

1. Introduction

Vesicode Cancer Biology Characterization Panel is a service based reagent kit simultaneously measuring 116 cancer related protein biomarkers. The markers in this panel have been selected to target membrane surface proteins of extracellular vesicles (**EVs**), while using 1 μ L of a 1:250 dilution of sample. The analytical performance of the product has been carefully validated and the results are presented below.

1.1 TECHNOLOGY

The Vesicode reagents are based on the *Proximity Barcoding Assay* (PBA) technology, where barcoded oligonucleotide labelled antibody probes are allowed to bind to their respective target proteins on the surface of EVs. These EVs-probe complexes are captured on a solid support via EVs – CTB recognition, allowing for washes to be performed and any unbound EVs to be removed. A reporter sequence is formed by a proximity dependent DNA polymerization event via PCR, which resulted from the hybridization of amplified barcoded circular oligonucleotide products to the oligonucleotide labelled probes bound on the capture EVs. This polymerization event results in the incorporation of the barcodes present in the system, which are subsequently detected via deep sequencing.

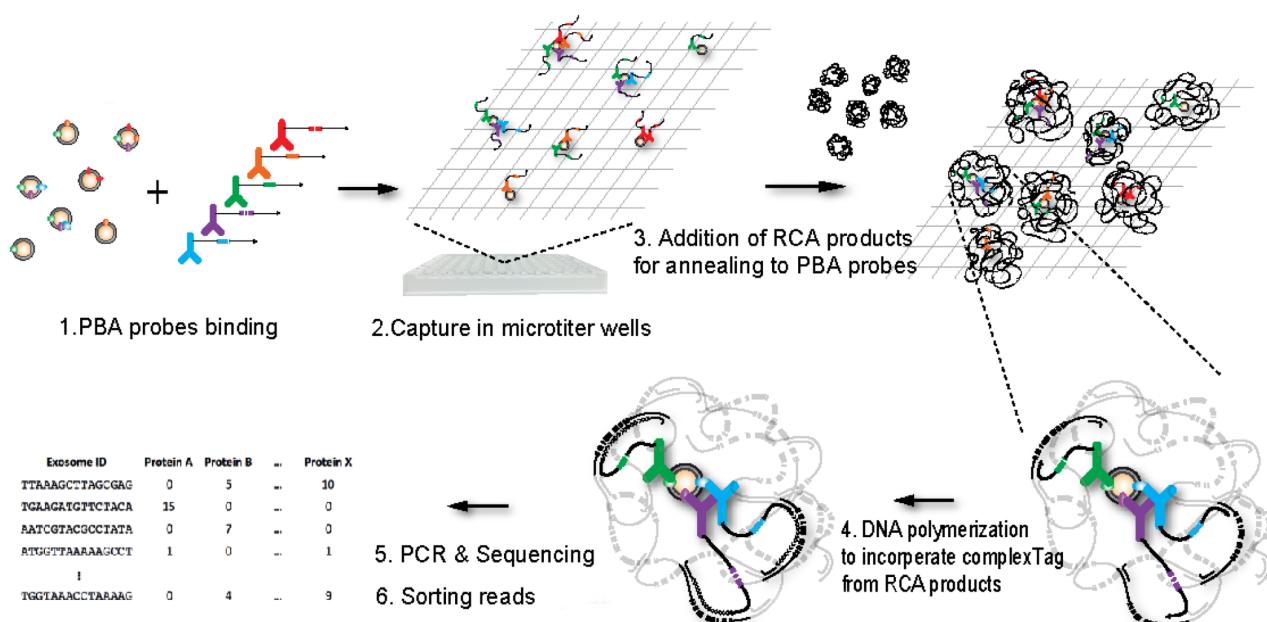
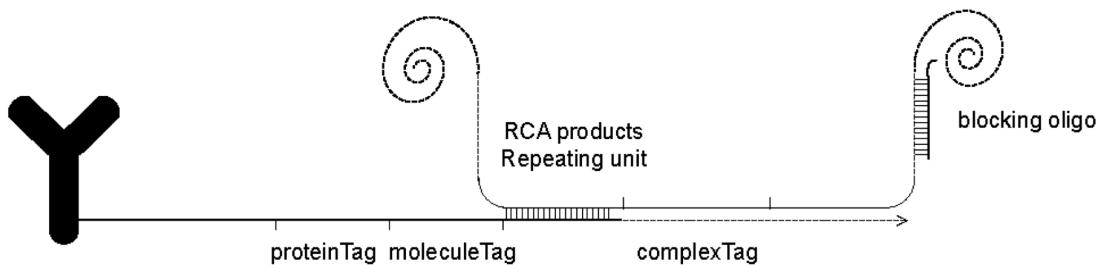
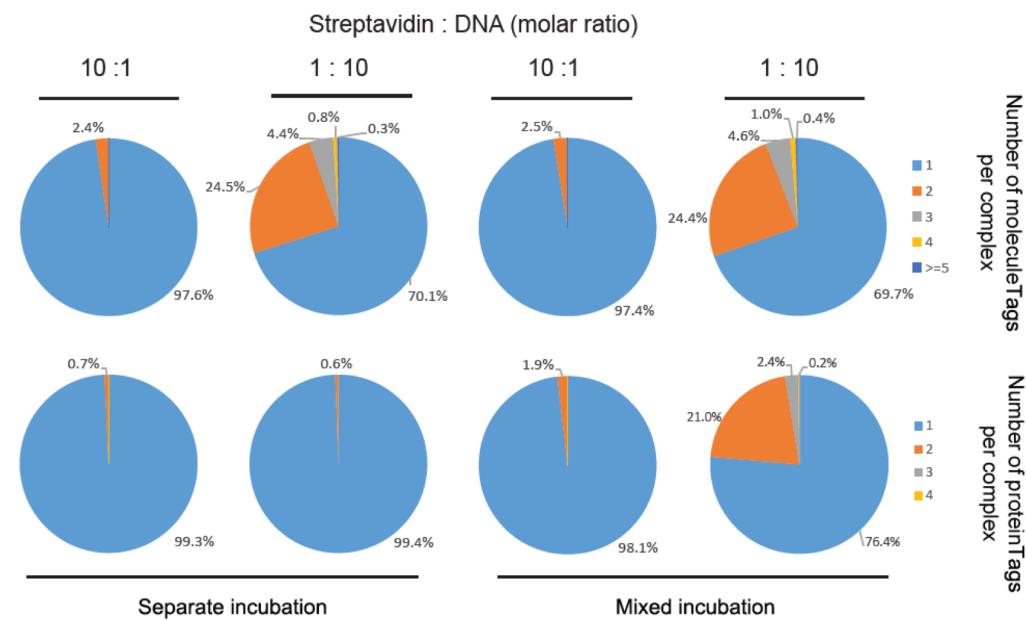
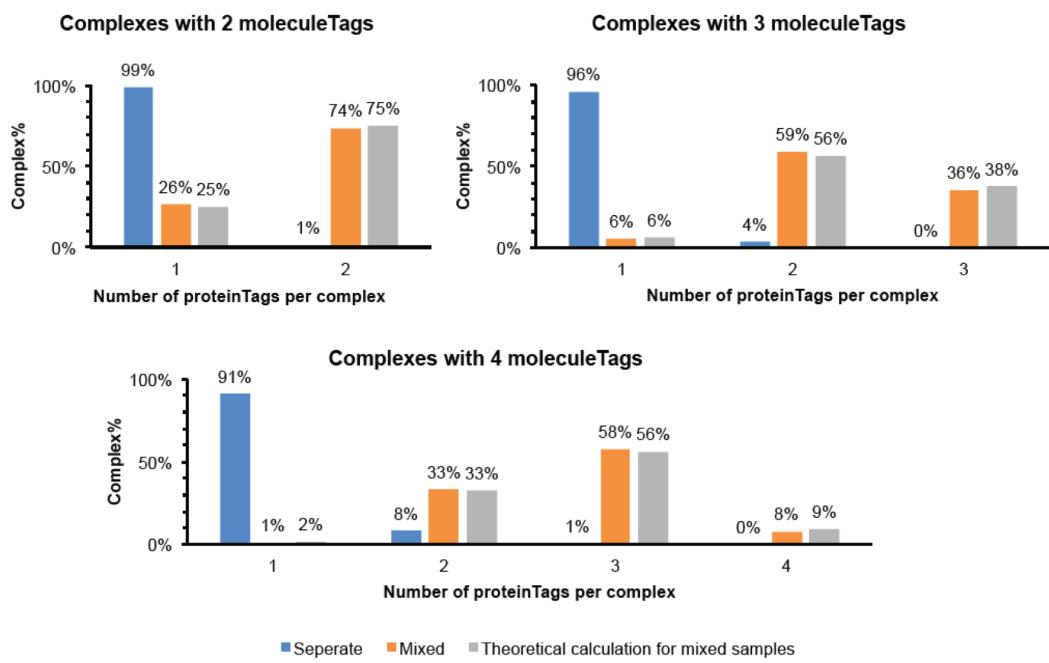


Fig.1 Vesicode's multiplex immunoassay procedure employs several steps: Probe incubation, Capturing, Hybridization, Polymerization and Sequencing.

1.2 QUALITY CONTROLS

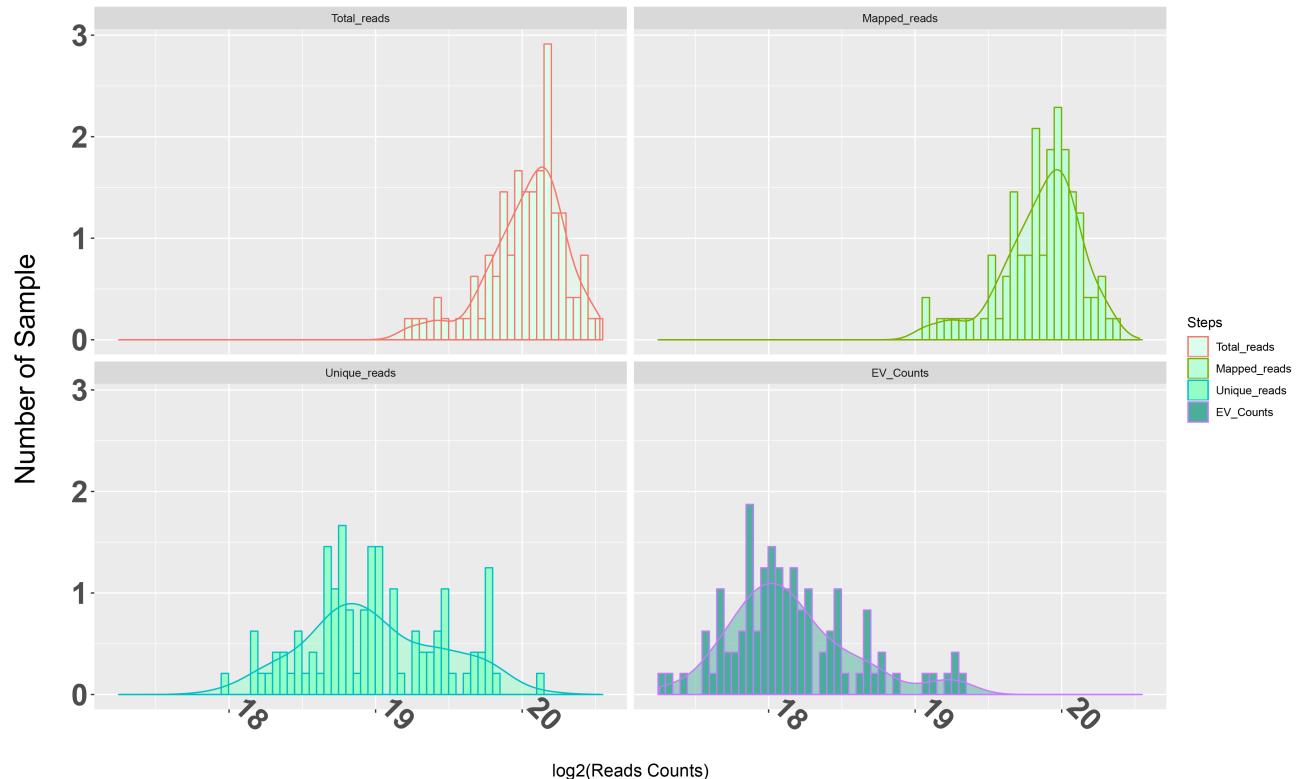
Internal and external controls have been developed by Vesicode for data and quality control purposes. These controls have been designed to enable monitoring of technical performance of the assay, and for the quality of the individual samples, providing information at each step of the Vesicode protocol (Figure 1). Controls performed to validate the analyses consists of immunoassay controls, extension controls and detection controls. The immunoassays control monitors all steps in the immunoreaction. The extension control monitors the extension and incorporation of the tags into the reporter molecule. Finally, the detection control monitors the sequencing readout of the method.

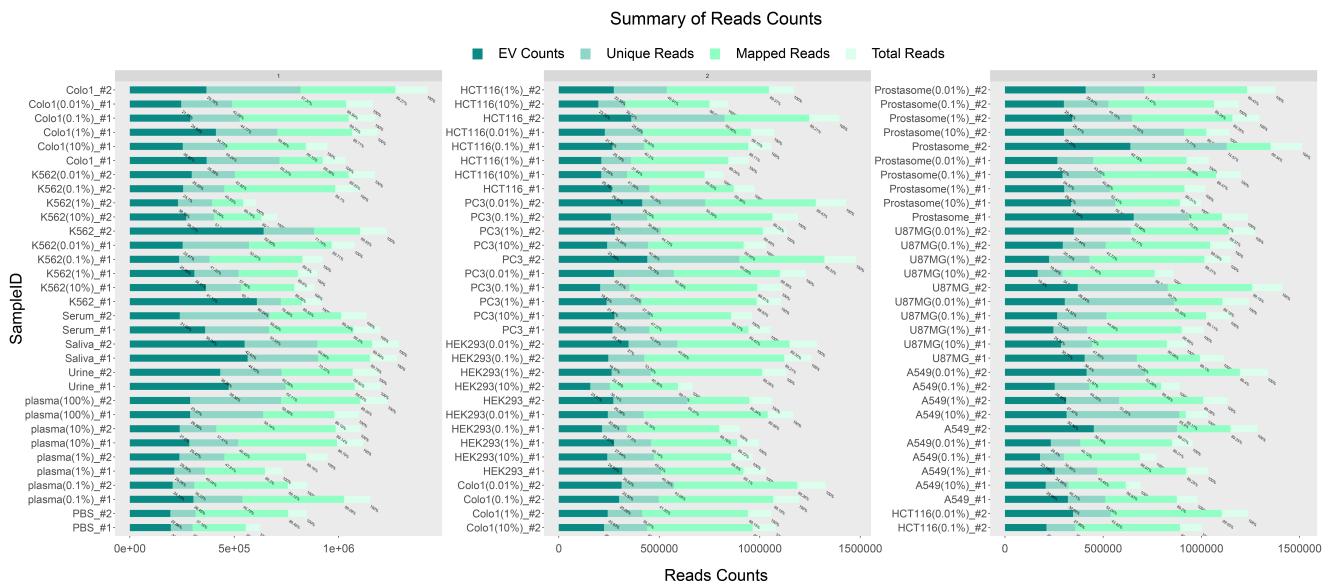




1.3 DATA ANALYSIS

the Density of Samples

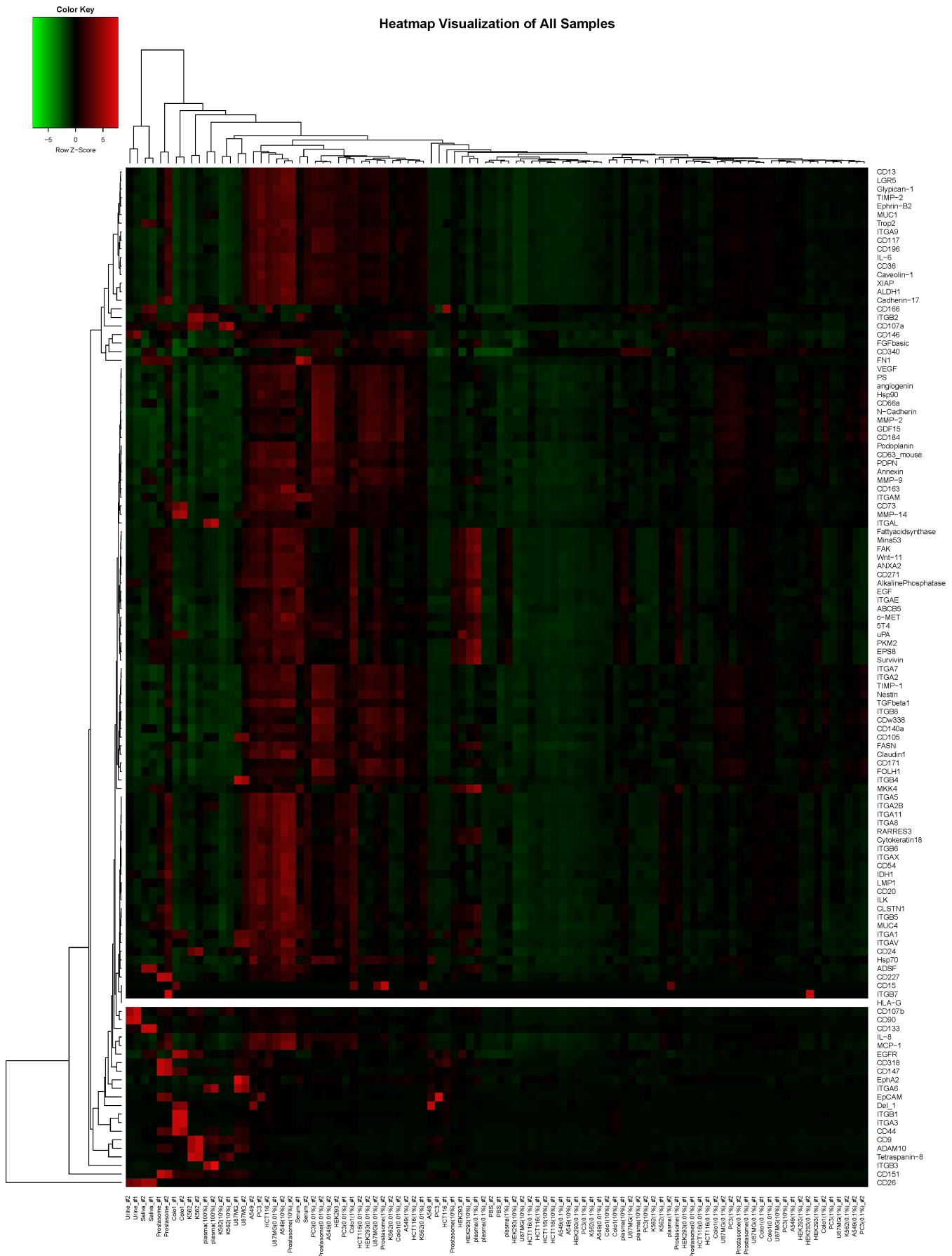




2. Performance characteristics

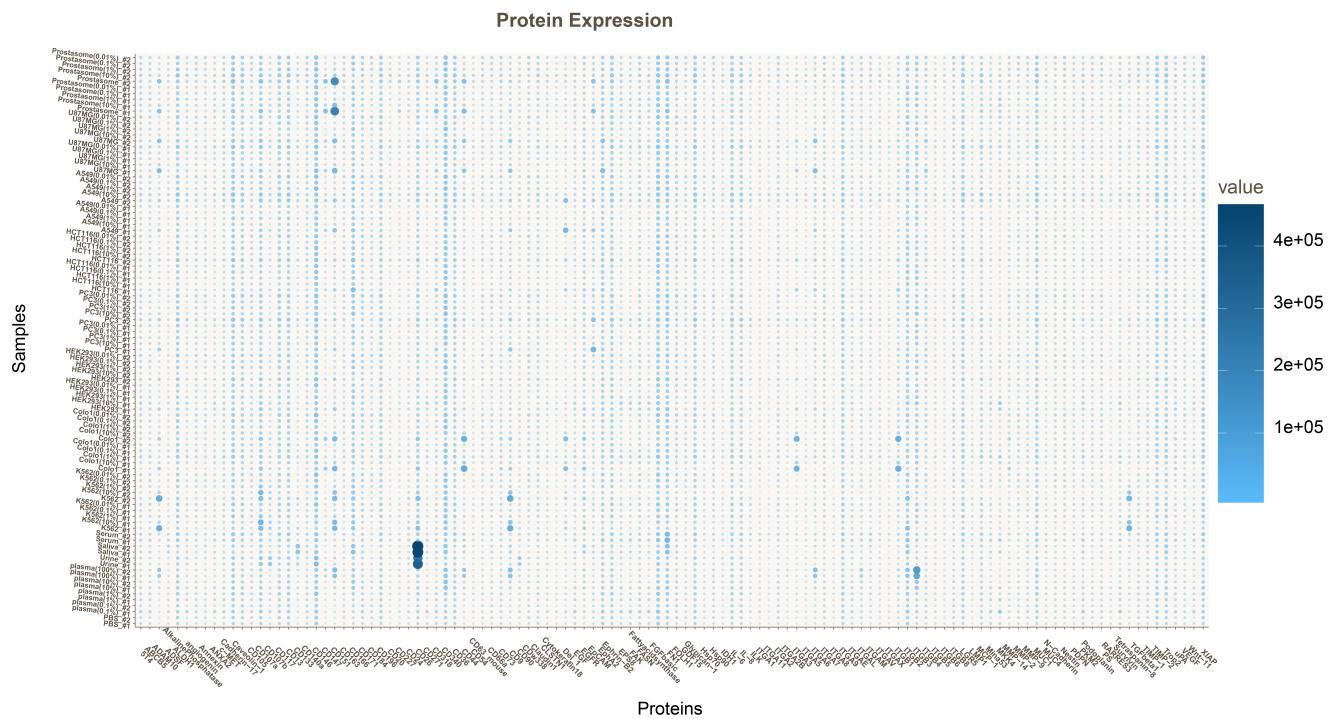
2.1 SAMPLE TYPES

The ability to use different sample types was evaluated with Vesicode's Cancer Biology Characterization Panel by spiking purified extracellular vesicles in Serum, Saliva, Urine, 0.1-100% plasma, and 0.1-100% cell lysate of a myriad of different cell types. Figure 7 shows a heatmap visualization of the expression of the targeted proteins across all sample types.

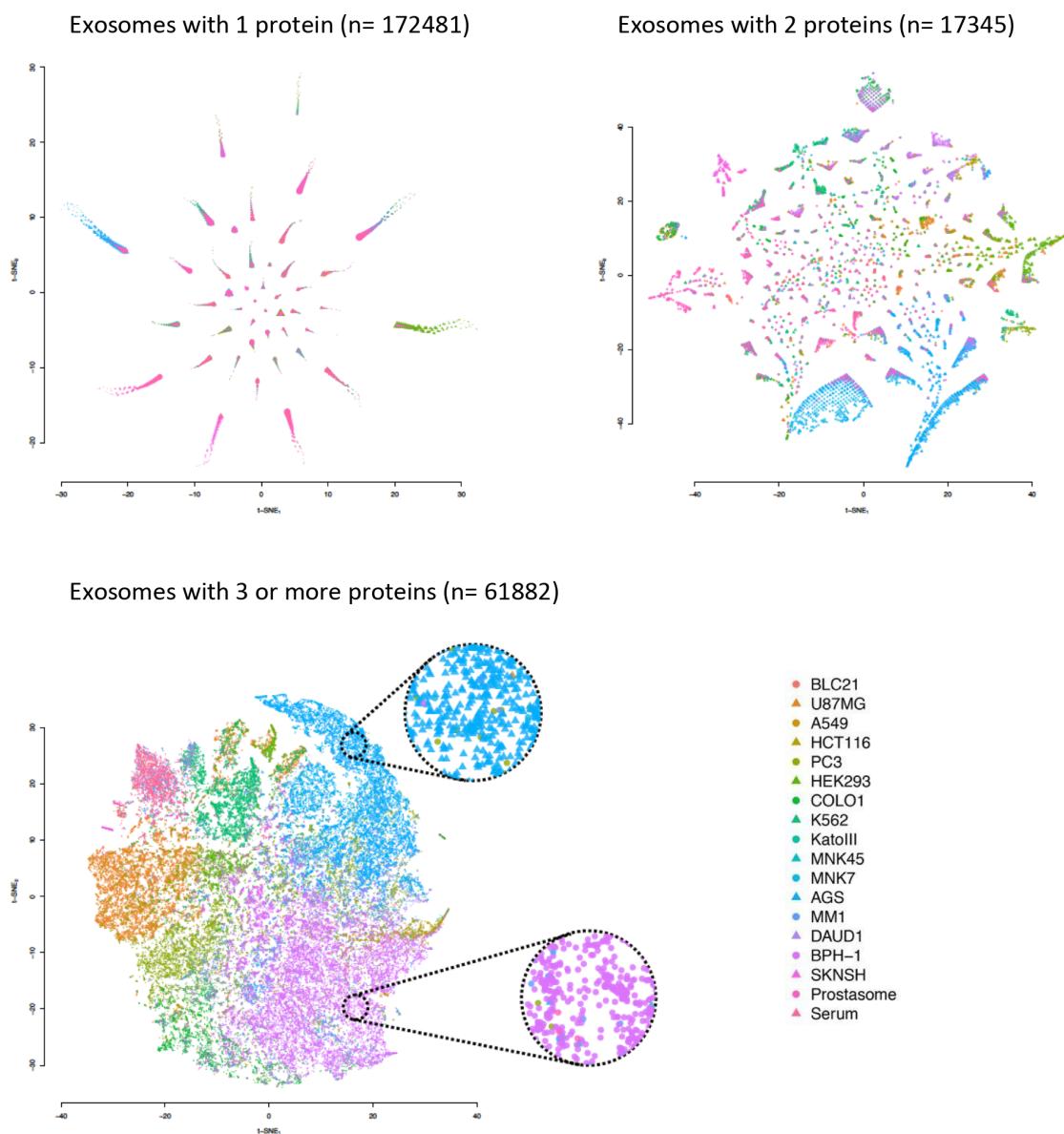


2.2 ANALYTICAL MEASUREMENT

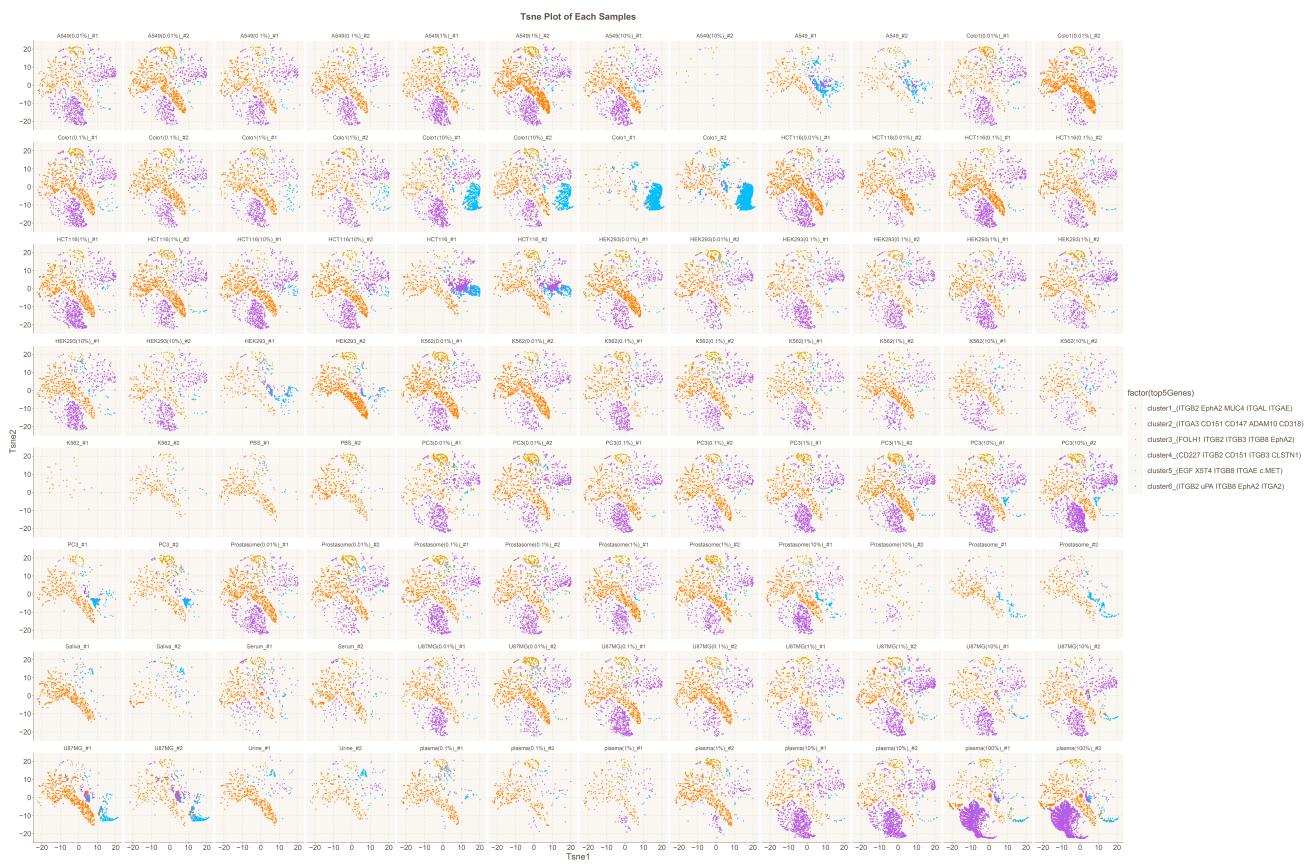
Figure 8 shows the distribution of protein measurement range defined by quantification and analysis of 116 cancer related targets present in a myriad of sample types.



2.3 PRECISION



2.4 ANALYTICAL SPECIFICITY



3. References
