Sequential smFISH (Allen Institute) Version 2

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Abstract

We have developed a multiplexed single molecule FISH protocol for use at the Institute. This protocol was optimized on human tissue, but will work on mouse tissue as well. It was adapted from Lyubimova et. al., Nature Protocols, 2013.

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Guidelines

Ensure that all reagents are in recombinant and RNAse-free format, as we have noticed RNA degradation in solutions that contain enzymes derived from whole organisms.

We filter every solution with a 0.2um syringe filter prior to use. This reduces background spots and dust that interfere with imaging of diffraction limited spots.

For the SDS treatment after fixation and permeabilization, be gentle when dropping SDS onto the section, as well as during washes. This treatment is relatively harsh and the tissue must be treated somewhat delicately.

Before start

Ensure all incubators and ovens are at the appropriate temperature prior to experiment.

Protocol

Tissue and Sectioning

Step 1.

10-14 um cryosections are taken from fresh-frozen tissue, which are collected on poly-lysinetreated

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#1 coverslips at room temperature (RT). After 5-10 min at RT, sections are placed at 4°C until sectioning is complete. At that point, proceed immediately to fixation and permeabilization.

DURATION
00:05:00 : RT

Fixation/Permeabilization

Step 2.

Post-fix sections for 15 min with 4% PFA @ 4 °C.

TEMPERATURE
4 °C : Post-fixing

REAGENTS
- 4% PFA by Contributed by users

DURATION
00:15:00 : Post-fixing

Fixation/Permeabilization

Step 3.

Wash with PBS (1/3)

REAGENTS
- PBS by Contributed by users

Fixation/Permeabilization

Step 4.

Wash with PBS (2/3)

REAGENTS
- PBS by Contributed by users

Fixation/Permeabilization

Step 5.

Wash with PBS (3/3)

REAGENTS
- PBS by Contributed by users

Fixation/Permeabilization

Step 6.

Permeabilize with cold methanol at -20 C for 10 min.

TEMPERATURE
-20 °C : Permeabilizing

DURATION
00:10:00 : Permeabilizing

Fixation/Permeabilization

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Step 7.

Air dry for 30 min in fume hood (Stopping point: store coverslips at -80C)

DURATION
00:30:00 : Air drying

Fixation/Permeabilization

Step 8.

Optional: Treat sections with 8% SDS/PBS for 10 minutes, followed by 3 – 5 rinses with PBSor 2XSSC

DURATION
00:10:00 :

Fixation/Permeabilization

Step 9.

Add 2ml 2X SSC

AMOUNT
2 ml : 2X SSC

REAGENTS
2X SSC by Contributed by users

Hybridization

Step 10.

Pre-heat hyb oven to 37 °C

TEMPERATURE
37 °C : oven

Hybridization

Step 11.

Place sections in hyb buffer without probes.

Hybridization

Step 12.

Add 4 ul probe 400ul hyb buffer.

AMOUNT
4 µl : probe

AMOUNT
400 µl : hyb buffer

NOTES

Specific to 6-well plate format – if using perfusion chamber, this volume can be reduced.

Hybridization

Step 13.

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Incubate at 37 C for 2H.

TEMPERATURE
37 °C : Incubation
DURATION
02:00:00 : Incubation

Wash

Step 14.

Add 2 ml wash buffer to each well.

AMOUNT
2 ml : wash buffer

Wash

Step 15.

Incubate at 37 C for 15 min.

TEMPERATURE
37 °C : Incubation
DURATION
00:15:00 : Incubation

Wash

Step 16.

Remove wash buffer.

Wash

Step 17.

Add 2 ml fresh wash buffer and incubate at 37 C for 15 min.

AMOUNT
2 ml : wash buffer
TEMPERATURE
37 °C : Incubation
DURATION
00:15:00 : Incubation

Wash

Step 18.

Replace wash buffer with fresh wash buffer + DAPI (final 5ug/mL) and incubate at 37 C for 15 min.

TEMPERATURE
37 °C : Incubation
DURATION
00:15:00 : Incubation

Wash

Step 19.

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GLOX buffer step if performing antibody stain

Wash

Step 20.

Mount and image or store at 4 C in 2XSSC until imaging session

TEMPERATURE

4 °C :

REAGENTS

2X SSC by Contributed by users

Stripping

Step 21.

65% formamide/2X SSC, 10 min X 3, 30 C

TEMPERATURE

30 °C :

REAGENTS

65% formamide/2X SSC by Contributed by users

DURATION

00:10:00 :

Stripping

Step 22.

Wash in 2XSSC (1/3)

Stripping

Step 23.

Wash in 2XSSC (2/3)

Stripping

Step 24.

Wash in 2XSSC (3/3)

Stripping

Step 25.

Following stripping, proceed to hybridization step.

GOTO

Hybridization step -> go to step #10

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Warnings

Please refer to the SDS (Safety Data Sheet) for hazard information and safety warnings.

Avoid exposure to formamide, DAPI

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