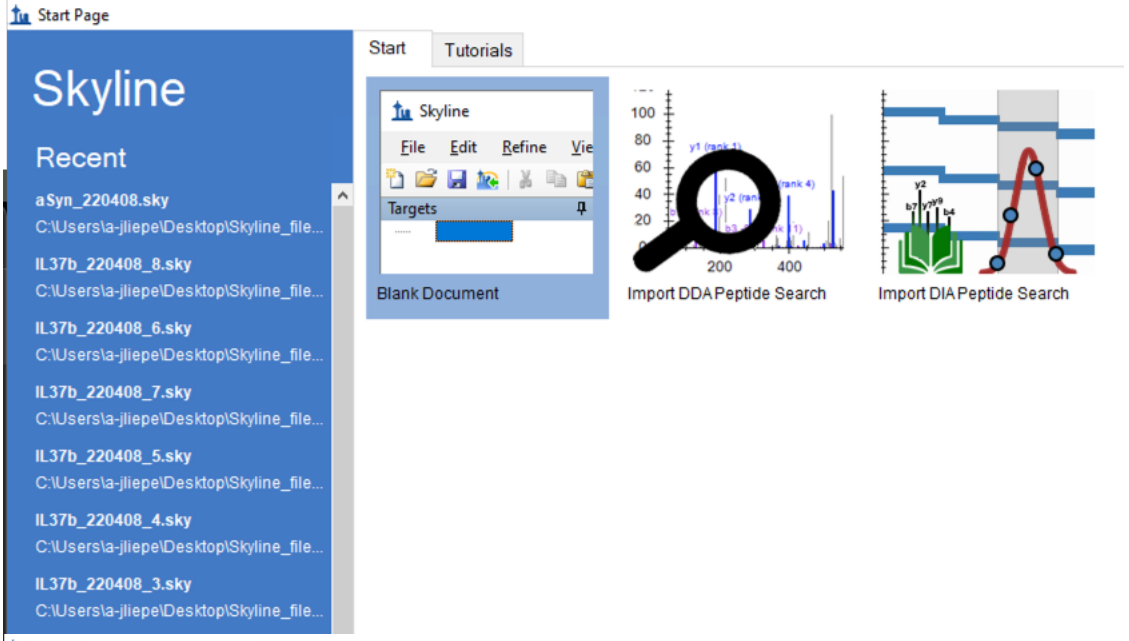
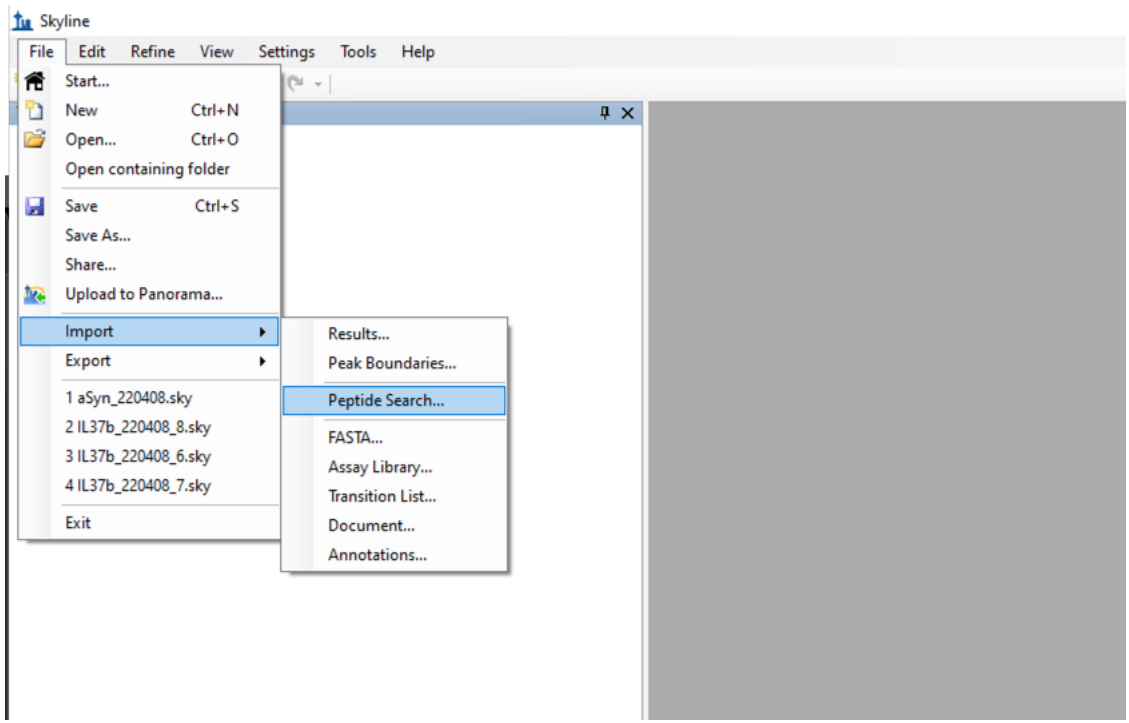
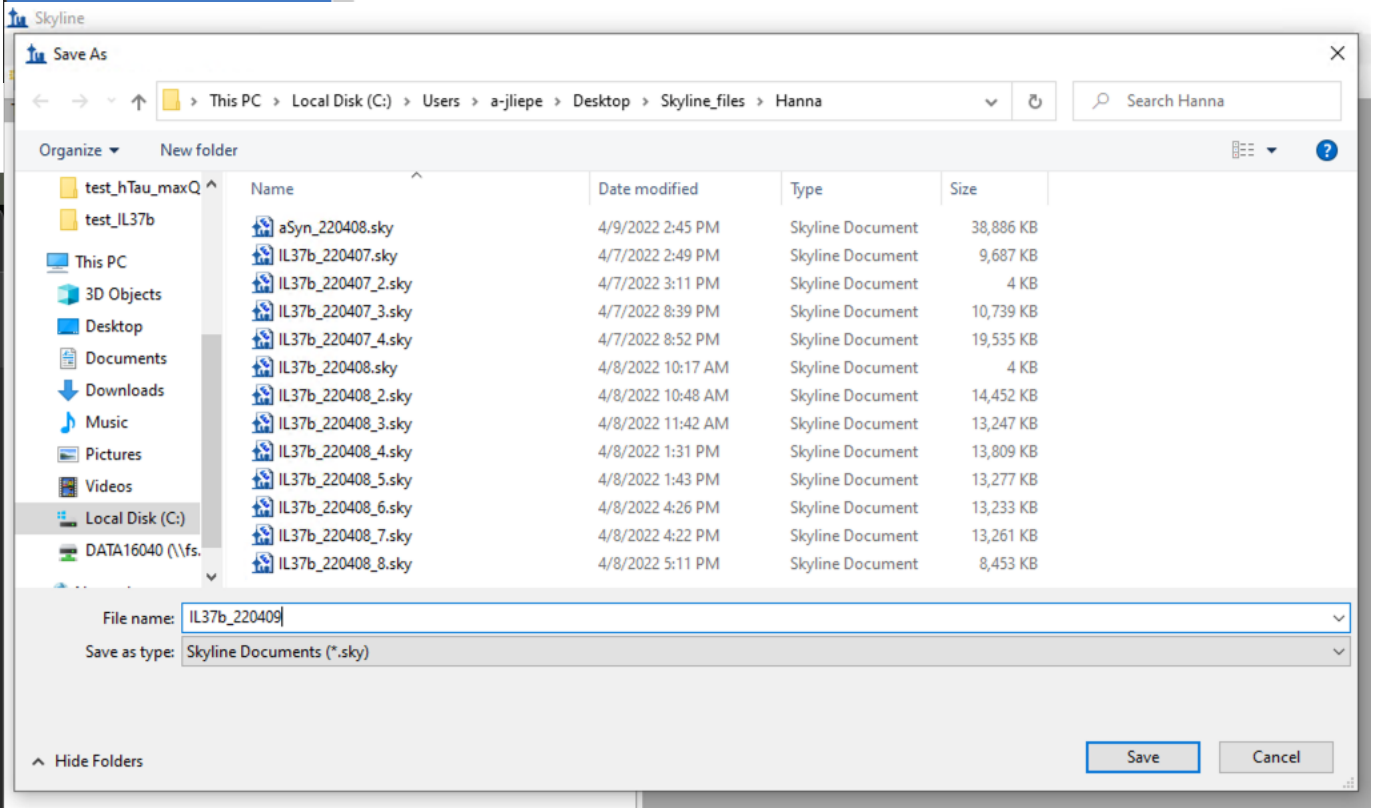
# Skyline tutorial

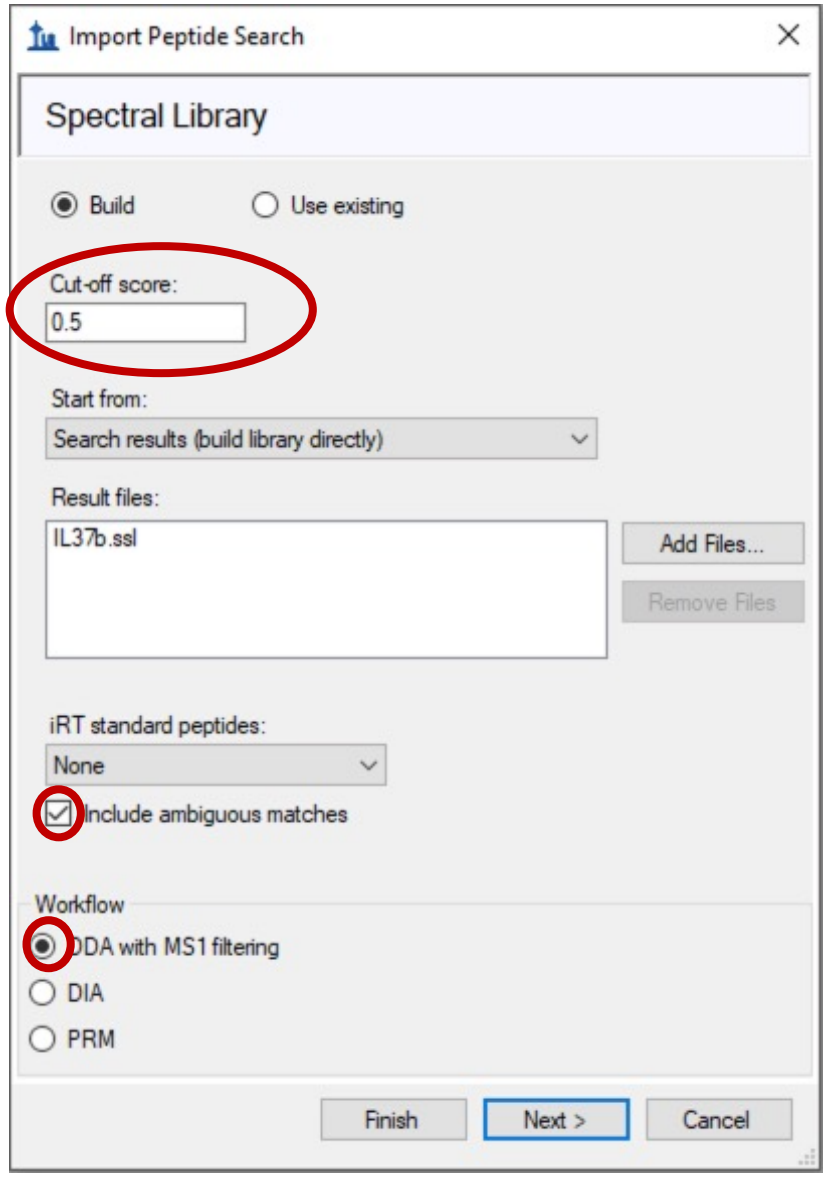
Relative quantification of inSPIRE assignments in aSPIre workflow



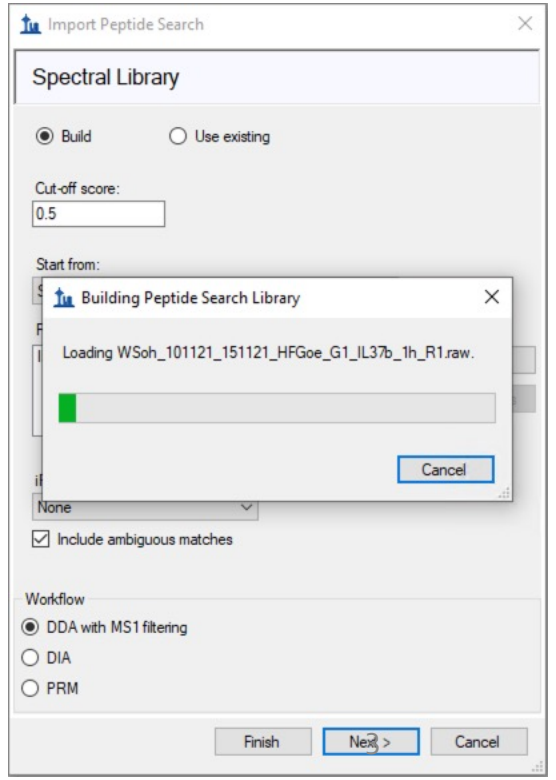



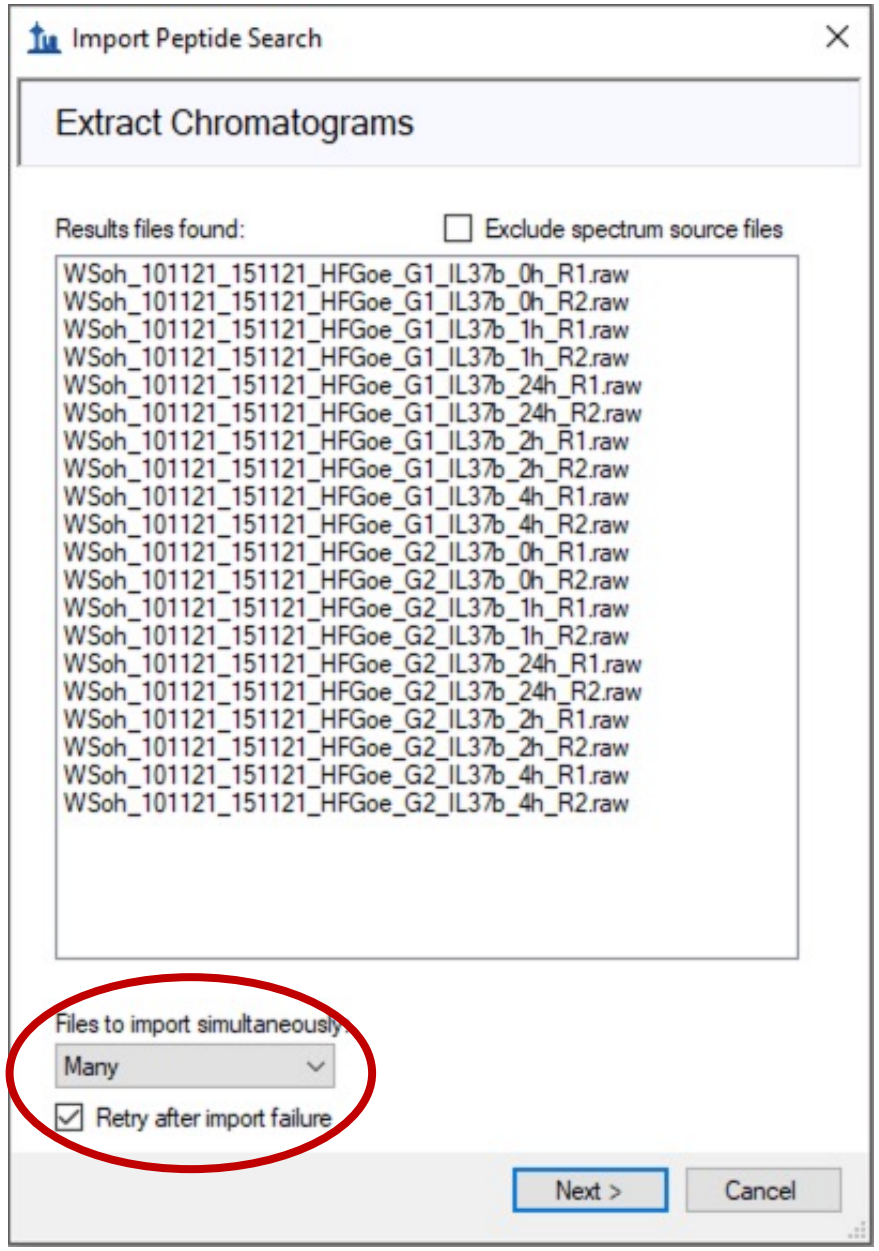

1. open a blank document and save it as .sky file
2. go to **File > Import > Peptide search**

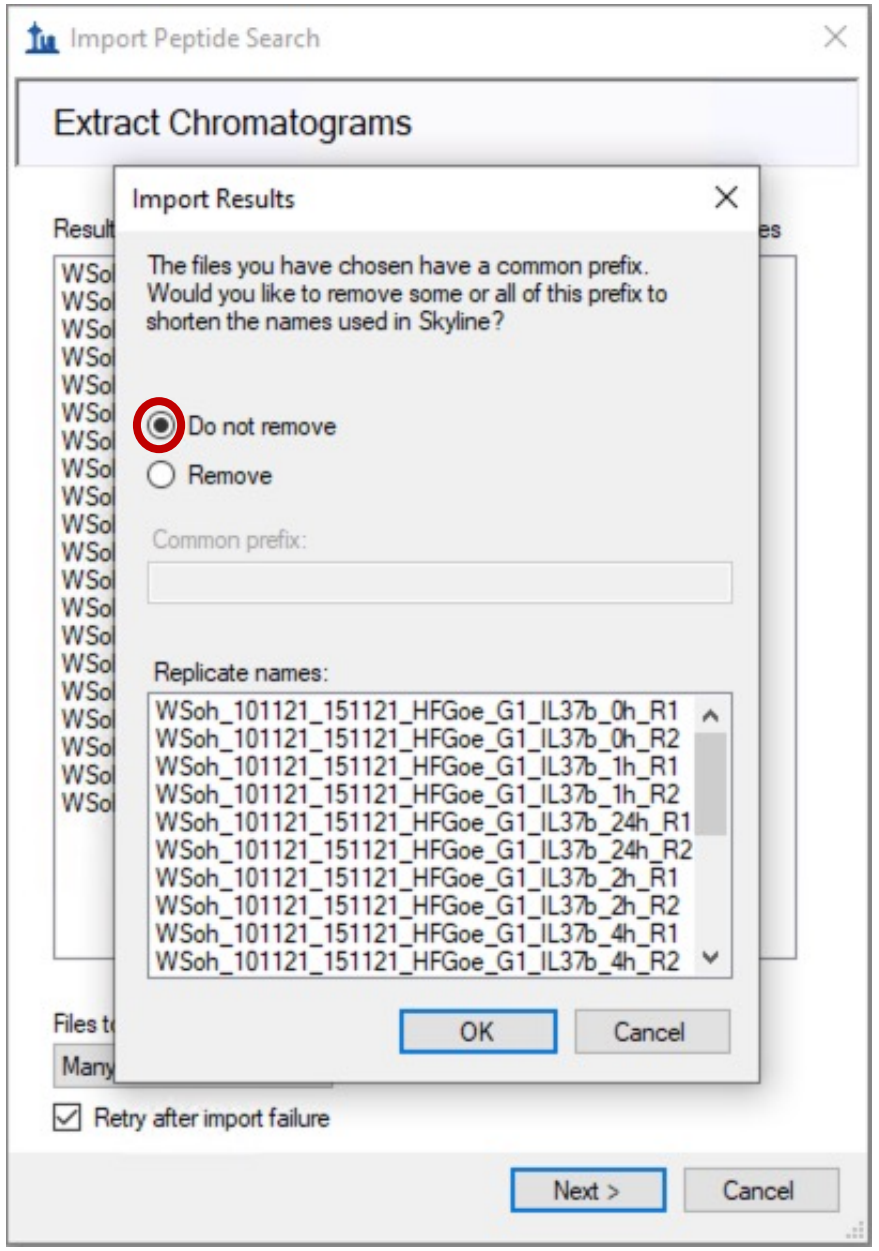


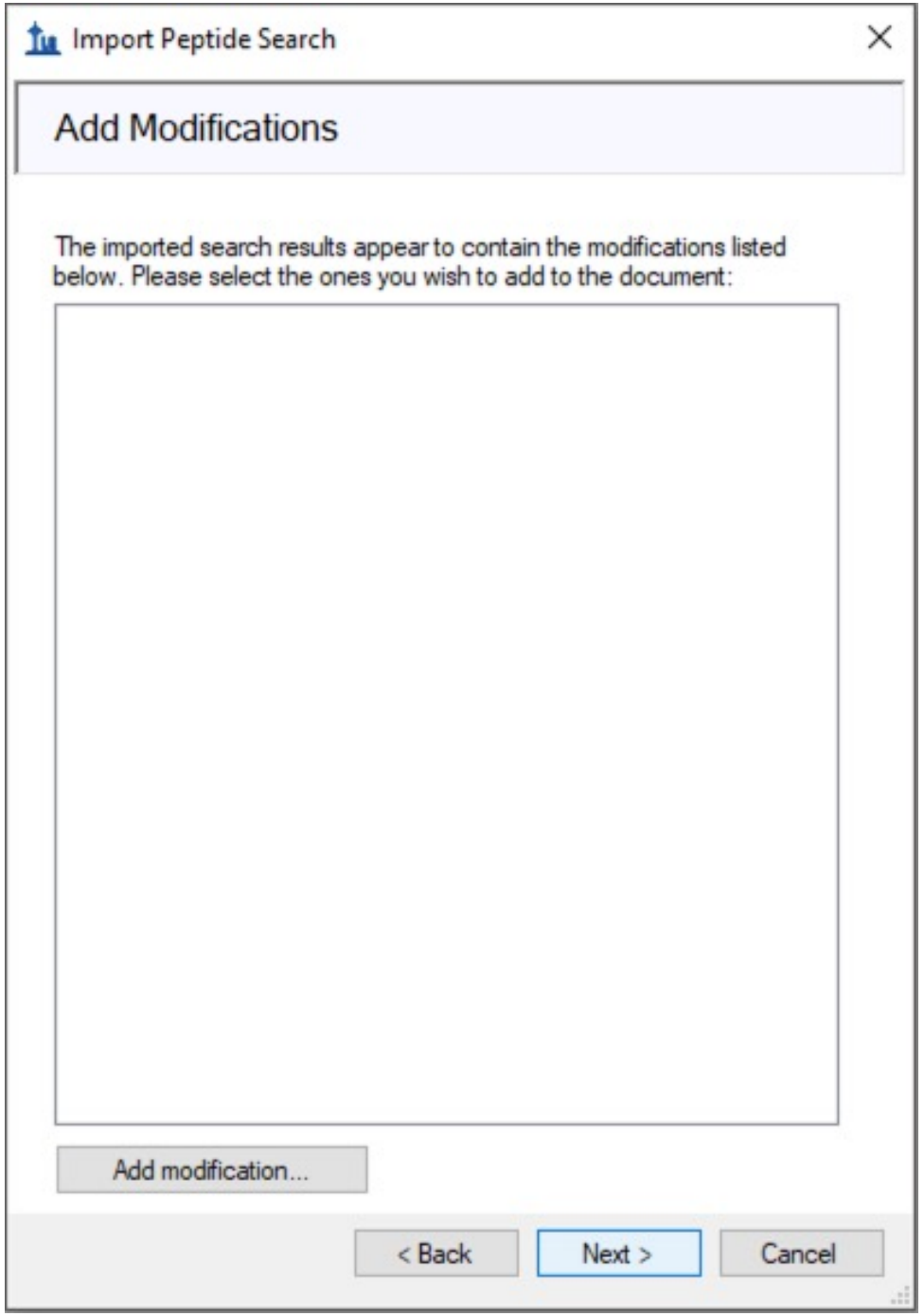

- set the cut-off score to 0.5. It is relying on the search engine's probability based score and will be automatically adjusted
- open the search results as .ssl table

**the .ssl has to be in the same folder as the .raw files!**

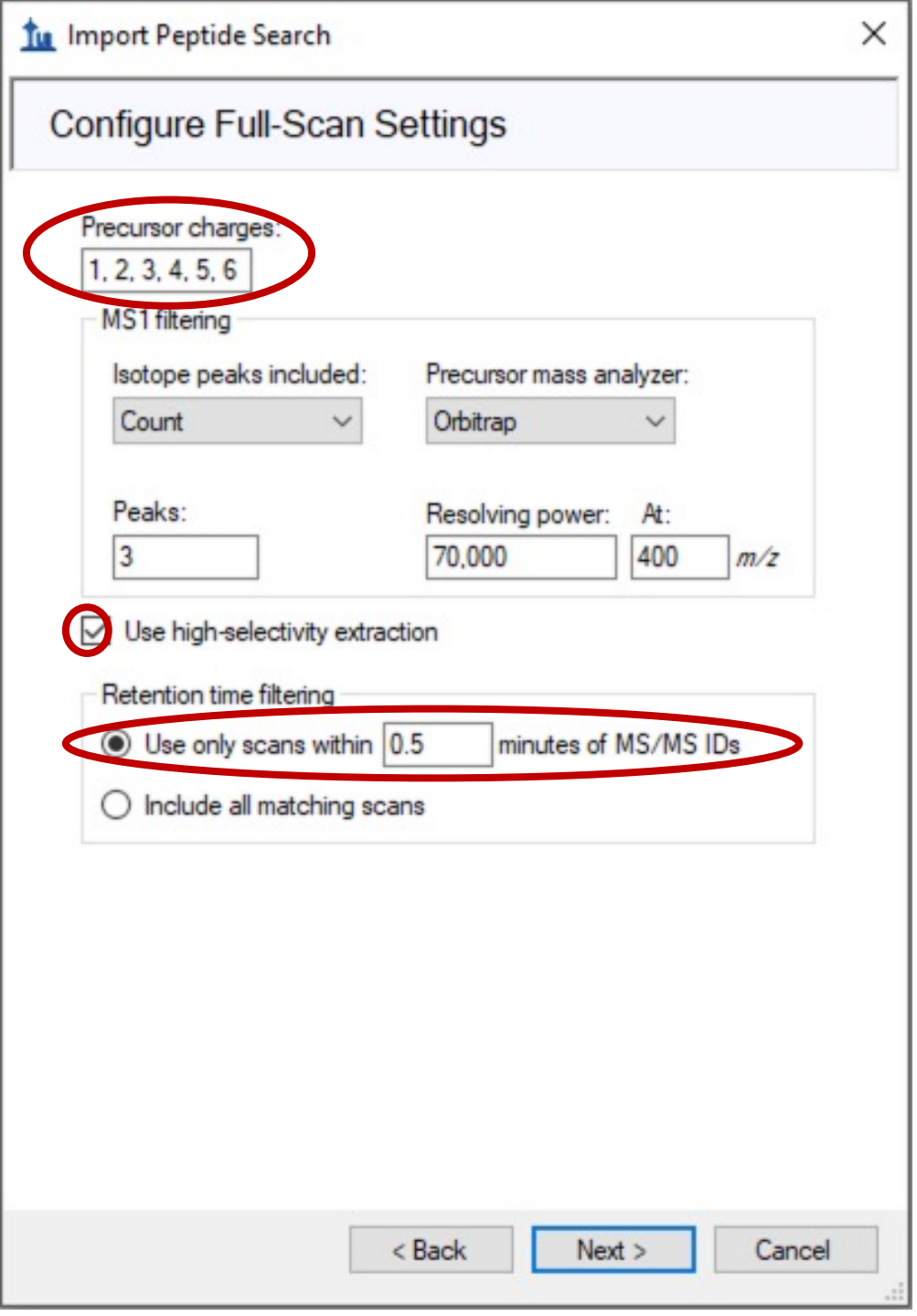



Import Peptide Search
Extract Chromatograms
Results files found:
Exclude spectrum source files
WSoh_101121_151121_HFGoe_G1_IL37b_0h_R1.raw
WSoh_101121_151121_HFGoe_G1_IL37b_0h_R2.raw
WSoh_101121_151121_HFGoe_G1_IL37b_1h_R1.raw
WSoh_101121_151121_HFGoe_G1_IL37b_1h_R2.raw
WSoh_101121_151121_HFGoe_G1_IL37b_24h_R1.raw
WSoh_101121_151121_HFGoe_G1_IL37b_24h_R2.raw
WSoh_101121_151121_HFGoe_G1_IL37b_2h_R1.raw
WSoh_101121_151121_HFGoe_G1_IL37b_2h_R2.raw
WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1.raw
WSoh_101121_151121_HFGoe_G1_IL37b_4h_R2.raw
WSoh_101121_151121_HFGoe_G2_IL37b_0h_R1.raw
WSoh_101121_151121_HFGoe_G2_IL37b_0h_R2.raw
WSoh_101121_151121_HFGoe_G2_IL37b_1h_R1.raw
WSoh_101121_151121_HFGoe_G2_IL37b_1h_R2.raw
WSoh_101121_151121_HFGoe_G2_IL37b_24h_R1.raw
WSoh_101121_151121_HFGoe_G2_IL37b_24h_R2.raw
WSoh_101121_151121_HFGoe_G2_IL37b_2h_R1.raw
WSoh_101121_151121_HFGoe_G2_IL37b_2h_R2.raw
WSoh_101121_151121_HFGoe_G2_IL37b_4h_R1.raw
WSoh_101121_151121_HFGoe_G2_IL37b_4h_R2.raw
Files to import simultaneously:
Many
Retry after import failure
Next >
Cancel


Import Peptide Search
Extract Chromatograms
Import Results
The files you have chosen have a common prefix. Would you like to remove some or all of this prefix to shorten the names used in Skyline?
Do not remove
Remove
Common prefix:
Replicate names:
WSoh_101121_151121_HFGoe_G1_IL37b_0h_R1
WSoh_101121_151121_HFGoe_G1_IL37b_0h_R2
WSoh_101121_151121_HFGoe_G1_IL37b_1h_R1
WSoh_101121_151121_HFGoe_G1_IL37b_1h_R2
WSoh_101121_151121_HFGoe_G1_IL37b_24h_R1
WSoh_101121_151121_HFGoe_G1_IL37b_24h_R2
WSoh_101121_151121_HFGoe_G1_IL37b_2h_R1
WSoh_101121_151121_HFGoe_G1_IL37b_2h_R2
WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1
WSoh_101121_151121_HFGoe_G1_IL37b_4h_R2
OK
Cancel
Retry after import failure
Next >
Cancel



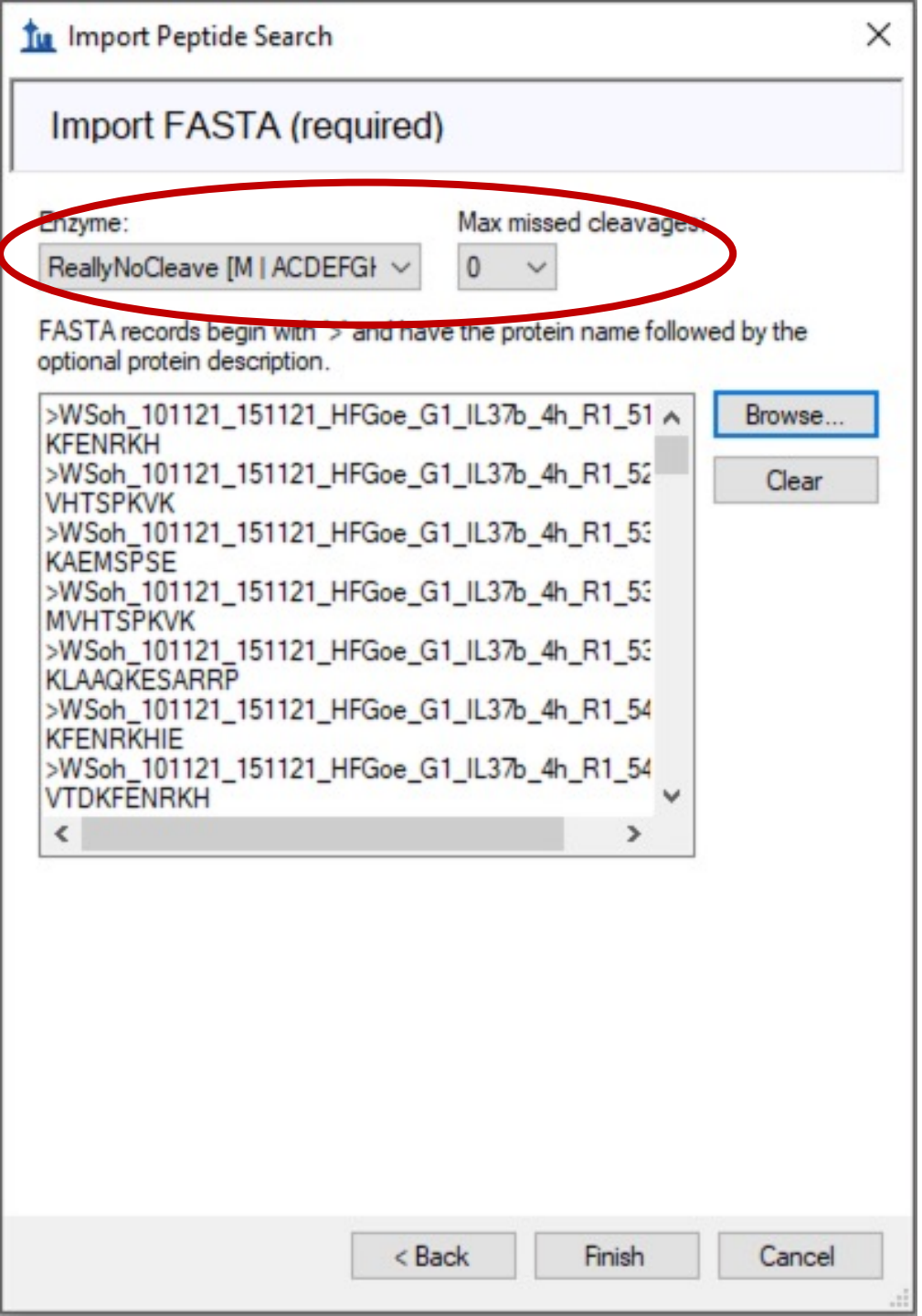

- do not add any new modifications here
- the modifications are already contained in the .ssl file and will be matched to the .raw files accordingly




- add precursor charge 1
- tick high-selectivity extraction

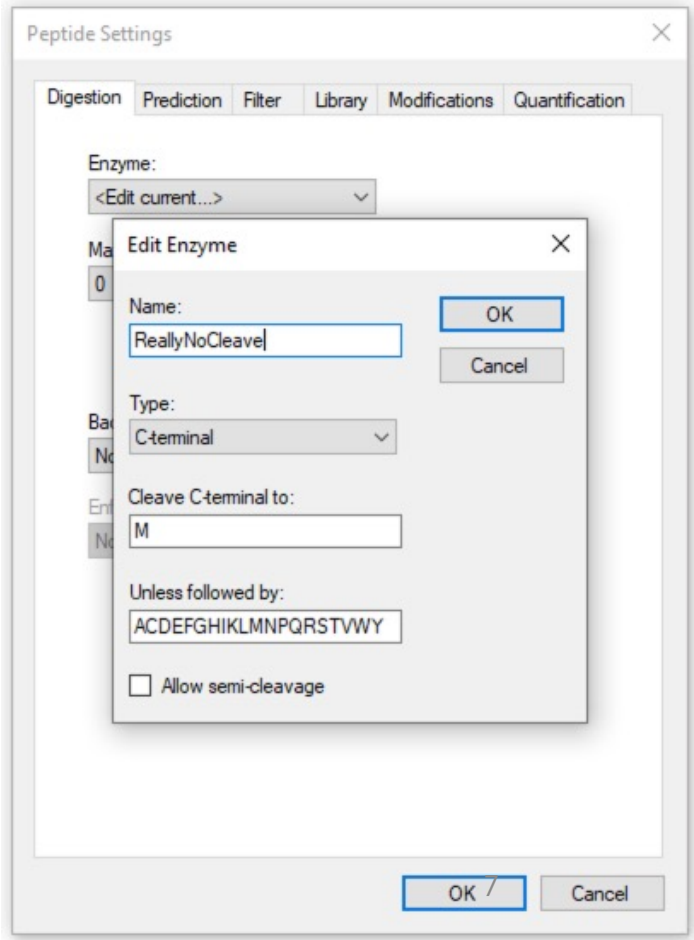
**modify the retention time window**

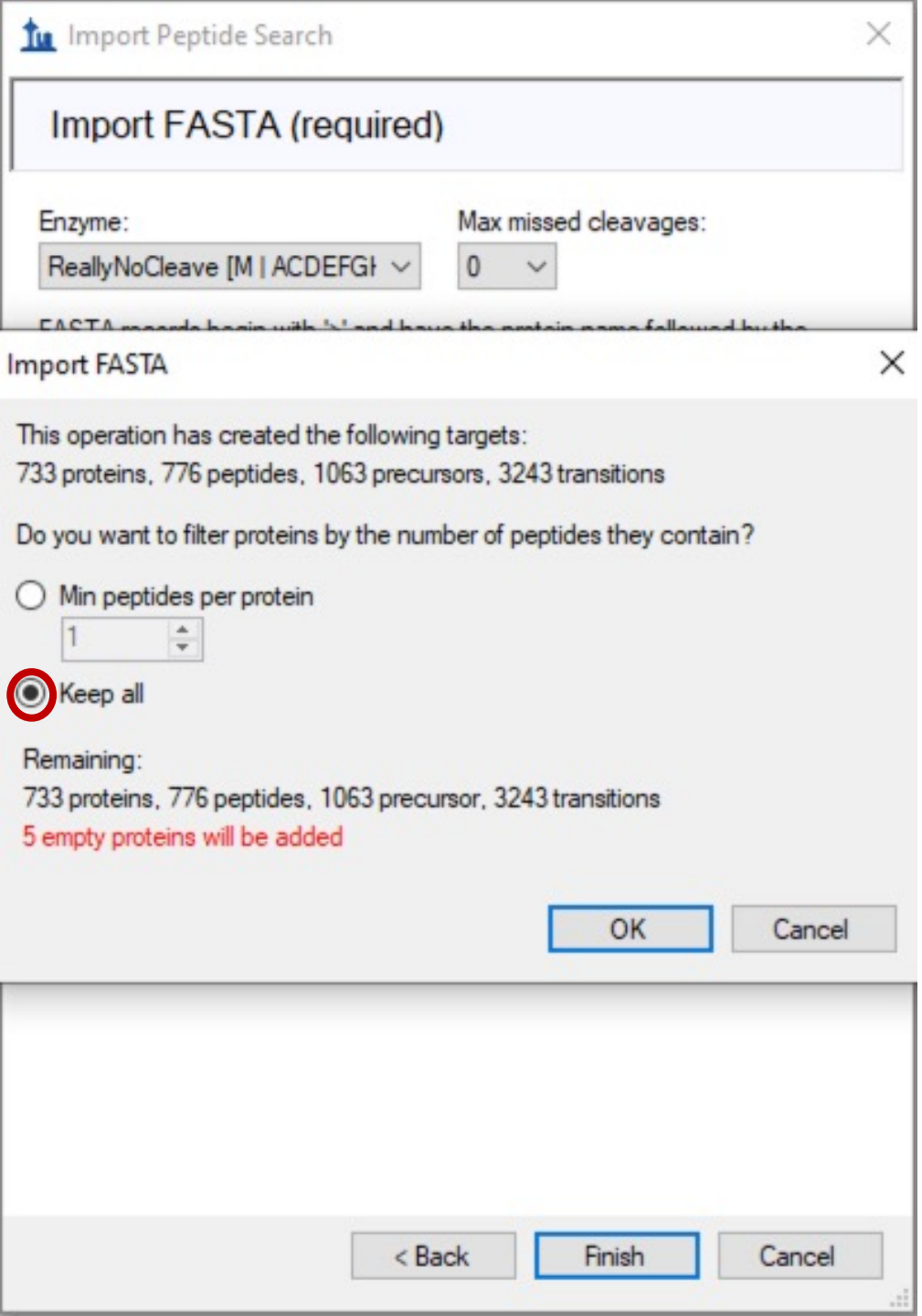
Output of the first script is the deviation of the measured from the predicted retention time. You can use this as a prior for the retention time window. Also look at the chromatigrams.

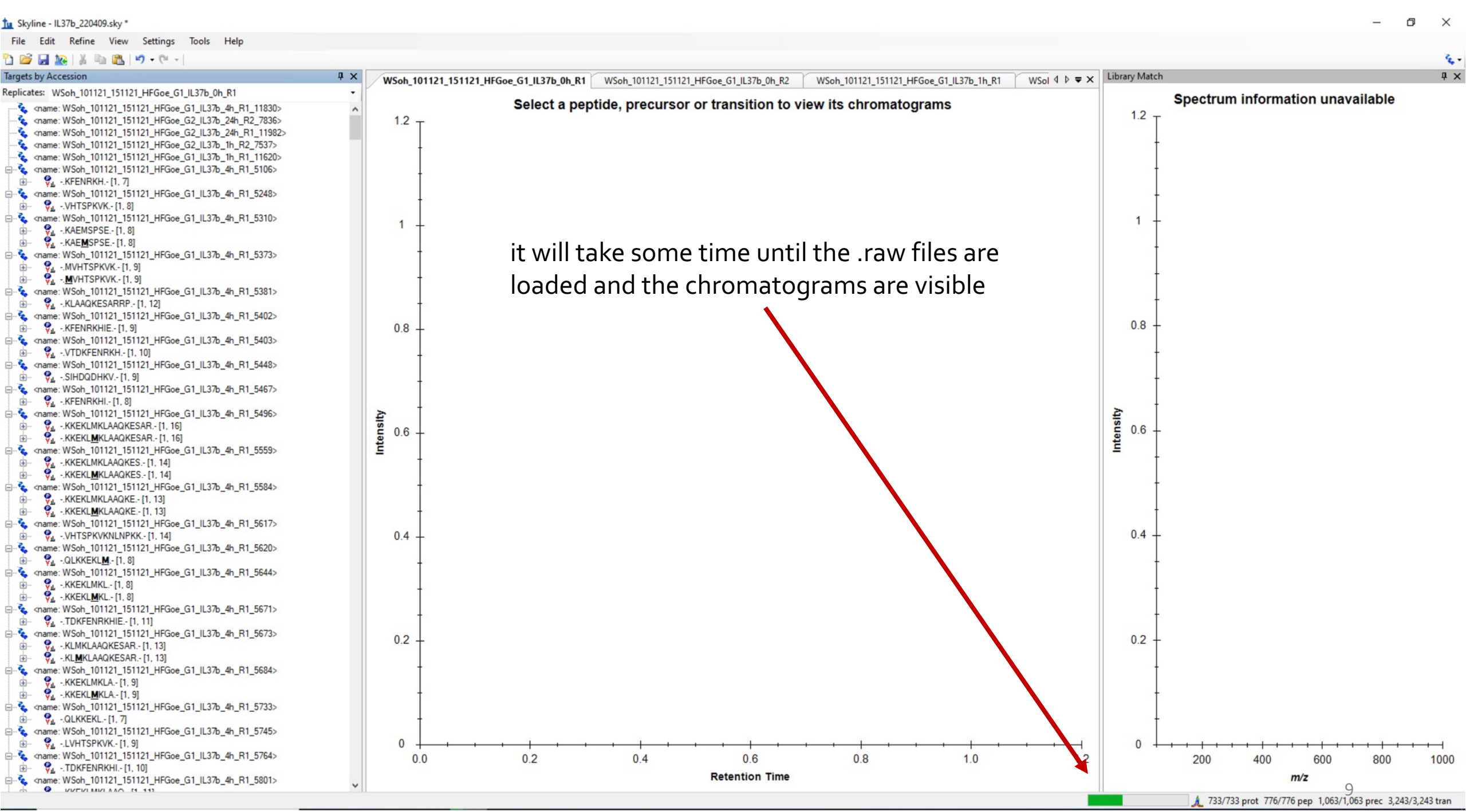


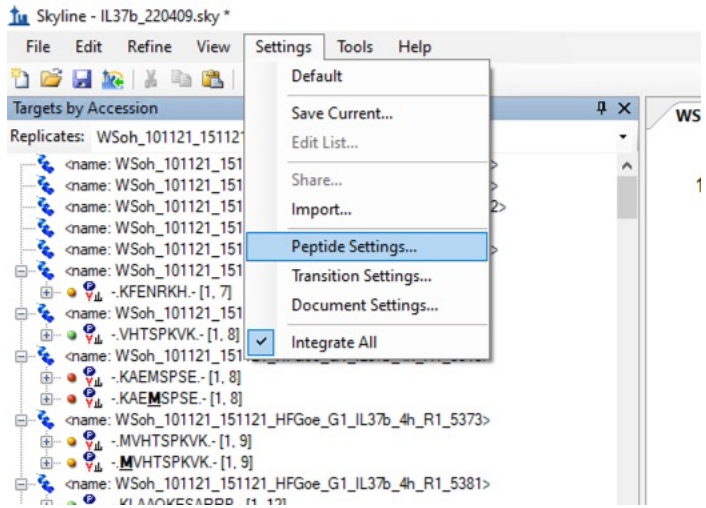

- add the enzyme “ReallyNoCleave”
- o missed cleavages
- load .fasta file with unique peptide sequences

specifications of the enzyme:
Settings > Peptide Settings

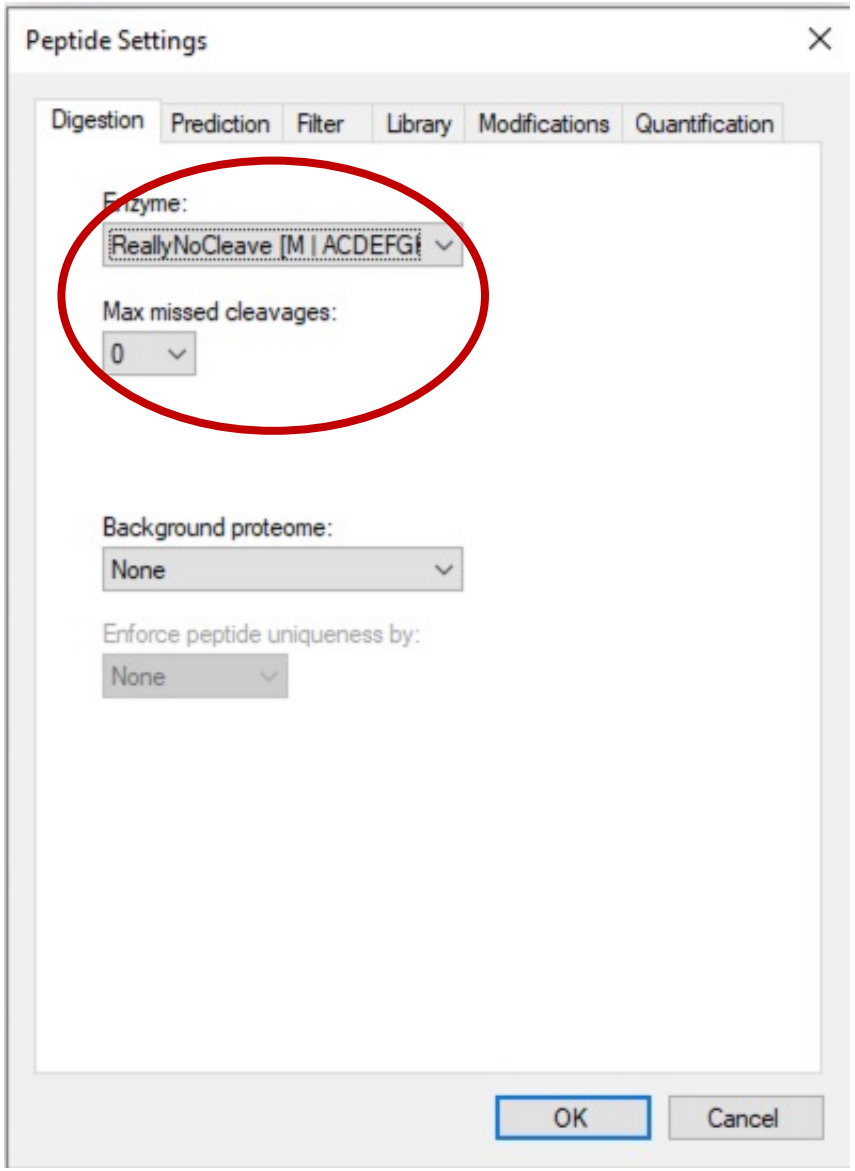


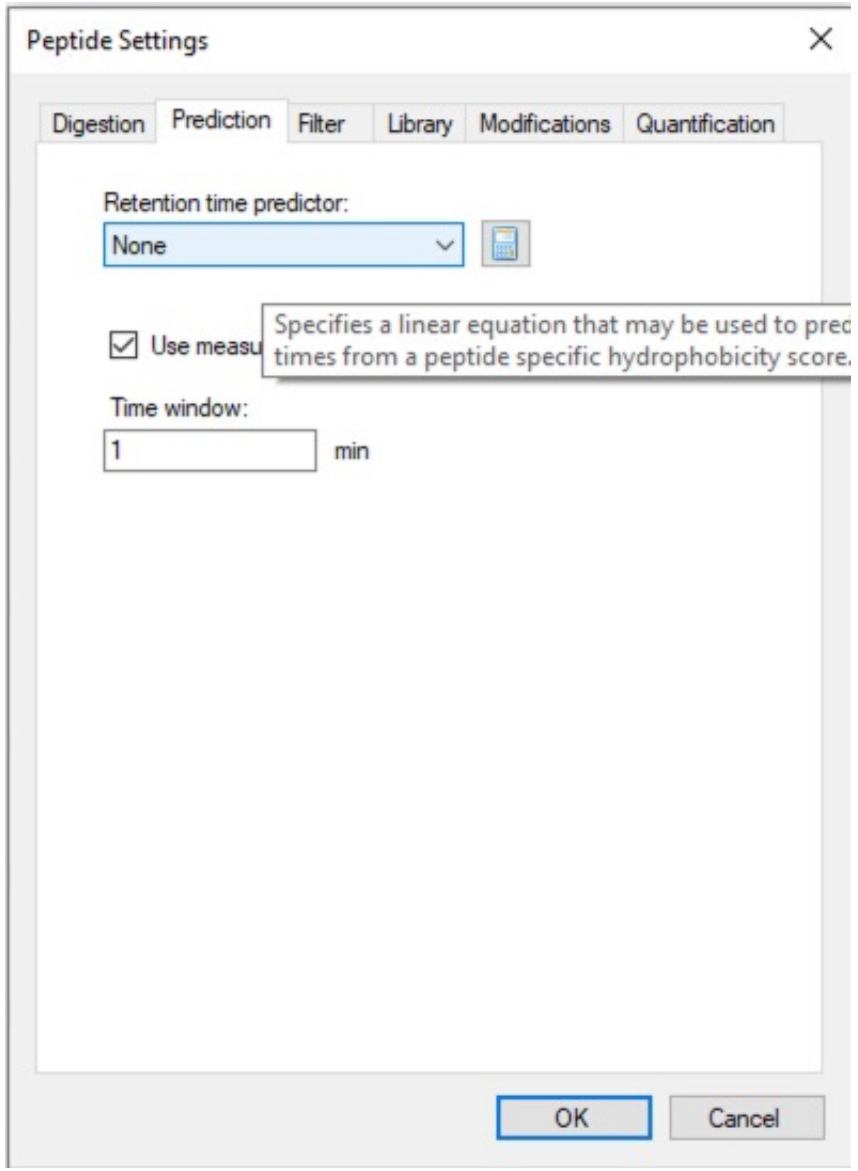

Import Peptide Search
Import FASTA (required)
Enzyme:
ReallyNoCleave [M | ACDEFGI
Max missed cleavages:
0
Import FASTA
This operation has created the following targets:
733 proteins, 776 peptides, 1063 precursors, 3243 transitions
Do you want to filter proteins by the number of peptides they contain?
Min peptides per protein
1
Keep all
Remaining:
733 proteins, 776 peptides, 1063 precursor, 3243 transitions
5 empty proteins will be added
OK
Cancel
< Back
Finish
Cancel

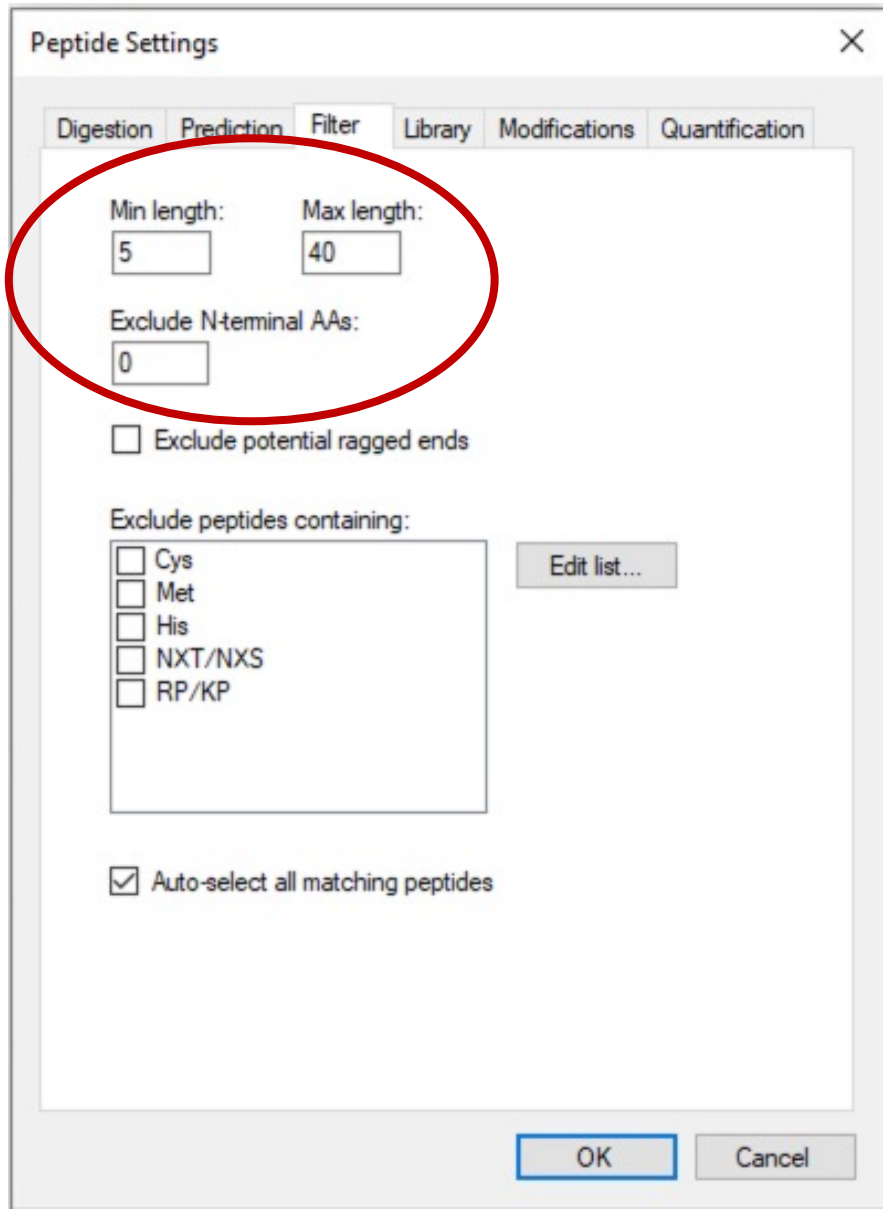

Skyline - IL37b_220409.sky *
File Edit Refine View Settings Tools Help
Targets by Accession
Replicates: WSoh_101121_151121_HFGoe_G1_IL37b_0h_R1
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_11830>
<name: WSoh_101121_151121_HFGoe_G2_IL37b_24h_R2_7836>
<name: WSoh_101121_151121_HFGoe_G2_IL37b_24h_R1_11982>
<name: WSoh_101121_151121_HFGoe_G2_IL37b_1h_R2_7537>
<name: WSoh_101121_151121_HFGoe_G1_IL37b_1h_R1_11620>
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5106>
-.KFENRKH.- [1, 7]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5248>
-.VHTSPKVK.- [1, 8]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5310>
-.KAEMSPSE.- [1, 8]
-.KAEMSPSE.- [1, 8]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5373>
-.MVHTSPKVK.- [1, 9]
-.MVHTSPKVK.- [1, 9]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5381>
-.KLAAQKESARRP.- [1, 12]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5402>
-.KFENRKHIE.- [1, 9]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5403>
-.VTDKFENRKH.- [1, 10]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5448>
-.SIHDQDHKV.- [1, 9]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5467>
-.KFENRKHI.- [1, 8]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5496>
-.KKEKLMKLAAQKESAR.- [1, 16]
-.KKEKLMKLAAQKESAR.- [1, 16]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5559>
-.KKEKLMKLAAQKES.- [1, 14]
-.KKEKLMKLAAQKES.- [1, 14]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5584>
-.KKEKLMKLAAQKE.- [1, 13]
-.KKEKLMKLAAQKE.- [1, 13]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5617>
-.VHTSPKVKNLNPKK.- [1, 14]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5620>
-.QLKKEKLM.- [1, 8]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5644>
-.KKEKLMKL.- [1, 8]
-.KKEKLMKL.- [1, 8]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5671>
-.TDKFENRKHIE.- [1, 11]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5673>
-.KLMKLAAQKESAR.- [1, 13]
-.KLMKLAAQKESAR.- [1, 13]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5684>
-.KKEKLMKLA.- [1, 9]
-.KKEKLMKLA.- [1, 9]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5733>
-.QLKKEKL.- [1, 7]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5745>
-.LVHTSPKVK.- [1, 9]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5764>
-.TDKFENRKHI.- [1, 10]
<name: WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_5801>
WSoh_101121_151121_HFGoe_G1_IL37b_0h_R1
WSoh_101121_151121_HFGoe_G1_IL37b_0h_R2
WSoh_101121_151121_HFGoe_G1_IL37b_1h_R1
WSol
Select a peptide, precursor or transition to view its chromatograms
it will take some time until the .raw files are loaded and the chromatograms are visible
Intensity
Retention Time
0
0.2
0.4
0.6
0.8
1
1.2
0.0
0.2
0.4
0.6
0.8
1.0
2
Library Match
Spectrum information unavailable
Intensity
m/z
200
400
600
800
1000
733/733 prot 776/776 pep 1,063/1,063 prec 3,243/3,243 tran

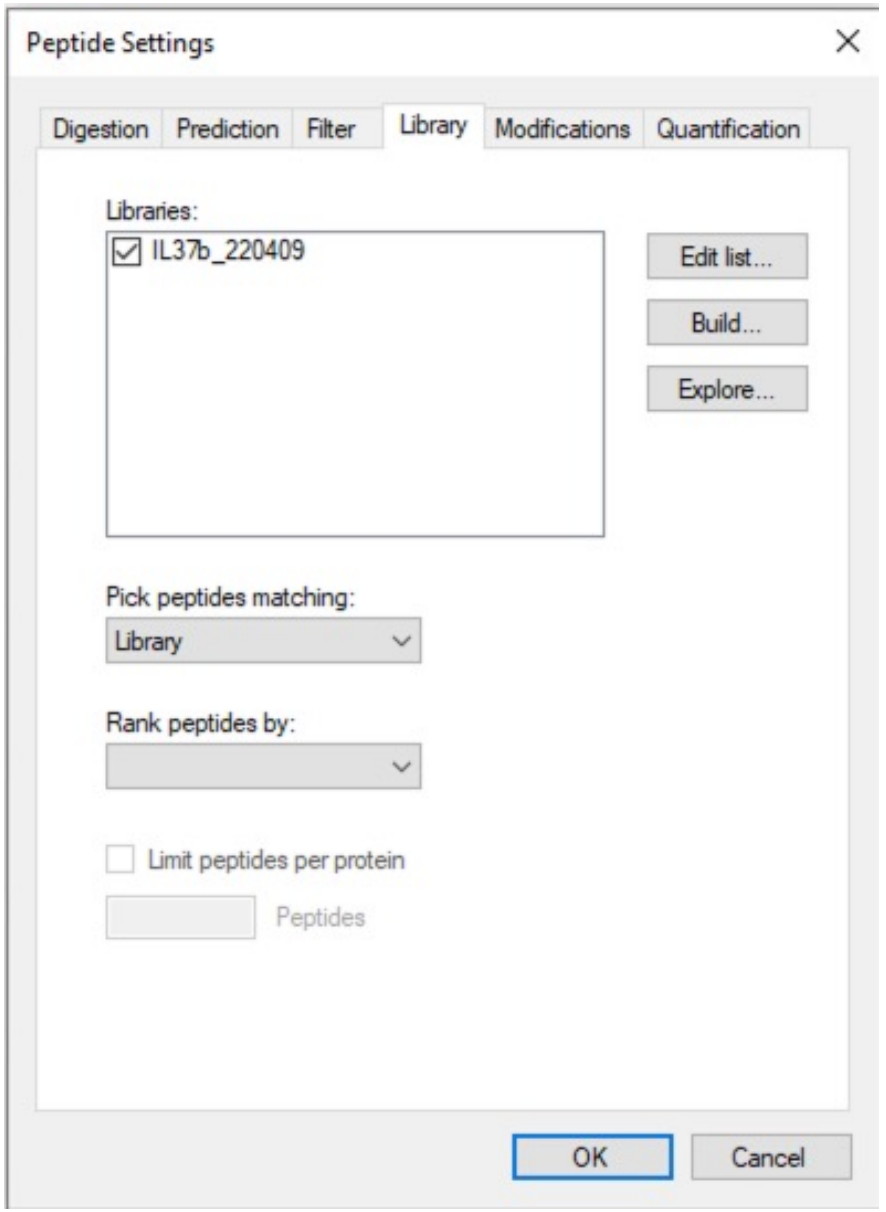


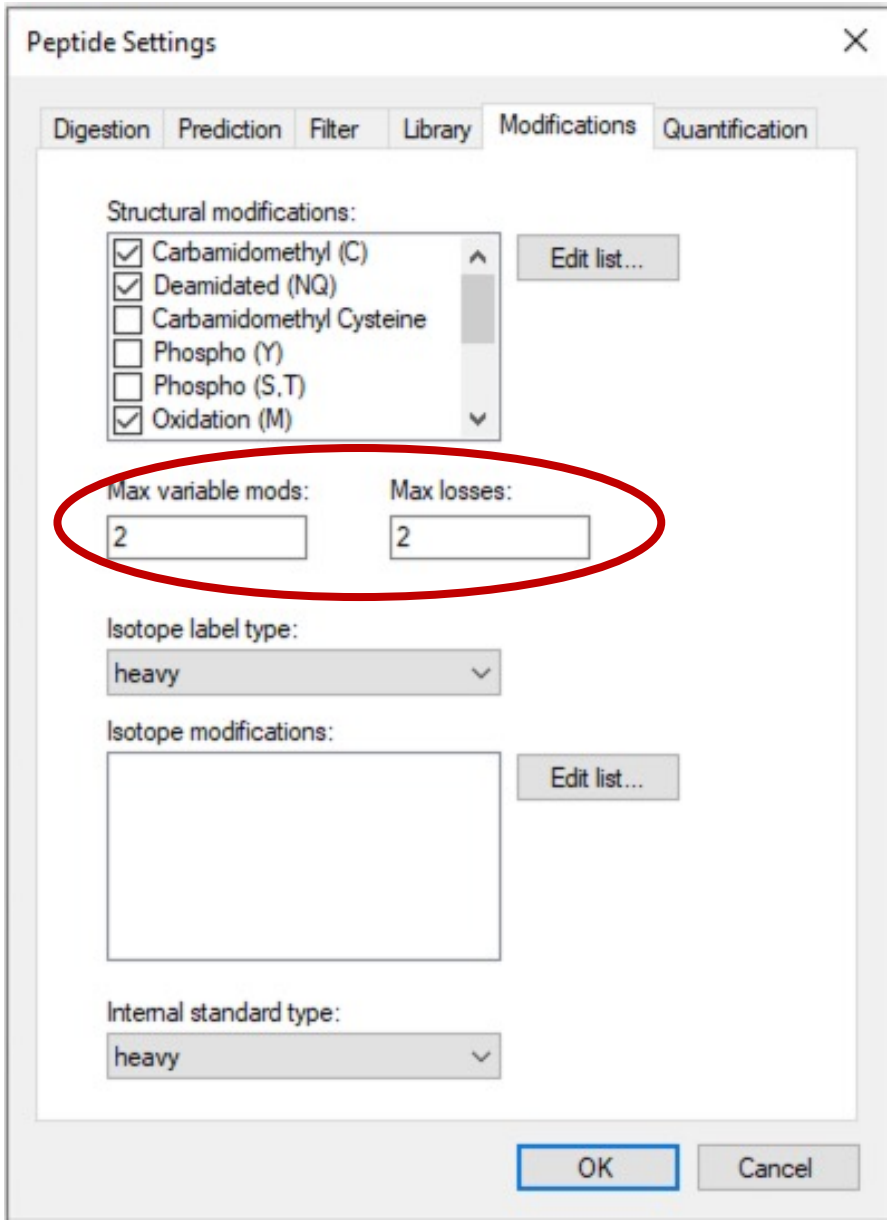

- make sure that **Settings > Integrate All** is ticked
- go to Settings > Peptide Settings and enter the following specifications

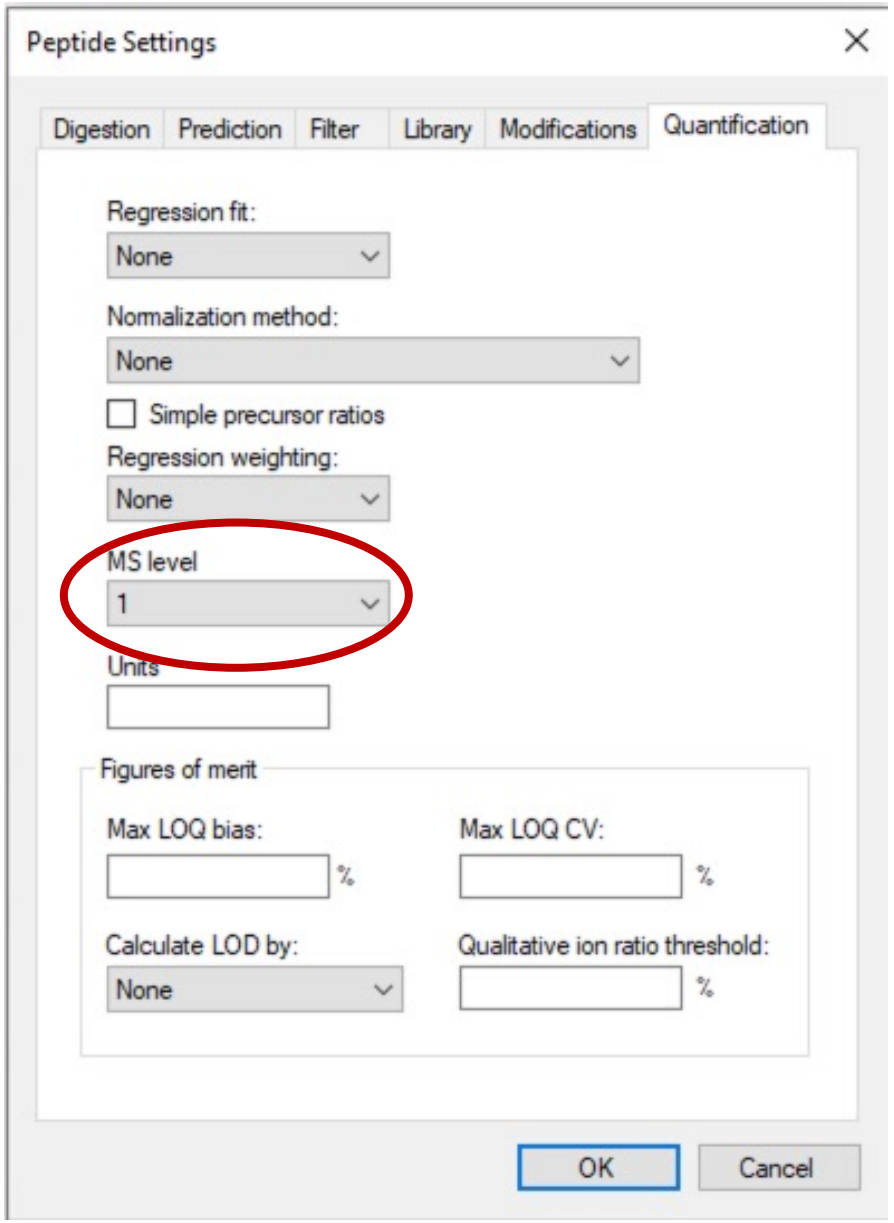


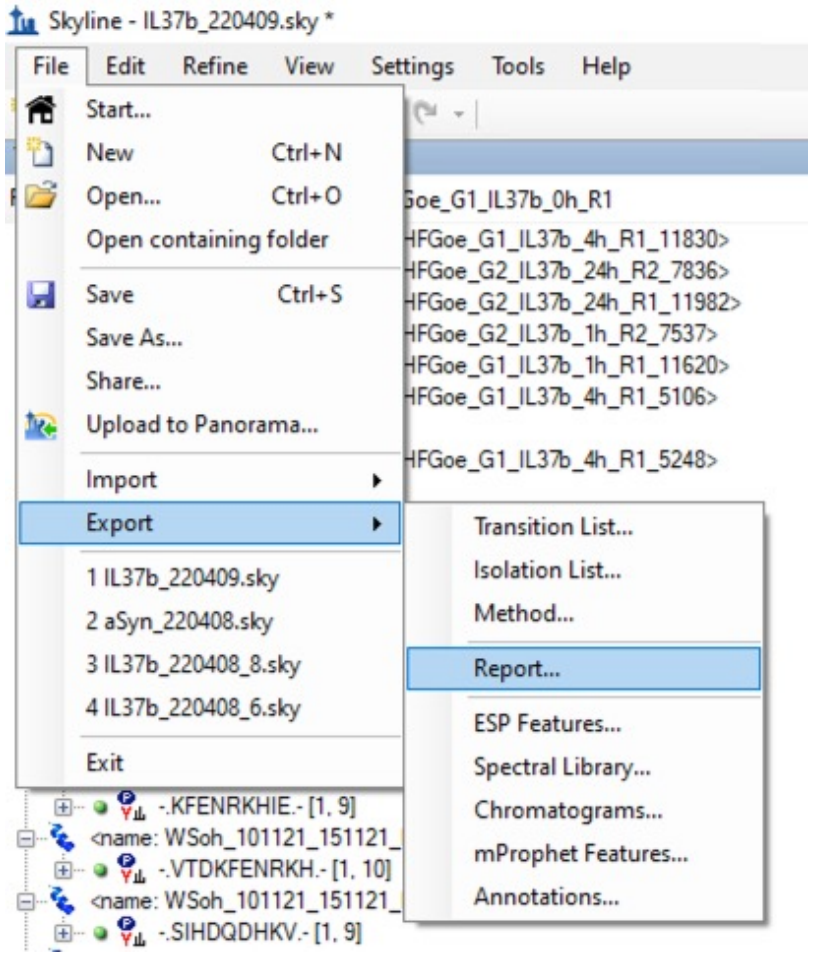

Peptide Settings
Digestion
Prediction
Filter
Library
Modifications
Quantification
Min length:
5
Max length:
40
Exclude N-terminal AAs:
0
Exclude potential ragged ends
Exclude peptides containing:
Cys
Met
His
NXT/NXS
RP/KP
Edit list...
Auto-select all matching peptides
OK
Cancel

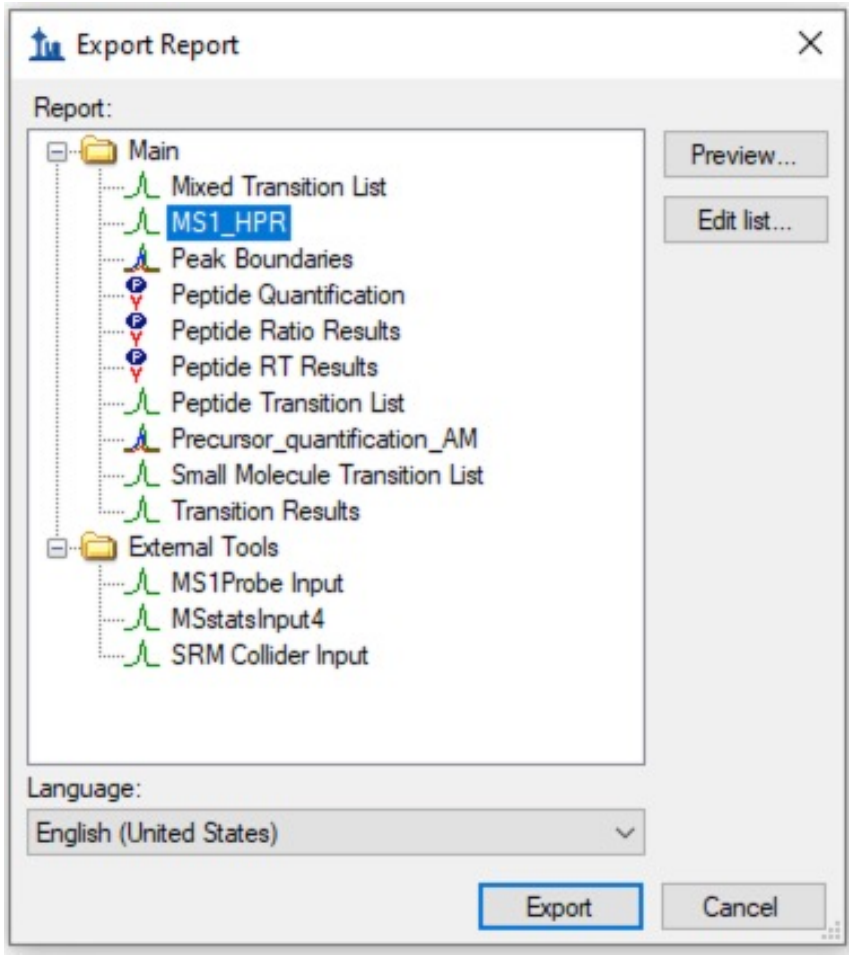

Peptide Settings
Digestion
Prediction
Filter
Library
Modifications
Quantification
Libraries:
IL37b_220409
Edit list...
Build...
Explore...
Pick peptides matching:
Library
Rank peptides by:
Limit peptides per protein
Peptides
OK
Cancel


Peptide Settings
Digestion
Prediction
Filter
Library
Modifications
Quantification
Structural modifications:
Carbamidomethyl (C)
Deamidated (NQ)
Carbamidomethyl Cysteine
Phospho (Y)
Phospho (S,T)
Oxidation (M)
Edit list...
Max variable mods:
2
Max losses:
2
Isotope label type:
heavy
Isotope modifications:
Edit list...
Internal standard type:
heavy
OK
Cancel


Peptide Settings
Digestion
Prediction
Filter
Library
Modifications
Quantification
Regression fit:
None
Normalization method:
None
Simple precursor ratios
Regression weighting:
None
MS level
1
Units
Figures of merit
Max LOQ bias:
%
Max LOQ CV:
%
Calculate LOD by:
None
Qualitative ion ratio threshold:
%
OK
Cancel

- you can view the chromatograms and MS2 spectra in the different .raw files in the GUI
- in order to export MS1 intensities, go to **File > Export > Report…**
- choose **MS1_HPR**



you can generate custom reports in Skyline. ***MS1_HPR*** contains the following info: