

PRIDE Information Template

PI Information:

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Project title:

Insert publication title here

Keywords:

Species, tissue types, sample prep, analysis type, disease/condition, etc.

Project description (5000 characters max):

Often the abstract of the publication, with a few additional details added from the background or methods sections if necessary to give a fuller understanding of the scope of the experiment.

Sample processing protocol (5000 characters max):

Information for this section will be largely taken from the methods section of a paper, but additional details about the instrument methods may be needed.

(Example text below)

Cell culturing, protein extraction, incubations and enrichments with bait proteins have been previously described in <insert citation here>.

Protein samples were run for 5 minutes into a 10% Bis-Tris Gel to remove impurities. Gel bands were excised after Coomassie-staining, washed twice for 30 min in 50 mM ammonium bicarbonate and 50% (v/v) acetonitrile, reduced in 10 mM DTT/100 mM ammonium bicarbonate, and alkylated in 55mM iodoacetamide and 100mM ammonium bicarbonate. Dehydrated gels were reswelled in digestion buffer containing 50 mM ammonium bicarbonate, 5 mM CaCl₂, and 12.5 ng/mL sequencing grade modified trypsin (Promega). Following an overnight incubation at 37C, extracted peptides were identified by tandem mass spectrometry using LTQ Velos linear ion trap mass spectrometers (ThermoFisher). Peptides were separated using an Agilent 1100 series capillary LC system (Agilent Technologies) and 0.5 3 250mm Zorbax SB-C18 column (Agilent Technologies). Data-dependent collection of MS/MS spectra used the dynamic exclusion feature of the instrument's control software (repeat count equal to 1, exclusion list size of 100, exclusion duration of 30 sec, and exclusion mass width of -1 to +4) to obtain MS/MS spectra of the five most abundant parent ions (minimum signal of 10,000) following each survey scan from m/z 350-2000. There was no averaging of microscans, a maximum MS1 inject time of 200 msec, a maximum MS2 inject time of 100 msec, and automatic gain control targets of 30,000 in MS1 mode and 10,000 in MS2 mode.

Data processing protocol (5000 characters max):

This section is often taken largely from the methods section. There may be more additional Proteomics-specific analysis details needed here than in the primary publication.

(Example text below)

For data analysis, databases of human sequences was downloaded from www.uniprot.org in June 2012 (for sample 1) or April 2013 (sample 2) and appended to 179 common contaminants. We used an appended sequence-reversed decoy database to estimate error thresholds, giving a total of 26,996 entries (sample 1) or 27,010 entries (sample 2). The database processing was performed with python scripts available at https://github.com/pwilmart/fasta_utilities. SEQUEST (version 28, revision 12, Thermo Fisher) searches for all samples were performed with trypsin specificity. Average parent ion mass tolerance was 2.5 Da. Average fragment ion mass tolerance was 1.0 Da. The ion series used in scoring were b and y. A static modification of +57 Da was added to all cysteine residues. A variable modification of +16 Da on methionine residues was also allowed with a maximum of 3 modifications per peptide. SEQUEST scores were combined into linear discriminant function scores to improve identification sensitivity, and discriminant score histograms created separately for each peptide charge state (1+, 2+, and 3+). Separate histograms were created for matches to forward sequences and for matches to reversed sequences for all peptides of 7 amino acids or longer. False discovery rates (estimated from decoy counts) were less than 5% for both peptide spectrum matches and proteins. Python scripts used for processing can be found at https://github.com/pwilmart/PAW_pipeline.

<Pull down menu categories>

(Note: some of these fields may be left blank, depending on the nature of the experiment)

Experiment Type:

Choose sample species here:

Choose tissue here:

Choose MS instrument here:

Choose cell type here:

Choose disease here:

Choose quantification method here:

Modifications: