Zebrafish spinal canal cilia staining **Mia Konjikusic**

- Euthanize embryos in high-dose Tricaine and fix in 4% sweet PFA (4% PFA, 4% sucrose in PBS) overnight at 4 °C.
- Wash embryos in PBSTr (1x PBS with 0.1% Triton X-100), 3x and distilled water 2x 5 min. each
- Incubate embryos in acetone for 7 min at −20 °C
- Wash again in distilled water 2x and in PBSTr 3x 5 min each
- Incubate in PBD blocking solution (1% BSA, 2% DMSO, 2% normal goat serum in PBST) for 1 hour
- Incubate embryos with a mouse GT335 glutamylated tubulin antibody primary antibody (1:500) diluted with PBD overnight at 4 °C
- Wash in PBD for 2x 10 minutes each then washed in PBSTr 3x 10 minutes each
- Add secondary antibodies (1:1000, diluted with PBD) overnight at 4 °C.
- Wash in PBD for 2x 10 minutes each then washed in PBSTr 5x 10 minutes each
- Mount embryos in low melt agar and image

Never remove all the liquid during washes, keep embryos fully submerged. Washes and antibody staining can go longer but extended times can lead to deterioration of the tissues.