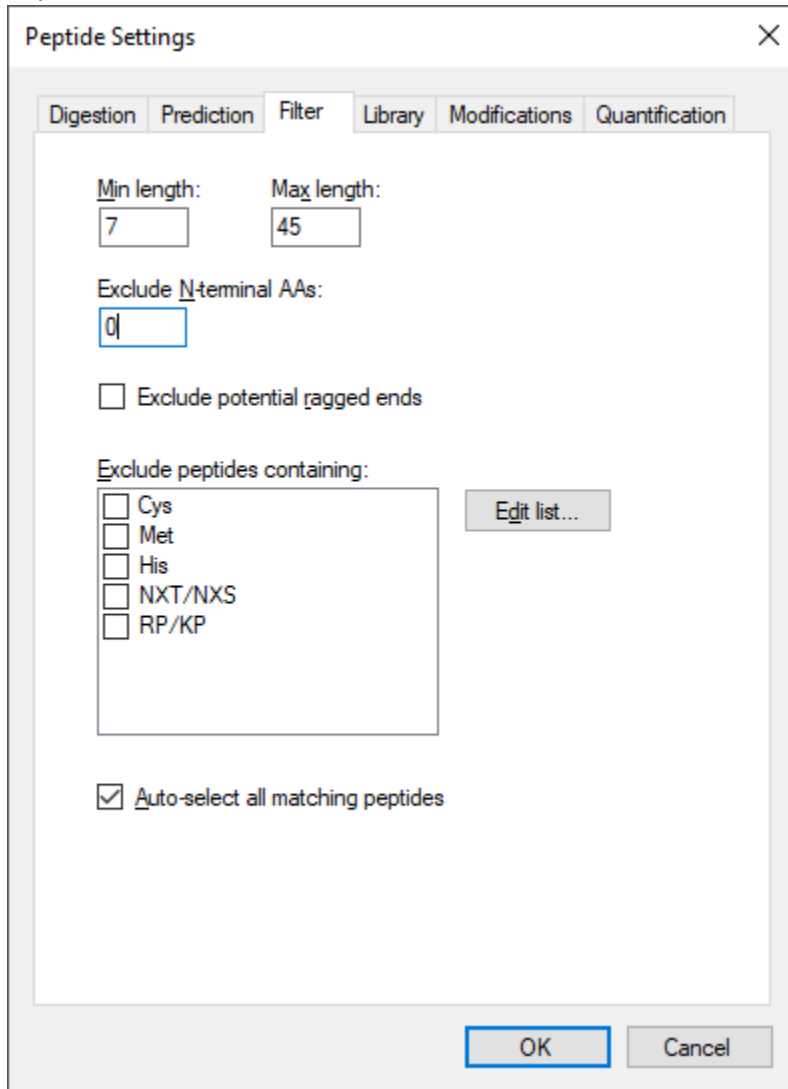


Selevsek Data Settings

- 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



The image shows a screenshot of the 'Peptide Settings' dialog box, specifically the 'Filter' tab. The dialog has a title bar with a close button (X) and a tabbed interface with five tabs: 'Digestion', 'Prediction', 'Filter', 'Library', and 'Quantification'. The 'Filter' tab is active. Inside the dialog, there are several settings:

- Min length:** A text box containing the number '7'.
- Max length:** A text box containing the number '45'.
- Exclude N-terminal AAs:** A text box containing the character 'Q'.
- Exclude potential ragged ends:** An unchecked checkbox.
- Exclude peptides containing:** A list of amino acids with checkboxes: Cys, Met, His, NXT/NXS, and RP/KP. All are currently unchecked.
- Edit list...:** A button next to the list of amino acids.
- Auto-select all matching peptides:** A checked checkbox.

At the bottom of the dialog, there are two buttons: 'OK' and 'Cancel'.

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

The image shows a software dialog box titled "Peptide Settings" with a close button (X) in the top right corner. The dialog has five tabs: "Digestion", "Prediction", "Filter", "Library", and "Modifications" (which is currently selected), and "Quantification".

Under the "Modifications" tab, there are several sections:

- Structural modifications:** A list box containing two checked items: "Carbamidomethyl (C)" and "Oxidation (M)". To the right of this list is an "Edit list..." button.
- Max variable mods:** A text input field containing the number "3".
- Max losses:** A text input field containing the number "1".
- Isotope label type:** A dropdown menu with "heavy" selected.
- Isotope modifications:** An empty list box. To its right is an "Edit list..." button.
- Internal standard type:** A dropdown menu with "heavy" selected.

At the bottom of the dialog, there are two buttons: "OK" and "Cancel".

- 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:

Start from:

Result files:

iRT standard peptides:

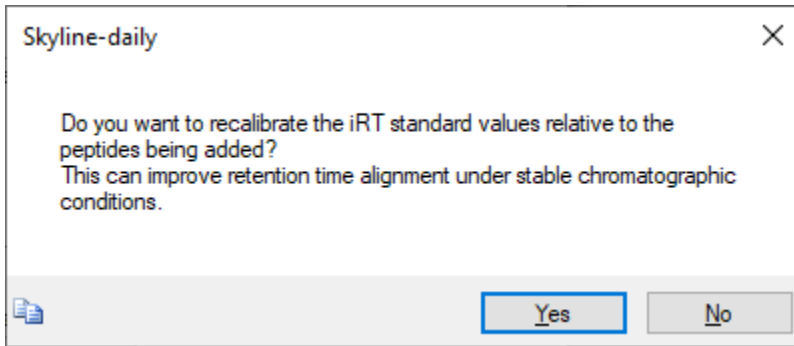
Include ambiguous matches

Workflow
 DDA with MS1 filtering
 DIA
 PRM

Add iRT Peptides

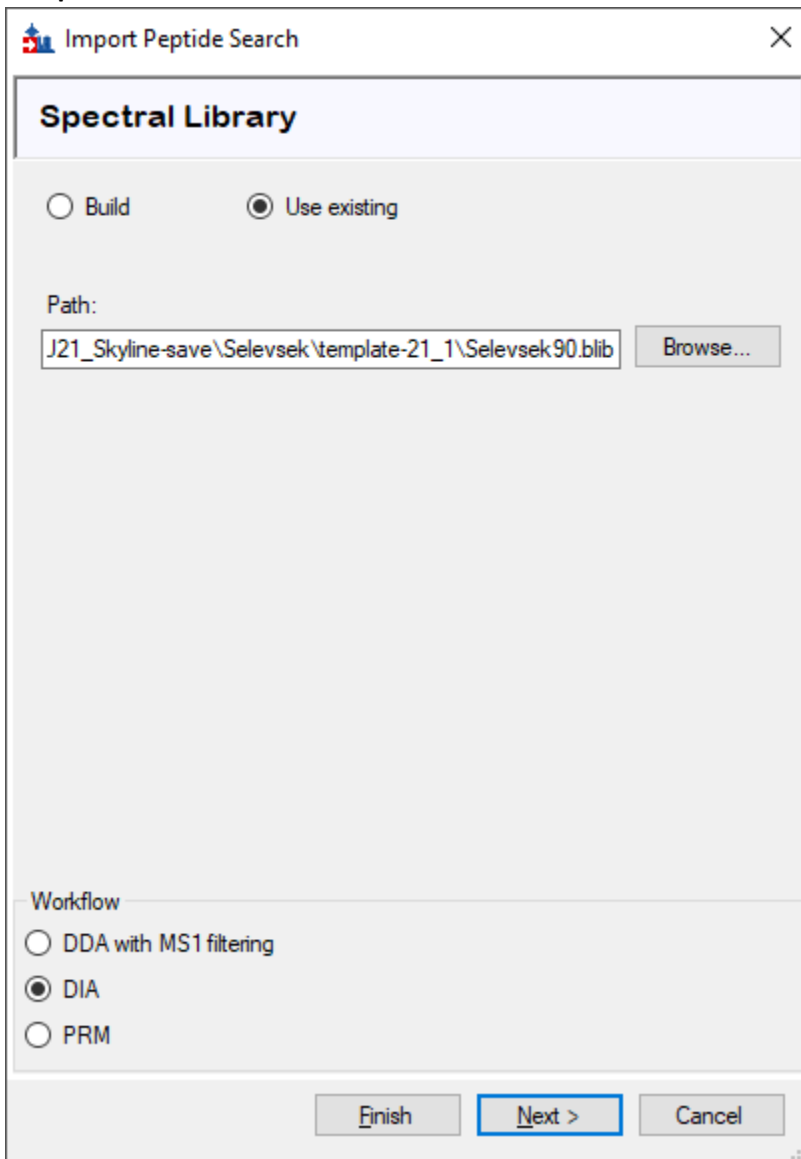
64487 new peptides will be added to the spectral library.
 1 run was successfully converted.

File	Points	Equation	R	Result
Selevsek blib	14	iRT = 1.778 * Measured RT - 6...	0.991	Success

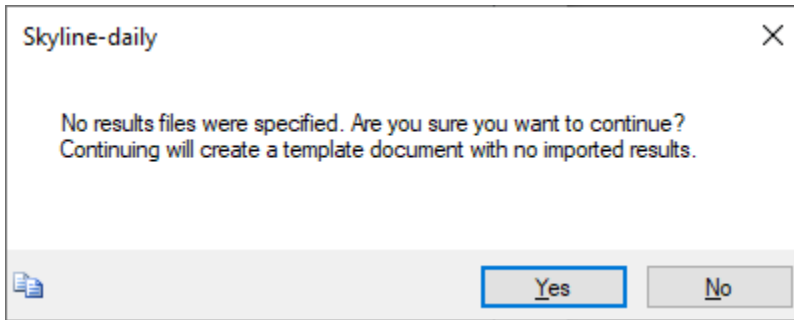


Yes! ... OK to ambiguous matches

- **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

The screenshot shows a software dialog box titled "Import Peptide Search" with a close button (X) in the top right corner. The main section is titled "Configure Transition Settings".

Fields and values:

- Precursor charges: 2, 3, 4
- Ion charges: 1, 2
- Ion types: y, b|
- Product ions from: ion 3 (dropdown)
- Product ions to: last ion (dropdown)
- Min m/z: 50 m/z
- Max m/z: 2000 m/z
- Use DIA precursor window for exclusion
- Ion match tolerance: 0.05 m/z
- Pick: 6 product ions
- 6 min product ions

Navigation buttons at the bottom: < Back, Next > (highlighted with a blue border), and Cancel.

- 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

Edit Isolation Scheme [Close]

Name: [OK] [Cancel]

Use results data isolation targets

Isolation width: Deconvolution:

Prespecified isolation windows [Calculate...] [Import...] [Graph...]

Measurement

Start	End	Margin
400	425	0.5
424	450	0.5
449	475	0.5
474	500	0.5
499	525	0.5
524	550	0.5
549	575	0.5

Deconvolution: Specify Margin Specify CE Range

Windows per scan:



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

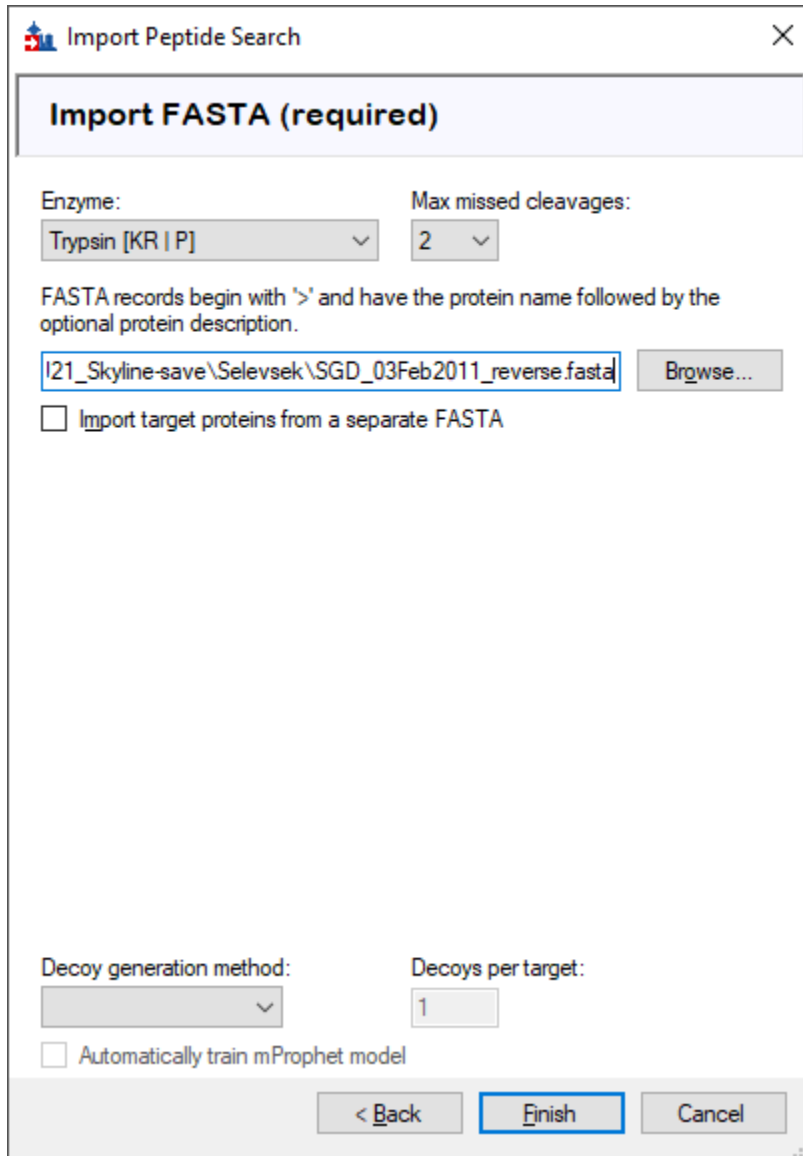
Include all matching scans

< Back

Next >

Cancel

- Import FASTA - Trypsin – 2 missed

The image shows a software dialog box titled "Import Peptide Search" with a close button (X) in the top right corner. The main heading is "Import FASTA (required)". Below this, there are two dropdown menus: "Enzyme:" set to "Trypsin [KR | P]" and "Max missed cleavages:" set to "2". A text box contains the file path "I21_Skyline-save\Selevsek\SGD_03Feb2011_reverse.fasta" with a "Browse..." button to its right. Below the text box is a checkbox labeled "Import target proteins from a separate FASTA" which is currently unchecked. At the bottom, there are two more dropdown menus: "Decoy generation method:" and "Decoys per target:" set to "1". Below these is another checkbox labeled "Automatically train mProphet model" which is also unchecked. At the very bottom, there are three buttons: "< Back", "Finish" (highlighted with a blue border), and "Cancel".

Import FASTA (required)

Enzyme: Trypsin [KR | P] Max missed cleavages: 2

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

I21_Skyline-save\Selevsek\SGD_03Feb2011_reverse.fasta Browse...

Import target proteins from a separate FASTA

Decoy generation method: Decoys per target: 1

Automatically train mProphet model

< Back Finish Cancel

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

Import FASTA

This operation has created the following targets:
4542 proteins, 45,106 peptides, 59,313 precursors, 355,878 transitions

Do you want to filter proteins by the number of peptides they contain?

Min peptides per protein
1

Keep all

Remove repeated peptides

Remove duplicate peptides

Remaining:
4542 proteins, 45,106 peptides, 59,313 precursor, 355,878 transitions

OK Cancel

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

Edit iRT Calculator

Name:

iRT database:

Regression type:

iRT standards:

	Target	iRT Value
▶	SSAAPPPPR	-27.60
	GISNEGQNASIK	-20.25
	HVLTSIGEK	-20.47
	DIPVPKPK	-7.58
	IGDYAGIK	-5.16

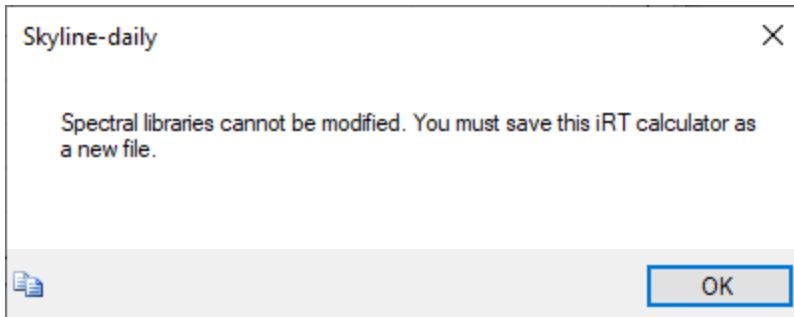
14 Standard peptides (11 required)

Other iRT values:

	Target	iRT Value
▶	GMAGGQHHR	-63.77
	KAALDTAK	-58.53
	NHTAHNQTR	-58.31
	SKNHTAHNQTR	-57.98
	KIAM[+15.99492]PQK	-57.76
	C[+57.02146]KGTVGNSHK	-57.73
	AKAEAEAK	-57.43
	SVDKTEK	-57.10
	RRHDDGSEK	56.87

64487 Peptides

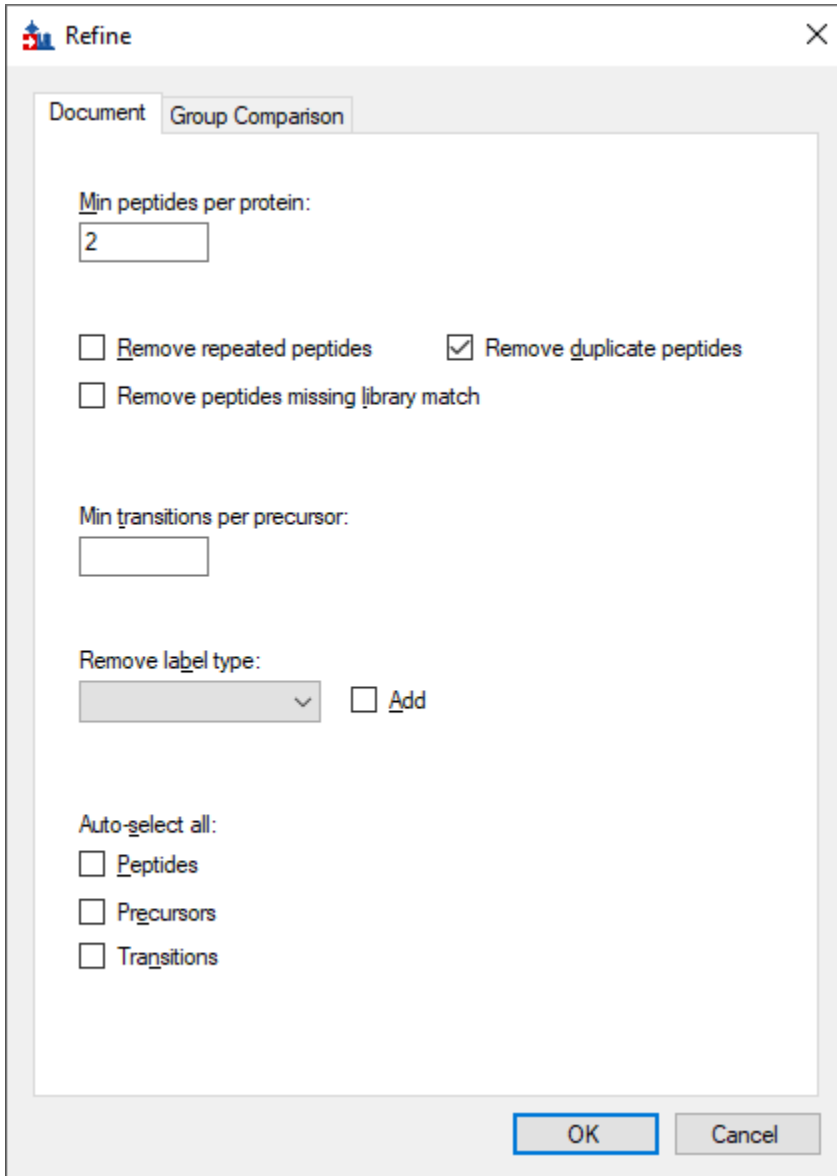
Bug: Click OK to save new .irtdb file



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_

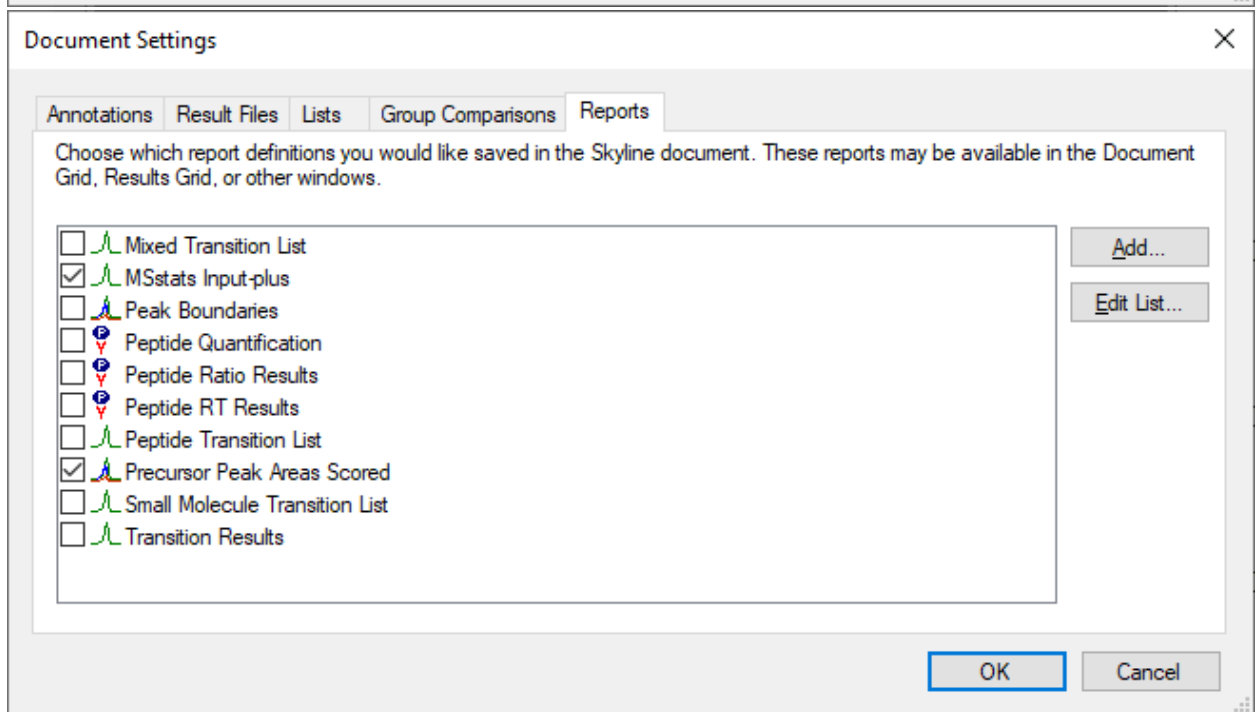
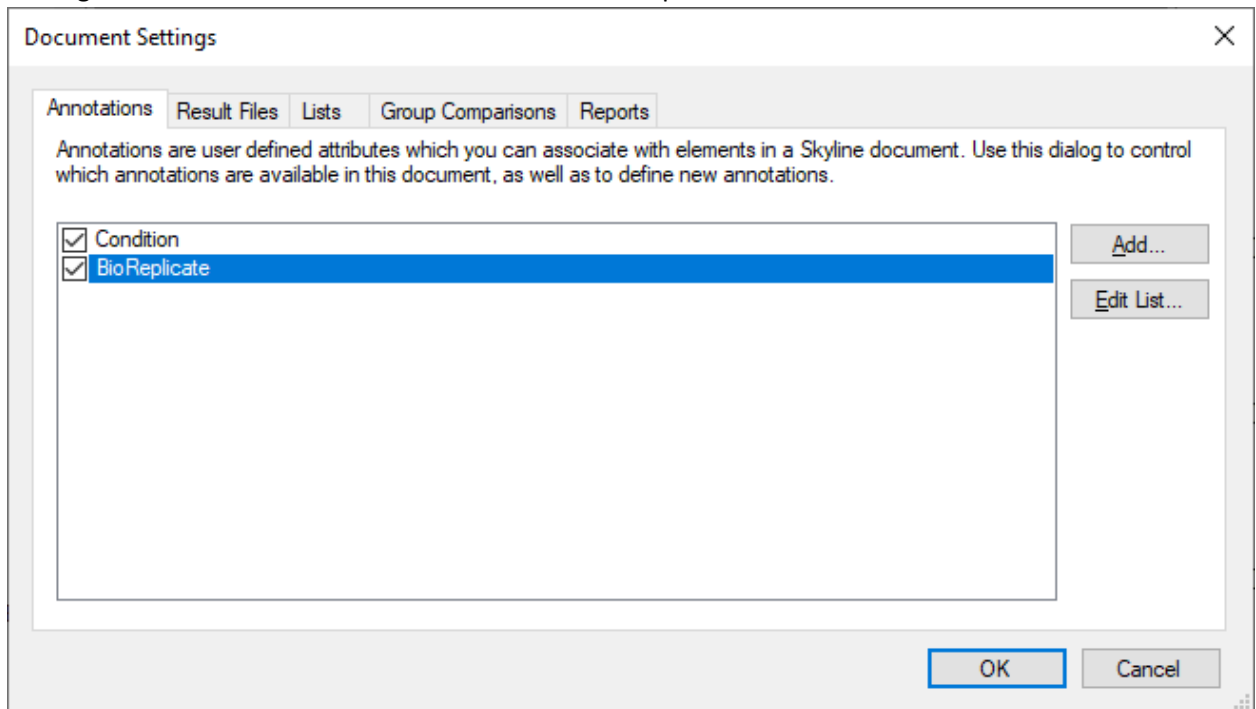
Targets

- Q0140
- Q0250
- R0020C
- R0030W
- R0040C
- reverse_YBR085W
- reverse_YNL250W
- YAL001C
- YAL002W
- YAL003W
- YAL005C

- Final count

1/3,597 prot 1/38,889 pep 1/50,764 prec 1/304,584 tran

- Settings > Document – add MSstats annotations and report



- Save and exit