Protocol 3: Microinjecting *A. longisetosus* Embryos

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Materials

Narishige XYZ micromanipulator
Narishige IM-300 Microinjector [version 8.2A]
Narishige's EG-44 capillary grinder
Sutter Instrument Inc. Model P-87 Flaming/Browning Micropipette Puller
Glass capillary tubes thin-wall, 1.0mm x .75 mm, 4" (A-M Systems, INC.: Catalog #: 615000)

I. Needle Preparation

1. Place a capillary tube in the needle puller. Pull on the following settings:
Pressure: 509
Heat: 560
Pull: 100s
Velocity: 40
Time: 115s

2. Grind the resulting needles under the following settings:
Speed: 8
Degree of grinding: 25°
Grinding time: 50s

3. Make sure that water is dripping onto the diamond stone at a constant rate. Place the needle on the needle holder, and lower the needle until it is just touching the stone.
** A good way of determining if it is touching the stone is that as soon as the capillary is in contact, the capillary will start to fill with water.

4. Lower the needle further, giving the fine-tuner a 1/8th turn. Grind for 50 sec. Check the needle under a microscope. Discard the needle if it has debris on it. A good needle should have a point with a length of roughly 10-15 µm (Figure 1). Place the needles in an UV cross-linker, and sterilize under high power for 10 minutes.

Figure 1: Diagram of the proper dimensions for the micro-needle.

II. Microinjection

1. Place the microscope slide containing the embryos (from Protocol 2, step 7) on the stage of an inverted microscope.

2. Before injections begin, make sure the microinjector is on the following settings:
Balance 4.0-4.5 psi
Injection time: 0.03s
Injection pressure: 35 psi

3. Fill the needles with 1 µl of liquid using a loading pipette (if using siRNA or dsRNA, spin the tube down for 5 minutes on maximum speed to filter out debris that could interfere with injections).

4. Place the needle on a micromanipulator and bring the needle into focus. It takes a bit of effort, but make sure the embryos and needle are in the same focal plane, as this ensures that the needle will hit the embryo in the proper place.

5. Using the microscope (not the micromanipulator), move the embryos to the needle. Before injecting, it is usually a good idea to pulse the injector outside of the embryo to ensure that nothing is blocking the tip of the needle.

6. The membrane should cave in where the needle is pressing it. Slowly move the needle into the embryo further, as the vitelline membrane is tough. When the needle has penetrated the embryo, pull out slightly so that the tip is just beneath the vitelline membrane. This may be seen more easily by increasing the power of the microscope. Avoid injecting into the yolk.

7. Pulse the fluid into the embryo until it can take no more pressure, and slowly remove the needle. If yolk is leaking from the embryo, you may need to adjust the injection time, or adjust your needle. Embryos do not usually survive if yolk is leaking.

8. Proceed to Protocol 2, step 9.