CELLECTA

# Cellecta GeoMx Service Guide

October 2020

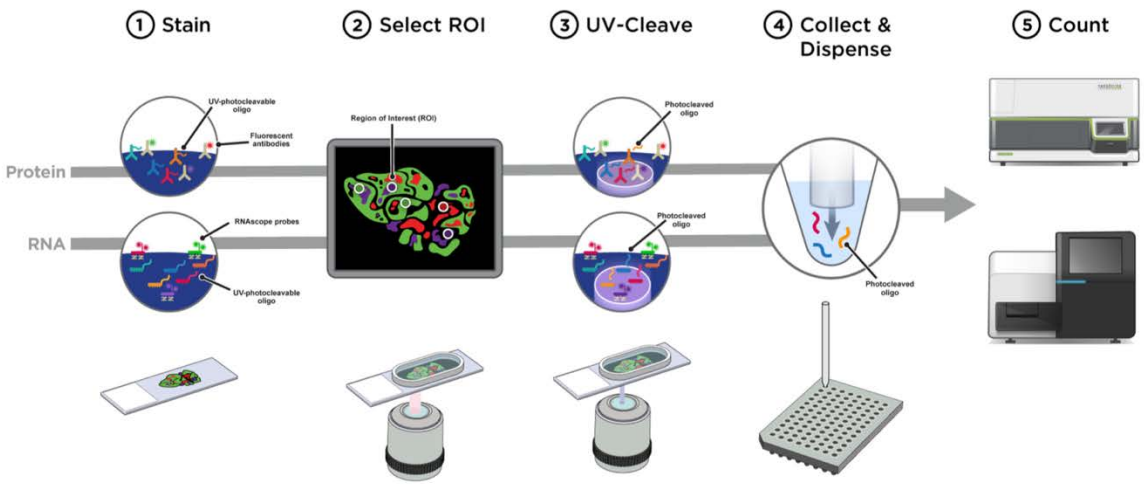
## Table of Contents

CELLECTA

# Overview

## GeoMx DSP Introduction

The Nanostring® GeoMx® Digital Spatial Profiler (DSP) is a new technology platform which enables spatial analysis of RNA and protein in tissue specimens on slides. Samples are stained with a large panel of pre-mixed biological probes--each incorporating a unique, UV-cleavable DNA barcode--and fluorescently-labeled morphology markers, used to visually or computationally elucidate tissue morphology. Using the fluorescent morphology markers for guidance, defined regions of interest (ROIs) are illuminated with UV light and then cleaved barcodes from these ROIs are counted by Illumina NGS sequencing. This allows for high-plex RNA from spatially resolved regions within the tissue. The resulting counts constitute an expression profile of key targets across that ROI and elucidate the biology specific to that region.




**Cellecta provides GeoMx spatial transcriptomic profiling as a custom service.** We expect to tailor the service to the unique needs of investigators and their projects. The sections below provide general requirements and describe the typical workflow and expected results. Specific details will be discussed with each investigator to optimize the study design and analysis plan.

CELLECTA

# Samples

Standard GeoMx profiling is done on formalin-fixed paraffin-embedded (FFPE) tissue sections. Typically, a customer would provide the unstained sections to Cellecta. If needed, we can recommend histology service labs that are able to process fixed tissue to generate the needed FFPE slides.

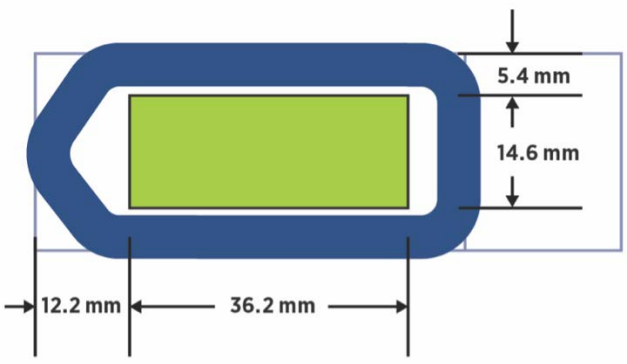
The GeoMx instrument can process up to 4 slides per run. This may suffice for some projects, others will use multiple runs to analyze a greater number of samples.

Here are some key points about the samples and their preparation for GeoMx analysis.

**Slide Preparation**

1. Unstained tissue sections should be 5 µm thick on Superfrost Plus slides.

Tissue sections must be placed in the Scan Area (the green area as shown in the slide diagram) in the center of the slide and be no larger than 36.2 mm long by 14.6 mm wide. They should not overlap with the slide gasket or the Tip Calibration area (this is the triangular region to the left of the green scan area in the slide diagram). If sections are larger than this size and/or placed off-center, the tissue located outside the Scan Area will not be scanned by the GeoMx DSP instrument.




2. Age of sections: Ideally, freshly cut tissue section samples (slides) are sent to Cellecta for analysis within 2 weeks of being cut.

3. Archival samples: Nanostring reports successful analysis of FFPE samples stored as blocks for years, cut and analyzed as per #2 above.

4. Tissue microarrays processed as FFPE sections can be analyzed as well as standard tissue sections.

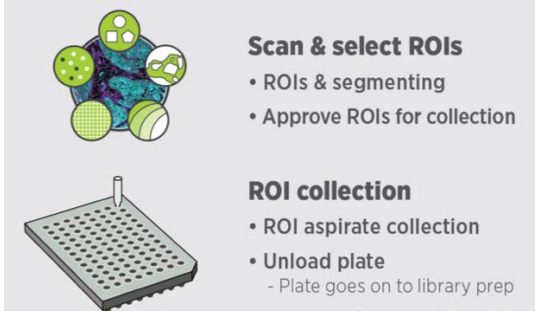
5. Depending on the experimental question, it may be possible to place 2 or 3 sections on the same slide for analysis

## Profiling Run

A typical experimental run is summarized in the figures below.

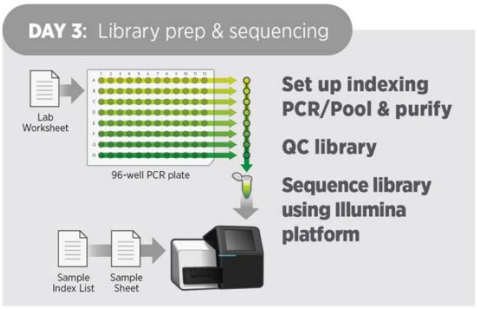
**Day 1.** The FFPE slides are baked and deparaffinized and incubated with the panel of probes overnight (e.g. the Cancer Transcriptome Atlas panel)

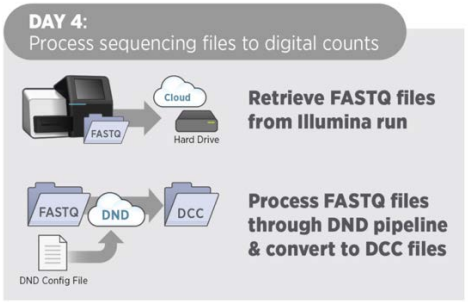

**Day 2.** The slides are washed and the morphology markers (antibodies, DNA stain) are applied. The slides are loaded into the GeoMx instrument and imaged to allow visualization of the antibody markers (e.g. pan-CK, anti-leukocyte CD45, et al.) and DNA stain (nuclei marker).

At this point the regions of interest (ROIs) for transcriptome profiling are selected. This critical step can be executed as per prior guidance from the investigator. Alternatively, a live web-based conference can allow the investigator to view the stained slide and offer real-time input into the ROI selection performed by Cellecta scientists.

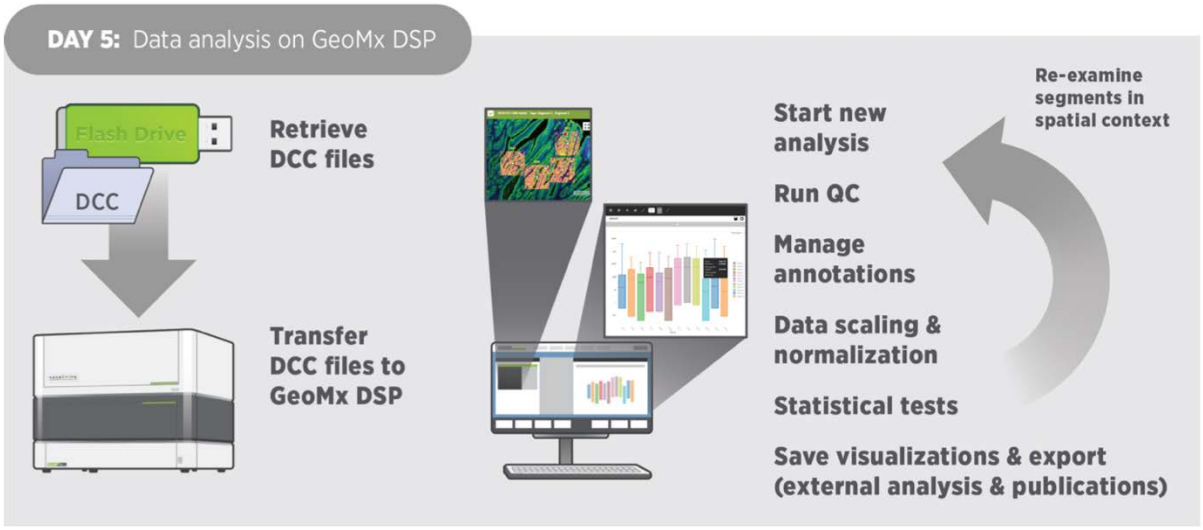
**Days 3 & 4.** The collected probe markers are processed into a library for Illumina NGS sequencing. The fastq files are processed to generate the DCC files used by GeoMx software for further analysis.




## Sequencing Data Analysis

**Day 5.** Analysis of gene expression within the ROIs is performed using the GeoMx software. The overall workflow includes QC analysis, scaling and normalization, quantitation of gene expression within ROIs, statistical analysis of differential expression between pairs of ROIs or groups of ROIs, and pathway analysis.




The analysis workflow is guided by prior discussions with the investigator that define the questions being addressed and the desired comparisons of different ROIs.

**Specific tools and options for the analysis include:**

- **Scaling**: The analysis can adjust counts to area or to the number of cells. Counts can be scaled to the geometric mean, median, or mean of the detected area or number of nuclei.

- **Normalization**: This type of data transformation balances the results between segments within an analysis using the counts from a specific set of probes. Q3 (3rd quartile of all selected targets) is the recommended normalization method. Additional options are normalization to Housekeeping genes, to Selected Probe sets, or to Custom Probes.

- **Ratio Builder**: This allows creation of ratios to estimate the relative expression of target probes. This can be used to compare expression of targets in a segment to the average of a group of segments delineated by tags, to compare expression of target between segment pairs from within an ROI, or to estimate relative expression of a target probe within a segment.

- **Statistical tests**: Two groups of the following tests may be run.
    - **Unpaired T- test**: useful for comparing two groups of independent samples
    - **Paired T- test:** useful for comparing two groups with a natural paired structure, e.g. pre- vs. post- treatment, or Tumor vs. Immune ROIs from the same collection of samples
    - **Mann-Whitney U-test:** useful for data that is extremely skewed or heavy-tailed
    - **Linear mixed models:** useful for data with repeated measurements from each sampling unit, e.g., multiple ROIs from each sample

- **Pathway Analysis:** This is available for datasets after a t-test or linear mixed model has been run and in datasets with 1000 or more targets

## Report

A typical workflow is completed with a document that reports the study design, procedures used, and the results obtained. Reports will be customized for the investigator's needs but will generally include both text and digital files that cover:

- **Summary** of the objectives and goals as discussed at the start of the project
- **Images** (and high-resolution image files) corresponding to the stained slides and ROIs of interest
- **Data analysis elements** including QC, normalization methods, expression count tables (also provided as files), differential gene expression and pathway analysis with statistical tests as appropriate and guided by the investigator

After review of the report, an investigator can perform additional data analysis using the gene count table file provided or request that Cellecta perform additional direct analysis using the GeoMx software.

###