

## Zorgl'hub Services

### - **Production of zebrafish embryos and adults**

Standard wild-type zebrafish (AB strain) can be provided by the ZEN facility.

### - **Zebrafish husbandry**

If you need to raise specific zebrafish strains for your project (such as transgenic lines or mutants, from external sources), you can rent tank space in a rack in the ZEN fish facility, dedicated to the project hub. Feeding and basic health monitoring will be included.

You will have to do your crosses and embryo collection. You will also have to perform genotyping, if necessary. We provide necessary training for this (see below).

### - **Support to developmental biology, cell biology and neuroscience projects (ZEN)**

→ We will help you design and conduct imaging and/or functional assays to address questions pertaining to development, cell biology or developmental neuroscience using zebrafish embryos and larvae. We possess or can import transgenic lines highlighting cell types or subcellular events. We will help in the planning of genetic tools, and train experimenters to functional and phenotypical analyses.

0. Advice on project design, evaluation of the strengths and weaknesses of the model
1. Raising and maintenance of necessary fish lines (includes obtaining authorization when GMO)
2. Help with tools design (morpholinos, constructs for gene overexpression or transgenic generation)
3. Initial training of a project lab member with (to be adjusted depending on the project):
  - . egg production
  - . embryo / larval staging
  - . microinjections into embryos
  - . embryo sorting and geno / phenotyping
  - . drug treatments
  - . microscopy observation, ISH and histology techniques
  - . sperm freezing and IVF

It is then expected that the follow-up experiments are conducted by project lab members inside the Zebrafish Neurogenetics Pasteur fish facility. It may be necessary (see below) that these project lab members obtain an authorization to experiment on animals.

4. Help with results interpretation and follow-up experimental design

The standard services are typically useful for gene expression analyses, functional assays in gene gain- and loss-of-function approaches, and phenotypical analyses.

#### Restrictions / Remarks:

- . 1 microinjection set-up available twice / week;
- . Project-specific molecular biology tools, drugs etc should be purchased by the project lab;
- . Projects needing work on late developmental stages (> 6 days-post-fertilization to adult) and conditional genetic techniques will be supported on a case by case basis.

### - **Support to immunology and infection projects (RIWO)**

→ After a discussion of the experimental design, we will generate a zebrafish infection model using your favourite microbe (up to P2+).

→ We will produce the required larvae (typically, transgenics highlighting a relevant leukocyte population) and microinject the pathogen. We will record the fate of your fluorescent microbe *in vivo* upon infection in the entire organism, and document the host innate immune response.

→ Optionally, we can combine this with a drug assay or manipulate immune cells or pathways; we also offer training in microbe microinjection.

0. Evaluation of the strengths and weaknesses of the model
  - Two points, in particular, deserve attention:
    - the temperature at which experiments will be conducted (zebrafish can be maintained between 24 and 32°C);
    - the availability of fluorescent microbes.
1. Survival curves/dose response assessment
  - Experimental microbe microinjection (24 larvae for two different doses) and 24 for CTRL non-injected fish. Follow them for 2 days post-injection (dpi) in the basic package (not requiring ethics committee approval)
2. Microbe dissemination
  - A) CFU/PFU Quantification of lysed larvae (5 larvae per condition and time point). NB lysates will be provided to the project lab members who will perform themselves the assays.
  - B) On living larvae, once a day, using a fluorescent microscopes (if the microbe is fluorescent)
    - a) Pattern of dissemination at the whole organism level
    - b) Evaluation of the microbe burden by fluorescent pixel count
  - C) Impact of infection on macrophage/neutrophil population (following daily 5 larvae per condition)
3. Inflammatory cytokine induction
  - A) Q-RT-PCR on 5 larvae per condition and time point
  - B) Whole-body localization of cytokine-expressing cells (using reporter fish)
4. Data analysis

We will provide a report including the imaging data and statistical analysis.

5. Optional tests
  - Infections may be followed for more than 2 days, but previous approval by the ethics committee is required (we will help you with the redaction of the demande d'autorisation)
  - Additional conditions (more doses / comparison of microbe strains / use of drugs) may be included
  - We may perform host manipulation (use of available mutants, or morpholino-mediated knock-down)
  - We offer training for microbe microinjection

#### Approximate costs

- |   |   |
|---|---|
| - production of embryos or adults             | 18 €/clutch (~100 eggs); 2 €/adult fish |
| - maintenance of fish lines for your projects | 0.60 €/tank/month                       |
| - training to embryo injection and analysis   | 164 €/half-day                          |
| - pathogen testing                            | 347 to 610 €                            |

These prices only cover running costs and apply to IP Units only.

To get a detailed quote, you'll have to submit your project to the Zorgl'hub committee.