

THE SERVICE PORTFOLIO OF THE ZEBRAFISH CORE FACILITY

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USING THE SERVICES OF THE ZEBRAFISH CORE FACILITY

The zebrafish core facility provides the competence and infrastructure to use the zebrafish model in research. Our aim is that internal, external, and corporate scientists can use our services within 7 days after applying.

To facilitate using the zebrafish animal model in research, we provide all necessary permits (see also information on ethical permits below), direct access to animals from common wildtype and transgenic strains, and running of complex research projects. Our equipment is free of charge.

Step-by-step how to become a user of the zebrafish core facility:

1. Book a meeting with the facility head Lars Bräutigam (lars.braeutigam@ki.se) to discuss the needs for your research project.
2. Get an introductory tour through our premises.
3. We will activate your access card
➔ You are ready to use our services and work hands-on with embryos up to 5 days after fertilization.
4. If you want to work with animals older than 5 days, you will need a zebrafish specific laboratory animal science education. KI provides both the theoretical and practical zebrafish LAS education which you can book by emailing las-edu@km.ki.se.

THE ZEBRAFISH CORE FACILITY AT KAROLINSKA INSTITUTET

The zebrafish core facility at Karolinska Institutet was established more than 18 years ago and moved into new state-of-the-art localities in 2020. We currently serve close to 100 internal, external, and corporate customers.

Seven staff offer everything from the provision of animals to project planning and running of complex research projects. Specialized staff, standardization of all procedures and continuous quality control ensure that the animals are kept to the highest ethical standards, and that the data we deliver is sound and reproducible.

In this document you will find information concerning the research pipelines we offer. The pipelines can of course be adjusted to your needs, and we work closely together with other core facilities to offer integrated services.

Please do not hesitate to contact us if you have any questions!

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Zebrafish core facility office: zebrafish-office@km.ki.se

Webpage: <https://ki.se/en/research/zebrafish-core-facility>

QUICKFACTS ABOUT THE ZEBRAFISH MODEL

The zebrafish is an established, ethically sound, and cost-effective animal model with proven clinical relevance concerning human physiology and pathology. Its genome is easily accessible and the availability of thousands of transgenic and mutant lines provides, together with unique *in vivo* imaging properties, an ideal platform for basic and applied research.

Following the principles of the 3R's should be part of any scientific study involving laboratory animals. Carrying out experiments in the zebrafish model can be an important aspect of adhering to the 3R principles as it may partially replace traditional rodent models and refine experiments carried out in these laboratory animals.

The embryonic development of the zebrafish is very rapid. Merely two days after fertilization, the cardiovascular system and most other organs are developed, and the embryo is freely swimming. Due to its extraordinarily fast development, many experiments can be performed on embryos younger than five days and importantly, those are not regarded as laboratory animals. *Therefore, no ethical license is required for experiments performed on embryos younger than 5 days.*

The transparency of zebrafish embryos allows to follow biological phenomenon such as migration of cancer cells or regeneration of tissues in a living organism and in real time. Due to their small size, zebrafish embryos can be kept in 96 well plates which makes them the only vertebrate model suitable for automated high-throughput screens such as xenotransplantation screens, lead-optimization, and pre-clinical toxicity studies.

Here is a list of recommended reviews on the zebrafish model:

The zebrafish as model in research: <https://pubmed.ncbi.nlm.nih.gov/35058207/>

The zebrafish as model for regeneration: <https://pubmed.ncbi.nlm.nih.gov/31540899/>

The zebrafish as model for cancer: <https://pubmed.ncbi.nlm.nih.gov/32251397/>

The zebrafish as model for drug discovery: <https://pubmed.ncbi.nlm.nih.gov/34117457/>

The zebrafish as model for cardiac disease: <https://pubmed.ncbi.nlm.nih.gov/35679360/>

INFORMATION ON ETHICAL PERMITS

Zebrafish embryos develop extremely fast, and most studies can be performed in animals younger than 5 days. Zebrafish younger than 5 days are not considered laboratory animals by both European and Swedish regulatory bodies and performing experiments in embryos younger than 5 days does neither require an ethical license nor a laboratory animal science course certificate.

In the zebrafish core facility, animals which do not show a phenotype can be housed under the facility license. Furthermore, common procedures can be performed under the ethical license of the zebrafish core facility.

Keeping zebrafish which do show a phenotype and/or performing non-common procedures require a personal ethical license.

STANDARDIZED RESEARCH PIPELINES

The zebrafish core facility has established standardized research pipelines which have been optimized based on published evidence and in-house knowledge. All pipelines can be modified and/or extended based on your needs. The zebrafish core facility works closely together with other core facilities and can therefore offer the integration of the pipelines into other platforms.

1) ZEBRAFISH STRAIN MANAGEMENT

1A) IMPORT AND EXPORT OF ZEBRAFISH LINES

The zebrafish core facility has all relevant permits to import zebrafish from within and outside the EU, and the facility takes care of the whole import process. Most lines are available from the European Zebrafish Resource center (<https://www.ezrc.kit.edu/>). All animals will be imported into our quarantine unit for biosafety reasons.

The zebrafish core facility has all relevant permits to export embryonic or adult zebrafish to other Swedish or European facilities and the facility takes care of the whole export process.

1B) DELIVERY OF EMBRYOS AND ADULTS

The zebrafish core facility houses close to 250 different wildtype and transgenic lines. Embryos and adults of wildtype and common transgenic lines can be delivered directly, animals of researcher-owned lines can be delivered after an agreement with the owner of the line.

Animals can be delivered on any day of the week; delivery of up to 3000 embryos per order is possible. Larger numbers can often be delivered after 2 weeks' notice.

1C) HUSBANDRY OF ZEBRAFISH LINES

The zebrafish core facility offers housing of wildtype, transgenic, and knock-out zebrafish strains for internal, external, and corporate users.

Our facility consists of different animal rooms following a barrier system which is similar to that in rodent facilities. The health of the animals is ensured by a tight health monitoring program which was established by our designated veterinarian based on the [FELASA/AALAS guidelines](#). Our biosafety plan is available upon request.

We have standardized procedures for breeding zebrafish, raising embryos and keeping adults. Furthermore, we have implemented a specific regeneration program that ensures minimal genetic drift.

All husbandry is performed by the core facility staff to ensure highest quality and reproducibility. All husbandry and associated procedures are standardized, and all SOPs are available on request. All animals including their detailed journals are registered in an electronic database accessible which is remotely accessible.

1D) GENOTYPING AND SCREENING OF ZEBRAFISH LINES

The zebrafish core facility offers a complete genotyping pipeline for transgenic and knock-out zebrafish strains. The pipeline consists of fin-clipping, single-housing of fin-clipped animals, PCR, and sequencing in collaboration with the KISEQ core facility, interpretation of the data and sorting of the fin-clipped animals.

The zebrafish core facility offers furthermore the screening of zebrafish embryos for the expression of fluorescent marker genes and raising of identified carrier fish.

1E) TATOOING OF ZEBRAFISH

The zebrafish core facility staff offers the implantation of visible elastomer tags under the skin of adult zebrafish which allows for long-term identification of individual animals. We have implemented a refined procedure published [here](#).

2) DELIVERY OF ANIMALS, TISSUES, AND ORGANS

The zebrafish core facility can deliver whole embryos at a precise developmental stage or tissues of adult zebrafish for analysis. Embryos and tissues can be delivered from common wildtype or transgenic lines or from researcher-owned lines after an agreement with the owner of the line. Fresh, snap-frozen, or fixed samples can be supplied.

The zebrafish core facility offers furthermore the embedding of embryos or organs of adult zebrafish for cryosectioning.

3) CRYOPRESERVATION AND IVF

The zebrafish core facility offers cryopreservation and IVF for both zebrafish lines housed at KI or imported from other zebrafish facilities in Sweden or EU.

The cryopreservation and IVF pipeline is based on the protocols published by the International Zebrafish Resource Center. The quality of the cryopreserved sperm is always analyzed with a test-IVF. A minimum of 10 cryo samples are stored in duplicates in ultra-low cryogenic freezers.

4) GENE ANALYSIS

4A) TRANSIENT GENE KNOCK-DOWN

Genes can easily be knocked down in the zebrafish using the morpholino technique which blocks translation or splicing of the targeted mRNA. The morpholino is injected into fertilized eggs from where it ubiquitously distributes, and phenotypes can be analyzed within a couple of hours.

For more information on the morpholino technique, please visit <https://www.gene-tools.com/>

The zebrafish core facility can assist with the design of the morpholinos and proper controls, the injection of morpholinos into wildtype and transgenic zebrafish embryos as well as the analysis of the phenotypes (see also the other pipelines below).

4B) ESTABLISHING OF KNOCK-OUT LINES

The zebrafish core facility can generate stable knock-out zebrafish lines. We use the CrispR-Cas9 system in combination with duplex-guidance RNAs, a system that can quickly and efficiently generate homozygous knock-out zebrafish. The system can be used both for transient approaches and for generating stable knock-out lines.

The zebrafish core facility can assist with the design of the guidance RNAs, the injection of the CrispR-Cas9 mixture as well as raising and genotyping of the animals.

The zebrafish core facility has established a standard CrispR-Cas9 protocol which allows to suppress the pigmentation in any wildtype or transgenic zebrafish line, see also [here](#).

4C) ESTABLISHING OF TRANSGENIC LINES

New transgenic zebrafish lines, for example expressing a fluorophore under the control of a tissue-specific promotor, can easily be established with the Tol2 technique. The zebrafish core facility can assist with the design and the injection of the constructs as well as the screening and raising of embryos.

5) TRANSPLANTATION OF CANCER CELLS

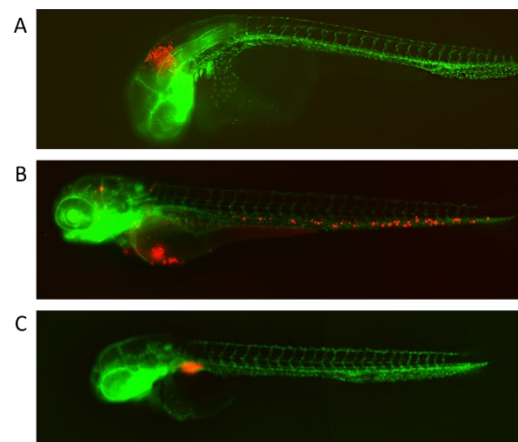
The zebrafish embryo is an ideal and clinically relevant model for xenotransplantation assays in order to study tumor growth, metastasis or the effect of small molecules on cancer cell survival. Due to the transparency of the embryo, the growth and destiny of the transplanted cells can easily be imaged in vivo and real time. The small size of the embryos allows for high-throughput pipelines (see also our high-content imaging pipeline).

Most transplantation assays deliver clinically relevant data for e.g. tumor growth or metastatic behavior before the embryo is 5 days old, hence no ethical permit is required. Cancer cells and primary material can be transplanted into various regions and tissues of the embryo including the central nervous system or the cardiovascular system.

The zebrafish core facility provides optimized and standardized protocols for preparation of the transplanted material, and we can transplant cells and materials into various organs and tissues of hundreds of embryos per hour. Our pipeline follows the published and standardized [ZeOncoTest](#).

The zebrafish core facility provides moreover a combined pipeline for transplantation of cancer cells, drug exposure and high content imaging. This pipeline delivers clinically relevant data in just 3 days and consists of:

- Transplantation of cancer cells or primary material into 2-day old embryos
- Screening for successfully transplanted embryos
- Exposure of transplanted embryos to small molecules
- Distribution of the transplanted and exposed embryos to 96-well plates
- Automated imaging up to 3 days post transplantation



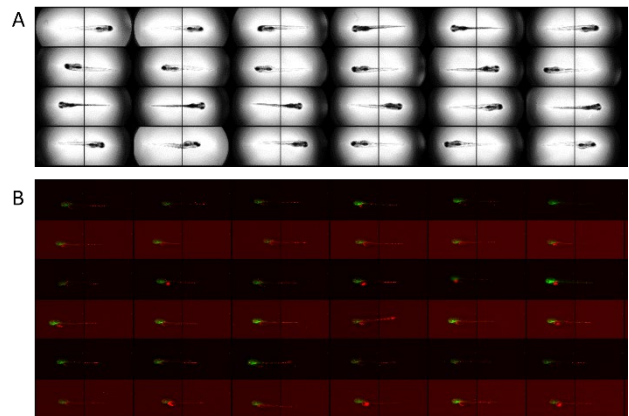
A: Cells transplanted into the central nervous system
B: Cells transplanted into the cardiovascular system
C: Cells transplanted into the PVP (=subcutaneously)

6) HIGH CONTENT IMAGING

The zebrafish core facility offers high-content imaging of zebrafish embryos mounted in 96-well plates. The embryos can either be imaged in fluorescent channels or in brightfield. Recording of videos and time-laps imaging is possible.

For high-content imaging we use the [Aquifer imaging system](#) specifically designed for high-throughput imaging of zebrafish.

The zebrafish core facility can provide custom-made 96-well plates with an agarose bed for ventral or dorsal imaging of zebrafish embryos, mounting of embryos in 96 well plates, and imaging.



A: Stable mounting of embryos in 96-well plates
B: HT-imaging of transplanted embryos, plate overview

7) DRUG EXPOSURE AND TOXICOLOGY STUDIES

The zebrafish core facility provides specific pipelines for exposing zebrafish embryos to small molecules.

The exposed embryos can be analyzed in various ways such as for morphological deviations, for effects on the cardiovascular system or for alterations of embryonic behavior. We also offer complete toxicological profiling based on the OECD guideline 236 (Fish embryo acute toxicity test). We do of course have the possibility to expose zebrafish embryos to toxic substances including CRM chemicals.

8) INFECTION STUDIES

The zebrafish core facility has a BSL-2 laboratory dedicated for exposing zebrafish to class-2 pathogens.

We have all necessary permits for using a range of class-2 pathogens in place and can assist with obtaining licenses for using additional pathogens if needed.

We can expose embryos to class-2 pathogens in various ways including immersion and injection into tissues and organs. We have furthermore the technical equipment to follow and image infected embryos in real time.

9) BEHAVIORAL ANALYSIS

The zebrafish core facility offers a range of specialized tools for zebrafish behavioral analysis. The equipment includes a Noldus Daniovision setup including the Ethovision software for high-throughput behavior analysis of zebrafish embryos and imaging systems for assessing adult zebrafish behavior.

The zebrafish core facility can assist with setting up and running pipelines for behavioral analysis.