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🌐 GeoMx RNA slide prep for fresh frozen human post-mortem brain tissue

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We use this protocol and it's working

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Abstract

This protocol largely follows the GeoMx manual slide preparation manual for RNA, with a few minor changes. This protocol also includes concentrations of antibodies, optimised on 10 um fresh frozen post mortem human brain tissue samples.

Guidelines

Cross-contamination of RNA should be avoided at all points. Regularly clean surfaces with RNase AWAY

Materials

REAGENTS

Leica tissue freezing medium

ChemCruz – Paraformaldehyde solution 4% in PBS sc-281692 – Santa Cruz Biotechnology

Gibco – PBS pH 7.4 – 70011-036

VWR – Formamide, deionized ULTRA PURE – CAS 75-12-7

Invitrogen Alexa Fluor™ 488 goat anti-chicken IgY (H+L) – REF – A11039

Invitrogen Alexa Fluor™ 647 goat anti-rabbit IgG (H+L) – REF – A21244

ATLAS ANTIBODIES – HPA014518 Anti-P2RY12 produced in Rabbit

Novus NBP1-05198 – Chicken anti-GFAP pAb

Invitrogen SYTO™ 83 orange fluorescent nucleic acid stain – REF – S11364

Invitrogen IHC Antigen Retrieval Solution 10X High pH – REF – 00-4956-58

ThermoFisher Scientific RNase AWAY – catalog # 7002

SIGMA – Glycine – G7126-100G

Promega – Tris base, molecular biology grade – REF – H5133

Sigma-Aldrich SSC Buffer 20x Concentrate – AM9763

Sigma-Aldrich Formalin solution, neutral buffered, 10% HT501128-4L

Invitrogen Proteinase K – REF – AM2548

EQUIPMENT

Morphy Richards – compact intellisteam 6L food steamer

ACD – HybEZ™ II Oven

Labnet international, inc. – MINI INCUBATOR

KIMTECH – 7558 2 ply wipes

BIO-RAD C1000 Touch™ Thermal Cycler

SciSpin Mini – centrifuge

Eppendorf Easypet 3

Cleaver scientific Stirring water bath

Grace Bio-Labs HybriSlips

Coplin jars – any manufacturer

Pipettes – any manufacturer

Superfrost charged slides –

Leica CM1950 cryostat

Tweezers - any manufacturer

Paintbrushes – any manufacturer

Serological pipettes - any manufacturer

GeoMx DSP - NanoString

Safety warnings

! Some chemicals used in this protocol are potentially harmful. Ensure that you read all safety instructions

Ethics statement

The protocols.io team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.



Prepare reagents

1 Prepare reagents for GeoMx pipeline prior to tissue preparation

A	B	C
Reagent	Dilution	Storage
70 % EtOH	Prepare 100 mL of 70 % ethanol by adding 30 mL of DEPC-treated water to 70 mL of 100 % ethanol. Change at least weekly	
50 % EtOH	Prepare 100 mL of 50 % ethanol by adding 50 mL of DEPC- treated water to 50 mL of 100% ethanol. Change at least weekly.	RT
1X PBS pH 7.4	Prepare 1 L of 1X PBS by combining 100 mL of 10X PBS and 900 mL of DEPC-treated water. Don't reuse.	4°C
10% neutral buffered formalin (NBF)	Caution: NBF is hazardous, handle with care and minimize inhalation risks. Work with 10% NBF in the fume hood.	RT
Proteinase K	Prepare 1 µg/mL by adding 10 µL of 20 mg/mL Proteinase K to 200 mL of 1X PBS made with DEPC-treated water Note: Should be prepared fresh for each run and not reused. Inaccurate concentration of Proteinase K will affect assay performance.	RT
NBF stop buffer	Prepare NBF stop buffer by adding 12.12 g Tris base and 7.5 g Glycine to 1 L DEPC- treated water. Do not reuse.	RT
2X SSC	Prepare 1 L of 2X SSC by combining 100 mL of 20X SSC and 900 mL of DEPC-treated water. Do not reuse.	RT
4X SSC	Prepare 1 L of 4X SSC by combining 200 mL of 20X SSC and 800 mL of DEPC-treated water. Do not reuse.	RT

Prepare Tissue Sections

1d



- 2 Remove frozen human brain tissue from  -80 °C freezer
- 3 In a biosafety cabinet, on dry ice, chip a 1 cm² piece of tissue from the middle frontal gyrus, extending from the white matter to the superficial cortex using a scalpel and tweezers
- 4 Decontaminate Leica CM1950 cryostat (and tweezers, paintbrushes (any accessories used to manipulate tissue sections)) using 70 % ethanol, RNase away and a UV decontamination cycle
- 5 Set chamber and specimen head temperatures on cryostat to  -20 °C
- 6 Transport tissue chips on  0 °C to cryostat.
- 7 Allow tissue chips to equilibrate within cryostat chamber for  00:30:00
- 8 Insert fresh razor blade into blade holder and secure with the blade clamping lever. Cover blade with safety guard until ready to section
- 9 Place cryostat specimen disc on the freeze shelf and cover the disc with tissue freezing medium.
- 10 Place tissue chip onto tissue freezing medium in the desired orientation (coronal with all cortical layers and white matter facing upwards)
- 11 Move disc to Peltier position on freeze shelf and activate the Peltier element (rapid freezing)
- 12 Allow tissue freezing medium to completely freeze before placing on the specimen head
- 13 Move the locking lever to orient the specimen so that the face of the block is parallel to the blade
- 14 Using a marker pen, mark out the GeoMx scan area on the back of Superfrost Plus charged slides

30m



- 15 Set cutting thickness to 10 μm
- 16 Trim into the tissue until the whole face of the block is present in each section (trimming in is also necessary for optimal RNA quality)
- 17 Using the anti-roll plate, generate a section free of wrinkles/creases. Paintbrushes can be used to gently flatten out tissue
- 18 Holding Superfrost Plus slide by the label, align the tissue section within the scan area (if using more than one section per slide, collect sections from the bottom of the slide, up towards the label)
- 19 Once aligned with scan area, lower slide onto tissue section, taking care not to press the section into the stage, place a finger on the back of the slide, underneath the section to ensure it adheres fully to the slide
- 20 Place slide (tissue facing upwards) onto freeze shelf
- 21 Remove specimen from specimen head and return to  -80 °C freezer before collecting more sections
- 22 Thoroughly clean cryostat chamber and stage with ethanol before mounting a new sample. Repeat the process until all samples have been collected

Expose RNA targets

16m

- 23 Remove slides from cryostat freeze shelf and submerge fully in RT 4 % PFA for

 00:10:00

10m

- 24 Wash slides 3x in 1X PBS ( 00:02:00 per wash)

2m

Tissue dehydration

58m

- 25 Turn on steamer and heat 1 staining jar of DEPC H₂O and 1 staining jar of 1X Tris EDTA to  100 °C (use  100 mL staining jars with transferrable slide racks for these steps to minimise time with steamer lid removed)

2m



- 26 Transfer slides to a slide rack and bake sections on slides in a 60 °C drying oven for 00:30:00 30m
- 27 Spray 70 % ethanol onto a kimwipe and clean the marker pen from the back of the slides 1m
- 28 Wash slides in 50 % ethanol for 00:05:00 5m
- 29 Wash slides in 70 % ethanol for 00:05:00 5m
- 30 Wash slides 2x in 100 % ethanol for 00:05:00 each wash 5m
- 31 Let slides air dry for 00:05:00 – during this time, transfer slides to slide rack that can fit inside staining jar 5m

Perform target retrieval

25m

- 32 Remove steamer lid and submerge slides in DEPC H₂O for 00:00:10 , then immediately transfer to 1X Tris EDTA for 00:20:00 20m 10s
- 32.1 NOTE - Antigen retrieval times may need optimisation – for 10 µm fresh frozen human brain samples, 20 minutes provided optimal results
- 33 When timer is finished, remove rack from steamer and immediately wash slides in room temperature 1X PBS for 00:05:00 . Slides can be stored in 1X PBS for 1 hour 5m
- 34 Heat water bath to 37 °C

Expose RNA targets

21m



35 Prepare proteinase K dilution – 0.1 µg/mL by adding 1 µL of 20 mg/mL Proteinase K to 200 mL of 1X PBS – place into a staining jar and add to water bath

1m

35.1 NOTE – Proteinase K concentration may need optimisation for different tissue types and thicknesses – for 10 µm fresh frozen human brain samples, 0.1 µg/mL provided optimal results

36 Once Proteinase K has reached 37 °C , incubate slides in Proteinase K for

15m

00:15:00 (or as optimised for other tissues/thicknesses)

37 During Proteinase K digest, ensure reagents for next steps are prepared

38 Wash slides in RT 1X PBS for 00:05:00 , proceed to next steps immediately

5m

Postfix—Preserve tissue morphology for soft tissues

20m

39 Post-fix the tissue by performing the following washes:

39.1 10 % NBF (00:05:00)

5m

39.2 NBF Stop Buffer 2x 00:05:00 each wash

5m

39.3 1X PBS (00:05:00) – slides can be stored in 1X PBS for 01:00:00 at

7h 5m

Room temperature or 06:00:00 at 4 °C

In-situ hybridisation (Overnight)

16h

40 Prepare hybridisation chamber – clean with RNase AWAY, wipe with Kimwipes. Add fresh Kimwipes to the chamber and dampen with DEPC treated water – make sure that the water does not pool

41 Prepare buffers for in-situ hybridisation – warm Buffer R to Room temperature before opening and thaw RNA detection probes, mix thoroughly by pipetting



- 42 Make the hybridisation solution according to the following table (n = number of slides NOT number of sections)
- 43 Remove slides from PBS one at a time, wipe away excess liquid with a fresh Kimwipe and place in hybridisation chamber.
- 44 Add  200 μ L hybridisation solution to each slide, ensuring no bubbles are introduced. It is preferable to remove some hybridisation solution, than have bubbles present
- 45 Gently apply a Grace Bio-Labs HybriSlip by setting one edge of the coverslip down on the slide and gradually laying the rest of the coverslip down to avoid bubbles
- 46 Once all HybriSlips have been applied, clamp slides into place, close the lid of the hybridisation chamber and insert into hybridisation oven. Incubate at  37 °C  Overnight (16-24 hours) 1d
- 47 Before removing slides from hybridisation oven, ensure the water bath is heated to  37 °C . Warm 100% formamide to  Room temperature before opening. Once at room temperature, prepare Stringent Wash by mixing equal parts 100 % formamide with 4X SSC. Fill 2 staining jars with Stringent Wash and heat to  37 °C in the water bath
- 48 Once Stringent Wash reaches  37 °C , remove slides from hybridisation oven and dip into 2X SSC, allowing the HybriSlips to slide off, proceed to Stringent Wash after  00:05:00 5m
- 49 Wash slides 2X in Stringent Wash ( 00:25:00 each wash) 25m
- 50 Remove slides from Stringent Wash and wash 2X in  Room temperature 2X SSC ( 00:02:00 each wash). Slides can be stored in 2X SSC for up to  01:00:00 1h 2m

Adding morphology markers

2h 15m



51 Prepare humidity chamber – clean with RNase AWAY. Line chamber with Kimwipes wetted with DEPC treated water

2m

51.1 NOTE – Morphology marker preparation will vary and should be empirically tested. Here we used unconjugated antibodies and thus modified the NanoString GeoMx protocol accordingly

52 Remove slides one at a time from 2X SSC, remove excess liquid with a Kimwipe and lay flat in humidity chamber

1m

53 Block sections in  200 μ L Buffer W for  00:30:00 with the lid closed

30m

54 Prepare morphology marker solution as per the table below

55

	A	B	C
	Reagent	Dilution	Volume
	Buffer W	-	(n) x 220 μ l
	GFAP	1:1000	(n) x 0.22 μ l
	P2RY12	1:100	(n) x 2.2 μ l

Morphology marker solution (n) indicates number of slides

56 Remove buffer W from slides by tapping on a Kimwipe, place back in humidity chamber and cover with  200 μ L morphology marker solution. Close the lid and incubate in humidity chamber for  01:00:00

1h

57 During incubation, prepare secondary antibody mixture as per the table below

58

	A	B	C
	Reagent	Dilution	Volume



	A	B	C
	Buffer W	-	(n) x 220 µl
	AF488	1:1000	(n) x 0.22 µl
	AF647	1:500	(n) x 0.44 µl

59 Wash slides 2x in 2X SSC

2m

60 Return slides to humidity chamber, cover with  200 µL secondary antibody mixture and incubate for  00:20:00

20m

61 During incubation, prepare nuclear stain as per the table below

62

	A	B	C
	Reagent	Dilution	Volume
	Buffer W	-	(n) x 220 µl
	SYTO83	1:1000	(n) x 0.22 µl

63 Wash slides 2x in 2X SSC

2m

64 Return slides to humidity chamber, cover with nuclear stain mixture and incubate for  00:15:00

15m

65 Wash slides 2x in 2X SSC, proceed to load slides onto the GeoMx DSP (see the GeoMx DSP Instrument User Manual) - <https://nanosttring.com/wp-content/uploads/2022/06/MAN-10152-01-GeoMx-DSP-Instrument-User-Manual.pdf>

If needed, slides can be stored in 2X SSC for 1 week at  4 °C



- 66 Once GeoMx DSP aspirates have been collected, proceed to library preparation as per the GeoMx DSP NGS Readout User Manual - <https://nanosttring.com/wp-content/uploads/2022/06/MAN-10153-01-GeoMx-DSP-NGS-Readout-User-Manual.pdf>