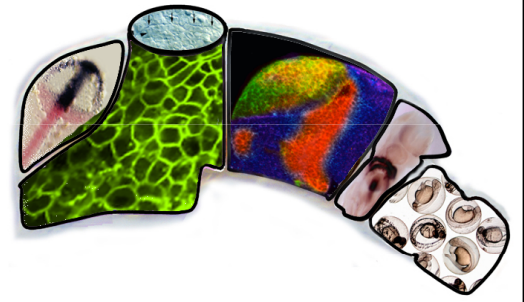


***In vivo* single cell manipulation in zebrafish embryos.**

The Houart Lab



Aim of the practical:

The zebrafish offers a set of advantages for the study of developmental neurobiology, from classical and molecular genetics to cell biology. This practical aims to illustrate the ease and precision at which cells can be moved inside the developing CNS of a given zebrafish embryo, or from a donor to a host embryo. This technique can be used to answer many kinds of biological questions, such as fate specification, cell-autonomy of a given defect, type of division of progenitors, cell behaviours (migration, circuit formation, synaptogenesis, ...).

The instructor will demonstrate the technique, showing transplant of a couple of GFP-tagged midbrain precursors from bud stage (late gastrula/early neurula stage) donor embryos into a wildtype host. Each student will have the time to practice the technique too. We encourage the students to try transplants relevant to their own research interests. We will be able to guide them in exploring the best way to use the technique in their research context. The transplants will spend the night in the 28°C incubator. The course participants will be able to analyse them the next morning, take pictures of the clones obtained and draw some conclusions on their results. Embryo mounting and dissection techniques will be taught.

Transplantation protocol

This protocol can be used to move cells from donor to host embryos at any developmental stage. It was designed to require as little preparation as possible to be able to do many transplants per session. The two most limiting factors are the quality and percentage of the methyl-cellulose and the sharpness and diameter of the capillary used for cell manipulation.

What is needed:

- A compound microscope equipped with long working distance objectives (10 and 20X), a fixed stage (or a micromanipulator fix on the stage), an external zoom placed in the head of the scope (1.5 and 2X).
- A standard micro-manipulator (allowing some fine control of movement)
- 1.2-1.55 mm diameter glass capillaries (without inner filament!)
- Micro-syringe (commercial rig on picture below or plastic syringe) linked to a needle holder (same holder as injection rig)
- 4 or 5 % methyl cellulose
- E2 medium with PenStrep (see recipe below)
- Glass slides
- 22 by 22 cover slips
- Pasteur pipettes
- 3 cm diameter Petri dishes
- Pairs of forceps (tweezers n°5)

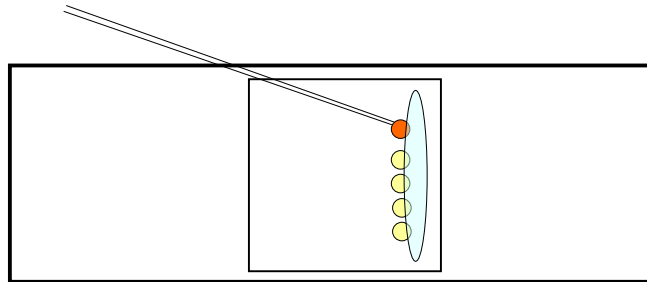
Protocol:

- Dechorionate the donor and host embryos: pronase treatment or by hand (recommended)
- Snip your pulled capillary with a pair of forceps, at the level required to give the diameter needed (bigger than a cell diameter and less than 2 – will vary with stage of embryo used). If needed, shape the tip of the capillary with a micro-forge.
Note: commercialised micro-needle (e.g. ES transfer tips from Eppendorf) is also an option.
- Place your needle in the holder of the microsyringe (or syringe) and the holder onto the micromanipulator. Adjust position of the needle under the objective.



- Place a cover slip on a glass slide (attached by capillarity using a small drop of water)
- Using a small diameter Pasteur pipette as a spreading tool, collect a bit of methyl cellulose by dipping the pipette into the tube of Methyl Cell. and then trace a line of methyl cell onto the cover slip (pale blue on illustration below).
- Using a clean Pasteur pipette, place one or two donor embryos (orange in figure below) and 4-6 hosts onto the cover slip, near the methyl cellulose strip.

- With the back of the tip of a pair of forceps, push gently the embryos against the methyl cellulose. While pushing them, orientate them such that the area in which you want to operate is outside of the cellulose. Push until around $\frac{1}{4}$ of the embryo is into the methyl cellulose (see below).



- Place the slide under the scope and adjust the position of the needle and the embryos such that the needle is close to the donor embryo under the objective.
- Penetrate the donor embryo at the desired location, using rapid and short movement of the stage.
- Using the micro-syringe, begin to pull cells out of the donor embryo (control on the syringe should be good enough to move one cell at a time and have stability of the cell position inside the capillary). Great control can be obtained, increasing the magnification using the decoupled zoom in the scope head.
- Take around 20-30 donor cells in the needle, then move out of the donor and place the needle near the first host embryo.
- Penetrate the host at the side desired for the transplant planned
- Push the precise number of cells you wish to give the host, using the microsyringe.
- Move to the next host and repeat the operation. Repeat the previous 2 steps until either you run out of donor cells (and therefore collect more from the donor embryo if needed) or have transplanted all hosts.
- When the whole row of hosts are transplanted, remove the cover slip delicately from the slide, using your fingers, only holding a very small corner of the cover slip away from the embryos. Place the cover slip inside a 3 cm diameter Petri dish. Make sure it is placed flat onto the bottom of the dish.
- Delicately pour a few ml of E2 + PenStrep medium on top of the embryos. Leave them to recover in a 28°C incubator. Never try to remove the embryos from the methyl cellulose, as it damages them irreversibly. The embryos will free themselves from it after a few hours in E2.
- The transplanted embryos can be used for time-lapse or other types of observation and experiments at the earliest 1 hour after surgery.

Recipes:

E2 medium (20X)

Part 1: Add 17.5 gr NaCl, 0.75 gr KCl, 2.4 gr MgSO₄, 0.41 gr KH₂PO₄, 0.12 gr Na₂HPO₄ to 1 litre of RO water. Autoclave

Part 2: Add 7.25 gr of CaCl₂ to 100 ml of RO water. Autoclave.

Part 3: Add 3 gr of NaHCO₃ to 100 ml of RO water. Autoclave.

Keep stocks refrigerated. To prepare a litre of 1XE2, combine 50ml of part 1, 2 ml of part 2 and 2 ml of part 3 and 946 ml of RO water.

4% methyl cellulose

Dissolve 4 gr of methyl-cellulose in 100 ml of fish water or E2 medium. Either wait until it is dissolved at room temp. (may take a couple of days) or zap for 10 seconds in the microwave oven (tricky! Time depends upon microwave oven age!)

Pen-Strep 500X solution

60 mg/ml of penicillin and 100 gr/ml of streptomycin, stored at -20°C