Novitch Lab
David Geffen School of Medicine at UCLA

In Ovo Electroporation of Chicken Embryos

Reagents to prepare before starting:
10X CMF (Tyrode's Saline without Ca^{++} or Mg^{++})
India Ink Solution
10X P/S (Penicillin-Streptomycin) in 1X CMF
DNA(s) to be electroporated
Pulled glass needles

10X CMF (Tyrode's Saline without Ca^{++} or Mg^{++})

80 g NaCl
2 g KCl
0.5 g NaH_2PO_4*2H2O or 0.384 g of anhydrous NaH_2PO_4
10 g Glucose
water to 1 L.
Autoclave or 0.2 μm filter to store
Scale accordingly to prepare smaller volumes.

India Ink Solution

for 1 ml:
100 μl India Ink (Pelikan #????, some other sources have been found to be toxic to the embryos)
900 μl 1x CMF 1x P/S
Prepare in a sterile eppendorf tube, vortex to mix, and spin briefly before use

10X P/S (Penicillin-Streptomycin) in 1X CMF

.1 ml 100X Penicillin-Streptomycin (what we use for tissue culture)
1 ml 10X CMF
8 ml ddH_2O
If all reagents are sterile, then you can just pipet each into a sterile tube. Otherwise, you should 0.2 μm filter this.

DNA solution to be electroporated

This will vary considerably depending on the experiment. The general parameters are to end up with 2-5 μg/μl of plasmid DNA in either ddH2O, TE pH 7.5, or 1X PBS with some Fast Green to visualize the DNA that you are injecting into the embryo (0.050 % final concentration of Fast Green is usually sufficient).

What I do is prepare the DNA solution with a final volume of 5-10 μl. To achieve this, I ethanol precipitate the DNA, and then resuspend it in the appropriate volume of water or buffer with the Fast Green mixed in.

Typically, for most misexpression studies, I use a 0.1-0.5 μg/μl concentration of the expression plasmid of interest. I then bring up the DNA concentration by adding a "neutral" source of DNA such as pBluescript to achieve the overall DNA concentration of 2-5 μg/μl.

Below is an example:

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Let's say that I want to have 0.5 µg/µl of pCIG (pCAGGS with Ires-nuclear EGFP) and nothing else. I would want to make a 10 µl solution containing 0.5 µg/µl of pCIG and 1.5-4.5 µg/µl pBluescript or similar plasmid to get the final concentration of DNA to 2-5 µg/µl.

Here is how it works if the stock plasmid concentration of pCIG is 1.2 µg/µl and pBluescript is 1.8 µg/µl:

for a 10 µl DNA mix with a final DNA concentration of 3 µg/µl:

$$\frac{10\ \mu l * 0.5\ \mu g/\mu l\ pCIG\ desired}{1.2\ \mu g/\mu l\ pCIG\ stock\ conc} = 4.17\ \mu l\ pCIG$$

$$\frac{10\ \mu l * 2.5\ \mu g/\mu l\ pBluescript\ desired}{1.8\ \mu g/\mu l\ pBluescript\ stock\ conc} = 13.9\ \mu l\ pBluescript$$

What I then do is bring up the volume of the mix to a convenient number (say 25 µl) with TE or ddH_2O, add 1/10 volume of 3M Na Acetate (pH 5.2), 2 volumes of 100% Ethanol, and then mix gently until the DNA falls out of solution. Spin at top speed in a microfuge for 10 minutes. Carefully pipette off the supernatant, being careful to not disturb the DNA pellet. Add 70% Ethanol to wash the pellet, spin again for 5 minutes, and carefully pipet off the ethanol. Air dry the pellet briefly at room temperature by placing the tube on its side or inverting at an angle on clean paper towels. Only dry the pellet for 5-10 minutes or until almost all of the liquid has evaporated and the pellet looks glassy. If you let the pellet dry too long, the DNA will be much harder to resuspend. Once dried, resuspend the pellet in 9.5 µl of ddH_2O or buffer. You will need to pipet up and down repeatedly to the DNA evenly mixed. Some people like to put it at 37˚C or hotter to facilitate its resuspension. Once resuspended, add 0.5 µl of a 1% Fast Green stock solution. I like to add the Fast Green last, as the DNA is easier to see in suspension without the green coloration. Briefly vortex and spin mixture to mix the Fast Green in.

DNA is now ready to be used for electroporation. You can store this at -20˚C for later use if necessary, but warm it to room temperature or 37˚C before injecting.

Visualizing the embryo

(optional) wipe the egg and instruments to be used with 70% ethanol, and wipe or air dry.

Using a syringe with a $\geqslant$ 23 gauge needle, remove some albumin from the blunt side of the egg incubated on its side. This lowers the embryo from the surface of the egg shell.

Cut open a window in the egg shell at the very top of the side that was placed upwards when the egg was incubated. You can do this by pecking a hole with forceps (don't waste your good sharp ones!), scissors, or a scapel blade. For scissors, what works nicely is to put a piece or two of scotch tape over the top of the egg, poke into the shell with the sharp point of the scissors, and then cut a square window. The tape keeps most of the small pieces of egg shell from falling on top of the embryo. Carefully remove the piece(s) of shell as to not disturb the embryo. If your window is not large enough to perform your manipulation, you can remove more shell as long as you don't allow too much of the egg white to leak out.

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Draw up some ink solution into a 1 ml syringe with a 26 gauge needle (with an L-shaped bend in the tip). Carefully, inject some ink under the embryo. By insert the needle bevel side up just outside of the ring of the embryo (where there are blood vessels forming at E2 and later) and then titling the tip of the needle upwards until you can almost see it underneath the periphery of the embryo (it's hard to see directly under the embryo). Push out some of the ink. Remove the needle and jiggle the egg a bit if you need to spread the ink more evenly. You should be able to see the embryo clearly. The ink will tend to diffuse away from the embryo, and you can inject more ink if necessary. Avoid poking under the embryo too many times, as this tends to break the yolk and make this leak out, and avoid poking the embryo or the developing blood vessels.