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RNAscope spatial transcript imaging in fresh-frozen mouse and human liver tissue

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We captured transcript distributions of select liver cell marker genes via in situ hybridization of specific targeting probes with the RNAscope Multiplex Fluorescent v2 Assay Protocol optimized for fresh-frozen samples. Our modifications to the commercial protocol included using half-concentration wash buffer (0.5X) for all wash steps downstream of probe incubation, and excluding the recommended protease step entirely. In mouse liver tissue, we spatially detected the transcripts for, ALB (Albumin), GLUL (Glutamine synthetase), and PTPRC (Protein Tyrosine Phosphatase Receptor Type C), and in human liver tissue, we spatially detected transcripts for GLUL, CD68 (Macrophage Antigen CD68), and LYVE1 (Lymphatic Vessel Endothelial Hyaluronan Receptor 1).

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RNAscope, liver, hubmap, mouse liver, human liver, transcriptomics, spatial FISH

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RNAscope® Multiplex Fluorescent Reagent Kit v2 Assay
RNAscope® Probe-Mm-Alb-C2 (ACD;Cat No.Cat No. 437691-C2)
RNAscope Probe-Mm-Glul (ACD;Cat No. 426231)
RNAscope® Probe-Mm-Ptprc-C3 (ACD;Cat No. 318651-C3).
RNAscope® Probe-Hs-GLUL-No-XMm (ACD;Cat No. Cat No. 511171),
RNAscope® Probe-Hs-CD68-C4 (ACD;Cat No. 560591-C4),
RNAscope® Probe-Hs-LYVE1 (ACD;Cat No. 426911).
Opal 520 Reagent (Perkin Elmer, FP1487001KT)
Opal 570Reagent (Perkin Elmer, FP1488001KT)
Opal 690 Reagent (Perkin Elmer,FP1488001KT).
4% paraformaldehyde (PFA)
phosphate buffered saline (PBS)
Ethanol (EtOH)

Tissue pretreatment

1 Tissue sections (8-10 µm thickness) were first post-fixed with 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS) and dehydrated in Ethanol (EtOH) immediately after fixation, immersed for 5 minutes at a time in 50% EtOH, 70% EtOH, 100% EtOH, and 100% EtOH an additional time.

2 Samples were then air-dried and treated with RNAscope® Hydrogen Peroxide Reagent for ten minutes at 23°C to 25°C and washed twice with deionized water. Importantly, we excluded the commercial protease step because tissue integrity was lost, and we could achieve stronger signal without any protease treatment.

RNAscope

3 These pretreated sample slides were incubated with pre-warmed target probes (20 nmol/L of each oligo probe) overnight (18-21 hours) at 40°C inside a HybEZ hybridization oven (ACD).

In mouse tissue, ALB was targeted with RNAscope® Probe-Mm-Alb-C2 (ACD;Cat No.Cat No. 437691-C2), GLUL was targeted with RNAscope Probe-Mm-Glul (ACD;Cat No. 426231), and PTPRC was targeted with RNAscope® Probe-Mm-Ptprc-C3 (ACD;Cat No. 318651-C3).

In human tissue, GLUL was targeted with RNAscope® Probe-Hs-GLUL-No-XMm (ACD;Cat No. Cat No. 511171), CD68 was targeted with RNAscope® Probe-Hs-CD68-C4 (ACD;Cat No. 560591-C4), and LYVE1 was targeted with RNAscope® Probe-Hs-LYVE1 (ACD;Cat No. 426911).

4 After overnight probe hybridization, samples were incubated in Amplifier 1 (preamplifier) (2 nmol/L) in hybridization buffer B (20% formamide, 5× SSC, 0.3% lithium dodecyl sulfate,10% dextran sulfate, blocking reagents) at 40°C for 30 minutes. Followed by two gentle washes in 0.5X Wash Buffer (0.05× SSC, 0.015% lithium dodecyl sulfate), two minutes each at room temperature.

5 Samples were incubated in Amplifier 2 (2 nmol/L) in hybridization buffer at 40°C for 15 minutes. Followed by two gentle washes in 0.5X Wash Buffer (0.05× SSC, 0.015% lithium

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dodecyl sulfate), two minutes each at room temperature.

6 Samples were incubated in Amplifier 3 (label probe) (2 nmol/L) in hybridization buffer C (5× SSC, 0.3% lithium dodecyl sulfate, blocking reagents) at 40°C for 15 minutes. Followed by two gentle washes in 0.5X Wash Buffer (0.05× SSC, 0.015% lithium dodecyl sulfate), two minutes each at room temperature.

7 Chromogenic detection was performed according to the commercial protocol utilizing a horseradish peroxidase (HPR) construct specific to each gene-dedicated imaging channel and a fluorescent Opal reagent of choice.

For the mouse sections, ALB was stained with Opal 520 Reagent (Perkin Elmer, FP1487001KT), GLUL was stained with Opal 570 Reagent (Perkin Elmer, FP1488001KT), and PTPRC was stained with Opal 690 Reagent (Perkin Elmer, FP1488001KT).

For the human sections, GLUL and LYVE1 were both stained with Opal 520 Reagent (Perkin Elmer, FP1487001KT), thus needing to be imaged in separate tissue sections, and CD68 was stained with Opal 570 Reagent (Perkin Elmer, FP1488001KT).

Each Opal reagent dye was diluted 1:1500 in RNAscope® Multiplex TSA Buffer.

8 Nuclei were stained with DAPI (4',6-diamidino-2-phenylindole) and coverslips were mounted over slides in Fluoro-Gel (EMS; 17985-10) and samples imaged by spinning disc confocal microscopy and Aperio Versa 8 fluorescent slide scanner.

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