^{15}N Stable Isotope Labeling Data Analysis

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Census[1] is a freely available quantitative software that can fully analyze ^{15}N-labeled data (http://fields.scripps.edu/).

The Census software provides researchers with unique algorithms such as enrichment ratio calculation, accurate prediction of isotope distribution upon enrichment, sample mixture error correction, outlier filtering and more (Figure 1). SILAM ^{15}N labeling shifts the mass of a peptide based on the number of nitrogen atoms present, which is a function of the amino acid sequence. In order to quantitate peptides, an algorithm needs to be able to calculate the mass shift for every peptide's sequence, which is easy to perform once the sequence is known. Most algorithms do not possess this capability and can only quantitate peptides when there is a set mass shift between peptides. In addition, Census will also calculate the atomic percent enrichment of ^{15}N for every peptide, as this can vary depending on a protein's longevity or turnover rate.

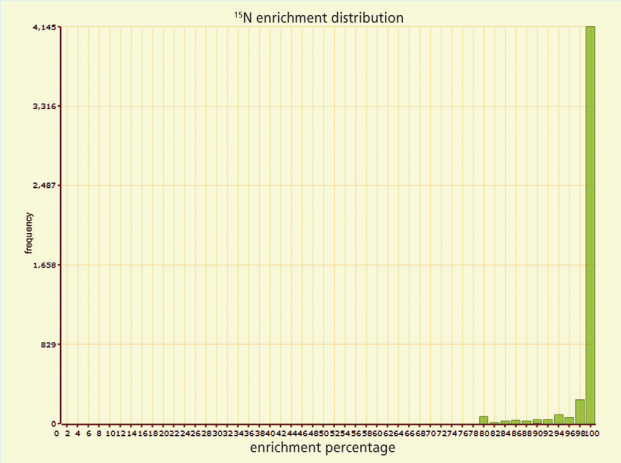

Figure 1. Enrichment calculation.

After protein identification, Census uses the amino acid elemental composition information to calculate corresponding isotopic distributions for both the light and heavy peptides. Ion intensities are extracted from spectral files using a user-defined mass accuracy tolerance to generate chromatograms from the m/z range surrounding both the unlabeled and labeled precursor peptides. Census then calculates peptide ion intensity ratios for each peptide pair using a linear least-squares correlation, which calculates the ratio and closeness of fit between the data points of the unlabeled and labeled ion chromatograms (Figure 2). To determine protein ratios, both mean and weighted means of peptide ratios were calculated upon peptide quality scores.

Integrated Proteomics Pipeline (IP2) is a proteomics data-analysis platform that provides comprehensive proteomics data analysis solutions from protein identification, quantification, modification analysis and multiple experiment analysis (Figure 3). The IP2 software supports the Census software and provides extended data analysis features with graphical tools. IP2 can analyze most quantitative data types, including ^{15}N stable isotope labeling.

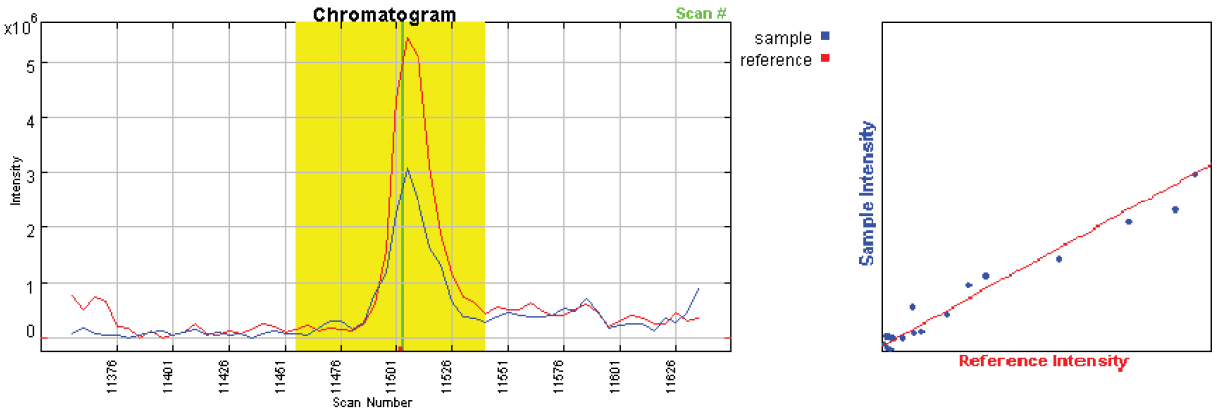

Figure 2. Census ratio calculation.

(continued)

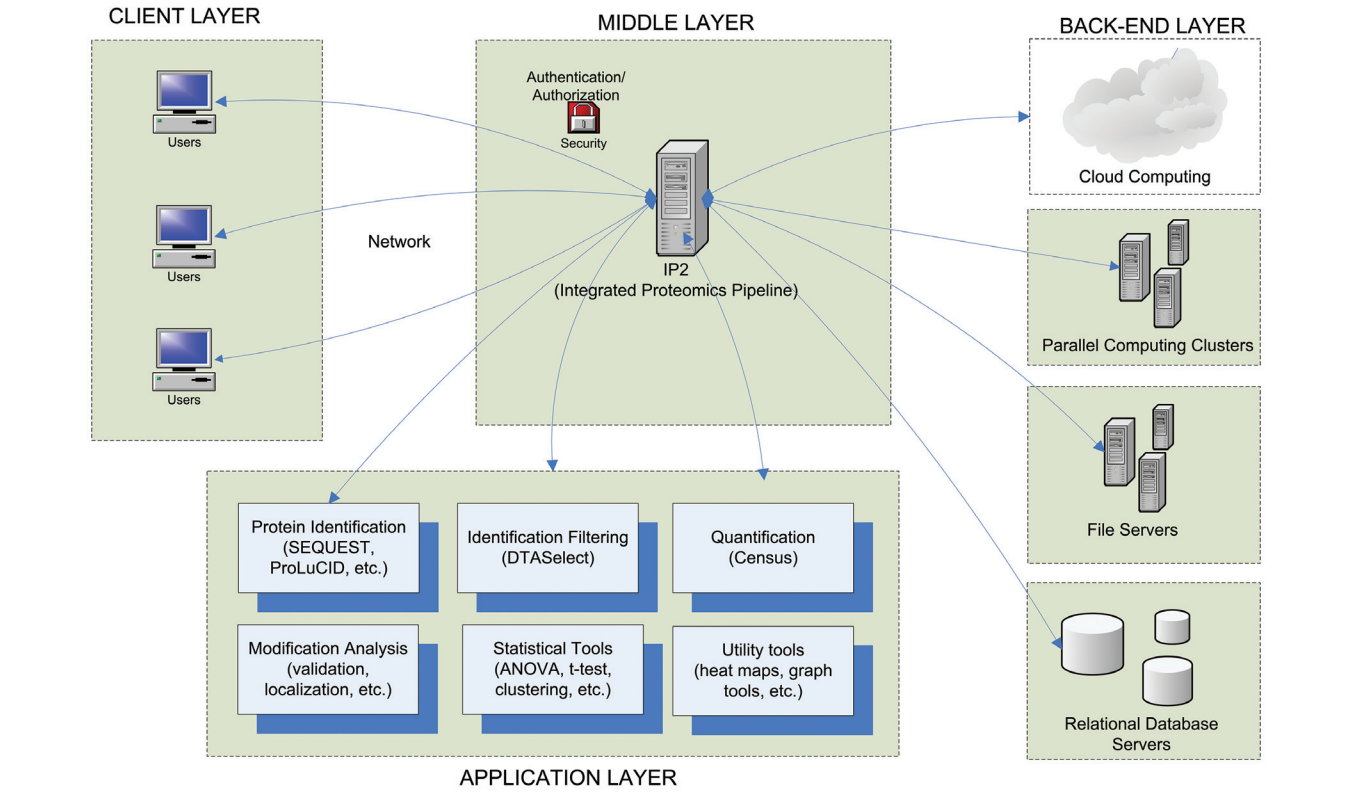

Figure 3. Overview of Integrated Proteomics Pipeline.

Users can effortlessly compare multiple samples to find significant proteins through various built-in statistical tools including *t*-test, ANOVA, clustering, post-hoc and more (Figure 4).

Additional information on Integrated Proteomics Pipeline (IP2) can be found at **www.integratedproteomics.com**.

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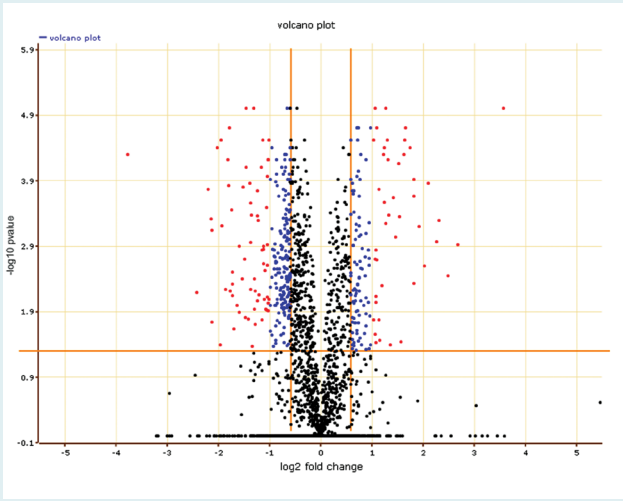

Figure 4. Finding significant proteins through multiple experiment comparison.

References

1. Park, S.K.; Venable, J.D.; Xu, T.; Yates, J.R. III. **2008**. A quantitative analysis software tool for mass spectrometry-based proteomics. *Nat Methods, 5*, 319-22.