PRG2009 study

Sample preparation:

1. Samples were dissolved in 800 μL Buffer A for Agilent MARS spin cartridge. Each sample was processed in 4 200 μL batches to deplete 7 major serum proteins following protocol provided by Agilent.
2. Unbound fractions from each sample were combined and concentrated with a 5kDa MWCO cartridge from Agilent to 200 μL each
3. Concentrated samples were reduced with DTT, methylated with iodoacetic amide following standard procedures
4. 2 μL ProteaseMAX 1% solution was added to each sample, mixed before tryptic digestion at 37 ºC over night.
5. Digestion was quenched with 10 μL 10% TFA.

LCMSMS analysis:

1. Tryptic digests were separated with Agilent Zorbax300SB C18 column (1.0 × 150 mm) at 50 μL/min flow. Gradient of 5-50% acetonitrile in 90 min.
2. Mass spectrometry analysis was carried out with a ThermoFinnigan LTQOrbitrap with data dependent analysis. For each full scan FT scan, the 5 most intense ions with charge >1 were analyzed in ion trap. After analysis, the ions were excluded from MSMS analysis for 30 sec.

Data Processing:

1. A fasta database containing human proteins, trypsin, and phospholase were generated through the Bioworks "FASTA database tool" and inserted as .fasta file into in-house database for mascot and Bioworks. In Bioworks, the database was indexed with 57.02150 as static modification on Cysteine and 15.99492 as differential mod on Met, full enzymatic digestion with Trypsin with 3 missed cleavages allowed. Peptide tolerance is 20 ppm and fragment tolerance 1.0

6. .raw files were submitted directly to Bioworks for search
7. .raw were converted to .mzxml then to .mgf with a program developed by Dr. Hua Xu at University of Illinois at Chicago (huaxu@uic.edu) before submission for Mascot searches.
8. Search results from Bioworks and Mascot were loaded into Scaffold 2.1.3 for protein ID and quantification through spectra count.