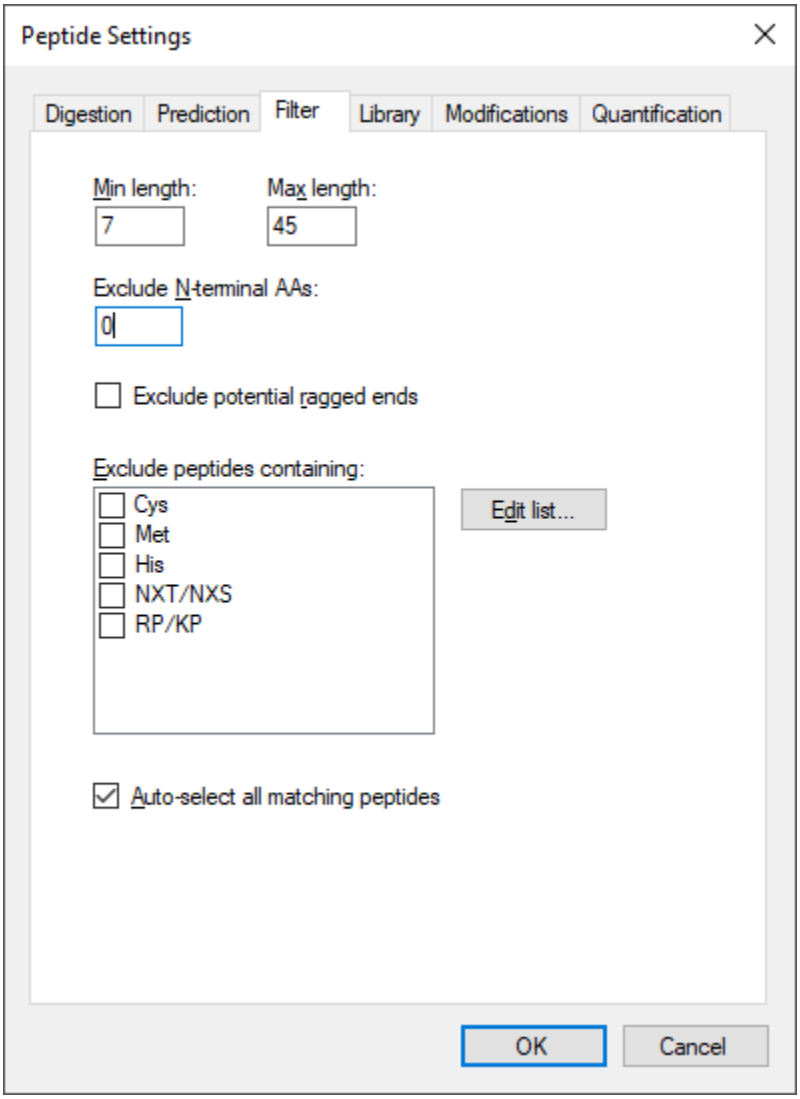
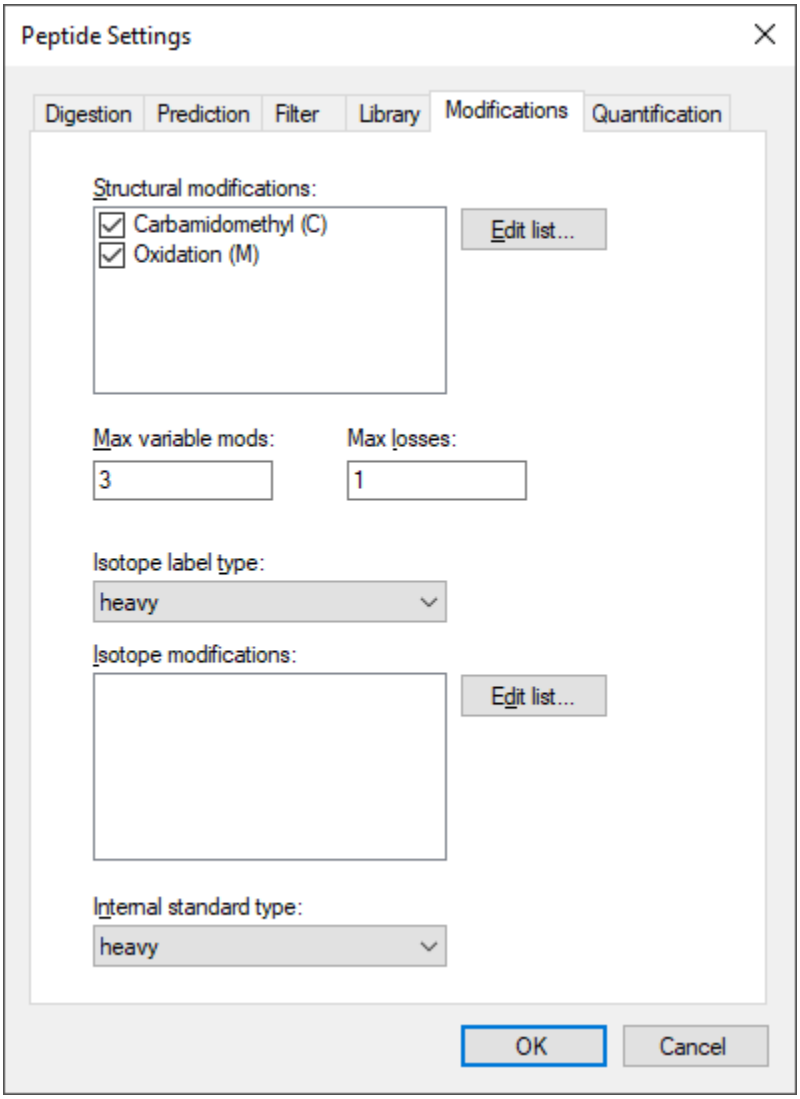
Selevsek Data Settings

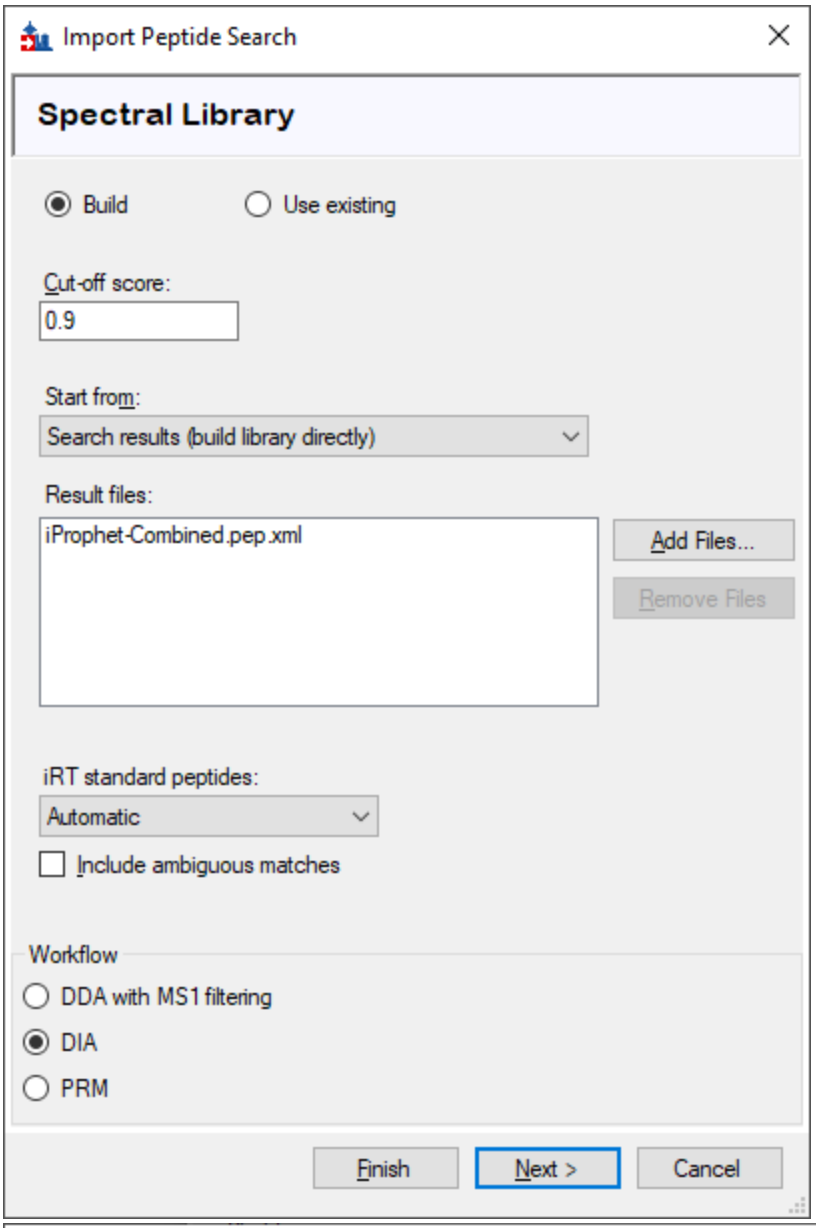
- Open reports\MSstats_annotations.sky – **if not already**
- New document – Settings > Default – No (New document again to clear Audit Log)
- Save as Selevsek.sky
- Peptide Filter – Min 7 to Max 45, Exclude 0

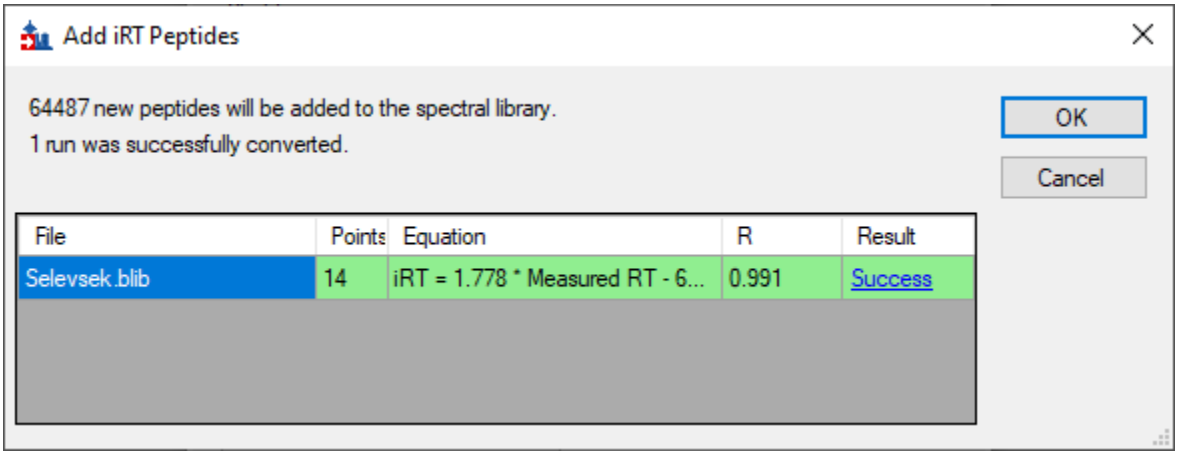


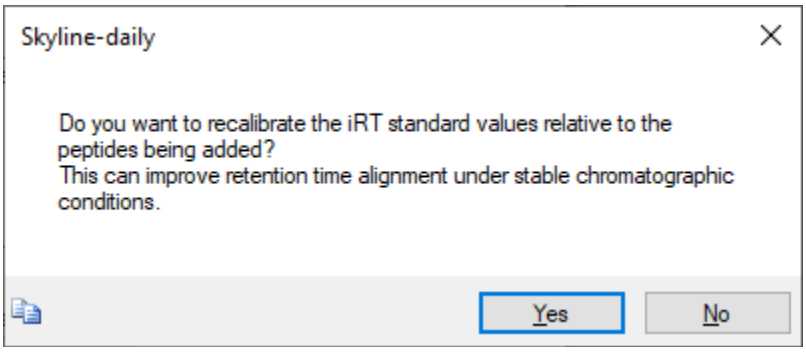
- Peptide Modifications – Carb (C), Oxi (M)



- File > Import > Peptide Search – DIA
- READ ONLY: Build a spectral library – cutoff = 0.9, iProphet-Combined.pep.xml, iRT = Auto (26 min redundant, 1.25 min to iRT)

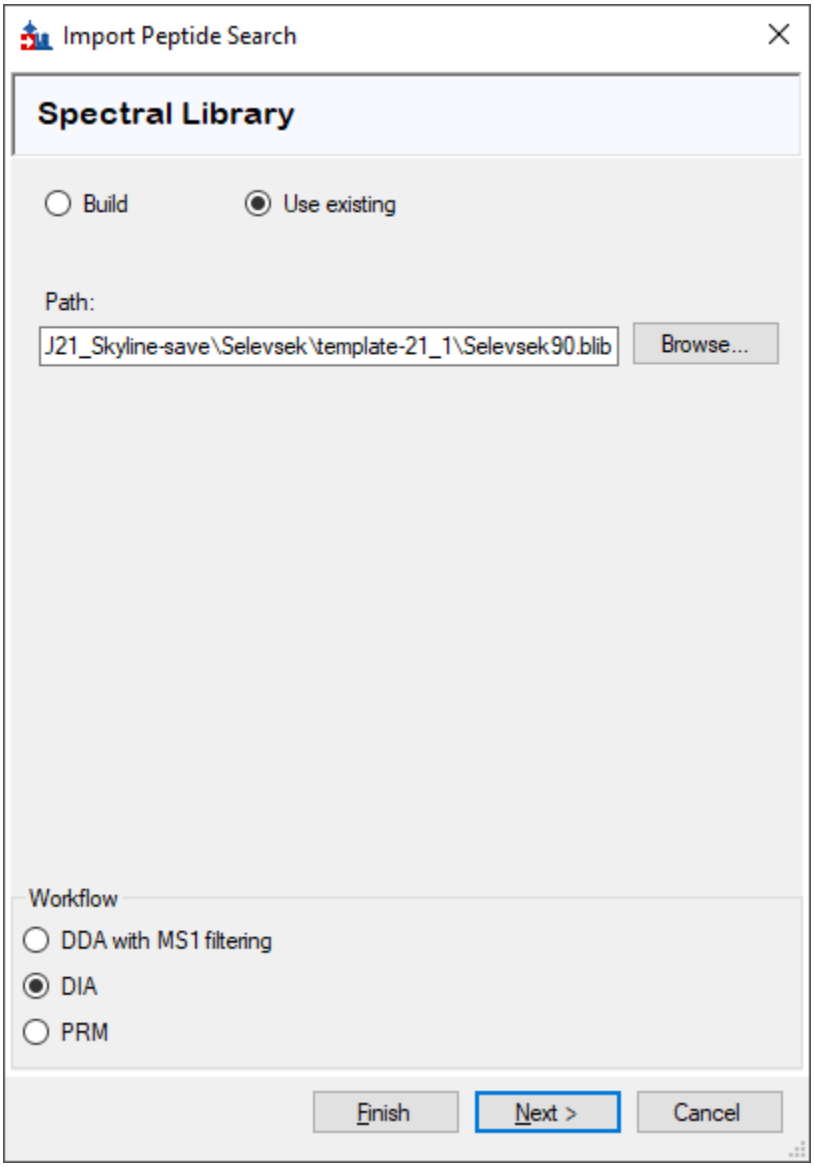

Import Peptide Search
Spectral Library
Build
Use existing
Cut-off score:
0.9
Start from:
Search results (build library directly)
Result files:
iProphet-Combined.pep.xml
Add Files...
Remove Files
iRT standard peptides:
Automatic
Include ambiguous matches
Workflow
DDA with MS1 filtering
DIA
PRM
Finish
Next >
Cancel


Add iRT Peptides
64487 new peptides will be added to the spectral library.
1 run was successfully converted.
OK
Cancel
File
Points
Equation
R
Result
Selevsek.blib
14
iRT = 1.778 * Measured RT - 6...
0.991
Success

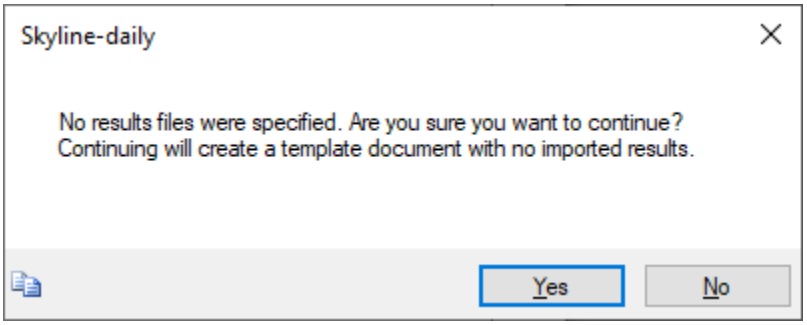


Yes! … OK to ambiguous matches

- **Use prebuilt Selevsek90.blib instead!!**

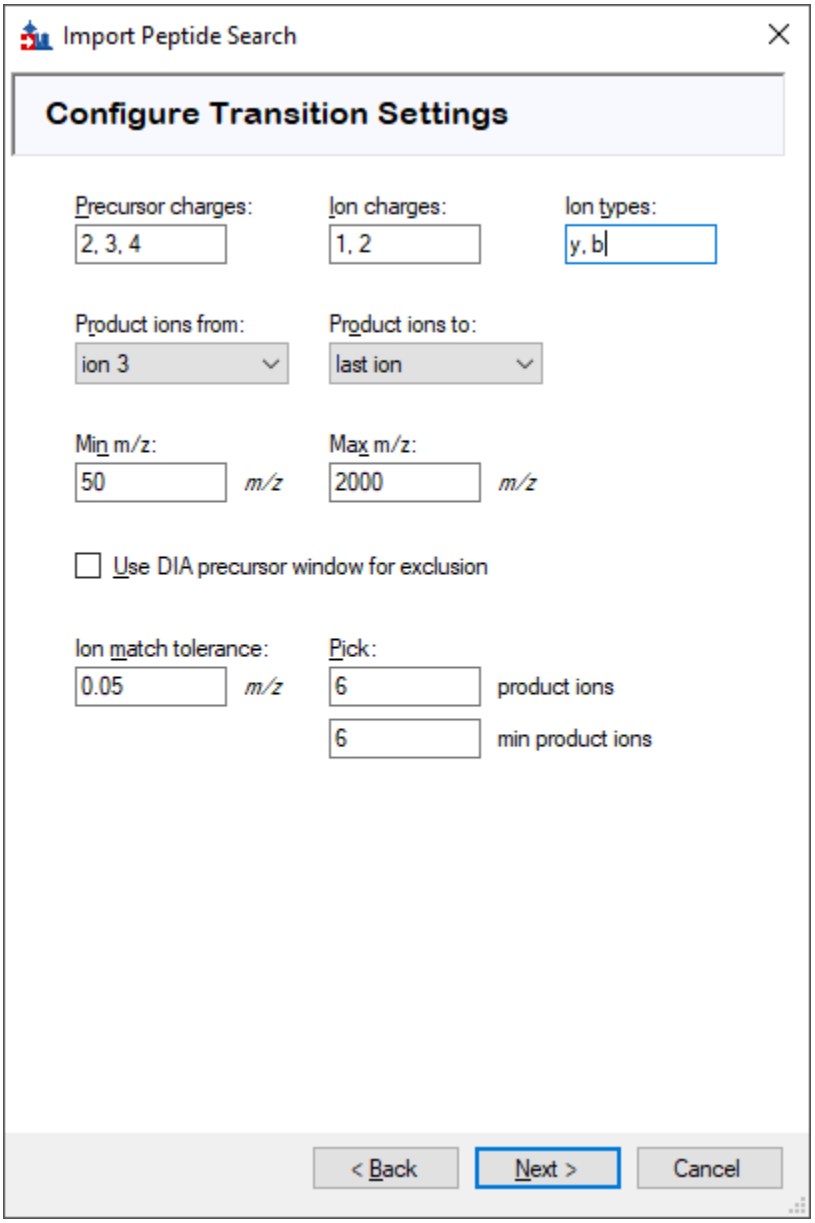


- Skip results files (to build template)



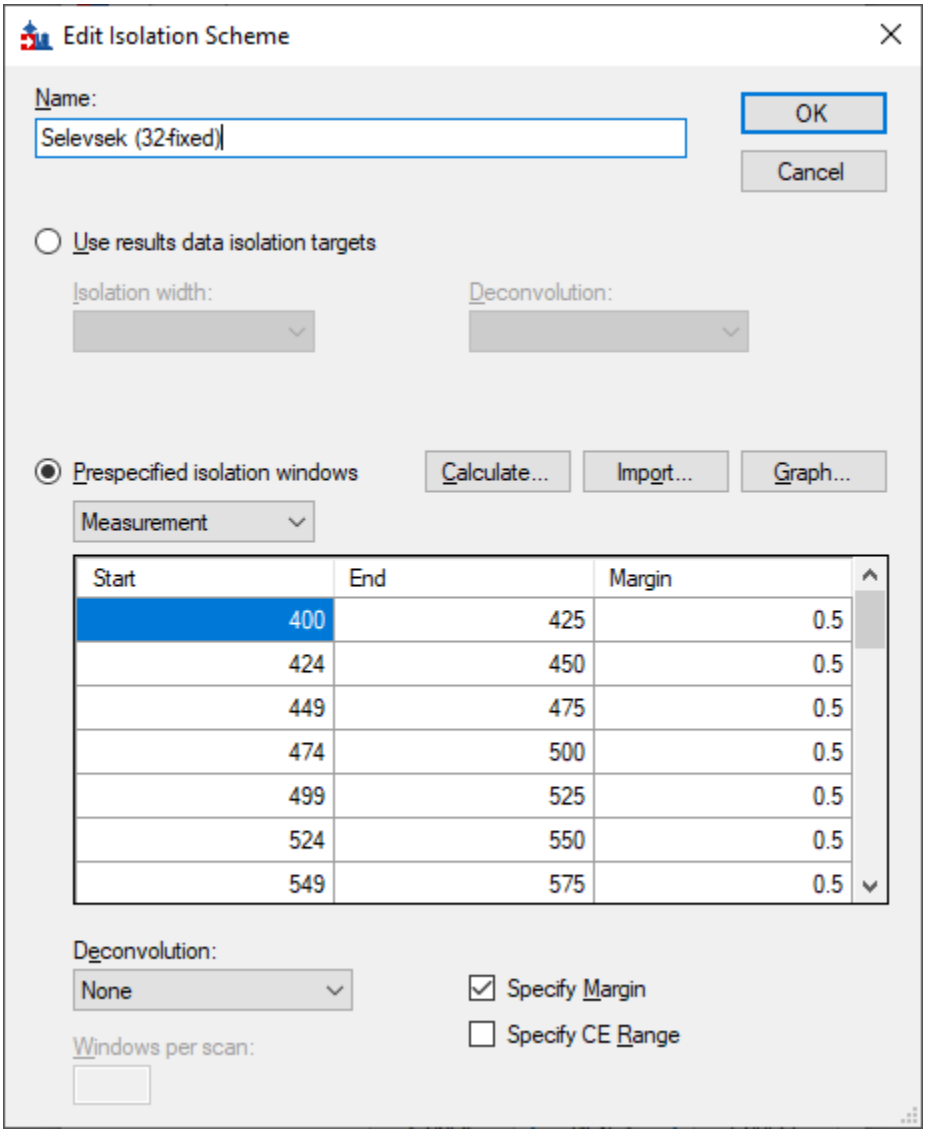
  Yes!
- Transition Settings – Pre: 2, 3, 4 and Ion: 1, 2 for Types: y, b – ion 3 to last ion
- Transition Instrument – 50 to 2000 m/z

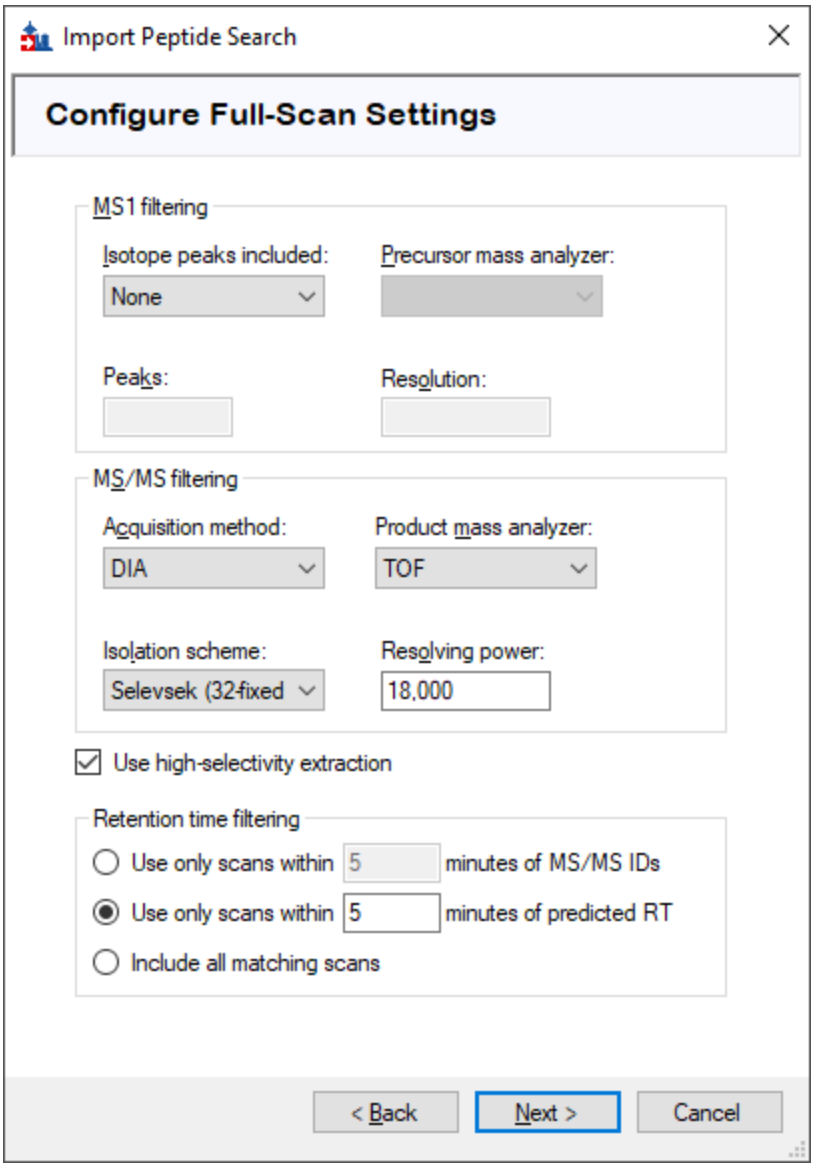
- Transition Library – Match: 0.05, Pick: 6, Min: 6, Filtered



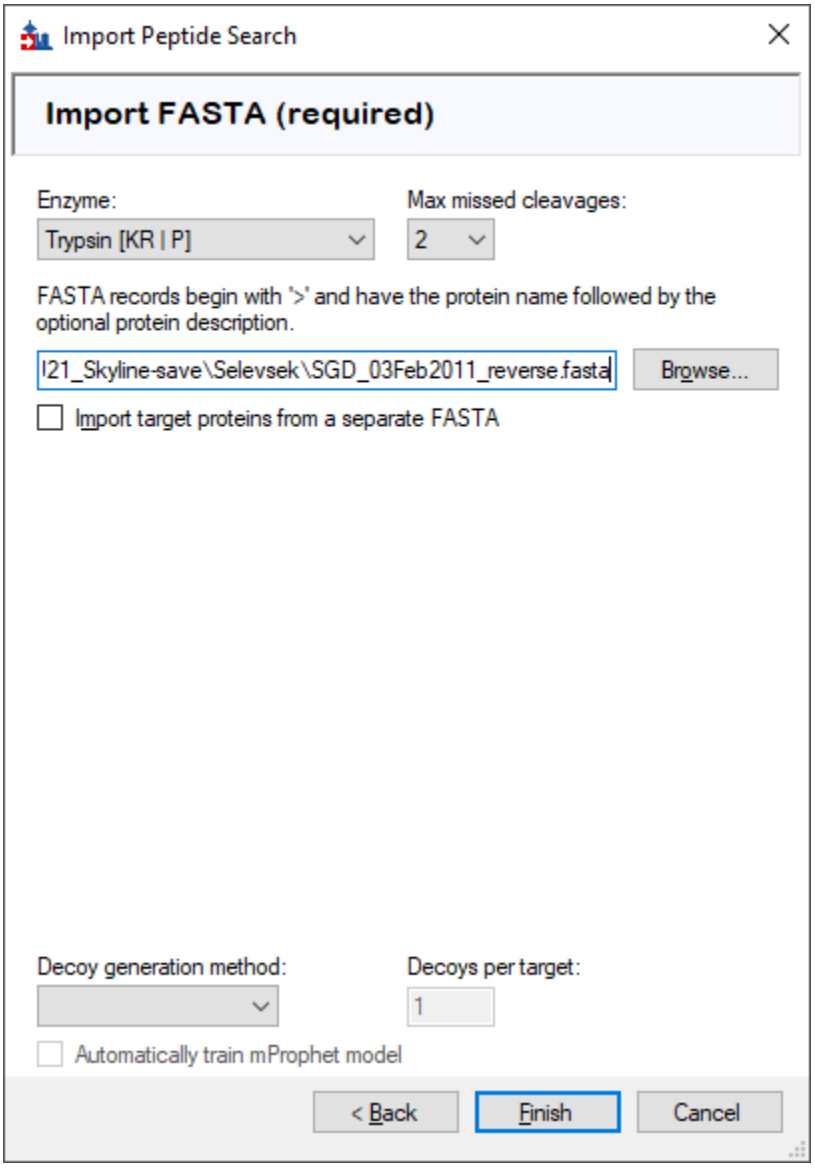
- Transition Full-Scan – DIA TOF, RP: 18,000, high-selectivity, Import Scheme, RT: 5

- Add “Selevsek (32-fixed)” isolation scheme – import from scans/mzML file

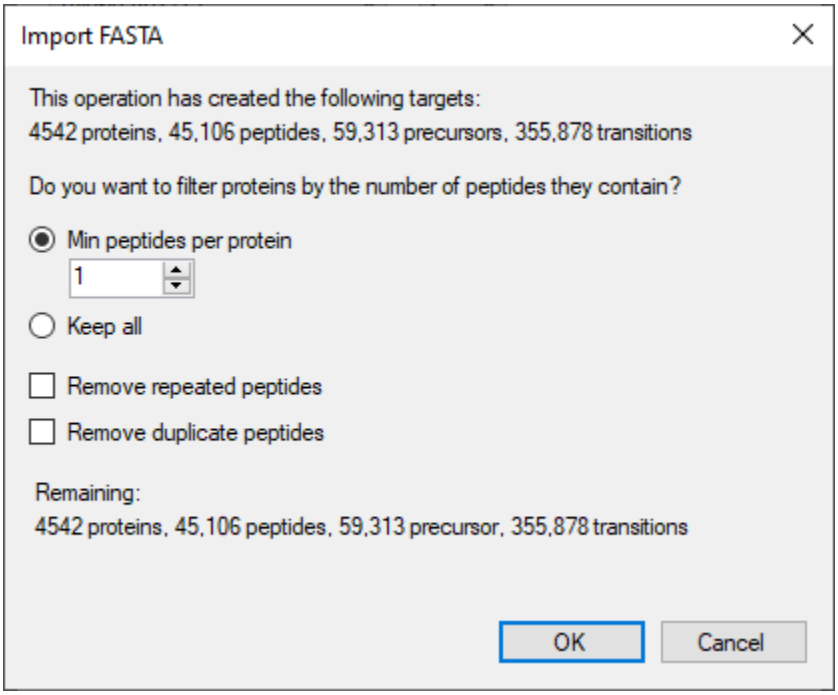



Import Peptide Search
Configure Full-Scan Settings
MS1 filtering
Isotope peaks included:
None
Precursor mass analyzer:
Peaks:
Resolution:
MS/MS filtering
Acquisition method:
DIA
Product mass analyzer:
TOF
Isolation scheme:
Selevsek (32-fixed
Resolving power:
18,000
Use high-selectivity extraction
Retention time filtering
Use only scans within 5 minutes of MS/MS IDs
Use only scans within 5 minutes of predicted RT
Include all matching scans
< Back
Next >
Cancel

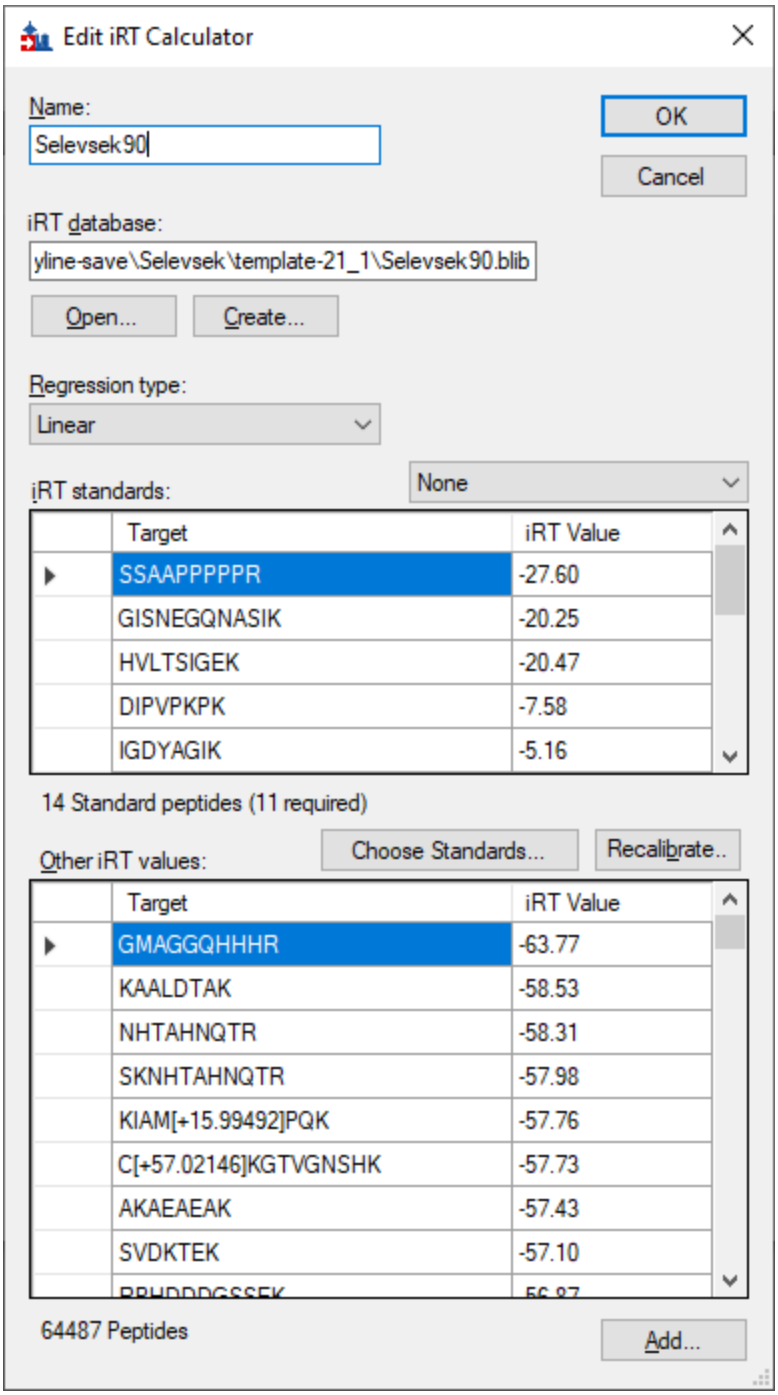
- Import FASTA - Trypsin – 2 missed



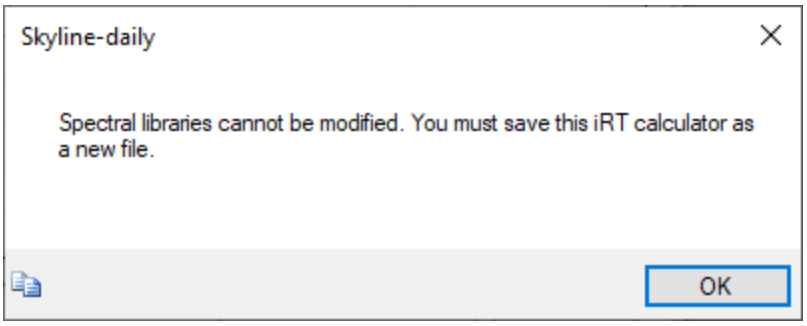
- Accept all peptides (to get around a bug) – click OK



- Settings > Peptide Settings – Prediction – click calculator > Add…
  Name: Selevsek 90 – Click Open – Type “S” and choose Selevsek90.blib – OK

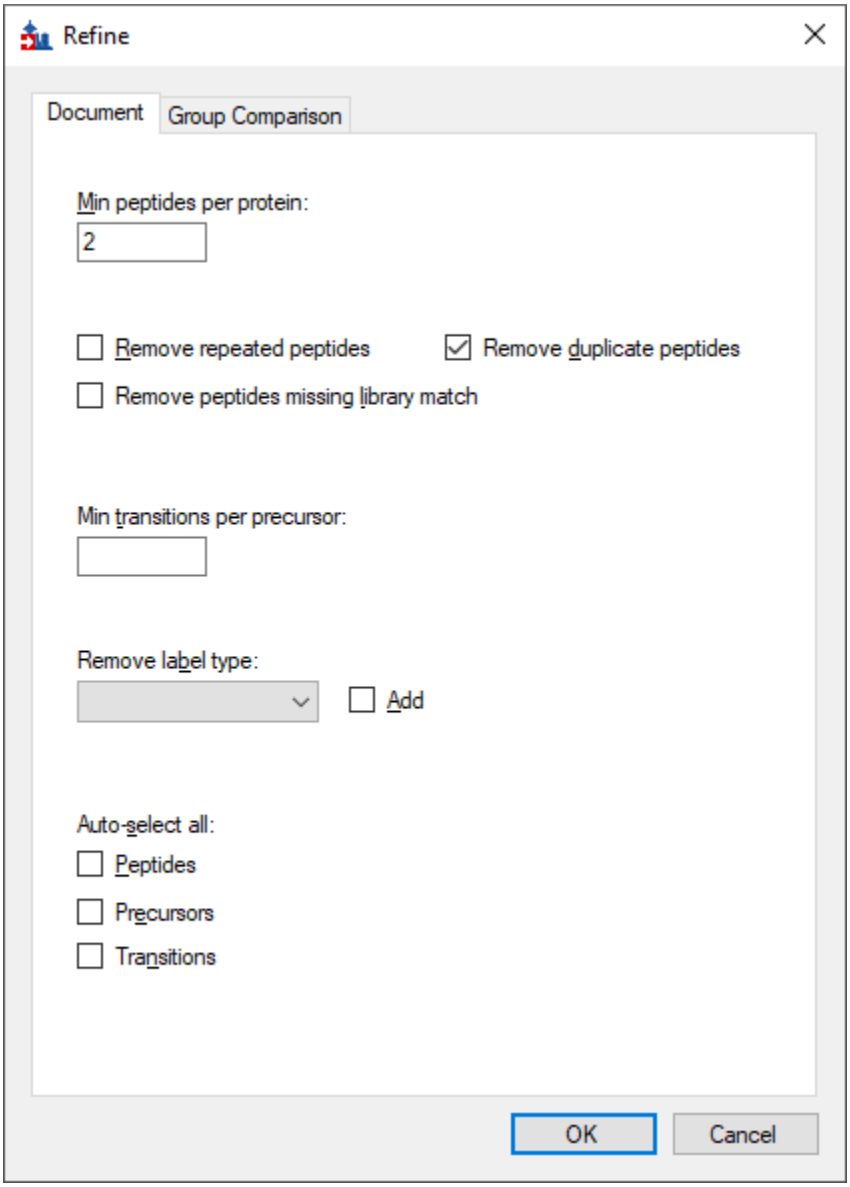


Bug: Click OK to save new .irtdb file

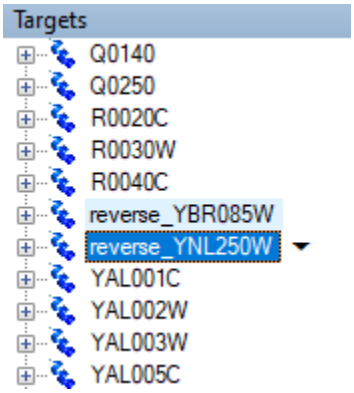


Type Selevsek90, and click Save
Click OK in Peptide Settings

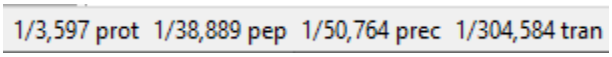
- Refine > Advanced – Min peptides per protein 2, Check Remove duplicate peptides



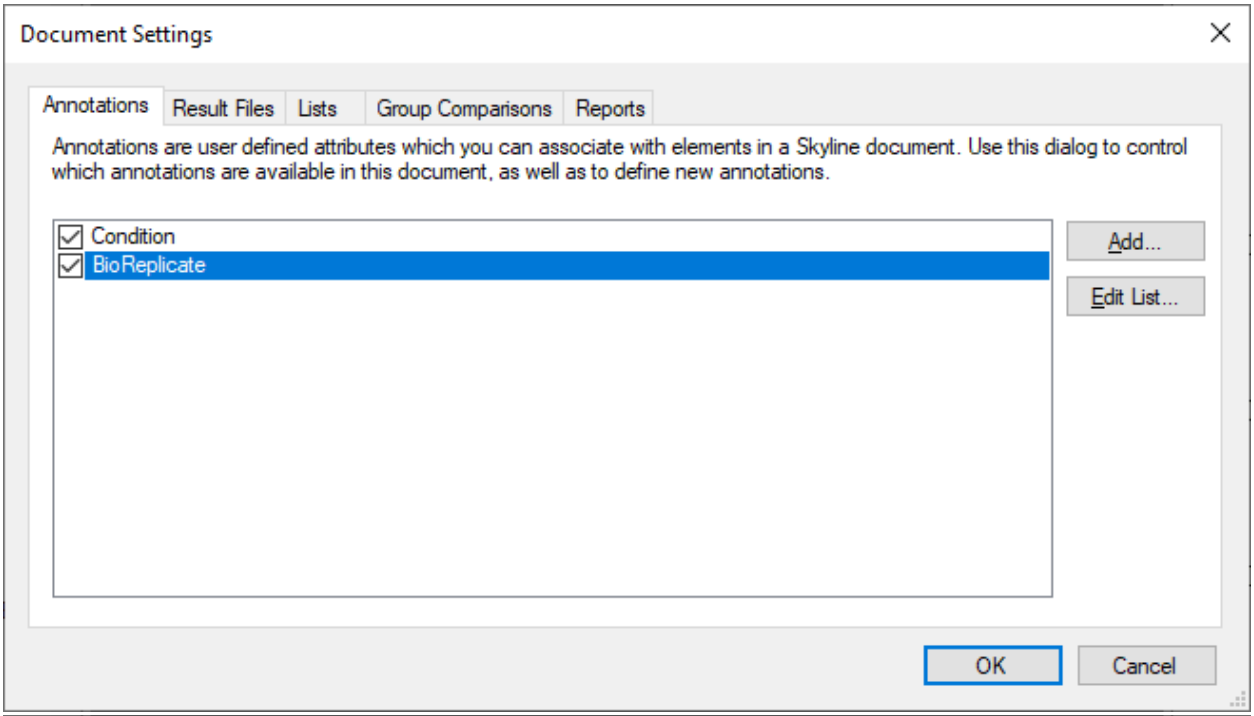
- Refine > Sort Proteins > By Name – delete reverse_

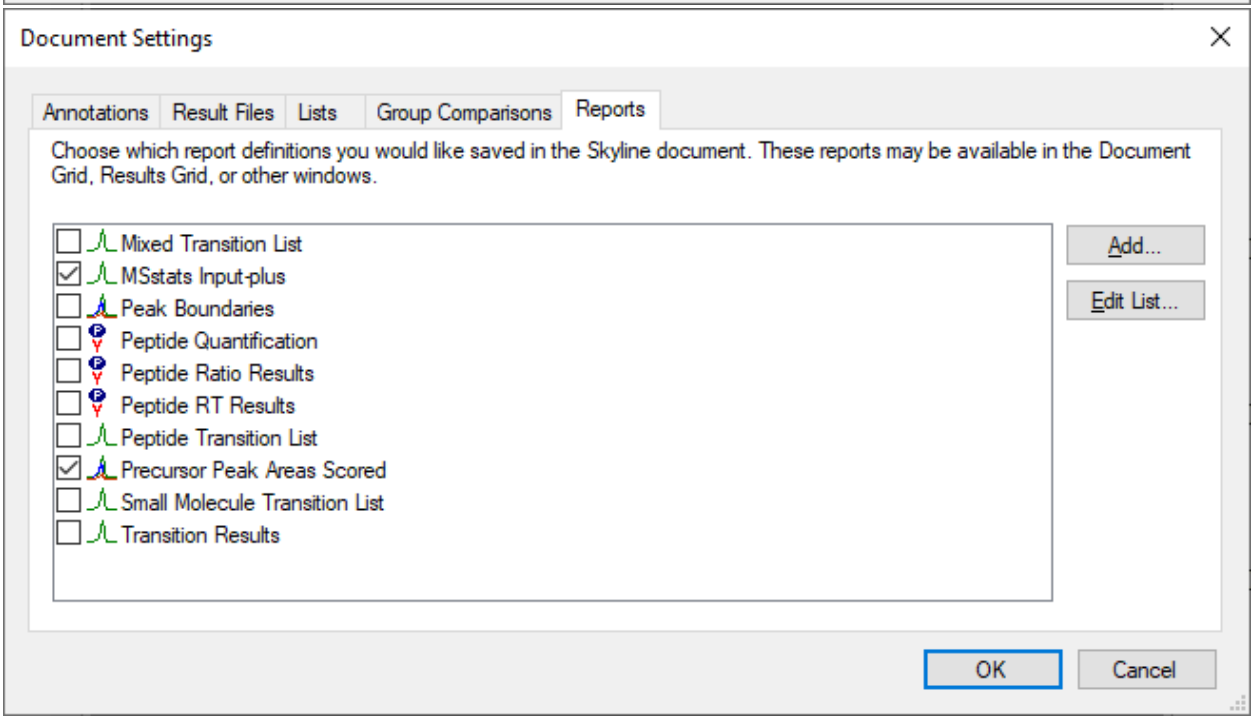


- Final count



- Settings > Document – add MSstats annotations and report





- Save and exit