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GeoMx Whole Transcriptome Atlas (WTA) Assay with H&E-Guided ROI Selection for FFPE Human Lung Tissue Microarrays (TMA)

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Kenichi Okuda[1], Edison T. Floyd[2], Nicholas C. Pankow[2], Anna T. Rogers[3], Adam D. Pfefferle[3], Gabriela De la Cruz[2], Gloria S Pryhuber[4], Jim Hagood[5]

[1]Marsico Lung Institute/Cystic Fibrosis Center, University of North Carolina at Chapel Hill;
[2]Pathology Services Core, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill;
[3]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill;
[4]University of Rochester Medical Center;
[5]Pediatric Pulmonology, Department of Pediatrics, University of North Carolina at Chapel Hill

Kenichi Okuda: Division of Pulmonary and Critical Care Medicine, Department of Medicine, University of North Carolina at Chapel Hill;
Adam D. Pfefferle: Department of Genetics, University of North Carolina at Chapel Hill;

Human BioMolecular Atlas Program (HuBMAP) Method Development Community
Tech. support email: **Jeff.spraggins@vanderbilt.edu**

Kenichi Okuda
University of North Carolina at Chapel Hill

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We use this protocol and it's working

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Abstract

This protocol describes the workflow for performing the NanoString GeoMx Digital Spatial Profiler Whole Transcriptome Atlas (WTA) assay on FFPE human lung TMA samples using:

- DAPI as the sole morphology marker
- H&E staining on serial sections for structural guidance
- GeoMx FFPE Slide Prep Kit (Buffer R/S) for RNA target retrieval
- Standard NanoString WTA probe hybridization, stringent washes, UV cleavage, NGS library preparation, and sequencing

This version is optimized for normal human lung tissue microarrays (TMAs), in which morphology may not be fully resolved with DAPI staining alone, and accurate ROI placement is achieved through virtual overlay of H&E-stained serial sections.

Guidelines

1. CDC. “COVID-19 Personal Protective Equipment (PPE) for Healthcare Personnel.” Centers for Disease Control and Prevention, 1 Jan. 2020, **www.cdc.gov/coronavirus/2019-ncov/downloads/COVID-19-PPE.pdf**.
2. WHO. “Personal Protective Equipment for COVID-19.” World Health Organization, World Health Organization, 5 May 2020, **www.who.int/medical_devices/priority/COVID_19_PPE/en/**.
3. University of Rochester Medical Center. COVID-19 Safety Training, University of Rochester Medical Center , 15 June 2020, rochester.csod.com/LMS/LoDetails/DetailsLo.aspx?loid=e8469bef-ae75-4d87-9ec1-f5d33e4f1519&query=%3fq%3dCOVID19+Safety+Training&isCompletionRedirect=true&loStatus=16‰num=1#t=1.

Materials

Materials and Reagents

A. NanoString reagents

- GeoMx FFPE Slide Prep Kit (Buffer R, Buffer S)
- Human Whole Transcriptome Atlas (WTA) NGS probe set
- GeoMx Instrument Buffer Kit + Collection Plate
- GeoMx Seq Code Pack (A/B/C/D depending on run)
- DAPI stain (NanoString-specified or equivalent nuclear dye)

B. General reagents

- Xylene
- 100%, 95% ethanol
- PBS (RNase-free)
- Proteinase K (per GeoMx FFPE Slide Prep Kit instructions)
- DEPC-treated water
- 2× SSC and 4× SSC solutions
- Formamide (100%) for stringent washes
- Coplin jars
- Grace Bio-Lab HybriSlip

C. NGS library preparation and sequencing reagents

(Per NanoString standard WTA protocol)

- 5× PCR Master Mix
- Seq Code Primer Plates
- Beckman Coulter AMPure XP beads
- Qiagen EB Buffer
- Agilent TapeStation D1000 High Sensitivity ScreenTape
- Qubit 1X dsDNA High Sensitivity kit
- Illumina sequencing reagents

Equipment

- GeoMx DSP instrument
- Hybridization chamber oven (37 °C)
- Thermocycler (with heated lid)
- Fluorescence slide scanner integrated in GeoMx
- Plate centrifuge
- Magnetic stand (96-well format)
- Bioanalyzer or equivalent
- Illumina sequencing platform (MiSeq, NextSeq, NextSeq2000)

Troubleshooting

Safety warnings

Working with human tissue potentially carrying infectious organisms: All laboratories should perform a site-specific and activity-specific risk assessment and follow standard precautions when handling clinical specimens. Personnel will adhere to safe work standard protocol, including N95 mask, face shield, lab coat, closed shoes and double gloves, shoe covers, hair net, and sleeve covers. All activity will be behind the shield of biosafety cabinet and/or with mask and safety glasses. Biosafety level 2 + practices will be followed, and the work performed in the designated lab space that is covered by annually updated institutional biosafety committee approved protocol. All institutional biosafety measures are followed in any manipulation of these human tissues.

Ethics statement

Human tissue collection for this protocol requires prior approval by the users' Institutional Ethics Board or equivalent ethics committee.

FFPE slide preparation

1 FFPE tissue samples used in this protocol were prepared according to the following protocols:

- 602.2 Donor Acceptance Criteria for URMC HTC HuBMAP and LungMAP Inclusion **dx.doi.org/10.17504/protocols.io.bjuxknxn**
- 603.3 & 604.5_URMC_HTC_Whole Lung and Lobe Processing **dx.doi.org/10.17504/protocols.io.biz7kf9n**
- 611.2 URMC HTC Formalin-Inflated, Paraffin-Embedded Human Lung Tissue (FFPE) **dx.doi.org/10.17504/protocols.io.bjttknnn**
- HuBMAP lung tissue sampling protocol for dissection of specific levels of airways and processing to FFPE **dx.doi.org/10.17504/protocols.io.j8nlk91jdv5r/v1**
- Lung, extra-pulmonary airway and extra-pulmonary pulmonary artery samples were selected as BRINDL FFPE processed blocks **dx.doi.org/10.17504/protocols.io.kxygxejwdv8j/v3**. Blocks were chosen to represent 9 regions of the lung and extra-pulmonary structures. The majority of the selected cases had a history consistent with healthy lungs, minimal or no medications, illness or smoking prior to donation.
- TMA preparation: FFPE tissue blocks from specific lung regions from 6 donors (age range 20-59 yo) were selected, cored with 3mm tool and used to make four TMAs using protocol **dx.doi.org/10.17504/protocols.io.yxmvmmd46v3p/v2**. The resulting FFPE TMA blocks, approximately 1 × 2 cm, were serially sectioned (5 micron) for use across multiple assay modalities. Adjacent sections were used for the GeoMx assay and the corresponding H&E guide slide.

2 **Bake**

- Bake TMA slides at 37°C overnight. Followed by bake at 60°C for 2 hours.

3 **Deparaffinization**

- Accomplished on the Bond III automated stainer using Bond Dewax Solution and 100% Ethanol.

4 **Antigen retrieval (GeoMx Kit Buffer R/S workflow)**

- Use the manufacturer instructions from GeoMx FFPE Slide Prep Kit:

1. Antigen retrieval accomplished on the Bond III automated stainer using epitope retrieval buffer 2 (Tris EDTA, pH9) (Leica, AR9640) at 100°C for 20 minutes.
2. Followed by Leica enzyme retrieval kit (AR9551) at a concentration of 1ug/ml in 1xDEPC PBS at 37°C for 15 minutes.

(All timings and conditions follow the standard workflow described in the GeoMx FFPE Slide Prep manual.)

5 **Post-fixation**

As in HTAN protocol section:

- 10% NBF (Electron Microscopy Sciences, 15740-04) for 5 min.
- Neutralization buffer for 5 min × 2.
- Washes in Bond Wash in between.
- Upon leaving the Bond Machine slides are placed in x1 DEPC PBS then proceed to Probe Hybridization.

WTA Probe Hybridization

6 **Prepare hybridization mix**
- Dilute WTA probe set per NanoString instructions (typically 1:10 in Buffer R + DEPC water).

7 **Apply to slides**
- Add ~200 µL of hybridization mix per slide.
- Apply HybriSlip carefully to avoid bubbles.

8 **Hybridize**
- Incubate 16 hours at 37 °C in hybridization chamber.

Stringent Washes

9 **Prepare stringent wash solution**
- Mix 4× SSC: 100% formamide = 1:1.
- Prewarm to 37 °C.

10 **Wash slides**
- Remove HybriSlip.
- Wash slides in stringent wash solution: 25 min × 2 at 37 °C.
- Wash in 2× SSC for 5 min × 2.

DAPI Staining (Morphology Marker)

11 **Prepare DAPI Working Solution**
Per NanoString concentration guidelines.

12 **Incubate**
- Apply DAPI to slides for 5–10 min at room temperature.
- Rinse briefly in PBS.
- Keep slides hydrated in PBS until loading into GeoMx. (Since no protein markers are used, this becomes the sole morphology channel during DSP scanning.)

Prepare Serial H&E Slide for ROI Guidance

13 **Scan H&E slide**

- A serial section adjacent to the DSP slide is stained with H&E according to standard histopathology procedures.
- Use any brightfield whole-slide scanner.
- Export image in TIFF/JPEG format.

14 **Align H&E to DAPI**

In external software (Aperio ImageScope and WebViewer):

- Import the H&E and DAPI images.
- Perform manual or software-assisted alignment.
- Annotate anatomical regions (pre-selection of ROIs)

These annotations guide ROI placement on the GeoMx instrument.

Loading Slides into GeoMx DSP Instrument

15 **Start new run**

- "New / Continue Run" in GeoMx software.
- Load slide with DAPI-only morphology.

16 **Select panel**

- Human Whole Transcriptome Atlas (WTA) RNA v1.0

17 **Set imaging channels**

- FITC/DAPI channel only

• *DAPI* as the fluorophore.
• Set exposure time per optimization (typically 20–80 ms).
• Select DAPI as focus channel.

ROI Selection Guided by H&E Overlay

18 **View DAPI morphology**

- Identify nuclei-dense areas, target structures, boundaries.

19 **Reference annotated H&E**

- Use the aligned H&E images to infer:

• Airway epithelium
• Submucosal gland acini
• Submucosal gland ducts
• Submucosa
• Terminal bronchiolar epithelium
• Pulmonary artery adjacent to terminal bronchioles
• Respiratory bronchiolar epithelium
• Pulmonary artery
• Alveoli
• Lymphatic ducts

- Smooth muscle
- Peribronchial regions
- Pleura
- Nerve

20 **Select and draw ROIs**

- Use circular, rectangular, or polygon ROIs (max 660 µm radius or 660×785 µm).
- Match ROI borders to H&E-identified structures.
- No segmentation masks are used (single-compartment ROIs).

21 **ROI acceptance criteria**

- Avoid folds or tissue tears.
- Avoid acellular regions.
- Ensure adequate nuclei count (>20 cells typically required by NanoString).

UV Cleavage and Collection

22 Follow NanoString instrument instructions:

1. UV-cleave WTA probes from each ROI.
2. Collect aspirates into the GeoMx 96-well collection plate.
3. Seal plate with permeable membrane if proceeding immediately, or non-permeable if storing at −20 °C.
4. Dry down at 65 °C up to 1 hour, confirm complete dryness.
5. Rehydrate with 10 µL nuclease-free water.

Library Preparation (Standard NanoString WTA Protocol)

23 **PCR Reaction Setup**

From the GeoMx-NGS Readout Library Prep protocol:

- 5× PCR Master Mix: 2 µL
- Seq Code Primer Mix: 4 µL
- Rehydrated DSP aspirate: 4 µL
- Total: 10 µL per reaction

PCR cycling (UDG + amplification):

- UDG incubation: 37 °C, 30 min
- UDG deactivation: 50 °C, 10 min
- Initial denature: 95 °C, 3 min
- 18 cycles:
  - 95 °C 15s
  - 65 °C 60s
  - 68 °C 30s
- Final: 68 °C 5 min

24 **Pooling and Cleanup**

- Pool 4 µL from each well.
- AMPure XP cleanup (1.2× ratio).
- Second cleanup of the eluate per protocol.

25 **QC**

Expected library size ~150 bp (TapeStation).

Sequencing

26
- Illumina platform (NextSeq2000)
- 2 × 27 bp paired-end, 100 cycles
- 5% PhiX spike-in
- Loading concentration per instrument (MiSeq, NextSeq, NextSeq2000) per protocol.

FASTQ files processed through GeoMx NGS DND Pipeline → generates DCC files.

Data QC and Normalization

27 **QC thresholds**

- Remove low-read ROIs (< recommended threshold)
- Remove ROIs with insufficient nuclei
- Ensure negative controls and non-template control within expected ranges

Normalization

- Upper Quartile (Q3) normalization recommended for WTA.

Data Output, Storage, Analysis and Publication

28 Data were exported to storage folder and uploaded to Globus Directory using HuBMAP schema for file organization prior to submission for standardized analysis and HuBMAP Portal publication. The data were also processed and analyzed by the HuBMAP-Lung TMC investigators for use in hypothesis testing and multi-modality integration.

Notes

29
- Ensure tissue remains hydrated between steps to avoid cracking.
- DAPI-only morphology needs H&E guidance for accurate spatial interpretation.
- Normal lung TMA may contain highly heterogeneous architecture, necessitating careful ROI selection.
- For low signal regions, verify:

• Hybridization quality
• Tissue section thickness (5 µm recommended)
• Correct use of Buffer R/S

Protocol references

DOI: dx.doi.org/10.17504/protocols.io.kqdg3×157g25/v1
DOI: dx.doi.org/10.17504/protocols.io.261gedwndv47/v2
DOI: dx.doi.org/10.17504/protocols.io.n92ldpb29l5b/v1
DOI: **dx.doi.org/10.17504/protocols.io.bjuxknxn**
DOI: **dx.doi.org/10.17504/protocols.io.biz7kf9n**
DOI: **dx.doi.org/10.17504/protocols.io.bjttknnn**
DOI: **dx.doi.org/10.17504/protocols.io.j8nlk91jdv5r/v1**
DOI: **dx.doi.org/10.17504/protocols.io.kxygxejwdv8j/v3**
DOI: **dx.doi.org/10.17504/protocols.io.yxmvmmd46v3p/v2**