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NEXT GENERATION PROTEOMICS

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Improved Library-Free Proteomics Analysis for dia-PASEF Using directDIA+ in Spectronaut

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Introduction

Data-independent acquisition (DIA) is increasingly being adopted as the default method for label-free discovery proteomics. This is supported by the possibility of skipping time consuming project-specific library generation. Spectronaut® 18 features an improved directDIA™ workflow, also known as directDIA+, which greatly improves the depth of proteome coverage using DIA proteomics.
Here we present directDIA+ performance for dia-PASEF projects in Spectronaut compared with DIA-NN 1.8.1 in terms of identification and ability to detect true candidates in controlled quantitative experiment.

Methods

Human plasma samples were measured using Biognosys' TrueDiscovery platform. In short, samples were depleted and downstream sample prep was performed in 96-well format. Samples were analyzed on a Thermo Scientific EASY-nLC 1200 coupled to a Thermo Scientific Orbitrap Exploris 480. A FAIMS Pro was connected to the Exploris 480. LC-MS DIA methods were optimized for the respective gradient lengths. DIA data was either directly searched using directDIA or analyzed with a library using Spectronaut (Biognosys).

Preliminary Data

We prepared a 4-species mixed proteome sample with two conditions (H. sapiens 1:1, S. cerevisiae 1:2, E. coli, 1:10, C. elegans 1.3:1).

For the quantitative experiment, each condition was acquired in triplicates using dia-PASEF on timsTOF HT with 30, 60, and 120 min gradients. The analysis were done using library free mode in Spectronaut 18 and DIA-NN 1.8.1 with the same protein database and FDR settings.

Comparison was based on overall identifications, precision, accuracy, and number of true candidates.

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Results

Spectronaut significantly outperforms DIA-NN for peptide precursor identification, whereas both software perform similarly at protein group level.

In order to assess true candidates' recovery, we applied unpaired t-test at the protein group level in both software. Subsequently candidates of differential abundance analysis were sorted by p-value and categorized as true and false positives based on the ground truth. SN 18 recovered more true candidates than DIA-NN 1.8.1 across all three gradient lengths.

Overall, the gap between the two software widens with increasing gradient length for the tested 15, 30, and 60 minutes methods.

Conclusions

Spectroanut's directDIA™ workflow provides in-depth analysis for dia-PASEF data with unmatched true candidates recovery.