

My document

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Microinjections

Ingredients

- 3-AT sea water
- PS-coated petri dish
- Pipette and tips
- Dejellied eggs
- Sperm
- Microinjection needle
- Injection solution

0.1 Preparation of injection solution and needle

Prepare the following injection solution, preferably the same day it is going to be used.

Ingredient	Final Quantity in 20 ul	Volume to be added
Linearised construct	100ng	Depends on construct concentration
gDNA	500ng	Depends on construct concentration
KCl (1M)	0.12M	2.4 ul
Glycerol (50%)	20%	8 ul
Control dye (10%)	0.25%	0.5 ul
ddH ₂ O	-	To add up to 20 ul

Linearised construct

This is restriction enzyme digested plasmid. For Anpep_1, digest with KpnI. Confirm digestion product with gel electrophoresis and use the PCR purification kit to remove restriction enzyme from the DNA solution.

gDNA

HindIII digested (overnight) and purified genomic DNA extracted from urchin epidermal tissue.

Control dye

Texas Red dye diluted to 10%.

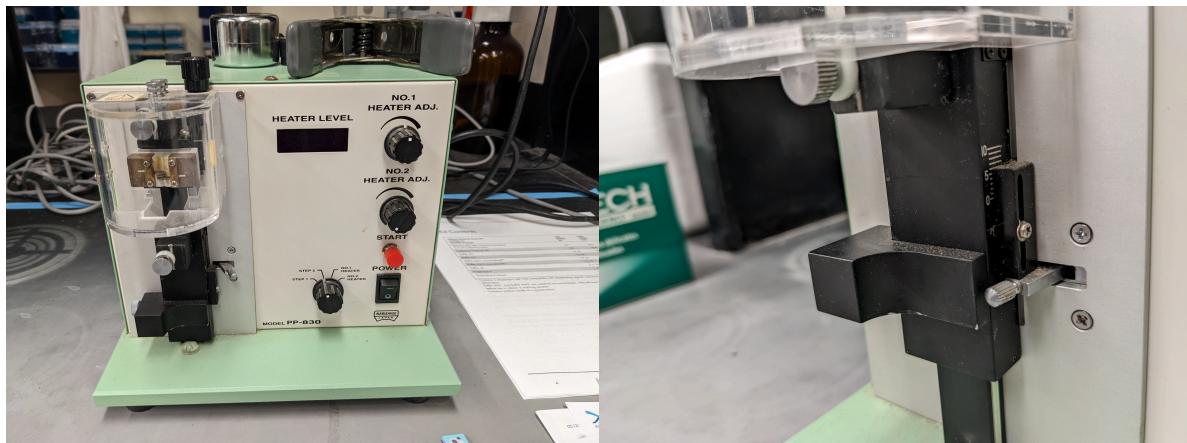
Injection solution should be centrifuged for 15 minutes at max speed immediately before filling the microinjection needle.

0.1.1 Preparation of the needle

⚠ Warning

Be careful never to touch either ends of the needle!

We used a Narishige puller, model PP-830



(a) Needle puller machine

(b) 6th grid setting

Figure 1: Needle puller set up

with the following settings:

Two stage: 67.4°C, 80.2°C.

Weight: 248.01 g



(a) Puller weights on scale

(b) Puller weights on the machine

Figure 2: Weights used for the puller

0.1.2 Filling the needle

To fill the needles, pipette up 0.5 ul of injection solution. Use a 10ul pipette tip. Make sure to get the liquid from the top/middle of the tube in order to avoid any precipitate stuck to the bottom/sides of the tube during the centrifugation.

Put a piece of clay on the side of a table and push the needle upside down into it. Fill needle by touching the end of the needle with the tip of the pipette and pushing out the liquid with the pipette. Make sure that the liquid enters the needle instead of just sitting on top. Wait a few minutes for the solution to reach the bottom of the needle.

Mount needle onto the macromanipulator:

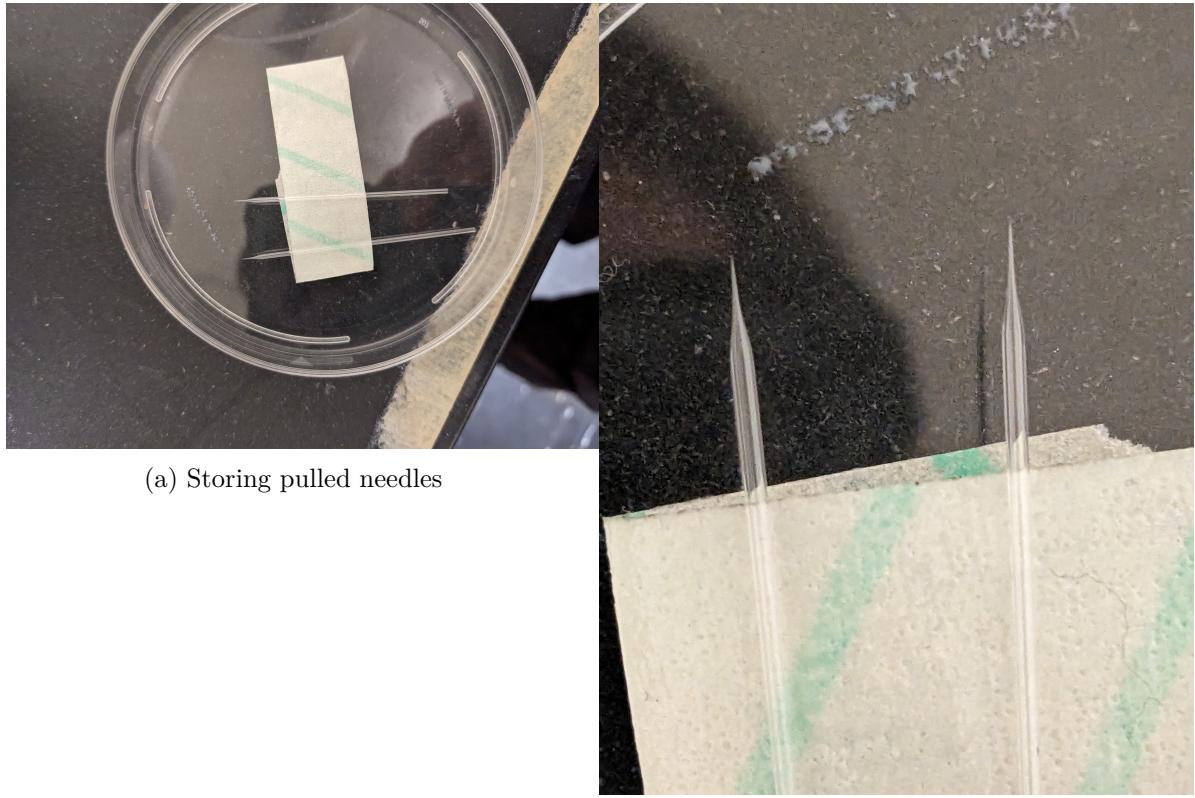


Figure 3: Pulled needles



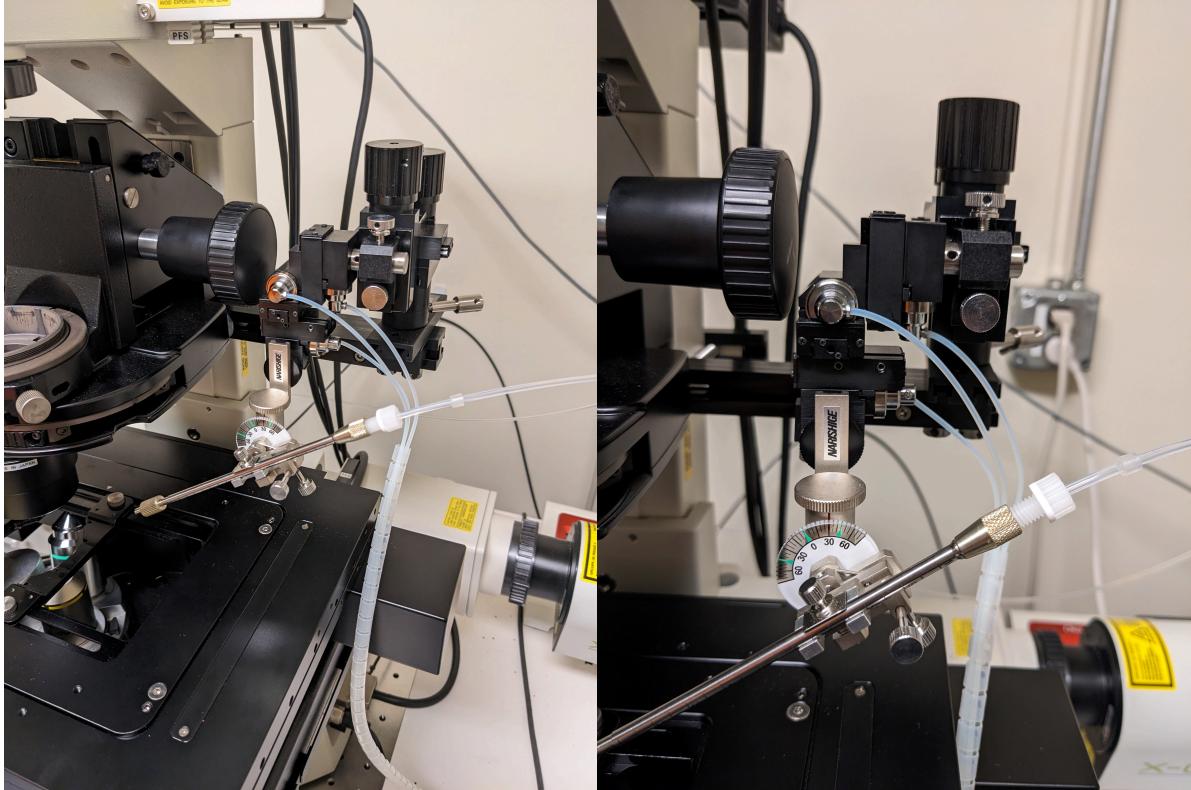
Figure 4: Filling needles



(a) Insert needle into needle holder, end first, make sure needle end is visible

(b) Needle in needle holder

Figure 5: Using the needle holder (aka grip head)



(a) Take off capillary holder (metal rod) and screw (b) Make sure that the capillary holder is at 30°
in needle holder

Figure 6: Setting up macromanipulator

0.2 The microinjection setup

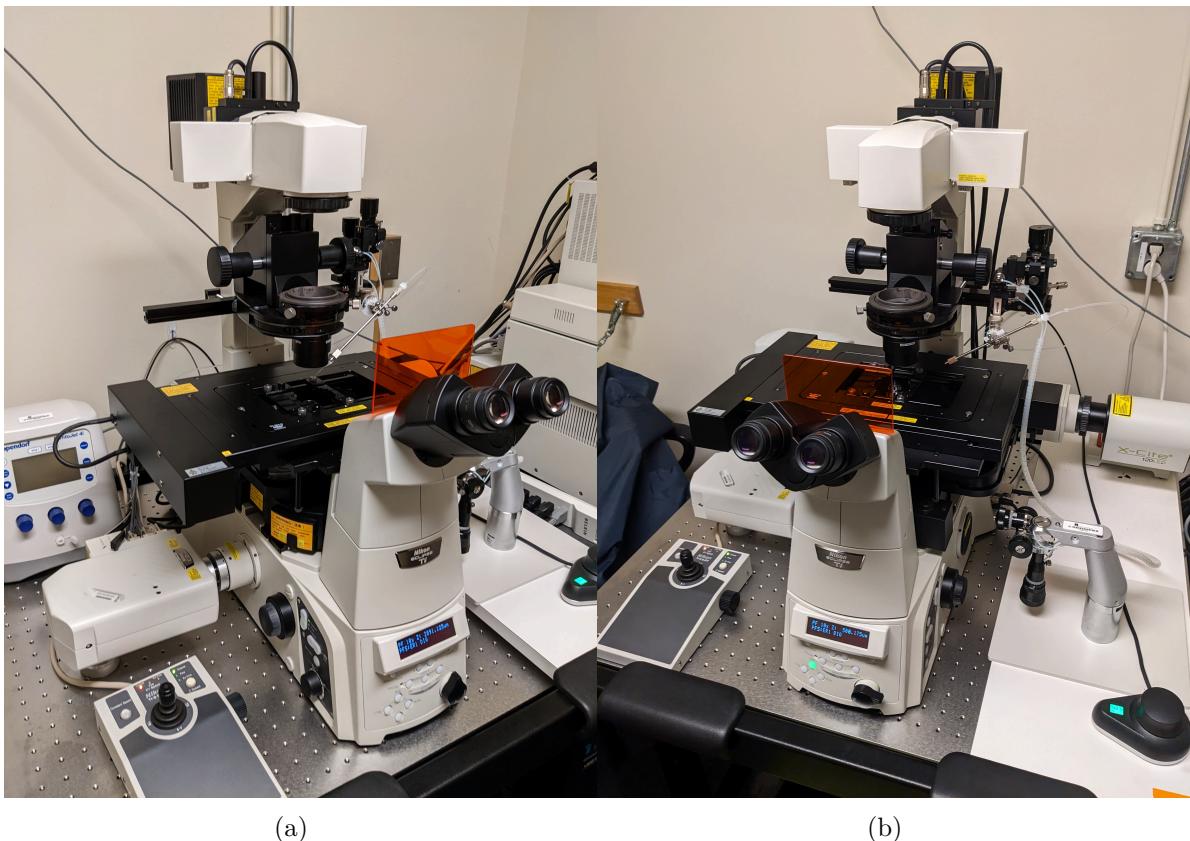


Figure 7: Confocal microscope set up

0.3 Preparing sea water

For embryonic development:

Prepare a beaker (1000 ml) with sea water (12°C, RO + instant ocean salt). Check for correct salinity (~33%)! Adjust salinity if needed. Place in 15°C fridge.

For during the microinjection:

Pour 25 ml of the filtered sea water into a 50 ml centrifuge tube and add 25ul of 1M 3-aminotriazole (3-AT) stock solution¹ to it. “Label as 3AT filtered sea water”. Place the 3AT sea water on ice.

¹3-AT stock solution: Prepare 1 M stock solution of 3-aminotriazole (3-AT, MW = 84.08) by dissolving 0.84 g of 3-AT in 10 ml of ddH₂O. This solution can be stored at 4 °C for up to 6 months.

0.4 Preparing the petri dish

Take a PS-coated petri dish², mark middle with a straight black line with marker on the outer side of the dish. Take a razor blade and make a cut parallel to the black line halfway between the black line and the edge of the dish on the inner side of the dish. This scratch will be important to break the injection needle to adjust the flow of solution as needed.

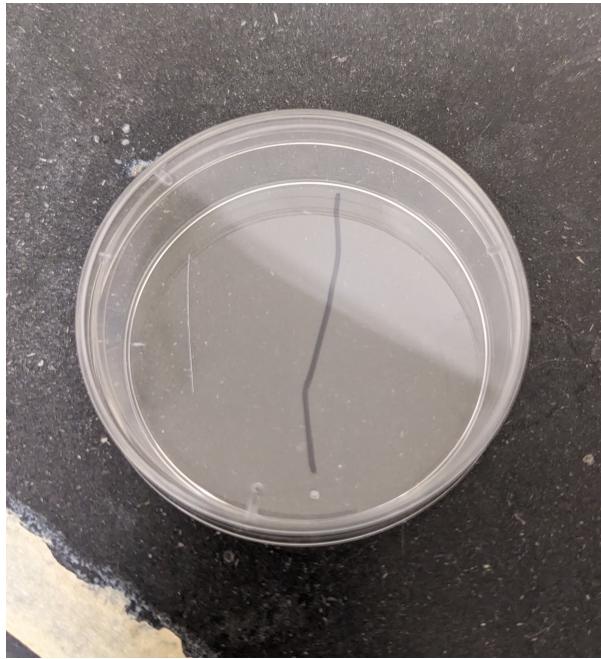


Figure 8: Prepared petri dish

Pipette 4 ml of 1 mM 3-AT sea water (prepared in the first step of “Collecting eggs and sperm”) into the PS-coated dish using a transfer pipette.

Place petri dish under microscope, such that the scratch mark is on the left.

Switch on the microscope and the FemtoJet 4i. Make sure to detach the injection tube first!

²PS coated dishes: Prepare 1% solution of protamine sulfate (PS) by adding 0.5 g of PS to 50 ml of deionized, distilled water (ddH₂O) in a 50 ml conical tube. Shake well at high speed on a bench shaker at room temperature for 1-2 hr to ensure complete dissolution of PS. This solution can be stored at 4 °C for at least 3 months (make sure to completely dissolve gel-like precipitate before each use). Take a sleeve of 60 mm x 15 mm polystyrene Petri dishes and lay out both lids and bottoms on the bench. Warm up PS solution to room temperature. Pour 1% PS solution in each dish (both bottoms and lids can be used) just enough to cover the surface, leave for at least 2 min. The leftover PS solution can be reused many times within 3 months when stored at 4°C. Place PS-treated dishes in a beaker filled with distilled water (dH₂O). Leave the beaker under running dH₂O for at least 10 min. PS-coated dishes can be used immediately or air dry for storage. Cover them to prevent dust accumulation. They can be stored at room temperature for 1 month.