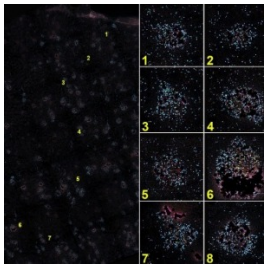
Mar 13, 2019 Version 3

Sequential smFISH V.3



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ZengU19 BICCN Grant[1]
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Protocol status: Working
We use this protocol and it's working

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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.

Attachments

DOCX

smFISH.docx

16KB

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.

Materials

STEP MATERIALS

- 4% PFA
- PBS
- PBS
- PBS
- 2X SSC
- 2X SSC
- 65% formamide/2X SSC

Protocol materials

- 4% PFA
- PBS
- PBS
- PBS
- 2X SSC
- 2X SSC
- 65% formamide/2X SSC
- 4% PFA
- PBS
- PBS
- PBS
- 2X SSC
- 2X SSC
- 65% formamide/2X SSC

Troubleshooting

Safety warnings

Please refer to the SDS (Safety Data Sheet) for hazard information and safety warnings.
Avoid exposure to formamide, DAPI

Before start

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

Tissue and Sectioning

1 10-14 um cryosections are taken from fresh-frozen tissue, which are collected on poly-lysinetreated #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.

00:05:00 RT

Fixation/Permeabilization

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

4% PFA

00:15:00 Post-fixing

4 °C Post-fixing

3 Wash with PBS (1/3)

PBS

4 Wash with PBS (2/3)

PBS

5 Wash with PBS (3/3)

PBS

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

-20 °C Permeabilizing

00:10:00 Permeabilizing

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

00:30:00 Air drying

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

00:10:00

9 Add 2ml 2X SSC

2X SSC

2 mL 2X SSC

Hybridization

10 **Pre-heat hyb oven to 37 °C**

37 °C oven

11 Place sections in hyb buffer without probes.

12 Add 4 ul probe 400ul hyb buffer.

4 µL probe

400 µL hyb buffer

Note

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

13 Incubate at 37 C for 2H.

37 °C Incubation

02:00:00 Incubation

Wash

14 Add 2 ml wash buffer to each well.

2 mL wash buffer

15 Incubate at 37 C for 15 min.

37 °C Incubation

00:15:00 Incubation

16 Remove wash buffer.

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

2 mL wash buffer

37 °C Incubation

00:15:00 Incubation

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

37 °C Incubation

00:15:00 Incubation

19 GLOX buffer step if performing antibody stain

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

4 °C

2X SSC

Stripping

21 65% formamide/2X SSC, 10 min X 3, 30 C

65% formamide/2X SSC

00:10:00

30 °C

22 Wash in 2XSSC (1/3)

23 Wash in 2XSSC (2/3)

24 Wash in 2XSSC (3/3)

25 Following stripping, proceed to hybridization step.

go to step #10 Hybridization step