Single cell protein profiling of immune cells within the tumor microenvironment using NanoString GeoMX™ Digital Spatial Profiler

**Summary**

GeoMX™ Digital Spatial Profiler (DSP) is a novel, highly multiplexed assay platform that digitally and spatially characterizes protein and RNA expression within intact tissue sections. Here, we present a proof-of-concept study on multiplexing single cell profiling using the Nanostirng GeoMX™ Digital Spatial Profiler (DSP), and next-generation sequencing (NGS) platform coupled with single cell DSP resolution to high throughput single cell profiling in intact FFPE tissue.

**Key results:**
- Single cell resolution in FFPE demonstrated using GeoMX™ DSP resolution, context information of each single cell preserved.
- Multiplexing protein expression analysis at single cell level.
- High reproducibility through various tumor types over 6 tumor tissues and close to 400 single cells analyzed.
- Single cell analysis using digital PCR quantification/heterogeneity of T cells in tumors.

**Key advantages of NGS readout of DSP:**
- More IO assays can be multiplexed and processed in a single NGS run.
- High throughput workflow with digital PCR extraction performed in plates and automation to automation.
- Single cell DSP technology accessible to any geneticist with competence with nanoscale NGS platforms for single cell analysis using digital PCR quantification/heterogeneity of T cells in tumors.

**NGS readout enhances the throughput of scDSP without altering sensitivity and DSP data resolution.**

The throughput of scDSP assay is up to 36x at a time, but NGS has much higher capacity for multiplexing analysis. To demonstrate that NGS readout increases throughput for scDSP analysis capacity, 384 scDSP samples were collected and divided into two sets. One set was hybridized in situ with Nanosting SNP assay kits with 8 individual runs. The other was analyzed with NGS with collapsing all the 384 samples into a single NGS run. The data below show that the NGS readout enhances throughput up to 384 per run without altering sensitivity, spatial resolution, or digital counting.

**Conclusions**

1. Successfully demonstrated that GeoMX™ DSP analyzes multiplexing protein expressions in single cells.
2. DSP-NGS readout was determined to be accurate, robust, high throughput based on reproducibility, cross platform concordance, and validation of digital counts.
4. High-throughput NGS readout enables large cohort discovery-based applications, including comprehensive differential expression profiling of single cells across entire tissue sections.

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Researchers interested in participating in NanoString’s technology access program for its Digital Spatial Profiling technology should contact us at TAP@nanosting.com.