LFQProfiler and RNP^{xl} – Open-source tools for label-free quantification and protein-RNA cross-linking integrated into Proteome Discoverer

Supporting Information

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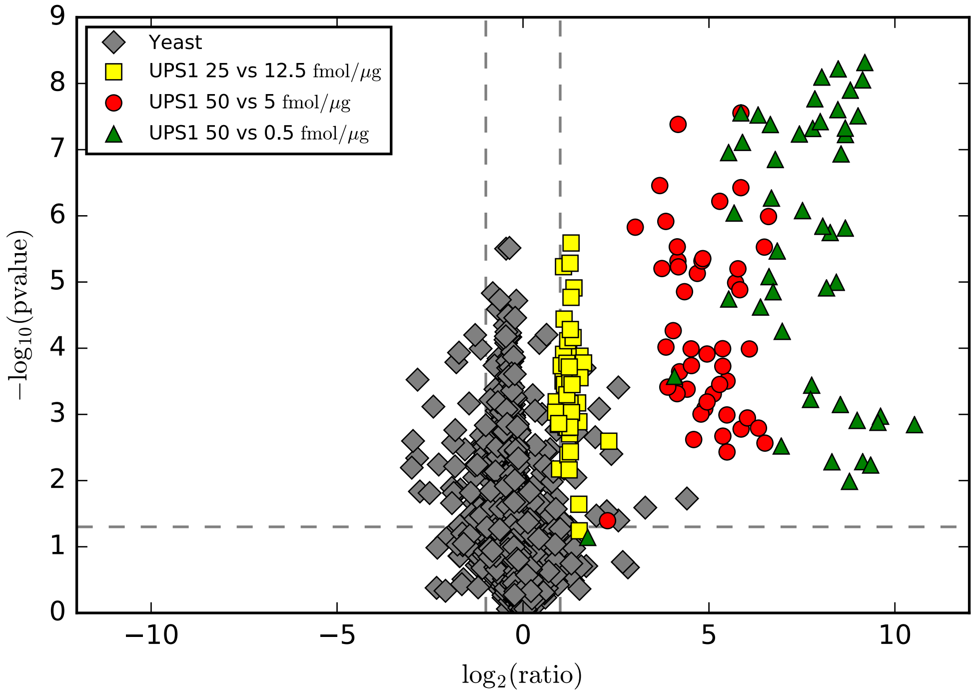
Table of contents:

- Supplementary Table S1: Parameter settings of PD nodes in the LFQProfiler benchmark (page S-2)

- Supplementary Figure S1: Volcano plot for LFQProfiler benchmarking results. (page S-3)

PD Node	Category	Parameter	Value
Spectrum Selector	Scan Event Filters	MS Order	Any
LFQProfiler FF	Feature Finding	Mass tolerance	6 ppm
LFQProfiler FF	Feature Finding	Charge Low	2
LFQProfiler FF	Feature Finding	Charge High	4
LFQProfiler FF	Feature Finding	Typical RT	40
LFQProfiler FF	Feature Finding	Minimum RT	5
LFQProfiler FF	Feature Finding	Averagine similarity	0.8
Sequest HT	Input Data	Protein Database	yeast_plus_ups.fasta
Sequest HT	Input Data	Enzyme Name	Trypsin/P (full)
Sequest HT	Tolerances	Precursor Mass Tolerance	10 ppm
Sequest HT	Tolerances	Fragment Mass Tolerance	0.6 Da
Sequest HT	Dynamic Modifications	1. Dynamic Modification	Oxidation / +15.995 Da (M)
Sequest HT	Dynamic Modifications (peptide terminus)	1. N-terminal Modification	Acetyl / +42.011 (N-Terminus)
Sequest HT	Static Modifications	1. Static Modification	Carbamidomethyl / +57.021 (C)
LFQProfiler	Feature Linking	Perform RT alignment	True
LFQProfiler	Feature Linking	Peptide min #runs	5
LFQProfiler	Feature Linking	Max RT difference [min]	1
LFQProfiler	Feature Linking	Max m/z difference	10 ppm
LFQProfiler	ID mapping	Max RT difference [min]	0.2
LFQProfiler	ID mapping	Max m/z difference	10 ppm
LFQProfiler	ID mapping	q-Value threshold	0.01
LFQProfiler	ID mapping	Protein database	Yeast_plus_ups.fasta
LFQProfiler	ID mapping	m/z reference	peptide
LFQProfiler	Intensity normalization	Method	median
LFQProfiler	Protein quantification	Protein-level FDR	0.01
LFQProfiler	Protein quantification	Use peptides	greedy
LFQProfiler	Protein quantification	Fido pre-filtering PEP threshold	0.2
LFQProfiler	Protein quantification	Top	5
LFQProfiler	Protein quantification	Averaging	sum
LFQProfiler	Protein quantification	Include all	True
LFQProfiler	Protein quantification	Filter charge	False
LFQProfiler	Protein quantification	Fix peptides	False

Supplementary Table S1: Parameter settings of PD nodes in the LFQProfiler benchmark. Parameters not mentioned here were set to default settings.



Supplementary Figure S1: Combined volcano plot for all three comparisons 50 vs 0.5 fmol / µg (green), 50 vs 5 fmol / µg (red), and 25 vs 12.5 fmol / µg (yellow) of spike-in protein standard. Yeast proteins are shown in gray. For each protein, the negative $\log_{10}$ of the p-value from Welsh's t-test for differential abundance between the two conditions is plotted against the corresponding $\log_2$ fold change.