MICROINJECTION OF RNAi

Required materials:

1cc insulin-gauge needles

MEM-HEPES

2% dbcAMP

siRNA, in 5 µl aliquots

35 mm Petri dish (catalog number: 35-1008)

Before beginning:

1. Collect oocytes according to protocol (see: *Oocyte Collection (and Whole mount staining)* protocol). Allow cells to recuperate from stress by incubating in MEM-$NaHCO_3$ before proceeding to microinjection. Do not leave oocytes in recovery medium for too long, otherwise zona pellucida will loosen and microinjection will be more difficult.

2. Adjust needles and break the tip of the injection needle.

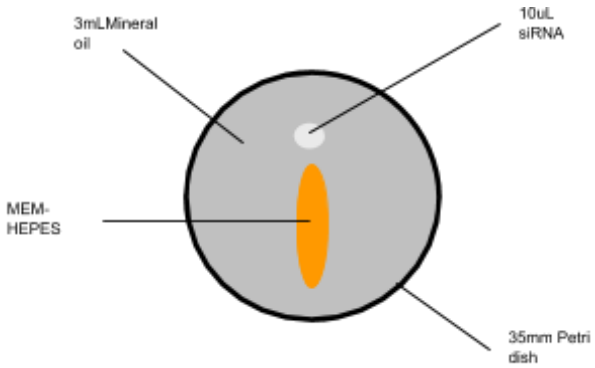
To prepare the dish:

Note: Dish should be prepared right before microinjection.

1. Pipette three drops of MEM-HEPES with 2% dbcAMP into the lid of the 35 mm Petri dish. Using pipette tip, merge three drops to give one vertically elongated drop.

2. Pipette two (2) 5 µl aliquots of siRNA above the drop of MEM-HEPES.

3. Cover entire dish with mineral oil (~3 ml). Dish should look like diagram shown below.



4. Prepare as many dishes as there are types of siRNAs to inject (e.g. one for *Cnot6*-siRNA and one for *Rspo1*-siRNA).

Microinjection:

1. Transfer oocytes from incubating dish to the top of MEM-HEPES drop in injection dish. Make sure to place the oocytes somewhere in the drop where it will be easy to tell the injected form the non-injected.

2. Mount dish on injecting apparatus.

3. Lower injection needle first to the edge of siRNA drop and fill it up with siRNA. Then bring it back to the top of the MEM-HEPES drop next to the oocyte.

4. Bring down the holding needle.

5. Make sure that oocytes and needles are all at the same focal level. This is achieved by making sure that the oocyte and both needles can be clearly seen and are able to touch each other. If not, adjust the focus until oocyte is maximally visible, and then adjust the positioning of the needles until they can both be seen as clearly as the oocyte. For holding needle, the inner lining of the lumen and the opening at the needle's tip should both be visible, as illustrated below. When properly aligned, the injection needle should visibly be able to enter the holding needle.

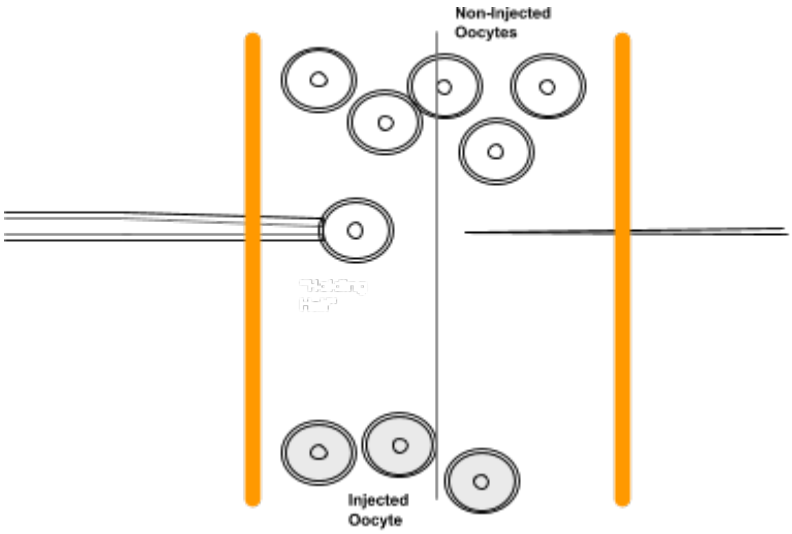
Caution: To avoid breaking the needles, do not lower them too deep in the dish!

6. Use the injection needle to move oocytes down next to the holding needle, where they will be aspirated and held in place.

7. Move injection needle to oocyte and pierce the cell. Make sure membrane is in fact perforated. Inject siRNA and take tip out of cell.

8. Use holding needle to move oocyte to the bottom of the drop.

9. Repeat for every oocyte.


Non-Injected
Oocytes
Injected
Oocyte