Ribosomal protein quantitation in sucrose gradient fractions Sample **Experimental sample Tryptic digest** Reference sample ¹⁵N-metabolically labeled Sucrose gradient fraction from ¹⁴N-Mixed, reduced, alkylated, preparation metabolically labeled cell lysate digested, PepClean purified cell lysate Data acquisition [Analyst] MS data SWATH-MS² data-independent acquisition [see attached] acquisition Raw data files .wiff files [vendor format] Chromatographic peak extraction [Skyline] Spectral library lon **Proteins of interest** MS² MRM-like transitions extracted for each product ion of interest. Extractions Consensus spectral library List of proteins of interest chromatogram limited to 5 minute window around expected retention time based on iRT calibration. [generated by SpectraST, [ribosomal proteins in Fig 3] 8 most intense product ions in spectral library extracted per precursor ion. see WF2] extraction **Extracted ion chromatograms** Ion intensity vs. retention time .skyd file Automated filtering [Python scripts] Spectral interference filtering [Skyline] Peak Extracted ion chromatograms filtered for exact mass error, deviation in Each transition hand-inspected for signal intensity, spectral expected retention time, spectral dot product between ¹⁴N and ¹⁵N, filtering and chromatographic interference spectral dot product with library spectra Transition intensity list [.csv] Peptide relative abundance [Python] Protein relative abundance [Python] ¹⁴N and ¹⁵N extracted ion chromatographic All ¹⁴N product ion intensities summed. All ¹⁵N For each protein, the median abundance product ion intensities summed. Ratio of ¹⁴N/¹⁵N intensity for each product ion, for each ratio across all children peptides reported. peptide, for each protein. intensity determined. Quantitation Relative abundance conversion [Python] **Relative protein** R-protein stoichiometry [.csv] For each fraction, initial protein abundance is reported relative to a cell lysate. This value was R-protein stoichiometry relative to a WT abundance [.csv] also calculated for a 70S particle purified from WT cells, which is assumed to bear stoichiometric 70S particle. See Figure 3 r-proteins. Each R-protein abundance is normalized using the ratio observed for the 70S particle, Relative to cell lysate to yield an R-protein stoichiometry relative to a 70S particle

Experimental

technique

Software

tool

Data file

Workflow 5