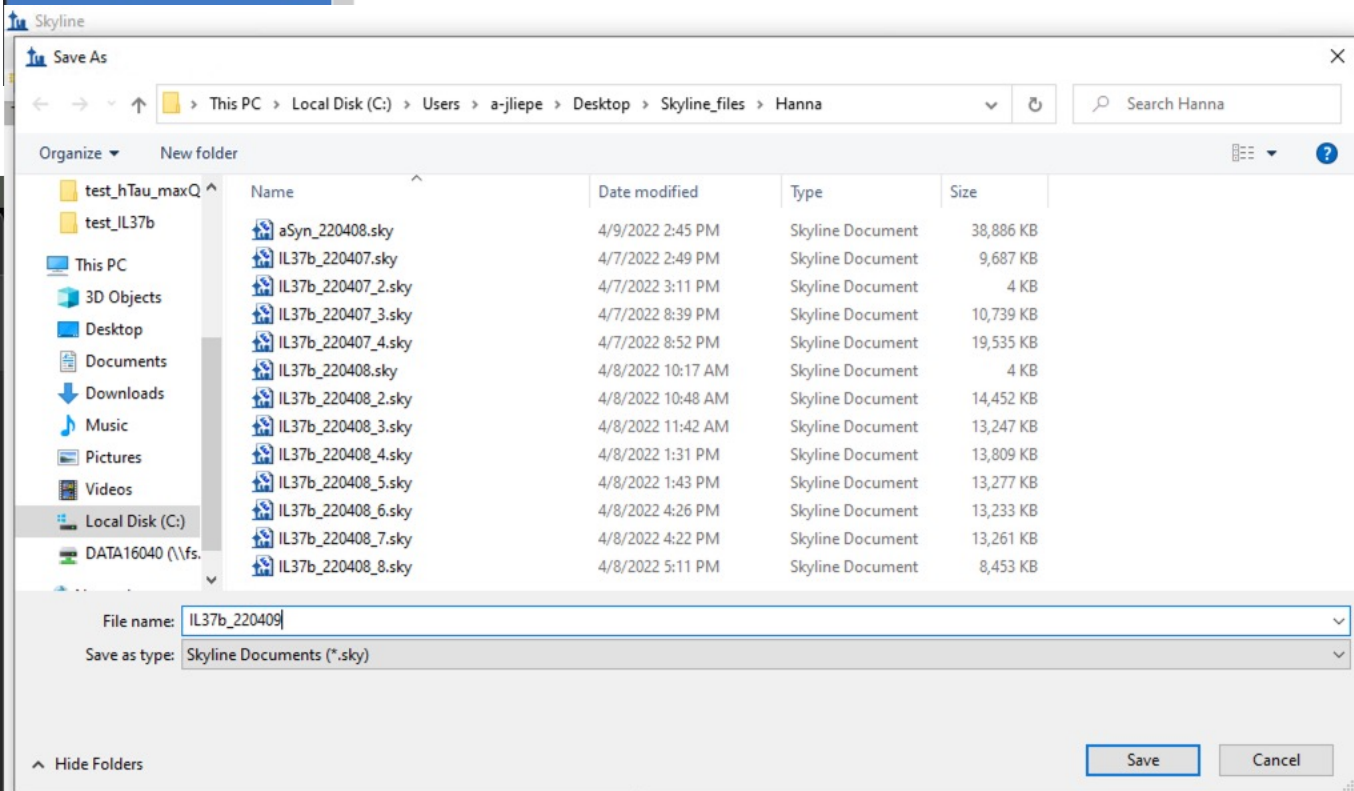
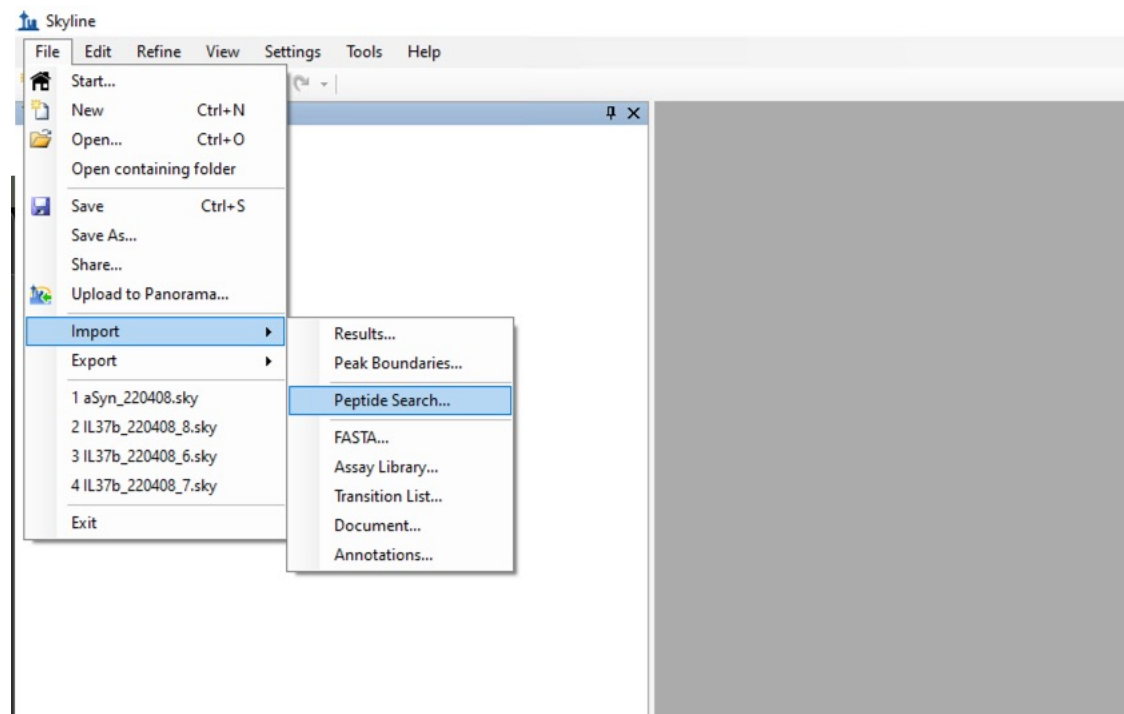
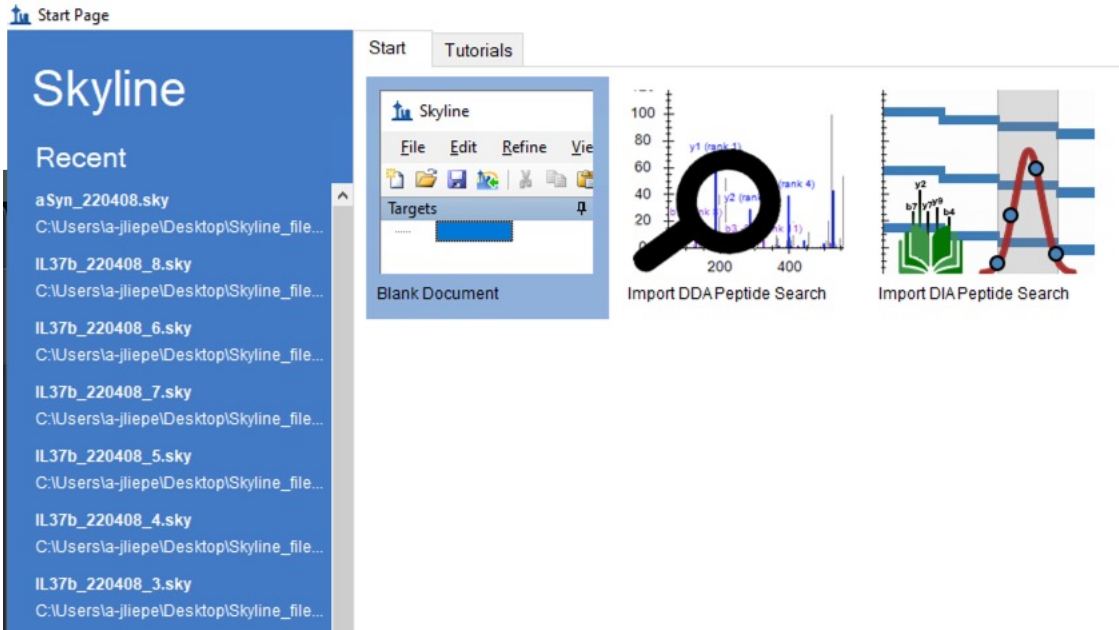


Skyline tutorial

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**

Import Peptide Search

Spectral Library

☒ Build ☐ Use existing

Cut-off score:
0.5

Start from:
Search results (build library directly)

Result files:
IL37b.ssl

Add Files...
Remove Files

iRT standard peptides:
None

☒ Include ambiguous matches

Workflow
☒ DDA with MS1 filtering
☐ DIA
☐ PRM

Finish Next > Cancel

- 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

Spectral Library

☒ Build ☐ Use existing

Cut-off score:
0.5

Start from:

Building Peptide Search Library

Loading WSoh_101121_151121_HFGoe_G1_IL37b_1h_R1.raw.

Cancel

None

☒ Include ambiguous matches

Workflow
☒ DDA with MS1 filtering
☐ DIA
☐ PRM

Finish Next > Cancel

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

Files to import simultaneously:
Many

☒ Retry after import failure

Next > Cancel

Import Peptide Search

Add Modifications

The imported search results appear to contain the modifications listed below. Please select the ones you wish to add to the document:

Add modification...

< Back 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

Import Peptide Search

Configure Full-Scan Settings

Precursor charges:
1, 2, 3, 4, 5, 6

MS1 filtering

Isotope peaks included: Count
Precursor mass analyzer: Orbitrap

Peaks: 3
Resolving power: 70,000
At: 400 m/z

☒ Use high-selectivity extraction

Retention time filtering

☒ Use only scans within 0.5 minutes of MS/MS IDs
☐ Include all matching scans

< Back Next > Cancel

- 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 chromatograms.

Import Peptide Search

Import FASTA (required)

Enzyme: ReallyNoCleave [M | ACDEFGI] Max missed cleavages: 0

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

>WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_51
KFENRKH
>WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_52
VHTSPKVK
>WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_53
KAEMSPSE
>WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_53
MVHTSPKVK
>WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_53
KLAAQKESARRP
>WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_54
KFENRKHIE
>WSoh_101121_151121_HFGoe_G1_IL37b_4h_R1_54
VTDKFENRKH

Browse... Clear

< Back Finish Cancel

- add the enzyme "ReallyNoCleave"
- 0 missed cleavages
- load .fasta file with unique peptide sequences

specifications of the enzyme:
Settings > Peptide Settings

Peptide Settings

Digestion Prediction Filter Library Modifications Quantification

Enzyme: <Edit current...>

Max missed cleavages: 0

Base modification: No

Enzyme modification: No

Edit Enzyme

Name: ReallyNoCleave OK Cancel

Type: C-terminal

Cleave C-terminal to: M

Unless followed by: ACDEFGHIKLMNPQRSTVWY

☐ Allow semi-cleavage

OK / Cancel

Import Peptide Search

Import FASTA (required)

Enzyme: ReallyNoCleave [M | ACDEFGI] Max missed cleavages: 0

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

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?

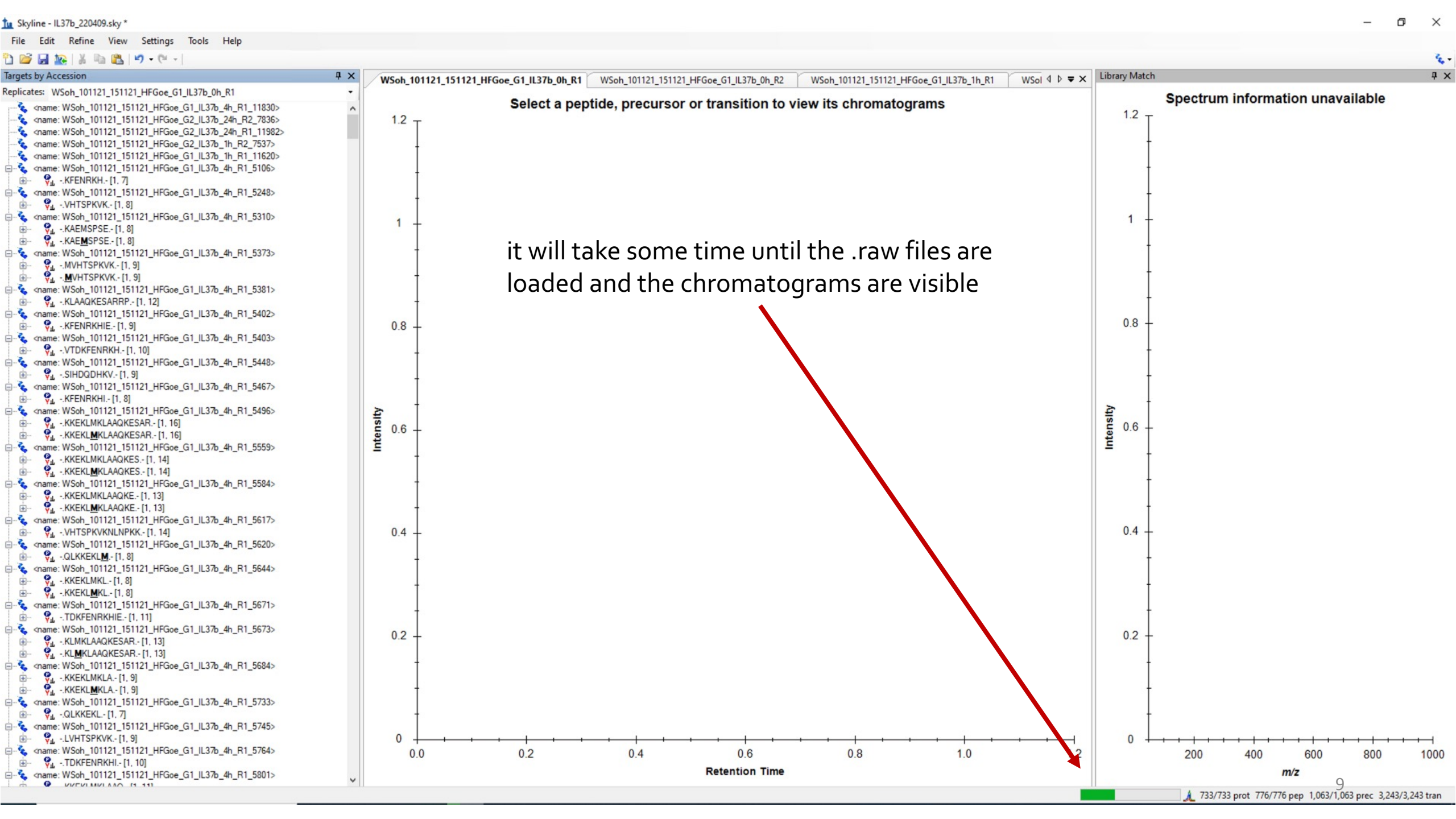
☐ Min peptides per protein
1

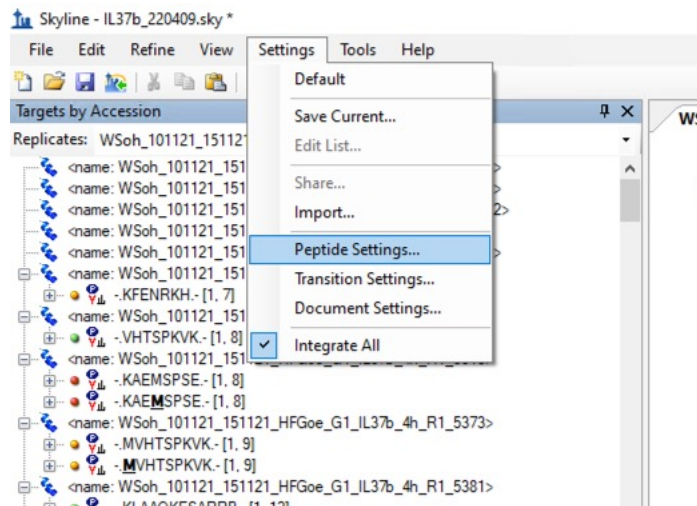
☒ Keep all

Remaining:
733 proteins, 776 peptides, 1063 precursor, 3243 transitions
5 empty proteins will be added

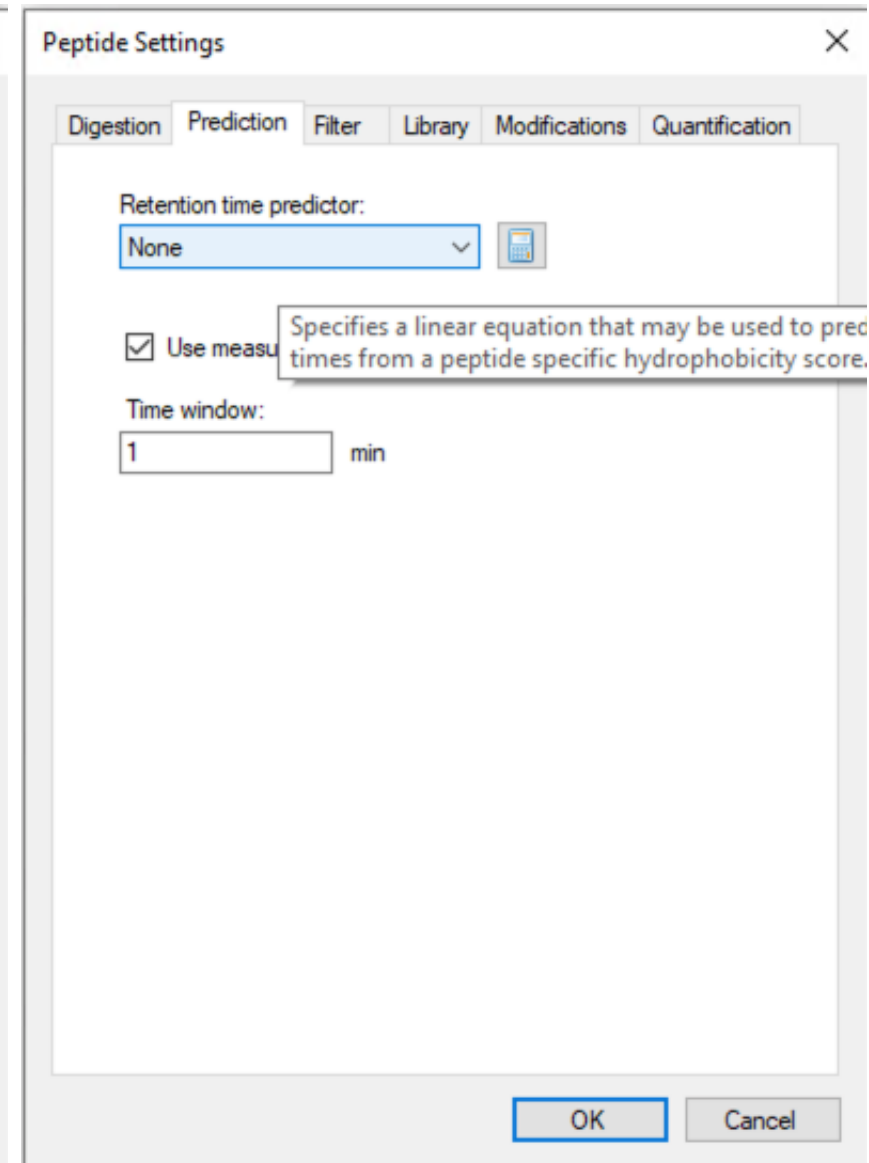
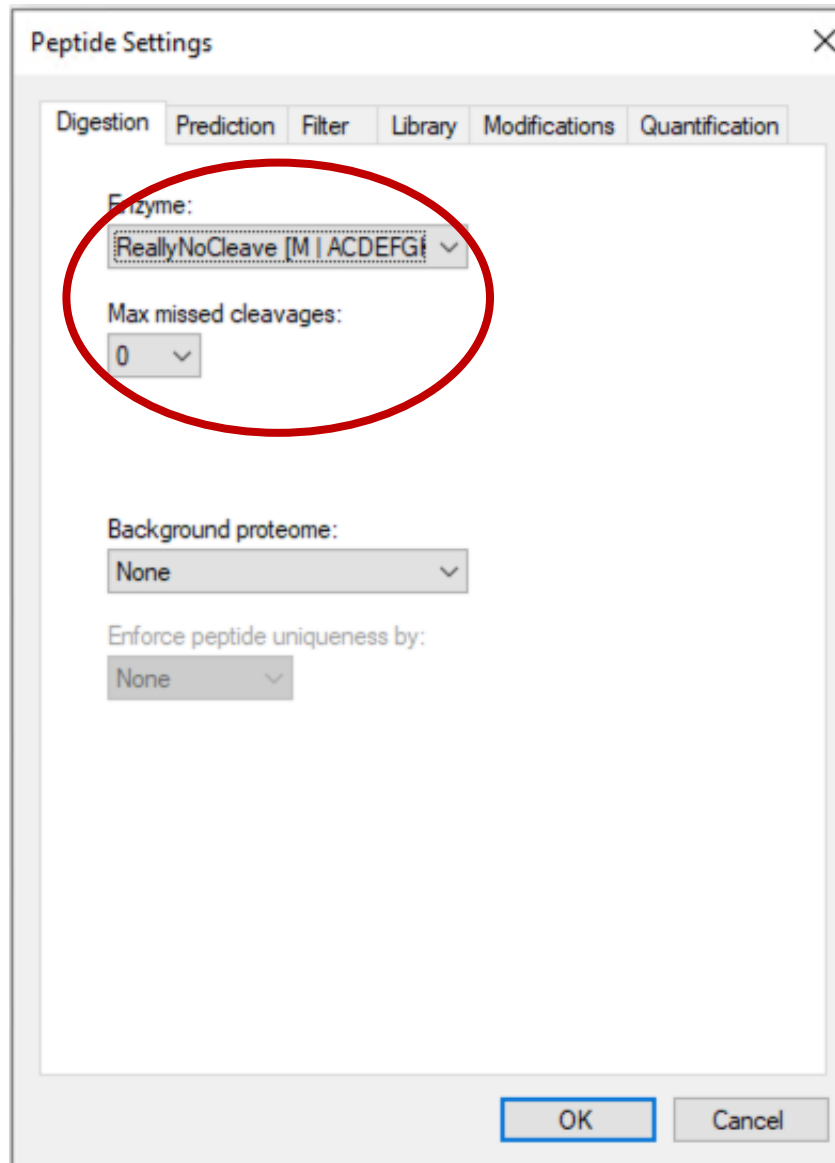
OK Cancel

< Back Finish Cancel





- 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

☒ 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

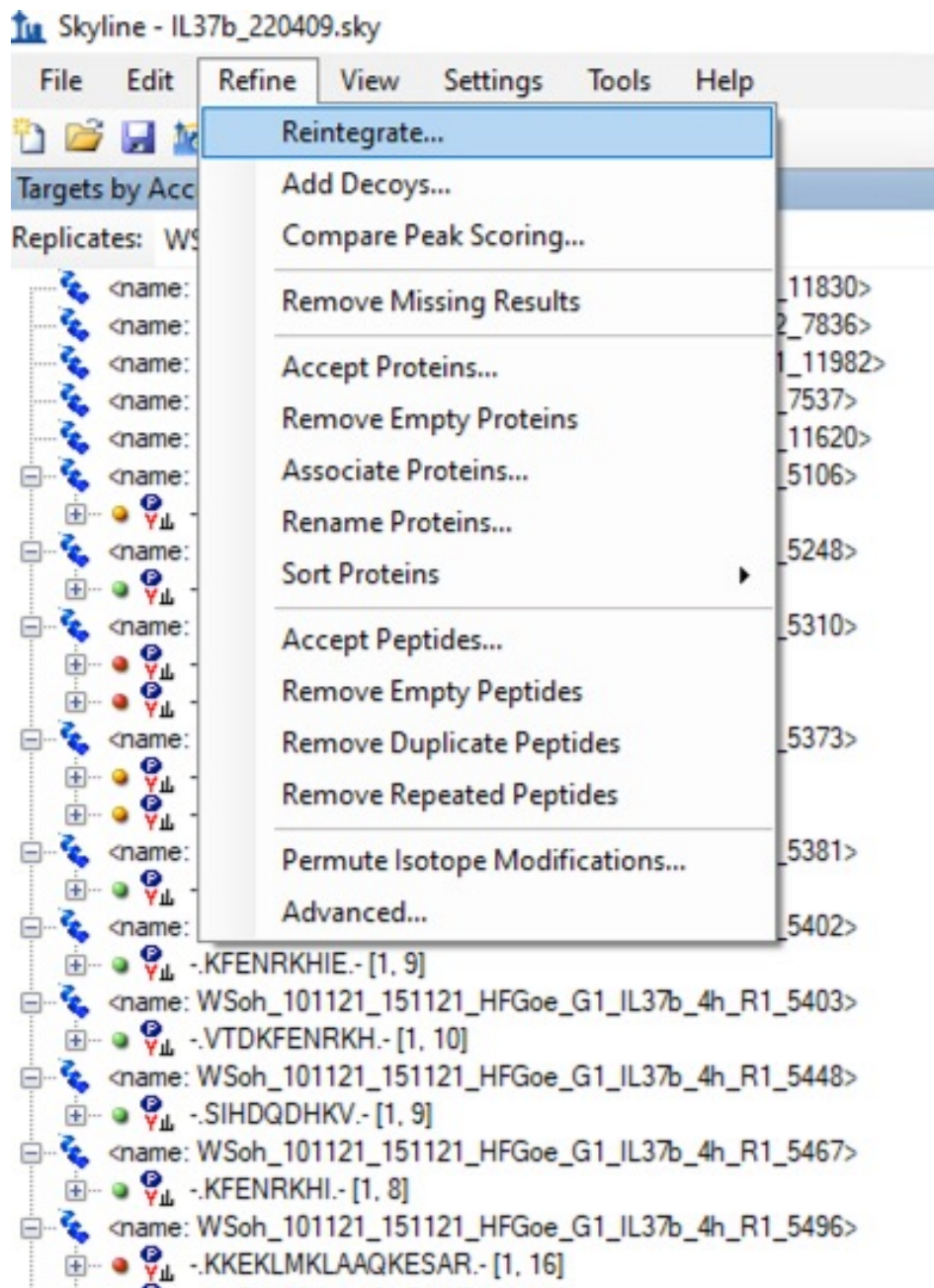
Max LOQ bias: %

Max LOQ CV: %

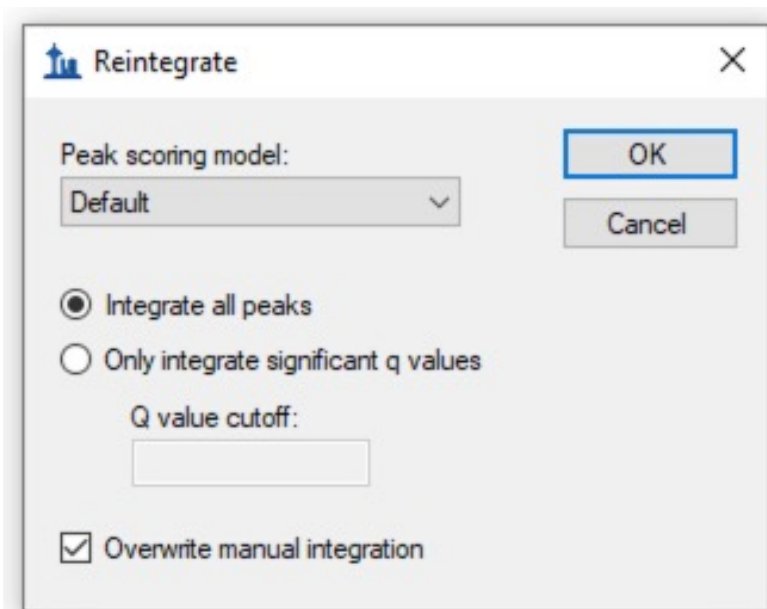
Calculate LOD by: None

Qualitative ion ratio threshold: %

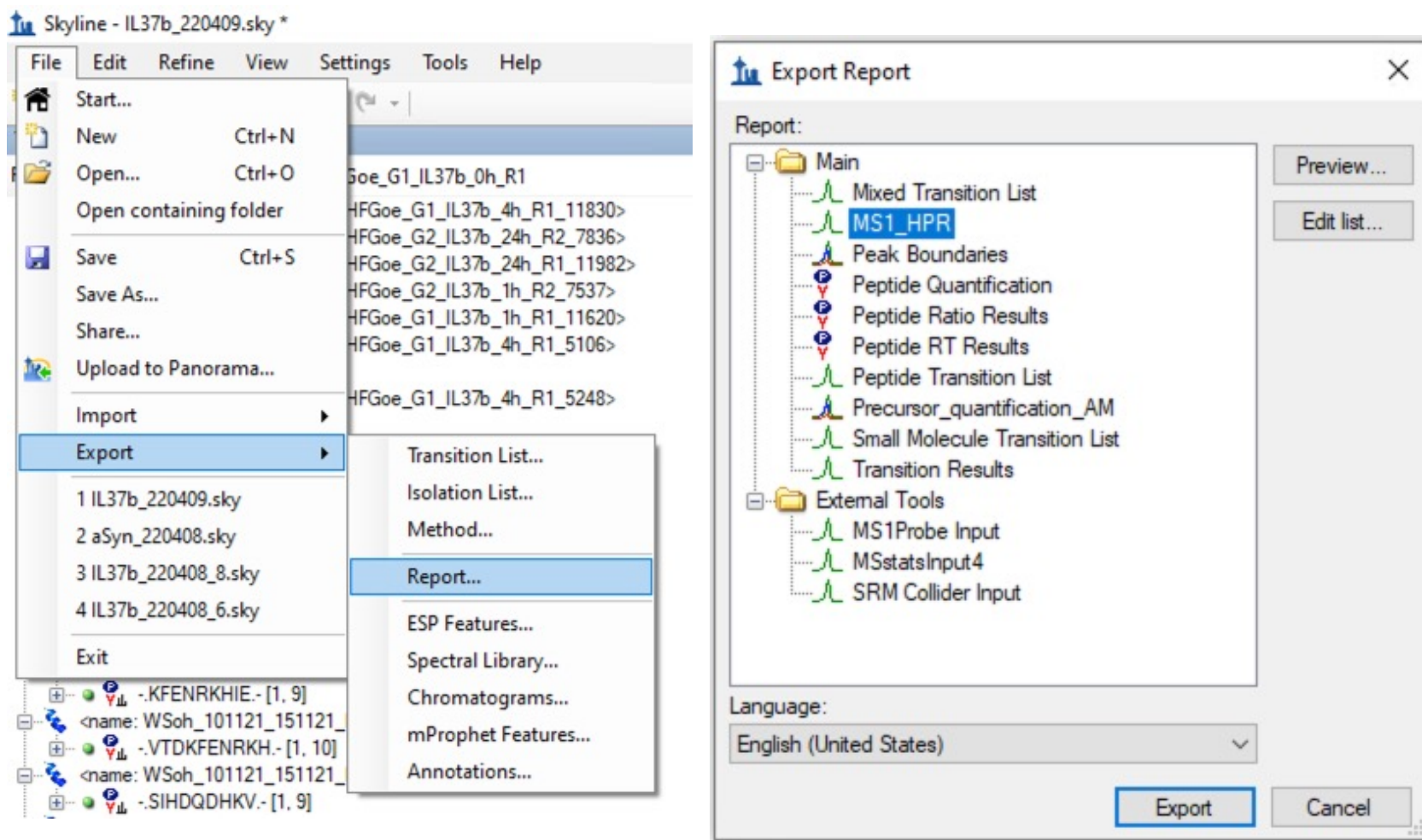
OK Cancel



after changing the peptide settings, go to **Refine > Reintegrate...**



- 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:

