Whole Mount smFISH with HCR Protocol

Optimized for *Aurelia* jellyfish, modified from Choi *et al.* (2014) ACS Nano, 8(5): 4284–4294
Last Updated 7/25/17
Document created by David Gold (www.DavidAdlerGold.com)

Antisense probe(s)	Sense probe(s)

Fixation:

☐ 1. Anesthetize animals in 7.3% MgCl2

☐ 2. Fix animals in 4% formaldehyde for 1 h @ room temperature (RT).

Acclimate from 7.3% MgCl2 to MeOH using 5 minute washes:

☐ 3. 25% MeOH
☐ 4. 50% MeOH
☐ 5. 75% MeOH
☐ 6. 100% MeOH

☐ 7. Store animals at -10°C

Rehydration:

5 minute washes at room temperature:

☐ 8. 60% MeOH 40% PBSTr
☐ 9. 30% MeOH 70% PBSTr
☐ 10. 100% PBSTr

11. Wash 3x in PBSTr over a 2-hour period at room temperature (RT): (1) ☐ (2) ☐ (3) ☐

Two-stage multiplexed in situ hybridization using DNA HCR Detection stage:

☐ 12. Pre-hybridize with 350 µL of probe hybridization buffer for 30 min at 45°C.

☐ 13. Prepare probe solution by adding 1 pmol of each probe (1 µL of 1 µM stock per probe) to 500 µL of probe hybridization buffer at 45°C.

☐ 14. Remove the pre-hybridization solution and add the probe solution.

☐ 15. Incubate the embryos overnight (12–16 h) at 45°C.

Remove excess probes by washing at 45 ◦C with 500 *µ*L:

- *Wash solutions should be pre-heated to 45 ◦C before use.*

☐ 16. 75% of probe wash buffer / 25% 5x SSCT for 15 min
☐ 17. 50% of probe wash buffer / 50% 5x SSCT for 15 min
☐ 18. 25% of probe wash buffer / 75% 5x SSCT for 15 min
☐ 19. 100% 5x SSCT for 15 min
☐ 20. 100% 5x SSCT for 30 min.

Amplification stage

☐ 21. Pre-amplify embryos with 350 µL of amplification buffer for 30 min at RT.

☐ 22. Prepare 30 pmol of each fluorescently labeled hairpin by snap cooling in 10 µL of 5x SSC buffer (heat at 95 ◦C for 90 seconds and cool to room temperature on the benchtop for 30 min).

☐ 23. Prepare hairpin solution by adding all snap-cooled hairpins to 500 µL of amplification buffer at room temperature.

☐ 24. Remove the pre-amplification solution and add the hairpin solution.

☐ 25. Incubate the embryos overnight (12–16 h) at room temperature.

Remove excess hairpins by washing with 500 µL of 5x SSCT at RT:
26. 2 x 5 min: (1) ☐ (2) ☐
27. 2 x 30 min: (1) ☐ (2) ☐
28. 1 x 5 min

RECIPIES (Make buffers fresh every 2-3 weeks)

PBSTr (50mL)
150 µl Triton X-100 in 50mL PBS

Probe hybridization buffer	**For 40 mL of solution**
50% formamide	20 mL formamide
5x sodium chloride sodium citrate (SSC)	10 mL of 20x SSC
9 mM citric acid (pH 6.0)	360 µL 1 M citric acid, pH 6.0
0.1% Tween 20	400 µL of 10% Tween 20
50 µg/mL heparin	200 µL of 10 mg/mL heparin
1x Denhardt's solution	800 µL of 50x Denhardt's solution
10% dextran sulfate	8 mL of 50% dextran sulfate
Fill up to 40 mL with ultrapure H2O	

Probe wash buffer	**For 40 mL of solution**
50% formamide	20 mL formamide
5x sodium chloride sodium citrate (SSC)	10 mL of 20x SSC
9 mM citric acid (pH 6.0)	360 µL 1 M citric acid, pH 6.0
0.1% Tween 20	400 µL of 10% Tween 20
50 µg/mL heparin	200 µL of 10 mg/mL heparin
Fill up to 40 mL with ultrapure H2O	

Amplification buffer	**For 40 mL of solution**
5x sodium chloride sodium citrate (SSC)	10 mL of 20x SSC
0.1% Tween 20	400 µL of 10% Tween 20
10% dextran sulfate	8 mL of 50% dextran sulfate
Fill up to 40 mL with ultrapure H2O	

5x SSCT	**For 40 mL of solution**
5x sodium chloride sodium citrate (SSC)	10 mL of 20x SSC
0.1% Tween 20	400 µL of 10% Tween 20
Fill up to 40 mL with ultrapure H2O	

50% dextran sulfate	**For 40 mL of solution**
50% dextran sulfate	20 g of dextran sulfate powder
Fill up to 40 mL with ultrapure H2O	