MS Bioworks
Biological Mass Spectrometry Solutions

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Project Report

Information

Client:	
Institute:	
Project Number:	
Date Submitted:	
Date Completed:	

Samples

Client identifier	MSB identifier	Organism
Control		*H. sapiens*
Test		*H. sapiens*

Objective

Identify and quantitatively profile the protein content of two submitted samples using the IP-works platform: SDS-PAGE, segmentation (10 gel segments per sample), in-gel digestion, LC/MS/MS and database searching. Data to be processed as Spectral Counts (SpC) and Normalized Spectral Abundance Factors (NSAF) and significant interactors determined.

Experimental Methods

Sample Preparation

20µL of the submitted sample was separated ~1.5cm on a 10% Bis-Tris Novex mini-gel (Invitrogen) using the MES buffer system. The gel was stained with coomassie and each lane was excised into ten equally sized segments.

Gel segments were processed using a robot (ProGest, DigiLab) with the following protocol:

- Washed with 25mM ammonium bicarbonate followed by acetonitrile.
- Reduced with 10mM dithiothreitol at 60°C followed by alkylation with 50mM iodoacetamide at RT.
- Digested with trypsin (Promega) at 37°C for 4h.

- Quenched with formic acid and the supernatant was analyzed directly without further processing.

Mass Spectrometry

Each gel digest was analyzed by nano LC/MS/MS with a Waters NanoAcquity HPLC system interfaced to a ThermoFisher Orbitrap Velos Pro. Peptides were loaded on a trapping column and eluted over a 75µm analytical column at 350nL/min; both columns were packed with Jupiter Proteo resin (Phenomenex). The mass spectrometer was operated in data-dependent mode, with MS performed in the Orbitrap at 60,000 FWHM resolution and MS/MS performed in the Velos. The fifteen most abundant ions were selected for MS/MS.

Data Processing

Data were searched using a local copy of Mascot with the following parameters:

Enzyme: Trypsin
Database: Uniprot Human (concatenated forward and reverse plus common contaminants)
Fixed modification: Carbamidomethyl (C)
Variable modifications: Oxidation (M), Acetyl (Protein N-term), Deamidation (NQ), Pyro-Glu (N-term Q)
Mass values: Monoisotopic
Peptide Mass Tolerance: 10 ppm
Fragment Mass Tolerance: 0.8 Da
Max Missed Cleavages: 2

Mascot DAT files were parsed into the Scaffold software for validation, filtering and to create a non-redundant list per sample. Data were filtered using a minimum protein value of 90%, a minimum peptide value of 50% (Prophet scores) and requiring at least two unique peptides per protein.

Results

A total of 893 human proteins were detected with two or more unique peptides at the protein-level false discovery rates indicated in the table below (based on forward/decoy database searching). Please refer to the accompanying Excel and Scaffold files for the full list of proteins and associated information. You can download the Scaffold file from:

ftp://75.144.89.5

Username: IP-works-MED26
Password: gGc6w4GM

The Excel file contains four worksheets:

Worksheet 1 (SpC) contains the full list of proteins, including known contaminants, and their unnormalized spectral counts (SpC). A brief overview of protein and peptide totals in each sample is shown here:

MSB Identifier	Control	Test
Total No. of Proteins	571	735
Total No. of Spectra Matching	13639	17026
Total No. of Unique Peptides	4203	6210
False Discovery Rate (%)	0.3%	0.0%

Worksheet 2 (Spc-CON) contains the full list of proteins minus known contaminants. These data were used in the subsequent NSAF calculation.

Worksheet 3 (NSAF) contains the conversion to Spectral Abundance Factor (SAF) and subsequent Normalized Spectral Abundance Factor (NSAF). This was based on the equation:

$NSAF = (SpC/MW)/\sum(SpC/MW)_N$

Where SpC = Spectral Counts
MW = Protein MW in kDa
N = Total Number of Proteins

NSAF values can be used to approximate relative abundance of proteins within a given sample, and relative abundance of a given protein between samples. A ratio of Test/Control is provided based on dividing the NSAF values.

Worksheet 4 (Interactors) shows those proteins determined the most significant interactors based on the following criteria:

1. Protein had at least 5 SpC in the Test sample.
2. Protein was not detected in the control sample OR
3. Protein was detected with a 4-fold or more increase based on dividing the NSAF values.

Based on these criteria we show 158 proteins that were unique or four-fold higher in the Test sample.