

### Import Peptide Search

#### Spectral Library

Build     Use existing

Start from:

**DIA raw (deconvolute, search, and build library)**

Files to search: Cut-off score: 0.95

File
231207_dia_mzml_rename\0013-23125-B1-ALL.mzML
231207_dia_mzml_rename\0014-23125-A1-ALL.mzML
231207_dia_mzml_rename\0014-23125-B1-ALL.mzML
231207_dia_mzml_rename\0015-23125-A1-THY.mzML
231207_dia_mzml_rename\0015-23125-B1-THY.mzML
231207_dia_mzml_rename\0016-23125-A1-ALL.mzML

iRT standard peptides:

None

Include ambiguous matches  
 Filter for document peptides

Workflow

DDA with MS1 filtering  
 DIA  
 PRM

Inputs = deconvoluted mzML files

### Import Peptide Search

#### Configure Transition Settings

Precursor charges:     Ion charges:     Ion types:

Product ions from:     Product ions to:

Min m/z:  m/z    Max m/z:  m/z

Use DIA precursor window for exclusion

Ion match tolerance:     Pick:  product ions  
 Ion match tolerance unit:     3 min product ions

### Import Peptide Search

#### Configure Full-Scan Settings

MS1 filtering

Isotope peaks included:     Precursor mass analyzer:

Peaks:     Mass Accuracy:  ppm

MS/MS filtering

Acquisition method:     Product mass analyzer:

Isolation scheme:     Mass Accuracy:  ppm

Use high-selectivity extraction

Retention time filtering

Use only scans within  minutes of MS/MS IDs  
 Use only scans within  minutes of predicted RT  
 Include all matching scans

Import Peptide Search

### Import FASTA (required)

Enzyme:  Max missed cleavages:

FASTA records begin with '>' and have the protein name followed by the optional protein description.

Import target proteins from a separate FASTA

Decoy generation method:  Decoys per target:

Automatically train mProphet model

Import Peptide Search

### Adjust Conversion Settings

DIA-Umpire

DIA-Umpire converts DIA spectra into pseudo-DDA spectra. Skyline builds a library by searching the DDA spectra but performs quantitation on the original DIA spectra.

Instrument preset:

- QExactive
- TripleTOF
- QExactive
- OrbitrapLTQ

Import Peptide Search

### Adjust Search Settings

Search engine:  
MSFragger

MS1 tolerance: 20 Unit: ppm  
MS2 tolerance: 20 Unit: ppm  
Fragment ions: b.y  
MS2 analyzer: Default  
Max variable mods: 3

Additional Settings

< Back Next > Cancel

Import Peptide Search

### Adjust Search Settings

Agree to MSFragger license

MSFragger is available freely for academic research, non-commercial or educational purposes under academic license. Other uses require a commercial license after the initial 60-day evaluation period that can be obtained by contacting Drew Bennett (andbenne@umich.edu) at the University of Michigan Office of Tech Transfer.

Working

Downloading MSFragger 3.4

Cancel

I would like to receive emails with updates in the future.  
 I would like to receive emails with updates in the future.

Accept and Download Cancel

< Back Next > Cancel

Import Peptide Search

### DDA Search

Search progress:

Show timestamps

```
[2024/02/21 08:42:57] Starting DIA-Umpire conversion
[2024/02/21 08:42:59] [0001-231030-A1-LT2 (1 of 41)] [step 1 of 7] assigning
spectra to DIA windows: 1/98373
[2024/02/21 08:43:00] [0001-231030-A1-LT2 (1 of 41)] [step 1 of 7] assigning
spectra to DIA windows: 5917/98373
[2024/02/21 08:43:01] [0001-231030-A1-LT2 (1 of 41)] [step 1 of 7] assigning
spectra to DIA windows: 12328/98373
[2024/02/21 08:43:02] [0001-231030-A1-LT2 (1 of 41)] [step 1 of 7] assigning
spectra to DIA windows: 15668/98373
[2024/02/21 08:43:03] [0001-231030-A1-LT2 (1 of 41)] [step 1 of 7] assigning
spectra to DIA windows: 18909/98373
[2024/02/21 08:43:04] [0001-231030-A1-LT2 (1 of 41)] [step 1 of 7] assigning
spectra to DIA windows: 22005/98373
[2024/02/21 08:43:05] [0001-231030-A1-LT2 (1 of 41)] [step 1 of 7] assigning
spectra to DIA windows: 25178/98373
[2024/02/21 08:43:06] [0001-231030-A1-LT2 (1 of 41)] [step 1 of 7] assigning
spectra to DIA windows: 28304/98373
[2024/02/21 08:43:07] [0001-231030-A1-LT2 (1 of 41)] [step 1 of 7] assigning
spectra to DIA windows: 31345/98373
[2024/02/21 08:43:08] [0001-231030-A1-LT2 (1 of 41)] [step 1 of 7] assigning
spectra to DIA windows: 34367/98373
[2024/02/21 08:43:09] [0001-231030-A1-LT2 (1 of 41)] [step 1 of 7] assigning
spectra to DIA windows: 37376/98373
```

Cancel Search

0%

< Back

Finish

Cancel

Import Peptide Search

### DDA Search

Search progress:

Show timestamps

```
window: 28/52
[2024/02/21 11:11:36] [0012-23125-A1-SAA (33 of 41)] [step 7 of 7] processing DIA
window: 29/52
[2024/02/21 11:11:45] [0012-23125-A1-SAA (33 of 41)] [step 7 of 7] processing DIA
window: 30/52
[2024/02/21 11:11:52] [0012-23125-A1-SAA (33 of 41)] [step 7 of 7] processing DIA
window: 31/52
[2024/02/21 11:11:56] [0012-23125-A1-SAA (33 of 41)] [step 7 of 7] processing DIA
window: 32/52
[2024/02/21 11:12:07] [0012-23125-A1-SAA (33 of 41)] [step 7 of 7] processing DIA
window: 33/52
[2024/02/21 11:12:12] [0012-23125-A1-SAA (33 of 41)] [step 7 of 7] processing DIA
window: 34/52
[2024/02/21 11:12:14] [0012-23125-A1-SAA (33 of 41)] [step 7 of 7] processing DIA
window: 35/52
[2024/02/21 11:12:16] [0012-23125-A1-SAA (33 of 41)] [step 7 of 7] processing DIA
window: 37/52
[2024/02/21 11:12:18] [0012-23125-A1-SAA (33 of 41)] [step 7 of 7] processing DIA
window: 38/52
[2024/02/21 11:12:25] [0012-23125-A1-SAA (33 of 41)] [step 7 of 7] processing DIA
window: 39/52
[2024/02/21 11:12:35] [0012-23125-A1-SAA (33 of 41)] [step 7 of 7] processing DIA
window: 41/52
[2024/02/21 11:12:40] [0012-23125-A1-SAA (33 of 41)] [step 7 of 7] processing DIA
```

Cancel Search

40%

< Back

Finish

Cancel

Skyline



Error

System.IO.IOException: WARNING: The output directory 'D:\dia\_mzml\231101\_dia\_mzml\_rename\crux-output' already exists.  
WARNING: The file 'D:\dia\_mzml\231101\_dia\_mzml\_rename\crux-output\percolator.log.txt' already exists and will be overwritten.

Command-line: C:\Users\wphipp5\AppData\Local\Apps\2.0\1QH7QGOD.Y1T\C5RQJ749.QML\skyl.tion\_2e441fc3bf6adc7f\_0017  
Working directory:  
Standard input:

at pwiz.Common.SystemUtil.ProcessRunner.Run(ProcessStartInfo psi, String stdin, IProgressMonitor progress, IProgressStatus& status)  
at pwiz.Skyline.Model.DdaSearch.MsFraggerSearchEngine.Run(Cancellation-TokenSource cancelToken, IProgressStatus status) in 0

Buttons: OK, More Info