**Plasmid Electroporation of HH13.5 Chick Neural Tubes**

PC = pulled-capillary; EP = electroporation; ET = electrode; NT = neural tube; PF = Parafilm; E = embryo; All Ringers should be sterile

Electroporation Prep

1. 70% EtOH-sterilize curved forceps, curved scissors, and a pair of fine forceps in an autoclaved TC glass petri dish (10 cm) for 5-10 mins.
2. Make sure the prongs on the electrode are 3 mm apart (from the inside edge of each prong). Then add enough EtOH in 14 ml bact. tube to fully submerge prongs.
3. Spray and wipe down work area with EtOH.
4. Attach a 1 mL syringe to a 25G 1½ in. needle. Bend the needle with the needle cap once at the base of the needle and again 1 in. from the tip of the needle. Bend so the opening of the needle faces the bend. Recap and set aside.
5. Attach a 5 mL syringe to an 18G 1½ in. needle and set aside for later albumin removal.
6. Add 10 mL Ringer’s into a 15 ml falcon tube in the biosafety hood. Then add 5-7 drops of filter-sterilized India Ink and set aside.
7. Fill a sterile 6 cm petri dish halfway with Ringers.
8. Fill a 50 ml centrifuge tube with 15 ml sterile Ringer’s, cap, and set aside.
9. Remove forceps, curved scissors, and fine forceps from 70% EtOH. Gently bang handle of each tool against edge of bench top to remove excess EtOH for faster drying. Place tools upright in a microfuge tube rack, then place tube rack in the laminar flow hood at least until the tools are completely dry.
10. Remove electrode from 70% EtOH (after at least 5 min. soaking) and lay on microfuge tube rack in the laminar flow hood to dry.
11. If a PC holder (made of tape) is not already set up, roll back a strip of packing tape on itself, so that the adhesive side is exposed all around. Attach that strip to the wall behind the microscope you’re working on.
12. Attach the roll of packing tape to the bench top for windowing eggs.
13. Place egg holder under the dissecting scope.
14. Prep/dilute plasmids appropriately for the experiment. Plasmids should contain a minimum final concentration of 0.1% Fast Green if visualization of the plasmid in the NT is necessary. Here we have been using 0.025% FG which seems to little to see any green color. The less FG the better as it can be toxic.
15. ET setup. Plug ET wires into EP power supply. Extend out a nearby fiber optic light guide to hang ETs from so they dangle inside 50 ml tube containing Ringers w/ only prongs submerged in Ringers. This is how they should be kept in between EPs. Turn on the power supply. Make sure it reads: 18V, 50 ms On, 100 ms Off, 5 pulses; and polarity switch points to ‘+’.
16. Take eggs out of incubator after 49 h incubation time for HH13.5. Eggs out at \_\_\_\_\_\_\_\_\_\_\_\_.
17. Remove 5 mL of egg whites from the eggs and discard into a beaker using the previously prepped 5 ml pipet.
18. Use the fine forceps to break off the tip of the PC so that an opening is created just large enough in diameter to allow plasmid to flow out, yet narrow enough to be inserted into the NT. Then attach the PC to a mouth pipet or place on the PC holder made of tape.
19. Pipette desired plasmid onto PF. Then use mouth pipette to suck up as much plasmid as possible and set aside. If working with multiple treatment types, use different PCs and areas of the PF to aspirate each plasmid. Be careful not to suck up air and create bubbles in the PC. Put remaining plasmid that could not be sucked into PC back in its PCR tube.
20. Fill 1 ml syringe with ink (diluted in Ringers). Suck the ink up from the 15 ml tube and expel it a few times to remove trapped air. Then suck up about 0.7 ml total.

NT Electroporations (Steps for a single embryo)

1. Cut open egg w/ curved scissors. Start cut by inserting blade of scissors into hole previously made from albumin removal. Cut a window that is about 1 cm in diameter to access the E.
2. Orient the egg in its holder so the tail end of the E is pointing at your right shoulder (for right-handers).
3. Insert the ink syringe into the yolk as far from the E as possible yet still on the topside of the yolk. Carefully move the tip of the needle directly below the E and inject enough ink (~50-100 μl) to stage the E and clearly see the tail NT. Move the egg as little as possible until EP to prevent ink dissipation.
4. Stage the E. Discard if not HH13.5 +/- ~0.5. If the head has not yet begun turning to the right (trunk flexure present) then the E is too young. If the head has completely flexured and even tilts down slightly, then for sure it is too old.
5. Inject plasmid into the caudal NT opening. With a mouth-pipet attached to a previously loaded PC, carefully poke the PC tip through membrane just above the caudal NT opening and insert it into the NT. Blow enough plasmid into the NT so it reaches the ideally the forelimb bud level at least. This can be seen by the NT spreading apart from the positive pressure applied from blowing or by the color of the Fast Green if used.
6. Quickly EP the E before plasmid can dissipate too much. Place wet ETs so the trunk region of the E is exactly in between the ETs and parallel with the ETs themselves. If the ETs get too close to the E, they can cause developmental deformities when a current is applied. Press the start button. Bubbles should be present when ETs are removed as a sign that current was generated.
7. Gently remove ETs and submerge in 6 cm petri containing Ringers. Press the start button 2-3 times to help remove the egg white.
8. Wipe the ETs gently with a clean Kimwipe and rehang them on the fiber optic light guide over the 50 ml tube containing Ringers.
9. Label the egg with a pencil describing the treatment type, HH stage, and other abnormalities if necessary.
10. Tape over the hole cut in the shell with packing tape. Make sure no major gaps exist in the folds of the tape that will allow air to enter and dry the egg overnight. Seal such folds with Scotch tape.
11. Place egg back on rack.
12. Repeat for other eggs using PCs containing the appropriate treatments. It is better to intersperse treatments than do all Es of one treatment before switching to another treatment.
13. Place eggs in the incubator at \_\_\_\_\_\_\_\_\_\_pm.
14. Remove eggs from the incubator at roughly 22 h later at \_\_\_\_\_\_\_\_.
15. Begin harvest as described on whole mount immuno protocol.