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Tissue-specific in vivo transformation of plasmid DNA in Neotropical tadpoles using electroporation V.1

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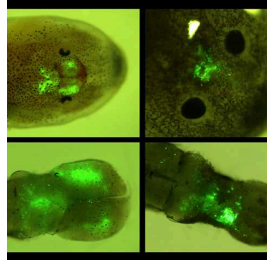
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We use this protocol and it's working

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Abstract

Electroporation is an increasingly common technique used for exogenous gene expression in live animals, but protocols are largely limited to traditional laboratory organisms. The goal of this protocol is to enable in vivo electroporation techniques in a diverse array of tadpole species. We explore electroporation efficiency in tissue-specific cells of five species from across three families of tropical frogs—poison frogs (Dendrobatidae), forest frogs (Aromobatidae), and glassfrogs (Centrolenidae). These species are well-known for their diverse social behaviors and intriguing physiologies that coordinate chemical defenses, aposematism, and/or transparency. Specifically, we examine the effects of electrical pulse and injection parameters on species- and tissue-specific transfection of plasmid DNA in tadpoles. After electroporation of a plasmid encoding green fluorescent protein (GFP), we found strong GFP fluorescence within brain and muscle cells that increases with the amount of DNA injected and electrical pulse number. We discuss species-related challenges, troubleshooting, and outline ideas for improvement. Extending in vivo electroporation to diverse amphibian species will offer a powerful approach to explore topics in genetics, behavior, and organismal biology.

Attachments



[Delia et al electrop...](#)

570.2MB

Guidelines


These parameters are a suggested starting point. Optimizing the protocol for species-specific applications to maximize transfection efficiency is recommended.

Materials

Ethyl 3-Aminobenzoate Methanesulfonate (Millipore Sigma Catalog #10521)
Sodium Bicarbonate (Millipore Sigma Catalog #S6014)
Josh's Frogs R/O Rx
Platinum Foil (Fisher Scientific Catalog #AA11509FF)
Lead Solder Wire (Amazon Catalog #B075WB98FJ)
Loctite Fun-Tak Mounting Putty Tabs (Amazon Catalog #1865809-12)
100 mm X 15 mm Petri Dishes (Fisher Scientific Catalog #FB0875713)
Serological Pipette (Fisher Scientific Catalog #12-567-600)
Electrical Tape (Fisher Scientific Catalog #19-047-280)
Micromanipulator (Sutter Catalog #MM-33)
Grass Instruments SD9 Square Pulse Stimulator
Disposable Paired 13 mm Subdermal Needle Electrodes (MFIMedical Catalog #RHL-RLSND121-1-0)
3.5" Replacement Glass Capillaries (Drummond Scientific Catalog #3-000-203-G/X)
Sutter Instrument Co P-97 (Sutter Catalog #P-P7)
Forceps (Fisher Scientific Catalog #12-000-157)
Mineral Oil (Millipore Sigma Catalog #M8410)
28 Gauge Metal Hub Blunt Point Needle (Fisher Scientific Catalog #14815616)
1 mL BD Disposable Syringe (Fisher Scientific Catalog #14-823-30)
Nanoject II Variable Volume Automatic Injector (Drummond Scientific Catalog #3-000-204)
pCMV-GFP (Addgene Catalog #11153)
Fast Green FCF (Millipore Sigma Catalog #F7258)
Kimwipes (Fisher Scientific Catalog #06-666)
Standard Disposable Transfer Pipettes (Fisher Scientific Catalog #13-711-7M)
Micro Detail Paint Brush
Stereomicroscope with a GFP filter

Troubleshooting

Safety warnings

 MS-222 is a respiratory irritant and the following personal protective equipment should be worn: labcoat, gloves and safety glasses.

Before start

Consult with your local animal ethics board prior to experimentation.



Anesthesia Preparation

5m

- 1 Mix 0.02g ethyl 3-aminobenzoate methanesulfonate (MS-222) and 0.08g sodium bicarbonate with 60 mL tadpole water
- 2 Store at 4C for up to one week

5m

Electrode Set-Up for Targeting Muscle Fibers

25m

- 3 Remove the tips from two 5 mL serological pipettes using scissors
- 4 Solder two ~ 5 mm X 8 mm pieces of platinum foil to separate electrical lead wires to make an electrode
- 5 Run one electrode wire through each cut serological pipette and secure it with electrical tape
- 6 Construct a platform out of clay evenly spread over the top of a Petri dish
- 7 Embed the anode into the clay with the foil exposed near the center of the Petri dish
- 8 Create a tadpole-sized (~ 1 cm) impression adjacent the anode foil

5m

5m

2m

2m

5m

1m

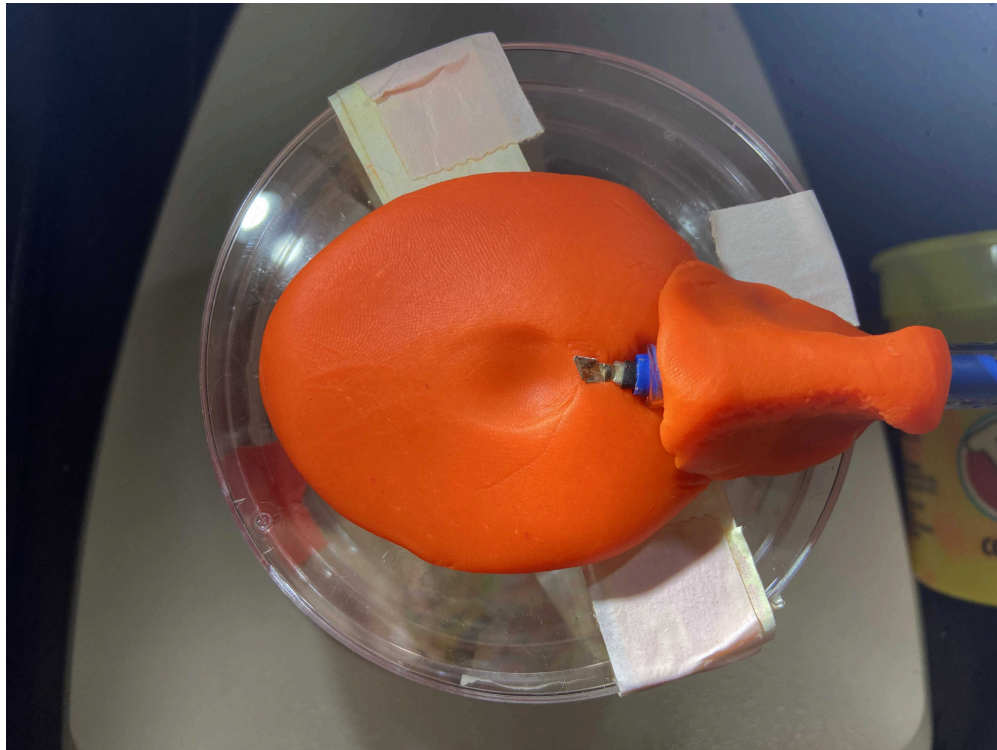


Figure 1. An anode embedded in a flat clay platform to hold tadpoles during the electroporation procedure.

- 9 Mount the cathode on a micromanipulator and bend the foil parallel to the anode

2m



Figure 2. A cathode mounted on a micromanipulator in position to make contact directly above the anode during the electroporation procedure.

- 10 Set the stimulator parameters to square wave, 1 pps, 0.1 ms delay, 5 ms duration, and 30-50 V

1m

Electrode Set-Up For Targeting Brain Cells

25m

- 11 Construct a platform out of clay molded into the shape of a hill
- 12 Fix the clay on the top of a Petri dish

5m

1m



Figure 3. A clay platform molded into the shape of a hill to hold tadpoles during the electroporation procedure.

- 13 Remove the tips from two 1 mL serological pipettes using scissors
- 14 Mount the serological pipettes side by side on the micromanipulator and fasten them together with electrical tape
- 15 Run one needle electrode wire through each cut serological pipette
- 16 Position the electrode needles ~ 1 mm apart and parallel to one another

5m

5m

2m

5m

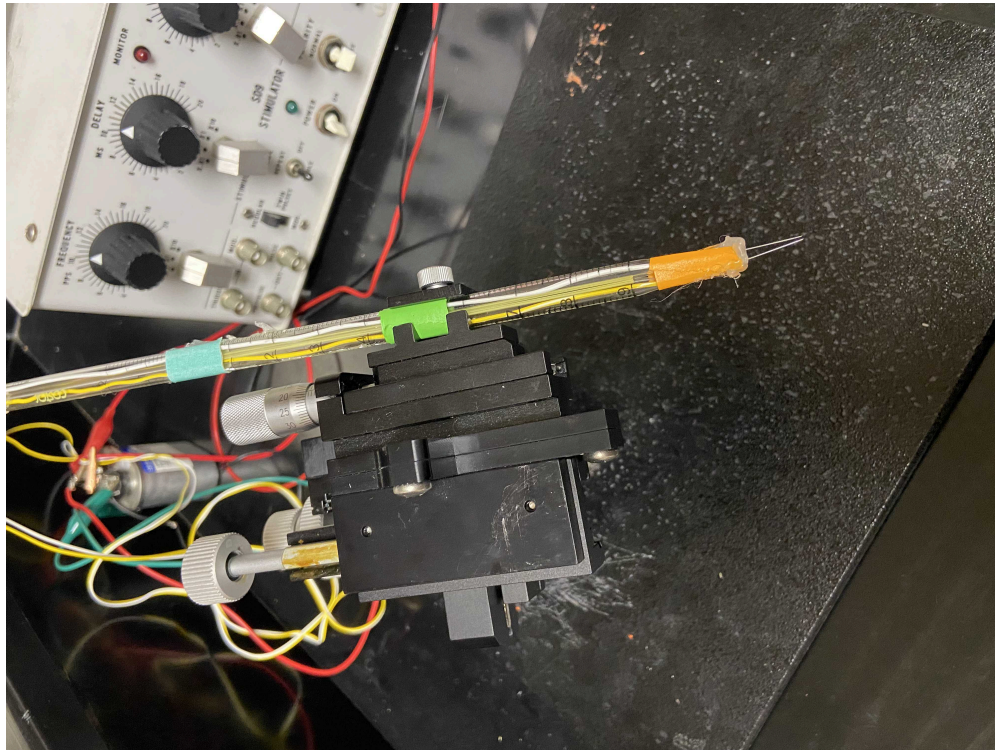


Figure 4. An assembled electrode mounted on a micromanipulator.

- 17 Set the stimulator parameters to 10 pps, 0.1 ms delay, 15 ms duration, and 30 V

1m

Note

These settings are a suggested starting point. Optimizing these parameters for each species, as the best pulse shape and settings may vary, is recommended.

Injection Set-Up

20m

- 18 Pull glass capillaries using a pipette puller
- 19 Break the pipette tip at an angle using forceps to create a beveled tip
- 20 Backfill the pipette with mineral oil using a 28-gauge needle and 1 mL syringe

5m

2m

2m



- 21 Place the pipette onto the injector plunger and tighten the collet 2m
- 22 Select an injection volume of 64.4 nl and set the injection rate to slow 2m
- 23 Empty enough mineral oil to load 2 uL of plasmid DNA solution 2m
- 24 Pipette 2 uL of plasmid DNA solution (0.25–0.27 μ g/ μ l) onto a piece of parafilm and mix with 0.2 uL 0.01% Fast Green 2m
- 25 Fill the pipette without introducing air bubbles 1m
- 26 Connect the electrode wires to the stimulator 1m
- 27 Place the platform under a dissection microscope with the electrode on one side and the injector on the other 1m

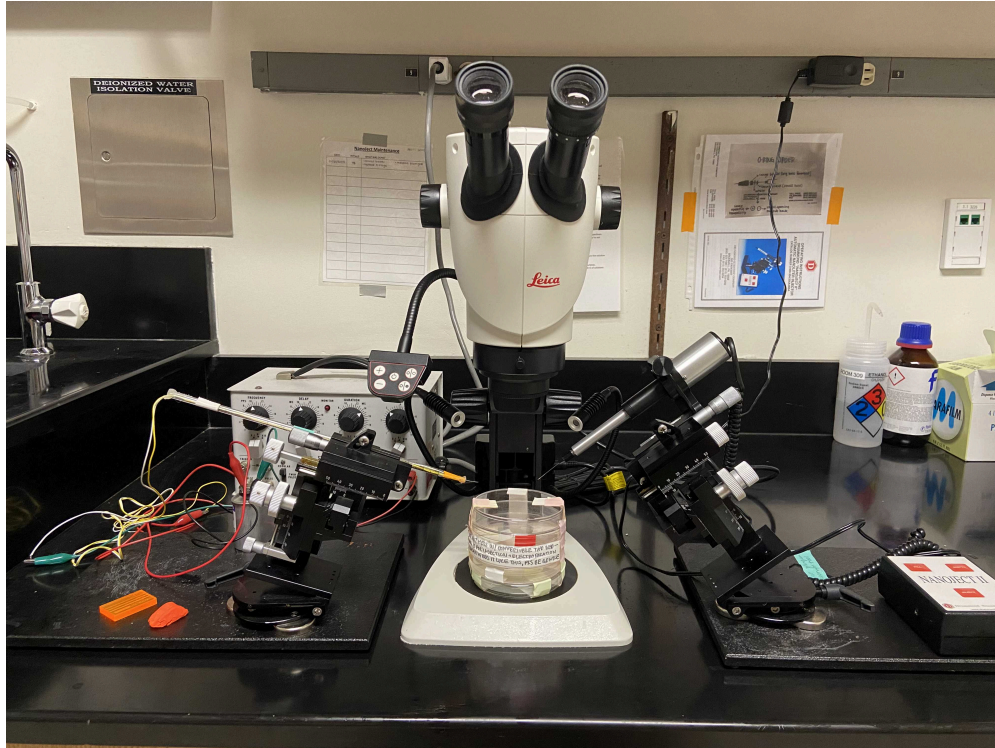


Figure 5. A dissection microscope focused on a tadpole injection platform that is placed between an electrode mounted on a micromanipulator and a microinjector mounted on a micromanipulator.

Electroporation

25m

- 28 Anesthetize the tadpole by placing it in a Petri dish of room temperature 0.03% MS-222 for 5 minutes 5m
- 29 Confirm the tadpole is fully sedated by checking for movement in response to stimuli 1m
- 30 Move the tadpole to the platform with a cut transfer pipette 2m
- 31 Adjust the position of the tadpole using a paintbrush 2m
- 31.1 For experiments targeting muscle fibers, place the tadpole flat on its side with its head in the depression and its tail on top of the anode



- 31.2 For experiments targeting brain cells, place the tadpole dorsal side up with its head in the depression
- 32 Orient the platform such that the head of the tadpole is facing toward the injector 1m
- 33 Lower the injector and insert the pipette into the target tissue 2m
- 33.1 For experiments targeting muscle fibers, insert the pipette into a tail myomere
- 33.2 For experiments targeting brain cells, insert the pipette into the brain ventricle
- 34 Inject the plasmid DNA with a 5-10 s interval between each injection 2m
- 34.1 For experiments targeting muscle fibers, deliver two injections
- 34.2 For experiments targeting brain cells, deliver three injections
- 35 Remove the pipette from the tadpole 1m
- 36 Orient the platform such that the head of the tadpole is facing toward the electrode 1m
- 37 Lower the electrode until it is in full contact with the target tissue 5m
- 37.1 For experiments targeting muscle fibers, the tail should lay on top of the anode and the cathode should press on the tail directly above the anode



Figure 6. Muscle electroporation in the tail of a *Ranitomeya imitator* tadpole.

- 37.2 For experiments targeting brain cells, the electrode should be touching the head on either side of the brain

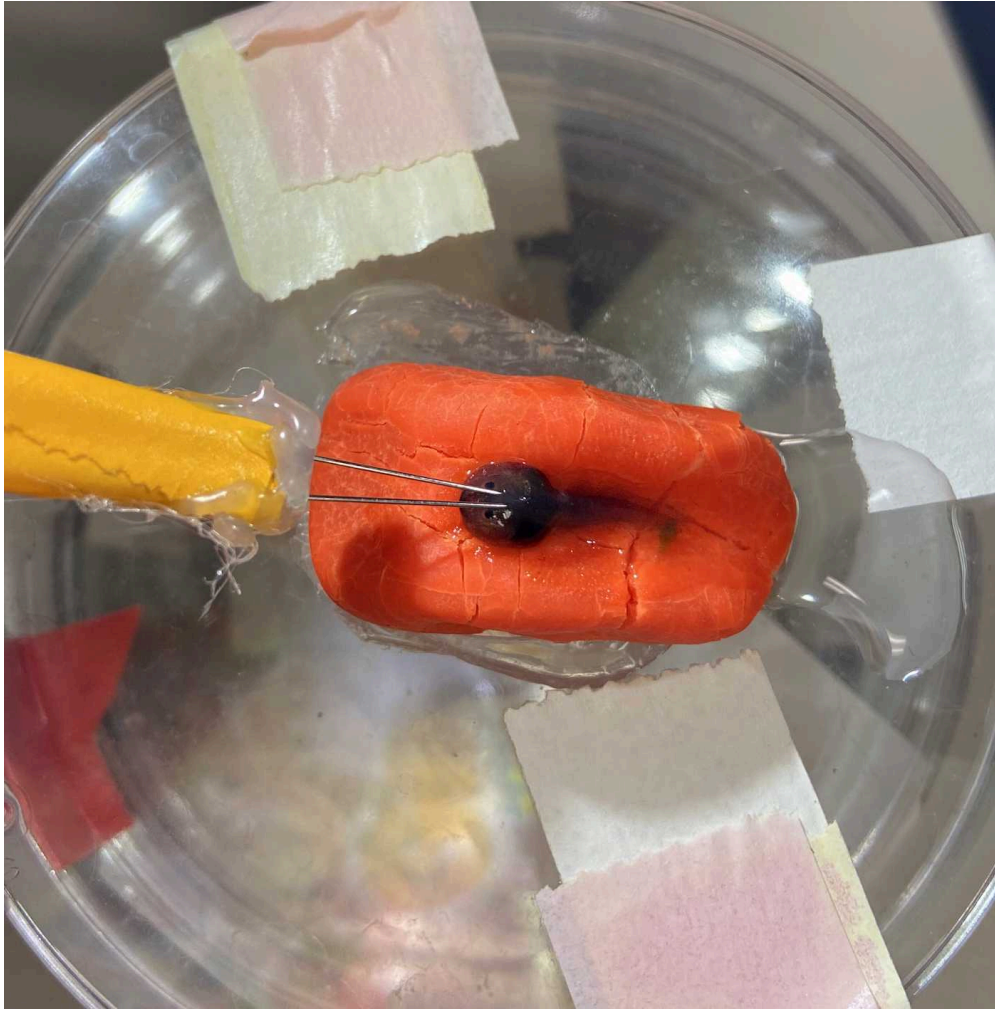


Figure 7. Brain electroporation in a *Ranitomeya imitator* tadpole with the electrode making contact with either side of the brain.

38 Deliver the electrical pulses

2m

Note

The pulse range for targeting brain cells is a suggested starting point. Optimizing the protocol for species-specific applications to maximize transfection efficiency is recommended.

- 38.1 For experiments targeting muscle fibers, deliver 4-8 double pulses with a 1 s interval between each set of pulses
- 38.2 For experiments targeting brain cells, deliver 4-10 pulses with a 1 s interval between each pulse



39 Transfer the tadpole to fresh tadpole water for several hours to recover

4h

40 Roughly 24 to 48 hours after electroporation, screen tadpoles for plasmid uptake by imaging GFP-positive cells

In Vivo Screening

20m

41 Anesthetize the tadpole by placing it in a Petri dish of room temperature 0.03% MS-222 for 5 minutes

5m

42 Move the tadpole to a new Petri dish with tadpole water and place under a stereomicroscope with a GFP filter

5m

43 Center the imaging field on the target tissue and capture the fluorescent image

10m

44 Transfer the tadpole to fresh tadpole water for several hours to recover

4h