Background:

WES is a capillary-based method of protein separation and immunodetection. It works through the same principals of a traditional western blot, sample separation, blocking, primary and secondary incubations and visualisation, however these steps are automated.

Sample Analysis

A WES plate (Figure 1A) is prepared following the provided instructions (SM-W004, ProteinSimple). Diluted samples are mixed with dithiothreitol and fluorescent master mix in a 8:1:1 ratio. Samples are denatured at 95°C for five minutes. The samples, antibody diluent, primary antibody, secondary antibody and luminol-peroxide mix are loaded into appropriate wells. A biotinylated ladder, antibody diluent, streptavidin-HRP and luminol peroxide are also loaded. After brief centrifugation of the plate, wash buffer is added.

The plate is then placed into the WES machine, whereby the load separation matrix is aspirated, and stacking matrix drawn up into each capillary. The ladder and samples are then loaded and lowered into running buffer. An electrical current is applied to the samples, and they are separated based on molecular weight. At the end of the separation, samples are immobilised by UV light, removing the lysis buffer and non-protein components. Then, the capillaries draw up the blocking solution, primary and secondary antibodies, flanked by wash steps. Finally, the chemiluminescent substrate is added into the capillaries, and the camera detects the levels of chemiluminescence at 9 different timepoints (1-512 seconds). The software then calculates the high dynamic range (HDR) of the peaks, which is calculated from the individual exposures and provides the largest dynamic range of the signal with the lowest background. The HDR peak is then used to calculate the area under the curve, which is analogous to the quantity of protein in the sample (Figure 1B).

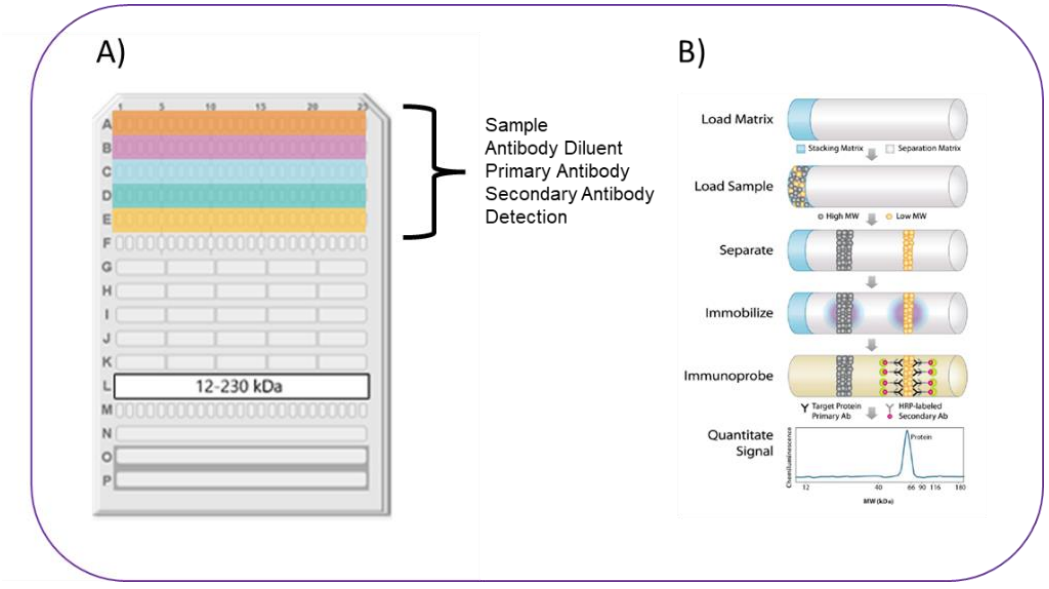

Figure 1: Schematic of A) Wes plate & B) size-based protein separation and detection on the WES machine. Taken from Protein Simple technical guide.

Normalisation with total protein:

To normalise the protein, we use a total protein assay provided by protein simple (DM-TP01). This kit works using the same principles as a Coomassie stain providing an indication of the protein concentration loaded into the assay. We validated that total protein is stable across treatments and measured total protein output at different protein concentrations to ensure we are in the linear part of the graph. The area under the curve of the protein of interest is divided by the area under the curve for the total protein to find the normalised relative protein levels.

Sample Data

A PFC SNAP25 and PSD95 (Jen Fletcher study)