The Single-Cell Western Has Arrived

Introduction

Milo enables scientists to perform single-cell resolution Westerns (scWesterns) for over 1,000 individual cells simultaneously, and in a fraction of the time of conventional Westerns. Researchers can now gain selective protein expression information for up to four protein targets in each cell, offering views into cell-to-cell variation within a complex sample.

Why Single-Cell Westerns?

Increase resolution

Many cell populations, such as tumor cells or differentiating stem cells are heterogeneous. Protein expression measurements made at the population level often overlook significant differences in expression between individual cells and the existence of cell subpopulations (**Figure 1**). Milo combines single-cell resolution with the power and versatility of Westerns. This approach allows simultaneous measurement of multiple proteins in over 1,000 single cells using commercially-available Western-validated antibodies.

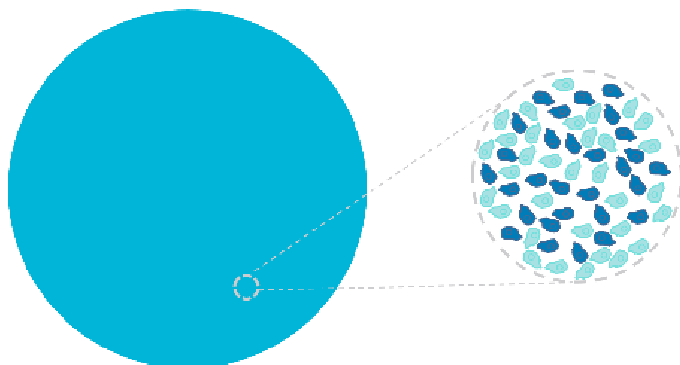

Figure 1: Bulk measurements cannot interrogate individual cells within a population. In contrast, a single-cell resolution Western reveals the presence of cellular subpopulations in a heterogeneous sample.

Validate Single-Cell RNA targets

Milo allows researchers to validate protein expression of mRNA transcripts identified using single-cell RNA-Seq. Up to 4 proteins can be probed simultaneously in each single cell. Milo can also be used to directly measure protein expression at the single-cell level in biological systems where RNA and protein expression do not correlate.

Detect challenging flow targets

Milo uses Western-validated antibodies and is able to simultaneously detect both surface proteins and internal proteins. Phosphorylated proteins, transcription factors, cytoplasmic, and nuclear proteins can all be detected. Since 10-100 times more antibodies are available for Westerns than for flow cytometry, Milo can detect hard-to-measure intracellular proteins or targets for which flow antibodies are not readily available.

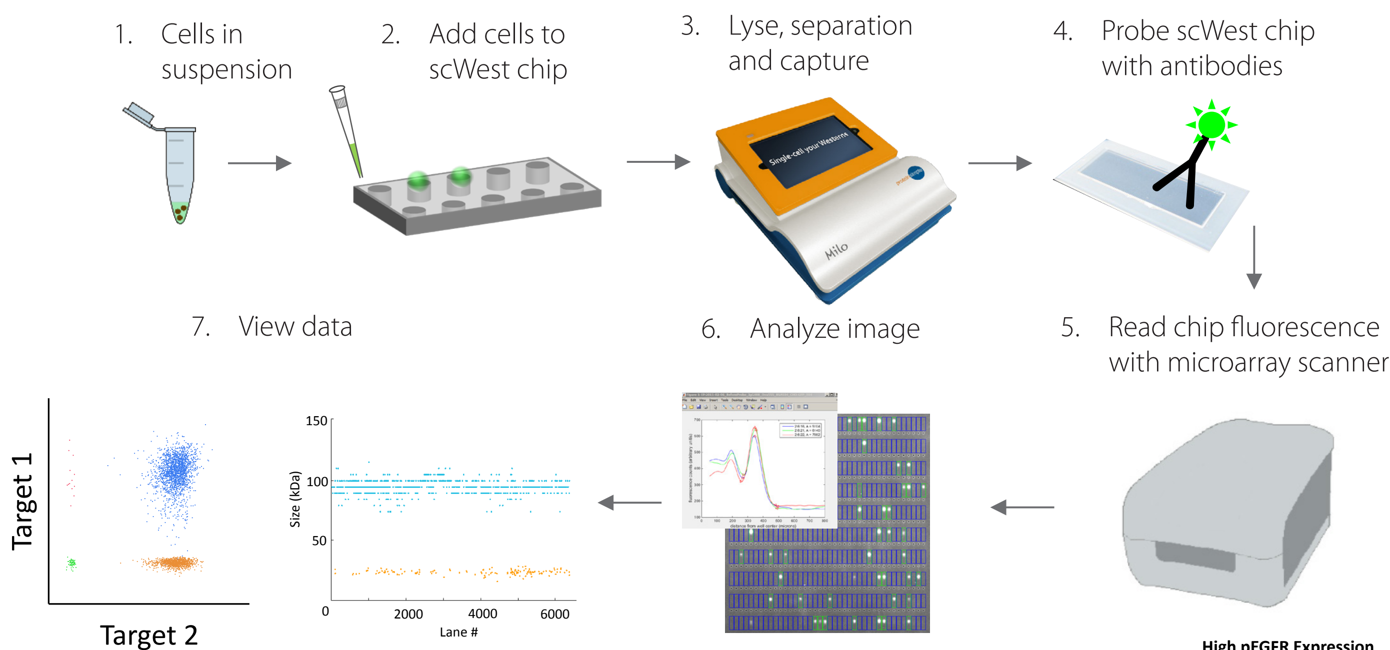

Figure 2: Single-Cell Western workflow and system components.

How does Milo Work?

The heart of the Single-Cell Western system is the scWest chip, which can perform Westerns on thousands of single cells in parallel in just four hours. The scWest chip captures 1,000 - 2,000 single cells and then performs size-based separation of the proteins in each single-cell lysate within a photoactive polyacrylamide gel. After separation, UV activation immobilizes protein bands in the gel. Chips are then probed with conventional primary and fluorescently-labeled secondary antibodies and imaged in a microarray scanner. The resulting image is analyzed using chip analyzer software and data is reported using standard visualization tools (**Figure 2**). Probed scWest chips can be dried and stored. Re-probing of archived chips is possible months later.

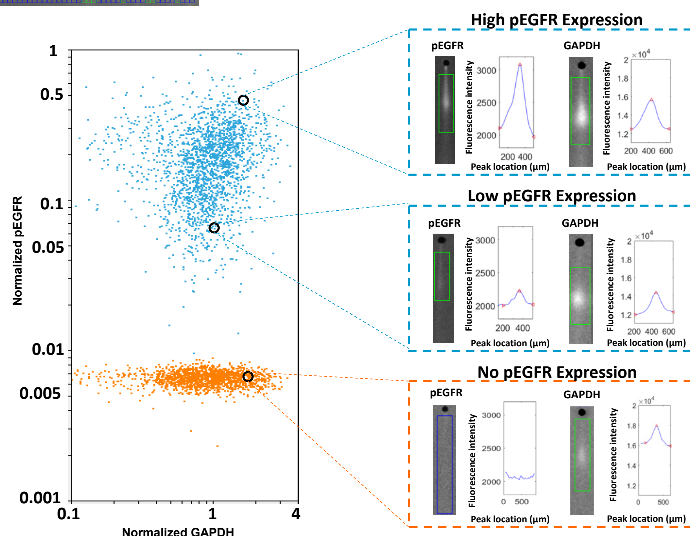

Figure 3: A 5-minute on-chip EGF stimulation induces pEGFR expression in A431 cells (blue) while unstimulated cells show no pEGFR expression (orange). Images of protein separations are shown (microwell at the top and electrophoresis in the downward direction) with characteristic peaks and representative intensity plots.

Example Single-Cell Western Measurements

Detect phosphoproteins

On-chip stimulation of cells can be used to study signaling pathways and probe for time-sensitive events such as phosphorylation. Many phospho-specific antibodies can have off-target binding, necessitating having both molecular weight and antibody binding information to make a specific measurement. Further, many phosphorylated forms of proteins involved in signal transduction do not have flow-validated antibodies and therefore cannot be measured at the single-cell level. **Figure 3** shows an scWestern scatter plot of pEGFR expression vs. GAPDH expression in stimulated and unstimulated cells. As in flow cytometry data, each point is a measurement from a single cell. However, unlike flow cytometry, each point is also associated with one or more separation images which can be inspected to verify that the signal is associated with a peak located at the correct molecular weight.

Subpopulations and heterogeneity

Conventional Westerns show only average protein expression in a sample. Milo reveals the existence of cell subpopulations and gives new insights into cell-to-cell heterogeneity (**Figure 4**). While flow cytometry measures single-cell heterogeneity, results are often confounded by off-target binding or variants/isoforms that are bound by the same antibody. In contrast, single-cell Westerns can distinguish off-target binding and protein variants/isoforms (**Figure 4 and Figure 5**).

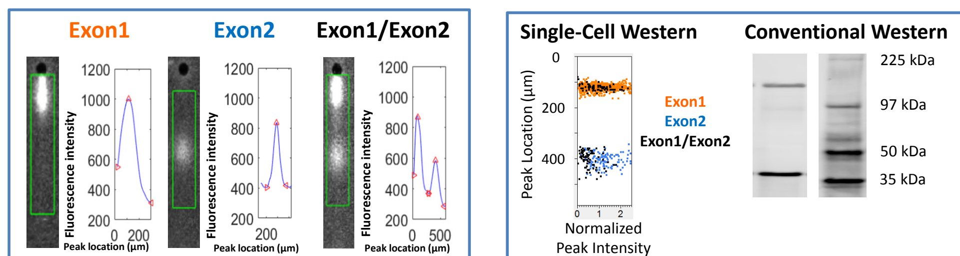

Figure 4: Milo reveals three subpopulations within the same population of cells. A single antibody has affinity to two protein variants (Exon1 and Exon2) in HEK293 cells. While conventional Westerns can show only the population average expression for Exon1 and Exon2, single-cell Westerns reveal three subpopulations: cells expressing only the large molecular weight variant Exon1 (top left, orange), only the small variant (top middle, blue), or both Exon1 and Exon2 (top right, black). Single-cell peak intensities plotted vs. separation distance (bottom) show the three populations and reveal cell-cell heterogeneity in protein expression.

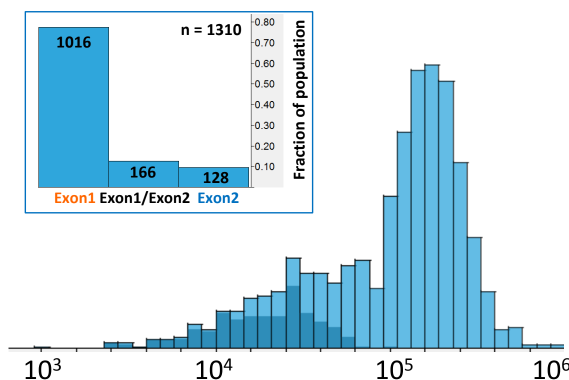

Figure 5: Histogram of total peak area (all targets). The light blue histogram shows the distribution of the total protein signal (Exon1 + Exon2) and represents what a flow cytometer would detect. The darker blue portion of the histogram indicates the ~20% of measurements that contain signal from the smaller molecular weight species (Exon2). If Exon1 were the desired protein target, off-target binding of Exon2 in flow would lead to ~20% false measurements. Inset: fraction of the cell population that has protein species Exon1 only, Exon1 and Exon2, and Exon2 only.

Toll-free: (888) 607-9692
Tel: (408) 510-5500
Fax: (408) 510-5599
info@proteinsimple.com
proteinsimple.com

© 2016 ProteinSimple. Milo, scWest, ProteinSimple and the ProteinSimple logo are trademarks and/or registered trademarks of ProteinSimple.