

## Harvesting Ascidian gametes

We use the European ascidian *Phallusia mammillata* because its eggs and early embryos are completely transparent and also because microinjected mRNAs encoding fluorescent fusion protein markers are translated in the unfertilized eggs and early embryos (Prodon et al., 2010).

*Ascidella aspersa* eggs and early embryos are also transparent and also translate well microinjected mRNAs encoding proteins coupled to GFPs and could be used for this type of project when *Phallusia* is not available (Levasseur & McDougall, 2000).

*Ascidella* has a more widespread distribution than *P. mammillata*, e.g., it can be collected at Woods Hole, USA as well as in Europe, and northern Japan (Nishikawa et al., 2014) although its gametes are not as abundant as those of *Phallusia* which can produce up to about 1 mL (500,000) unfertilized eggs all arrested at metaphase I of meiosis.

1. Dissect the hermaphroditic animals by cutting through their tunics between the siphons with a razor blade or scissors. (see video collection of gametes)

Use thumbs to pry open the tunic and remove the soft-bodied ascidian. Lay animal so that egg and sperm are facing up on a paper towel to absorb water.

2. Pierce the overlying oviduct and collect the eggs using plastic or glass pipettes, massaging the oviduct to empty all the eggs.

Deposit the eggs directly to 4.5 ml fresh filtered sea water in a 5 cm Petri dish and store them at the appropriate temperature (16°C for *Ciona* and 18°C for *Phallusia*).

We use natural seawater supplemented with 5 mM TAPS pH buffer (adjust seawater pH to 8.2 with HCl and NaOH).

3. Then collect sperm from the underlying spermiduct using a pipette or a needle and syringe.

First absorb most of the moisture from the animal, then puncture the sperm duct with a needle to collect the sperm.

Concentrated sperm can be stored several days at 4°C in Eppendorf tubes. *Phallusia* eggs can be efficiently fertilized by sperm from the same individual (therefore one should take care not to contaminate eggs with sperm while collecting gametes), whereas self-fertilization is inefficient in *Ciona*, so it is necessary to open at least two animals.

4. It is also possible to obtain immature oocytes from ovary tissue in order to study oocyte maturation and polarity. We have analysed this process for *Ciona* (Prodon et al., 2006).