Step-by-Step Tutorial for Integrating Contaminant FASTA and Spectral Libraries in Various DDA and DIA Proteomics Software Platforms

Mass spectrometry-based proteomics is challenged by the presence of contaminant protein background signals. During data analysis, contaminant FASTA libraries allow the search algorithm to distinguish between peptides with similar retention times and *m/z*. Here, we generated universal contaminant FASTA and spectral libraries that can be used for both data-dependent acquisition (DDA) and data-independent acquisition (DIA) proteomics, available to download at: https://github.com/HaoGroup-ProtContLib, and ProteomeXchange (#PXD031139) These new contaminant libraries have been shown to reduce false identifications, increase protein IDs, and do not influence protein quantification for both DIA and DDA workflows. We modified the contaminant FASTA library to contain a "Cont" prefix before each UniProt identifier, simplifying the process of searching and removing contaminant proteins prior to statistical analysis.

In this tutorial, we describe how to use our new contaminant FASTA and spectral libraries with various DDA and DIA software platforms.

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(PDF is available on BioRxiv and the Hao Lab Website: https://blogs.gwu.edu/haolab/research/publication/)

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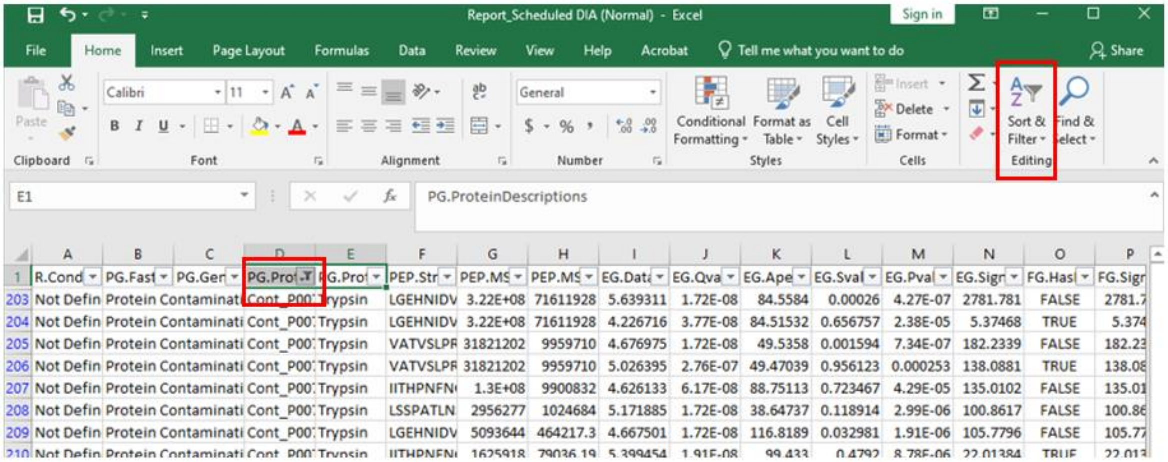
1. Brief Description of Contaminant Libraries

Exogenous contaminant proteins originated from reagents and sample handling are often shared in most bottom-up proteomic experiments. Although widely used for DDA proteomics, the list of common protein contaminants from MaxQuant and cRAP list have not been updated for years, containing deleted Uniprot IDs, sample-specific interference proteins that are incorrectly listed as contaminants, and commercially available human protein standards which are not contaminant proteins. Therefore, we first built a new contaminant FASTA library by manually merging the available contaminant lists online, updating their Uniprot entry IDs, deleting noncontaminant proteins, searching for new contaminant proteins on Uniprot, and combining them into a new FASTA file. Our new contaminant FASTA library contains 381 contaminant proteins including all human keratins and skin-derived proteins, common bovine contaminants from cell culture and affinity columns, various proteolytic enzymes, affinity tags, and other contaminants. When compared to the MaxQuant and cRAP contaminant lists, our new FASTA library is up-to-date for all Uniprot IDs and contains an additional 166 contaminant proteins. This new FASTA library can be used for both DDA and DIA proteomics. We also added a "Cont_" prefix in each contaminant entry in the FASTA library, allowing contaminant proteins to be easily filtered and removed in the result files.

2. Removing Contaminant Proteins from Result Files.

1.1. Launch the results file in Microsoft Excel. In the "Home" tab, click on "Sort & Filter" and then "Filter".

1.2. Navigate to the Protein ID column and type in "Cont_".



E1 | PG.ProteinDescriptions

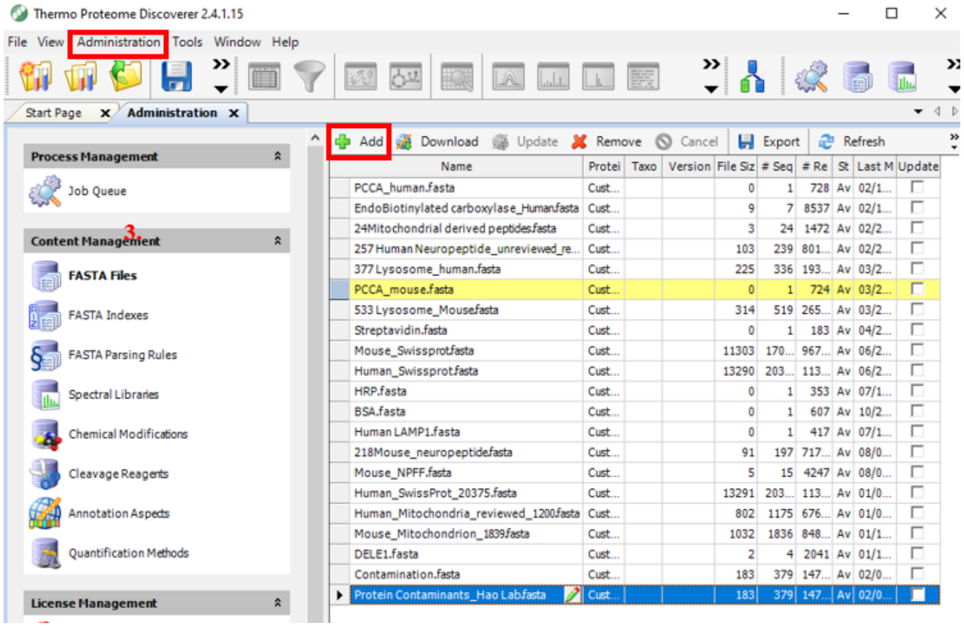
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	R.Cond	PG.Fast	PG.Ger	PG.Pro	G.Pro	PEP.Str	PEP.MS	PEP.MS	EG.Data	EG.Qva	EG.Ape	EG.Sval	EG.Pval	EG.Sign	FG.Has	FG.Sign
203	Not Defin	Protein Contaminati	Cont_P00	Trypsin		LGEHNIDV	3.22E+08	71611928	5.639311	1.72E-08	84.5584	0.00026	4.27E-07	2781.781	FALSE	2781.7
204	Not Defin	Protein Contaminati	Cont_P00	Trypsin		LGEHNIDV	3.22E+08	71611928	4.226716	3.77E-08	84.51532	0.656757	2.38E-05	5.37468	TRUE	5.374
205	Not Defin	Protein Contaminati	Cont_P00	Trypsin		VATVSLPR	31821202	9959710	4.676975	1.72E-08	49.5358	0.001594	7.34E-07	182.2339	FALSE	182.23
206	Not Defin	Protein Contaminati	Cont_P00	Trypsin		VATVSLPR	31821202	9959710	5.026395	2.76E-07	49.47039	0.956123	0.000253	138.0881	TRUE	138.08
207	Not Defin	Protein Contaminati	Cont_P00	Trypsin		IITHPNFN	1.3E+08	9900832	4.626133	6.17E-08	88.75113	0.723467	4.29E-05	135.0102	FALSE	135.01
208	Not Defin	Protein Contaminati	Cont_P00	Trypsin		LSSPATLN	2956277	1024684	5.171885	1.72E-08	38.64737	0.118914	2.99E-06	100.8617	FALSE	100.86
209	Not Defin	Protein Contaminati	Cont_P00	Trypsin		LGEHNIDV	5093644	464217.3	4.667501	1.72E-08	116.8189	0.032981	1.91E-06	105.7796	FALSE	105.77
210	Not Defin	Protein Contaminati	Cont_P00	Trypsin		IITHPNFN	1625918	79036.19	5.399454	1.91E-08	99.433	0.4792	8.78E-06	22.01384	TRUE	22.013

1.3. This will select all contaminant proteins. Evaluate the selected proteins to ensure that they are not biologically relevant based on custom sample types. Remove contaminant proteins prior to statistical analysis.

3. Using Contaminant FASTA in DDA Software

3.1. Proteome Discoverer for DDA

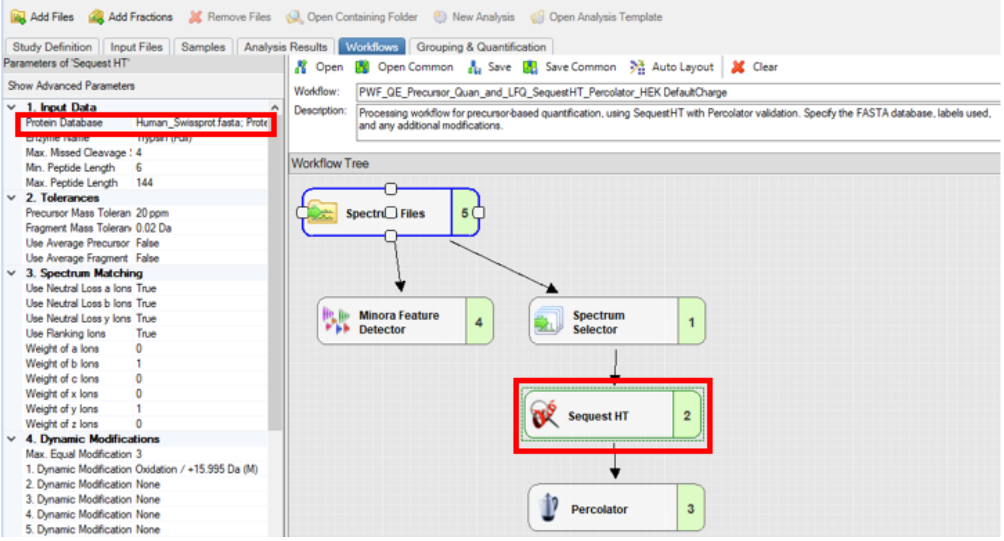
3.1.1. Click the “Administration” tab and select “Maintain Fasta Files”. Click “Add” and then select “Protein Contaminants_Hao Lab.Fasta”.



Name	Protei	Taxo	Version	File Siz	# Seq	# Re	St	Last M	Update
PCCA_human.fasta	Cust...			0	1	728	Av	02/1...	□
EndoBiotinylated carboxylase_Humanfasta	Cust...			9	7	8537	Av	02/1...	□
24Mitochondrial derived peptidesfasta	Cust...			3	24	1472	Av	02/2...	□
257 Human Neuropeptide_unreviewed_re...	Cust...			103	239	801...	Av	02/2...	□
377 Lysosome_human.fasta	Cust...			225	336	193...	Av	03/2...	□
PCCA_mouse.fasta	Cust...			0	1	724	Av	03/2...	□
533 Lysosome_Mousefasta	Cust...			314	519	265...	Av	03/2...	□
Streptavidin.fasta	Cust...			0	1	183	Av	04/2...	□
Mouse_Swissprotfasta	Cust...			11303	170...	967...	Av	06/2...	□
Human_Swissprot.fasta	Cust...			13290	203...	113...	Av	06/2...	□
HRP.fasta	Cust...			0	1	353	Av	07/1...	□
BSA.fasta	Cust...			0	1	607	Av	10/2...	□
Human LAMP1.fasta	Cust...			0	1	417	Av	07/1...	□
218Mouse_neuropeptidefasta	Cust...			91	197	717...	Av	08/0...	□
Mouse_NPFF.fasta	Cust...			5	15	4247	Av	08/0...	□
Human_SwissProt_20375.fasta	Cust...			13291	203...	113...	Av	01/0...	□
Human_Mitochondria_reviewed_1200fasta	Cust...			802	1175	676...	Av	01/0...	□
Mouse_Mitochondrion_1839fasta	Cust...			1032	1836	848...	Av	01/1...	□
DELE1.fasta	Cust...			2	4	2041	Av	01/1...	□
Contamination.fasta	Cust...			183	379	147...	Av	02/0...	□
Protein Contaminants_Hao Labfasta	Cust...			183	379	147...	Av	02/0...	□

3.1.2. Click on the “Sequest HT” tab in the processing workflow in a study file. For protein database, select both the “Protein Contaminants_Hao Lab” and organism FASTA for your sample.

NOTE: The protein contaminant FASTA file must be included in data processing step to reduce protein/peptide false identifications.



Parameters of 'Sequest HT'

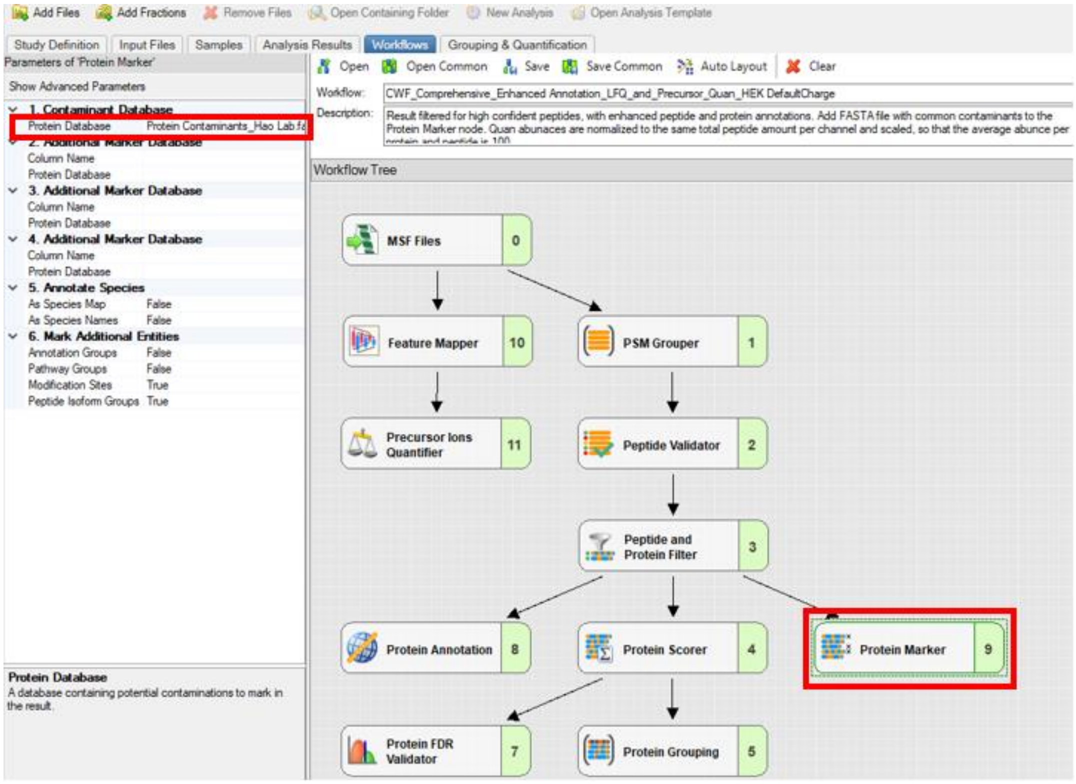
Show Advanced Parameters

1. Input Data	
Protein Database	Human_Swissprot.fasta; Prote
Enzyme Name	Trypsin (Full)
Max. Missed Cleavage :	4
Min. Peptide Length	6
Max. Peptide Length	144
2. Tolerances	
Precursor Mass Toleran	20 ppm
Fragment Mass Toleran	0.02 Da
Use Average Precursor	False
Use Average Fragment	False
3. Spectrum Matching	
Use Neutral Loss a Ions	True
Use Neutral Loss b Ions	True
Use Neutral Loss y Ions	True
Use Flanking Ions	True
Weight of a Ions	0
Weight of b Ions	1
Weight of c Ions	0
Weight of x Ions	0
Weight of y Ions	1
Weight of z Ions	0
4. Dynamic Modifications	
Max. Equal Modification	3
1. Dynamic Modification	Oxidation / +15.995 Da (M)
2. Dynamic Modification	None
3. Dynamic Modification	None
4. Dynamic Modification	None
5. Dynamic Modification	None

Workflow: PWF_QE_Precursor_Quan_and_LFQ_SequestHT_Percolator_HEK DefaultCharge

Description: Processing workflow for precursor-based quantification, using SequestHT with Percolator validation. Specify the FASTA database, labels used, and any additional modifications.

3.1.3. Select your consensus step workflow. Under the “Protein Marker” tab, include the contaminant FASTA in the contaminant database. This will create a separate column in the result file marking contaminant proteins.



Parameters of 'Protein Marker'

Show Advanced Parameters

1. Contaminant Database
- Protein Database: Protein Contaminants_Hao Lab.fa

2. Additional Marker Database
- Column Name
- Protein Database

3. Additional Marker Database
- Column Name
- Protein Database

4. Additional Marker Database
- Column Name
- Protein Database

5. Annotate Species
- As Species Map: False
- As Species Names: False

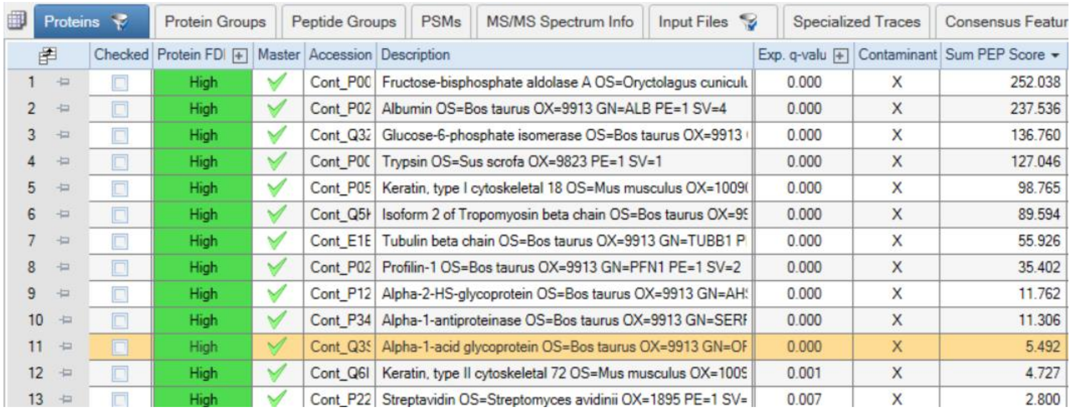
6. Mark Additional Entities
- Annotation Groups: False
- Pathway Groups: False
- Modification Sites: True
- Peptide Isoform Groups: True

Protein Database
A database containing potential contaminations to mark in the result.

Workflow: CWF_Comprehensive_Enhanced Annotation_LFQ_and_Precursor_Quan_HEK DefaultCharge

Description: Result filtered for high confident peptides, with enhanced peptide and protein annotations. Add FASTA file with common contaminants to the Protein Marker node. Quan abunaces are normalized to the same total peptide amount per channel and scaled, so that the average abunce per protein and peptide is 100.

3.1.4. Contaminant proteins can be filtered using the accession column or separate contaminant column.



	Checked	Protein FDI	Master	Accession	Description	Exp. q-valu	Contaminant	Sum PEP Score
1	□	High	✓	Cont_P00	Fructose-bisphosphate aldolase A OS=Oryctolagus cuniculu	0.000	X	252.038
2	□	High	✓	Cont_P02	Albumin OS=Bos taurus OX=9913 GN=ALB PE=1 SV=4	0.000	X	237.536
3	□	High	✓	Cont_Q32	Glucose-6-phosphate isomerase OS=Bos taurus OX=9913	0.000	X	136.760
4	□	High	✓	Cont_P00	Trypsin OS=Sus scrofa OX=9823 PE=1 SV=1	0.000	X	127.046
5	□	High	✓	Cont_P05	Keratin, type I cytoskeletal 18 OS=Mus musculus OX=1009	0.000	X	98.765
6	□	High	✓	Cont_Q5H	Isoform 2 of Tropomyosin beta chain OS=Bos taurus OX=9	0.000	X	89.594
7	□	High	✓	Cont_E1E	Tubulin beta chain OS=Bos taurus OX=9913 GN=TUBB1 P	0.000	X	55.926
8	□	High	✓	Cont_P02	Profilin-1 OS=Bos taurus OX=9913 GN=PFN1 PE=1 SV=2	0.000	X	35.402
9	□	High	✓	Cont_P12	Alpha-2-HS-glycoprotein OS=Bos taurus OX=9913 GN=AH	0.000	X	11.762
10	□	High	✓	Cont_P34	Alpha-1-antiproteinase OS=Bos taurus OX=9913 GN=SERP	0.000	X	11.306
11	□	High	✓	Cont_Q3S	Alpha-1-acid glycoprotein OS=Bos taurus OX=9913 GN=OF	0.000	X	5.492
12	□	High	✓	Cont_Q6I	Keratin, type II cytoskeletal 72 OS=Mus musculus OX=1009	0.001	X	4.727
13	□	High	✓	Cont_P22	Streptavidin OS=Streptomyces avidinii OX=1895 PE=1 SV=	0.007	X	2.800

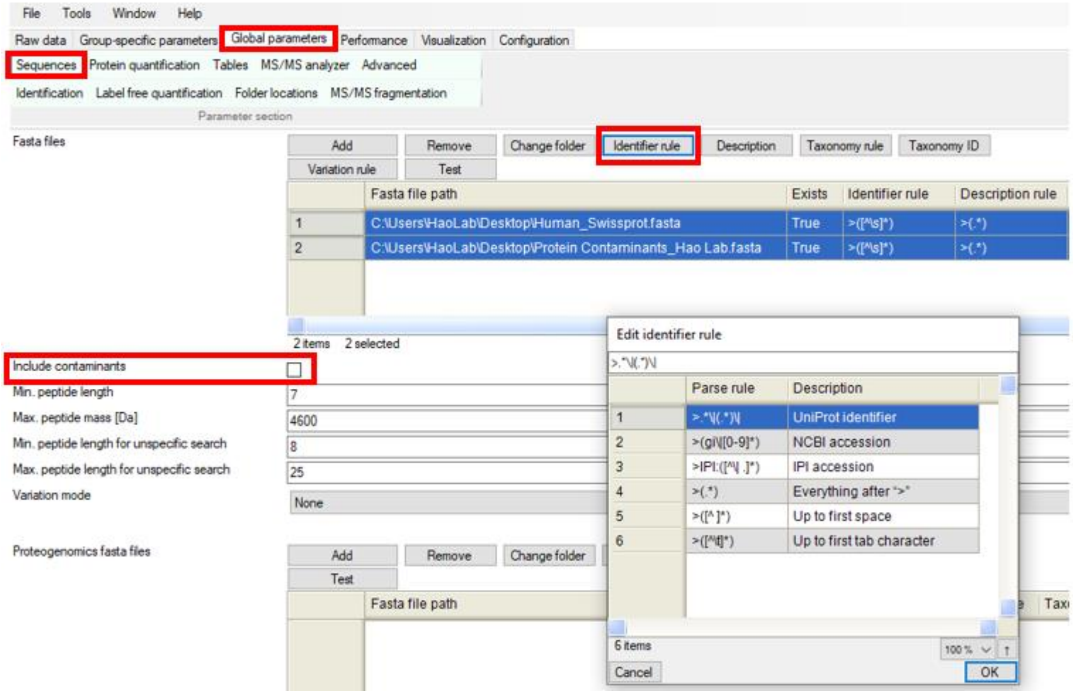
3.2. MaxQuant for DDA

3.2.1 Launch MaxQuant. Load *.raw* files. Click the “Global parameters” tab and then select “Sequences”.

3.2.2 Select the “Protein Contaminants_Hao Lab.Fasta” and then click on “Identifier rule”. Select “UniProt Identifier”.

3.2.3 Unselect “Include contaminants”.

NOTE: Including the existing MaxQuant contaminant database will not affect results. However, contaminant proteins from the new FASTA will not be marked in the contaminant column in the MaxQuant results file, which may lead to confusion. Contaminant proteins are marked in the UniProt ID column with the prefix “Cont_” as described on page 2.



Fasta files

	Fasta file path	Exists	Identifier rule	Description rule
1	C:\Users\HaoLab\Desktop\Human_Swissprot.fasta	True	>([^\s]*)	>(.*)
2	C:\Users\HaoLab\Desktop\Protein Contaminants_Hao Lab.fasta	True	>([^\s]*)	>(.*)

2 items 2 selected

Include contaminants ☐
Min. peptide length 7
Max. peptide mass [Da] 4600
Min. peptide length for unspecific search 8
Max. peptide length for unspecific search 25
Variation mode None

Proteogenomics fasta files

Fasta file path

Edit identifier rule

>.*\|(.*)\|

	Parse rule	Description
1	>.*|(.*)|	UniProt identifier
2	>(gi|[0-9]*)	NCBI accession
3	>IPI:([^| .]*)	IPI accession
4	>(.*)	Everything after “>”
5	>([^]*)	Up to first space
6	>([^\t]*)	Up to first tab character

6 items 100 %

4. Building Contaminant Protein Spectral Libraries

To establish comprehensive contaminant protein spectral libraries for DIA proteomics, we created a series of contaminant-only samples using various proteolytic enzymes, affinity purification beads and fetal bovine serum (FBS) that are commonly used for cell culture medium. Contaminant Protein Spectral Library is available to download in Github and ProteomeXchange (#PXD031139). For proteomics software that allows the input of multiple spectral libraries, our contaminant spectral library and custom proteomics data can be included together. For software that only allows one spectral library input, an integrated spectral library can be built using our contaminant-only raw data and custom proteomics data. We have tested that the integrated spectral library performs similarly to two separate libraries. Either method is better compared to the results analyzed without the contaminant library.

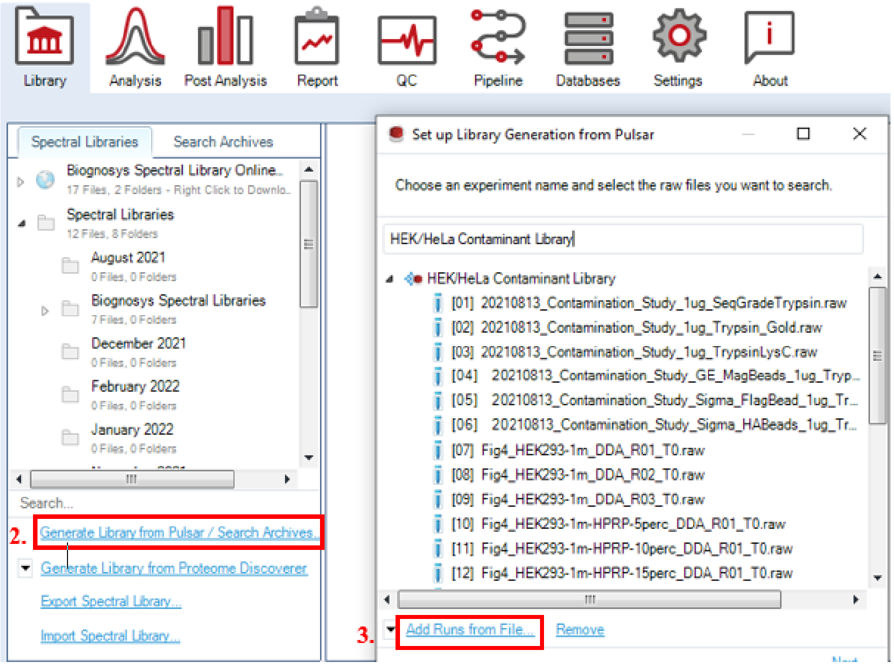
4.1. Building a Spectral Library in Spectronaut

4.1.1. Launch Biognosys Spectronaut and select the "Databases" tab. Import the "Protein Contaminants_Hao Lab.Fasta".

4.1.2. Select the "Library" tab. Click "Generate Library from Pulsar/Search Archives".

4.1.3. Select "Add Runs from File" to add *.raw* files.

Note: The *.raw* files from our universal contaminant-only experiment can be included to ensure the accurate detection and inclusion of contaminant spectra within the library.



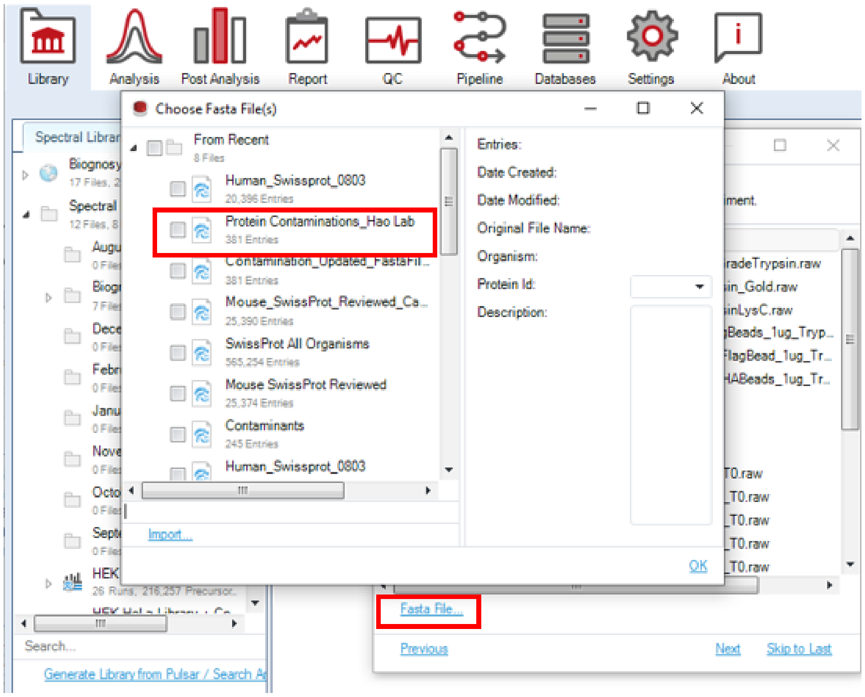
Set up Library Generation from Pulsar

Choose an experiment name and select the raw files you want to search.

HEK/HeLa Contaminant Library

HEK/HeLa Contaminant Library
[01] 20210813_Contamination_Study_1ug_SeqGradeTrypsin.raw
[02] 20210813_Contamination_Study_1ug_Trypsin_Gold.raw
[03] 20210813_Contamination_Study_1ug_TrypsinLysC.raw
[04] 20210813_Contamination_Study_GE_MagBeads_1ug_Tryp...
[05] 20210813_Contamination_Study_Sigma_FlagBead_1ug_Tr...
[06] 20210813_Contamination_Study_Sigma_HABeads_1ug_Tr...
[07] Fig4_HEK293-1m_DDA_R01_T0.raw
[08] Fig4_HEK293-1m_DDA_R02_T0.raw
[09] Fig4_HEK293-1m_DDA_R03_T0.raw
[10] Fig4_HEK293-1m-HPRP-5perc_DDA_R01_T0.raw
[11] Fig4_HEK293-1m-HPRP-10perc_DDA_R01_T0.raw
[12] Fig4_HEK293-1m-HPRP-15perc_DDA_R01_T0.raw

4.1.4. Click “Next” and then “Fasta File.” Select the “Protein Contaminants_Hao Lab.Fasta”. Select the remaining settings to build the desired library.



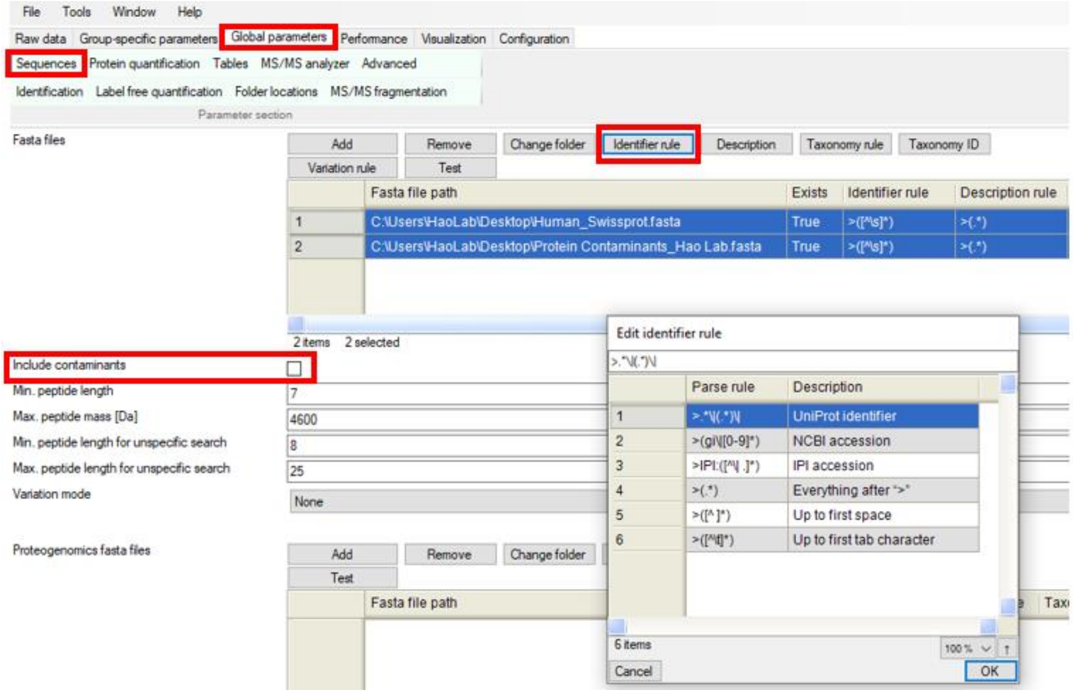
4.2. Building a Spectral Library in MaxQuant

4.2.1 Launch MaxQuant. Load *.raw* files. Click the “Global parameters” tab and then select “Sequences”.

4.2.2 Select the “Protein Contaminants_Hao Lab.Fasta” and then click on “Identifier rule”. Select “UniProt Identifier”.

4.2.3 Unselect “Include contaminants”.

NOTE: Including the existing MaxQuant contaminant database will not affect results. However, contaminant proteins from the new FASTA will not be marked in the contaminant column in the MaxQuant results file, which may lead to confusion. Contaminant proteins are marked in the UniProt ID column with the prefix “Cont_” as described on page 2.



	Fasta file path	Exists	Identifier rule	Description rule
1	C:\Users\HaoLab\Desktop\Human_Swissprot.fasta	True	>([^\s]*)	>(.*)
2	C:\Users\HaoLab\Desktop\Protein Contaminants_Hao Lab.fasta	True	>([^\s]*)	>(.*)

2 items 2 selected

Include contaminants ☐

Min. peptide length 7

Max. peptide mass [Da] 4600

Min. peptide length for unspecific search 8

Max. peptide length for unspecific search 25

Variation mode None

Edit identifier rule

>.*\|(.*)\|

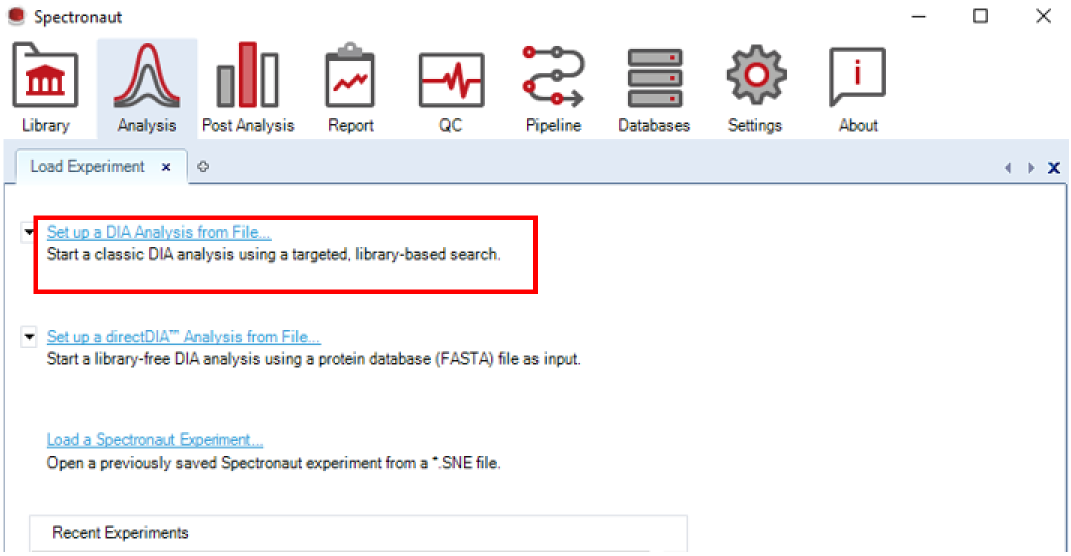
<table>
<tr><th></th><th>Parse rule</th><th>Description</th></tr>
<tr><td>1</td><td>>.*\|(.*)\|</td><td>UniProt identifier</td></tr>
<tr><td>2</td><td>>(gi\|[0-9]*)</td><td>NCBI accession</td></tr>
<tr><td>3</td><td>>IPI:([^\| .]*)</td><td>IPI accession</td></tr>
<tr><td>4</td><td>>(.*)</td><td>Everything after ">"</td></tr>
<tr><td>5</td><td>>([^]*)</td><td>Up to first space</td></tr>
<tr><td>6</td><td>>([^\t]*)</td><td>Up to first tab character</td></tr>
</table>

6 items

5. Using Contaminant FASTA and Spectral Libraries in Library-based DIA

5.1. Spectronaut for Library-based DIA.

5.1.1. Launch Biognosys Spectronaut. Select “Set up DIA Analysis from File”.



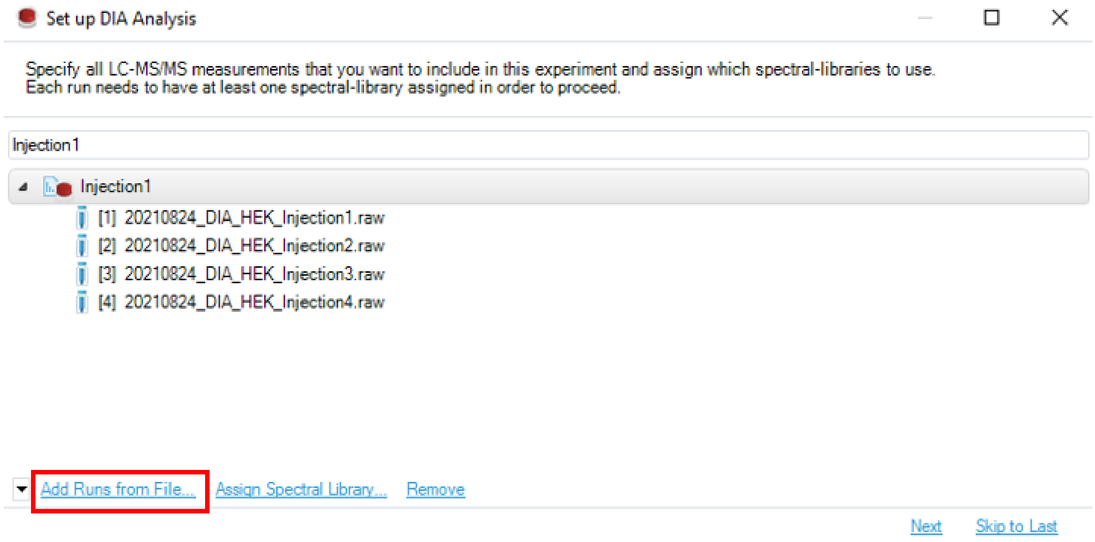
Set up a DIA Analysis from File...
Start a classic DIA analysis using a targeted, library-based search.

Set up a directDIA™ Analysis from File...
Start a library-free DIA analysis using a protein database (FASTA) file as input.

Load a Spectronaut Experiment...
Open a previously saved Spectronaut experiment from a *.SNE file.

Recent Experiments

5.1.2. Load the *.raw* files for the study.



Specify all LC-MS/MS measurements that you want to include in this experiment and assign which spectral-libraries to use.
Each run needs to have at least one spectral-library assigned in order to proceed.

Injection1

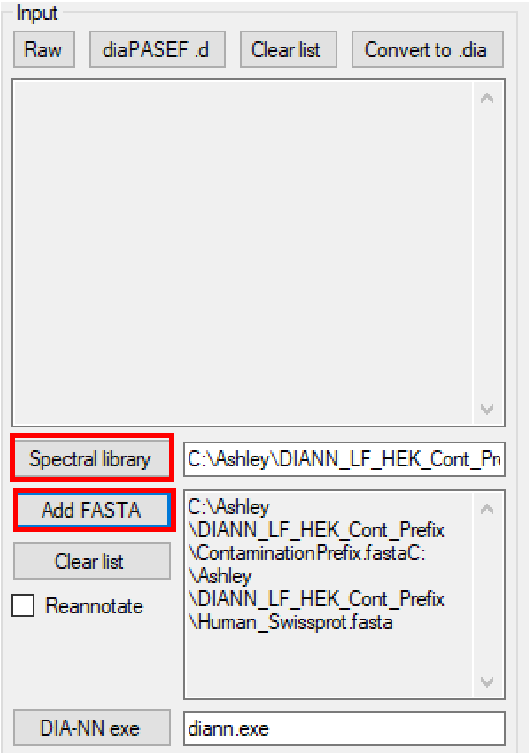
Injection1
- [1] 20210824_DIA_HEK_Injection1.raw
- [2] 20210824_DIA_HEK_Injection2.raw
- [3] 20210824_DIA_HEK_Injection3.raw
- [4] 20210824_DIA_HEK_Injection4.raw

5.1.3. Select the contaminant-containing spectral library and contaminant FASTA used during library creation.

5.2. DIA-NN for Library-based DIA.

5.2.1. Launch DIA-NN. Click “Spectral library” and add the contaminant FASTA that was built using Spectronaut.

5.2.2. Load the *.raw* files. Under “Add FASTA” select the appropriate FASTA libraries to build the spectral library.



C:\Ashley\DIANN_LF_HEK_Cont_Pr

C:\Ashley
\DIANN_LF_HEK_Cont_Prefix
\ContaminationPrefix.fastaC:
\Ashley
\DIANN_LF_HEK_Cont_Prefix
\Human_Swissprot.fasta

□ Reannotate

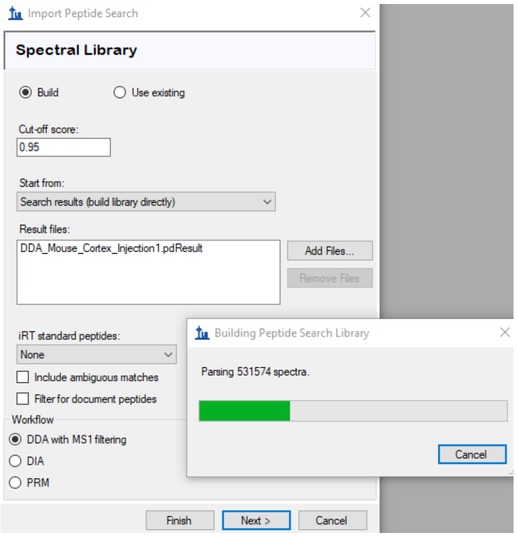
diann.exe

5.3. Skyline for Library-based DIA.

5.3.1. Launch Skyline (version 21.2) and open a "Blank Document".

5.3.2. A spectral library can be built by selecting "File", "Import" and then "Peptide Search."

5.3.3. Import the *.pdResult* file from Proteome Discoverer or *msms.text* file from MaxQuant. Select "Next" to build the peptide search library.


Import Peptide Search

Spectral Library

● Build ○ Use existing

Cut-off score: 0.95

Start from: Search results (build library directly)

Result files: DDA_Mouse_Cortex_Injection1.pdResult

iRT standard peptides: None

□ Include ambiguous matches

□ Filter for document peptides

Workflow

● DDA with MS1 filtering

○ DIA

○ PRM

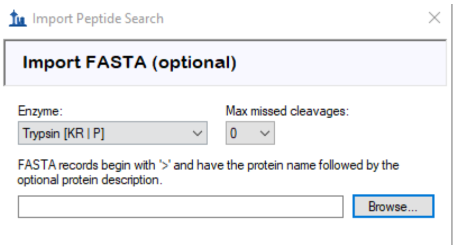
Building Peptide Search Library

Parsing 531574 spectra.

5.3.4. Select the appropriate .raw files and click "Next".

5.3.5. Select the FASTA File and then "Finish".

NOTE: Only a single FASTA library can be imported. The contaminant FASTA file will need to be combined with the organism FASTA.


Import Peptide Search

Import FASTA (optional)

Enzyme: Trypsin [KR | P]

Max missed cleavages: 0

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

5.3.6. Library-based DIA analysis can be conducted using established Skyline workflows. However, the conjoined FASTA file used to build the library should be included during data analysis.

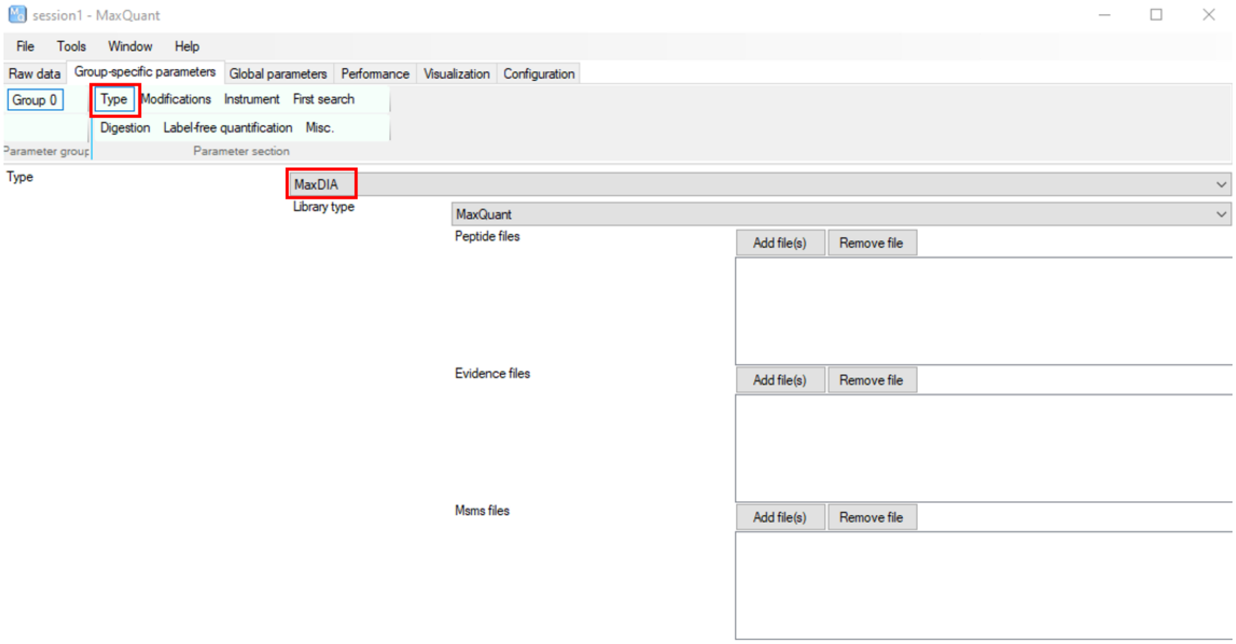
5.4. MaxDIA for Library-based DIA.

5.4.1. Launch MaxQuant. Load .raw files. Click the "Global parameters" tab and then select "Sequences".

5.4.2. For library-based DIA proteomics, you must include the same contaminant and organism specific FASTA files used to generate the spectral library. Select the "Protein Contaminants_Hao Lab.Fasta" and the organism specific UniProt FASTA file. Click on "Identifier rule" and select "UniProt Identifier".

5.4.3. Select the "Group-Specific Parameters" tab. Click "Type" and select "MaxDIA" from the drop-down menu.

5.4.4. Import the peptide, evidence and msms.*txt* file for library-based DIA.

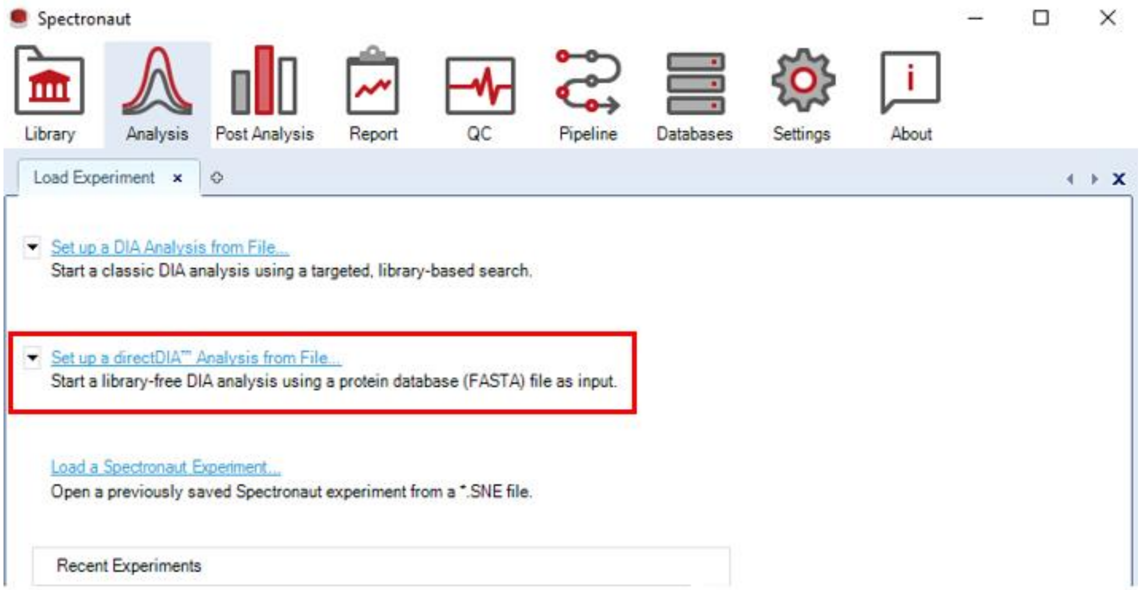


Type: MaxDIA
Library type: MaxQuant

6. Using Contaminant FASTA in Library-free DIA

6.1. Spectronaut for library-free DIA

6.1.1. Launch Spectronaut. Click “Set up a directDIA Analysis from File”.



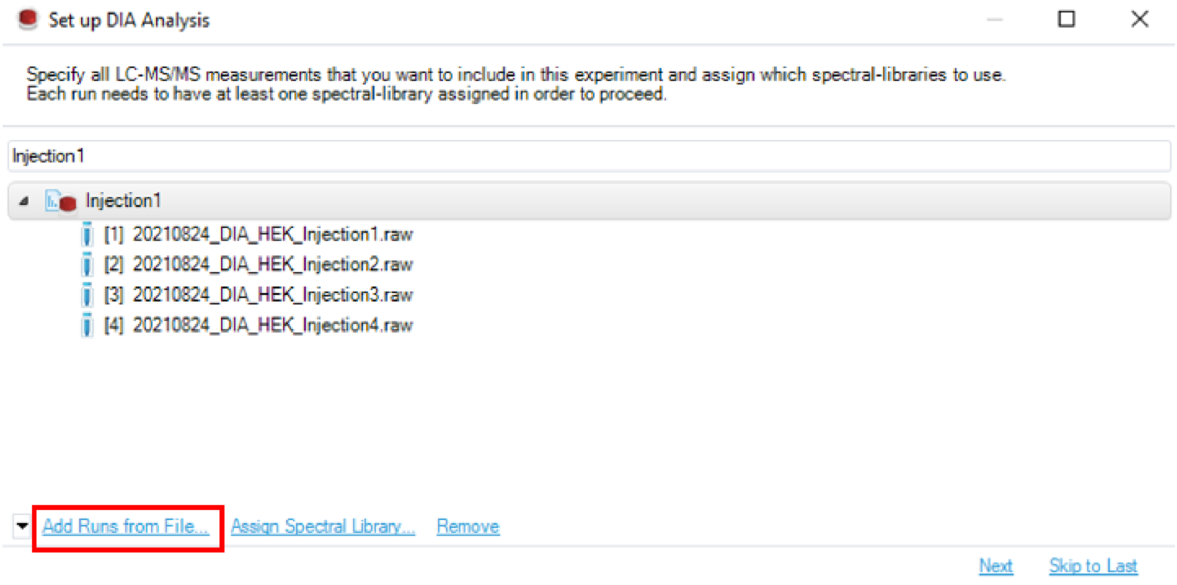
Set up a DIA Analysis from File...
Start a classic DIA analysis using a targeted, library-based search.

Set up a directDIA™ Analysis from File...
Start a library-free DIA analysis using a protein database (FASTA) file as input.

Load a Spectronaut Experiment...
Open a previously saved Spectronaut experiment from a *.SNE file.

Recent Experiments

6.1.2. Load the *.raw* files for the study.



Specify all LC-MS/MS measurements that you want to include in this experiment and assign which spectral-libraries to use.
Each run needs to have at least one spectral-library assigned in order to proceed.

Injection1

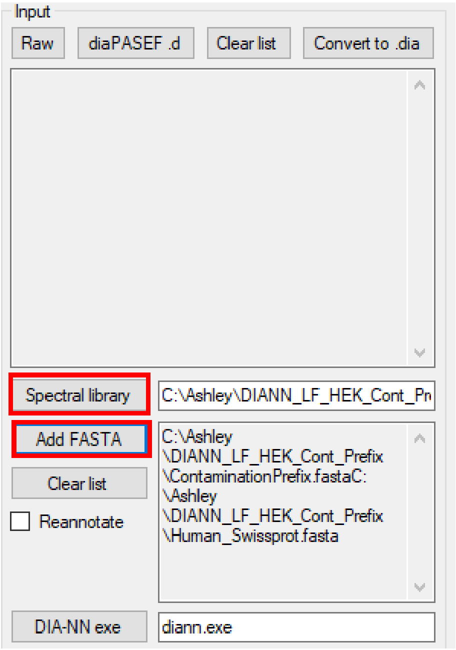
Injection1
[1] 20210824_DIA_HEK_Injection1.raw
[2] 20210824_DIA_HEK_Injection2.raw
[3] 20210824_DIA_HEK_Injection3.raw
[4] 20210824_DIA_HEK_Injection4.raw

6.1.3. Select the contaminant FASTA and organism FASTA.

6.2. DIA-NN for library-free DIA

6.2.1. Launch DIA-NN. Click “spectral library” and add the contaminant library that was built using Spectronaut.

6.2.2. Under “Add FASTA” select the appropriate FASTA libraries to build the spectral library.



Spectral library: C:\Ashley\DIANN_LF_HEK_Cont_Pr

Add FASTA:
C:\Ashley
\DIANN_LF_HEK_Cont_Prefix
\ContaminationPrefix.fastaC:
\Ashley
\DIANN_LF_HEK_Cont_Prefix
\Human_Swissprot.fasta

□ Reannotate

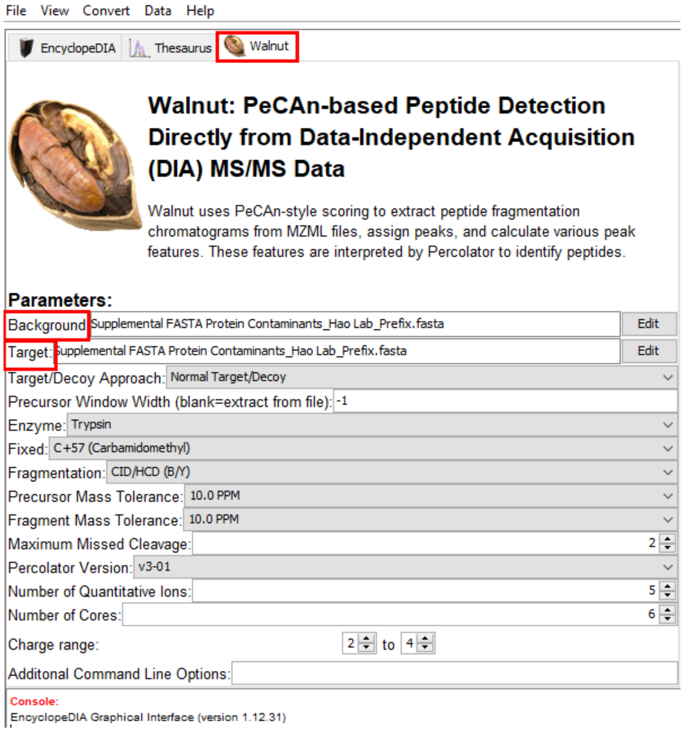
DIA-NN exe: diann.exe

6.3. PECAN for library-free DIA

6.3.1. Launch EncylopeDIA (version 1.12.31). Select the Walnut tab.

6.3.2. Import the contaminant FASTA library to the “Background” and “Target” sections.

NOTE: Only a single FASTA library can be imported into the workflow. The Hao Lab Contaminant library must be combined with your organism FASTA database.



Walnut: PeCAn-based Peptide Detection Directly from Data-Independent Acquisition (DIA) MS/MS Data

Walnut uses PeCAn-style scoring to extract peptide fragmentation chromatograms from MZML files, assign peaks, and calculate various peak features. These features are interpreted by Percolator to identify peptides.

Parameters:

Background: Supplemental FASTA Protein Contaminants_Hao Lab_Prefix.fasta
Target: Supplemental FASTA Protein Contaminants_Hao Lab_Prefix.fasta
Target/Decoy Approach: Normal Target/Decoy
Precursor Window Width (blank=extract from file): -1
Enzyme: Trypsin
Fixed: C+57 (Carbamidomethyl)
Fragmentation: CID/HCD (B/Y)
Precursor Mass Tolerance: 10.0 PPM
Fragment Mass Tolerance: 10.0 PPM
Maximum Missed Cleavage: 2
Percolator Version: v3-01
Number of Quantitative Ions: 5
Number of Cores: 6
Charge range: 2 to 4
Additonal Command Line Options:

Console:
EncyclopeDIA Graphical Interface (version 1.12.31)