

# Direct ribosomal protein quantitation in sucrose gradient fractions

## Sample preparation

**Experimental sample**  
Sucrose gradient fraction from  $^{14}\text{N}$ -metabolically labeled cell lysate

**Tryptic digest**  
Mixed, reduced, alkylated, digested, PepClean purified

**Reference sample**  
70S particle purified on sucrose gradient from  $^{15}\text{N}$ -metabolically labeled cell lysate

## MS data acquisition

**Data acquisition [Analyst]**  
SWATH-MS<sup>2</sup> data-independent acquisition [see attached]

**Raw data files**  
.wiff files [vendor format]

## Ion chromatogram extraction

**Proteins of interest**  
List of proteins of interest [selected list of r-proteins – see Fig S3B]

**Chromatographic peak extraction [Skyline]**  
MS<sup>2</sup> MRM-like transitions extracted for each product ion of interest. Extractions limited to 5 minute window around expected retention time based on iRT calibration. 8 most intense product ions in spectral library extracted per precursor ion.

**Spectral library**  
Consensus spectral library [generated by SpectraST, see WF2]

**Extracted ion chromatograms**  
Ion intensity vs. retention time  
.skyd file

## Peak filtering

**Spectral interference filtering [Skyline]**  
Each transition hand-inspected for signal intensity, spectral and chromatographic interference

**Automated filtering [Python scripts]**  
Extracted ion chromatograms filtered for exact mass error, deviation in expected retention time, spectral dot product between  $^{14}\text{N}$  and  $^{15}\text{N}$ , spectral dot product with library spectra

## Quantitation

**Transition intensity list [.csv]**  
 $^{14}\text{N}$  and  $^{15}\text{N}$  extracted ion chromatographic intensity for each product ion, for each peptide, for each protein.

**Peptide relative abundance [Python]**  
All  $^{14}\text{N}$  product ion intensities summed. All  $^{15}\text{N}$  product ion intensities summed. Ratio of  $^{14}\text{N}/^{15}\text{N}$  intensity determined.

**Protein relative abundance [Python]**  
For each protein, the median abundance ratio across all children peptides reported, normalized that of bL24, which is expected to bind stoichiometrically in all samples

**Experimental technique**

**Software tool**

**Data file**

**Relative protein abundance [.csv]**  
A relative protein abundance .csv file. Plotted as a heatmap in Fig S3B