Injecting Genetic Material in Zebrafish Embryos

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[Abstract] Zebrafish is a convenient model for transient genetic manipulations such as morpholino-mediated gene knockdown and DNA/mRNA-based genetic overexpression. These genetic approaches do not involve the challenge of making transgenic animals, and their effect can last up to a week. This protocol describes the detailed steps of injecting nucleic acids in to zebrafish embryos.

Materials and Reagents

1. Zebrafish
2. Oil
3. Zebrafish mating boxes (Aquatics)
4. Mineral oil (Sigma-Aldrich, catalog number: M5904)

Equipment

1. Bench
2. Forceps
3. Injection
4. Micrometer slide
5. Micropipette puller (Sutter Instrument)
6. Microinjection system Pico-injector (Harvard Apparatus)
7. Benchtop microscope (Leica Microsystems)
8. Micrometer slide (3B Scientific)
9. Glass capillary tubing (Thermo Fisher Scientific)
10. Benchtop microcentrifuge (Biotool)

Procedure

A. The night before:

Set up 10-15 pairs of fish, keeping the male and female separated from one another. By doing this, the fish won't mate until you are ready for them, but they will still be amenable to mating.

Pull the needle using the needle puller according to the manufacturer's instructions.

B. The morning of injections:

1. Put fish together (in the fish room)

   In each of 3-5 single boxes, combine the male and female fish so that the female will begin to lay eggs (egg laying will begin in 15-45 min, and the same fish may lay eggs more than one time).
2. Filling needles (in the injection room)
   a. Hang needle above bench.
   b. Spin RNA/cDNA/dye sample in benchtop microfuge so that any debris will go to the bottom of the tube.
   c. Using a gel-loading tip, remove 1-1.5 µl of sample from tube and load into back end of needle.
   d. Wait for capillary action to carry sample to needle tip.

C. Adding embryos to injection dish

1. Collect eggs from the fish you have put together, labeling the dish with the date and type of fish and take them to the injection room.
2. Add embryos to injection dish and use forceps to push them into the individual rows.
3. Using forceps, rotate embryos so that the yolk is facing the future direction of the needle.
4. Remove enough E3 so that the embryos do not float, but be careful not to let them dry out.
5. Set this dish aside and break the needle.

D. Breaking needles

6. Turn on air supply and the injection apparatus.
7. Set the Pinj to 25-30 kPa (turn "inject" knob to change) - the higher the P, the better it is for breaking the needle.
8. Make sure the Pout and Pbal are slightly positive (0.1-0.2) (turn "balance" knob, if needed).
9. Place a drop of oil on micrometer slide and set this aside.
10. Insert filled needle into micromanipulator.
11. Find the end of the needle under the microscope (this will likely require you to move the needle with the micromanipulator until it comes into the field of view).

12. Adjust the microscope to the highest magnification, keeping the end of the needle in view.
13. Using forceps, clip end of needle. (This takes practice to clip the needle in the correct place!).
14. Put micrometer slide in field of view.
15. Put tip of needle in oil on slide so that it is positioned over markings. Do not let the tip of the needle touch the slide.
16. Adjust the injection time to 6-8 msec.
17. Step on the injection pedal ≥ inject into oil and look at droplet size.
18. Adjust Pinj and injection time and continue to inject into the oil until the droplet is 0.12-0.15 mm.
19. The drop size can be larger, but it is more likely to be toxic to the embryo.
20. You are now ready to inject embryos.

    Do not leave the broken needle tip exposed to the air for too long, or the RNA/cDNA/dye may dry and clog the tip of the needle. You may put the tip of the needle into a dish with water/E3 to keep it wet, but be sure to check that the water is not going into the needle, or that your sample is not leaking into the water!

    Note: If the tip of the needle does clog, put the needle tip in some water/E3 and push the "clear" button. This will sometimes remove the clog.

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