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Spatially resolved detection of T cell receptor clonality elucidates spatial relationships between TCR expression, immune infiltration and cancer-associated pathways

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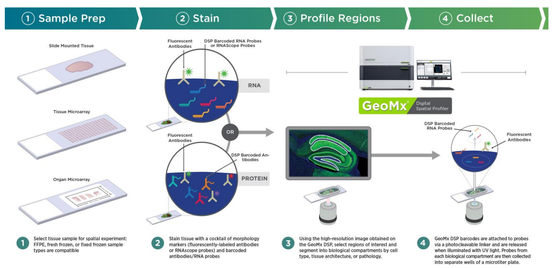
Abstract

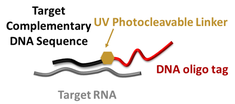
As T cells mature, genes encoding T cell receptor (TCR) segments are somatically recombined to generate a diverse repertoire of receptors specific to unique antigens. The resultant TCR diversity, and subsequent clonal expansion events, are critical in understanding the adaptive immune response to pathogens and cancers. Many methods have been developed to determine specific clonotypes and overall TCR diversity present in various tissues; however, nearly all fail to capture spatial orientation and arrangement of T cells engaging with their microenvironment. Here, we present an expanded TCR profiling panel for the GeoMx® Digital Spatial Profiler that can be combined with the GeoMx Cancer Transcriptome Atlas (CTA) or Human Whole Transcriptome Atlas (WTA), representing the first spatial assay for simultaneous quantification of all functional TCR constant, variable and joining segments *in situ*. We validated the performance of the TCR probe pool in inflamed tonsil and cell pellet arrays. We next used the GeoMx TCR spike-in panel to characterize intra- and inter-patient TCR heterogeneity in a cohort of 68 T cell lymphomas. T cell lymphomas are characterized by a dominant clone, corresponding to the tumor, and a population of potentially tumor-targeting T cells. Our results demonstrate the ability to link the spatial context of TCR segment expression in both malignant and non-cancerous T cells with the presence of other immune cells and cancer-associated pathways. Together, the combination of our TCR spike-in panel with the CTA or WTA illuminates T cell phenotypes, signaling pathways, population dynamics, and transcriptomic changes, yielding an unparalleled view of the T cell response in any context.

GeoMx® DSP enables direct *in situ* expression profiling



The **GeoMx Digital Spatial Profiler (DSP)** contains a tunable micromirror array which enables profiling of distinct tissue compartments, complex morphological structures, and cell populations dispersed throughout a tissue.

RNA Analysis: Antisense Detection Probe



Tagged Oligonucleotide Chemistry

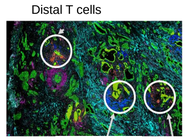
GeoMx Digital Spatial Profiler (DSP) uses oligonucleotides which hybridize to target mRNAs to quantitatively read out DNA tags which are selectively released *in situ* by specifically shining UV light into certain regions of the tissue

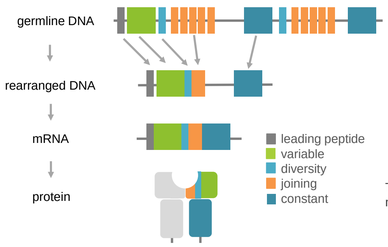
TCR Spike In Panel enables *in situ* expression profiling of TCRs

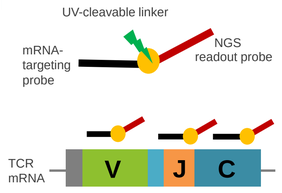
The human TCR spike in panel covers all **variable** and **joining** gene segments for alpha, beta, gamma, delta chains, and:

- Addresses spatial heterogeneity of TCR clonality and tissue response in FFPE
- Is designed to be added to other NGS readout panels (CTA or WTA)
- Has minimal target site cross-reactivity

	TCR profiling panel (Alpha program)
TCR chains	α, β, γ, δ
TCR segments	V & J
Total TCR targets	203
Positive controls	6 T cell markers
Negative controls	50 probes



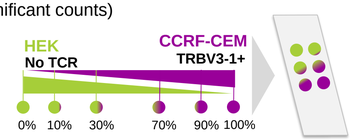




TCR spike in panel detects the correct TCR in an FFPE cell pellet array

Profiling a cell pellet array containing a dilution of the T cell lymphoma cell line (CCRF-CEM) into an epithelial cell line demonstrated

- detection of known TCRs expressed by CCRF-CEM
- high specificity (other TCRs not detected with significant counts)



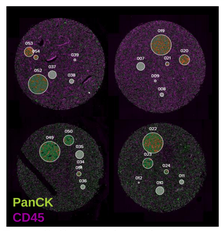


Figure 3: Cell pellets containing different densities of CCRF-CEM. Cells stained with CD45 are shown in purple, panCK in green.

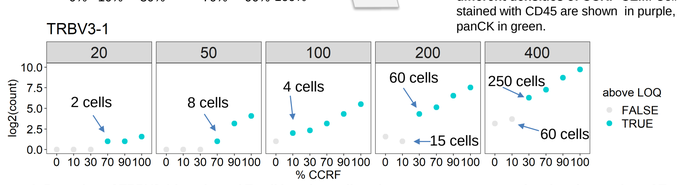


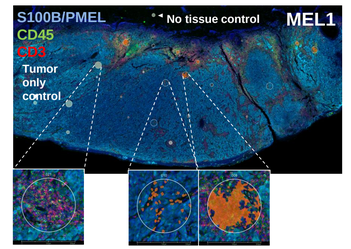
Figure 1: **Detection of TRBV3-1 in a titrated T cell lymphoma line shows counts are proportional to the number of T cells present.** Counts of TRBV3-1 in a titrated cell pellet array as percent of CCRF-CEM cells increases across ROIs 20, 50, 100, 200, and 400uM in diameter. Cell numbers shown are approximate.

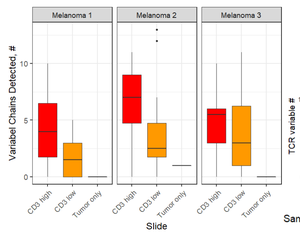
Spatial resolution of TCRs in three melanoma samples

Melanoma samples (n = 3) were profiled with the TCR spike in panel to evaluate detection of TCR chains within T-cell segmented regions as well as within tumor only regions of interest (ROIs)

- 25 ROIs were selected in each tissue for profiling with the TCR spike in panel
- Little to no evidence of TCR detection in tumor-only ROIs
- Cell number independent of detection of TCRs
- Detection of γ/δ TCR chains observed in two of the three melanomas along with sample-specific expanded variable chains

ROI Types	Segmentation Strategy	Diameter
CD3 high	9 segmented 3 geometric (no segmentation)	4 x 50um 4 x 100 um 4 x 200 um
CD3 low	9 segmented 3 geometric (no segmentation)	4 x 50um 4 x 100 um 4 x 200 um
Tumor	1 geometric	1 x 100 um





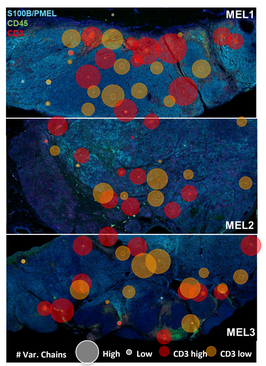
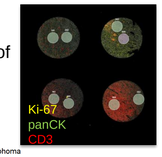


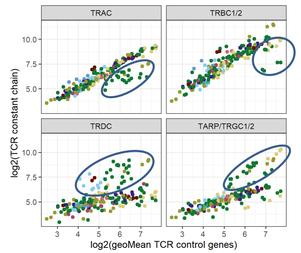
Figure 2: Spatial Arrangement of Variable Chains & Clonal Heterogeneity. Spatial detection of TCR chains across multiple ROI strategies. Spatial localization and diversity of T-cell variable chain utilization shown for each sample.

Transcriptomic characterization of γ/δ-derived subset within T cell lymphoma cohort

A cohort of T cell lymphomas (n=68) were profiled with the TCR spike in panel

- 184 ROIs selected across all cores
- Enrichment of γ/δ TCR chains and depletion of α/β chains observed in a subset of samples





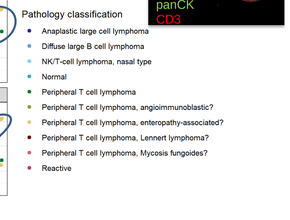


Figure 3: Correlation of TCR constant chains with TCR spike-in control genes. Circled samples are enriched for gamma/delta constant chains and depleted for alpha/beta constant chains.

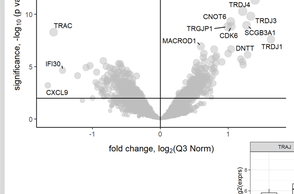


Figure 4: Cores enriched for gamma/delta show enrichment for related genes. Genes on the right of x=0 are enriched in gamma/delta-rich samples; for example, *DNTT* is involved in T-cell maturation and V(D)J recombination. Genes to the left of x=0 are enriched in alpha/beta-rich samples.

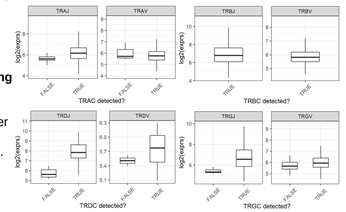


Figure 5: Detection of corresponding V and J segments correlates with detection of TCR constant chains. Samples are divided based on whether each constant chain was detected. *TRBC1/2* was detected in all samples.

Conclusions

- Together, the TCR spike-in panel and GeoMx RNA atlas panels enable *in situ* profiling of specific cell types and their neighboring cells
- T-cell receptor gene segments were confirmed to be sensitively and specifically captured by GeoMx
- Diverse T-cell populations detected from melanoma, along with sample-restricted detection of γ/δ T-cells
- GeoMx simultaneously differentiated α/β- from γ/δ-derived T cell lymphomas on a tissue microarray and identified genes enriched in one subtype vs. the other
- Studies are ongoing to validate individual probe performance and benchmark against TCR sequencing technologies