Mass spectrometry data processing

Softwares

1. Proteome Discoverer version 2.2
2. Mascot version 2.4
3. MS Excel, Python or MATLAB

Steps

1. Search raw MS data files with Proteome Discoverer version 2.2 (Thermo Fisher Scientific) against the human SwissProt database using Mascot version 2.4 (Matrix Science) with following parameters.
    a. Mass tolerance of 10 ppm for precursor ions and 20 mmu for fragment ions
    b. Minimum peptide length set to six amino acids, and upto two tryptic miscleavages allowed.
    c. Fixed modifications for cysteine carbamidomethylation, TMT-labeled lysine and TMT-labeled peptide N-termini.
    d. Dynamic modification for Phosphorylation of serine, threonine, tyrosine and oxidation of methionine.
    e. ptmRS module to localize phosphorylation sites
2. Export Peptide spectrum matches (PSMs) file from the search file.
   Note: Following data processed can be done in MS Excel or Python
3. Filter data with following parameters to yield high confidence identification and quantification
    a. Rank = 1 and Search Engine Rank = 1
    b. Mascot ion score > 18
    c. Isolation interference < 30%
    d. PSMs with >95% localization probability (ptmRS probabilities) for all sites
    e. For ATM/ATR substrate analysis, include peptides that contain ‘SQ’ or ‘TQ’ motif.
    f. For pY analysis, include peptides with at least one pY
    g. For sup analysis, apply filters a-c.
4. Sum up TMT reporter ion intensities for each phosphopeptide.
5. Normalize phosphopeptide quantification using the median relative quantification of peptides from the supernatant analysis to correct for small variation in sample input.