In Vivo Electroporation

Materials:

- Genetronics BTX T820 Square wave generator:settings at 8-12 volts, 3 pulses and length of 99 at (LV) 99msec/500V.
- 3 mm gold plated stainless steel electrodes (Genetronics model 514)
    - Alternative: fashion desired shape and length using 0.5 or 0.25 mm diameter platinum or silver wire. Use heat shrink or nail polish to insulate electrodes.
- 100mm- 1.0 mm OD x 0.5 mm ID glass capillary tubes (pulled on KOPF vertical pipette holder model 720 with settings of 17.4 heat and 2 solenoid).
- DNA or Morpholino: DNA ≥ 1μg/μl in TE or PBS 1x (Ca & Mg free).
    - Morpholino at 500μM concentration (Gene-tools 300nm fluorescent tagged SD or Non-SD). Diluted to final concentration with either water or PBS 1x.
- Locke solution: 0.85 g NaCl, 0.042 g KCl, 0.03 g $NaHCO_3$, 0.024 g $CaCl_2$, 0.1 g Glucose, 100 mls dd H_2O.
- Pelikan Fount India ink solution: approx. 2-3 drops per 10mls sterile Locke solution. Filter through Nalgene 0.22 filter.
- Mouth pipette holder (Leica)
- Two 3 cc syringes
- #5 biological forceps
- Tungsten needle
- 16G 1 1/2 and 26G 1/2 needles
- Non toxic tape: Scotch 3/4" 600

Procedure:

Incubate chicken or quail eggs on their side prior to electroporation (this time varies according to desired H&H stage). Spray and wipe down egg surface with 70% ETOH. Using 16 gauge needle extract 3 cc's of albumen from the small end of the egg (seal hole with small piece of tape). Also use the 16 gauge needle to start a pilot hole in the center of the egg. Use forceps to break open a hole to access the embryo. To visualize the vitelline membrane, inject ink solution with 26 gauge needle underneath the embryo (insert needle under the embryo from outside the blood ring). Using a sharp tungsten needle, tease the vitelline membrane away from the area around the embryo (enough to place the electrodes on both sides of the embryo).

Load the DNA/Morpholino into the glass pipette by either back filling with microloader tips or drawing up the solution after the needle is mounted in the pipette holder. Back the tip of the glass pipette to desired diameter (be careful not to have tip larger then neural tube diameter). Inject DNA/Morph into the lumen of the neural tube making sure to not penetrate ventrally. Injecting in an anterior to posterior direction starting from the rhomencephalon works well when targeting the trunk neural tube. Place one to two drops of sterile Locke solution on the embryo or wet electrodes in egg albumin prior to each electroporation (optional). Settle the electrodes 1-2 mm on both sides of the embryo and electroporate (should see bubbles if it worked properly). The micro-pipette, and electrodes can be held by hand or put into a micro-manipulator.

Carefully remove electrodes and seal the egg with tape. Place the egg back into the incubator on its side. The embryos can be checked for DNA/Morph uptake 4-6 hours after electroporation with the appropriate fluorescent microscope.

Note: Electrodes should be wiped down between each electroporation with ddH2O. After each session clean with soft bristle tooth brush and toothpaste.