A simple and integrated workflow for deep proteomic and transcriptomic analysis of sorted cell populations

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In addition to the long standing efforts to understand and manipulate the immune system in the treatment of autoimmune and other immune-related diseases, it is increasingly becoming a direct target for cancer therapeutics. The immense heterogeneity in immune cell phenotype and function and corresponding role in health and disease requires increasingly sophisticated analytical approaches to analysis. Recent advances in flow and mass cytometry have greatly expanded the number of immune cell parameters that can be interrogated, resulting in an improved understanding of the immune system heterogeneity. These technologies, however, remain limited in the number and types of analytes that can be examined in a single clinical sample. Expanding these parameters will enable the discovery of novel biology, potentially leading to the discovery of new therapeutic targets and biomarker signatures.

The NanoString nCounter® platform enables the highly multiplexed digital detection of both RNA and protein from a single biological specimen. Specifically, the nCounter® Vantage 3D™ RNA:Protein Immune Cell Profiling Assay interrogates 30 cell surface proteins and 770 immune-related RNA starting with as few as 20,000 cells in suspension. We demonstrate here the development of a streamlined workflow that seamlessly integrates standard immune cell flow sorting with downstream nCounter® analysis (Figure 1).

By co-staining PBMC with both fluorescently-labeled and DNA-barcoded antibodies, CD8+, CD3+, CD4+ T cells, and CD19+ B cells were isolated by flow cytometry followed by analysis of additional proteins and 770 RNA from each sorted population on the nCounter platform. Demonstrating the value of this workflow in analyzing rare cell populations, the number of target cells were titrated to determine the sensitivity of the workflow (Figure 2). Without the requirement for additional molecular biology methods, such as RNA purification or sequencing library construction, this method is ideally suited for incorporation into any cell sorting workflow.

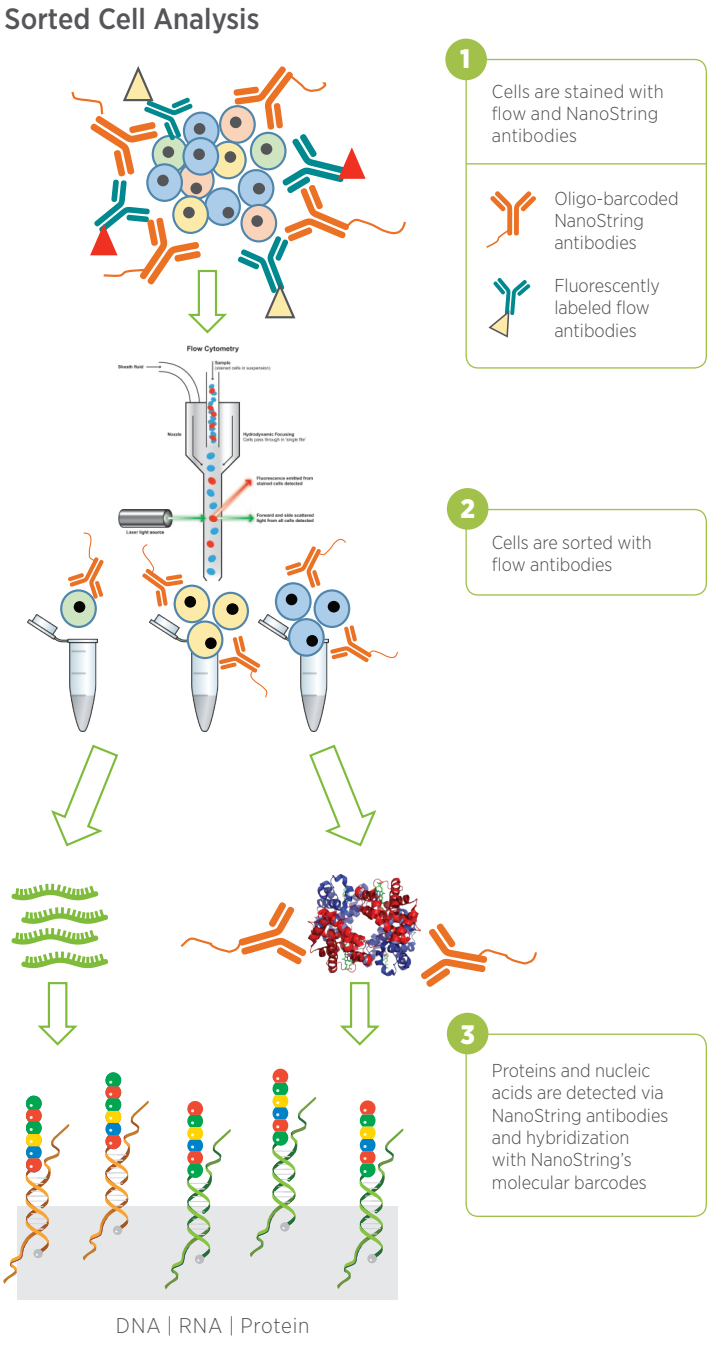

FIGURE 1 Workflow for the isolation and deep proteomic and genomic analysis of defined cell sorted populations.

High Parameter Protein Analysis from Low Cell Inputs

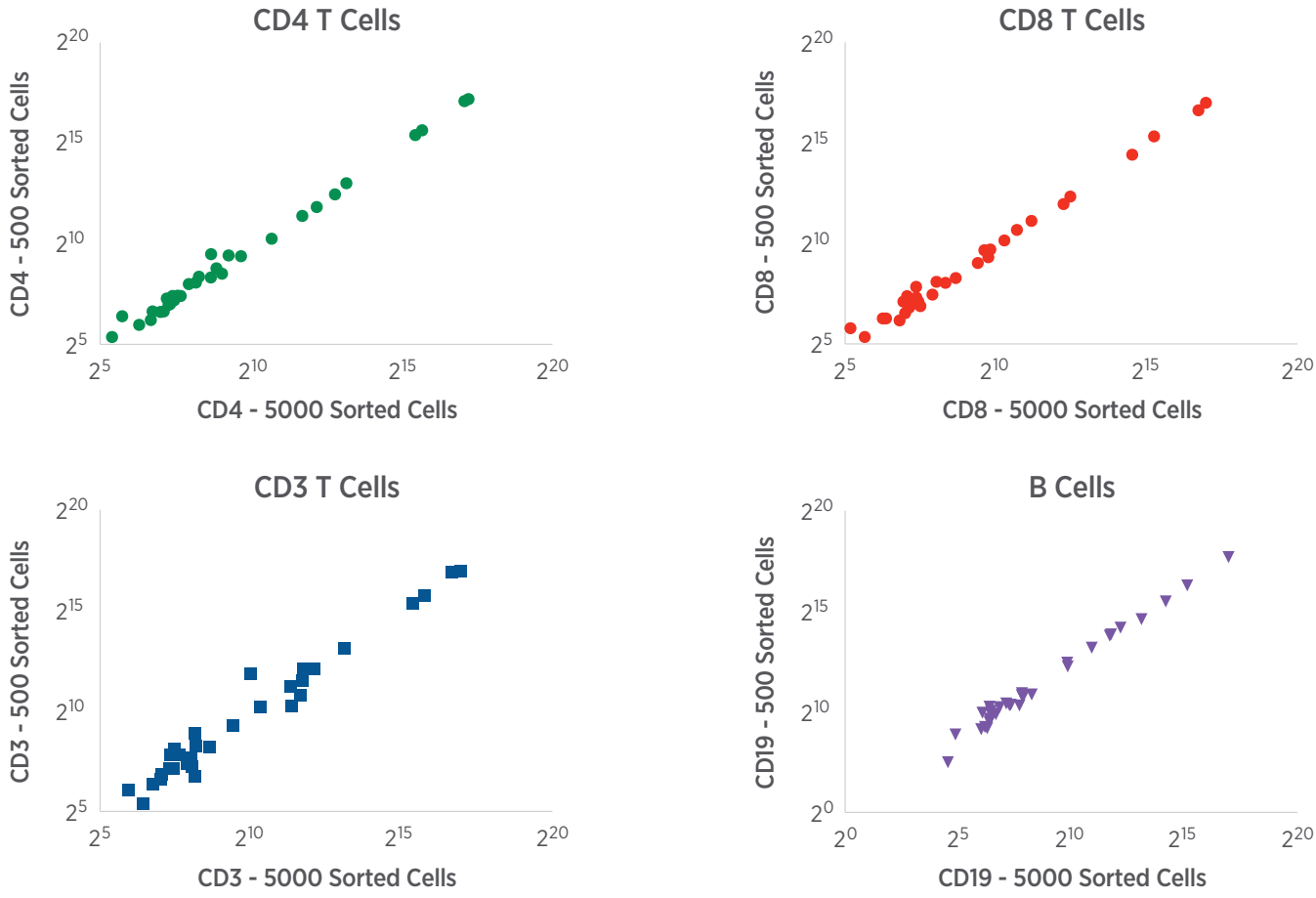

FIGURE 2 Correlation of the expression of 30 protein markers in defined immune cell populations across different low cell inputs. Values on X and Y axis are normalized counts.

References

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