

PROTEOMICS

Supporting Information for Proteomics

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A guided tour of the Trans-Proteomic Pipeline

- (1) requires vendor DLLs
- (2) third-party software included in TPP

Supplementary Material

SILAC sample preparation. *S. cerevisiae* strain JLY1 (*Mat alpha his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 arg4::KANMx4*) was grown to OD600 ~1.0 in minimal media supplemented with arginine and lysine of either normal isotopic distribution or isotopically heavy arginine ($^{13}\text{C}_6$, $^{15}\text{N}_4$) and lysine ($^{13}\text{C}_6$, $^{15}\text{N}_2$). Cells were frozen in liquid nitrogen and disrupted with a Retsch PM100 mixer mill. Powder was suspended in 25mM ammonium bicarbonate and cleared by centrifugation. Protein concentration was determined by BCA assay (Pierce). Lysate from yeast grown in heavy or light amino acids was mixed in a light to heavy ratio of 1 to 1. The mixture contained a total of 0.5 mg of yeast protein. The mixture was reduced with 2 mM TCEP for 30 min at room temperature and alkylated with 10 mM iodoacetamide for 30 min at room temperature. Trypsin (Promega) was added at a ratio of 1:100 w/w for overnight digestion at 37 °C. Digestion was verified by PAGE. The mixture was dried by evaporative centrifugation and sample was suspended in 1 ml of 1% ACN, 0.1% formic acid in water. The sample was loaded to a C18 clean up column (Waters) and washed with the loading buffer, and eluted in 45% ACN, 0.1 % formic acid. The mixture was again dried by evaporative centrifugation and resuspended in 200 μl of 0.1% Formic acid. 5 μl was loaded for each mass spectrometry analysis. Mass spectrometry was performed as previously described in Klimek et al.[1].

Reference

1. Klimek, J., Eddes, J. S., Hohmann, L., Jackson, J. *et al.*, The standard protein mix database: a diverse data set to assist in the production of improved Peptide and protein identification software tools. *J. Proteome Res.* 2008, **7**, 96-103.