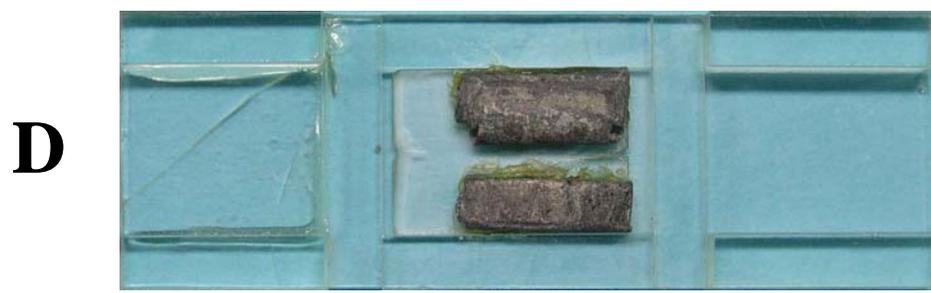
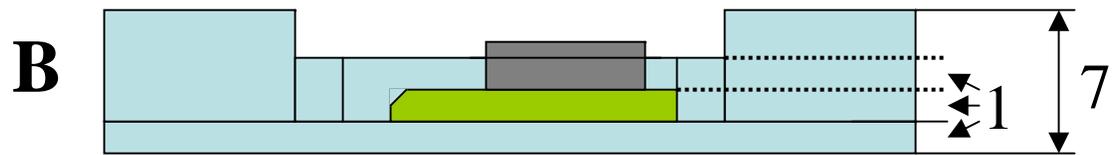
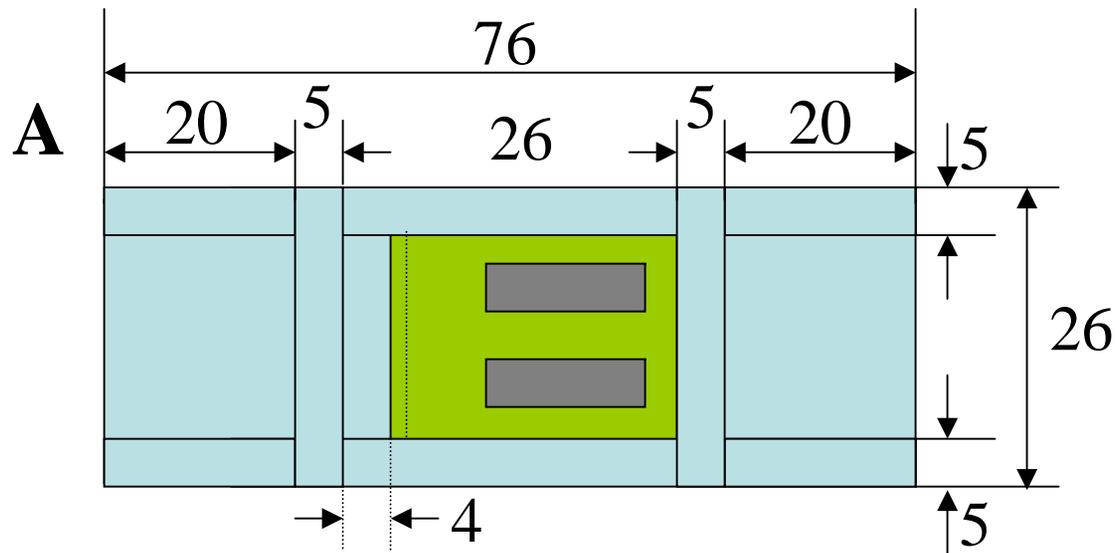


## **Appendix A7-2.**

### **Microinjection to medaka embryos (upright microscope method)**

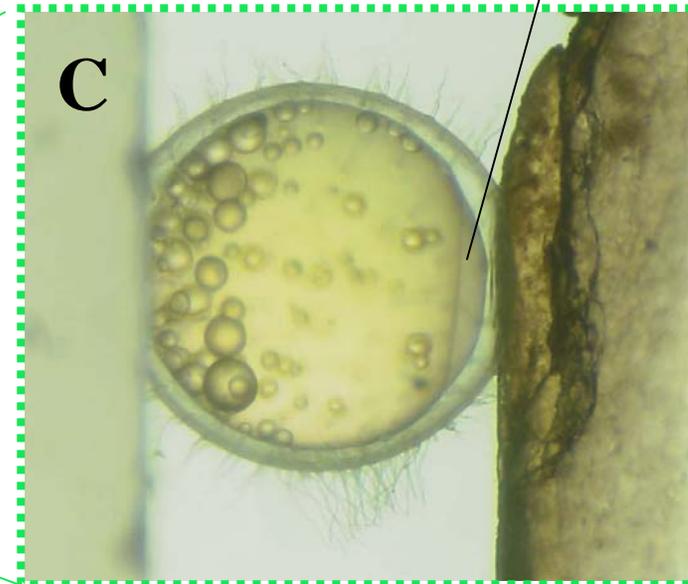
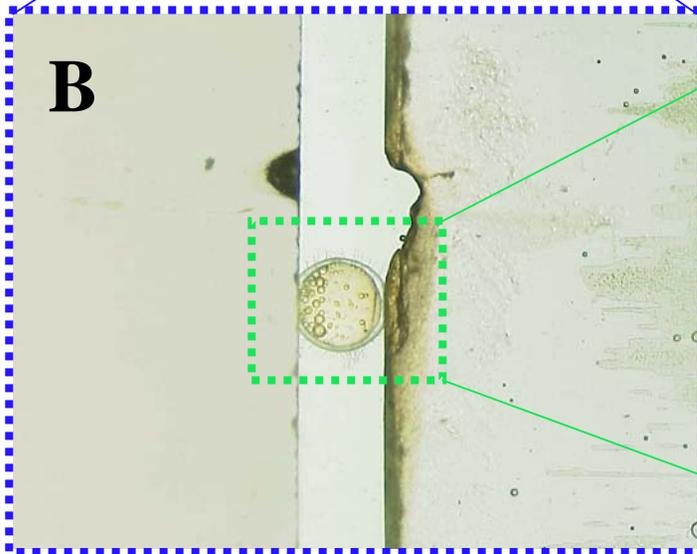
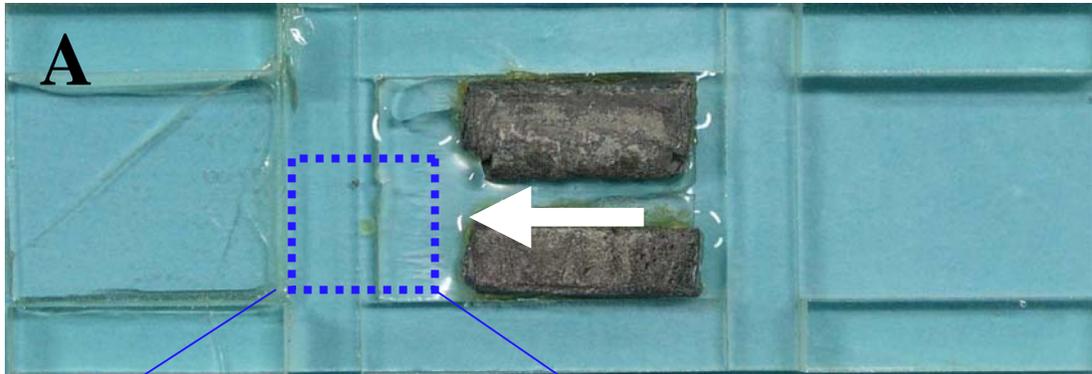
The procedure for the microscope (upright type) method is almost same as that for the stereo microscope (binocular) version (A7-1). In this appendix, critically different parts are shown. For the detailed procedure, please refer to the M7 movies.

1. Egg holder (glass type)
2. Egg orientation
3. Preparation of a needle
4. Opening the tip of a needle
5. Entire procedure (Flowchart)



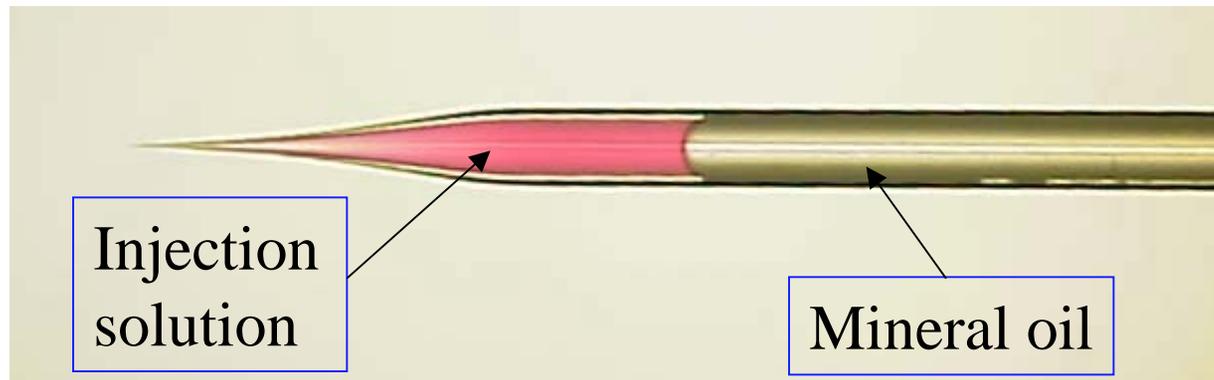
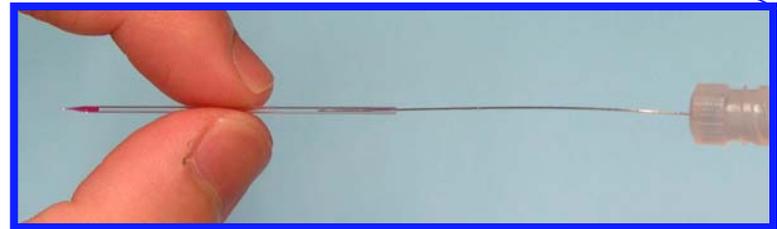
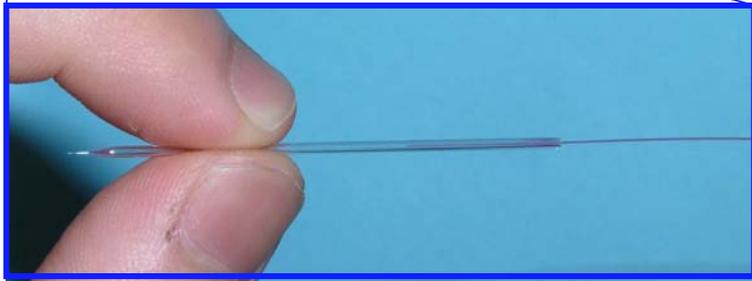
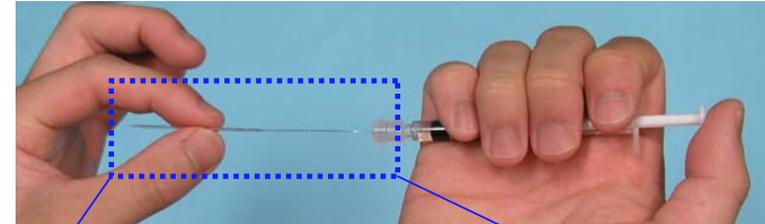
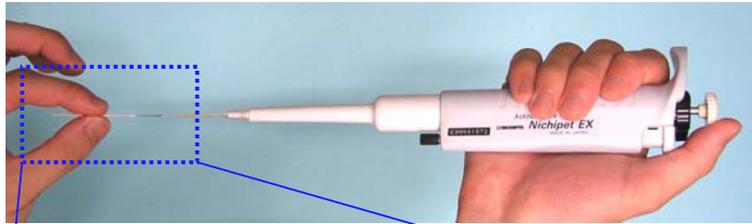
**A typical egg holder for an upright microscope**

The egg holder is made of a sliding glass plate and glass sticks. (“A” indicates the top view and “B” indicates the side view. The sizes are represented in mm.) “C” consists of a glass plate with a block of lead as weight. The weight is made of soldered metal (an alloy of lead and tin). “D” and “E” are photographs of the egg holder.



### **Egg orientation**

Orient the cytoplasm of the egg to where the needle (white arrow in A) will arrive, and fix the egg in the groove of the egg holder. Refer to movie M7-1d.



### **Preparation of a needle: Filling with injection solution and mineral oil**

Fill the tip of the needle with injection solution from the open end of the needle using a microloader tip. If using a hydraulic injector, add mineral oil with a microsyringe (stainless needle) after filling with injection solution.

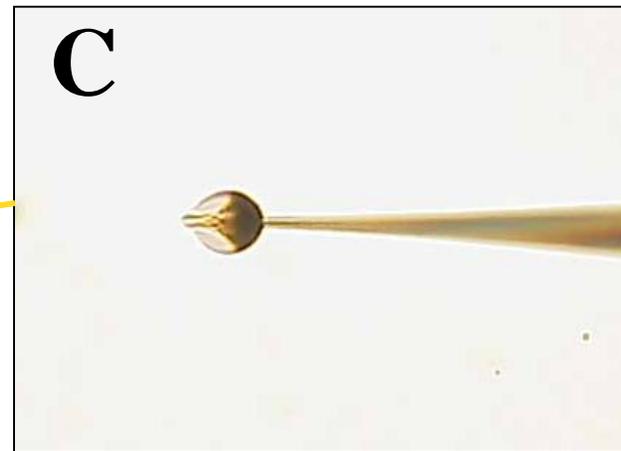
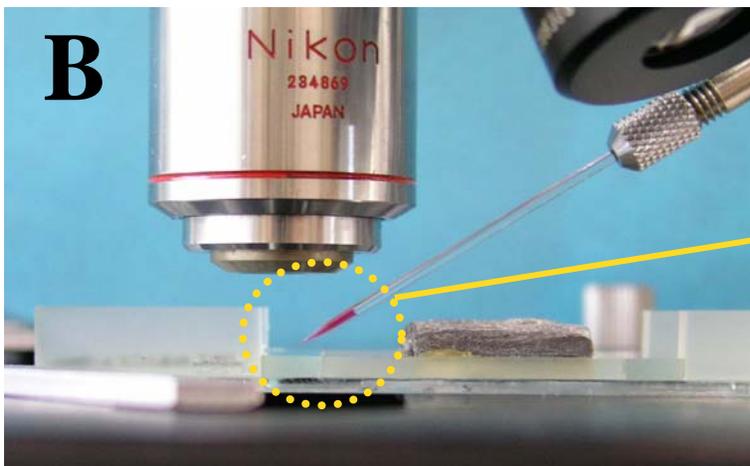
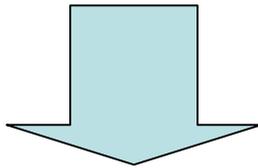
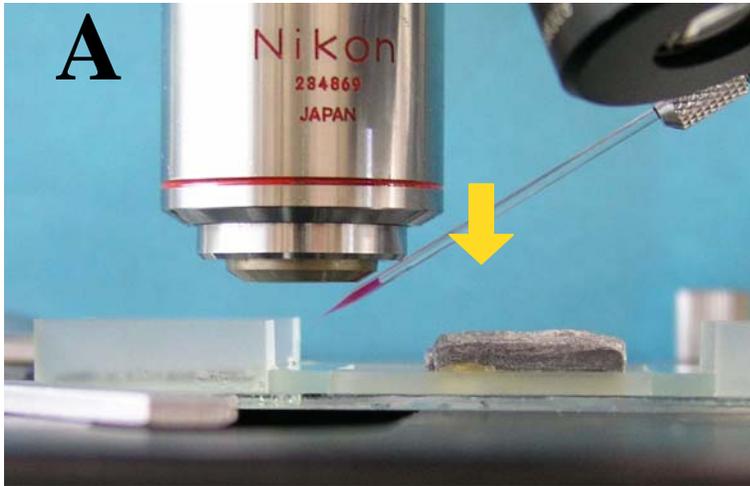
## Opening the tip of a needle

Fill a needle with injection solution and mineral oil and then attach the needle to the needle holder on the microscope. Then, fix the egg holder on the microscope stage.

A: Gradually lower the needle close to any part of the glass surface of the egg holder.

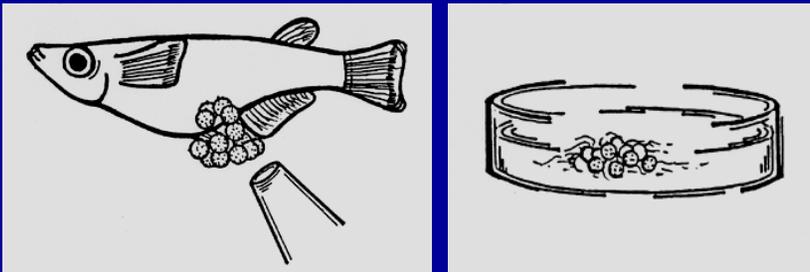
B: Slowly and carefully, place the tip of the needle in contact with the glass surface. When the tip touches the glass surface, it will be broken and opened.

C: You can confirm the opening by leakage of injection solution from the tip to the glass surface.

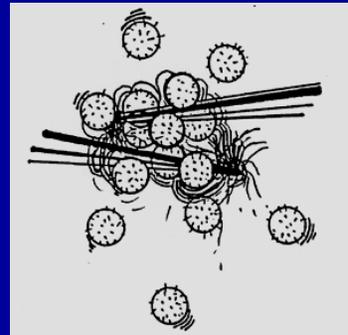


**Microinjection :** With a fine glass capillary, solution is directly introduced into the embryo

1. Collect eggs within 20 minutes after fertilization.



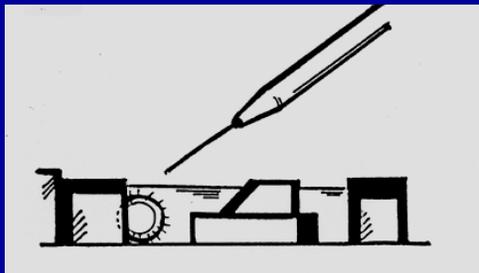
2. Remove attaching filaments.



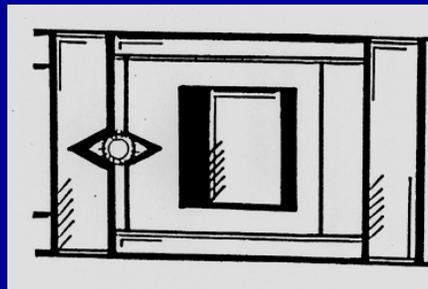
3. Incubate at 6°C to reduce development speed.  
(in case of *O. latipes*)



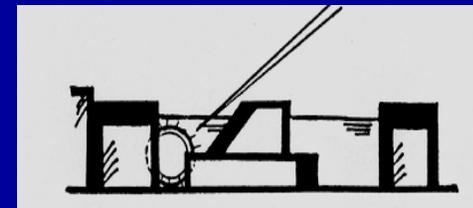
4. Put an egg on the egg holder and orient cytoplasm to the needle.



5. Fix the egg in the groove.



6. Inject DNA.



7. Incubate injected eggs.

