Transgenesis by microinjection of *Phallusia* eggs

Injection is a convenient way of transgenesis in ascidians and can be used to introduce cRNA, plasmidic DNA, protein (GFP), morpholino antisense oligo, or CrispR sgRNAs constructs.
It is also possible to inject combinations of them (i.e. protein+RNA; plasmid DNA+RNA+protein; etc…) without compromising embryonic development.

1. We use an inverted Olympus IX70 with left mounted stage control, right hand mounted three way Narishige hydraulic micromanipulator, and an air compressor providing the compressed air for a Narishige IM300 injection box.

2. Our microinjection method is as described previously with some small modifications (Sardet et al., 2011; McDougall et al., 2014). The mounting chamber is made of Perspex. Briefly, the injection chamber is 73 mm long, 25 mm wide and 4 mm thick. The central reservoir is 1mm thick and has a 15 mm by 15 mm piece removed that will make a reservoir for approx. 400 µl of seawater once the coverslips are attached (*see* video n°1*: Wedge, eggs loading, RNA tube*).

5. First the wedge is prepared as in *video n°1* (make sure that GF-coated glass surfaces are pointed towards each other).

6. Silicon is applied to both sides of the injection chamber where the coverslips will later be attached.

7. The coverslip with the wedge is attached first to the flat side of the mounting chamber with the "wedge" facing what will be the seawater reservoir (*see* video n°1).

6. Next, using a binocular microscope, 50 to 100 eggs are transferred into the wedge by mouth pipette (capillary action pulls the seawater and eggs into the wedge so long as a small enough volume is applied).

7. Then a coverslip is applied to the other side to make a sandwich with the wedge and eggs in the centre (*see* video n°1).

8. Next the reservoir between both coverslips is filled up with TAPS filtered sea water carefully so as not to dislodge the eggs.

9. Then place the mounting chamber into a large enough Petri dish on two pads of water-soaked tissue paper to create a humid environment so that the 400µl reservoir does not evaporate and store at 16°C until required.

10. Once the eggs have been prepared for microinjection prepare the mRNA Filling tube. Pipette 1µl RNAase free mineral oil into a "Filling Tube" prepared previously *(see video n°2: Make a RNA tube).* Then pipette 1µl RNA into the same Filling tube then pipette a further 1µl oil making an oil-mRNA-oil sandwich.

Attach this to the underside of the injection chamber with a little VaLaB (*see* video n°1). Note, we keep these Filling tubes with mRNA in them for up to about 6 weeks at 4°C and can reuse the same 1µl of mRNA several times over that 6 week duration.

11. Place the mounting chamber with eggs and wedge onto the stage and advance the microinjection needle towards the injection chamber. We use filament-free glass needles to prepare the microinjection needles (pulled from GC100T10 pipettes) using a Narishige PN-30 horizontal puller.

12. First break the tip of the needle against the Filling tube then suck up some mRNA from the Filling tube into the injection pipette using negative pressure. (We inject using the 10x objective and suck up enough mRNA to fill the field of view, keeping the meniscus in view at all times).

13. Set the balance on the IM300 so that the meniscus is stable.

14. Advance the microinjection needle towards the egg (see video n°3: egg injection).

15. Insert the needle into the center of an egg and apply a brief 10 ms pulse of negative pressure to break the plasma membrane. Then inject the mRNA into the center of the egg (see video n°3).

16. Remove the injection needle slowly until it is almost out of the egg, then remove it very rapidly to avoid killing the egg (we use the left hand stage control for the fast removal).

17. Push those injected eggs out the edge of the wedge using the microinjection needle. Once sufficient eggs have been injected collect them with a mouth or hand-held suction pipette and transfer to a GF-coated Petri dish containing TAPS filtered seawater and store at 16°C until required (we sometimes keep injected eggs overnight but not for longer).