in situ hybridization performed on the peri-rhopalial tissue of a scyphozoan jellyfish

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Bouchard C, Boudko DY, Jiang RHY (2019) A SLC6 transporter cloned from the lion's mane jellyfish (Cnidaria, Scyphozoa) is expressed in neurons. PLoS ONE 14(6): e0218806. doi: 10.1371/journal.pone.0218806

1 Fix tissue 2 h, RT, in freshly prepared 4% PF in 0.1 M PB (pH 9.5) + 0.42M NaCl + 2 mM MgSO4

1.1 Phosphate Buffer pH 9-9.5: made from phosphate dibasic (No pH adjustment required)

1.2 PBS: 100 mM pH 7.5, 150 mM NaCl

2 Wash 3X 5' in PBST, pH 7.5

2.1 PBST: PBS +0.1% Tween

3 Dehydration in PBS/methanol 3:1 → 1:1 → 1:3 10 min each then 100% methanol 5 min to ON in -20C.

4 Rehydration in PBS/methanol 1:3 → 1:1 → 3:1 10 min each then PBST, 10 min.

5 Heat treatment in 1 ml PBST, 5 min. Bain-marie at 95C with stopper. First 2 min, tissue should be resuspended vigorously every 20 sec, every 60 sec during the rest of incubation in order to prevent glueing of specimens. Snap-cool tubes on ice.

6 Wash 2X in PBST

7 Incubation 5 min in TEAA

7.1 TEA: 0.1 M triethanolamine. Dissolve powder in water. Adjust pH at 7.8 with 5 N NaOH.
TEAA: 5 ml TEA + 12, 5 ul glacial acetic acid. Acetic acid must be added to TEA just before the incubation with tissues.

8 Wash 2X 10 min in PBST

9 Post-fixation in 4% PF in PBST pH 7.5 for 20 min.

10 Wash 3X in PBST, 10 min each

11 Saturation of unspecific binding with 75 ul PBST/HS-DNA (0,5 mg/ml) for 10 min

11.1 PBST/fish sperm: 0, 5 mg/ml fish sperm DNA in PBST. This DNA was made from herring and cod sperm and has a molecular weight distribution of 150-3000 bases.

12 Addition of the same amount of HybMix, incubate 5 min

12.1 HybMix:
50% formamide
5 x SSC
1mg/ml HS-DNA
0, 1% Tween 20

13 Prehybridization in 150 ul HybMix for 10 min RT

14 Prehybridization in 150 ul HybMix for 2h at 45C

15 Hybridization in 1 ml HybMix + DIG-probes sense or antisense for 1 day and 2 nights at 40C. Before use, denature probe in HybMix at 70C for 10 min and snap-cool on ice.

16 2 wash in 1 ml of Wash I 60 min at 45C

16.1 Wash I:
50% formamide
2 x SSC
0, 1 % Tween 20

17 4 wash in 1 ml of Wash I, 15 min at 45C

18 Wash in PBST 20 min.

19 Saturation in PBST/10% NGS, 2h in fridge with shaking

19.1 Blocking solution: 10 % normal goat serum in PBST

20 Incubation in anti-DIG-AP-conjugated antibody diluted 1:2000 in PBST containing 1 % NGS/ 4C over the weekend with shaking. (preadsorption was not necessary).

21 Wash 4X in PBST for 20 min.

22 Incubation 3X 10min in AP-Buffer (containing 10mM MgCl2)

22.1

22.2

23 Substrate reaction with NBT/BCIP solution

24 Stop reaction with PBST/50 mM EDTA. 3X 5min

24.1

24.2

24.3

24.4

24.5

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26

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27.1