Video Article

Transplantation of GFP-expressing Blastomeres for Live Imaging of Retinal and Brain Development in Chimeric Zebrafish Embryos

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Abstract

Cells change extensively in their locations and property during embryogenesis. These changes are regulated by the interactions between the cells and their environment. Chimeric embryos, which are composed of cells of different genetic background, are great tools to study the cell-cell interactions mediated by genes of interest. The embryonic transparency of zebrafish at early developmental stages permits direct visualization of the morphogenesis of tissues and organs at the cellular level. Here, we demonstrate a protocol to generate chimeric retinas and brains in zebrafish embryos and to perform live imaging of the donor cells. The protocol covers the preparation of transplantation needles, the transplantation of GFP-expressing donor blastomeres to GFP-negative hosts, and the examination of donor cell behavior under live confocal microscopy. With slight modifications, this protocol can also be used to study the embryonic development of other tissues and organs in zebrafish. The advantages of using GFP to label donor cells are also discussed.

Video Link

The video component of this article can be found at https://www.jove.com/video/1924/

Protocol

Day 1. Preparation

1. Set up fish crosses

Set up pairwise crosses of zebrafish in the evening. To prevent random mating before the desired time, use dividers to separate the female and male fish in the mating cassettes. GFP-expressing transgenic fish are used as a source of donor blastomeres. The pt104 transgenic line (Zou *et al.*, 2008), used here, expresses GFP ubiquitously under the control of the EF1 promoter. GFP expression allows visualization of donor cells by live imaging. Other transgenic fish lines that express suitable fluorescent proteins may be used for specific needs.

2. Prepare wedge-shaped agarose wells for embryo holding

Pour melted 1% agarose, prepared in E3 egg water (Westerfield, 2007), into a Petri dish. Float a plastic mold that has wedge-shaped protrusions on the agarose solution and let the agarose solution completely solidify. Remove the mold carefully and immerse the agarose bed with E3 water.

3. Prepare transplantation needles

Use a vertical Needle puller (David KOPF Instruments) at power setting 11.5 to make needles by pulling glass capillary pipettes (World Precision Instrument). Good needle tips should taper with long shafts. Grind the needle tips at a 45-degree angle with a Micro Grinder (Marishige) until beveled openings of 40-45μm in inner diameter are obtained (Fig. 1A). To clean up the needle tips, attach a hose to the blunt end of the needle, connect the hose to a syringe, and then suck up and release a 10% hydrofluoric acid solution until all visible debris is removed. Next, wash the tips with distilled water, followed by 100% ethanol. Air-dry the needles until all ethanol evaporates. It is important to ensure that the tips are completely dry: residual ethanol will ruin the needles during the high-temperature polishing step. To soften and polish the rough edges of the needle tips, bake the needle tips with the heat generated by the filament of a Micro Forger (Marishige). Do not let the needle tips touch the filament during polishing. The amount of electricity passing though the filament and the distance between the filament and the needle tips can be adjusted to attain desired heating conditions. The temperature should not be too high: this will melt and deform the needle tips. After the rough edges are smoothened, carefully touch the heated filament with the needle tip and then gently pull the filament away from the needle to generate a pointed end (Fig. 1B). A sharp, smooth, and clean needle is critical for obtaining intact donor blastomeres and for not damaging the host embryos.

4. Prepare an embryo-positioning probe

Pull a glass pipette over a gas flame to obtain a fine glass probe with a smoothened end. This probe will be used to position the embryos in the proper orientation prior to transplantation.

Day 2. Blastomere transplantation

1. Collect and dechorionate embryos

The next day, remove the dividers in the cross cassettes to allow fish to mate. Place the embryos in a Petri dish filled with E3 water. Then, dechorionate embryos by manually tearing away the chorions from the embryos with a pair of fine forceps. Use a glass pipette with bent shaft to carefully transfer the dechorionated embryos into agarose wells. Avoid any contact of the embryos with the air, which cause the embryos to burst. Let the embryos develop at 28.5 °C in the agarose wells until 3 hours post fertilization, or hpf.

2. Set up a transplantation apparatus

While the embryos develop, set up a transplantation apparatus by connecting the transplantation needle to the mineral oil-filled micro-pumping system. Make sure that the system does not leak and no air bubbles are trapped in the system. Back fill the entire transplantation needle with mineral oil, except for the 0.5 - 1 µl of space at the tip opening.

3. Transplantation of blastomeres

To transplant blastomeres, use a glass positioning probe to orientate the blastomere side of the 3-4-hpf embryos upwards. Then, gently insert the transplantation needle into a donor embryo and slowly suck up 5-20 blastomeres into the needle. Insert the blastomere-filled needle into a host embryo and release the blastomeres slowly. The site at which the blastomeres are released will influence where their progeny localize at later developmental stages. To analyze retinal and brain development, release cells at the animal pole. During transplantation, make sure that the needle tip does not contact air. To minimize mechanical damages, host embryos shall be left in the agarose wells at 28.5 °C until the next day. Then, transfer the mosaic embryos to 24-well plates pre-coated with 0.5 ml of 1% agarose, with one embryo in each well. 1 ml of E3 egg water is added to each well. To prevent bacterial infections, add 10 µl of 100X penicillin and streptomycin to the E3 egg water.

Day 3. Embedding and live imaging of mosaic embryos

1. Embedding embryos

Prepare 1.5% low melting point agarose (LMP) in E3 egg water and keep the solution in a 30 °C water bath. Transfer a mosaic embryo to a FluoroDish tissue culture dish with a glass bottom of 0.17mm in thickness (World Precision Instruments). Remove excess E3 water to leave the embryo just covered by a minimal amount of E3. Add 30-50 µl melted 1.5% LMP to the embryo. Quickly position the eye or the brain as close to the glass bottom as possible before the agarose solution solidifies. After the solution cools down, add additional agarose solution to further secure the embryos in place. Then, immerse the solidified agarose block with 2-3 ml E3 egg water.

2. Confocal Imaging

For confocal microscopy, a water-immersion inverted objective (Olympus, PlanApo, 40 X/0.90 WLSM, Japan) with large focus depth is used. A drop of water is added onto the objective and then place a FluoroDish tissue culture dish on the objective. To image development, perform time-lapse imaging by taking pictures of the embryos every 5 minutes. Depending on the nature of experiments, the time intervals between shots should be adjusted.

Representative results

Figure 2 illustrates a representative 24-hpf mosaic zebrafish. GFP expression highlights the donor cells in green. The retinal neuroepithelial cells span the entire thickness of the retinal wall.

The movie shows the movements of donor cells in the host brain at 24 hpf. Notice cells morphologies change over time.

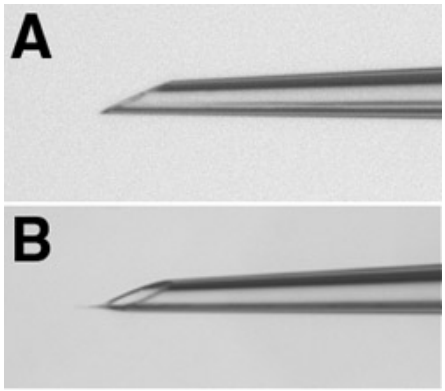

Figure 1. Shapes of the transplantation needle tips. A. The beveled opening of the tip of a needle was free of debris after hydrofluoric acid washes. B. A pointed end was generated at the needle tip.

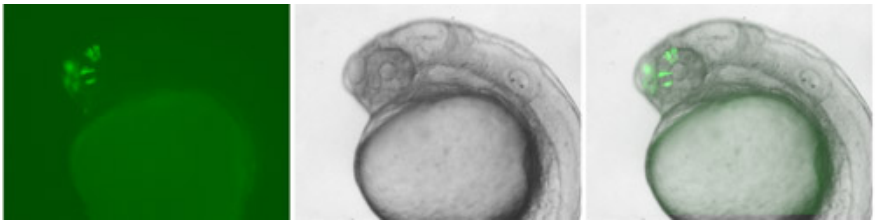

Figure 2. Visualization of GFP-expressing donor cells in a chimeric zebrafish embryos at 24 hpf. The left panel shows the GFP signals in the brain and retina. The middle panel is a bright field picture of the embryo. The right panel shows the merged image.

Movie 1. Live imaging of GFP positive donor cells demonstrated the extensive changes in the morphologies of brain neuroepithelial cells during development. The individual frames of the movie were collected every 5 minutes between 24 and 25 hpf. Click here to see the movie.

Discussion

In this video, we demonstrate a method for blastomere transplantation to analyze the retinal and brain development in zebrafish. This protocol can also be used to analyze the development of other tissues and organs by simply releasing the donor blastomeres to corresponding locations in the host embryos. The labeling with GFP molecules yields a cleaner background than labeling with fluorescent dye-conjugated Dextran (Ho and Kane 1990). This is because the debris of Dextran dyes from dead donor cells lasts much longer than GFP, thus inferring with imaging (Catalano *et al*., 2007). In addition, the GFP-positive donor cells are healthier than those containing Dextran dyes.

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