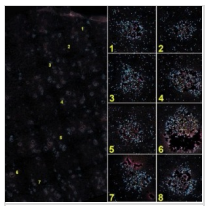

Feb 15, 2022

Sequential smFISH Allen Institute

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Human Cell Atlas Method Development Community

CZI Spatial Transcriptomics Protocol Repository

Jennie Close

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_Allen Institute.docx

DOI

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protocol ,

Jan 08, 2018

Feb 15, 2022

9482

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

TE buffer pH 8, 100ml **Sigma**

Aldrich Catalog #93283-100ML

Deionized Formamide, 500 ml **Sigma**

Aldrich Catalog #4650-500ML

DAPI (4,6-diamidino-2-phenylindole) **Sigma**

Aldrich Catalog #32670-5MG-F

RBS 35 Concentrate **Invitrogen - Thermo**

Fisher Catalog #27950

tRNA from E.Coli MRE 600 **Sigma**

Aldrich Catalog #10109541001

Dextran Sulfate Sodium Salt **Sigma**

Aldrich Catalog #D8906-5G

Glucose Oxidase **Sigma**

Aldrich Catalog #G2133-10KU

Catalase **Sigma**

Aldrich Catalog #C3515-10MG

Trolox **Sigma**

Aldrich Catalog #238813-1G

Glucose **Sigma**

Aldrich Catalog #G8270 - 1KG

Buffers and Solutions:

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Imaging Buffer
Imaging buffer stock (can be stored at 4°C)
0.4g glucose
48.5mL nuclease free water
1mL 1M Tris-HCl
500µL 5M NaCl

Enzymes/Trolox (per 5mL of imaging buffer, added right before use)
5µL glucose oxidase (3.7mg/mL stock)
8.78µL catalazse (7mg/mL stock

Wash buffer
5 ml 20X SSC
10 ml Formamide
35 ml deionized, nuclease free water

Hybridization buffer
7.3 ml deionized, nuclease free water
1 ml 20X SSC
1 g Dextran Sulfate
(rotate on tube rotator until Dextran dissolves)
then add:

1 ml Formamide (can substitute 10% ethylene carbonate for formamide)
500 ul tRNA stock (20 mg/ml)
100 ul RVC stock (200 mM)
40ul BSA stock (50 mg/ml)

Avoid exposure to formamide, DAPI

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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.

Fixation/Permeabilization

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

3 ▪ Wash with PBS 3X

4 ▪ Permeabilize with room temperature isopropanol 3 min

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

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

7 Add 2ml 2X SSC

Hybridization

8 Place sections in hyb buffer without probes for 5 min.

9 Add probes to 400ul hyb buffer at a final concentration of 2ng/ul* (specific to 6-well plate format – if using perfusion chamber, this volume can be reduced)

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*We store a working 200ng/ul stock of probes in the dark at 4C. These are diluted 1:100 for hybridizations, but this may need to be adjusted depending on the probe.

10 Incubate at 37 C for 2H

Wash

11 Add 2 ml wash buffer to each well, incubate at 37 C for 15 min

12 Remove wash buffer

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

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

15 (GLOX buffer step if performing antibody stain)

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

Stripping

17 65% formamide/2X SSC, 10 min X 3, 30 C

18 3 washes in 2XSSC

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Imaging

19 Add enzymes to Imaging Buffer just prior to imaging.

20

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