High abundance transcript staining in mouse gut

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PER Info:
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Hiprfish:
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Materials

- dHTP/dDTP Mix (depending on whether G or C is excluded from the extension sequence)
  - dATP 100mM, NEB, 6ul
  - dTTP 100mM, NEB, 6ul
  - dCTP/dGTP 100mM, NEB, 6ul
  - H2O ultrapure, RNAse and DNAse free, 82ul
- Extension Mix (on ice)
  - ThermoPol Reaction buffer 10x, NEB, 10ul
  - MgSO4 100mM, NEB, 10ul §
  - dHTP/dDTP 6mM each base, 10ul §
  - H2O ultrapure, RNAse and DNAse free, 55ul
- Polymerase
  - Bst DNA polymerase LF 8,000U/ml, NEB, 10ul §
- Oligos
  - Cleaning hairpin 200uM, 2ul (either Clean.G or Clean.C depending on which base is excluded from the extension sequence)
  - Hairpin 200uM, 1ul §
  - HiPR-FISH Probe 200um total, 2ul
- Gel
  - Invitrogen E-gel EX 2%
  - DNA step ladder 25bp, 1ul
- Sectioned sample in paraffin
- Xylene substitute
- 100% EtOH

- 95% EtOH
- 1x PBS
- Oven at 60˚C
- Wash containers: 50ml falcon tubes or slide rack and coplins jars
- Sodium borohydride
    - Danger: produces hydrogen gas when encouters water. If ignites, do not put out with water, use powder. Toxic as well.
- PBS 1x at 4˚C
- Isopropanol 99%
- lysozyme 10mg/ml in Tris-HCL 10mM pH7.5
- biorad frameseal chambers
- probes (200uM)
    - encoding probes with flanking regions
        - 16s rRNA Probes
        - Transcript specific probes
    - branching probes complementary to flanking regions for flanking probes
    - adapter probes complementary to branching probes
    - fluorescent readout probes complimentary to adapter probes
- hybridization buffer (100ul)
    - 20x SSC 10ul
    - Denhardt's solution 10ul
    - Dextran Sulfate 20ul
    - 50%w/v Ethylene Carbonate 20ul
    - 1% SDS 1ul
    - Oligo probes Xul
    - RNase free water 39-Xul
- wash buffer (heated to 48˚C)
    - 5M NaCl 2.15ml
    - 1M Tris-HCl 1ml
    - 0.5M EDTA 0.5ml
- resin
    - invitrogen prolong glass antifade mountant
- cover glass no. 1.5

Procedure

Probe Extension

1. Prep PCR tubes
    1. Add 1ul extension hairpin to PCR tube.
2. Make extension mix
    1. Make dHTP/dDTP mix.
    2. Make extension mix on ice excluding polymerase.
    3. Add Cleaning hairpin.
    4. Add polymerase.
3. Transfer extension mix to PCR tube with hairpin and mix (Final volume 98µl).
    1. Incubate 15min at 37˚C.
4. Add Probe Oligos and mix.
    1. Incubate 60min § at 37˚C.
    2. Deactivate 20min at 80˚C.
    3. Cool to 4˚C.
5. Gel assessment
    1. Dilute 1ul sample 1:4 in H2O.
    2. Load 20ul of the resulting mix to a gel.
    3. Dilute 1ul of original probe solution 1:100,000 in H20 and load 20ul.
    4. Also load 20ul of a 50bp ladder diluted 1:20.
    5. Run Gel on invitrogen module until short sequence band separation on the ladder is clear.
    6. Image the gel. Bands should appear at the same length as the original probe and/or at a few bp longer (hairpin), and at 100-500 bp longer (extended probe). The extended probe is often weak and streaky

Deparaffinization and autofluorescence quenching

1. Place slide in 60˚C oven for 1hr.
2. Wash slide in the following sequence
    1. Xylene 15min x2
    2. 100% EtOH 5min
3. Measure 0.5g Sodium borohydride and add to ~25ml PBS 1x at 4˚C on ice in the fume hood. Top PBS to 50ml and leave cap off or loose in hood.
4. Wash sample in PBS and add to chilled 1% Sodium borohydride solution for 30min.
5. Wash slide with Isopropanol 99% and transfer to PBS 1x at RT to wash. Add Isopropanol to sodium borohydride in waste container. Leave waste cap loose until off-gassing is complete (~1day).

Permeabilization

1. incubate cells/tissue with lysozyme for 1hr at 37˚C

2. Wash in PBS
3. Wash in 100% EtOH.

rRNA FISH and mRNA FISH

1. Incubate slides with encoding probes in hybridization buffer
    1. 12hrs at 46˚C.
    2. Wash in wash buffer for 15min at 48˚C.
    3. Rinse in EtOH and allow to dry.
2. Incubate slides with branching probes in hybridization buffer
    1. 1hr at 46˚C.
    2. Wash in wash buffer for 15min at 48˚C.
    3. Rinse in EtOH and allow to dry.
3. Incubate slides with adapter and readout probes in hybridization buffer
    1. 30min at RT.
    2. Wash in wash buffer for 15min at 48˚C.
    3. Rinse in EtOH and allow to dry.
4. Pipette $15\mu l$ resin onto sample and apply coverglass