May 14, 2024

NanoString GeoMx DSP TMA-TNP Phase 4 WTA assay

Forked from NanoString GeoMx DSP TMA-TNP protein assay

DOI
dx.doi.org/10.17504/protocols.io.kqdg3x157g25/v1

Jinho Lee[1,1], Gabriel Zangirolani[1], Koei Chin[2], Heidi S Feiler[3], Christopher Corless[1]

[1]Knight Diagnostics Laboratory, OHSU; [2]OHSU; [3]Knight Cancer Institute, OHSU

NCIHTAN OMS Atlas

Jinho Lee
OHSU

DOI: dx.doi.org/10.17504/protocols.io.kqdg3x157g25/v1

Protocol Citation: Jinho Lee, Gabriel Zangirolani, Koei Chin, Heidi S Feiler, Christopher Corless 2024. NanoString GeoMx DSP TMA-TNP Phase 4 WTA assay. **protocols.io https://dx.doi.org/10.17504/protocols.io.kqdg3x157g25/v1**

Protocol status: Working
We use this protocol and it's working

Created: December 12, 2023

Last Modified: May 14, 2024

Protocol Integer ID: 92220

Keywords: NanoString, GeoMx, Digital Spatial Profiler, HTAN, TMA, DSP, cell profiling, cell type

Funders Acknowledgement:
Human Tumor Atlas Network
Grant ID: NIH NCI U2C
CA233280

Abstract

This protocol outlines the NanoString GeoMx Digital Spatial Profiler Whole Transcriptome Atlas (DSP WTA) assay that was applied in the Human Tumor Atlas Network (HTAN) Tissue MicroArrary (TMA) -TransNetwork Project (TNP).

The TMA-TNP evaluates various characterization and analytics methodologies on a large array of breast tumor samples representing a broad spectrum of disease state and subtype. A commercially available anonymized breast tumor TMA was purchased and serial sections were distributed. Participating HTAN Centers characterized the FFPE specimens using various imaging platforms and generated a spatially resolved cell type/state census using each center's method of choice. Data was recorded in a common repository to enable joint analysis.

The protocol that immediately precedes this one for TMA-TNP Phase 4 can be found at: **dx.doi.org/10.17504/protocols.io.ewov1o7wolr2/v1**. It describes FFPE block serial sectioning, slide processing and TMA sample distribution. Two compartments (Tumor and Stroma) in each TMA core were analyzed to determine cell-to-cell interactions in the tissues.

Materials

1. Materials
Slide Baking Oven
Antigen retrieval steamer
Humidity chamber
Hybridization chamber oven
Grace Bio-Labs hybriSlip

2. Reagents
Xylene
Ethanol
TBS
TBS-T
PBS
Formamide
Citrate buffer pH9.0
10% Neutral Buffered Formalin (NBF)
NBF stop buffer
DEPC-treated water
20x SSC
Proteinase K

3. Nanostring reagents
GeoMx Instrument Buffer kit / Collection plate (Item no. 100474)
FFPE slide prep kit (Buffer R / Buffer S) (Item no. 121300313)
Human Whole Transcriptome Atlas NGS probe (Item no. 12141102)
Solid tumor Morphology kit (Anti-PanCK / Anti-CD45 / SYTO-13) (Item no. 121300301)
GeoMx Seq Code Pack_AB (Item no. 121400201)

FFPE slide sample preparation

1h 59m

1 FFPE slide preparation

1.1 Bake FFPE slides at 60 °C for Overnight . 1d

1.2 Deparaffinize by sequential incubation with Xylene (00:03:00 twice), 100% EtOH (00:01:00 twice) and 95% EtOH (00:01:00). 9m

1.3 Briefly rinse the slides with PBS and incubate with 1x Tris EDTA antigen retrieval buffer (pH 9.0) for 00:15:00 (default condition for breast cancer tissue) at 100 °C in a steamer. 15m

1.4 Wash the slides with PBS for 00:05:00 (1 time). 5m

1.5 place the slides in a coplin jar and expose RNA targets with Proteinase K at [M] 0.1 picomolar (pM) 0.1 microgram/mL, default condition for 00:15:00 at 37 °C in waterbath 15m

1.6 Wash the slides with PBS for 00:05:00 (1 time) and proceed to the next step immediately 5m

Postfixation

1h 59m

2 Preserve tissue morphology for soft tissues

2.1 Transfer the slides in a coplin jar containing [M] 10 % volume NBF (neutral buffered formalin) buffer and incubate them for 00:05:00 5m

2.2 Transfere the 10slides in a coplin jar containg NBF stop buffer (Tris base 24.5 g and glycine 15 g in water 2 L) and incubate them for 00:05:00 twice 10m

2.3 Wash the slides with PBS for 00:05:00 5m

in situ hybridization 1h 18m

3 RNA target hybridization

3.1 Prepare hybridization solution with Buffer R (warm at Room temperature before opening) and thaw RNA detection probes on ice. Before use, mix thorouhgly by pipetting. Once thawed, refrigerate at 4 °C for up to 3 months

3.2 Mix RNA probes at 1:10 dilution (eg. mix 25 µL of RNA probe mix in 200 µL of Buffer R and 25 µL of DEPC-treated water to make up to final volume 250 µL per slide)

3.3 Clean all equipment (especially for the hybridization chamber) with RNase AWAY and allow to dry or rinse with DEPC-treated water to avoid contamination

3.4 Wet Kimwipes with 2x SSC or DEPC-treated water and place them in the hybridization chamber to keep the slides from drying out

3.5 Add 200 µL of hybridization solution to each slide and gently apply a Grace Bio-Labs hybriSlip onto the slide. Avoid the formation of air bubbles on the tissue

3.6 Incubate the slides in the hybridization chamber oven at 37 °C for 16:00:00 (up to 24:00:00) 1d

Stringent washes

4 Off-target probe removal

4.1 100% Formamide (FA) solution should be adjusted to Room temperature before preparing the stringent washing solution. Mix 4x SSC and 100% FA in equal volume and prewarm the solution in two coplin jars at 37 °C in water bath

4.2 Gently remove the hybriSlip covers from the slides. Transfer slides in the stringent washing solution and wash twice at 37 °C for 00:25:00 in water bath. 25m

4.3 Wash slides with 2x SSC for 00:05:00 twice 10m

Tissue staining

5 Staining with morphological markers

5.1 Block the slides with Buffer W for 00:30:00 at Room temperature 30m

5.2 Freshly prepare visualization markers (PanCK and CD45, Alexa-fluorescent conjugated) in 1:40 and Syto13 (1:10) dilution with Buffer W to a volume of 220ul per slide.

5.3 Incubate slides and visualization markers for 01:00:00 at Room temperature 1h

5.4 Wash the slides with 2x SSC for 00:05:00 twice 10m

Sample loading to GeoMx

6 Sample loading onto DSP GeoMx

6.1 Log onto GeoMx software and start with **"New / Continue Run".**

6.2 After loading slides with the collection plate information, select (or create) the Readout group for NGS project. DSP is ready to scan slides. 30m

6.3 A slide scan name is created and panel/visualization marker information is selected as below: 10m

In the **Probe Reagent Kit** field, select **Human NGS Whole Transcriptome Atlas RNA V1.0**

1. Select the FITC/525 nm, Cy5/568 nm and Texas Red/615 nm channels.
2. For **FITC/525 nm, select SYTO 13** as fluorophore, DNA as biological target and enter 50 as exposure time.
3. For **Cy5/568 nm, select Alexa 532 as fluorophore, PanCK** as biological target and enter 300 as exposure time.
4. For **Texas Red/615 nm, select Alexa 594 as fluorophore, CD45** as biological target and enter 300 as exposure time.
5. Select FITC/525 nm as focus channel

6.4 When the scan area for each slide has been adjusted with sensitivity setting, select **Scan**.

ROI selection and sample collection

7 ROI selection and AOI segmentation

7.1 After scanning is done, each color channel intensity is adjusted to show visualization markers along with tissue or cell line property

7.2 Each ROI is determined and selected by pathologist's guide, and drawn with circle (maximum 660um radius), rectangle (maximum 660x785um) or polygonal shape (maximum 660x785um). Due to the limitation of scan area in the slide loading slot, two same TMA slides were used to cover half of 88 cores in each slide (88 cores were embedded and slightly shifted to either left or right side of slide)

7.3 In the segment menu, 2 segmentation classes (Tumor, stromal and others) are added and parameters are set in the following order:

For Tumor segmentation (Segment 1), Alexa 532 (PanCK) is set to positive ("+") and the others set to ignore ("0") for the tumor collection

For stromal segmentation (Segment 2), Alexa 532 (PanCK), Alexa 594 (CD45) and FITC 525 (SYTO 13) were set to ignore ("0") to collect all other regions

Then click **Generate Segments**.

7.4 Once all segments are automatically generated, each channel parameter needs to be manually re-adjusted with pathologist's input to confirm if the segmentation is correctly done.
Caution: less than 20 cells in each segment is removed from collection due to threshold for low signal.

7.5 Once all AOI segmentation is complete, Exit Scan Workspace button icon is clicked to approve ROI selection and samples are collected in 96-well plate.

NGS library preparation and sequencing

1h

8 Sample sequencing

8.1 The sample collection plates were completely dried out at 65 °C for 01:00:00 and shipped to the genomics core at Dana Faber Cancer Institute for NGS library preparation and sample sequencing

1h

8.2 FASTQ raw sequencing files were generated and converted to DCC (Digital Count Conversion) files which can be imported to DSP for the analysis

QC and data normalization

9 QC DSP data and normalization

9.1 Select and queue the slides to analyze using **"New Analysis"** in the DSP server.

9.2 Determine the New Analysis file name and save it in the designated folder.

9.3 Open an analysis file and perform the **QC** with default parameters except Negative control (4 or lesser), No template PCR control (NTC count, 1,000-10,000) and minimum nuclei counts (50-100)

9.4 (Optional) QC passed samples are processed with Biological probe QC (only applying to CTA)

9.5 QC data were filtered with low expression across all segments. Choose 5-10% of segments frequency or higher of Limit of Quantification (LoQ) or both.

9.6 Upper Quartile (Q3) normalization from all target were performed after filtering out low expression targets (Q3 normalization method is recommended since larger number of targets may increase the noise of geomean normalized data) and data was exported in csv format