

# Spatially-resolved measurement of intratumoral T-cell Receptor diversity in Melanoma with the GeoMx Digital Spatial Profiler

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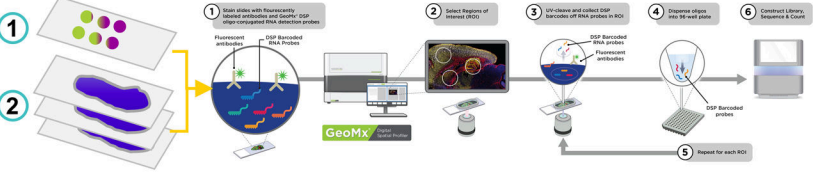
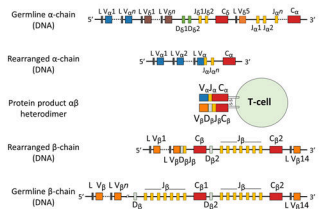
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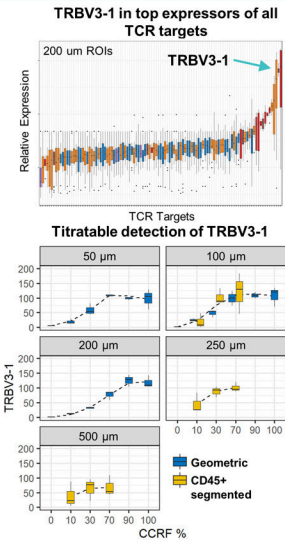
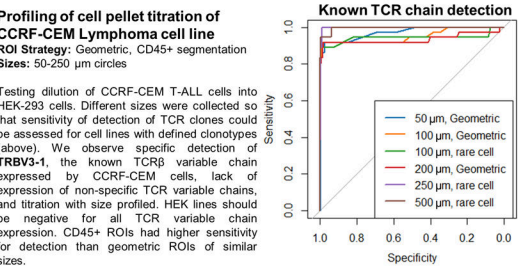
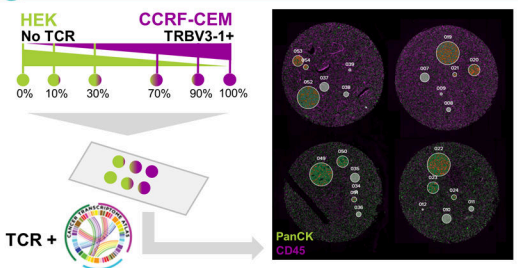


## Rationale for developing TCR specific readout for GeoMx DSP

- Diversity & clonality of the T-cell Receptor (TCR) repertoire is an important biomarker of active immune response in cancer
- The physical distribution of T-cells within the microenvironment reflects the status of the ongoing immune response and potential to respond to immunotherapy
- GeoMx digital spatial profiling can potentially be used to characterize TCR utilization by profiling constant and variable regions of  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  TCRs in addition to gene expression
- We show proof-of-principle data on the sensitivity and specificity of our approach using a series of cell pellet arrays
- Further, we demonstrate detection of multiple TCR variable regions in a series of melanoma tumors, sampling from multiple regions within the tissues yielding insights about the TCR repertoires prevalence and distribution within tumors

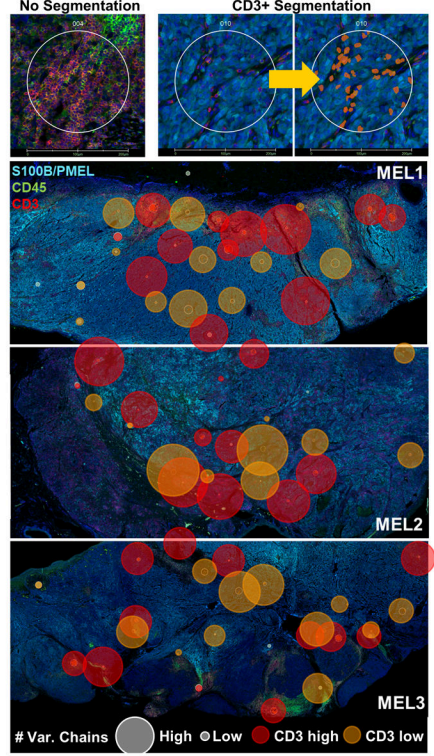


## 1 Sensitive and specific detection of TCR variable chains from cell pellet arrays



## 2 GeoMx enables spatially resolved determination of TCR chain utilization and estimated clonality

**Clonality and Diversity Assessment by GeoMx**  
TCR chain utilization was characterized for 3 T-cell infiltrated primary melanoma samples using circular ROIs with or without segmentation based on CD3+ staining. ROIs were placed in regions with either high CD3+ staining, low CD3+ staining or tumor regions with no CD3+ staining to assess TCR chain expression. Bubbles show low (0) to high (13) unique TCR variable chains detected within the ROI.



**Defining Patient-specific TCR clonotypes**  
TCR probes against all variable and constant segments, as well as targets for CD3, CD4, CD8, and TRAT1 were assessed relative to background (top right). For each ROI (x-axis) we observe consistent detection of constant regions & CD3/4/8 targets, but unique patterns of TCR variable regions across patients, with common variable chains restricted to each patient.



## Conclusions

- TCR probes developed for use in combination with GeoMx NGS assays:
- Show strong concordance with known cell line expression
- Have low background signal across cell lines and in tumor only ROIs
- Identify diverse variable chain distributions across distinct regions in melanoma samples
- Enable characterization of TCR chain utilization in a spatially resolved manner

