Brain Ventricle Injection Protocol

Materials Needed:
Tricaine
Agarose coated petri dish
Yellow or white pipetteperson tips
Pulled capillary needle with fiber (used for injections)
Rhodamine-dextran, or other similar dye
Hairloop
Fine watchmaker's forceps

Place dechorinated embryos in agarose coated petri dish filled with E3 and just enough tricaine solution to anesthetize embryos and prevent movement. Use pipette tip to make wells in agarose bottom. Use hairloop to orient embryos in wells, tails into wells, so that anterior brain is to the left, posterior brain to the right. Place pulled capillary needle filled with dye into micromanipulator holder. Break off tip with forceps. Line up the needle pointing to the left, parallel with the brain. Inject needle into hindbrain ventricle, being careful not to go through the brain and into the yolk, if your purpose requires injecting into the ventricle region only. It is easiest to penetrate into the hindbrain ventricle, as the dorsal hindbrain tissue is extremely thin. The most difficult part is puncturing the epidermis, so take care once you puncture through that you pull the needle back or move the embryo dish to the left to compensate. Inject dye with pressure.

Image embryo with both light and fluorescent microscopy. To superimpose images: Open both images in Photoshop. In fluorescent image: 1) adjust red color if necessary (Image: Adjustments: Replace Color, or Image: Adjustments: Levels), 2) replace black color with white, 3) select all, copy, 4) paste onto light microscopy image, 5) in layer window. select "multiply."