

## Electroporation in Chick Embryos

1. Culture fertilized chick eggs (Charles River Lab, 1-800-772-3271) on their sides at 38.5°C for 33~38 hours. Mark the orientation (top).
2. Spray and wipe egg surface with 70% EtOH.
3. Make a small hole in the pointed end of eggs with scissors. Remove 3 ml of albumen with 20-gauge needle, then seal with melted paraffin or small tape.
4. Open a small window in the center of the eggs with scissors (FST #14061-10) to access the embryo.
5. To visualize the embryo, inject India ink solution (1:20 in Tyrode's solution) with 26-gauge needle underneath the embryo (optional if you can find the embryo).
6. Make a pinhole in the anterior tip of the neural tube (this is not necessary for st9-10 embryos).
7. Inject plasmid DNA (1  $\mu\text{g}/\mu\text{l}$  in TE or PBS with 0.5% Trypan Blue) with a glass micropipette into the neural tube lumen. Inject from the metencephalon to the mesencephalon. Make sure not to penetrate ventrally.
8. Put one or two drops of PBS on the embryo and place the electrodes on both sides of the embryos, touching the vitelline membrane. Activate charge and pulse sequences. Clean electrodes with dH<sub>2</sub>O immediately after each electroporation.
9. Seal eggs with tape and incubate eggs. Detect expression at 8-48 hours after transfection (maximal expression at 8-24 hours).

**Electroporation Settings:** 25V, 50ms/950ms, 3x

Electroporator: BTX ECM<sup>®</sup> 830 ([www.btxonline.com](http://www.btxonline.com)) or CUY-21 ([www.protechinternational.com](http://www.protechinternational.com)).

Parameters: 15-25V, 50 ms pulse length, 5 pulses. Reduce pulse length to 10 ms to get scattered expression.

Electrode: BTX Genetrodes Model 514 (3 mm tip, #10-001850-02), or 516 (1mm tip, #10-002509-01) with 4 mm gap.

Electrode holder: BTX Genetrode Holder (BTX, #10-002560-01).