

# qMS-based quantitation of pulse-labeling

## Sample preparation

## MS data acquisition

## File conversion, PSM search

## Isotope envelope quantitation

## Peak filtering

## Quantitation

**Experimental sample**  
Lysate or sucrose gradient fraction  
 $^{14}\text{N}$ -metabolically labeled and then pulsed to 50%  $^{15}\text{N}$

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

**Reference sample**  
 $^{15}\text{N}$ -metabolically labeled cell lysate or 70S sucrose gradient fraction

**Data acquisition [Analyst]**  
DDA data-dependent acquisition [see WF1]

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

**File conversion [MSConvert]**  
Peak picking or profile mode

**Converted data files**  
Peaks picked

**Spectral library generation**  
[Comet, PeptideProphet, iProphet, SpectraST] see WF2 for details

**Converted data files**  
 $\text{MS}^1$  profile mode

**Spectral library**  
Contains retention time information for each peptide identified. Limited to ribosomal proteins

**Isotope envelope abundance [.csv]**  
For each protein, for each peptide, for each isotope species, an abundance is reported.

**$\text{MS}^1$  Isotope envelope quantitation [Massacre]**  
 $\text{MS}^1$  isotope envelopes extracted in m/z and retention time region specified by the spectral library. Isotope envelope fit to theoretical distribution given peptide composition and isotope labeling (0%  $^{15}\text{N}$ , 50%  $^{15}\text{N}$ , or 100%  $^{15}\text{N}$ ).

**Peptide filtering [Python scripts]**  
Each isotope fit is hand-inspected for chromatographic and spectral interferences and is further filtered for exact mass error, relative mass error between species, and retention time drift.

**Filtered abundance [.csv]**  
For each protein, for each peptide, for each isotope species, an abundance is reported.

**Pulse incorporation calculation [Python]**  
For each peptide, ratio of pre-pulse ( $^{14}\text{N}$ ) to post-pulse (50%  $^{15}\text{N}$ ) calculated. For each protein, median peptide ratio reported and plotted (Fig 2D)

**Protein relative abundance [Python]**  
For each peptide, fraction of pre-pulse ( $^{14}\text{N}$ ) to total ( $^{14}\text{N}$  + 50%  $^{15}\text{N}$ ) calculated. For each protein, median peptide fraction labeling reported and plotted. Curves fit to pool size equations from Chen et al. (Fig 2E)

**Synthesis & degradation [Python]**  
For each peptide, ratio of post-pulse (50%  $^{15}\text{N}$ ) to standard ( $^{15}\text{N}$ ) [synthesis] or pre-pulse ( $^{14}\text{N}$ ) to standard ( $^{15}\text{N}$ ) [degradation] calculated. For each protein, median peptide ratio reported (Fig S2D, S2E)

**Experimental technique**

**Software tool**

**Data file**