# Supplementary Note 1: DIA Data Analysis Without Spectral Libraries

This tutorial is a practical guide for how to use the Encyclopedia software suite to build chromatogram libraries for DIA-MS. We have a GUI-based workflow and also a command line workflow. We've included options for visualizing the results of the Encyclopedia analysis in Skyline or in Encyclopedia itself.

#### Citations

MSconvert (<u>https://www.nature.com/articles/nbt.2377</u>)

A cross-platform toolkit for mass spectrometry and proteomics. Chambers MC et al. *Nat Biotech* 30, 918-920 (2012). doi.org/10.1038/nbt.2377 Encyclopedia (<u>https://www.nature.com/articles/s41467-018-07454-w</u>)

Chromatogram libraries improve peptide detection and quantification by data independent acquisition mass spectrometry. Searle BC et al. *Nat Comm* 9, 5128 (2018). doi.org/10.1038/s41467-018-07454-w

You will need:

- MSConvert from Proteowizard: *Windows only!* 
  - <u>http://proteowizard.sourceforge.net/download.html</u>
- EncyclopeDIA suite (\*.jar file): command line and cross-platform GUI
  - <u>https://bitbucket.org/searleb/encyclopedia/wiki/Home</u>

To exactly replicate the results here, you will also need:

- RAW data files from the tutorial HeLa dataset (MSV000084531)
  - 2018may16\_hela\_window\_size\_test\_BCS\_hela\_narrow\_1.raw
  - 2018may16\_hela\_window\_size\_test\_BCS\_hela\_narrow\_2.raw
  - 2018may16\_hela\_window\_size\_test\_BCS\_hela\_narrow\_3.raw
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  - o 2018may14\_hela\_window\_size\_test\_BCS\_hela\_wide\_400\_1000\_1.raw
  - 2018may14\_hela\_window\_size\_test\_BCS\_hela\_wide\_400\_1000\_2.raw
  - 2018may14\_hela\_window\_size\_test\_BCS\_hela\_wide\_400\_1000\_3.raw
- FASTA of the Uniprot human reference proteome (reviewed; 20,350 entries)

#### SUMMARY: Three steps for DIA-MS analysis by chromatogram library

- 1. Convert .raw files to .mzML using MSConvert
- 2. Build library using Walnut or XCorDIA in EncyclopeDIA
- 3. Search wide-window data with library from step 2 using EncyclopeDIA

#### Appendix: Visualization options

- A. Skyline
- B. EncyclopeDIA viewer
- C. Viewing ELIB files with DB Browser for SQLite

# **GUI-BASED WORKFLOW**

# 1. Convert .raw files to .mzML using MSConvert

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**! NOTE**: Make sure to have "peakPicking" as the first filter

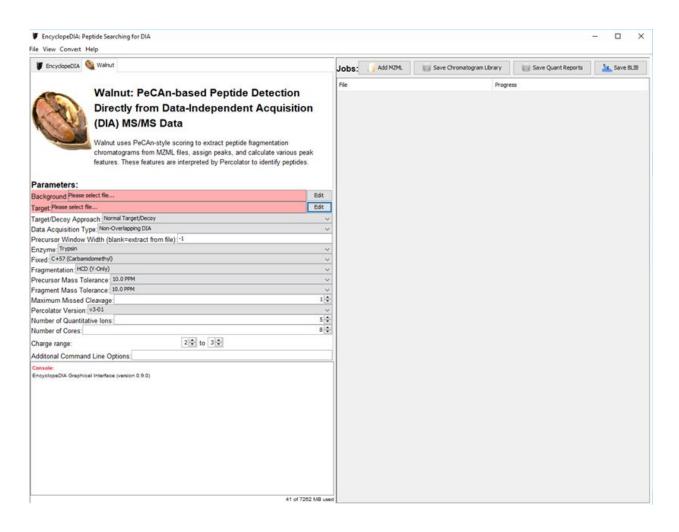
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! NOTE: If the MS1 data is acquired as a SIM scan, an acquisition mode generally used for producing chromatograms, you must have another box checked ("SIM as spectra", bottom left) so the SIM data are written to the mzML file as spectra and not as a wide extracted ion chromatogram. If so, your MSConvert should look like the screenshot below:

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# 2. Build chromatogram library using Walnut in EncyclopeDIA



2.1 On the left hand side right underneath the Walnut icon, where it says **Parameters**, find the "**Background**" field and click the corresponding "**Edit**" button to select the background fasta file. The background fasta file should basically be the reference fasta for your model system (*E.coli*, yeast, human, etc). Here, we'll use an example experiment in Hela, so we've downloaded the human reference proteome from Uniprot, and navigated the file explorer to that downloaded fasta file.

EncyclopeDIA: Peptide Searching for DIA					>
File View Convert Help					
🖉 EncyclopeDIA 🥸 Walnut		Jobs: Add MZML	Save Chromatogram Library	Save Quant Reports	Save BLIB
Walnut: PeCAn-based Peptide Detection Directly from Data-Independent Acquisit (DIA) MS/MS Data Walnut uses PeCAn-style scoring to extract peptide fragmentation chromatograms from MZML files, assign peaks, and calculate various features. These features are interpreted by Percolator to identify pepti	tion s peak	Fie	Progr	55	
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Target/Decoy Approach: Normal Target/Decoy	~				
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Enzyme Trypsin	~				
Fixed C+57 (Carbamidomethyl)	~				
Fragmentation HCD (Y-Only)	~				
Precursor Mass Tolerance 10.0 PPM	~				
Fragment Mass Tolerance: 10.0 PPM	~				
Maximum Missed Cleavage:	1 🗘				
Percolator Version v3-01	~				
Number of Quantitative Ions:	5 🜩				
Number of Cores:	8 🗘				
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48 0	of 7262 MB used				

2.2 Again under "**Parameters:**", just underneath the "**Background**" field, which should now contain the filename of that organism fasta, click the "**Target**" field corresponding "**Edit**" button. Navigate to a fasta of your target search proteome. Here, I'm interested in the whole proteome (no specific subcellular fraction like mitochondria) so I'll select the same fasta that I used in the Background field.

**! NOTE:** More about the Target/Background fasta: For experiments looking at a "whole proteome" (lysates, for example), both the Target and the Background fasta are the same file (the yeast reference fasta, human reference fasta, etc). For experiments where subcellular fractionation was performed or where you're only interested in some subset of the proteome, use a "Background" fasta of the whole organism and a "Target" fasta just of the proteins you're interested in (for example, a mitochondrial isolation might use the human proteome for a Background fasta, and a MitoCarta fasta that only includes mitochondrial proteins).

# **! NOTE** Both Background and Target files are .fasta format. PeCAn users may recall processing a fasta to get a list of peptides for input, but Walnut includes the in silico digest step so you can just give Walnut the .fasta

2.3 Set the remaining parameters if you have experiment-specific details that deviate from the defaults (for example, a different digestion enzyme than the default trypsin, or a different fragmentation type than HCD, etc)

2.4 In the top right, where it says "**Jobs**:", click "**Add MZML**". Navigate the file explorer to your converted gas phase fractionated library files from step 1. Select all the gas phase fractionated library MZMLs and click "**Open**". The MZML files you selected should now appear under the "**Jobs**" buttons. You can monitor progress using the GUI.

EncyclopeDIA: Peptide Searching for DIA						- 🗆 ×
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🔰 EncyclopeDIA 🚳 Walnut		Jobs:	Add MZML	Save Chromatogram Library	Save Quant Reports	Save BLIB
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Target:human_20350entries_20191016_uniprot-proteome_UP000005640+reviewed_yes.fasta	Edit					
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Data Acquisition Type: Non-Overlapping DIA	~					
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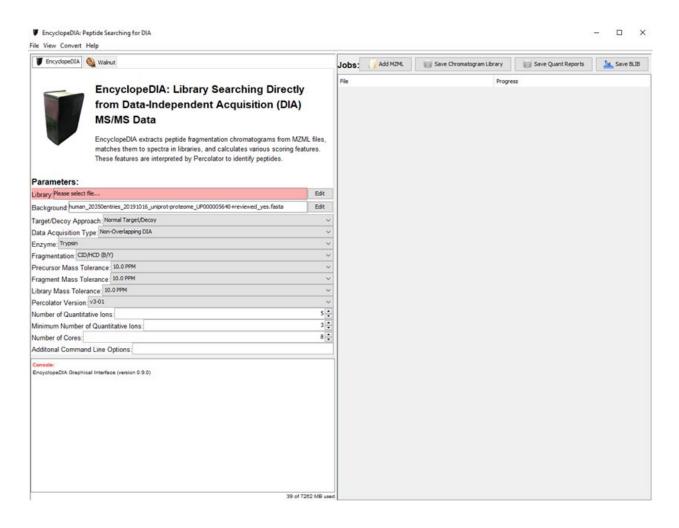
2.5 When the six gas phase fractionated files have finished running, click **"SAVE CHROMATOGRAM LIBRARY**" and give your library some descriptive filename.

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					est_BCS_hela_narrow		Wrote 1506 peptides identified at 1.0% FDR		
Walnut uses PeCAn-style scoring to extract peptide fragmentation					est_BCS_hela_narrow		Vrote 720 peptides identified		
chromatograms from MZML files, assign peaks, and calculate various pe		Read 2018may16_hela_window_size_test_BCS_hela_narrow Write Library 2018may16_hela_window_size_test_BCS_hela				N	Wrote 249 peptides identified	at 1.0% FDR	
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				w size test BCS hela narrow	Wrote 5808 peptides identified		
(DIA) MS/MS Data		Read 2018ma	y 16_hela_windo	w_size_test_BCS_hela_narrow	Wrote 3640 peptides identifier	d at 1.0% FDR	
		Read 2018ma	ay 16_hela_windo	w_size_test_BCS_hela_narrow	Wrote 1506 peptides identified		
Walnut uses PeCAn-style scoring to extract peptide fragmentation				w_size_test_BCS_hela_narrow	Wrote 720 peptides identified		
chromatograms from MZML files, assign peaks, and calculate various				w_size_test_BCS_hela_narrow	Wrote 249 peptides identified		
features. These features are interpreted by Percolator to identify peptid	les.	Write Library	20 tonay to jiele	a_window_size_test_BCS_hela	31642 peptides identified a	LUNIPUK	
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Quant Extracting 1182.8 to 1184.8 m/z (42.03993 to 90.1653 min)							
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Quant Extracting 1194.8 to 1196.8 m/z (75.44697 to 77.06919 min)							
Quant Extracting 1196.8 to 1198.8 m/z (86.37902 to 87.17902 min)							
Quant Extracting 1198.8 to 1200.8 m/z (63.334446 to 64.134445 min)							
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Writing global target/decoy peptides: 31642/315, pi0: 0.832605	10						
Writing global target/decoy proteins: 3983/39	~						

# 3. Search wide-window data with chromatogram library from Step 2

3.1 Close and reopen the EncyclopeDIA GUI to clear EncyclopeDIA's cache/history



3.2 Within EncyclopeDIA GUI (not Walnut), on the left hand side under "Parameters:" across from the "Library" field, click the "Edit" button. Using the file explorer, select the .elib file you just saved in Step 2.5

	Save Quant Reports
EncyclopeDIA: Library Searching Directly from Data-Independent Acquisition (DIA) MS/MS Data	

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These features are interpreted by Percolator to identify peptides.

Library:2018may16\_hela\_window\_size\_test\_BCS\_hela\_narrow\_1-8\_LIBRARY.elb

Target/Decoy Approach Normal Target/Decoy

Data Acquisition Type Non-Overlapping DIA

Background human\_20350entries\_20191016\_uniprot-proteome\_UP000005640+reviewed\_yes.fasta

Parameters:

Enzyme: Trypsin

Fragmentation CID/HCD (B/Y) Precursor Mass Tolerance 10.0 PPM

Percolator Version v3-01 Number of Quantitative Ions

Number of Cores Additonal Command Line Options Console: EncyclopeDIA Graphical Interface (version 0.9.0)

Fragment Mass Tolerance: 10.0 PPM

Minimum Number of Quantitative Ions:

Library Mass Tolerance: 10.0 PPM

3.3 Underneath the "Library" field, across from the "Background" field, click the corresponding "Edit" button and select the appropriate Background file (should be the same fasta you used in 2.1 above!)

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FincyclopeDIA: Peptide Searching for DIA

File View Convert Help

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from Data-Independent Acquisition (DIA)	Read	d 2018may14_hela_window	_size_test_BCS_hela_wide_4			
MS/MS Data EncyclopeDIA extracts peptide fragmentation chromatograms from MZM matches them to spectra in libraries, and calculates various scoring feat These features are interpreted by Percolator to identify peptides.						
arameters:						
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ta Acquisition Type Non-Overlapping DIA	~					
zyme: Trypsin	~					
agmentation: CID/HCD (B/Y)	~					
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3.4 In the top right, next to "Jobs", click the "Add MZML" button and select all of the wide-window .mzML files that were acquired using this narrow-window library file.

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🖉 EncyclopeDIA 💊 Walnut		Jobs:	Add MZML	Save Chromatogram Libra	ry Save Quant Reports	Save BLI
		File			Progress	
EncyclopeDIA: Library Searching Direct	ly			w_size_test_BCS_hela_wide_4	Wrote 26704 peptides identifier	
from Data-Independent Acquisition (DIA	)			<pre>w_size_test_BCS_hela_wide_4 w_size_test_BCS_hela_wide_4</pre>	Wrote 26768 peptides identifie Wrote 28179 peptides identifie	
MS/MS Data	,	1000 2010	1071 (J 100 J 11 100		1100 2017 9 0000 001000	
monilo Data						
EncyclopeDIA extracts peptide fragmentation chromatograms from M matches them to spectra in libraries, and calculates various scoring fo These features are interpreted by Percolator to identify peptides.						
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arget/Decoy Approach Normal Target/Decoy	¥					
Data Acquisition Type: Non-Overlapping DIA	Ŷ					
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ragmentation: CID/HCD (B/Y)	~					
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ragment Mass Tolerance: 10.0 PPM	~					
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Percolator Version: v3-01	~					
lumber of Quantitative Ions:	5 🗘					
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lumber of Cores:	8 🛟					
dditonal Command Line Options:						
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2uart Extracting 952.7 to 964.7 m/z (31.683819 to 106.2789 min) 2uart Extracting 964.7 to 976.7 m/z (27.967362 to 106.43288 min)						
Juant Extracting 970.7 to 989.7 miz (28.921316 to 104.176630 min) Juant Extracting 987.0 to 00.7 miz (26.9370 to 105.50418 min) Juant Extracting 1000.7 to 1012.7 miz (48.200123 to 104.92002 min)						
Writing Encyclopedia ELIB from 2018may14_hela_window_size_test_BCS_hela_wide_400_1000_3.mzML (28 inbite) Writing 28179 peptides to entries table	1174					
Witing 28179 peptides to peptidequants table						
inished writing to Encyclopedia ELIB at Tue Nov 19 04:32:08 EST 2019 Witing local target/decoy peptides: 28179/5427, pi0: 0.0295764						
Witing local target/decoy proteins 3848/38						
inished analysis! 28179 peptides identified at 1.0% FDR (4.5 minutes)						
Fror deleting temp file!	÷					

3.5 Click **"Save Quant Reports"** to perform a final experiment-wide FDR correction and export peptide quant, and protein quant.

- Select "Save Chromatogram Library" to build a file with bonus information like integration boundaries.
  - This is only applicable if you do not want to retention time-align across the MZML files (for example, if your MZMLs are fractionated in a way such that you don't expect to sample the same peptides in each file)
- Select "Save Quant Reports" to get peptide/protein quantitation matrices in the form of a tsv.
  - Pick this if your MZMLs were the experimental samples you want to post-process.
  - If you are following this workflow as-is, this is what you should pick!
- Select "Save BLIB" to build a spectral library file that Skyline can use.
  - This option is effectively depreciated now that Skyline reads ELIB file formats

dopeDIA 🧕 Walnut Jobs:	Add MZML	Save Chromatogram Library	Save Quant Reports	A Save BLIE	
Fie	1.6.				
En avelana DiA di ibaran Osana bira Direcato	IBmauld hals windo	w_size_test_BCS_hela_wide_4	Vrogress Wrote 26704 peptides identified at 1.0% FDR		
David 20		w_size_test_BCS_hela_wide_4	Wrote 26768 peptides identified at 1.0% FDR Wrote 26768 peptides identified at 1.0% FDR		
		w size test BCS hela wide 4	Wrote 28179 peptides identified		
MS/MS Data	rary 2018may14_hel	a_window_size_test_BCS_hela	28824 peptides identified at	1.0% FDR	
EncyclopeDIA extracts peptide fragmentation chromatograms from MZML files, matches them to spectra in libraries, and calculates various scoring features. These features are interpreted by Percolator to identify peptides.					
ters:					
18may16_hela_window_size_test_BCS_hela_narrow_1-8_LIBRARY.elb Edit					
nd:human_20350entries_20191016_uniprot-proteome_UP000005640+reviewed_yes.fasta Edit					
coy Approach Normal Target/Decoy v					
uisition Type Non-Overlapping DIA v					
Trypsin 🗸 🗸					
ation: CID/HCD (B/Y) v					
Mass Tolerance: 10.0 PPM v					
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r Version: v3-01 v					
f Quantitative lons: 5					
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f Cores 8 +					
Command Line Options:					
ze pepidento pepidenta tacienti					
173 indegrated library entries because the RT interver could not find any fragment ions. 115 Indegrated library entries because the RT interver could not find any fragment ions. 115 Indegrated library entries because the RT interver could not find any fragment ions. 115 Indegrated library entries because the RT interver could not find any fragment ions. 115 Indegrated library entries because the RT interver could not find any fragment ions. 115 Indegrated library entries because the RT interver could not find any fragment ions. 115 Indegrated library entries because the RT interver could not find any fragment ions. 115 Indegrated library entries because the RT interver could not find any fragment ions. 115 Indegrated library entries because the RT interver could not find any fragment ions. 115 Indegrated library entries because the RT interver could not find any fragment ions. 115 Indegrated library entries because the RT interver could not find any fragment ions. 115 Indegrated library entries because the RT interver could not find any fragment ions. 115 Indegrated library entries because the RT interver could not find any fragment ions. 115 Indegrated library entries because the RT interver could not find any fragment ions. 115 Indegrated library entries because the RT interver could not find any fragment ions. 115 Indegrated library entries because the RT interver could not find any fragment ions. 115 Indegrated library entries because the RT interver could not find any fragment ions. 115 Indegrated library entries because the RT interver could not find any fragment ions. 115 Indegrated library entries because the RT interver could not find any fragment ions. 115 Indegrated library entries the RT interver could not find any fragment ions. 115 Indegrated library entries the RT interver could not find any fragment ions. 115 Indegrated library entries the RT interver could not find any fragment ions. 115 Indegrated library entries the RT interver could not find any fragment ions. 115 Indegrated library entries					
rce files					
rds processed					
rds processed					
rds processed					
rds processed					
rds processed					
It number of tragments in over or 21642 peptides					
iting protein report for 3503 protein groups/					
rds processed coassing 65046 records, found 21682 quantitative unique peptides. Writing reports tr number of fragments in 5041 of 21682 peptides ting peptide report for 62164 unique peptides!					

**!** NOTE Encyclopedia is determining a lot of information about the DIA experiment. Important details include peptide detections, fragment refinement, and peak boundaries. We'll import that information into Skyline next so that we can visualize the results; however, there's also a visualizer built right into Encyclopedia. See Appendix for details.

! NOTE Encyclopedia's ELIB output can be used in Skyline for visualizing DIA-MS experiments. See Appendix for details.

# **COMMAND LINE WORKFLOW**

#### 1. Convert .raw files into .mzml using MSConvert

For non-overlapping windows:

msconvert.exe -v --zlib --64 --mzML --filter "peakPicking true 1-" \*.raw

For overlapping windows:

msconvert.exe -v --zlib --64 --mzML --filter "peakPicking true 1-" --filter "demultiplex optimization=overlap\_only" \*.raw

NOTE: data acquired on Lumos instruments needs an extra flag (--simAsSpectra) to convert precursor scans correctly:

```
msconvert.exe --zlib --64 --mzML --filter "peakPicking true 1-"
--filter "demultiplex optimization=overlap_only" --simAsSpectra
*.raw
```

! NOTE If your file conversion is going slow, you probably aren't using a current version of MSconvert!

#### 2. Build chromatogram library using Walnut (command line)

2.1. Searching DIA data against a FASTA with Walnut: for a full list of options and default values for your version of EncyclopeDIA:

```
java -jar encyclopedia.jar -walnut --help
```

The parameters you may want to change include the enzyme used to prepare the samples,

```
$ java -Xmx<GB_OF_MEM>G -jar encyclopedia.jar -walnut \
    -i <MZML_IN> \
    -f <BACKGROUND_FASTA> \
    -t <TARGET_FASTA> \
    -acquisition DIA \
```

```
-enzyme <ENZYME> \
-frag <FRAGMENTATION> \
-ftol <FRAGMENT_TOLERANCE> \
-ftolunits <FRAGMENT_TOLERANCE_UNITS> \
-ptol <PRECURSOR_TOLERANCE> \
-ptolunits <PRECURSOR_TOLERANCE_UNITS> \
-minCharge <MIN_CHARGE> \
-maxCharge <MAX_CHARGE>
```

With a typical DIA setup (trypsin digest, Orbitrap instrument, demultiplexing the RAW file overlapping windows with MSConvert), the command usually looks like this:

```
$ java -Xmx8G -jar encyclopedia.jar -walnut \
    -i DIA_narrow_run_400to500mz.mzML \
    -f human.fasta \
    -t human.fasta \
    -acquisition DIA \
    -enzyme trypsin \
    -frag YONLY \
    -ftol 10.0 \
    -ptolunits ppm \
    -minCharge 2 \
    -maxCharge 3
```

Running Walnut produces several results files, including:

<MZML\_IN>.dia <MZML\_IN>.mzML.pecan.txt.log <MZML\_IN>.mzML.features.txt <MZML\_IN>.mzML.pecan.txt <MZML\_IN>.mzML.pecan.decoy.txt

2.1. Merge Walnut results into a chromatogram library file (.elib): this command must be run within the same directory as the search command from 2a. You have to be in the same directory because EncyclopeDIA will look for the result files in the current

directory in order to compile them into one ELIB, so if the result files aren't there, Walnut has nothing to compile.

For a full list of options and default values for your version of EncyclopeDIA:

```
java -jar encyclopedia.jar -libexport --help
```

OUTPUT\_LIBRARY\_NAME: (filename) Any name of your choice. Entering **narrow\_merged** here would result in a final output of narrow\_merged.elib (or narrow\_merged.blib).

ALIGN\_SPECTRA?: (true or false) You will likely want to set this to false for this step as you're probably doing a narrow isolation gas phase fractionation. In general, if each of your mzML acquisitions collect an identical precursor range, this should be true -- otherwise it should be false.

USE\_BLIB\_FLAG: To export an elib, leave this blank. To export a .blib, set to: -blib

Typical run:

```
$ java -Xmx<GB_OF_MEM>G -jar encyclopedia.jar -libexport \
    -i <INPUT_DIRECTORY> \
    -o <OUTPUT_LIBRARY_NAME> \
    -a <ALIGN_SPECTRA?> \
    <USE_BLIB_FLAG> \
    -f <BACKGROUND_FASTA> \
    -t <TARGET_FASTA> \
    -ftol <FRAGMENT_TOLERANCE> \
    -ftolunits <FRAGMENT_TOLERANCE_UNITS>
```

Example:

```
$ java -Xmx8G -jar encyclopedia.jar -libexport \
    -i ./ \
    -o narrow_merged \
    -a false \
    -f human.fasta \
    -t human.fasta \
    -ftol 10.0 \
    -ftolunits ppm
```

# 3. Search wide-window data with chromatogram library from step 2 using EncyclopeDIA (command line)

For a full list of options and default values for your version of EncyclopeDIA:

```
java -jar encyclopedia.jar --help
```

LIBRARY\_ELIB\_FILE: This should be the result from your narrow library search in step 2.b.ii, e.g. **path/to/your/narrow\_merged.elib** 

Typical run:

```
$ java -Xmx<GB_OF_MEM>G -jar encyclopedia.jar \
    -1 <LIBRARY_ELIB_FILE> \
    -i <MZML_IN> \
    -f <BACKGROUND_FASTA> \
    -t <TARGET_FASTA> \
    -acquisition DIA \
    -enzyme <ENZYME> \
    -frag <FRAGMENTATION> \
    -ftol <FRAGMENT_TOLERANCE> \
    -ftolunits <FRAGMENT_TOLERANCE> \
    -ptol <PRECURSOR_TOLERANCE> \
    -minCharge <MIN_CHARGE> \
    -maxCharge <MAX_CHARGE>
```

Output files:

<MZML\_IN>.dia <MZML\_IN>.mzML.elib <MZML\_IN>.mzML.encyclopedia.txt <MZML\_IN>.mzML.encyclopedia.txt.delta\_rt.pdf <MZML\_IN>.mzML.encyclopedia.txt.log <MZML\_IN>.mzML.encyclopedia.txt.rt\_fit.pdf <MZML\_IN>.mzML.encyclopedia.txt.rt\_fit.txt <MZML\_IN>.mzML.features.txt
<MZML\_IN>.mzML.first\_round.txt

# Appendix

Settings > Peptide Settings.

# Importing peptide detections into Skyline

4a. Prediction: Check "Use measured retention times when present", Time window=2

4b. Filter: Min length=3, Max length=40, no excluded amino acids checked

Settings > Transition Settings.

Transition Settings		× Tra	nsition Settings			×
Prediction Filter Library Instrument	Full-Scan	F	Prediction Filter	Library Ins	trument Full-Sc	an
Precursor mass:	Product ion mass:		Peptides Sm	all Molecules		
Monoisotopic ~	Monoisotopic ~		Precursor cha	arges: Ion	charges:	lon types:
Collision energy: Thermo TSQ Quantii ~	Declustering potential:		2, 3 Product ion	1,	2	<u>y</u>
			From:		To:	
Optimization library:	Compensation voltage:		ion 3	~	/ last ion	~
None 🗸	None 🗸		Special ior	10 F		
Use optimization values when pre	sent			129L 129H 130L 130H	~	Edit List
			Use DIA pro		w for exclusion ansitions	
	OK Cancel					OK Cancel

rediction	Filter	Library	Instrument	Full-Scan	
lon match	n tolera	ince:			
0.005		m/z			
🗹 lf a lib	orary sp	ectrum is	available, pie	sk its most intense ions	8
Pick:					
5		product io	ns		
I		minimum p	product ions		
O From	filtered	ion charg	es and types		
O From	filtered	ion charg	es and types	plus filtered product id	ons
From	filtered	product in	ons		

ransition Settings X	Transition Settings
Prediction Filter Library Instrument Full-Scan	Prediction Filter Library Instrument Full-Scan
Min m/z: Max m/z:	MS1 filtering
50 m/z 1500 m/z	Isotope peaks included: Precursor mass analyzer:
Dynamic min product m/z	None V
Method match tolerance m/z:	Peaks: Resolution:
0.005 m/z	
Firmware transition limit: Firmware inclusion limit:	Isotope labeling enrichment:
	MS/MS filtering
Min time: Max time:	Acquisition method: Product mass analyzer:
min min	DIA $\checkmark$ Centroided $\checkmark$
	Isolation scheme: Mass Accuracy:
	Results only V 10 ppm
	Use high-selectivity extraction
	Retention time filtering
	Use only scans within 2 minutes of MS/MS IDs
	O Use only scans within 5 minutes of predicted RT
	Include all matching scans
OK Cancel	OK Cancel

4c. Prediction: Precursor/Product ion mass="Monoisotopic"

4d. Filter: lon charges=1,2 lon types="y"\*, From=Ion 3, To=last ion, no special ions \*Set "ion types" to reflect how you searched the data in Encyclopedia!

4e. Library: Ion match tolerance=0.005 m/z, check "If a library spectrum is available, pick its most intense ions", pick=5 product ions, select "From filtered product ions"

4f. Instrument: "Min m/z=50, Max m/z=1500, Method match tolerance m/z=0.005

4g. Full-Scan (MS1): "Isotope peaks included=None"

4h. Full-Scan (MS/MS): "Acquisition method=DIA, Product mass analyzer=Centroided, Isolation scheme=Results only, Mass Accuracy=10 ppm, check "Use only scans within "2" minutes of MS/MS IDs

#### Load the FASTA database and the ELIB library from EncyclopeDIA into Skyline.

4i. Open Settings/Peptide Settings/Digestion. Select "<Add...>" for Background proteome

Digestion	Prediction	Filter	Library	Modifications	Quantification	
Enzyr						
-	sin [KR   P]					
пур	ant front i 1					
Max	missed cleav	ages:				
1	$\sim$					
Back	ground prote	ome:				
			~			
mou						
None	9					
None	e t					
None yeas mous	e t se					
None yeas mous <ado< td=""><td>e t se 1&gt; t current&gt;</td><td></td><td></td><td></td><td></td><td></td></ado<>	e t se 1> t current>					
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None yeas mous <ado< td=""><td>e t se 1&gt; t current&gt;</td><td></td><td></td><td></td><td></td><td></td></ado<>	e t se 1> t current>					

4j. Set Name="[yournamehere]", then click "Create..." and type in a descriptive name for the soon-to-be-created Proteome File and click "**Save**". Select "Add File..." to add the background fasta you were just using in the Encyclopedia suite.

Peptide Settings		×			
Digestion Prediction Fi	Iter Library Modifications Qu	uantification			
Enzyme:			-		
🗽 Edit Background Proteom	e	×			
Name:		ОК			
yeast_bg_proteome	]				
Proteome file:		Cancel			
Open Create	📩 Create Background Protec	ome			×
FASTA files:					
No miles.	← → × ↑ 📙 « agi	ng_yeast → iron-pa	thway 🗸 진	Search iron-pathw	/ay P
	Organize 👻 New folde	r			
	Attachments ^	Name	Date modified	Туре	Size
	Broad Institute	Rproj.user	9/11/2018 3:36 PM	File folder	
0)	Documents	data	9/11/2018 2:46 PM	File folder	
Click the 'Open' button to choos the 'Create' button to create a ne	Documents 1	aresults	10/2/2018 3:21 PM	File folder	
	Papers				
	🤱 Lindsay Pino				
	This PC				
	🐂 Libraries				
	👝 fuckyoubcs (H:)				
	afed				
	inactive				
	novo				
	novo_8v12mzo				
	novo_qc				
	📕 Seagate Backup 🗸				
	File name: I'm_ty	ping_a_name_for_th	is_proteomefile		~
18923	Save as type: Proteo				~
15-72				1.32	
277	∧ Hide Folders			Save	Cancel
				- Y	

4k. Open Settings/Peptide Settings/Library. Select "Edit list…", then "Add…". Set the name to be "[yournamehere]" and select "Browse…" to select the .elib that you saved in EncyclopeDIA from the wide-window search (not the gas phase fractionated, narrow window library). "Ok" out to the Peptide Settings.

Peptide Set	tings					×	
Digestion	Prediction	Filter	Library	Modifications	Quantification	4	
Librar	ies:						
💁 Edit Librar	ries			×	Edit list		
Libraries:					Build		
wasko_1016-8	387			Add	Explore		
			C	Copy			
				Edit			
			R	lemove			
				Up			
				Down			
			F	leset			
		ОК		Cancel			
	Edit Libr	rary					×
	Name:						ок
-	youma	mehere					incel
-	Path:						
						Bron	wse
	Spectra	al Library	Links:				
	Peptide	Atlas	NIST	<u>GPM</u>			
	🗹 Us	e explici	t p <mark>eak b</mark> o	unds			

4I. Check the new "[yournamehere]" library and uncheck all other libraries. Click "Explore..." to view it

! If you get a pop up warning that 'Peptide settings have been changed. Save changes?' then click "Yes"

4m. Check "Associate proteins" and click "Add All...". Select "Add to all matching proteins" and "Include all peptides" and hit "OK".

4n. If you have more than one library (\*.elib) for your experiment, you must select each of those libraries from the dropdown menu in "Spectral Library Explorer" and repeat step 4m for each.

40. Save the Skyline file!

4p. Import .mzML data into Skyline

*!* Tip: Use the .mzml files, not the .raw files! If you prefer using the .raw files for some reason, you will need to set the windowing scheme parameters in the "MS/MS filtering" box under the "Full Scan" tab in Skyline's "Transition Settings".

#### Skyline: Export report for MSstats

To load the MSstats report format, install the MSstats external tool. Alternatively, you can quickly build a custom report using the little black binoculars to find whatever fields you're interested in exporting.

4q. File > Export > Report...

4r. Edit list... > Add...

4s. Make sure to name your report ("View Name:"), and check the fields you want the report to include. Binoculars are at the top left, next to the Redo arrow, and will find fields by name.

# EncyclopeDIA data visualizers

# EncyclopeDIA ELIB Browser

V EncydopeDIA 🚳 Walnut		Jobs: 🥠 Add MZML	Save Chromatogram Library	Save Quant Reports	Save BLIB
EncyclopeDIA: Library Searching Directly from Data-Independent Acquisition (DIA) MS/MS Data EncyclopeDIA extracts peptide fragmentation chromatograms from MZI matches them to spectra in libraries, and calculates various scoring fea These features are interpreted by Percolator to identify peptides.	ML files,	Fie	Pro	gress	
Parameters:					
ibrary_2018may16_hela_window_size_test_BCS_hela_LIBRARY_1-8.elib	Edit				
Background:human_20350entries_20191016_uniprot-proteome_UP000005640+reviewed_yes.fasta	Edit				
arget/Decoy Approach: Normal Target/Decoy	~				
Data Acquisition Type: Non-Overlapping DIA	~				
Enzyme: Trypsin	~				
ragmentation: HCD (Y-Only)	~				
Precursor Mass Tolerance 10.0 PPM	~				
ragment Mass Tolerance 10.0 PPM	~				
ibrary Mass Tolerance: 10.0 PPM	~				
Percolator Version: v3-01	~				
lumber of Quantitative lons:	5 🛟				
Animum Number of Quantitative Ions:	3 🛟				
lumber of Cores:	8 📮				
Additonal Command Line Options:					
Console: IncyclopeDIA Graphical Interface (version 0.9.0)					

The top left under "View", select the "Launch ELIB Browser" option

Edit
Peptide Protein Retent TC Score
Select a peptide1

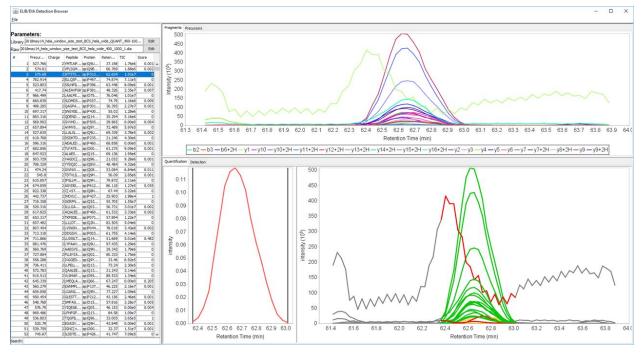
Next to the "Library" field, click the "Edit" button and navigate the explorer to the "Save Chromatogram Library" file you just saved. Once the library has loaded, you should have a populated target list like this:

ELIB/DIA Detection Browser		_	1	
		_		
Parameters: Library 2018may14_hela_window_size_test_BCS_hela_wide_QUANT_400-100 Ed				
Library 2018may14_hela_window_size_test_BCS_hela_wide_QUANT_400-100 Ed Raw Please select file Ed				
# Precur Charge Peptide Protein Retent TIC Score				
💮 Please wait 🗙				
Reading Library				
	Select a peptide!			
	MAKEN BARDON			
Search:				

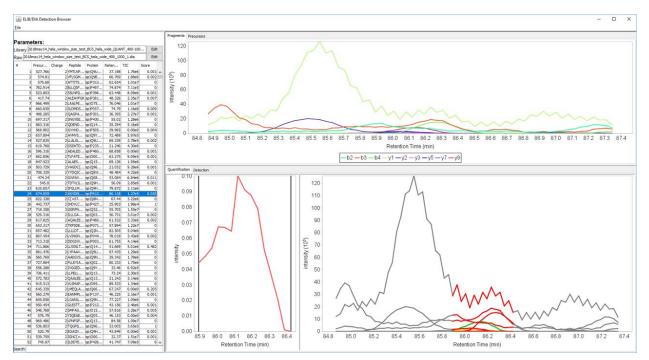
ELIB/DIA Detection Br	owser			
Eile				
Parameters:				
Library 2018may14_hela_wi	ndow_size_test_BCS_heli	_wide_QUAN	VT_400-100.	Edit
Raw Please select file				Edit
# Precur Charg	e Peptide Protein	Reten	TIC S	Score
1 527.766	2 YMTLNP sp Q9U		1.76e6	0.001
2 574.81	2 VFLSGM sp/Q98	66.769		0.002
3 575.68	3 ATTITS sp P313 2 ELLQSF sp P497		1.01e7 7.11e5	0
5 523.803	2 SSLNP11 sp  P 396		8.09e6	0.001
6 417.74	2 ALEAVFGK sp  P381		2.35e7	0.007
7 966.499 8 660.839	2 LAALPE sp  075 2 SLDMDS sp  P057		1.01e7 1.16e8	0.009
9 498.285	2 QAGPA sp 9301		2.27e7	0.001
10 697.317	2 SNGYEE sp/P430	. 55.02	1.28e6	0
11 883.316 12 569.902	2 QDEND sp  Q14 3 GVVHD sp  P505		5.16e6 0.00e0	0.004
12 569.902	2 AVMVS sp Q9Y		0.00e0 5.97e5	0.004
14 527.835	2 LLALGL splQ9U	69.339	3.78e5	0.002
15 619.768	2 DSDKTD sp  P235		4.30e6	0
16 596.316 17 682.856	2 AEALED sp P460 2 TLFATE sp 000		0.00e0 9.09e5	0.001
18 847.923	2 ALAES sp/Q15		1.95e6	0.001
19 503.729	2 VAGDC[ sp]Q96		9.28e6	0.001
20 708.329 21 474.24	2 YYDQIC sp Q8W 2 GWNW sp Q08		4.32e6 6.84e6	0.011
22 545.8	2 TDTVLIL sp/Q9H		2.85e6	0.001
23 610.857	2 IFGLUM sp Q9H		2.11e6	0
24 674.859	2 ASVDEL sp P612	. 86.118	1.27e5	0.055
25 822.338 26 442.737	2 C[+57 sp)Q8N		3.22e6 1.98e4	0
27 719.358	2 DHOVLC sp P427 3 GGRPN sp Q52		1.55e7	0
28 529.316	2 ILLLGA sp Q03	56.731	3.01e7	0.002
29 617.825	2 AQALEE sp  P 460		3.33e6	0.002
30 653.317 31 857.482	3 TKPSDE sp/P071 2 LLLLDT sp/Q2N		1.22e7 5.04e6	0
32 807.454	2 LVINGN sp P044.		3.43e8	0.002
33 713.318	2 DOGSW sp P003	. 61.755	4.14e6	0
34 711.866 35 881.476	2 LLSSSLT sp/Q14 2 LYPAAV sp/Q9U		5.01e6	0.482
35 881.476	2 AAEGVS sp Q90		1.29e6 1.79e6	0
37 727.884	2 FLLEYTA sp Q02	80.333	1.75e6	0
38 558.288	2 IVGGED sp/Q9Y		6.92e5	0
39 736.411 40 572.783	2 UPELL sp Q13 2 QAALEE sp Q13		2.30e5 3.14e6	0
41 915.513	2 VLSMAP sp  095		1.34e6	0
42 645.339	2 LMEQLA sp  Q66		0.00e0	0.205
43 560.279	2 EANMPL sp P137		2.16e7	0.001
44 609.858 45 950.454	2 LGANIL sp)Q9N 2 GLESTT sp)P212		1.09e6 2.46e6	0.001
46 548.768	2 SMFAG sp 015	\$7.816	3.28e7	0.005
47 576.79	2 YIQEAE splQ05		0.00e0	0.004
48 969-486 49 536.803	2 LPNFGF sp Q 13 2 TQGP1L sp Q96	84.58	1.09e7 3.65e5	0
50 520.79	2 EGAIIV sp/Q9H		0.00e0	0.001
51 539.759	2 IGNC[+ sp 000	32.37	1.51e7	0.001
52 745.87	2 ILDOTE sp P426	41.747	7.09e5	0 4
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Next to the "Raw" field, click the "Edit" button to select one of the RAW files analyzed in that chromatogram library.

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3 575.68	3 ATTITS splp3		54 1.01e7	0.002	
4 782.914	2 ELLQSF splP4		74 7.11e5	0	
5 523.803	2 SSLNP1L splP3		H8 8.09e6	0.001	
6 417.74	2 ALEAVEGK spip3		26 2.35e7	0.007	
7 966.499	2 LAALPE sp)07		146 1.01e7	0	
8 660.839	2 SLDMDS sp IP0		79 1.16e8	0.009	
9 498.285	2 QAGPA splP3		95 2.27e7	0.001	
10 697.317 11 883.316	2 SNGYEE sp/P4 2 QDEND sp/Q1		02 1.28e6 94 5.16e6	0	
11 883.316	3 GV/HD sp P5		54 5.16e6 65 0.00e0	0.004	
12 569.902	2 AVMVS splQ5		65 0.00e0 89 5.97e5	0.004	
14 527.835	2 LLALGL sp  QS		39 3.78e5	0.002	
15 619.768	2 DSDKTD sp P2		46 4.30e6	0	
16 596.316	2 AEALED sp P4		58 0.00e0	0.001	
17 682.856	2 TLFATE sp (OC		75 9.09e5	0.001	
18 847.923	2 ALAES splQ:		.36 1.95e6	0	
19 503.729	2 VAGDC[ sp]QS		52 9.28e6	0.001	
20 708.329 21 474.24	2 YYDQIC sp Q8 2 GWNW sp Q0		84 4.32e6 84 6.84e6	0.011	
21 4/4.24 22 545.8	2 TDTVLIL sp QS		09 2.85e6	0.001	
23 610.857	2 IFGLLM spiQS		72 2.11e6	0.001	
24 674.859	2 ASVDEL sp P6		18 1.27e5		Select a peptide!
25 822.338	2 C[+57 sp)Q8		44 3.22e6	0	
26 442.737	2 DHOVLC sp/P4	7 25.9	03 1.98e4	1	
27 719.358	3 GGRPN sp  Q		05 1.55e7	0	
28 529.316	2 ILLLGA sp QQ		31 3.01e7		
29 617.825	2 AQALEE sp  P4		32 3.33e6	0.002	
30 653.317 31 857.482	3 TKPSDE sp/P0 2 LLLLDT sp/Q2		94 1.22e7 05 5.04e6	0	
32 807.454	21.VINGN spiP0		19 3.43e8	0.002	
33 713.318	2 DOGSW sp P0		55 4.14e6	0	
34 711.866	2 LLSSSLT sp/Q1		69 5.01e6	0.482	
35 881.476	2 LYPAAV sp QS	J 97.4	35 1.29e6	0	
36 560.769	2 AAEGVS sp  QS		42 1.79e6	0	
37 727.884	2 FLLEYIA sp QQ		133 1.75e6	0	
38 558.288	2 IVGGED sp QS		46 6.92e5	0	
39 736.411	2 LLPELL sp  Q1 2 QAALEE sp  Q1		24 2.30e5	0	
40 572.783 41 915.513	2 QAALEE sp Q1 2 VLSMAP sp Q3		43 3.14e6 33 1.34e6	0	
42 645.339	2 LMEQLA sp Q6		47 0.00e0	0.205	
43 560.279	2 EANMPL sp P1		25 2.16e7		
44 609.858	2 LGANIL sp)QS		27 1.09e6	0	
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48 969.486	2 LPNFGF sp  Q1		58 1.09e7	0	
49 536.803	2 TQGPIL sp/QS		05 3.65e5	1	
50 520.79	2 EGAIIV sp Q9		49 0.00e0	0.001	
51 539.759 52 745.87	2 IGNC[+ sp 00 2 ILDOTE sp P4		37 1.51e7 47 7.09e5	0.001	
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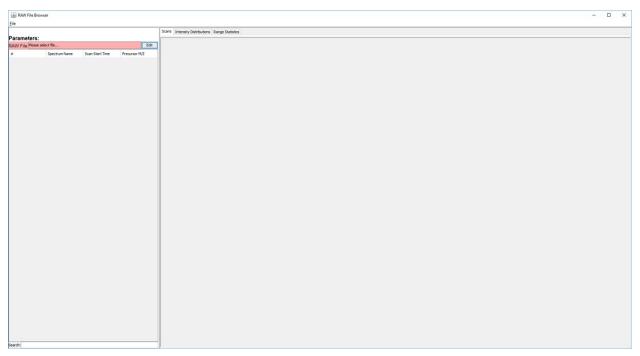
Above is an example of a "good" peptide, where Encylopedia found lots of interference-free fragments (all the fragment traces in the bottom right are green). The fragment ion chromatograms shown in red don't follow the average profile of the peak group.



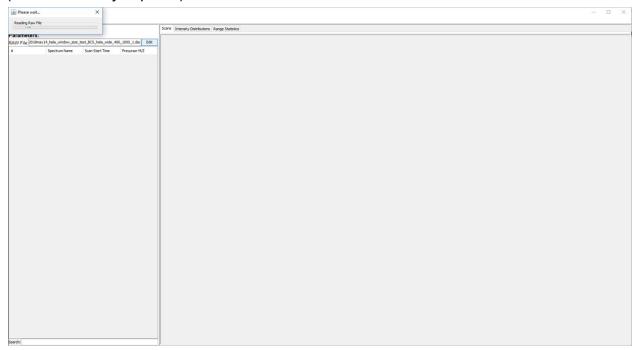
Above is an example of peptide that was detected but doesn't look quantitative.

# EncyclopeDIA RAW File Browser

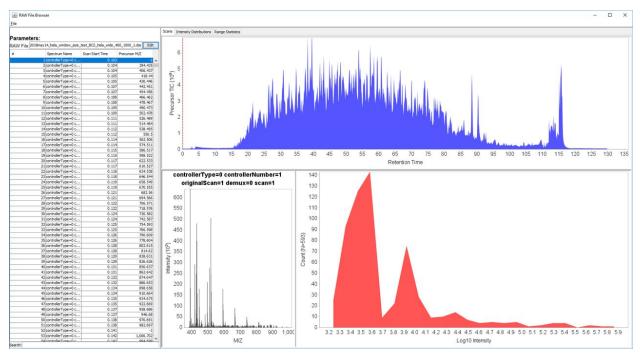
Top left under "View", select "Launch RAW File Browser"



Select a "RAW" file that has been previously analyzed with the Encyclopedia suite (Walnut or Encyclopedia)



last updated 16 Dec 2019



The target list on the left lists the Scan Number (#), SpectrumName, Scan Start Time, and Precursor M/Z.

On the top above the graphics, there are three tabs. The "Range Statistics" gives some valuable information about the DIA method like the Ion Inject Time across each precursor window and across retention time bin:

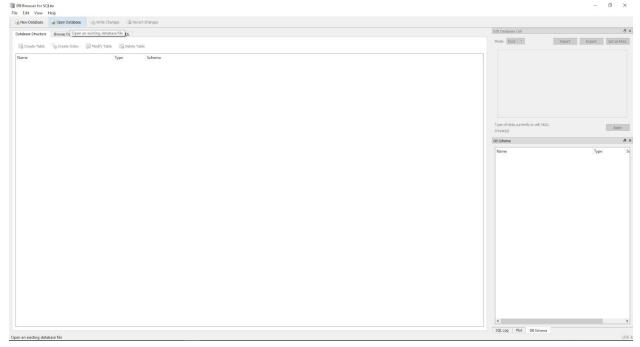
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### Viewing ELIB files with DB Browser for SQLite

The \*.elib files that Encyclopedia builds are SQL databases, which are kind of like multi-tab Excel files but fancier. To view these files and browse the data stored in the elib, follow the steps below:

Download DB Browser for SQLite here: https://sqlitebrowser.org/

Open DB Browser for SQLite Navigate to "Open Database" button on top left



At the bottom of the "Choose a database file" pop-up next to the "File Name" field, select "All files" from the file type dropdown

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Navigate to the appropriate directory and select the elib file you want to view. Click .Open"

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! It might take a minute to load. The window should look like this once it's loaded:

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> i entries	CREATE TABLE entries (PrecursorMz double not null, PrecursorCharge int not null, PeptideModSeq string not null, PeptideSeq string not null, Copies int not null, RTInSeconds double not null, Score		
> i fragmentquants	CREATE TABLE fragmentquants ( PrecursorCharge int not null, PeptideModSeq string not null, PeptideSeq string not null, SourceFile string not null, IonType string not null, IonIndex int not null, Frag		
> 🧰 metadata	CREATE TABLE metadata (Key string not null, Value string not null)		
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> iii peptidetoprotein	CREATE TABLE peptidetoprotein (PeptideSeq string not null,isDecoy boolean, ProteinAccession string not null)		
> III proteinscores	CREATE TABLE proteinscores (ProteinGroup int not null, ProteinAccession string not null, SourceFile string not null, QValue double not null, MinimumPeptidePEP double not null, ISDecoy boolean n		
> i retentiontimes	CREATE TABLE retentiontimes (SourceFile string not null, Library float not null, Actual float not null, Predicted float not null, Delta float not null, Probability float not null, Decoy boolean, PeptideMo		
Indices (16)		Type of data currently in cell: NULL	
Key_Metadata_index	CREATE INDEX 'Key_Metadata_index' on 'metadata' (Key' ASC)	0 byte(s)	App
PeptideModSeq_PrecursorCharge_Source	CREATE INDEX 'PeptideModSeq_PrecursorCharge_SourceFile_Entries_index' on 'entries' (PeptideModSeq'ASC, 'PrecursorCharge'ASC, 'SourceFile'ASC)	0.0912(0)	
PeptideModSeq_PrecursorCharge_Source	CREATE INDEX 'PeptideModSeq, PrecursorCharge, SourceFile, Fragments_index' on 'fragmentquants' (PeptideModSeq' ASC, 'PrecursorCharge' ASC, 'SourceFile' ASC)	DB Schema	
PeptideModSeq_PrecursorCharge_Source_	CREATE INDEX 'PeptideModSeq_PrecursorCharge_SourceFile_Localizations_index' on 'peptidelocalizations' ('PeptideModSeq' ASC, 'PrecursorCharge' ASC, 'SourceFile' ASC)		
PeptideModSeq_PrecursorCharge_Source	CREATE INDEX 'PeptideModSeq_PrecursorCharge_SourceFile_Peptides_index' on 'peptidequants' ('PeptideModSeq' ASC, 'PrecursorCharge' ASC, 'SourceFile' ASC)	Name	Type
PeptideModSeq_PrecursorCharge_Source	CREATE INDEX 'PeptideModSeq_PrecursorCharge_SourceFile_Scores_index' on 'peptideScores' ('PeptideModSeq' ASC, 'PrecursorCharge' ASC, 'SourceFile' ASC)	<ul> <li>Tables (9)</li> </ul>	
PeptideSeq_Entries_index	CREATE INDEX 'PeptideSeq_Entries_index' on 'entries' ('PeptideSeq' ASC)	> i entries	
PeptideSeq_Fragments_index	CREATE INDEX 'PeptideSeq_Fragments_index' on 'fragmentquants' ('PeptideSeq' ASC)	> i fragmentquants	
PeptideSeq_Localizations_index	CREATE INDEX 'PeptideSeq_Localizations_index' on 'peptidelocalizations' ('PeptideSeq' ASC)	> 🔟 metadata	
PeptideSeq_PeptideToProtein_index	CREATE INDEX 'PeptideSeq_PeptideToProtein_index' on 'peptidetoprotein' ('PeptideSeq' ASC)	> peptidelocalizations	
PeptideSeq_Peptides_index	CREATE INDEX 'PeptideSeq_Peptides_index' on 'peptidequants' (PeptideSeq'ASC)	> iii peptidequants	
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PrecursorMz_Entries_index	CREATE INDEX 'PrecursorMz_Entries_index' on 'entries' (PrecursorMz' ASC)	> 🔲 peptidetoprotein	
ProteinAccession_PeptideToProtein_index	CREATE INDEX 'ProteinAccession_PeptideToProtein_index' on 'peptidetoprotein' ('ProteinAccession' ASC)	> i proteinscores	
ProteinAccession ProteinScores index	CREATE INDEX 'ProteinAccession ProteinScores, index' on 'proteinscores' (ProteinAccession' ASC)	> i retentiontimes	
ProteinGroup_ProteinScores_index	CREATE INDEX 'ProteinGroup, ProteinScores, index' on 'proteinscores' ('ProteinGroup' ASC)	<ul> <li>Indices (16)</li> </ul>	
Views (0)		Key_Metadata_index	
Triggers (0)		PeptideModSeq_PrecursorCharge	Source
		PeptideModSeq_PrecursorCharge	Source
		PeptideModSeq_PrecursorCharge	Source
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		PeptideModSeq_PrecursorCharge	Source
		PeptideSeq_Entries_index	
		PeptideSeq_Fragments_index	
		PeptideSeq_Localizations_index	
		PeptideSeq_PeptideToProtein_inde	ex
		PeptideSeq_Peptides_index	
		PeptideSeq_Scores_index	
		PrecursorMz_Entries_index	
		ProteinAccession_PeptideToProtei	in_index
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		ProteinGroup_ProteinScores_index	4
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Click on "Browse Data" tab at top under the "Open Database" button.

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		PrecursorCharge			Copies	RTInSeconds	Score	assEncodedLeng		nsityEncodedLer							
1	Filter	Filter	Filter	Filter	Filter	Filter	Filter	Filter	Filter	Filter	Filter	Filter	Filter	Filter			
7	739.34951480	2	SQDSYPGSPS	SQDSYPGSPS	1	2229.4104003	0.0147205004	72	BLOB	36	BLOB	36	x <b>&amp;&amp;@</b> ? <b>&amp;</b> kw	2211.421630			
7	766.92889455	2	NIC[+57.0214	NICQFLLEVGIVK	1	6027.0522460	0.0258984006	64	BLOB	32	BLOB	32	BLOB	6004.581054			
8	842.41328255	2	IHMPAALVSNL	IHMPAALVSNL	1	3511.0832519	0.0009796069	160	BLOB	80	BLOB	80	BLOB	3493.22436			
	65 <b>8.3</b> 4331230	2	ANAVFEWHITK	ANAVFEWHITK	1	3324.0947265	0.0051113101	112		56	BLOB	56	BLOB	3306.22265			
	712.03006369	3	WKPWEPI VEF	WKPWEPLVEE	1	4436.6674804	0.0043321000	240		120		120	BLOB	4418,406731			
	747.38705555			SCVITYLAQV			0.0056147598			76		76		4024.54467			
	867.49545230									84	BLOB	84			Type of data currently in cell: NULL		R
				AGADIIITYFAP			0.0016375599							6203.90478!	0 byte(s)		
	876.92558780		GGDPTKEPEP	GGDPTKEPEP	1	2960.7556152	0.0003493140	168	BLOB	84	BLOB	84		2939.32348	DB Schema		
2	729.89557480	2	QLWGLLIEETEK	QLWGLLIEETEK	1	5128.2675781	0.0069805700	104	BLOB	52	BLOB	52	BLOB	5109.80419!	Name	T	Туре
7	789.41613830	2	YEISSVPTFLFFK	YEISSVPTFLFFK	1	5882.1791992	0.0015772900	128	BLOB	64	BLOB	64	BLOB	5863.45605 <sup>,</sup>	Tables (9)     entries		
9	912.46233180	2	ENYLEENLLYA	ENYLEENLLYA	1	4878.9047851	0.0054434901	104	BLOB	52	BLOB	52	BLOB	4857.025390	> fragmentquants		
-	913.06350580	2	LAVLVALILEQ	LAVLVALIILEQ	1	6365.6689453	0.0006269380	152	BLOB	76	BLOB	76	BLOB	6346.81542!	> metadata > peptidelocalizations		
	506.98372636	3	LLIHOSLAGGIL	LLIHOSLAGGIL	1	3630.6269531	0.0003216550	192	BLOB	96	BLOB	96	BLOB	3612,694824	> peptidequants		
	473.58731269	2	IVALCEDUMPER	LVHFEPHMRPR	1	1574 7770541	0.0035781299	144		72		72	BLOB	1553.07202:	> peptidescores > peptidetoprotein		
	838.42849880									72	BLOB	72		2114.92529;	> proteinscores		
				IQGSAGEISTS			0.0135725000								> indices (16)		
(	693.03241702	3	KEELMFFLWA	KEELMFFLWA	1	5591.1748046	0.0017114599	216	BLOB	108	BLOB	108		5572.48242:	<ul> <li>Key_Metadata_index</li> </ul>		
4	579.31368480	2	VETFSGVYKK	VETESGVYKK	1	1759.9394531	0.0035321300	104	BLOB	52	BLOB	52	BLOB	1741.86413!	PeptideModSeq_Precision		
	598.28747855	2	LWVAC[+57.0	LWVACYNGGR	1	2884.1545410	0.0039093401	96	BLOB	48	BLOB	48	BLOB	2866.13476!	PeptideModSeq_Prece PeptideModSeq_Prece		
2	743.86989130	2	WIPPSEATSQ	WIPPSEATSQ	1	3189.234375	0.0111840004	104	BLOB	52	×00000	52	BLOB	3174.995112	PeptideModSeq_Preck	ursorCharge_Source	
1	813.38865230	2	NNHLIEEINNEMR	NNHLIEEINNEMR	1	3275.0422363	0.0109200999	104	BLOB	52	BLOB	52	BLOB	3253.69848	PeptideModSeq_Precu PeptideSeq_Entries_in		
9	900.11419052	3	FLDTDTIC[+5	FLDTDTICYRV	1	4663,7099609	0.0068768500	152		76		76	BLOB	4641.89453:	PeptideSeq_Fragment	ts_index	
	582.26439330			EVDEGAWETK			0.0080174598			32		32		2019.39721	PeptideSeq_Localization PeptideSeq_PeptideTo		
	499.24220980			FIASTGMDR	-		0.0063471300			36	BLOB	36		1929,19628!	PeptideSeq_Peptides_i	index	
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	884.97066830			IWTKDGNLAS			0.0971783995		BLOB	44	BLOB	44		3232.76611:	ProteinAccession_Pep	tideToProtein_index	
6	549.30702730	2	HTMMFSATEPK	HTMMFSATEPK	1	3110.0251464	0.0423033013	80	BLOB	40	BLOB	40	×00;00:0	3092.13037:	ProteinAccession_Prot ProteinGroup_Protein		

Under the "Browse Data" tab, select the table you want to view from the "Table:" dropdown menu. For example, the meta data:

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	proteinscores retentiontimes					BLOB	80	BLOB	3493.22436					
	658.34331230 2	ANAVFEWHITK	ANAVFEWHITK 1	3324.0947265	0.0051113101 112	BLOB	56	BLOB	56	BLOB	3306.22265			
	712.03006369 3	WKPWEPLVEE	WKPWEPLVEE 1	4436.6674804	0.0043321000 240	BLOB	120	BLOB	120	BLOB	4418.406731			
	747.38705555 2	SC[+57.0214	SCVITYLAQV 1	4046.5668945	0.0056147598 152	BLOB	76	BLOB	76	BLOB	4024.54467:			
	867.49545230 2	AGADIIITYFAP	AGADIIITYFAP 1	6226.4223632	0.0016375599 168	BLOB	84	BLOB	84	BLOB	6203.90478!	Type of data currently in cell: NULL 0 byte(s)		
	876.92558780 2	GGDPTKEPEP	GGDPTKEPEP 1	2960.7556152	0.0003493140 168	BLOB	84	BLOB	84	BLOB	2939.32348	D8 Schema		
	729.89557480 2	QLWGLLIEETEK	QLWGLLIEETEK 1	5128.2675781	0.0069805700 104	BLOB	52	BLOB	52	BLOB	5109.80419!	Name	Type	
	789.41613830 2	YEISSVPTFLFFK	YEISSVPTFLFFK 1	5882.1791992	0.0015772900 128	BLOB	64	BLOB	64	BLOB	5863.45605	✓		
	912.46233180 2	ENYLEENLLYA	ENYLEENLLYA 1	4878.9047851	0.0054434901 104	BLOB	52	BLOB	52	BLOB	4857.025390	> entries > fragmentquants		
	913.06350580 2	LAVLVALIILEQ	LAVLVALIILEQ 1	6365.6689453	0.0006269380 152	BLOB	76	BLOB	76	BLOB	6346.81542!	> metadata > peptidelocalizations		
	506.98372636 3	LLIHQSLAGGIL.	LLIHQSLAGGIL 1	3630.6269531	0.0003216550 192	BLOB	96	BLOB	96	8L08	3612.694824	> peptidequants		
	473.58731269 3	LVHFEPHMRPR	LVHFEPHMRPR 1	1574.7779541	0.0035781299 144	BLOB	72	BLOB	72	BLOB	1553.07202:	> peptidescores > peptidetoprotein		
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	579.31368480 2	VETESGVYKK	VETFSGVYKK 1	1759.9394531	0.0035321300 104		52		52	BLOB	1741.86413	Key_Metadata_index PeptideModSeq_PrecursorCh	arne Source	
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	743.86989130 2		WIPPSEATSQ 1		0.0111840004 104		52	×00000	52		3174.995112	PeptideModSeq_PrecursorCh PeptideModSeq_PrecursorCh		
	813.38865230 2		NNHLIEEINNEMR 1		0.0109200999 104		52	BLOB	52	BLOB	3253,69848(	PeptideModSeq_PrecursorCh PeptideSeg_Entries_index	arge_Source	
	900.11419052 3	FLDTDTICI+5	FLDTDTICYRV 1		0.0068768500 152		76		76		4641,89453	PeptideSeq_Fragments_index		
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	499.24220980 2	FIASTGMDR	FIASTGMDR 1		0.0063471300 72		36	BLOB	36	BLOB	1929.19628	PeptideSeq_Peptides_index     PeptideSeq_Scores_index		
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Choosing the "metadata" table will display information such as the parameter settings when Encyclopedia was run, the TIC for each raw file in the chromatogram library/quant report, etc

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					> intries		
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	-percolatorVersionNumber	3			Key_Metadata_index PeptideModSeq_Pre		
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last updated 16 Dec 2019