

Video Article

Time-lapse Live Imaging of Clonally Related Neural Progenitor Cells in the Developing Zebrafish Forebrain

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Abstract

Precise patterns of division, migration and differentiation of neural progenitor cells are crucial for proper brain development and function^{1,2}. To understand the behavior of neural progenitor cells in the complex *in vivo* environment, time-lapse live imaging of neural progenitor cells in an intact brain is critically required. In this video, we exploit the unique features of zebrafish embryos to visualize the development of forebrain neural progenitor cells *in vivo*. We use electroporation to genetically and sparsely label individual neural progenitor cells. Briefly, DNA constructs coding for fluorescent markers were injected into the forebrain ventricle of 22 hours post fertilization (hpf) zebrafish embryos and electric pulses were delivered immediately. Six hours later, the electroporated zebrafish embryos were mounted with low melting point agarose in glass bottom culture dishes. Fluorescently labeled neural progenitor cells were then imaged for 36 hours with fixed intervals under a confocal microscope using water dipping objective lens. The present method provides a way to gain insights into the *in vivo* development of forebrain neural progenitor cells and can be applied to other parts of the central nervous system of the zebrafish embryo.

Protocol

1. Preparation of Zebrafish Embryos

1. Two days before electroporation, set up mating cages of wild type fish using dividers to separate males from females.
2. One day before electroporation, pull the dividers in mating cages from the previous day.
3. Collect embryos and incubate 50 fertilized embryos in 30 ml embryonic medium containing 0.003% phenylthiourea (PTU) at 28.5°C.
4. On the day of electroporation, dechorionate the embryos manually with fine forceps (Inox 5, Dumont Electronic, Switzerland) when the embryos reach the developmental stage of 22hpf. Then transfer the embryos to 10ml electroporation ringer's³ (180 mM NaCl, 5mM KCl, 1.8 mM CaCl₂, 5 mM Hepes pH 7.2) containing 0.003% PTU.
5. Add 420 µl tricaine stock (4mg/ml) to the 10ml electroporation ringer's to anesthetize the embryos.

2. Preparation for Electroporation

1. Glass injection needles were pulled from capillaries (1.2 mm OD, 0.9 mm ID, with filament) on a Flaming-Brown P897 puller (Sutter Instruments, Novato, CA, USA). The needle tip was broken off with forceps to get a sharp end as shown in figure 1D. The diameter of the tip should be around 10 µm and the taper angle should be around 30 degree.
2. Prepare 0.8% and 1% low-melting point agarose (Shelton Scientific, Inc. catalog #IB70050) in electroporation ringer's solution. Aliquot the agarose solution in 2 ml microcentrifuge tubes. Keep the aliquots in a heat block at ~37°C.
3. The Gal4/UAS system is used to drive gene expression in neural progenitor cells of electroporated embryos. Two plasmids are used: plasmid EF1α-GFF-PT2KXIG, in which Gal4FF is driven by the ubiquitous promoter EF1α, and plasmid UAS-E1B-EGFP-PT2KXIG.
4. Prepare EF1α-GFF-PT2KXIG and UAS-E1B-EGFP-PT2KXIG plasmid DNA using GenElute™ HP Plasmid Midiprep Kit (Sigma).
5. The plasmids are then concentrated by sodium acetate/ethanol precipitation to the final concentration of 1-2 µg/µl in 10 mM Tris-Cl (PH 8.5).
6. Mix the two plasmids and dilute with nuclease-free H₂O to a final concentration of 500 ng/µl for each plasmid. Addition of phenol red (diluted 1/10 total volume) to the solution is preferred for visualization of delivery. Keep the DNA solution on ice.
7. Right before electroporation, backload the injection needle with 2 µl DNA solution and adjust the injection volume to 2 nl.

3. Electroporation

1. A stereo dissection microscope (Zeiss Stemi 2000 with a maximum magnification of 50x), an air pressure injector (Narishige IM 300 microinjector), two micromanipulators (WPI M3301R, World Precision Instruments, Sarasota, FL, USA), and an electric stimulator (SD9 square pulse stimulator, Grass Tleafactor, West Warwick, Rhode Island, USA) are set up as shown in Figure 1A.
2. To mount the embryos, immerse 2 embryos in 1% low-melting point agarose. Then immediately remove the embryos from the agarose with a glass pipette.
3. Place the embryos on an inverted plastic Petri dish lid in individual drops of agarose. Orientate the embryos gently with a fiber probe to a dorsal-up position before the agarose solidifies so that the forebrain is accessible to both the electrodes and the microinjection needle (Figure 1B). Embryos must be mounted in individual agarose drops.
4. Manipulate the electrodes with the left micromanipulator. Insert the electrodes into the agarose as shown in Figure 1B. Custom platinum iridium parallel bipolar electrodes 125 µm in diameter and spaced 500 µm apart are used for electroporation (FHC, catalog #PBSA0575).
5. Insert the microinjection needle into the forebrain ventricle and inject 4nl DNA mix, as flowing of red fluid and the swelling of the ventricle can be observed.

6. Immediately, one 28.5 V pulse lasting 1 millisecond was applied manually. Then reverse the polarity and apply one more 28.5 V pulse lasting 1 millisecond. This will result in bilateral mosaic labeling of the forebrain neural progenitor cells.
7. After electroporation of 10 embryos, add 5ml embryonic medium to cover the embryos and peel the agarose with a microsurgical knife to release the embryos.
8. Transfer the electroporated embryos to a fresh dish of 30 ml embryonic medium containing 0.003% PTU and incubate at 28.5°C.

4. Mounting and Time-lapse Live Imaging

1. Around 4 hours after electroporation, the EGFP signal is observable if viewed under an epifluorescent dissecting microscope.
2. Five hours after electroporation, select the embryos with highly mosaic but strong EGFP expression in the forebrain region.
3. Transfer the selected embryos to 10ml embryonic medium containing 0.003% PTU.
4. Add 420 μ l tricaine stock (4mg/ml) to the 10ml embryonic medium to anesthetize the embryos.
5. To mount the embryos, immerse 1 embryo in 0.8% low melting point agarose. Then immediately remove the embryo from the agarose with a glass pipette and place the embryo on the center of a 35mm glass bottom culture dish (MatTek corporation, Part# P35GC-1.5-10-C, www.glass-bottom-dishes.com).
6. Orientate the embryos gently with a fiber probe to a dorsal-up position before the agarose solidifies.
7. Place the dish properly on the temperature controlled stage of the confocal microscope as shown in figure 1C (We use a Nikon C1 spectral confocal microscope with up-right objectives). Adjust the temperature to 28.5°C.
8. Add 3ml 28.5°C preheated embryonic medium containing 0.003% PTU to cover the embryo. The embryo is now ready for imaging.
9. Perform time-lapse live imaging with a fixed interval using a water dipping objective.

5. Representative Results

Shown in Figure 2 are selected frames of a time-lapse confocal live imaging of the embryo electroporated of EF1 α -GFF + UAS-E1B-EGFP at 22 hpf.

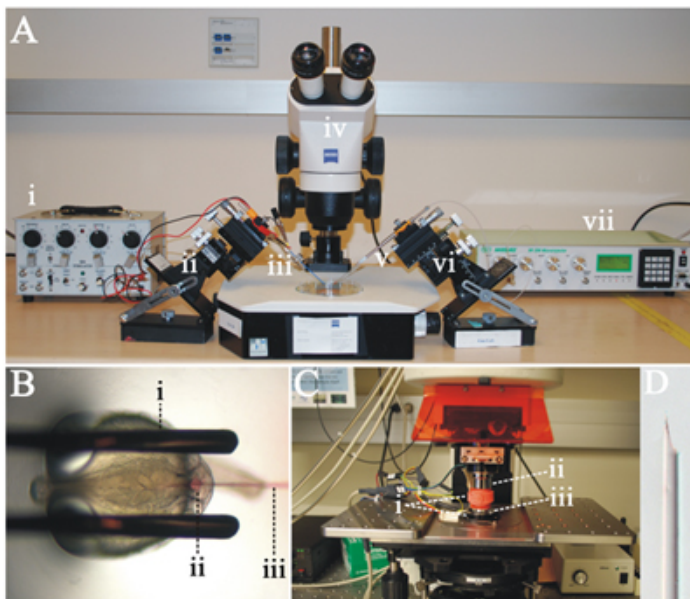


Figure 1. Apparatus set-up. (A) The assembly of electroporation equipments: the Grass SD9 square pulse stimulator(i), the left micromanipulator (ii), the electrodes (iii), the Zeiss dissecting microscope (iv), the injection needle (v), the right micromanipulator (vi) and the Narishige IM 300 microinjector (vii). (B) The relative position of the electrodes (i), the forebrain ventricle (ii) and the injection needle (iii). Note the red-colored DNA solution has been injected into the forebrain ventricle. (C) The assembly of live imaging on Nikon C1 confocal microscope: the temperature controller (i), the water dipping objective len (ii) and the electroporated embryo embedded in low melting point agarose in a glass bottom culture dish (iii) (D) Image of a pulled and cut needle for microinjection of DNA.

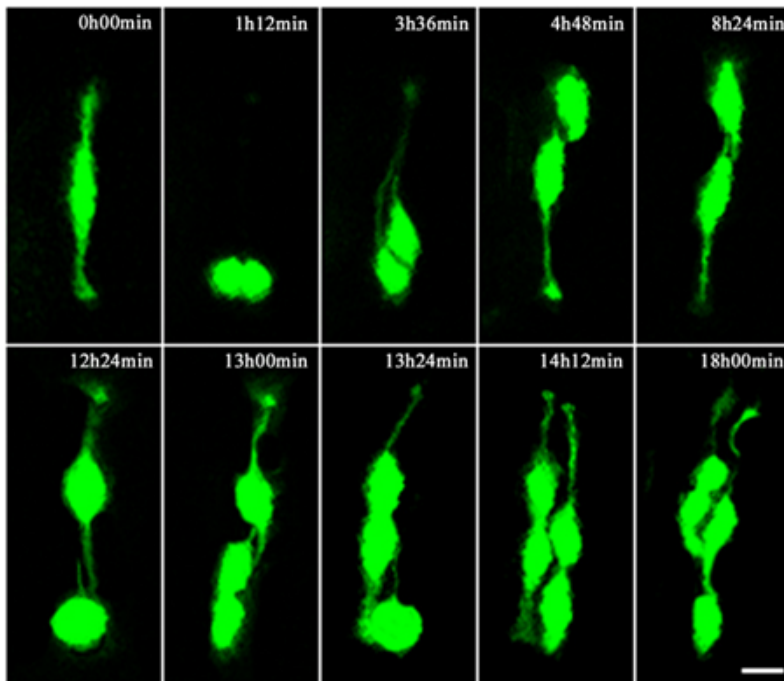


Figure 2. Selected frames of an 18-hour time-lapse confocal live imaging of the embryo electroporated with EF1 α -GFF + UAS-E1B-EGFP at 22 hpf. Ventricle surface is on the bottom side of each image frame. Scale bar represents 10 μ m.

Discussion

In this video, we demonstrate a method for time-lapse live imaging of neural progenitor cells at a clonal level in the developing zebrafish forebrain. We tested and modified the existing electroporation protocols⁴⁻⁶ to genetically and fluorescently label individual neural progenitor cells. A lineage tree composed of clonally related progeny cells can be established since only a few cells were sparsely labeled with a relative low voltage of electroporation. In addition to EGFP, the neural progenitor cells can be subcellularly labeled with various fluorescent proteins by simply replacing EGFP with other fluorescent markers. For example, if EF1 α -GFF was co-electroporated with UAS-E1B-membrane-EGFP and UAS-E1B-H2B-mRFP, membranes of individual progenitor cells will be labeled with green fluorescent protein while nucleus will be labeled with red fluorescent protein. Most of the labelled progenitor cells are healthy as evidenced by the general normal development of the electroporated embryos and few apoptosis observed during live imaging. It is also critical to use a low concentration of agarose to mount the embryo and use a minimal laser power during confocal live imaging to keep the cells healthy. The present technique can also be applied to other parts of the central nervous system such as the hind brain and the spinal cord. However, this technique can not be applied on embryos younger than 18 hpf, since the DNA constructs have to be injected into the ventricle for electroporation.

Disclosures

No conflicts of interest declared.

Acknowledgements

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