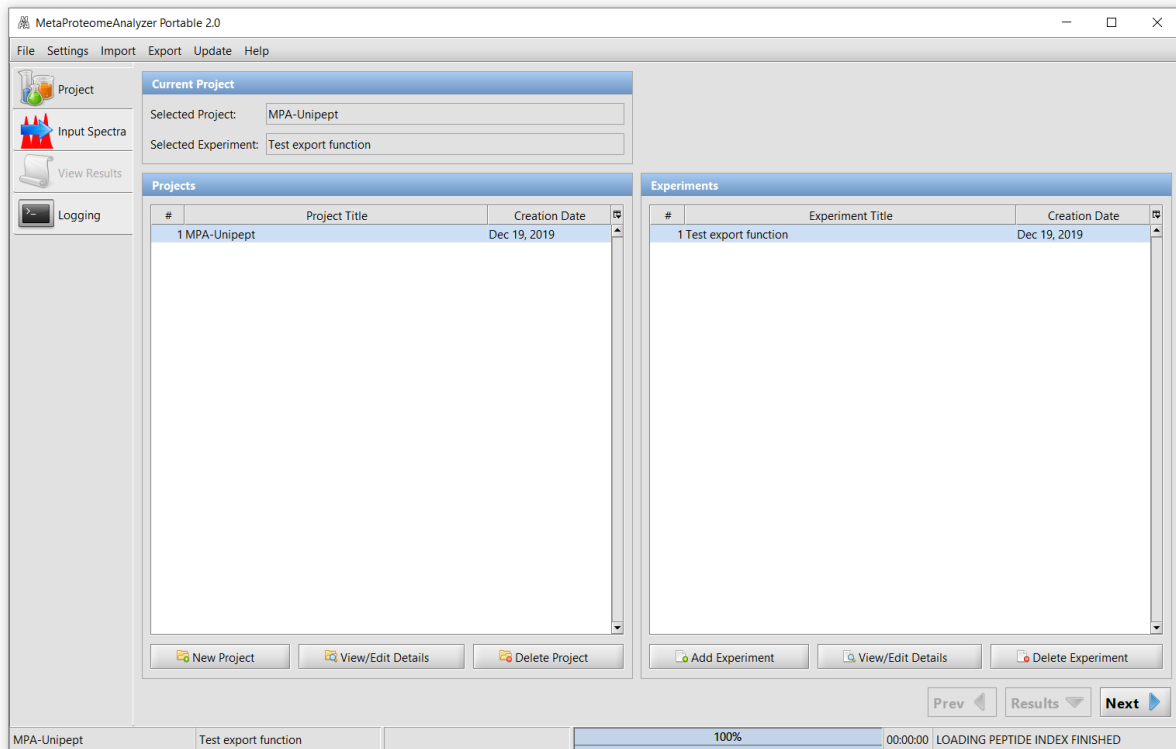


## Supplementary “Connecting MetaProteomeAnalyzer and PeptideShaker to Unipept for seamless end-to-end metaproteomics data analysis”

For experienced MPA users: steps 1 - 4 are identical for any MPA workflow. The added functionality is described in step 5.

Step 1: Create a project (click New Project) and an experiment (Add Experiment), click Next.



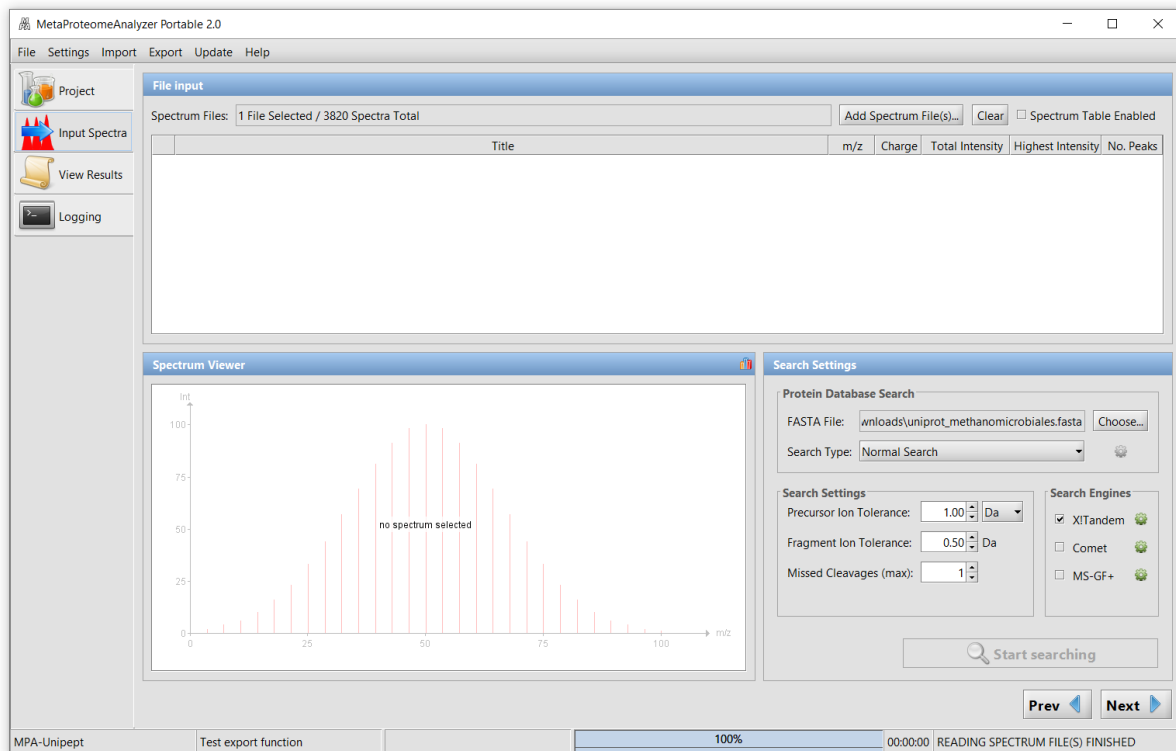
Step 2: Add a Spectrum file (dataset), a FASTA database of protein sequences, and fill in the search parameters. In this mini tutorial we used the Ebendorf data set on the GitHub page, performed a normal search and used X!Tandem as search engine. We allowed one missed cleavage.

Dataset:

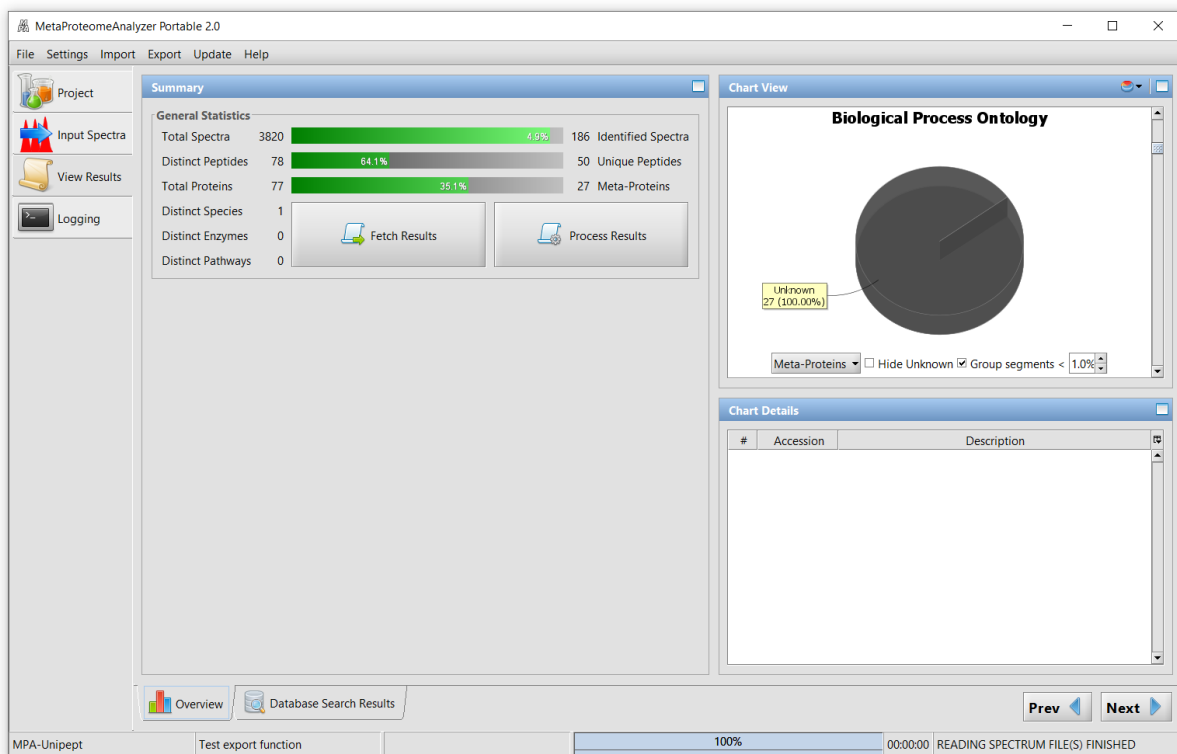
<https://github.com/compomics/meta-proteome-analyzer/raw/master/test/de/mpa/resources/Ebendorf1.zip>

FASTA database:

[https://github.com/compomics/meta-proteome-analyzer/raw/master/test/de/mpa/resources/fasta/uniprot\\_methanomicrobiales.fasta](https://github.com/compomics/meta-proteome-analyzer/raw/master/test/de/mpa/resources/fasta/uniprot_methanomicrobiales.fasta)



Step 3: Click on Fetch results.



Step 4: Click on Process results. Check the maximum PSM FDR and adapt if necessary. In this tutorial, we change the FDR to 0.01.

The screenshot displays the MetaProteomeAnalyzer Portable 2.0 software interface. A 'Result Processing Settings' dialog box is open, allowing users to adjust various parameters for data processing. The dialog is divided into three main sections: 'Scoring', 'Taxonomy Definition', and 'Meta-Protein Generation'. In the 'Scoring' section, the 'Maximum peptide-spectrum match FDR' is set to 0.01. The 'Taxonomy Definition' section shows 'Peptide-to-Protein Taxonomy' and 'Protein-to-Meta-Protein Taxonomy' both set to 'by common ancestor'. The 'Meta-Protein Generation' section has 'Generate Meta-Proteins' checked, with a 'Peptide Rule' set to 'have at least one peptide in common'. Other options like 'Consider Leucine and Isoleucine Distinct', 'Cluster Rule', and 'Taxonomy Rule' are currently unchecked. The background shows the 'Summary' panel with general statistics (3820 Total Spectra, 186 Identified Spectra) and a 'Chart View' showing a 'Biological Process Ontology' pie chart. The 'Chart Details' panel is empty. The status bar at the bottom indicates 'MPA-Unipept', 'Test export function', '100%', '00:00:00', and 'POPULATING TABLES FINISHED'.

MetaProteomeAnalyzer Portable 2.0

File Settings Import Export Update Help

Project  
Input Spectra  
View Results  
Logging

Summary

General Statistics

Total Spectra 3820 4.9% 186 Identified Spectra

Distinct Peptides  
Total Proteins  
Distinct Species  
Distinct Enzymes  
Distinct Pathways

Result Processing Settings

Scoring

Maximum peptide-spectrum match FDR 0.01

Taxonomy Definition

Peptide-to-Protein Taxonomy by common ancestor

Protein-to-Meta-Protein Taxonomy by common ancestor

Meta-Protein Generation

☒ Generate Meta-Proteins

☒ Peptide Rule have at least one peptide in common

☐ Consider Leucine and Isoleucine Distinct

Max. Peptide Sequence Distance 0

☐ Cluster Rule in UniRef100

☐ Taxonomy Rule on superkingdom level or lower

Restore Defaults OK Cancel

Chart View

Biological Process Ontology

Unknown 27 (100.00%)

Meta-Proteins ☐ Hide Unknown ☒ Group segments < 1.0%

Chart Details

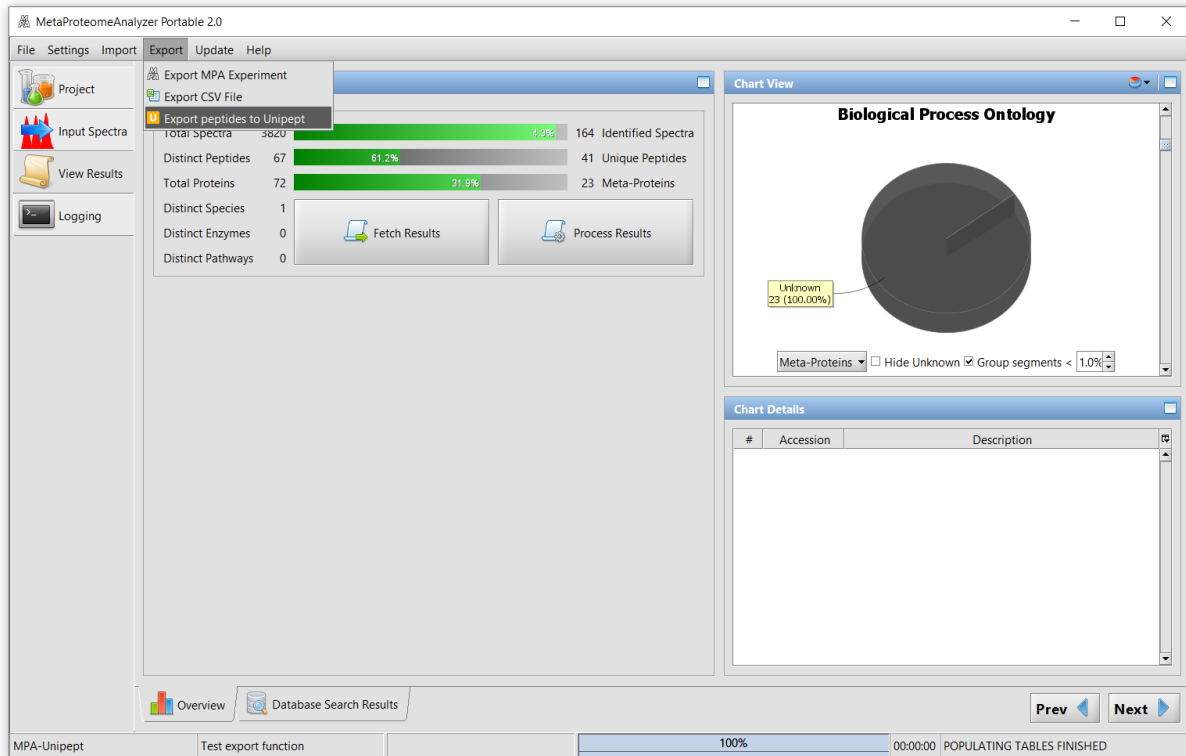
#	Accession	Description
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Overview Database Search Results

MPA-Unipept Test export function 100% 00:00:00 POPULATING TABLES FINISHED

Prev Next

Step 5: the peptides on 1% FDR are fetched and ready to be exported to Unipept. Click Export > Export peptides to Unipept.



Step 6: Unipept is opened in the browser with the identified peptides. Choose the preferred search settings for Unipept and click on Update. The taxonomic and functional results can now be viewed in the browser or exported to a .csv file.

