Fluorescent Embryo staining protocol

1. Fix embryos according to standard methods (i.e., dechorionating in 50% bleach, fixing in PFA-heptane, MeOH popping, store at -20 degrees)
2. When ready to perform staining, remove embryos from the freezer and transfer to an eppendorf tube (I use the 0.6 mL tubes for staining- it helps to cut down on the amount of antibody used).
3. Rinse embryos at least 3x in PBT-BSA, allowing them to settle each time.
4. Rock embryos 30' (or longer) in PBT-BSA at RT.
5. Add primary antibody, diluted in PBT-BSA (for anti-HA I used anti-rat 1:500)
6. Rock embryos overnight in primary antibody dilution at 4 degrees
7. The next day, remove and save the primary antibody for later use (anti-HA can be used a total of 2x)
8. Rinse embryos 3x in PBT-BSA
9. Wash embryos 3x for 10' in PBT-BSA
10. Add secondary antibody, diluted in PBT-BSA. For fluorescent stains, dilute the antibodies from the freezer 1:400 in PBT-BSA. From this point forward, keep the tubes in the dark, either covering with foil or using a black box
11. Rock embryos in the dark at RT for at least one hour
12. Rinse embryos 3x in PBT
13. Wash embryos 3x for 10' in PBT
14. Following the last wash, remove as much PBT as possible. Add three drops of Vectashield to the embryos in the tube. They will float to the top of the Vectashield
15. Let embryos stand in the dark for at least an hour at RT, or until they have sunk in the Vectashield (I usually let them sit overnight at 4 degrees in the Vectashield, they should sink by then)
16. Transfer the embryos+Vectasheild mixture to a bridged slide using a P1000 tip. For bridged slides, I create a slide with a bridge two Scotch tape layers thick.
17. Cover with a 1.5 coverslip (in case you want to do confocal work on these), and keep in a slide case until ready for viewing.