copmans et al., 2016)
Touch response	2 – 5 dpf	Rohon-Beard neurons, CoPA interneurons, CiD interneurons in the rostral spinal cord (Pietri, 2009; Umeda et al., 2016)	Ion channelopathies (Ribera and Nüsslein-Volhard, 1998)
Startle response (vibration)	2 – 5 dpf	Mauthner neurons in the brain stem (Wilson, 1959)	Startle disease (Ganser et al., 2013), effects of anesthetics (Bedell et al., 2018)

## EZRC outreach – Standardizing zebrafish work

Participation or co-organization in workshops on:

- **reference diets**
- **fish facility management**
- **euthanasia** (Köhler et al., 2017).
- **severity assessment** (Bert et al., 2016).



Articles:

- Cryopreservation as a means of **reducing animal experiments** (Geisler et al., 2017).
- **Developmental stages** to be considered experimental animals (Strähle et al., 2012).
- White paper on standards for zebrafish **husbandry** (Baier et al., 2014).

European Society for Fish Models in Biology and Medicine (**EuFishBiomed e.V.**) (Strähle et al., 2012).

- **International Zebrafish and Medaka Course (IZMC)**

- Co-organized by EZS and EZRC
- Training course for students, graduates, postgraduates and PIs
- Theory: **Legal aspects, ethics, fish biology, alternative fish models**
- Practical: **Husbandry, breeding, anesthesia, fin clipping, euthanasia**
- FELASA accredited
- 6 courses per year for approx. 20 participants each

- **Hands-on cryopreservation training**

- 1 day, 2 participants
- Scheduled on request
- Approx. 6 courses per year



# Thanks

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