HCR protocol whole mount 5/20/21

Modified from Choi et al, 2018

Day 0:

Fix samples in 4% PFA overnight at 4C and then wash and store in 100% Methanol in the freezer until use

Day 1:

1. Rehydrate embryos in MeOH/PBST (0.1% Tween) for 5 minutes each at RT: 75% MeOH, 50% MeOH, 25% MeOH, 5x PBST
2. Permeabilize the embryos/larvae with 30 ug/ml ProtK for 45 min at RT for 5 dpf larvae (1.5 ul of 20 mg/ml ProtK into 1 ml of PBST)
    - Try a range of permeabilization times to optimize for your stage/your probe
3. Rinse quickly with PBST 2 x and postfix in 4% PFA for 20 minutes
4. Rinse with PBST (X 5) to remove fix
5. Transfer embryos to single PCR tubes and add 125 ul of probe hybridization buffer at 37C for at least 30 minutes rocking or rotating
6. Prepare probe solution (0.5 ul of 2 uM stock HCR probe into 125 ul of probe buffer or 2 ul of our IDT stock into 150 ul)
7. Incubate samples at 37C overnight or longer shaking/rotating

Day 2:

1. Wash larvae 4x 15 min with probe wash buffer at 37C
2. Wash larvae 2x for 5 min with 5x SSCT at RT
3. Add 125 ul of amplification buffer for at least 30 min at RT
4. Prepare hairpins separatedly (1 ul of h1 and 1 ul of h2 per 100 ul of amplification buffer at 95C for 90 s then snap cooled in dark drawer at RT for 30 min)
5. Add snap cooled hairpins to RT amplification buffer and add to the embryos and incubate rocking overnight at RT

Day 3:

1. Wash larvae 2x 5 min with 5x SSCT at RT
2. Wash larvae 2x for 30 min with 5x SSCT
3. Store samples at 4C in dark before microscopy