Mass Spectrometry Facility Authentication of Key Biological and/or Chemical Resources

The quality of resources used to conduct research is critical to the ability to reproduce the results. To acquire reproducible measurements, proper functioning of the instrumentation during data acquisition and systematic processing of the data are essential.

Quality Control and Assessment for LC-MS/MS-Based Discovery Proteomics

Performance (chromatography, mass accuracy, and sensitivity) of high resolution high mass accuracy LC-MS/MS Orbitrap instrumentation is assessed prior to sample analysis by Mass Spectrometry Facility staff using commercially available peptide standards and Hela cell digests (ThermoScientific). The instrumentation is cleaned and calibrated weekly or prior to large-scale time-intensive experiments. Identification of peptides and protein groups is based on forward and reversed database searches using a false positive rate of <1% at the protein, peptide, and modified peptide levels. Due to the propensity for false positives and ambiguous site assignment of post-translational modifications, the fragmentation patterns (MS/MS) of modified peptides of interest, are manually confirmed prior to site-directed mutagenesis, antibody generation, or publication. For global identification of post-translationally modified peptides, the search results are ranked by score and the tandem mass spectra are inspected to determine the threshold for identification of modified peptides. The probability of site assignments within a peptide are reported for each potential site of modification.

For large-scale proteomic experiments requiring database searching, normalization, and quantitative processing across many LC-MS/MS runs simultaneously, the MaxQuant platform is employed. Raw LC-MS/MS data uploaded into MaxQuant are searched to provide peptide identification, inference of the proteins or protein groups present, assignment of sites of post-translational modifications, and extraction of quantitative information at the peptide, protein, and modification-site levels *(Nat Biotechnol*. 2008;26(12):1367-72. PMID: 19029910; *Mol Cell Proteomics*. 2014;13(9):2513-26. PMC4159666). The resulting text files are processed and evaluated using the Perseus computational platform which provides visualization of run to run reproducibility, distribution of the quantitative data, statistical tests, and a mechanism for bioinformatic interrogation of the functional relevance of regulated sites of post-translational modification (*Nat Methods*. 2016;13(9):731-40 PMID: 27348712). The MaxQuant text files are also loaded into programs, such as PTX-QC, to rapidly evaluate the reproducibility, efficiency, and robustness of the chromatography and data acquisition (*J Proteome Res*. 2016;15(3):777-87. PMID: 26653327). These ancillary programs quickly reveal the presence of contaminants (including mycoplasma), technical errors that may have occurred during sample preparation, poor chromatography in one or more analyses, inefficient instrument parameters, and systematic errors that can impact the reproducibility of quantitative measurements. These features are not typically evident in the simplified reports given to investigators.

For label free quantitation based on chromatographically resolved peak intensities, a minimum of 4 biological replicates are recommended per treatment condition. Replicates are analyzed in a block-randomized format with intervening blanks to prevent carry over. Application of label free proteomics to identify protein interactions by affinity enrichment-MS (after BioID, immunoenrichment, drug pulldowns) is performed under the assumption that most of the proteins identified are non-specific binding proteins which form the baseline to distinguish proteins that are enriched with the bait (*Mol Cell Proteomics*. 2015 Jan;14(1):120-35. PMC4288248). For SILAC-based quantitation, 3 biological replicate experiments included a label-swap control are recommended. Efficient metabolic incorporation of isotopically labeled amino acids (>95%) is confirmed by LC-MS/MS prior to performing the experiment. For TMT-based quantification, instrument parameters and operation are assessed using the triple KO TMT standard (*J Am Soc Mass Spectrom*. 2016 Oct;27(10):1620-5. PMC5018445). Personnel in the Facility are attuned to looking for and addressing any discrepancies in

performance of the autosamplers, nanoLC systems, mass spectrometers, nitrogen generators, and other components necessary for proper functioning of the instrumentation.

Antibodies

Authentication of antibodies used to enrich post-translationally modified peptides for subsequent modification-specific proteomic experiments is the responsibility of the project PI. For large-scale experiments, purchase of the same lot of antibody is recommended to avoid batch effects.

Peptides

Custom, isotopically-labeled, synthetic peptides with or without post-translational modifications are ordered from Sigma or JPT Peptide Technologies with the following specifications: >98% purity as determined by analytical HPLC, amino acid analysis for lyophilized peptide concentration, and aliquots of a specified quantity in one-time use vials. Mixtures of isotopically labeled synthetic peptides and phosphopeptides for spiking into samples as internal controls and evaluate reproducibility are purchased from ThermoScientific.