THE ZEBRAFISH CORE FACILITY HANDBOOK

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THE MANAGEMENT OF THE ZEBRAFISH CORE FACILITY

The zebrafish core facility is since many years one of the core facilities at Karolinska Institutet and part of Comparative Medicine. The core facility supports internal and external research groups with specialized services around the zebrafish animal model. Below you find the relevant contact information:

Office of the zebrafish core facility:	zebrafish-office@km.ki.se	
Lars Bräutigam, Facility head:	lars.braeutigam@ki.se	phone: 08 524 87 316
Chiara Zullian, designated veterinarian:	chiara.zullian@ki.se	phone: 08 524 87 310
Elisabet Andersson, animal welfare officer:	elisabet.andersson@ki.se	phone: 08 524 87 258

GETTING ACCESS TO THE ZEBRAFISH CORE FACILITY

If you want to start using the zebrafish core facility, send an email to the office and book a meeting. After the meeting and a thorough introduction to the facility premises, rules and regulations, your KI card will be activated to the rooms of the zebrafish core facility.

You, as the holder of the activated card, must follow the rules and regulations presented in this document. In case you break our rules, your access will be suspended for one month. If you repeatedly break rules, your access will be suspended indefinitely.

NB: the activated KI card is personnel and must not be borrowed to anybody else.

NB: do not bring any visitor into the rooms of the zebrafish core facility without informing the head of the facility first.

HEALTH MANAGEMENT OF THE ZEBRAFISH CORE FACILITY

The zebrafish core facility has developed a detailed biosafety plan that minimizes the risk of introducing and spreading of pathogens in the zebrafish core facility. The following gives a short overview on our health management plan:

- Directed movement of staff, researchers, and animals.
- Dedicated personal protective equipment (PPE).
- Barrier system with each barrier having specific access requirements.
- Advanced zebrafish strain management plan.
- Advanced health control by sampling live food, sentinels, sludge and random animals 4 times per year based on the FELASA recommendations.

The current health report for our barriers can be requested from the zebrafish office.

IMPORT OF FISH INTO THE ZEBRAFISH CORE FACILITY

Zebrafish embryos and adults, the latter solely for cryopreservation, can be imported from other Swedish laboratories and the European stock center in Germany (EZRC). Importing from other suppliers might be possible, but will require a special import permit from the Swedish authorities. The sending laboratory must be able to provide a health certificate not older than 6 months. The zebrafish core facility management may stop an import procedure if no health certificate is provided, or unwanted pathogens have been detected in the sending fish facility.

If you would like to import zebrafish, please write an email to zebrafish-office@km.ki.se

All imports must go through quarantine. Hence it can take up to 6 months from receiving embryos until the line is being ready to use in the main fish room. All zebrafish strains will be included in an “open strain database” that is visible to the users of the zebrafish core facility. This strain database facilitates collaborations and can avoid unnecessary, time-consuming, and costly imports. Importantly, the open strain database will only reveal the genotype, not the owner of the line. The zebrafish core facility will not grant access to any personal strain without written consent from the strain owner. If you need to keep your zebrafish strain(s) confidential, please contact the director.

AVAILABLE ZEBRAFISH STRAINS

The zebrafish core facility houses approximately 200 different genotypes. The most common genotypes are provided by the core facility (see also below), genotypes owned by research groups are often readily shared between research groups.

The following strains are owned by the zebrafish core facility and available to every user:

AB (commonly used wildtype line):

The AB line is derived from two lines, A and B, purchased by Streisinger at different times from a pet shop in Albany, Oregon. The original A and B lines probably originated from a hatchery in Florida. Haploid offspring from individual females of the AB line were screened for healthy, good-looking embryos, and those females were used to make future generations by crossing them to unselected males. The AB line was maintained in this manner by the Oregon labs from the 1970's to the 1990's. This procedure reduced the number of lethal mutations so that this line can be used for parthenogenesis.

TL (commonly used wildtype line):

Homozygous for leot1 and lofdt2. Obtained from a dealer and kept by raising mixed eggs from different egg lays of well-laying females. leot1 is a recessive mutation causing spotting in adult fish, also known as tup. lofdt2 is a dominant homozygous viable mutation causing long fins. This is not the line used in the Sanger zebrafish sequencing project.

Casper (zebrafish line without pigments):

Published in PMID: 18371439; genotype: roy $^{-/-}$;nacre $^{-/-}$

Tg(fli1a:EGFP) (zebrafish line with endothelial cells/blood vessels expressing GFP):

Published in PMID: 12167406

Tg(cmlc2:EGFP) (zebrafish line expressing GFP in cardiomyocytes):

Published in PMID: 12950077

Tg(gata1:dsRed) (zebrafish line expressing dsRed in erythrocytes):

Published in PMID: 14608381

Tg(mpeg1:gal4-UAS:NTR-mCherry) (zebrafish line in which macrophages can be depleted):

Depletion of macrophages with 10 mM MTZ; Published in PMID: 32764590

Tg(mpeg1:gal4-UAS:NTR-mCherry) x Tg(mpo:GFP)

See above; additionally, neutrophils expressing GFP)

Tg(mpo:GFP) (zebrafish line expressing GFP in neutrophils)

Tg(UAS:GFP;collagen2:mCherry) (zebrafish line for screening Gal4 driver lines)

Constitutively expresses mCherry in brain and spinal cord; expresses GFP when crossed with active Gal4

THE ROOMS OF THE ZEBRAFISH CORE FACILITY

The following list includes a short description of our rooms and the general rules that apply.

THE INJECTION LABORATORY

The injection laboratory is the main wet laboratory of the zebrafish core facility. Experiments with toxic chemicals can be performed here. The following rules apply:

- Users have 24/7 access to the injection laboratory
- Users must wear laboratory shoes, lab coat and gloves at all times
- No visitors are allowed, except those approved by the facility head beforehand
- Consumables, small equipment, and reagents from your laboratory can be taken into the injection lab and stored in your labelled personal box
- No Styrofoam iceboxes can be taken into the injection laboratory. Please change to a green thermal box and new ice (both provided)
- You must place your mobile phone into a disposable plastic bag (provided)
- Nothing that has been to barrier C must be brought into the injection lab.
- Before handling toxic chemicals including CMR chemicals submit a written risk-assessment and project plan to the office

THE MAIN FISH ROOM

In the main fish room, most of the wildtype and transgenic lines are housed. Fin-clipping of those lines is performed at a dedicated area in the main fish room. The following rules apply:

- Users have access to the main fish room only if staff is present
- Users must change to specific lab shoes, lab coats and fresh gloves before entering
- Users may work at the dedicated fin-clip bench
- Users are not allowed to handle housing aquaria or fish outside the dedicated fin-clip bench
- Consumables, small equipment, and reagents can be taken to the fin-clipping bench only
- Nothing that has been to barrier C must be brought into the main fish room
- Mobile phones and other small electronic equipment cannot be taken into the main fish room
- No Styrofoam iceboxes can be taken into the injection laboratory. Please change to a green thermal box and new ice (both provided in entry room of the injection lab)

THE OPEN-ACCESS LABORATORY

The “open-access laboratory” can be used to house fish for experiments or fish with unknown health status. Moreover, experiments with embryos from the quarantine unit can be performed here. The following rules apply:

- All animals housed in the open-access unit must be housed under a personal ethical permit.
- Zebrafish strains can be housed and fin-clipped in the open-access laboratory. Eggs can from those stocks can be bleached and raised in the main fish room or quarantine room.
- The staff is feeding and checking all fish in the open-access laboratory. Other duties on request.
- Users have unlimited access to the open-access room (lights on 6:00, lights off 20:00)
- Depending on the procedure performed, users must change to working clothes and/or lab coats and wear red lab shoes and gloves.
- Users must wait minimum 12 hours after visiting the open-access laboratory before entering any room of barrier B or higher. As an alternative a shower and complete change of outer clothes is possible. Lockers and shower are available in the core facility.
- Cell phones and other small electronic equipment must be put in a disposable plastic bag before taken into the open-access laboratory.
- Consumables, small equipment, and reagents from your laboratory can be taken into the researcher’s lab and stored in labelled boxes.
- Nothing that has been to the open-access laboratory must be brought into the injection lab or main fish room
- No Styrofoam iceboxes can be taken into the injection laboratory. Please change to a red thermal box and new ice (both provided)
- Before handling toxic chemicals including CMR chemicals submit a written risk-assessment and project plan to the office

THE BSL-2 / HIGH-CONTAINMENT LABORATORY

The BSL-2/high-containment laboratory is a dedicated laboratory that allows handling and injection of bacteria and viruses. Additionally, experiments involving CMR chemicals can be performed here. Users have access to the BSL-2 unit after an introduction. A risk-assessment and project plan must be submitted prior to running experiments.

OTHER ROOMS OF THE ZEBRAFISH CORE FACILITY

Besides the above-named rooms, the zebrafish core facility has a quarantine unit, a rotifer culturing room, a dish room, a SPF fish room and a dry lab. Users do not have access to these rooms.

INFORMATION ON ETHICAL PERMITS

Zebrafish embryos younger than 5 days are considered unlikely to experience pain, suffering or lasting harm and are excluded from the normative on animal testing by the EU directive 2010/63/EU and the corresponding Swedish legislation L150. Therefore, no ethical permission is required for experiments on zebrafish embryos younger than five days. For housing of zebrafish older than 5 days as well as running experiments involving zebrafish older than 5 days, the following rules apply:

Housing of wildtype zebrafish

All wildtype zebrafish lines can be housed under the ethical permit of the zebrafish core facility.

Housing of transgenic or knock-out zebrafish that do not show a phenotype

All transgenic or knock-out zebrafish lines that do not show a visible can be housed under the ethical permit of the zebrafish core facility.

Housing of Transgenic or knock-out zebrafish that do show a phenotype

All transgenic or knock-out zebrafish lines that do show a visible phenotype (such as impaired survival, morphological or behavioural differences) must be housed under a user-specific ethical permit.

Running experiments

Experiments involving zebrafish embryos older than 5 days must be covered by user-specific ethical permit. That applies also if the experiment is started before 5 days and ended > 5 days.

Establishing new transgenic and/or knock-out strains

The zebrafish core facility can establish new transgenic or knock-out lines for users under the ethical permit of the facility. If a phenotype becomes visible in the first generation (F0), then the user will need to apply for a user-specific ethical permit to be able to continue breeding.

Ethical permits for picking up zebrafish larvae/adults

If you need to pick up larvae or adults for experiments, the animals will be moved from the facility ethical permit to your personal ethical permit on the day of pick-up. You need to document all experiments according to the Swedish regulations and terminate the larvae as “used in experiment” in tick@lab.

The license holder is responsible for following the terms of their ethical permission as well as for documentation in tick@lab. We also point out that all experiments involving embryos older than 5 days must be performed in the premises of the zebrafish core facility if not granted otherwise by the ethical committee.

WRITING AN ETHICAL PERMIT

General Information about how to apply for an ethical permit is found on http://ki.se/en/km/ethic-review-of-animal-experiments. The zebrafish core facility can provide a template for an ethical permit including basic procedures.

INFORMATION ON THE REQUIREMENT OF A LABORATORY ANIMAL COURSE

Users that only handle and perform experiments in zebrafish embryos younger than five days are encouraged but not required to take a zebrafish laboratory animal course. Handling or experimenting with zebrafish older than five days requires a valid course certificate. Course certificates which are issued by another Swedish Universities may be valid once registered in the KI education database. Course certificates of international Universities may be valid, but the theoretical part including Swedish law and regulations must additionally be completed. For more information and registration for the fish animal science course contact las-edu@km.ki.se.

ORDERING OF EMBRYOS AND SERVICES

All orders are placed in ilabs. Go to https://karolinska.corefacilities.org/account/login, log in with your KI credentials and search for the zebrafish core facility. Please note that your PI must give you access to a project number in ilabs so that services can be invoiced.

- Eggs can be provided both on weekdays and on weekends.
- Matings can be timed to produce eggs between ca 6:00 and 9:30 on weekdays and between 8:00 and 9:30 on weekends.
- All orders must come in at least Thursday at 12:00 the week before delivery.
- We have a limited number of mating slots per day – first come first serve.

KEEPING EMBRYOS IN THE INCUBATOR

- All embryos ordered as “non-sorted” will be placed inside the “todays orders” incubator. *The user must sort the embryos during the first day, any unsorted embryos will be discarded at 24 hpf.*
- All embryos ordered as “sorted” will be placed in the “user incubator”.
- All manipulated (*e.g*. microinjected) embryos are placed in the “user incubator”.
    - *Every plate must be labelled with name, date and stock number. If this information is missing, the staff will discard the embryos.*
- If you wish to keep your embryos longer than 5 days in the incubator, write the ethical permit number on the plate. Plats without ethical permit number will be discarded > day 5

KEY INFORMATION ON WORKING WITH EMBRYOS

Zebrafish embryos are raised in E3 medium at 28.5 C and room air (no CO_2 incubator). It is very important to ensure that the embryos have optimal conditions to grow and develop, otherwise embryos might develop slower, irregular or die. Keep in the following in mind:

- Never more than 50-60 embryos in a 10 cm dish
- Never more than 200-300 embryos in a 20 cm dish
- Always remove unfertilized eggs during the first day of development

KEY INFORMATION ON HOUSING AND MATING ZEBRAFISH

The zebrafish core facility houses both core-facility owned as well as researcher-owned strains. The section below gives an overview on the core facility strategy on zebrafish housing, regeneration, and animal welfare.

HOUSING ZEBRAFISH

In general, the following rules apply for housing zebrafish.

- 2 tanks with 20 fish each are kept per zebrafish strain
- Males and females are housed together
- A minimum of 10 fish must be housed together long term (> 7 days). If needed, distinguishable play-mate fish are added to the tanks by the staff

ZEBRAFISH STRAIN MANAGEMENT

In order to minimize the spreading of diseases, a strain-management plan has been established. The key-points include:

- Zebrafish are sacrificed with 1.5 years of age; carrier fish or other special fish that must be kept longer can be moved to the quarantine unit and kept for up to 2 years if not stated otherwise in the researcher's ethical permit.
- The core facility staff informs the line owner well in advance before the regeneration of a strain.
- The regeneration will be invoiced as package-price; the regeneration is compulsory.

MATING ZEBRAFISH

The following rules apply for setting up matings

- Zebrafish can be mated when the gender is visible, at approx. 3 months of age
- Zebrafish can be mated with 7 days intervals
- Outcrosses can only be set up if the individuals are of approx. the same size or older than 4 months

SINGLE HOUSING OF ADULT ZEBRAFISH

In some circumstances, zebrafish can be housed individually for a restricted period.

- Fin-clipped fish can be housed individually until the result of the genotyping is available, however maximum 7 days
- Single housing of zebrafish for longer than 7 days is only possible when “play-mate” fish are added to the tanks so that there is a minimum of 10 fish per tank. The addition of the playmates is done by the staff of the core facility.

CRYOPRESERVATION AND IVF OF ZEBRAFISH STRAINS

The zebrafish core facility offers cryopreservation, long-term storage and IVF of zebrafish strains housed in the zebrafish core facility or other national and international facilities. For more information and orders please write an email to zebrafish-office@km.ki.se.

THE USAGE OF CHEMICALS IN THE ZEBRAFISH CORE FACILITY

For all zebrafish experiments involving CMR chemicals other than PTU (phenylthiourea), a written risk assessment and project plan must be submitted to the office. The use of methylene blue as additive for E3 has been phased out and E3 containing methylene blue will only be provided in small amounts for specific experiments.

WASTE HANDLING AND DISPOSAL PROCEDURES IN THE ZEBRAFISH CORE FACILITY

All users must follow the rules below:

- Any left-over embryos are placed in the designated area in the injection lab or open-access unit
- Any laboratory waste is disposed of into the yellow waste bins
- Any laboratory waste contaminated with small amounts of toxic chemicals is disposed of into the yellow bins
- Sharps are disposed of into the sharps container
- Any liquids containing PTU are disposed of in the dedicated PTU-waste container

USEFUL LINKS

www.zfin.org webpage of the zebrafish international resource center. Source of mutant/transgenic lines

www.ezrc.org webpage of the European zebrafish resource center. Source of mutant/transgenic lines

https://www.zdmsociety.org/ webpage of the zebrafish disease model society

https://zhaonline.org/ webpage of the zebrafish husbandry organization

IN CASE OF AN ACCIDENT

In case of an accident, users should contact the public health-care provider (consultation at “Vårdguiden”, phone 1177).

Cuts and abrasions should be treated to their severity. Minor cuts should be treated with lab first aid kit, major cuts must be evaluated by a medical specialist. Do not force bleeding.

Accidents that occur in the Zebrafish Core facility should immediately be reported to the director of the zebrafish core facility and an electronic report should be submitted to https://internwebben.ki.se/sv/rapportera-incident.

RESPONSIBILITY STATEMENT

I hereby confirm that I have read and understood all the terms and conditions to work in the premises of the zebrafish core facility and that I agree to all the routines. I acknowledge that if I do not follow the routine procedures and guidelines my access to the zebrafish core facility will be terminated. Furthermore, I acknowledge that if I allow other people to use my access card to enter the zebrafish core facility my access to the zebrafish core facility will be terminated.

Please sign electronically with eduSign and send to lars.braeutigam@ki.se