embryo injection through the chorion Aaron Putzke

1) The night before set up fish to squeeze. It's easier to inject more embryos when they are synchronized, and since you want to inject as early as possible, you can load the wells of the injection dish as they are swelling. They should all be injected before the first cell division to maximize the chance of integration into the genome (for stable transgenic lines).

2) Have two injection plates on hand. I prefer injection molds with the square-bottom wells. As the chorion swells the embryo gets wedged into the wells and won't move. With the beveled-edge molds the embryos can move around as you try to inject, which slows down the process and also makes it a bit harder to get the needle to go into the chorion.

3) BAC DNA: I inject at 100 ng/μL. Higher than that gets really sticky and I've found that above or below that concentration decreases the number of positively labeled embryos on the following day. With BAC DNA just use a few μL. Throw out what you don't use from that. The BAC DNA stock should be stored (long-term) in small aliquots (10μL) at -80°C. Once thawed, short-term it is good for about 6 months at 4°C. Do not re-freeze BAC DNA (it's too big and degrades too easily in the process).

4) For needles you can go with shorter, slightly wider (tend to go through the chorion more easily) which is the following settings on the needle puller: heat 735, pull 90, velocity 50, and time 120 (just use the non-labeled program for changing these settings - don't change any of the set programs). This may need to be adjusted if a new filament is put in the puller. However, I have since found that using needles pulled with program 9 (Moens) work very well and tend to clog less. They are more flexible, but for me, are more successful. Try both and use the settings that work best for you. Break and test the needles under the highest magnification. Test each needle in oil, once broken, to make sure that approximately 1nl of DNA (~5 ticks of the reticle gives a bolus that is ~1nL in volume) is injected each time the pedal is pressed.

5) After fertilizing a clutch of eggs, once the chorions begin to swell, load them carefully into the wells. Ideally there should be no, or very little, space in between them so that the amount of movement while injecting them is minimized.

6) I inject at the 1.6 magnification setting. Introduce the needle so that it is touching the chorion and just making an indent but is not piercing the chorion. Lightly finger-tap the knob controlling the advance/withdrawal of the needle. This should pop the needle through the chorion. Advancing the needle by turning the knob often just squishes the embryo. Next, use the same procedure to pierce the embryo. I try to orient the embryo so that the needle is coming up into the cytoplasm from just below the yolk/cytoplasm interface (see below). Embryos seem to survive more often when injected this way, as opposed to going straight into the cytoplasm from the top. It may help to use a very small amount of phenol red mixed with the DNA to see where the DNA is injected (0.1μL in 6μL of DNA). Withdraw the needle slowly due to the fact that the sticky BAC DNA will stick to the needle and pull out of the embryo if you go too fast.