Project Report

Information

Client:	John Q. Sample
Institute:	Sample U
SBI Project Number:	SBI-XXXX

Samples

Client Identifier	SBI Identifier
S1	29555
S2	29257
S3	29258
S4	29259
S5	29260
S6	29261

Objective

Protein profiling of the submitted sample using custom sample preparation, LC-MS/MS, data analysis and reporting.

Experimental Methods

Sample Preparation

The protein concentration of each sample was determined by Qubit fluorometry (Invitrogen). 10µg of each sample was processed by SDS-PAGE using a 10% Bis Tris NuPage mini-gel (Invitrogen) in the MES buffer system. The migration window (2cm lane) was excised and in-gel digestion performed using a ProGest robot (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

Half of the digested sample was analyzed by nano LC-MS/MS with a Waters NanoAcquity HPLC system interfaced to a ThermoFisher Q Exactive. Peptides were loaded on a trapping column and eluted over a 75µm analytical column at 350nL/min using a 2hr reverse phase gradient; both columns were packed with Luna C18 resin (Phenomenex). The mass spectrometer was operated in data-dependent mode, with the Orbitrap operating at 60,000 FWHM and 17,500 FWHM for MS and MS/MS respectively. 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/P
Databases: SwissProt Human (concatenated forward and reverse plus common contaminants)
Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M), Acetyl (N-term), Pyro-Glu (N-term Q), Deamidation (N,Q)
Mass values: Monoisotopic
Peptide Mass Tolerance: 10 ppm
Fragment Mass Tolerance: 0.02 Da
Max Missed Cleavages: 2

Mascot DAT files were parsed into Scaffold (Proteome Software) for validation, filtering and to create a non-redundant list per sample. Data were filtered using at 1% protein and peptide FDR and requiring at least two unique peptides per protein.

Results

Mass Spectrometry

Scaffold files for this study containing all search results, coverage maps, peptide lists, product ion data and gene ontology data are available for download from our Dropbox account – download link to the specified folder(s) will be included in a separate email.

Details of protein identification data can be found in the Excel workbook accompanying this report, SBI-XXXX.xls. The Excel file contains five worksheets:

Protein Report contains the full list of identified proteins and their molecular weight and spectral counts (SpC). A summary is presented in the table below:

	29555	**29257**	**29258**	**29259**	**29260**	**29261**
Total number of proteins identified	127	94	111	278	366	344
Total number of spectra matching	2320	1696	1914	3676	4535	4225
Total number of unique peptides	1114	767	901	2189	2781	2549

Protein Report – CON as above with contaminant proteins removed.

NSAF Calc 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 Molecular weight 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.

MSC v AFSC contains the comparison of the MSC and AFSC. A fold change is presented based on dividing the average NSAF values calculated for each group.

T-Test as above with a minimum of three spectral count values greater than 0 in at least one of the groups. A T-Test is performed on these values. There are 98 proteins with a p-value less than 0.05. Of these 79 are binary differences and 4 have a fold change greater than four.