MS Bioworks
Protein Mass Spectrometry Services

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Quant-works SILAC Service (MSB-21)

Metabolic labeling methods such as SILAC (stable isotope labeling by amino acids in cell culture) enable global protein quantitation using light and heavy peptides present in the mass spectrometry data. Growing cells in media containing heavy lysine and arginine allows for a fixed mass shift to be incorporated into all proteins; this allows the pairwise comparison of control (light) and treated (heavy) samples that are mixed together prior to processing. The ability to multiplex two or three samples per analysis allows for increased throughput and cost savings in quantitative proteomics experiments along with improved relative quantitation. The MS Bioworks Quant-works SILAC service (MSB-21) provides a comprehensive platform including:

- Sample preparation (cell lysis and protein quantitation)
- Heavy label incorporation check
- Sample mixing, fractionation, and trypsin digestion
- LC/MS/MS with 20h instrument time on a Orbitrap Velos Pro or Q Exactive
- Data processing and data analysis in MaxQuant
- Detailed report

This service note summarizes a study with MCF10A cells which were treated with an AKT kinase inhibitor A-443654. Cells were treated with vehicle (control; light) or A-443654 (treated; heavy), lysed and mixed 1:1 following protein quantitation. Proteins were separated by SDS-PAGE into 20 fractions. Each fraction was analyzed by LC/MS/MS on an Orbitrap Velos Pro tandem mass spectrometer with a 1h gradient per fraction (a total of 20h instrument time). Data were processed using MaxQuant searching against Swissprot Human. The ratio of heavy-to-light was calculated for each protein.

Results

A total of 5,093 proteins were detected with two or more unique peptides with a false discovery rate (based on forward/decoy database searching) of 1% at the protein level. 14 proteins were up-regulated two-fold or greater with treatment, 73 proteins were down-regulated two-fold or greater with treatment. This is represented below with Log2 fold-change plotted, the top six up-regulated proteins are listed:

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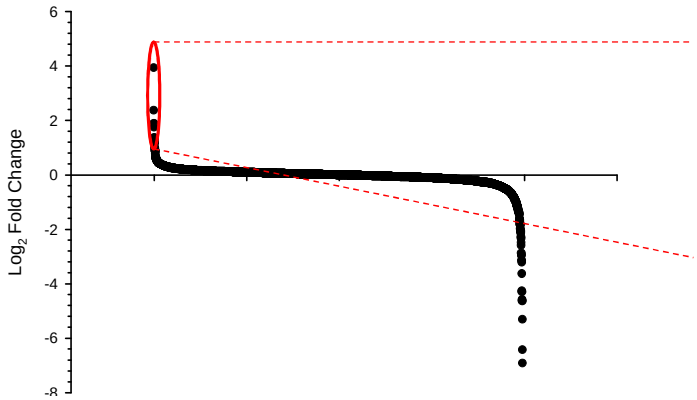
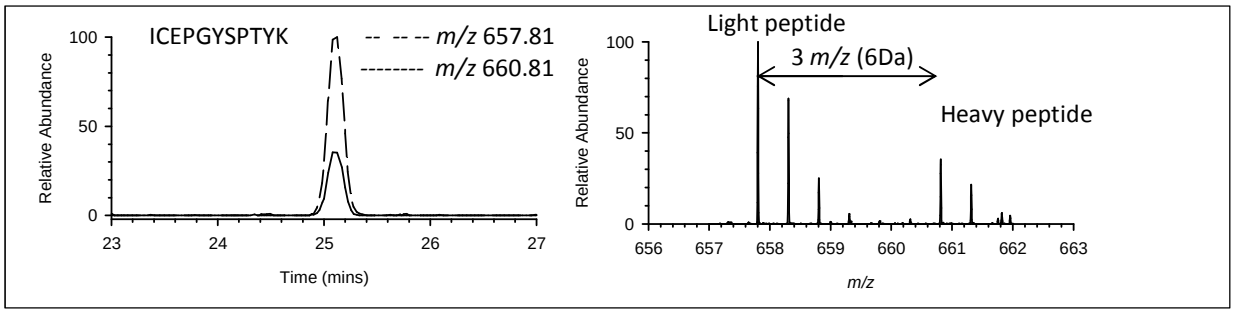


Gene Name	Log$_2$ Fold Change
ATG2B	3.920
FARS2	2.349
CEP152	1.859
WDR46	1.827
LTBP1	1.722
CDC42EP4	1.352

There were 369 phosphosites quantitated as part of the same experiment, the levels of cathepsin B, a cysteine protease known to be involved in cancer progression and invasion, decreased fivefold as a result of drug treatment. The LC/MS selected ion chromatogram and corresponding summed spectrum for the light and heavy phosphopeptide are shown below:



Quant-works SILAC service provides a powerful platform for quantitative proteomics in biomarker discovery and drug development. SILAC services can be ordered directly from our website at www.msbioworks.com.

Service ID	Cost ($)
MSB-21	4100

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