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Tissue preparation and tissue imaging for MERFISH

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We use this protocol and it's working

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Abstract

MERSCOPE Sample preparation protocol and imaging protocol

Materials

General Reagents:

- 32% paraformaldehyde (PFA) – (Electron Microscopy Sciences, 15714S)
- 10x PBS, DNAse, RNAse free - (Thermofisher, AM9625)
- Sucrose - (Sigma Aldrich, S9378)
- Ethanol (EtOH) – (Sigma, E7023)
- DNAse, RNAse free water
- Tissue-Tek OCT compound (VWR, 4583)
- RNAse inhibitor, murine – (NEB, M0314S)

MERSCOPE Reagents:

- MERSCOPE Sample Prep Kit (Vizgen, 10400012) containing:
- o Sample Prep Buffer
- o Formamide Wash Buffer
- o Sample prep clearing premix
- o Gel embedding premix
- Custom 500 gene panel premix
- MERSCOPE Slide Box (Vizgen, 10500001)

Perfusion and sectioning of brain

1 C57Bl6 male mice were housed in groups until 2-3 months of age.

2 4% PFA solution was made from 32% PFA concentrate in 1x PBS.

3 Mice were perfused first with 25 mL of chilled 1x PBS (DNAse, RNAse free) followed by 50 mL of chilled 4% PFA. Brains were extracted and post-fixed in PFA for 16:00:00 . 16h

4 Brains were transferred to 30% Sucrose until fully submerged.

5 Brains were then submerged in OCT solution and frozen on dry ice.

6 10um coronal sections of ventral midbrain were obtained with a cryostat and placed onto specialized MERSCOPE slides.

MERSCOPE sample preparation

7 Each step of the following protocol was performed in RNAse free conditions using RNAse away and RNAse free solutions.

8 Each brain slice to be imaged was first placed into a 60mm petri dish and washed 3 times in 5 mL 1x PBS for 00:05:00 . 5m

9 Add 5 mL of 70% EtOH, cover the petri dish with parafilm and incubate at 4 °C for 16:00:00 to permeabilize the tissue. 16h

10 Aspirate the EtOH and wash 1 time with 5 mL Sample Prep Buffer.

11 Aspirate the sample prep buffer and incubate with 5 mL of Formamide Wash Buffer at 37 °C for 00:30:00 . 30m

12 Aspirate as much of the formamide wash buffer from the dish and slide without disturbing the tissue. Then add 50 µL of the custom MERSCOPE gene panel mix. Place a small piece (2cm x 2cm) of fresh parafilm on top of the tissue section without disturbing it or introducing air bubbles.

13 Clean the outside of the petri dish with 70% EtOH and place into a tissue culture incubator (humidified, 37 °C) for 36-48 hours

14 Remove the parafilm and add 5 mL of formamide wash buffer. Incubate at 47 °C for 00:30:00 . 30m

15 Aspirate the wash buffer and add another 5mL formamide was buffer to the petri dish. Continue incubating at 47 °C for another 00:30:00 . 30m

16 Wash 1 time with 5 mL sample prep buffer for 00:05:00 . 5m

17 Prepare Gel Coverslips by spraying with RNAse away and cleaning with a kimwipe. Then wash with 70% EtOH and clean again with a kimwipe followed by lens cleaning paper. Add 100 µL of Gel Slick solution to the coverslip and allow to evaporate at room temperature (approximately 10 minutes).

18 Prepare the gel embedding solution by adding 5mL Gel embedding premix to 25uL of 10% Ammonium persulfate solution and 2.5 µL of TEMED. Retain 100 µL of the embedding solution into a separate Eppendorf.

19 Aspirate the sample prep buffer and add the remaining 5 mL of gel embedding solution to the petri dish. Incubate at room temperature for 00:01:00 . 1m

20 Aspirate the gel embedding solution and add 50 µL of the retained solution directly on top of the tissue section. With forceps, place the cleaned glass coverslip on top of the tissue (gel slick coated side facing the tissue). Allow the gel embedding solution to spread across the entire coverslip and aspirate any excess solution. Incubate at room temperature for 01:30:00 to allow the gel to solidify. 1h 30m

21 Carefully remove the coverslip without disrupting the tissue.

22 Warm the clearing solution at 37 °C for 00:30:00 prior to use. For each sample, prepare 5 mL of clearing solution by adding 50uL of Proteinase K. Add the clearing solution to the petri dish and incubate at 47 °C for 24:00:00 . 1d 0h 30m

23 Once the tissue has fully cleared, wash the petri dish with sample prep buffer (approximately 3 times, 5 mL for 00:05:00 on rotation). Add DAPI and PolyT solution to the petri dish and incubate at room temperature for 10 minutes. Wash extensively with sample prep buffer and prepare the sample for imaging. 5m

Imaging 1h

24 Imaging cartridges are first thawed in 37 °C water bath for 01:00:00 before imaging. After a complete thaw is finished, puncture a hole in the fluid container and add 100 µL of RNAse inhibitor, pipetting up and down 10 times to mix the contents. 1h

25 Slides are loaded into the slide holding contraption and connected to the Merscope input and output tubing. The imaging cartridge is inserted to the device and imaging fluid is pulled through the slide holder.

26 4x images of the brain tissue is collected and the area of interest is circled with the Vizgen imaging software. The device is allowed to run over the course of 24-48 hours until all regions of interest are imaged.