HCR v3 (Choi et al., 2018)

Pre-gaming: Fix samples in fresh 4% PFA in PBS overnight at 4C. Next day, rinse a few times in PBS, and directly embed them in Tissue Tek OCT or NEG-50 and freeze blocks to be stored at -80C (can last for months). Cryosection at desired thickness (usually 14-20 um for zebrafish samples) onto superfrost plus slides and allow slides to air dry in slide box at RT for a few hours to overnight before storage at -80C.

Day 1:

1) Air dry slides for at least 30 minutes
2) Fix tissue in fresh 4% PFA in PBS for 10 to 15 minutes
3) ProtK in PBS (1 ug/ml: 1 ul into 20 ml) for 5 to 10 minutes depending on tissue thickness. I do 5 minutes for 14 um thick sections
4) Rinse in PBS 2x
5) Refix in PFA for 5 minutes at RT
6) Ethanol dehydration 5 minutes each: 50%, 70%, 100%, 100% Ethanol
7) Let slides air dry for 5 minutes at RT
8) Put slides in probe hybridization buffer at 37C for at least 10 minutes
9) Prepare probe solution (0.4 ul of 2 uM stock HCR probe into 100 ul of probe buffer) (can get away with half the amount of probe)
10) Add probe solution to slides and cover in parafilm. Incubate slides at 37C O/N in a moist chamber

Day 2:

1) Remove excess probe by washing 4 x 5 minutes with wash buffer at 37C
2) Wash samples 2 x 5 minutes with 5X SSCT (0.1% Tween)
3) Put samples in amplification buffer for 30 minutes at RT
4) Prepare hairpins separately - 3 ul of 3 uM stock
    - heat at 95C for 90 seconds and cool to RT in a dark drawer for 30 minutes
5) Add snap cooled hairpins to 300 ul of RT amplification buffer
6) Remove amplification buffer and add hairpin solution. Cover with parafilm and leave in the dark at RT overnight

Day 3:

1) Wash slides in 2 x 5 min in 5x SSCT at RT
2) Wash slides 2 x 5 min in 5x SSC at RT
3) Dry slide and add antifade mounting reagent and cover slip