Labeling Individual Neurons in the Brains of Live *Xenopus* Tadpoles by Electroporation of Dyes or DNA

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Cold Spring Harb Protoc; doi: 10.1101/pdb.prot077149

Protocol

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This protocol describes the targeted introduction of fluorophore in the form of a dye or genetic material into single cells. This method has the advantage of producing true single-cell chimeric animals in which to study the effects of overexpression or knockdown of a gene in an otherwise entirely wild-type background.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

RECIPE: Please see the end of this article for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

DiI (0.01%–0.05% [w/v] in absolute ethanol)

Other color lipophilic dyes such as DiD or DiO are also available.

Fluorescent dextran (5% [w/v] in 0.1 M phosphate buffer or water)

Alexa Fluor-conjugated dextrans are more expensive but provide better photostability and less phototoxicity than traditional xanthene dyes such as FITC and TRITC. It is economical to use the former for experiments and the latter for practice.

MS-222 (0.02% [w/v] in 10% Steinberg's rearing medium) (Sigma-Aldrich)

The solution can be stored for 1 mo at 4°C.

Plasmid DNA (0.1–5.0 µg/µL)

An endotoxin-free plasmid purification kit is recommended for plasmid preparation. DNA solutions can be kept indefinitely at –20°C.

Steinberg's rearing medium <R>

Xenopus laevis tadpoles (at appropriate stage of development)

Imaging is facilitated in tadpoles from an albino strain, which are transparent.

Equipment

Coarse micromanipulator (e.g., Narishige M-152)

Electrical stimulator capable of producing square wave pulses at a range of amplitudes (0–100 V) and frequencies (0–200 Hz) (e.g., SD9 [Grass Technologies] or Axoporator 800A [Molecular Devices])

Adapted from *Imaging in Developmental Biology* (ed. Sharpe and Wong). CSHL Press, Cold Spring Harbor, NY, USA, 2011.

Cite this article as *Cold Spring Harb Protoc*; 2013; doi:10.1101/pdb.prot077149

Fine brush

Fixed stage upright microscope (e.g., Olympus BX51WI) with long-working-distance air objectives

For the optic tectum, a 20× objective provides a useful level of magnification.

Glass micropipettes

These should resemble patch pipettes with 0.5–1.0-µm tips to give ~10 MΩ resistance when filled with standard recording internal solution. If the tips are too long, they will bend and break on the skin of the tadpole. Borosilicate standard wall pipettes with filament (1.5 mm OD, 0.86 mm ID), pulled on a Sutter P-97 puller using a box filament, are recommended. For DiI iontophoresis and either dextran or DNA electroporation, tip shape is critical to avoid labeling multiple cells and to prevent clogging. A good tip can be reused in multiple tadpoles in one session but should not be saved for later use.

Laboratory tissues (e.g., Kimwipes)

Silver wire leads (0.25-mm diameter, ~4-cm length, soldered onto a standard copper wire)

Transfer pipettes (plastic)

METHOD

For single-cell electroporation, the tip of a fluorophore-filled glass micropipette is placed against the cell to be electroporated (under visual guidance provided via an upright microscope), and current is passed through the pipette. This transiently disrupts the plasma membrane and ejects the fluorophore.

1. Prepare the labeling solution containing an appropriate fluorescent dye or plasmid DNA.

   Solutions of fluorescent dyes can be stored for at least 1 mo at –20°C, although they are generally best made fresh from powder. Care should be taken to minimize exposure of fluorescent dyes to light at all times.

2. Backfill the pipette with the labeling solution. Mount the pipette on a micromanipulator set up on an upright microscope.

3. Connect the circuit as follows: Positive lead of stimulator → silver wire → labeling solution in micropipette → [gap] → tadpole → moist tissue → silver ground wire → negative lead of stimulator.

   For electroporation with DNA, the polarity of the stimulator must be reversed: DNA is negatively charged and will move toward the positive electrode. To monitor for blockage of the pipette tip, a 10-kΩ resistor can be inserted in series anywhere along the circuit, and an oscilloscope can be used in parallel to measure the potential drop across the resistor.

4. Anesthetize a tadpole by immersing it in 0.02% MS-222 for ~1 min.

5. Transfer the tadpole onto the stage of the microscope with a large-bore dropper pipette (e.g., a plastic transfer pipette with the tip cut back to ~5-mm diameter). Carefully orient the tadpole dorsal side up on the moist tissue by gently sliding it with a fine brush.

6. Use the microscope and micromanipulator to visually advance the micropipette into the brain of the tadpole, positioning the tip directly adjacent to the cell to be labeled.

7. Apply pulses from the stimulator.

   Settings for amplitude and pulse duration must be set empirically for each tissue and pipette shape. A good starting point is three to five pulses with an amplitude setting of ~30–80 V on the Grass stimulator. For DiI, use pulse durations of 1–10 msec; for dextrans, use pulses of 10–100 msec; and for DNA electroporation, good transfection efficiency is achieved by delivery of three to five brief (~1 sec) bursts of 1-msec pulses at a frequency of 200 Hz.

   See Troubleshooting.

8. Withdraw the pipette.

   The pipette can be removed immediately after electroporation and repositioned to electroporate another site. We typically target four sites per animal to improve the yield of single cells.

   See Troubleshooting.

9. Using a dropper, transfer the tadpole to fresh 10% Steinberg's rearing medium.

10. Perform image analysis.

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TROUBLESHOOTING

Problem (Step 7): DiI or DNA solution accumulates and blocks the tip.

Solution: It is often possible to rescue the pipette by temporarily reversing the polarity of current through the pipette and delivering one or two brief pulses. Note that a broken tip will entirely prevent efficient labeling with DiI or DNA. Dextran solutions almost never clog the pipette tip.

Problem (Step 8): The DiI solution sticks to the outside of the pipette and leaves a track of dye along the pipette path.

Solution: The best way to avoid this is to apply negative current before retracting the micropipette from the injection site. This dilutes the dye in the pipette tip and prevents leakage and clogging.

DISCUSSION

An epifluorescence microscope allows the dye injection to be monitored during and after labeling. Although it is very beautiful to watch the dye spread throughout the cell, it is critical to remember that looking at the cell with the fluorescent light during the labeling process is one of the easiest ways to kill it. It is recommended that the labeled cell be observed very briefly and only with neutral density filters. For DNA electroporation, there is no instant feedback regarding labeling success because GFP expression takes many hours to develop. For this reason, it can be useful to practice single-cell electroporation with a dextran solution before attempting DNA electroporation.

The success rate in labeling single isolated neurons is slightly lower for DNA electroporation than for dye iontophoresis, but cells imaged with GFP typically remain brighter and healthier over many days of imaging, ultimately resulting in a higher yield of cells that can be used for analysis. If multiple injection sites are used for each animal, the success rate for labeling single neurons in each animal can approach 80%–90%. This method has been used to label cells in *Xenopus* tadpoles between stages 39 and 50 (Nieuwkoop and Faber 1956). In principle, it should be useful in older and younger animals. However, as the animal gets older, the thicker skin becomes more difficult to penetrate without breaking the electrode tip. This problem can be addressed by making a tiny hole in the skin through which the electrode can be inserted. It is worth noting that lipophilic dyes like DiI can be used to label single cells even in fixed material. For further details regarding fluorescence imaging in *Xenopus* neurons, see **In Vivo Time-Lapse Imaging of Neuronal Development in *Xenopus*** (Ruthazer et al. 2013).

RECIPE

Steinberg's Rearing Medium

Reagent	Final concentration
NaCl	58.2 mM
KCl	0.67 mM
$Ca(NO_3)_2$	0.34 mM
$MgSO_4$	0.78 mM
HEPES	50 mM

Use at 10% strength and adjust the pH to 7.4.

ACKNOWLEDGMENTS

This work was supported by the Canadian Institutes of Health Research, National Alliance for Research on Schizophrenia and Depression, National Institutes of Health, and National Science Foundation. We thank the following individuals for generously providing plasmids: G. Patterson

and J. Lippincott-Schwartz (PA-GFP), R. Tsien (mCherry), K. Mural (EGFP-f), and C. Cepko (LNL-GFP, Cre recombinase).

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Ruthazer ES, Schohl A, Schwartz N, Tavakoli A, Tremblay M, Cline HT. 2013. In vivo time-lapse imaging of neuronal development in *Xenopus*. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top077156.

Cite this article as *Cold Spring Harb Protoc*; 2013; doi:10.1101/pdb.prot077149