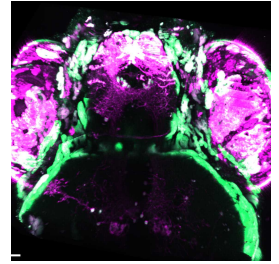


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Bulk in vivo electroporation (single cell)

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Anya Suppermpool¹

¹University College London

FishFloorUCL



Anya Suppermpool

UCL

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Abstract

This protocol will allow you to express single (or near single/mosaic) cell expression in the tectum of larvae. This protocol is adapted from (Hoegler and Horne, 2010; Nikolaou et al., 2015). If you would like to do focal-electroporation (into cell) to trace projections/neurites please see (Henriques et al., 2019) or any older Bianco paper.

Electroporation is a way to deliver plasmids or vectors into living embryos. For my experiments, I want to see larvae/neurons at 6-9dpf so I electroporate at 3dpf. But if you wish you do younger see Hoegler (24hpf) - likely that you need to adapt some things as younger larvae are curled and will be more fidget-y.

Note: depending on what you want to express/and how much of it, electroporation time may differ. For example, AS wants one-neuron expression which gets roughly 10-30% of electroporated fish (depending on expression see Inject and electroporate section). AS then wants say 15-20 positive embryos $\Rightarrow$ meaning 150-200 embryos electroporated $\Rightarrow$ taking roughly 13:00-17:30/18:00 (5hr) long.



Equipments

- 1 Stimulator SD9 (Grass Instruments) - borrow from Isaac Bianco (Ask Isaac before use!)
- 2 Electroporation penTM
- 3 Micro glass needle (0.58mm inside diameter, Sutter Instrument, Germany, BF100-58-15) pulled using a micropipette puller (Model P-87 Sutter Instrument, Germany) – normal injection needle
- 4 Petridish (100 mm × 20 mm/normal size) lid + to contain your embryos
- 5 Glass slide
- 6 Water Bath at 42C (to keep agarose warm)
- 7 25mL plastic pipette to mount fish (or can use glass pipette but because glass has smaller opening/diameter agarose tends to get stuck more quickly so need a glass of hot/warm water to store/de-agarose the glass pipette
- 8 Forceps (fine) and scalpels (want straight edge/45degree not curved blade for precisions)
- 9 Pipette tips microloader (for loading plasmid into needle AND orienting larvae)
- 10 Pressure injection system (microinjector) and needle manipulator
- 11 Dissection microscope

Reagents

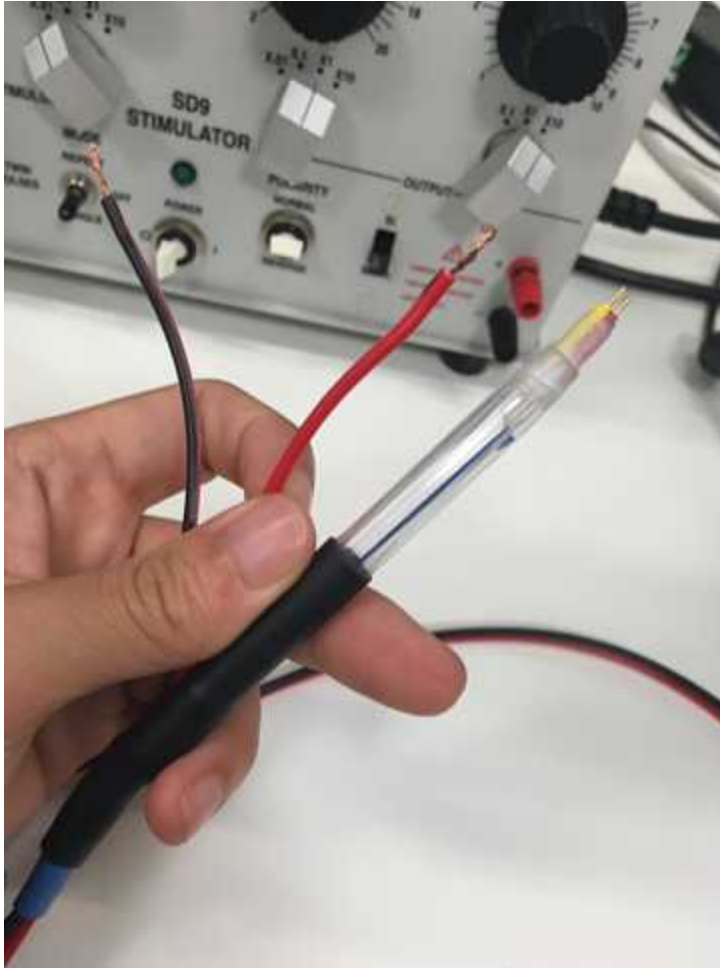
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- 13 Fish water and tricaine 25x (communal fridge)
- 14 Low-melting agarose in fish water (methylene blue-free)
 - 14.1 For electroporation use low so 1-1.5%
 - 14.2 For imaging (live) AS use 2-3%
 - 14.3 AS: use agarose in glass bottles not eppendorf tubes as this holds heat better, holds more + plus can be stored in water baths to keep temperature.
- 15 DNA plasmid of interest and/ or transgenic line (if using Gal4/UAS system)
 - 15.1 AS electroporate Gal4 (FoxP2.A:Gal4FF) into Tg(UAS:FingR.PSD95)* stable line. AS only did this way as UAS:FingR lines (due to negative feedback mechanisms needed to be 1: in a stable line/genomically integrated, 2: ideally in higher copies). But you can do whichever in theory that scientifically suits your experiment. E.g. Nikolaou et al co-electroporated Gal4 and UAS:GCaMP.
- 16 DNA plasmid (>500ng/uL, best at 1ug/uL)
- 17 Tol2 transposase mRNA (>150ng/uL)**
 - 17.1 tol2 transposase mRNA was in vitro transcribed from the NotI-linearized pCS-TP6287 plasmid (plasmid# 1151 Wilson lab database, UCL) using an SP6 mMACHINE Kit (Ambion, USA). RNA was purified using RNA Clean & Concentrator Kits (Zymo Research, USA).
- 18 Electroporation buffer (180 mM NaCl, 5 mM KCl, 1.8 mM CaCl₂, 5 mM HEPES, pH 7.2)
 - 18.1 AS make this in 2X and dilute fresh everytime pre-electroporation (with fish water tricaine to make 0.02% Tricaine (1X, MS-222, Sigma-Aldrich)

19 Phenol red (0.03%)

Making Electroporation Pen™

20 Constructing electroporation electrodes pen



21 Use wire cutter to cut plastic cover off exposing wire (wire end of the electrode)

22 Electrode should be about 1mm apart (to fit larvae head in between)

23 Run electrodes+wires through shell of a BIC/BIRO pen (other brand are available)

- 24 Cover the wire with shell of a BIC pen for economy
- 25 Secure/waterproof the gap between two electrodes and pen shell using epoxy-resin and parafilm
- 26 Use heat-shrink tubing to hold electrode wires together as well as connect between wires and pen shell.

How to mount fish for electroporation

- 27 Pre mounting, screen your larvae to have the correct UAS/Gal4 background
 - 27.1 AS: incross UAS:FingR so screen for the strongest expressing FingR+ larvae (background Gal4). As found that stronger expression yields higher electroporated fish (30%) vs regardless of expression level (10%).
 - 27.2 AS: Electroporate at 3dpf.
- 28 Tricaine (1X) larvae that you would like to mount. One batch at a time as mounting/injecting-electroporating/unmounting may take a while each round. AS typically do two plates(lids) at once and each lid containing 8 larvae (16 larvae per round in total) the whole round takes about ~25 minutes.
- 29 When larvae are definitely anesthetized mount larvae (one at a time onto the lid with glass slide with the agarose). Note: onto the plastic lid and not onto the glass slide. Glass slide only there to align the larvae.
 - 29.1 How AS mount: *make sure* agarose is warm enough (if not you will boil the embryo). To do this, AS put agarose onto back of AS' hand to check temperature + if using glass pipette putting pipette into agarose bottle, pipette should NOT steam up if the correct temperature.
 - 29.2 Pipette up larvae using glass/plastic pipette with as little water as possible and place larvae into glass bottle with agarose.
 - 29.3 Pick that larvae up using pipette and drop a little droplet of agarose-containing larva onto the surface of petridish lid (open side up).
 - 29.4 Quickly and gently use trimmed microloader to orient the larva dorsal up.

29.5 Repeat this for the rest of your larvae on your lid (AS recommend 10-12 larvae)

30 Now you have (say 5) larvae in droplets of agarose on your dish lid. Once they have solidified (but not too long that larvae hypoxic and gets ill so within 3mins max as there's other steps after this) you can use your trimmed microloader to move the larva-droplet into position along and perpendicular to glass slide (facing away) (See Fig 3)

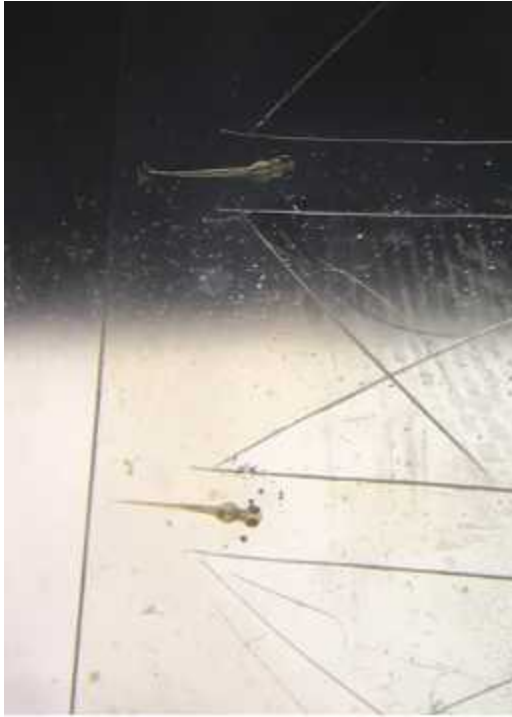


Fig 3

31 Pour agarose along these droplet into a line, mobilizing larvae to the glass slide and glass slide to the dish lid.

32 Once agarose has solidify, fill the half of the petridish lid with larvae with electroporation buffer (with 1X traicaine)

33 Cut agarose along larvae (both) side of body (Figure) and also in front of larvae head for needle and electrode to access (as much as you can)

34 Repeat this for the rest of your larvae/dish

Inject and electroporate

35 Stimulator settings: Duration: 5ms, Power: 85V, Frequency: 200Hz, Delay:0

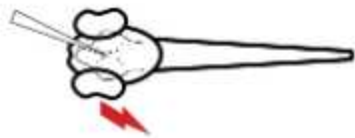
36 Prepare solution and load into injection needle.

36.1 AS in needle mix: FoxP2.A:Gal4FF (500ng/uL) + Tol2 mRNA (10-30ng/uL) + phenol red (0.03%) to help see solution in needle

37 Calibrate needle to inject 1nL using graticule ruler

38 Inject solution into larvae' midbrain ventricle (AS do 5-8nL).

38.1 You should see the ventricle distends slightly



38.2 Guess the more you inject the more chance of expression but AS found that 8nL max as larvae can get edema.

39 Electroporate immediately!

39.1 Five 5ms trains of 85 V voltage pulses at 200 Hz were

39.2 DNA solutions begins to diffuse immediately to other areas of the larvae, so electroporate as soon as possible (within 5-10seconds) post injection. Electroporate after injection of each embryo.

39.3 Obviously take out the needle from the ventricle before electroporate



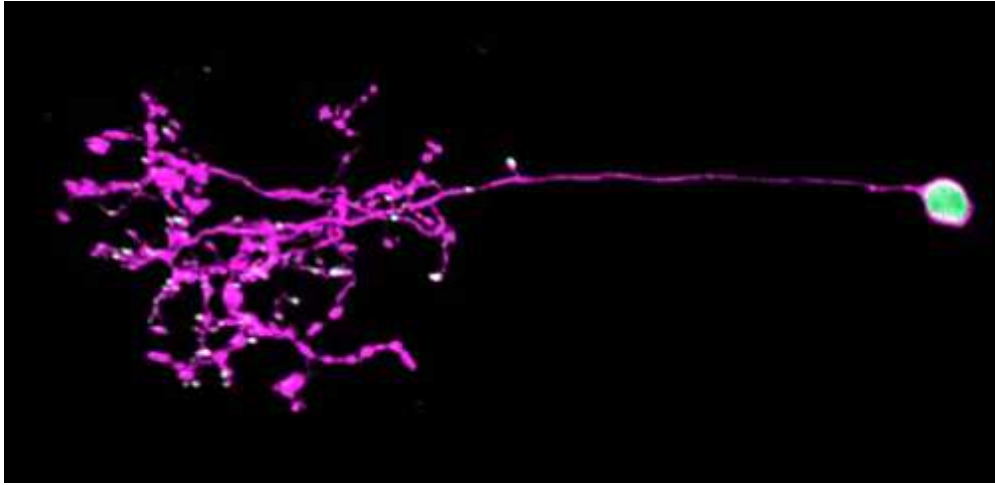
- 40 Position electroporator penTM such that the positive electrode (red) is lateral and slightly dorsal to the hemisphere of the optic tectum targeted.
- 40.1 orientation of the electric field used here will specifically target only one hemisphere (right or left) of the developing optic tectum
- 40.2 AS: although because AS adapted using Tol2 mRNA, AS also sometimes see expression on the other hemisphere. ** so maybe don't use Tol2 if you want to restricted to one side
- 41 Obviously *DO NOT TOUCH* embryo with electrode. This will harm/kill them.
- 42 Repeat injection/electroporation to next larvae

How to unmount fish

- 43 Once done with all your larvae. Pour out electroporation buffer and replace with normal fish water. Wait 10-15mins for fish to recover
- 44 Free embryo from agarose by using forceps to gently hold agarose along the body (Do not touch the actual embryo) and scalpel to cut agarose from the head. Larvae should swim out freely.

Screen for your result

- 45 Screen under table top microscope (if your expression is high enough). AS has to screen under confocal microscope to see single cells



Protocol references

Hoegler, K. J. and Horne, J. H. (2010) 'Targeting the zebrafish optic tectum using in vivo electroporation', Cold Spring Harbor Protocols, 5(7), pp. 1–7. doi: 10.1101/pdb.prot5463.

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