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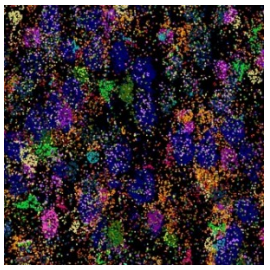
osmFISH



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Human Cell Atlas Metho... CZI Spatial Transcriptom... 1 more workspace

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External link: http://linnarssonlab.org/osmFISH/

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We use this protocol and it's working

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Abstract

osmFISH is a cyclic single molecule fluorescent *in situ* hybridization protocol used to quantify the expression level of specific transcripts in tissue sections by direct labeling of individual RNA molecules. The number of transcripts quantified in each round correspond the numbers of fluorophores available in the microscope setup. In order to quantify a large number of genes, osmFISH provides a method to remove the probes/labelling (stripping) from their targets and get the tissue ready for the next round of labeling - imaging - stripping.

Even though the osmFISH protocol has been developed to perform multiple smFISH rounds, it can also be used as a quick and simple method for one-round smFISH. Furthermore, the protocol can be further extended to include more complex encoding/barcoding shemes that can be used to resolve a larger number of targets.

Attachments

PDF

Codeluppi et al 2018...

39MB

Guidelines

The volume of the wash steps are determined by the volume of the flow cell. At least replace the volume once. The wash volume we use is double that of the flow cell. So 2 × 500µl = 1ml per wash, in our case.

Stellaris RNA FISH probes were orderd from Biosearch Tecnologies

Troubleshooting

Safety warnings

The protocol requires the handeling of PFA, SDS and formamide. Please read the safety data sheet on these chemicals and handle accordingly. Furthermore the imaging buffer Slow Fade Diamond contains Phenol.

Before start

Please reed our considerations about the imaging and hybridization chamber setup on our **website**.

Prepare the Hybridization mix. We tipically make 10-40ml, aliquot and freeze at -20°C untill use.

Aliquot the formamide in 200ml aliquots and freeze at -20°C. At the start of each week we thaw wat we need that week.

Tissue Collection

1 Transcardally perfuse a mouse with cold oxygenated artificial spinal fluid solution.

2 Quickly dissect out the brain on a cold surface.

3 Place the brain in cryomold and fill it up with Tissue-Tek O.C.T. (Sakura)

4 Snap freeze the sample by lowering the cryomold in a slush of isopentane and dry ice. Make sure to prepare the slush before dissection and wait to use it until the bubbling stops.

5 Store sample at -80°C.
-80 °C

Coverslip Cleaning

6 Empty a box of coverslips (#1.5) in a beaker. Rinse coverslips in distilled water two times for 20 min.

7 Incubate the coverslips in concentrated nitic acid for 24 hours.

8 Rinse coverslips in water 4 times for 1 hour.
01:00:00 Rinse 1
01:00:00 Rinse 2
01:00:00 Rinse 3
01:00:00 Rinse 4

9 Fill the beaker with distilled water and sterilize the coverslips by autoclaving.

10 Wash coverslips once with 95% ethanol and store them indefinitively in 95% ethanol.

Coverslip Functionalization

11 Place the coverslips to use in a rack and air dry to remove the ethanol.

12 Prepare 2% (3-Aminopropyl)triethoxysilane, APS (v/v)(Sigma) in acetone in a staing jar.
Make sure to use fresh APS that has been stored in a dry environment.
Dip the rack with the coverslips for 1 minute in the solution, followed by a 1 minute dip in RNAse free water.
00:01:00 Silanization
00:01:00 Water

13 Air dry the coverslips and store them in a dessicated envirionment.

Tissue sectioning

14 Cryocut 10µm thick sections of the fresh frozen tissue and mount the section on the functionalized coverslips. In order to remove the electostatic charge of the coverslip clean one side of the coverslip with a kimwipe wetted with ethanol.

15 Make a fixing chamber by cutting a hole in a piece of parafilm and place it in a petri dish. The parafilm functions as a spacer to separate the tissue section from the bottom of the petri dish. The number of chambers matchs the number of sections that will be cut. Place a drop of 4% PFA in the a clean petri dish inside the hole in the parafilm. Directly after capture, fix the section with 4% PFA for 10minutes by placing the tissue section face down on the PFA drop.

Suggestion
We saw that PFA soultion prepared from powder causes lower background compared to commercial PFA solutions. However, we didn't test all the solutions available in the market.
00:10:00 Fixation

16 After fixation transfer the glass to a clean petri dish and rinse twice with PBS.

17 Remove the excess of PBS and dry the slide with a tissue without touching the tissue.

18 Dehydrate the slide in >1ml of isopropanol for 3 minutes. Followed by air drying.

00:03:00 Dehydrate

19 Store the slide at -80°C untill use.

-80 °C

Flow cell setup

20 Assemble the sample in the flow cell.

We use a custom flow cell that was manufactured by **Microliquid**. A flow cell facilitates the automation. Please see our notes on the osmFISH **website**.

test_render_chamber_6.png

osmFISH First Round

21 Rehydrate the tissue for 5 minutes with 2X SSC buffer.

22 Clear the tissue by incubating the tissue with 4% SDS in 200mM boric-acid pH 8.5 4 times for 5 minutes.

00:05:00 Wash 1

00:05:00 Wash 2

00:05:00 Wash 3

00:05:00 Wash 4

Note

Adjust the volume of the buffer to the volume of the flow cell. At least replace the liquid once. The wash volume that we use is double that of the flow cell. So 2x 500ul = 1ml

23 Wash the section with SSC 2X 5 times.

24 Wash the tissue twice with Tris EDTA buffer pH 8.

25 Perform a heat shock by placing the sample at 70°C for 10 min.

70 °C

00:10:00 Heat shock

26 Promptly wash 3 times with SSC 2X

27 Incubate the tissue with hybridization mix without probes for 5 minutes at room temperature.

Hybrization mix without probes:
2X SSC (Sigma)
10% (w/v) Dextran sulfate (Sigma)
10% (v/v) Formamide (Ambion)
1 mg/ml E. coli tRNA (Roche)
2mM Ribonucleoside vanadyl complexes (RVC) (Sigma)
0.2 ml/ml Bovine Serum Albumin (Sigma)
00:05:00 Pre-hybridization

28 Incubate the tissue with hybridization mix with probes for 4 hours at 38.5°C.

Hybrization mix with probes:
2X SSC (Sigma)
10% (w/v) Dextran sulfate (Sigma)
10% (v/v) Formamide (Ambion)
1 mg/ml E. coli tRNA (Roche)
2mM Ribonucleoside vanadyl complexes (RVC) (Sigma)
0.2 ml/ml Bovine Serum Albumin (Sigma)
250nM Probes (Sellaris, LGC Biosearch technologies). ~48 probes/target. The final concentration of each probe set needs is 250nM.

38.5 °C Hybridization temperature
04:00:00 Hybridization time

Note

The hybridization temperature is a sensitive parameter and can depend on the calibration of the incubator. Please, make a test to optimize the temperature for your specific incubator. If there is too much background increase the temperature. If there is too little staining decrease the temperature.

29 Wash 4 times 15 minutes with 20% formamide in 2X SSC at 38.5°C. Add a nuclear staining (ex. Hoechst, Dapi) if needed.
38.5 °C Stringency wash temperature
00:15:00 Stringency wash 1

00:15:00 Stringency wash 2
00:15:00 Stringency wash 3
00:15:00 Stringency wash 4

30 Wash with 2X SSC.

31 Inject Slow Fade Diamond imaging buffer (Thermo). Do not use hardening mounting media in the flow cell. You can use hardening mountine media if the coverslips will be mounted on a slide before imaging.

Imaging

32 Perform imaging of the region of interest.
It is important to save the field of views that are imaged in order to repeat the imaging of the same region. For an extensive discussion on the imaging setup and repeated imaging of the same tissue section please see our **website**.

osmFISH Repeat Round

33 Wash 5 times with SSC 2X to remove the mounting media.

34 Strip the probes off of their targets by washing 3 times 10 minutes with 65% formamide in 2X SSC at 30°C.
30 °C
00:10:00 Stip 1
00:10:00 Strip 2
00:10:00 Strip 3

35 Wash 5 times with SSC 2X

36 Optional: verify if the signal is stripped by imaging.

37 Incubate the tissue with Hybridization mix without probes for 5 minutes at room temperature.

Hybrization mix without probes:
2X SSC (Sigma)

10% (w/v) Dextran sulfate (Sigma)
10% (v/v) Formamide (Ambion)
1 mg/ml E. coli tRNA (Roche)
2mM Ribonucleoside vanadyl complexes (RVC) (Sigma)
0.2 ml/ml Bovine Serum Albumin (Sigma)
00:05:00 Pre-hybridization

38 Incubate the tissue with Hybridization mix with probes for 4 hours at 38.5°C.

Hybrization mix with probes:
2X SSC (Sigma)
10% (w/v) Dextran sulfate (Sigma)
10% (v/v) Formamide (Ambion)
1 mg/ml E. coli tRNA (Roche)
2mM Ribonucleoside vanadyl complexes (RVC) (Sigma)
0.2 ml/ml Bovine Serum Albumin (Sigma)
250nM Probes per target (Sellaris, LGC Biosearch technologies)

38.5 °C Hybridization temperature
04:00:00 Hybridization time

Note

The hybridization temperature is a sensitive parameter and can depend on the calibration of the incubator. Please, make a test to optimize the temperature for your specific incubator. If there is too much background increase the temperature. If there is too little staining decrease the temperature.

39 Wash 4 times 15 minutes with 20% formamide in 2X SSC at 38.5°C. Add a nuclear staining (ex. Hoechst, Dapi) if needed.
38.5 °C Stringency wash temperature
00:15:00 Stringency wash 1
00:15:00 Stringency wash 2
00:15:00 Stringency wash 3
00:15:00 Stringency wash 4

40 Wash with 2X SSC.

41 Inject Slow Fade Diamond imaging buffer (Thermo). Do not use hardening mounting media in the flow cell. You can use hardening mountine media if the coverslips will be

mounted on a slide before imaging.

Imaging Repeat Round

42 Perform imaging of the region of interest.

Repeat

43 Repeat the "osmFISH Repeat Round" (Steps 33-42) untill all targets have been labeled

go to step #33