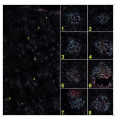

Mar 13, 2019

Working

# Sequential smFISH

Version 3

ZengU19 BICCN Grant[1]

[1]Allen Institute

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BICCN

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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.


smFISH.docx

PROTOCOL STATUS

**Working**

We use this protocol in our group and it is working

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.

STEPS MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| 4% PFA | | |
| PBS | | |
| PBS | | |
| PBS | | |
| 2X SSC | | |
| 2X SSC | | |
| 65% formamide/2X SSC | | |

SAFETY WARNINGS

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

Avoid exposure to formamide, DAPI

BEFORE STARTING

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**

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**