Chicken embryo electroporation : spinal cord

Material :

70% ethanol, PBS, nuclease-free water, 0.22 um filter, 20 ml syringe, 18G needles, small scissors, large plastic vial, egg holder, scalpel, Silver Femtotips (Eppendorf), platinum electrode 4-6.2 mm long, large transparent tape, dissection microscope + cold light illumination

Eggs : WYVERKENS
Pypaenshoek 47
1500 Halle
Tel: +32 2 356.57.97
gert.wyverkens@telenet.be

Order until Wednesday morning, available from Friday morning on
Closed on Monday

Transporter : Snelkoerier info@snelkoerier.be
Warande 104, 1500 Halle (België)
+32 2 306 70 90
+32 474 50 70 90 / +32 496 50 70 90

Before starting the experiment :

- store the eggs between 8 and 14°C (max: 10 days)

- incubated eggs for 60h before electroporation at 38°C in a humid incubator

- sterile filter 50 ml PBS on a 0.22 um filter (to be added on the embryos after electroporation)
- dilute expression plasmid in water at 0.2 to 1 ug/ul; add ±1/10 of 2% Trypan blue
- spin down the DNA solution on a SpinX Costar column for 5' at 13.000 RPM
- place the electrodes into non-sterile PBS
- under microscope, break the tip of the Femtotip with the scalpel (!careful when removing the cap!)
- load the Femtotip (10-15ul) from the bottom; wait ±10' for the solution to reach the tip
- most of the time: re-break the tip of the Femtotip and check with the injector

- wash all the eggs in 70% ethanol (don't rotate or shake the eggs to keep the embryo on the top)
- puncture the bottom (round side) of the eggs with closed scissors
- keep the 18G needle as vertical as possible and remove ±2 ml of albumen; discard

Injection and eletroporation :

- put an egg on the holder, open a square window (±1cm^2) on the top of the shell with the scalpel
- locate the embryo and rotate the egg to place it in the middle of the window, head to the right
- under the microscope : locate the hindbrain and the spinal cord; determine the HH stage
- inject for 0.5 – 2s at ±5-10 psi, 1-3 times (the solution must fill the lumen of the neural tube)

- ASAP : place the electrodes on both sides of the embryo in the region of interest
  electroporate 2 times at 25V – 30 ms x3 with intervals of 1'' (mode LV; unipolar)
  add 2 drops of sterile PBS onto the electroporated embryo
- put a piece of transparent tape on the window
- label the egg (stage, DNA, [], other), electroporate all the eggs and put back into the incubator

<u>Collect of the embryos</u> (after 6-15h for a direct regulation; 24h or later for downstream effects) :

- open the window, discard the dead embryos
- cut the membrane around the living embryos and transfer to PBS; dissect out the membranes
- under the fluorescence microscope, check for GFP and cut the regions of interest
- fix max. 1h in 4%PFA; wash in PBS, protect in sucrose and freeze in OCT in the -80°C

Laboratory of Neural Differentiation (NEDI) - Animal Molecular and Cellular Biology (AMCB)
Louvain Institute of Biomolecular Science and Technology (LIBST) – UCLouvain
place Croix du Sud 5, B-1348 Louvain-la-Neuve
Phone : +32 10 473471
Email : Frederic.Clotman@uclouvain.be