

1-1-2009

Tissue Targeted Embryonic Chimeras: Zebrafish Gastrula Cell Transplantation

Elizabeth R. Deschene
Smith College

Michael J. Barresi
Smith College, mbarresi@smith.edu

Follow this and additional works at: https://scholarworks.smith.edu/bio_facpubs



Part of the [Biology Commons](#)

Recommended Citation

Deschene, Elizabeth R. and Barresi, Michael J., "Tissue Targeted Embryonic Chimeras: Zebrafish Gastrula Cell Transplantation" (2009). Biological Sciences: Faculty Publications, Smith College, Northampton, MA. https://scholarworks.smith.edu/bio_facpubs/202

This Article has been accepted for inclusion in Biological Sciences: Faculty Publications by an authorized administrator of Smith ScholarWorks. For more information, please contact scholarworks@smith.edu

Video Article

Tissue Targeted Embryonic Chimeras: Zebrafish Gastrula Cell Transplantation

Elizabeth R. Deschene¹, Michael J. Barresi¹

¹Department of Biological Sciences, Smith College

Correspondence to: Michael J. Barresi at mbarresi@email.smith.edu

URL: <https://www.jove.com/video/1422>

DOI: [doi:10.3791/1422](https://doi.org/10.3791/1422)

Keywords: Developmental Biology, Issue 31, zebrafish, microinjections, gastrula-stage, transplantation, forebrain

Date Published: 9/11/2009

Citation: Deschene, E.R., Barresi, M.J. Tissue Targeted Embryonic Chimeras: Zebrafish Gastrula Cell Transplantation. *J. Vis. Exp.* (31), e1422, doi:10.3791/1422 (2009).

Abstract

Certain fundamental questions in the field of developmental biology can only be answered when cells are placed in novel environments or when small groups of cells in a larger context are altered. Watching how one cell interacts with and behaves in a unique environment is essential to characterizing cell functions. Determining how the localized misexpression of a specific protein influences surrounding cells provides insightful information on the roles that protein plays in a variety of developmental processes. Our lab uses the zebrafish model system to uniquely combine genetic approaches with classical transplantation techniques to generate genotypic or phenotypic chimeras. We study neuron-glia cell interactions during the formation of forebrain commissures in zebrafish. This video describes a method that allows our lab to investigate the role of astroglial populations in the diencephalon and the roles of specific guidance cues that influence projecting axons as they cross the midline. Due to their transparency zebrafish embryos are ideal models for this type of ectopic cell placement or localized gene misexpression. Tracking transplanted cells can be accomplished using a vital dye or a transgenic fish line expressing a fluorescent protein. We demonstrate here how to prepare donor embryos with a vital dye tracer for transplantation, as well as how to extract and transplant cells from one gastrula staged embryo to another. We present data showing ectopic GFP+ transgenic cells within the forebrain of zebrafish embryos and characterize the location of these cells with respect to forebrain commissures. In addition, we show laser scanning confocal timelapse microscopy of Alexa 594 labeled cells transplanted into a GFP+ transgenic host embryo. These data provide evidence that gastrula staged transplantation enables the targeted positioning of ectopic cells to address a variety of questions in Developmental Biology.

Video Link

The video component of this article can be found at <https://www.jove.com/video/1422/>

Protocol

Part 1: Alexa 594 Labeled Embryos

1.1 Microinjection Plate and Needle Preparation

1. Making microinjection plates

1. Prepare injection plates comprised of three to five 1-mm troughs within an agarose mold using a technique by Westerfield, 2007¹. These troughs will snugly hold the embryos in a line for efficient and systematic microinjections. Prior to making the plates, take three 1-mm, 4-inch long non-filament capillaries and carefully break them in half. Using superglue, glue two of these 2-inch long capillaries side-by-side on a flat surface. Glue the third capillary on top of these two, nestled in the groove, creating a pyramid shape with the three capillaries. Allow to dry and repeat until enough "trough molds" are constructed.
2. Pour 20-25mL (5mm depth) of molten 1.5% agarose in embryo medium (EM) [5mM NaCl, 0.17mM KCl, 0.33mM CaCl₂, 0.33mM MgSO₄ and 0.00003% methylene blue] into a 100mm petri dish. Immediately place three to five of the previously made pyramid-shaped trough molds to the bottom of the plate ensuring the flat side of the mold is touching the bottom of the petri dish and completely covered by agarose.
3. When the agarose has solidified, the glass capillary molds must be taken out. To remove, cut out a large agarose square containing the three capillary molds. Gently dislodge the agar on the periphery of the cut square and discard. Flip the square agar piece over and, using fine forceps, completely remove the trough molds. Pour molten agarose into the petri dish around the square to hold the newly molded wells in place. Let set. Plates can be parafilm sealed and stored for weeks at 4 °C. Discard at signs of any growth in the agar.

2. Needles for microinjections.

1. Use a Micropipet capillary puller make the necessary injection needles out of 1-mm, 4-inch glass capillaries with internal filament. Needles were pulled using the Flaming/Brown Micropipet Puller with a heat of 550, pull force of 120, velocity of 200, and a time/delay of 200.

1.2 Embryo Collection and Microinjection Apparatus Setup

1. Fertilized eggs are immediately collected after they are laid and maintained in a petri dish filled with EM. Injection of fluorescent dextrans is ideal at the one-cell stage and should be completed by the 16-cell stage².
2. Using a wide tip glass pipette, expel embryos into the wells of a room temperature injection plate filled with EM. Gently wedge the embryos into the bottom of the troughs with clasped blunt forceps. Embryos whose chorion or yolk have been compromised should be discarded.
3. Load the injection needle with 1-2 μ l of diluted fluorescent Alexa 594 solution (5% by weight in 0.2 M KCl). Allow the dextran to travel to the tip of the injection needle by capillary action due to the embedded filament running the length of the injection needle.
4. Attach the injection needle to the capillary holder of a stereotactic micromanipulator.
5. Adjust pressure on the microinjector apparatus to begin with 40-50 Psi and pulse duration of 500msec.
6. Gently graze the tip of the injection needle to break the seal with forceps (ideal diameter is 0.05-0.15mm). Using a stereomicroscope, expel the dextran onto a micrometer with mineral oil atop of it to calibrate the needle. Modify the bolus size by adjusting the air pressure, duration of the pulse and the size of the needle tip. A bolus size equivalent to a volume of 0.8-1nl should be maintained throughout injections³. Often tips will become clogged with yolk particles; adjusting the pressure or breaking the needle tip may alleviate the problem, otherwise a new injection needle must be loaded.

1.3 Microinjections of Fluorescent Alexa 594

1. Under high magnification, starting from one end of the trough, smoothly pierce the chorion and cell membrane of embryos to enter into the yolk. Inject the dextran solution just under the cell. Make sure to remove the injection needle with the same smooth movement used to enter the embryo. Move the petri dish to position the next embryo inline and continue injecting down the length of the trough. Check the pressure and the bolus size periodically. Be careful not to move or bend the injection needle when it is in the embryo as this is likely to fatally damage the embryo and/or break the injection needle.
2. Gently push the embryos out of the troughs with forceps. Transfer them to a petri dish filled with antibiotic Pen/Step EM as described in Nusslein-Volhard and Dahm (2002) (1ml 6.5M CaCl₂, 1ml 3.5M NaHCO₃, 25ml 20x EM and 10ml of 60mg/ml penicillin, 100mg/ml streptomycin stock) in preparation for later transplantations. Incubate the embryos at 28.5 °C and monitor for unfertilized eggs or embryo death.

Part 2: Gastrula Stage Transplantations

2.1 Dechorionating and Transplant Plate Preparation

1. *Dechorionating plates.* Dechorionated embryos that have not completed epiboly require a soft bottom plate so as not to have their yolk adhere and tear on the plastic. The day prior to transplants, using the 1.5% agarose solution previously described, make dechorionating plates by pouring 5-10 mL of molten agarose into a 100mm petri dish. Only a thin layer of agarose (2-3mm) is needed to cushion the embryos during dechorination. Let cool.
2. *Transplant plates.* Specialized plates with separated wells need to be made as described in⁴. Pour 30ml of the 1.5% agarose solution into a 100mm petri dish and insert a transplant well mold. The mold should contain 104 triangular divots each comprised of three 90 degree sides and one 45 degree angled side. The wells are designed to hold two embryos side-by-side (Fig. 1A, purple box). Take care not to trap air bubbles under the mold. Let the agarose harden. Removing the mold reveals a sunken area containing the square wells with a sloped edge.

2.2 Embryo and Transplant Apparatus Setup

1. *Positioning of donor and host embryos*
 1. Development of hosts and donors need to be monitored to maintain near age-matched pairings. To do this, embryos can be raised at varying temperatures (25-31 °C) to slow down or speed up development respectively.
 2. Both host and donor embryos should be hand dechorionated on an agar-coated dechorionating plate 1 hour prior to the desired age. In the case of gastrula stage transplants this would need to be completed by 5hpf.
 3. Prior to shield stage, at 5.5hpf host and donor embryos are loaded into a transplant plate filled with antibiotic EM. Embryos are loaded into the individual 1-mm wells using a glass pipette. An example is as follows: the first row is filled with donor embryos (18 total, two embryos per well) and the following two rows are filled with host embryos (one embryo per well, 9 embryos per row).
2. *Setting up the transplant apparatus*
 1. Transplants are conducted on the Zeiss Lumar fluorescent stereo microscope that has fully automated, joystick controlled zoom and focus manipulation. While transplants can be done without this automation of the microscope it does dramatically increase ease and efficiency especially when a high number of transplants is desired.
 2. Set the Eppendorf *AirTram* to the middle location of its scale. Connect up the *AirTram* (Fig. 1A, red box) tubing to the capillary holder, and secure the holder to the Eppendorf automated micromanipulator (Fig. 1A). This micromanipulator is fully automated and joystick controlled. Again, this automation is not completely necessary but significantly improves ones ability to navigate the needle around and into the embryo. Alternatively, many researchers prefer the *OilTram* that is said to offer smoother control of cell collection; however, we have had no difficulties with the *AirTram*.
 3. The capillaries used were sterile Eppendorf *TransferTips (ES)* that have a 15 m beveled opening at the tip and a 20 degree bent angle 1mm from the tip (Fig. 1A green box).

2.3 Gastrula Stage Cell Transplants

1. At 6hpf, using the micromanipulator gently graze the embryo with the tip of the capillary to rotate the embryo in position for cell extractions such that its midline and shield are visible and opposing the tip of the capillary. Aspirate a small amount of EM into the capillary to prevent any chance of cells contacting the air-water interface, which would damage the cells.

2. Based on the zebrafish fate map, targeting cells to the forebrain requires the transplantation of cells at the midline exactly between the animal pole and the shield⁵. Gently pierce the ectoderm of donor embryos (Rhodamine injected or GFP transgenic embryos) in this location. The thickness of cells between the ectoderm and underlying yolk in the gastrula is thin; therefore, use caution not to impale the yolk as this will often lead to death within hours. Using the *AirTram* aspirate cells slowly into the capillary. Always maintain visibility of the water line. Slight agitation of the tip while in the donor will help to loose cell-cell contacts. Approximately 10-50 cells from the donor embryo should be extracted before it is removed from the embryo.
3. Remove the capillary from the donor, similarly orient the host, and pierce and expel the cells into the exact same location between the animal pole and shield of host embryos.
4. After transplantation, the donor and host embryos are removed from the wells, separated and gently placed on an agar coated petri dish filled with antibiotic EM. Leaving embryos in the transplant wells increases mortality. Incubate embryos at 28.5 °C.

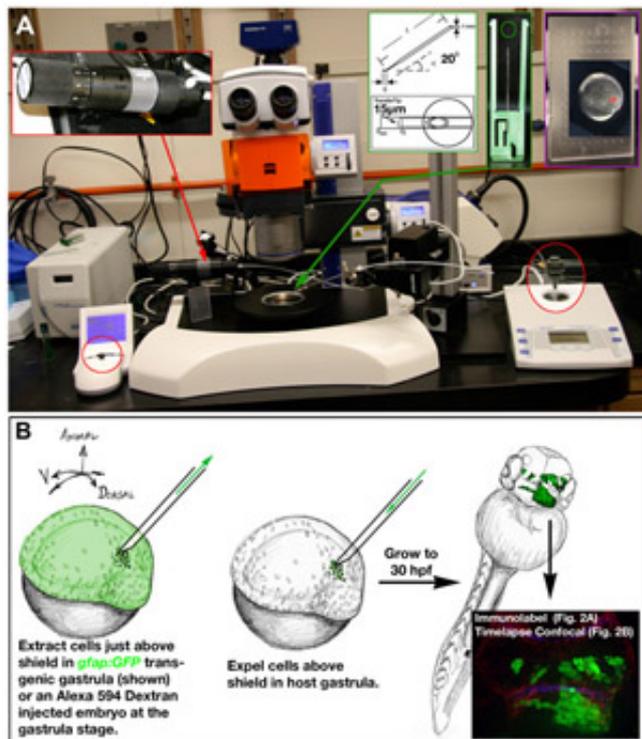
2.4 Visualizing and Imaging Embryos

1. *Mounting fixed and live embryos.*
 1. Host embryos derived from transgenic GFP or Rhodamine injected embryos can be visualized and imaged alive or as fixed embryos. We present here examples of both fixed and live imaging options. Fix embryos were sacrificed at 30hpf using 4% paraformaldehyde in 0.1M phosphate buffer (PB) overnight at 4 C⁻¹.
 2. Rinse embryos 2x and then wash 3 x 5 minutes in 0.1M PB.
 3. In the example we provide, fixed hosts were immunolabeled for all axons with antibodies directed against Acetylated Tubulin (α -AT) as described previously⁶.
 4. Place embryos in 75% glycerol, sink at room temperature and then store at 4 °C.
 5. Prepare a slide to mount two embryos. Create two petroleum jelly square outlines on a slide that match the inside shape and diameter of a square coverslip.
 6. Deyolk an embryo and for a frontal view of the forebrain, dissect off the forebrain by cutting perpendicularly across the midbrain. Place this tissue with a minimal amount of glycerol within the petroleum well. Orient the tissue into the appropriate position and place a coverslip over the specimen.
 7. Live specimen were mounted in a 0.75% agarose solution made up in a 4% solution of Tricaine (in EM) onto glass bottom culture dishes. Prior to the agarose solidifying, the embryo is manipulated with a tungsten needle to correctly orient it for imaging. For our analysis we oriented the forebrain directly against the coverslip.
2. *Imaging*
 1. Both fixed and live hosts were imaged using the Leica SP5 Laser Scanning Confocal Microscope. Z-Stacks were acquired and 3- or 4-dimensional image processing was completed using *Volocity* software.

Results:

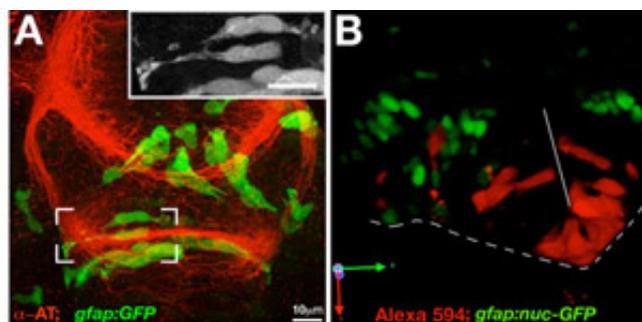
In an effort to address the role of astroglial cells in the forebrain it is necessary to to both visualize these cells and target different genetic manipulations to small clusters of diencephalic and telencephalic cells. To generate these types of chimeric embryos, in which some portion of the astroglial population within the embryo is different whether by genotype or phenotype, we used a multifaceted approach that combines the use of GFP transgenic embryos, which labels astroglia, with the use of gastrula-staged cell transplantation. To specifically target our clones to the diencephalon, we utilized the zebrafish gastrula fate map to selectively extract cells at the midline equidistant from the animal pole and the shield⁵ (Fig. 1B). These extracted *tg[gfap:gfp]* cells were then transplanted into the same location in a wild type host gastrula, which was then raised to 30hpf, and immunolabeled for all axons (α -Acetylated Tubulin) as reference landmarks for forebrain anatomy. As an example shown here, confocal imaging of one host embryo reveals isolated GFP+ cells throughout the telencephalon and diencephalon (Fig. 2A). The full morphology of these GFP+ cells can be observed in this clonal assay, revealing that most of these cells take on the characteristic radial glial morphology, where the soma is located adjacent to the ventricular zone and a large end foot terminates a radial process at the pial surface (Fig. 2A, inset). Three-dimensional rendering of the Z-stack of these collected optical slices clearly showed the position of these cells with respect to the labeled axons (Movie 1).

Another approach commonly used for visualizing transplanted cells is to initially microinject a fluorescent cell lineage dye, such as Alexa 594, into the yolk of a one-cell staged wild type or GFP transgenic embryo. As an example we injected Alexa 594 into wild type embryos at the one-cell stage. We allowed them to develop to shield-stage gastrula and then carried out forebrain targeted transplantation as mentioned above, but instead we transplanted into *tg[gfap:nuc-gfp]* transgenic host gastrula. Imaging of the dorsal telencephalon by Laser Scanning Confocal microscopy revealed fluorescent red clusters of donor cells amongst GFP+ nuclei within the forebrain (Fig. 2B). We further analyzed this host by collecting Z-stacks every three minutes over the course of two hour with the Laser Scanning Confocal. 4-dimensional rendering of this timelapse using *Volocity* software (Improvisation) shows dynamic cellular movements of the rhodamine cell membranes and the GFP+ nuclei (Movie 2).



[Click her to see a larger version of figure 1.](#)

Figure 1: Transplant apparatus and schematic of experimental methods. A) Apparatus used to conduct gastrula-stage transplants. The Zeiss Lumar fluorescent stereo microscope was used to conduct these transplants. It has joystick controlled focus and zoom (left red circle). The Transferman NK was used to manipulate the position of the capillary which is also joystick controlled (right red circle). During setup 60-80 embryos are placed into individual agar wells previously made in a 100mm petri dish with a plastic mold (purple outlined box). The transplant capillary (*TransferTip*) manufactured by eppendorf has a 15µm inner diameter opening and a 20 degree angled tip (green outlined box). The aspiration of cells is controlled with Eppendorf's *AirTram* (red outlined box). B) Illustration of gastrula stage transplantation procedure from donor into host embryos. Cells are extracted from *tg[*gfap:GFP*]* embryos (shown here) or Alexa 594 injected (as described in protocol) donor embryos. Cells are removed from the midline halfway between the shield and animal pole, and transplanted into the same region of host embryos. Hosts are fixed and imaged using Laser Scanning Confocal Microscopy.



[Please click here to see a larger version of figure 2.](#)

Figure 2. Example of gastrula stage transplants within the zebrafish forebrain. A) A frontal view of wild type host embryos with transplanted *tg[*gfap:gfp*]* cells in the diencephalons and telencephalon of the zebrafish forebrain. Axons are labeled in red (α -Acetylated Tubulin antibody) and transplanted cells are labeled in green (endogenous GFP expression). Embryos are 30 hpf. Inset shows radial glial morphology of GFP+ cells. B) Dorsal view of the telencephalon of a live *tg[*gfap:nuc-GFP*]* host embryo with Rhodamine fluorescing transplanted cells (red). Nuclei of host embryos are labeled in green (GFP). Dashed line outlines the anterior surface of the forebrain. Solid line indicates the ventricular zone. Scale bar is 10µm.

Movie 1. The relationship of ectopically labeled radial glial cells amongst forebrain commissures. Cells from a gastrula staged *tg[*gfap:GFP*]* embryo were transplanted into a non-GFP transgenic wild type line. A Laser Scanning Confocal Z-Stack was collected and then processed for 3-D rendering using *Volocity* software. Initially, the image is a frontal view of the zebrafish forebrain at 30hpf, and it has been flipped horizontally as compared to Figure 2B. Axons (α -Acetylated Tubulin, red) within the anterior commissure (AC, top) and post-optic commissure (POC, bottom) are seen. The green cells are the GFP+ transplanted cells that took root in the forebrain and generated clear radial glial morphology. As the movie progresses it focuses in on two radial glial cells possessing end feet that contact the POC. [Click here to Download Movie 1.](#)

Movie 2. Timelapse imaging of transplanted Alexa 594 Labeled Cells in the forebrain. Cells from a gastrula previously injected with Alexa 594 were transplanted into *tg[gfap:nuc-gfp]* transgenic embryos. This movie represents a 3-D projection of a 2h timeseries, in which Z-stacks were taken every 5 minutes. Z-Stacks were collected on a laser scanning confocal microscope, and 4-D renderings completed using *Volocity* software. Transplanted cells are labeled with Alexa 594 (red), and GFP+ nuclei are green. As this timelapse runs, the 4D image starts with a dorsal view of the forebrain at 30hpf and will rotate about the X-axis, as clear movement is detected in the cell membranes of all transplanted cells and in the GFP+ nuclei as well. [Click here to Download Movie 2.](#)

Discussion

Generating chimeric embryos is a powerful tool that addresses many research questions within several model systems, namely the fruit fly (*D.melanogaster*), worm (*C.elegans*), zebrafish (*D.erio*) and mouse (*M.musculus*). We argue that the zebrafish model system is uniquely suited for generating chimeras in a relatively easy, fast and versatile way. The zebrafish is a vertebrate that develops outside the mother making it significantly more accessible as compared to the mouse model. Zebrafish are also significantly larger than flies or worms, which simplifies embryological manipulations such as cell transplantation. In addition, the ability to combine cell transplantation procedures with transgenic techniques enables a large array of possible experiments in which gene function can be manipulated in a localized tissue-specific manner. For instance, small clones of GFP+ or Rhodamine labeled cells enable the characterization of full cell morphologies that are often lost in a homozygous transgenic donor or are impossible to visualize due to differential subcellular labeling with common antibodies. Furthermore, by using transgenically tagged or fluorescently filled cells, we can track donor cells in the live zebrafish embryo over time. Timelapse imaging of individual cells provides a novel analysis of cellular behavior in the context of the whole organism.

Our lab also combines the use of heat shock inducible transgenic lines with cell transplantations such that certain axon guidance cues can be misexpressed in donor cells following an elevation in incubation temperature (data not show). This technique is an extremely powerful and direct approach to locally misexpress a protein of interest in a spatial and temporal-specific manner, which allows us to see how the specific protein affects development. In our case, this technique can extend our knowledge behind the function of Slit-Robo guidance cues in the positioning of commissures and glial cells at the midline⁵.

Other approaches to locally misexpressing genes such as focal UV uncaging and localized heatshock tools (laser or soldering iron), do offer alternative ways to manipulate the expression of genes and markers in a small cluster of cells^{7,9}; however, cell transplantation establishes a final environment of analysis that is free of any trauma induced by UV, laser or heat making cell transplantation a more controlled experimental approach. In conclusion, we have found cell transplantation procedures to be a crucial part of our neurodevelopmental biology research. Generating clones of cells with different properties is a critical tool for the developmental biologist in addressing fundamental questions of cell behaviors, gene function, and cell autonomy.

Disclosures

The authors have nothing to disclose.

Acknowledgements

We would like to thank members of the Barresi Lab for their support and helpful comments on this manuscript. We thank Alexander Workman for his constant technical assistance as well as the animal care staff for their help in maintaining the Smith College Zebrafish colony. We also thank Mike Hallacy, Rudi Rottenfusser, and Carl Zeiss Microimaging for lending some microscopy equipment for the film of this protocol. This work was supported by an N.S.F. funded research grant, 0615594.

References

1. Westerfield, M., *The Zebrafish Book, A guide for the laboratory use of zebrafish (Danio rerio)*. 5th ed. (University of Oregon Press, Eugene, 2007).
2. Nüsslein-Volhard, C. and Dahm, R. ed., *Zebrafish, A Practical Approach*. (Oxford University Press, 2002).
3. Graeden, E. and Sive, H., Live imaging of the zebrafish embryonic brain by confocal microscopy. *J Vis Exp* (26) (2009).
4. Gilmour, D. T., Jessen, J.R., Lin, S., in *Zebrafish, a practical approach.*, edited by C. and Dahm Nusslein-Volhard, R. (Oxford University Press, 2002), Vol. 1, pp. 121.
5. Woo, K., Shih, J., and Fraser, S. E., Fate maps of the zebrafish embryo. *Curr Opin Genet Dev* 5 (4), 439 (1995).
6. Barresi, M. J., Hutson, L. D., Chien, C. B., and Karlstrom, R. O., Hedgehog regulated Slit expression determines commissure and glial cell position in the zebrafish forebrain. *Development* 132 (16), 3643 (2005).
7. Kozlowski, D. J., Murakami, T., Ho, R. K., and Weinberg, E. S., Regional cell movement and tissue patterning in the zebrafish embryo revealed by fate mapping with caged fluorescein. *Biochem Cell Biol* 75 (5), 551 (1997);
8. Halloran, M. C. et al., Laser-induced gene expression in specific cells of transgenic zebrafish. *Development* 127 (9), 1953 (2000);
9. Hardy, M. E., Ross, L. V., and Chien, C. B., Focal gene misexpression in zebrafish embryos induced by local heat shock using a modified soldering iron. *Dev Dyn* 236 (11), 3071 (2007).