

## GeoMx Digital Spatial Profiler (DSP) nCounter Protein Assay SOP

### Version 1.0

#### 1. Technical platform

The GeoMx® Digital Spatial Profiling (DSP) technology is a novel platform developed by NanoString. This product relies upon antibodies coupled to photocleavable oligonucleotide tags. After the incubation of antibodies to slide-mounted tissue sections, cell types of interest with fluorescent morphology markers to create fluorescent images for selecting region of interest (ROIs). The oligonucleotide tags are released from discrete regions of the tissue via UV exposure. Released tags are quantitated in an nCounter® and counts are mapped back to tissue location, yielding a spatially resolved digital profile of analyte abundance.

This SOP describes the step-by-step process to perform the DSP assay at TMP-IL.

#### 1. **Analyte(s):**

- 1) Morphology Biomarker: PanCytokeratin, CD45
- 2) Profiling Core and modules:
  - i. *GeoMx Immune Cell Profiling Core*: Beta-2-microglobulin; CD3; CD56; CTLA4; GZMB; PD-1; CD11c; CD4; CD68; Pan-cytokeratin; HLA-DR; PD-L1; CD20; CD45; CD8; Fibronectin; Ki-67; SMA; Negative controls (Ms IgG2a, Ms IgG1, and Rb IgG); Positive controls (S6, Histone H3 and GAPDH).
  - ii. *GeoMx IO Drug Target Module*: 4-1BB; LAG3; ARG1; OX40L; B7-H3; STING; GITR; TIM-3; IDO1; VISTA
  - iii. *GeoMx Immune Activation Status Module*: CD127; CD25; CD27; CD40; CD44; CD80; ICOS; PD-L2
  - iv. *GeoMx Immune Cell Typing Module*: CD14; CD163; CD34; CD45RO; CD66b; FAPalpha; FOXP3
  - v. *GeoMx Pan-Tumor Module*: MART1, NY-ESO-1, S100B, Bcl-2, EpCAM, Her2, PTEN, ER-alpha, PR
  - vi. *GeoMx Cell Death Module*: BAD, BCL6, BCLXL, CD95/Fas, GZMA, NF1, Cleaved Caspase 9, p53, PARP, BIM
  - vii. *GeoMx PI3K/AKT Signaling Module*: Phospho-AKT1 (S473), Phospho-GSK3B (S9), Phospho-GSK3A (S21)/Phospho-GSK3B (S9), INPP4B, PLCG1, Phospho-PRAS40 (T246), Phospho-Tuberin (T1462), Pan-AKT, MET
  - viii. *GeoMx MAPK Signaling Assay*: BRAF, EGFR, Phospho-c-RAF (S338), Phospho-JNK (T183/Y185), Phospho-MEK1 (S217/S221), Phospho-p38 MAPK (T180/Y182), Phospho-p44/42 MAPK ERK1/2 (T202/Y204), pan-RAS, p44/42 MAPK ERK1/2, Phospho-p90 RSK (T359/S363)

#### 2. Reagents, controls, and calibrators

##### Reagents

- GeoMx DSP nCounter Reagent Kits
  - GeoMx DSP Collection Plate (Cat# 100473)
  - GeoMx DSP Instrument Buffer Kit (Cat# 100474)
  - GeoMx Protein Slide Prep Kit (Cat# 121300312)
  - GeoMx Hyb Code Pack Protein (Cat# 121300401)
  - nCounter Master Kit (Cat# 100052)
  - GeoMx Solid Tumor TME Morphology Kit (Cat# 121300301)
- GeoMx DSP Protein Panels
  - GeoMx Immune Cell Profiling (Cat# 121300101)
  - GeoMx IO Drug Target Assay (Cat# 121300102)
  - GeoMx Immune Activation Status Assay (Cat# 121300103)
  - GeoMx Immune Cell Typing Assay (Cat# 121300104)
  - GeoMx Pan-Tumor Assay (Cat# 121300105)
  - GeoMx Cell Death Assay (Cat# 121300112)
  - GeoMx PI3K/AKT Signaling Assay (Cat# 121300113)
  - GeoMx MAPK Signaling Assay (Cat# 121300111)
- Leica BOND Reagents
  - BOND Research Detection System (includes 6x 30 mL Open Containers) (Cat# DS9455)
  - BOND Epitope Retrieval Solution 1-1L (RTU) (Cat# AR9961)
  - BOND Dewax Solution – 1L (RTU) (Cat# AR9222)
  - BOND Wash Solution 10X Concentrate – 1L (Cat# AR9590)

## Equipment

- GeoMx Digital Spatial Profiler from NanoString
- BOND RX Fully Automated Research Stainer from Leica Biosystems

### 3. Semi-Automated Steps (*GeoMx nCounter Slide Preparation User Manual*)

Deparaffination, epitope retrieval, Incubation and counterstaining

#### 1) **Leica Automated slide preparation** (*GeoMx nCounter Slide Preparation User Manual*)

- i. Bake at 60°C for 30 minutes
- ii. BOND Dewax Solution (AR9222) at 72°C with 3 changes
- iii. 100% Alcohol with 3 changes
- iv. Epitope Retrieval solution (ER1, pH9, AR9961) at 100°C for 20 minutes
- v. Buffer W (NanoString) incubation for 60 minutes

#### 2) **Primary Antibody incubation** (*GeoMx nCounter Slide Preparation User Manual*)

- i. Unload the slides from the instrument and Draw hydrophobic barrier around the tissue
- ii. Apply high-plex oligo-coupled antibody cocktail of protein panels + morphology markers to the tissue
- iii. Place the slide in humidifier chamber and Incubate at 4 °C overnight.
- iv. Stringently wash with wash buffer to remove any excess cocktail solution.
- v. Apply DNA counterstaining with SYTO 13 (Nanostring)

### 4. **DSP Scanning, ROI selection and AOI collection** (*GeoMx nCounter DSP Instrument User Manual*)

- i. Scan the slide at low resolution: Use visible wavelength low-plex imaging to establish tissue “geography”.
- ii. Scan the slide at high resolution (20X): Select Regions-of-Interest (ROIs) for high-plex profiling and segment Area-of-Illumination (AOIs) as needed for digital profiling.
- iii. AOIs are collected in collection plate

5. **AOI Hybridization and nCounter Readout** (*GeoMx nCounter Readout User Manual*)

1) **AOI hybridization** (*GeoMx nCounter Readout User Manual*)

- i. Combine Core probe R and module probe Rs and Add probe U and hybridization buffer to Probe Rs mixture
- ii. Transfer an aliquot of mixture to each GeoMx code pack (A – H) and Dispense each GeoMx HybCode Master Mix across a row of a new plate and transfer DSP aspirate to the plate
- iii. Hybridize overnight at 67 °C for 16 hours

2) **Readout on nCounter MAX** (*GeoMx nCounter Readout User Manual*)

- i. Load hybridization products to Prep Station and transfer analytes to a cartridge
- ii. Load the cartridge to Digital Analyzer and download nCounter readout file.
- iii. Upload readout files to DSP and Map back to tissue section.

**Notes:**

- 1) Protein Panels varies among projects, which can be up to 7 panels.
- 2) ROI selection and AOI segmentation are following experimental design.
- 3) Customized Morphology Markers can be developed by project design.

**Reference:**

<https://www.nanostring.com/geomx-online-user-manual>

GeoMx nCounter Slide Preparation User Manual

GeoMx nCounter DSP Instrument User Manual

GeoMx nCounter Readout User Manual



MAN-10087-08\_GeoMx-nCounter\_Slide\_Pr



SEV-00065-07\_GeoMx-nCounter\_DSP\_Instru



MAN-10089-07\_GeoMx\_nCounter\_Readou

**Immunohistochemistry and Digital pathology Laboratory**

**GeoMx DSP scientist leads:**

*Wei Lu, MD, PhD Sr. Research scientist.*

**Director: Luisa Maren Solis Soto, MD Assistant Professor MD Anderson Cancer Center**

**Signature (Luisa M. Solis Soto)**

**Date: 09/06/2022**