

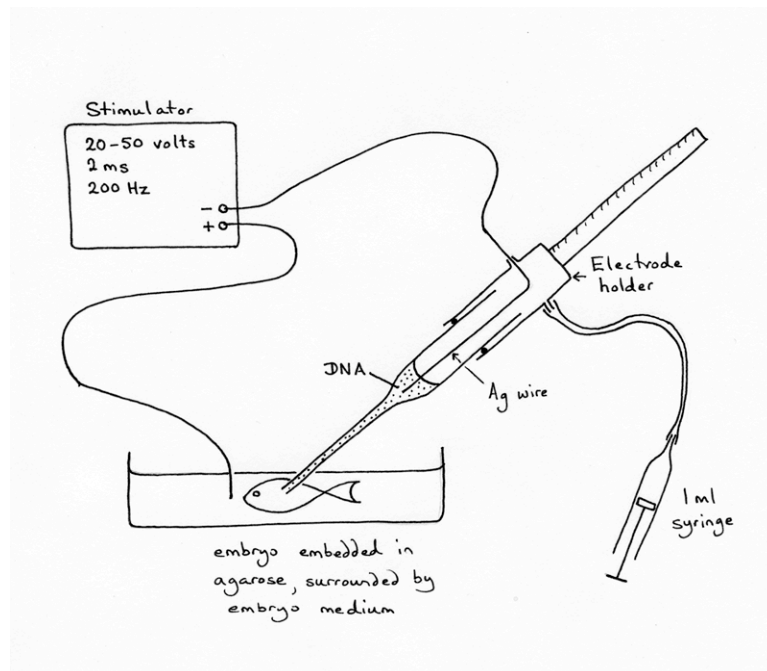
Focal electroporation of DNA into zebrafish nervous system (Marcel Tawk and Jon Clarke, UCL)

This technique was originally devised for use in *Xenopus* larvae and cultured tissue slices by Holly Cline's lab in Cold Spring Harbor and some of the following text is cribbed from their excellent website (<http://www.cshl.org/labs/cline/sce.html>). We've made a few minor modifications to get it to work in zebrafish.

Focal electroporation is a technique developed to deliver genes into small clusters of cells or individual cells within intact tissues, although it may also be applicable to cells in culture. Targeting transcription to individual cells or cells clusters is achieved by restricting both the DNA and the electric field required for electroporation to the 1-3 μm tip of a glass micropipette.

This technique utilizes electroporation in which a brief external high voltage pulse induces sufficient transmembrane potential to disrupt the electrostatic forces maintaining lipid bilayer structure, causing the temporary formation of small pores in the cell membrane. DNA and other charged molecules are then electrophoretically transferred into the cell through these pores. Following pulse termination, pores reseal over 10s to 100s of ms.

Focal electroporation is a powerful transfection method since it readily allows the delivery of multiple genes each carried by independent plasmids into targetted cells or specific regions of the embryo or larvae. In addition, it can be used to transfer macromolecules besides DNA into cells, including RNA, proteins, morpholinos, dyes and drugs. While charged molecules are actively electrophoresed into cells with higher efficiency, non-charged molecules may also diffuse from the micropipette into cells through the pores.



This is what we do for 24h or older embryos.

We pronase embryo in one of two ways to help get electrode through tough elastic skin

1. anaesthetise fish and then dunk in pronase (5mg/ml embryo medium) for about 1min
2. 3 quick rinses in embryo medium
3. embed embryo in low melt agarose
4. cut out window in agarose to expose target area

or for a slightly more aggressive approach to a smaller area of embryo

1. anaesthetise fish and embed in low melt agarose
2. cut out window to expose target area
3. add drop of pronase (this time 20mg/ml) into window for approx 1 min
4. rinse 3 times in embryo medium

This pronase step is not required for older embryos as the skin becomes less elastic and electrodes can be pushed through this layer more easily.

For the electroporation:

1. You need to pull a patch-like pipette. We follow the instructions on the Sutter website and using thin-walled, 1.2mm with filament glass we pull pipettes with tips of approximately 1 to 3 μm .
2. back-fill with 1 - 2ul DNA (1 $\mu\text{g}/\text{ul}$ distilled water).....use one of those very long thin pipette tips to get this right down to the shoulder of the glass pipette
3. secure into a microelectrode holder with long silver wire connector that reaches down into the DNA (keep this silver wire clean by gently scraping it with edge of glass slide or emery paper.....do this at least every session to get rid of crud)
4. apply a tiny bit of positive air pressure to back of pipette via a 1ml syringe (move syringe plunger just 0.1ml)
5. attach back of microelectrode to negative pole on stimulator (we use a GRASS SD9)..... you need to be able to deliver 2ms square pulses of approx 20 - 50 volts (for DNA) or 2-5 volts (for fluorescent dextrans) at a rate of 200Hz
6. attach other pole of stimulator to another silver wire which is placed in the embryo medium surrounding fish.....it may help to place this wire so that it is opposite the cells you want to electroporate, so the electric fields will run directly through the target cells.....but it also works if you don't pay attention to its location
7. manipulate electrode tip through skin, evl, whatever and into target tissue.....we do all of this on a fixed stage injection microscope with x20 extra long working distance objective, but a good high mag dissecting scope would be fine for many things

8. give 2 or 3 blasts of pulses that lasts for about 1 second each (we don't measure this accurately, just hold the button down and guess).....quite probably different tissues need different stimulus regimes
9. polarity of pulses is criticalthe microelectrode should be attached to negative terminal for DNA
10. DNA takes something like 6 to 10 hours before expression but we haven't measured this accurately

Also works fine for RNA, fluorescently tagged morpholinos and fluorescent dextrans. Dextrans are small and mobile so they require potential differences of only 2-5 volts (rather than the 20-50 volts needed for DNA).

Often this technique will transfect a single cell but not always, and using larger electrode tips will hit more cells.

We will demonstrate this technique by electroporating either a GFP plasmid or fluorescent dextran.

References

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