RNA Scope on neonatal mouse cochlear tissue

generally a good idea to fill some of the wells around your sample wells with MQ H2O so that the 96 well plate acts as its own humidifying chamber

Amplification and detection (Day 2 cont'd)

Reagents Needed: RNAscope 2.5 HD Detection Reagents – RED (Cat# 322360)
Vector Labs Low pH antigen unmasking solution (H-3300)

NOTE: again it is critical that the tissue be completely covered by all of the solutions for all of the steps.
NOTE: we have shortened the washes for the protocol here at BIE, but in general if you increase the time and number of washes, you can get better signal to noise

coverslip. **DO NOT** expose the tissue to EtOH or any other organic solvents, or mounting media containing ethanol or other organics as this will dissolve the Fast RED. **NOTE:** If signal is faint, you may be able to increase it by lengthening the AMP5 step. AMP6 may also be lengthened to 15 min.

Immunofluorescence

NOTE: If performing immunofluorescence subsequent to Fast RED reaction, it is important to note that the Fast Red has an emission spectra that extends into the far-red channel and so any antigens you wish to detect with Alexa-647 antibodies (or similar) will have to have a good signal to noise ratio as the Fast Red can cause bleedthough in this channel. If you are attempting to show co-localization, it would be best for the protein of interest to be labeled in the blue or green channels.

Wash tissue 2X in PBS.

Apply your primary antibodies in 0.05% TritonX in PBS and incubate for 15hrs (i.e. overnight) at 4°C. Wash 3X in PBS. **NOTE:** Lina Jensen in Alan Cheng's lab recommends not using blocking buffer as it seems to worsen rather than improve immunostaining after RNAscope. We have found that immunostaining with all of our antibodies so far works very well without performing a blocking step.

Apply secondary antibodies in 0.1% TritonX in PBS and incubate at RT in the dark for 2h.

Wash 3X in PBS.

Optional: Incubate in Hoechst at 1:1500 for 25 min. Then wash 3 x 5 in in PBS

Mount coverslips on slides using 50% glycerol in PBS (or other aqueous mounting media) and seal the edges with clear nail polish. NOTE: If you used the FastRED, do not use mounting media that contains EtOH or other organic solvents as it will dissolve the FastRED.

NOTES:

GFP/EGFP/tdTomato are mostly quenched by the in situ procedure so endogenous fluorophores can only be simultaneously detected via immunostaining. Abcam's anti-GFP (rabbit, IP grade, or the chicken anti-GFP) and rabbit anti-mCherry are excellent antibodies for this.

It is important to use the low pH antigen unmasking solution instead of the kit wash buffer as the kit wash buffer contains LDS detergent and may disintegrate the tissue or we often find cause it to stop sticking to the slide if you are doing sections. If you are not doing mouse cochlea, but have a thicker tissue with more ECM, you may want to use the kit wash buffer instead.

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