

Quant-works TMT Service (MSB-20)

Chemical labeling methods such as TMT (Tandem Mass Tags, Pierce) enable global protein quantitation using reporter ions present in the MS/MS data. The ability to multiplex up to six samples per analysis allows for increased throughput and cost savings in quantitative proteomics experiments. The MS Bioworks Quant-works TMT service (MSB-20) provides a comprehensive platform including sample preparation (lysis and protein quant), digestion, labeling, peptide-level fractionation, LC/MS/MS, data processing and data analysis. This service note summarizes a controlled study with K562 cells in which a 1:1, 1:2 and 1:5 mixture was created and analyzed.

Experimental

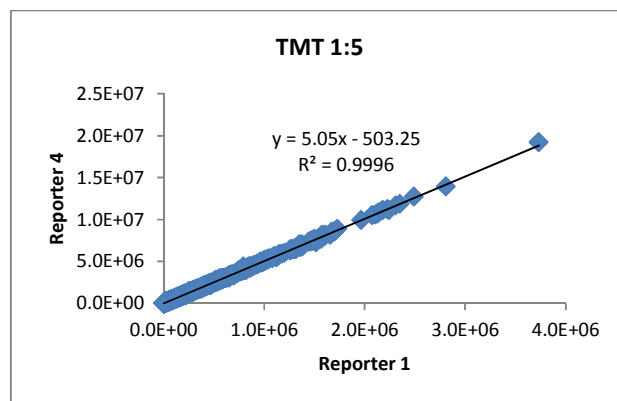
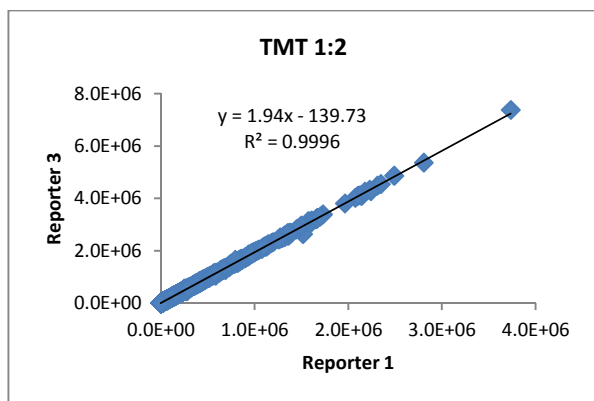
K562 cells were lysed and solubilized in TEAB. A 200 μ g aliquot was digested with trypsin and portions were labeled according the following table:

Label	Amount (μ g)
128	20
129	20
130	40
131	100

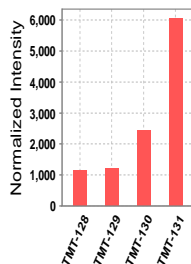
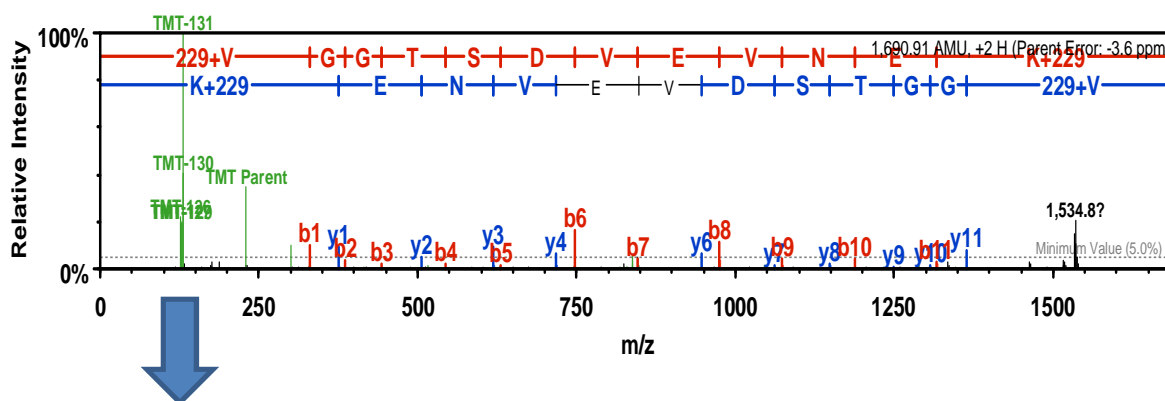
Labeled peptides were pooled and separated by SCX into 10 fractions. Each fraction was analyzed by LC/MS/MS on Q Exactive tandem mass spectrometer in HCD mode with a 2h gradient per fraction (a total of 20h instrument time). Data were searched against Swissprot Human and processed for reporter ion intensities using MaxQuant.

Results

A total of 3,593 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. The ratio between the reporter ions was determined for each pairwise comparison; the intensity values of the 1:2 and 1:5 pairs are plotted below. Excellent agreement with the expected ratios was observed, with the mean and median Log₂ fold-change both determined as 0.96 and 2.34, respectively with 8% and 4% CV, respectively.



An example product ion spectrum showing the TMT reporter ion ratios (expanded below) allowing quantitation between samples and concomitant backbone fragmentation allowing protein identification:



Quant-works TMT service allows for in-depth, multiplexed global protein quantitation in proteomics studies. The service encompasses all stages of the experiment from sample preparation through to data analysis.

Service ID	TMT Plex	Cost (\$)
MSB-20	6	7875

MS Bioworks TMT service can be ordered through www.piercenet.com or www.msbioworks.com