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Version 2

LC-MS/MS Proteomics Data Searching for PASEF DIA and DDA V.2

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Protocol status: Working

We use this protocol and it's working



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Disclaimer

Scaffold DDA and Spectronaut are paid software

Abstract

The following are search parameters for DIA and DDA PASEF bottom-up, unlabeled proteomics data collected on timsTOF mass spectrometers.

Before start

1. Scaffold DDA viewer can be downloaded for free here: www.proteomesoftware.com

DDA Searching with Scaffold sDDA version 6.7.4

- 1 A new project was made within Scaffold DDA version 6.7.4 with all samples loaded simultaneously
- 2 Data were searched within sDDA using MSFragger with the default parameters recommended by sDDA using the human uniprot database containing 50% decoys
- 3 With these initial filters, data were filtered within sDDA with 5% peptide and 5% protein FDR with a 2 peptide minimum
Data was then additionally filtered down to 1% peptide and 1% protein FDR with a 2 peptide minimum to ensure quality reports
- 4 Exports were generated from sDDA GUI view:
 - Samples report
 - Peptide report
 - Spectra report by protein group

DIA Searching with Spectronaut version 20.3

- 5 Create a new project and load all DIA raw files into Spectronaut version 20.3
- 6 Search the data within spectronaut as a "library free" analysis bases on the human uniprot database with fully default parameters
- 7 Export the following pre-loaded report formats
 - Pivoted BGS report
 - Pivoted Protein report