

Starfish oocytes for microinjection

Species: *Asterina miniata* (batstars, California, Bodega Marine Station; Marinus Inc., Long Beach)

Oocyte preparation:

- punch a 4 mm diameter hole in the tough skin of the animal on one side of the arm close to the madreporite (every arm can be used twice, but it is good to go around the animal clockwise from the madreporite and use each arm once only until all arms are used once).
- If the animal is female yellow/orange ovaries will be close to the surface. In a male the gonads are white.
- From a female take a piece of the ovary out (~ 10 mm in length) with a curved tweezer and put it into calcium free artificial sea water (cfASW) in a small beaker. In the cfASW shred the ovary with two tweezers into small pieces or use scissors to mince it finely. Allow the oocytes and follicle cells to flow out of the ovary for 2-3 min. This is speeded up by swirling the beaker.
- When most oocytes are in suspension, swirl the beaker and decant the oocyte suspension into a fresh beaker leaving the ovary tissue behind. Wait for another 5-15 min for the follicle cells to detach. Check periodically on a microscope.
- When most follicle cells are sheared from the egg replace the cfASW by regular ASW. Too long incubation in cfASW kills the eggs!
- Oocytes should now be stored between 18-22 °C and can be used for 12 h for microinjection.