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Extending Capabilities of Real-Time Database Search on the Orbitrap Eclipse Tribrid Mass Spectrometer for Multiplexed Proteomics

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Abstract

Introduction

The Real-Time Search (RTS) feature implemented on the Orbitrap Eclipse grants the ability to generate peptide spectrum matches (PSMs) from MS2 spectra during acquisition via online database search. Only upon confident identifications, the instrument may be triggered to proceed with SPS-MS3. By using the RTS feature, the acquisition efficiency would be largely improved while the precision and accuracy are well maintained.

Purpose

To evaluate recent improvements to RTS based TMT quantitation workflow including: 1) Close-out to prevent acquisition of excess MS3s for proteins which have already reached a sufficient depth of quantitative sampling, 2) Online false discovery rate (FDR) assessments to predicate MS3 events on real-time PSM-level FDR estimates.

Results

The RTS-MS3 workflow increased quantification accuracy and the number of quantified features compared to SPS MS3 methods. New capabilities of RTS, Close-out and FDR filtering, further contributed to more quantified IDs and better quantification performance.

Materials and Methods

Sample Preparation

Thermo Scientific™ Pierce™ TMT11plex Yeast Digest Standard was resuspended in buffer (5% Acetonitrile in 0.1% TFA) to a final concentration of 250ng/µl.

Ecoli peptides of different ratios (0:1:2:4:8) was spiked into an equal amount of Hela peptides and labeled with TMTpro 16 plex.

LC/MS Analysis

TMT 11 plex Yeast digest standard(500 ng) was loaded onto a 50 cm EASY-Spray™ column (ES803A) and separated by 50min gradient using a Thermo Scientific™ Easy-nLC™ 1000 at 250 nL/min flow rate.

For TMTpro 16 plex 2 proteome (human and E.coli) samples, an Ultimate 3000 RSLC nano system was used with a 50 cm EASY-Spray™ column (ES803A) at 300nL/min flow rate. One µg of sample was loaded and followed by a 120min-gradient separation.

Both experiments were performed on an Orbitrap Eclipse Tribrid mass spectrometer. Data acquisition was carried out on the ICSW 3.4.

Data Analysis

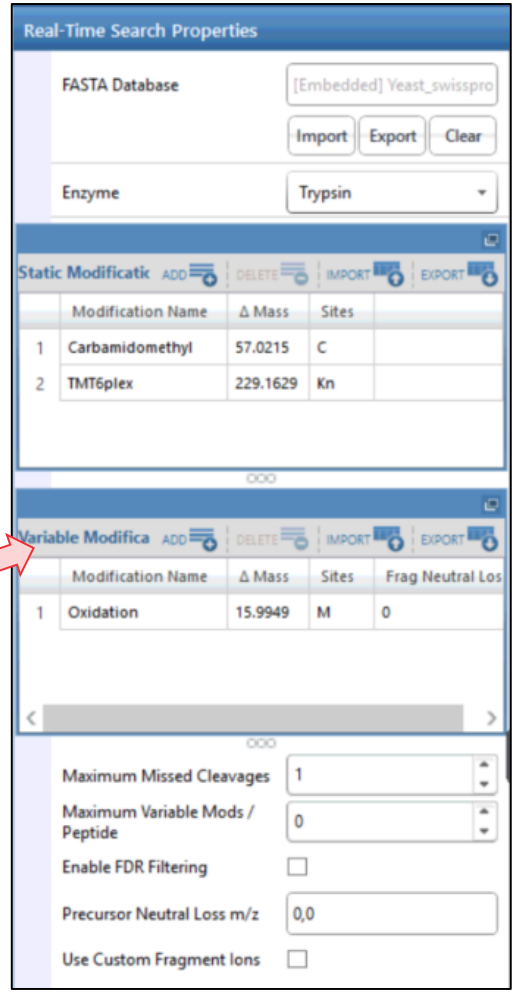
MS^2 spectra were analyzed during RTS by the Comet search algorithm. Close-out and FDR filtering were setup in corresponding experiments.

Post-acquisition data analysis was performed by Sequest HT in Thermo Scientific™ Proteome Discoverer™ Software 2.5.

Materials and Methods

MS Method

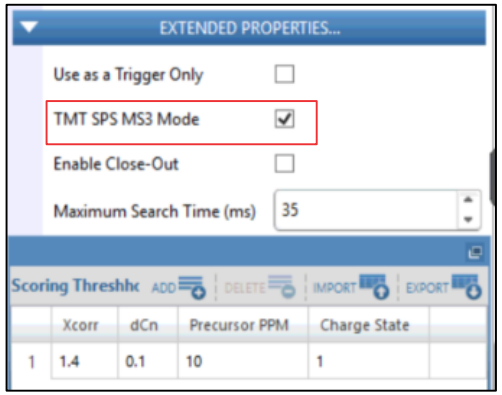
Real-Time Search Setup



Real-Time Search Extended Properties

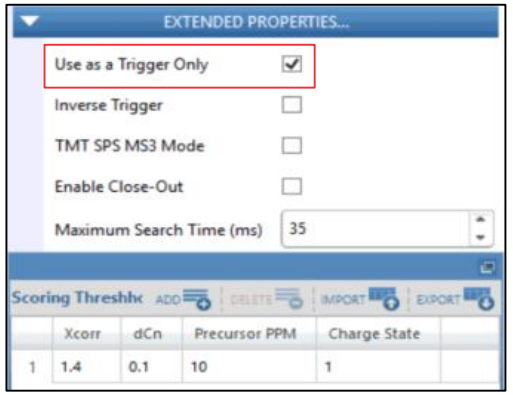
Real-Time Search

(SPS selection of TMT-containing fragments)



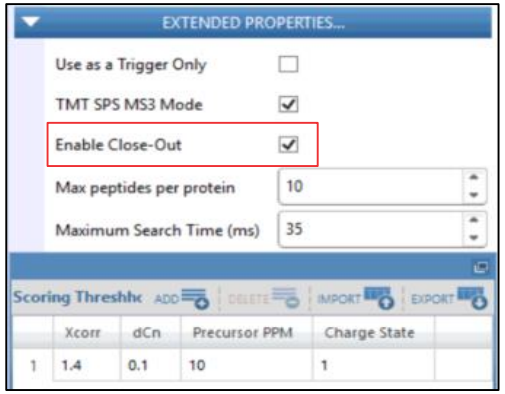
RTS Trigger-Only

(SPS selection of top abundant fragments)



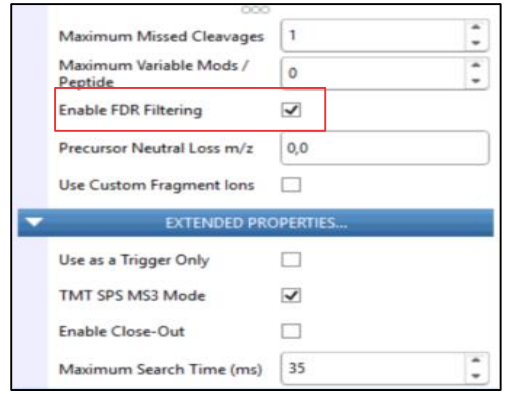
RTS Close-Out

(Limit number of peptides identified for each protein)



RTS FDR

(MS3 triggered by real-time PSM-level FDR estimates)



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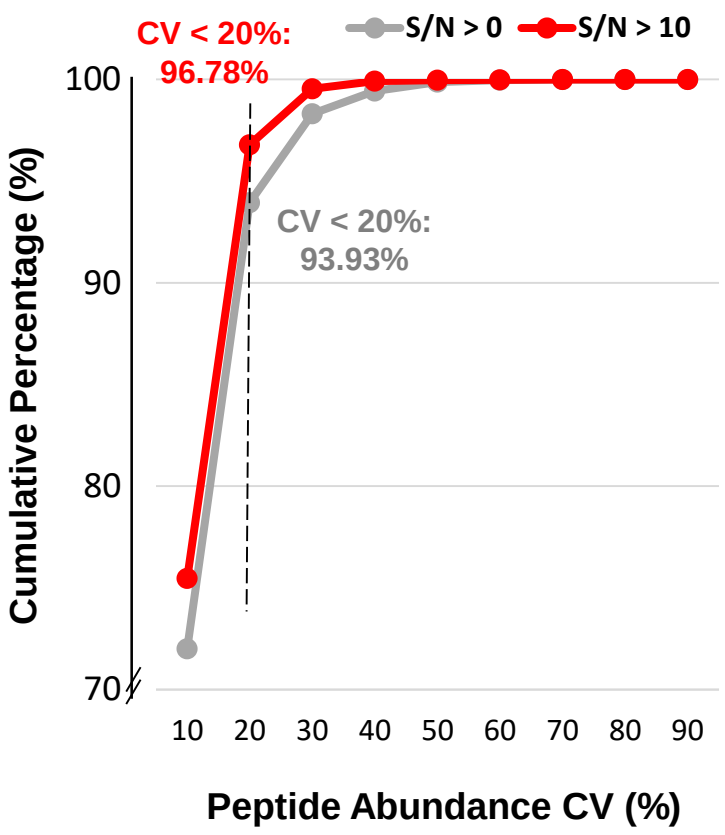
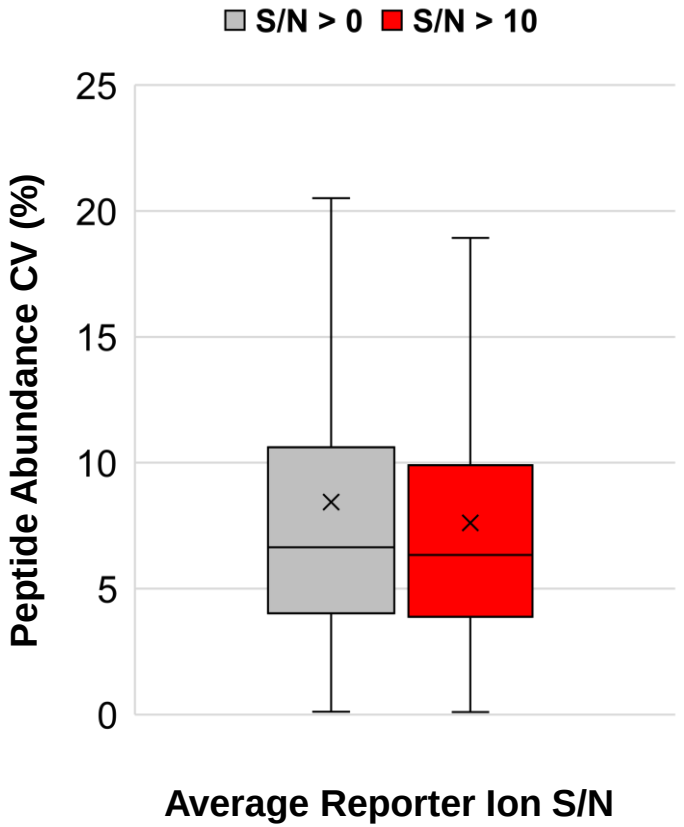
Proper Threshold Applied in Data Analysis to Ensure Data Quality

RTS-SPS-MS3 Quantitative Precision at Different Reporter Ion S/N Level

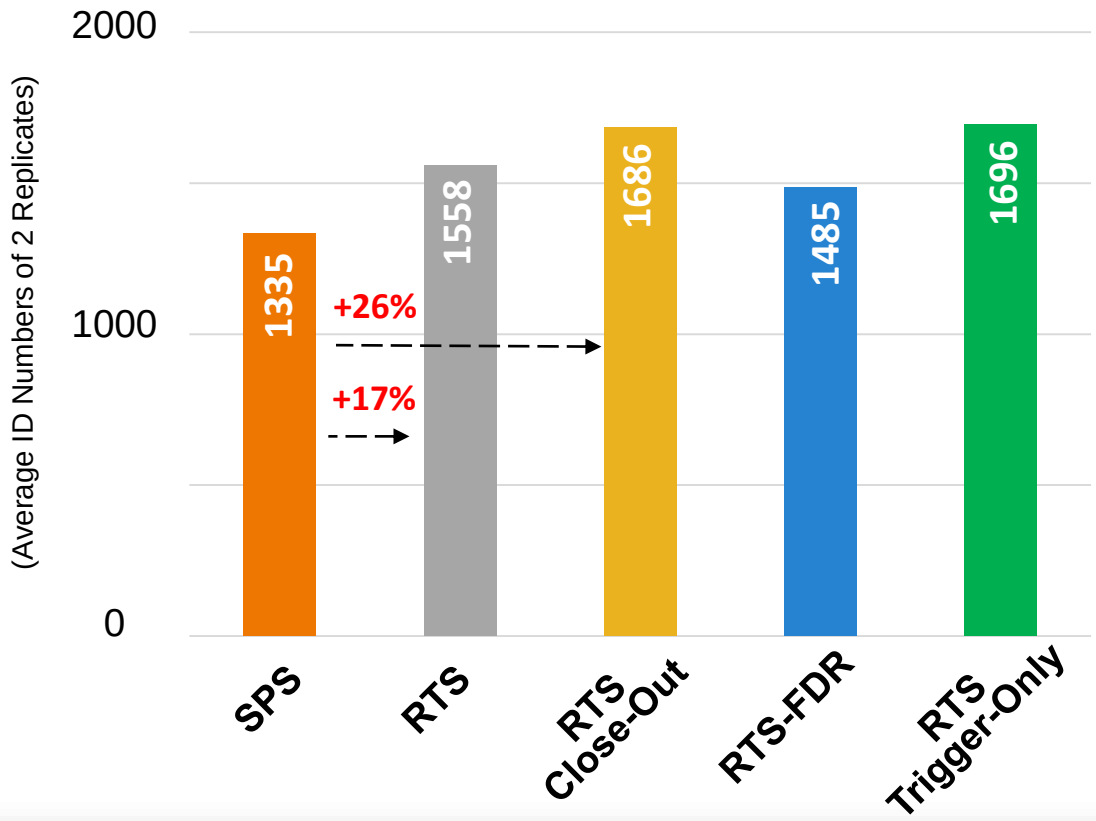


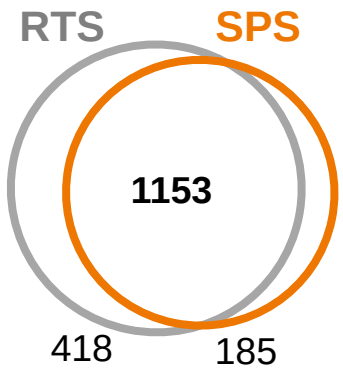


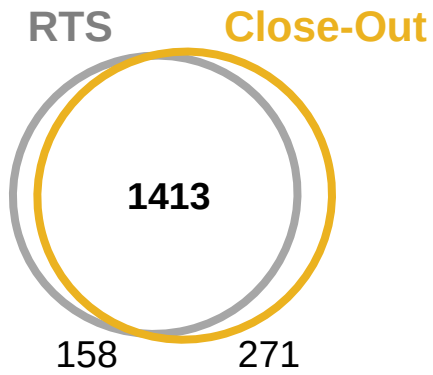
- In multiplexed quantitative analysis, the quality of reporter ions could largely influence the quantitation performance.
- We applied the new feature of 100% ”Minimum Channel Occupancy” in PD 2.5 software to ensure the dataset completeness.
- By increasing the threshold of average reporter ion signal-to-noise ratio, the precision of quantitative analysis is improved.

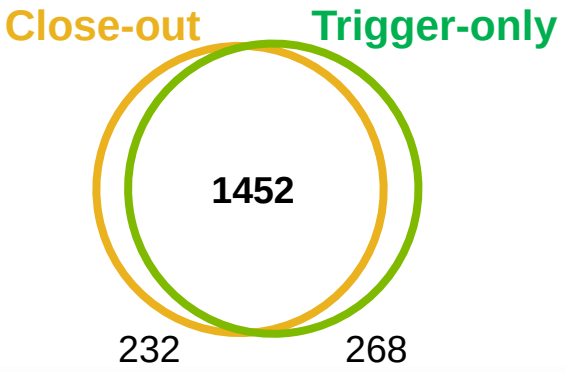
Quantitation Performance of Extended Features in RTS-SPS-MS3

of Quantified Proteins (Average Reporter Ion S/N > 10)









- Real-Time Search improved number of quantified proteins by **17%,** which is further boosted up to **26%** by Close-out.
- Close-Out and Trigger-Only quantified similar number of proteins but each feature owns unique IDs.

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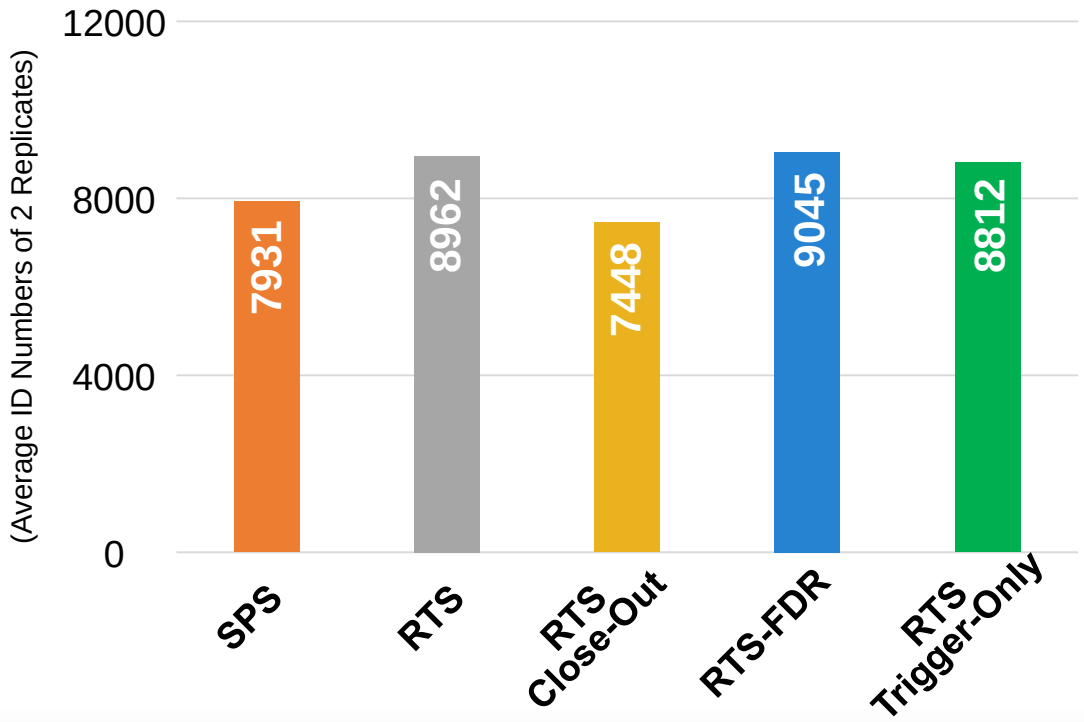
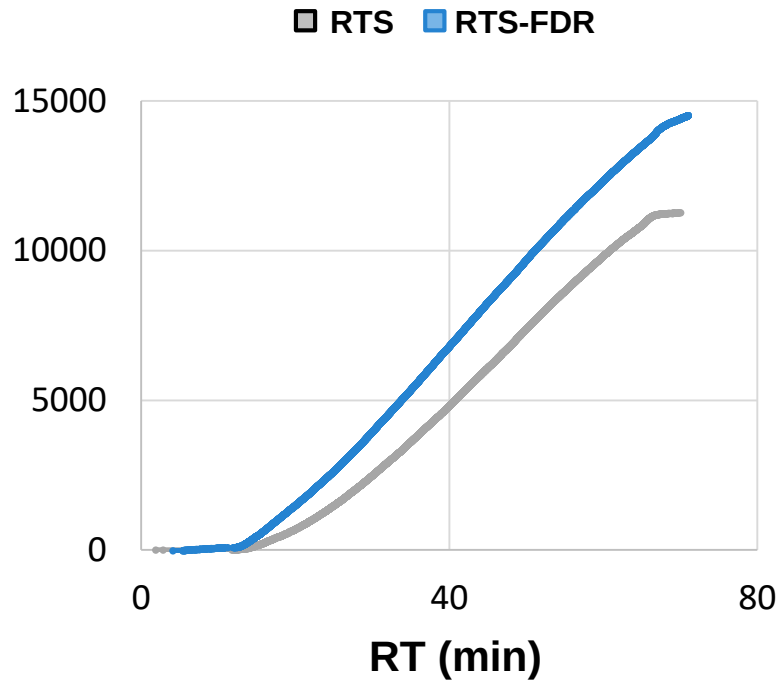
Quantitation Performance of Extended Features in RTS-SPS-MS3

of Quantified Peptides
(Average Reporter Ion S/N > 10)



of Quan Spectra
(Average Reporter Ion S/N > 10)



- RTS and RTS-FDR improved number of quantified proteins by **13%** and **14%**, compared to SPS-MS3.
- FDR filter enabled more quan spectra in addition to RTS.

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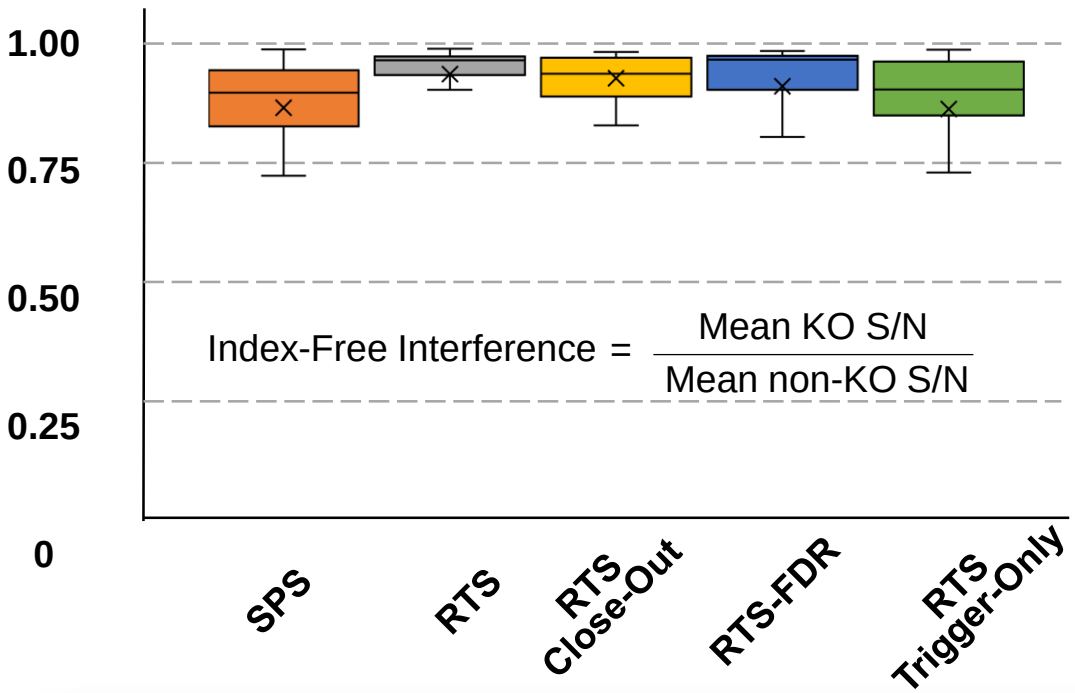
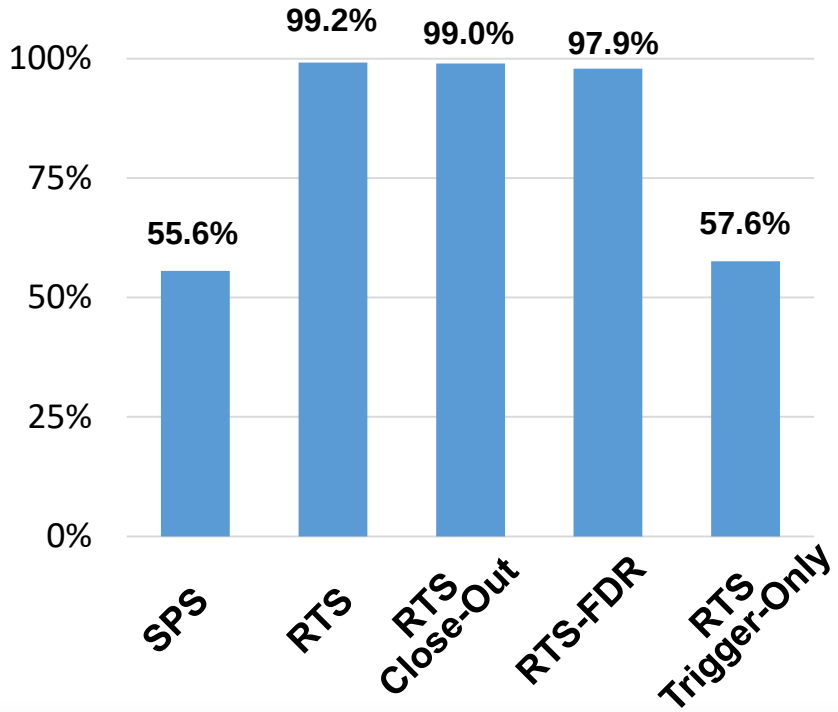
Quantitation Performance of Extended Features in RTS-SPS-MS3

Index-Free Interference
(Met6 Channel; Average Reporter Ion S/N > 10)



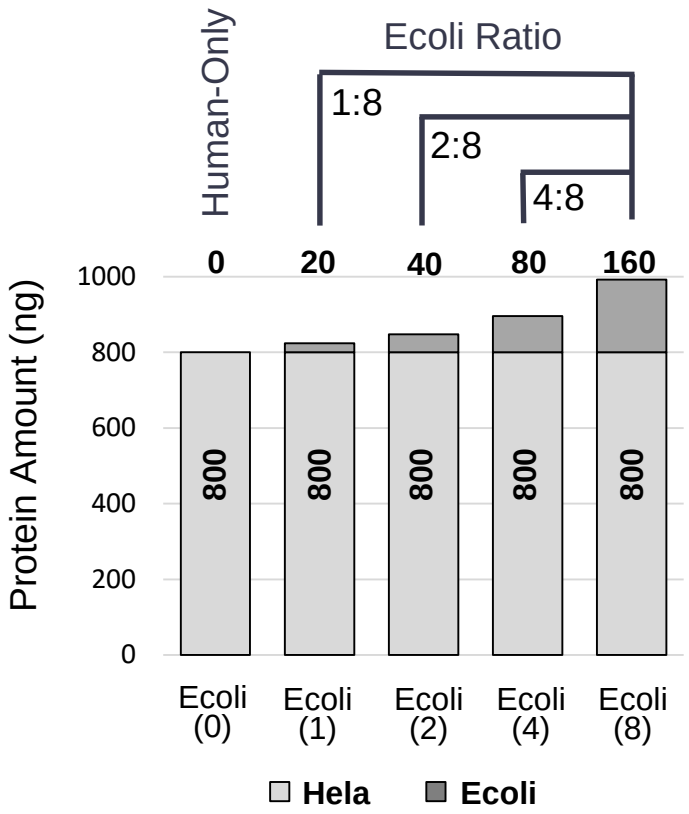
SPS Mass Matches >65%
(Average Reporter Ion S/N > 10)



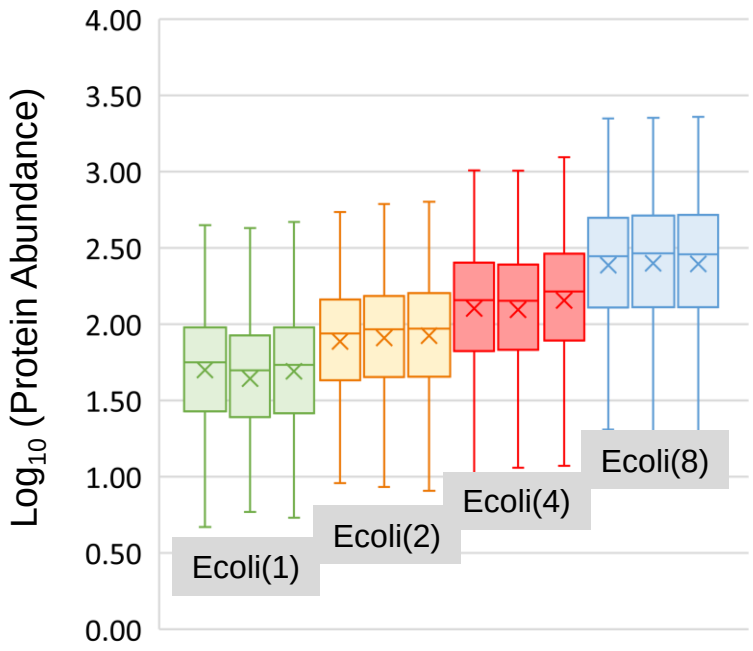
- RTS "TMT mode" option (RTS-informed selection of TMT containing fragments for SPS isolation) exhibited better quantitation performance compared to abundance dependent methods (SPS, RTS-TriggerOnly).
- CloseOut and FDR filter improved quantitation IDs while still maintained the outstanding quantitation performance.

Human:Ecoli Mixed Proteome Quantification

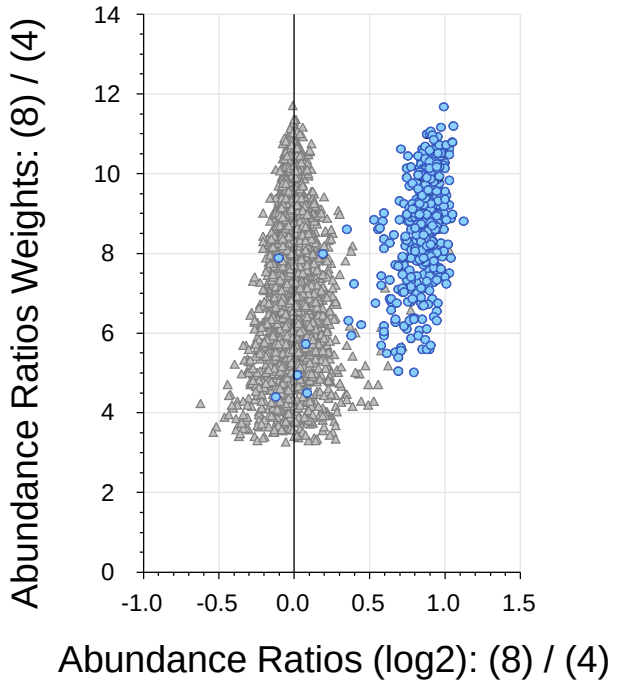
Experiment Design



High Reproducibility



Quantitation Accuracy



- RTS and RTS-Close Out improved number of quantified proteins by **25%** and **30%**, compared to SPS-MS3

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Conclusions

- Real-time Search feature on Orbitrap Eclipse, benefiting from the online database search, enhanced the quantification accuracy and the number of quantified features compared to SPS MS3 methods
- The new capabilities in the RTS-MS3 workflow extended its capabilities for TMT quantitation.
  - Close-Out improved number of quantified proteins.
  - FDR filter increased number of quantified peptides and quan spectra.
- Both new capabilities achieved high accuracy and precision in quantitation performance.