Embryo antibody staining with amplification of the Cut staining

- Collect eggs
- Dechorionate eggs in bleach 2-4min
- Wash with distilled water
- Fix in 4% FA + methanol 20min
- Remove the formaldehyde solution and wash with methanol 3 times
- Keep at –20°C in methanol if necessary.

Coloration

- Incubate with primary antibodies ON at 4°C or 1h at RT (mouse anti-Cut, DSHB, 1/1000, plus other primary antibodies)
- 3 washes 20min each in PBT
- Incubate with secondary antibodies (anti-mouse-biotin, 1/1000, plus other secondary antibodies) for 1h
- 3 washes 20min each in PBT. Meanwhile, 30min before the next step, prepare the streptavidine-HRP solution by mixing solutions A+B at 1/100 dilution each in PBT (Vectastain, kit ABC)
- Incubate with streptavidine-HRP for 1h (500uL in each tube)
- 3 washes 10min each in PBT. Remove as much PBT as you can after the last wash
- Incubate with the TSA-biotin solution (NEN, 4uL TSA-biotin in 200uL of amplification buffer) for the HRP reaction. Usually for 10min (try also 8-14min to get various staining intensities). Tubes should also be rotating during this step.
- Quick wash in PBT
- 3 washes 10min each in PBT
- Incubate with streptavidine-Alexa568 or streptavidine-Alexa488 (Molecular Probes, 1/1000) for 30min
- 3 washes 10min each in PBT

Mounting

- Remove as much PBT as you can
- Put 55uL of Vectashield mounting medium in the tube and aspirate the embryos as they come up with a P200 pipette tip that has been cut at its tip.
- put the liquid on a slide, cover with coverslip