Single Molecule FISH on Mouse Tissue Sections

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Tissue preparation

Solutions and Reagents

10X PBS (Ambion #AM9625)

4% formaldehyde or paraformaldehyde in PBS

Cryoprotecting solution:

- 4% formaldehyde/paraformaldehyde
- 30% sucrose
- Made in PBS

OCT compound (LabTek)

70% Ethanol, chilled

0.1% poly-L-Lysine (Sigma #P8920)

All solutions are prepared on nuclease-free water in new plastic flasks or clean/baked glass bottles.

Procedure

- Fix the tissue immediately after dissection in cold 4% formaldehyde/paraformaldehyde in PBS (fixative volume at least 5x the tissue volume). Flush the tissue with the fixative if possible. Incubate at 4ºC for 3 hours with gentle agitation.
- Move the fixed tissue into pre-chilled cryoprotecting solution, incubate overnight at 4ºC with gentle agitation.
- Label plastic molds for frozen blocks, fill them with OCT compound, let chill on ice for a few minutes.
- Quickly rinse the tissue with ice-cold PBS. Place pieces of the tissue into the molds filled with cold OCT, position them as desired, and then freeze quickly by placing the mold dry ice. Wrap frozen blocks in foil and store at -80ºC forever.

- It is possible to use regular adhesive glass slides for tissue section mounting, but we prefer 22x22 mm glass coverslips #1, which can be washed in 6-well plates. To avoid peeling off of the tissue sections during washes perform the following pre-treatment of the coverslips: Wash the coverslips in 2% RBS35 (or similar detergent) for 15 min with sonication, rinse with Millipore water, then wash twice with 100% ethanol for 15 min with sonication, let air dry (can also flame dry but carefully). Coat the clean dry coverslips in poly-L-lysine (dilute 0.1% stock solution 1:10 in nuclease-free water) for 30 min at room temperature. Dry coverslips over-night and use them the next day. Coverslips can be placed in metal racks (EMS #72241-01) in a glass beaker (EMS #70312-23) for all washing, drying and coating steps.
- Cut 5-10 μm frozen tissue sections using your favorite cryotome settings. Typical sections are 6 μm, keeping the temperatures of the sample and the stage around -20ºC.
- Mount the section onto the coated coverslip (it will melt momentarily and adhere to the glass), air dry for ~5 minutes, then fix in 4% formaldehyde in PBS for 15 min at room temperature. Alternatively, place the tissue-mounted coverslips into 6 well plates placed on dry ice with lids closed. After freezing, the coverslips with mounted sections can be stored at -80ºC for a while). To thaw, pour RT (or slightly warmer) 4% formaldehyde in PBS over the frozen coverslips and fix for 15 min on a bench top. If you're using 22x22 mm coverslips, all washes can be done in 6-well plates, solutions poured carefully on a side of a well, not to dislodge sections from the glass. If you are using glass slides, use histology glass wash jars, baked over-night at 200ºC.
- Rinse the coverslips with 1X PBS, then replace with cold 70% ethanol. Incubate at 4ºC for a minimum of 2 hours before proceeding to hybridization. The coverslips can be kept in 70% ethanol in a fridge for days, up to two weeks (could be longer).

Hybridization and washes

Solutions and Reagents

Hybridization buffer: (stored in -20C, prepare 750ul stocks of this; 150 μl needed per slide):

Reagent	Final concentration	Stock	For 10 ml
Dextran sulfate (Sigma #D8906)	10%	Powder	1g

Formamide (Ambion #AM9342) (stored in 4°C, bring to RT before opening)	10%	100% liquid	1 ml
E.coli tRNA, Roche (Sigma #R4251) (stored in -20°C)	1mg/ml	20 mg/ml	500 µl
SSC (Ambion # AM9765)	2X	20X	1 ml
BSA (Ambion #AM2616)	0.02%	5% (50mg/ml)	40µl
Vanadyl-ribonucleoside complex, (NEB S1402S)	2mM	200mM	100µl
Nuclease free Water (Ambion #AM9932)			7.3 ml

Dissolve the Dextran sulfate in water at room temperature with agitation first (it takes about 30 min), then add the rest of the reagents. Freeze in aliquots, store at -20ºC.

Wash buffer (can be stored on bench in RT)

Reagent	Final concentration	Stock	For 500 ml
Formamide	10%	100% liquid	50 ml
SSC(Ambion # AM9765)	2X	20X	50 ml
Nuclease-free Water			400 ml

Concentration of Formamide in Hybridization buffer and Wash Buffer can be increased if GC-content is very high.

Procedure

Optional: Proteinase K treatment: Discard the Ethanol and add 2XSSC, leave for 5-10 minutes. Replace with 2XSSC+proteinase K (1:2,000 to 1:20,000 dilution of the stock which is Ambion #AM2546, 20mg/ml). Leave for 10 minutes. Wash twice with 2XSSC for 5 minutes. Discard and add Wash buffer.

- Rehydrate the sections by replacing the 70% ethanol with the Wash buffer. Replace with a fresh change of the Wash buffer, let stand for 3-5 min.
- Thaw an aliquot of hybridization buffer. Mix well. Add the probe in TE to the buffer, a final probe concentration of 0.1ng/μl (~1:3000 from the stock probes) in the hybridization buffer usually works well, but the optimal concentration should be determined experimentally. Vortex buffer before use.
- If you're using 22x22mm coverslips, pipette 150μl drops of the hybridization mix onto a clean piece of parafilm for each sample (spread the parafilm on the bottom of a 15mm petri dish).
- Take the coverslips from the wash wells with fine forceps, pat a corner and wipe the back with a paper towel, place the coverslip section-down onto the drop of the Hybridization mix.
- Incubate overnight at 30^{o}C. Don't seal the petri dish with parafilm to allow for drying. Protect from light.
- Pipette wash buffer (~50-100 μl) to the edge of the coverslip to peel the parafilm off without damaging the sample. Wash twice in the Wash buffer for 30 min at 30^{o}C. For the second wash add 1:200 of Dapi 10ug/ml (Sigma #D9564) to the wash buffer. Pre-warm buffer+Dapi for 30 min together with samples to increase reaction kinetics.

Mounting and imaging

Solutions and Reagents

20X SSC (Ambion #AM9765)

10% Glucose in Nuclease-free water

1M TRIS (pH 8.0 Ambion #AM9856)

3.7 mg/ml Glucose Oxidase (Sigma G2133-10KU), stored at -20^{o}C

(Dissolve in RNA-FREE sodium acetate in PH 5.2, Amresco 50mM (stock is 3.0M, catalog # E521-100ML) or Ambion, put in 37C for an hour until completely dissolved. Important, after diluting to 50mM make sure PH remained 5.2 and correct if needed, make sure never to put PH meter electrode inside to prevent RNAse contamination.)

Catalase suspension (Sigma 3515-10mg), stored at +4ºC

GLOX buffer:

Reagent	Final concentration	Stock	For 10 ml	For 50 ml
TRIS (pH 8.0, Ambion M9856)	10mM	1M	100μl	500μl
SSC	2X	20X	1 ml	5 ml
Glucose	0.4%	10%	400μl	2ml
Nuclease-free Water			8.5 ml	42.5 ml

Procedure

- Prepare GLOX buffer, add to the sections, let stand for a few minutes. (Sections can be stored in GLOX buffer at 4°C)
- In a separate tube, prepare the anti-bleach mounting medium: add 1μl of the Glucose oxidase and 1μl of the Catalase (vortex the suspension before pipetting) to 100μl of GLOX buffer, mix.
- Wipe the back of the coverslips and dry the edges. Add 10-15μl of anti-bleach solution onto the sections; place a circular coverslip (EMS #72228-01 15mm diameter) on top of the sample. Use Watman paper to suck off as much of the excess liquid as possible to avoid sliding of the circular coverslip (which will damage the tissue).
- Put a gasket (Grace Biolabs #JTR20-0.5) on a VWR rectangular microscope slide (#16004-422). Mount the sample onto the coverslide with the gasket, with the circular coverslip inside the chamber created by this construction.
- Image within a day from hybridization.