VERSION 2

AUG 15, 2018

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.snnedde

Protocol Citation: Jennie Close, Zoe Maltzer 2018. Sequential smFISH (Allen Institute). **protocols.io** <https://dx.doi.org/10.17504/protocols.io.snnedde>

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Protocol status: Working
 We use this protocol and it's working

Created: Aug 15, 2018

Last Modified: Aug 15, 2018

PROTOCOL integer ID:
 14766

Keywords: smFISH

Sequential smFISH (Allen Institute) V.2

Jennie Close¹, Zoe Maltzer¹

¹Allen Institute for Brain Science

BICCN

Allen Institute for Brain Science

Stephanie Mok

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

[smFISH_Allen
Institute.docx](#)

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 Contributed by users
PBS Contributed by users
PBS Contributed by users
PBS Contributed by users
2X SSC Contributed by users
2X SSC Contributed by users
65% formamide/2X SSC Contributed by users
4% PFA Contributed by users
PBS Contributed by users
PBS Contributed by users
PBS Contributed by users
2X SSC Contributed by users
2X SSC Contributed by users
65% formamide/2X SSC Contributed by users

SAFETY WARNINGS

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

BEFORE START INSTRUCTIONS

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-lysine-treated #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 Contributed by users

00:15:00 Post-fixing

4 °C Post-fixing

- 3 Wash with PBS (1/3)

PBS Contributed by users

- 4 Wash with PBS (2/3)

PBS Contributed by users

- 5 Wash with PBS (3/3)

PBS Contributed by users

- 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 PBS or 2XSSC

00:10:00

9 Add 2ml 2X SSC

2X SSC Contributed by
users

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 Contributed by
users

Stripping

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

65% formamide/2X SSC Contributed by
users

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
undefined