

Preparation

View → text zoom → large

Set properties text size to 14 point

CONTEXT

- We are going to use Skyline to generate the isolation list to be used for our DIA method

Transition Settings – Full Scan

Transition Settings

Prediction | Filter | Library | Instrument | Full-Scan

MS1 filtering

Isotope peaks included: Precursor mass analyzer:

Peaks: Resolving power: At: m/z

Isotope labeling enrichment:

MS/MS filtering

Acquisition method: Product mass analyzer:

Isolation scheme: Resolving power: At: m/z

Retention time filtering

Use only scans within minutes of MS/MS IDs

Use only scans within minutes of predicted RT

Include all matching scans

OK Cancel

Click this last

Edit isolation scheme

Edit Isolation Scheme

Name:

Use results data isolation targets

Isolation width: *m/z* Deconvolution:

Asymmetric

Prespecified isolation windows

Start	End

Deconvolution:

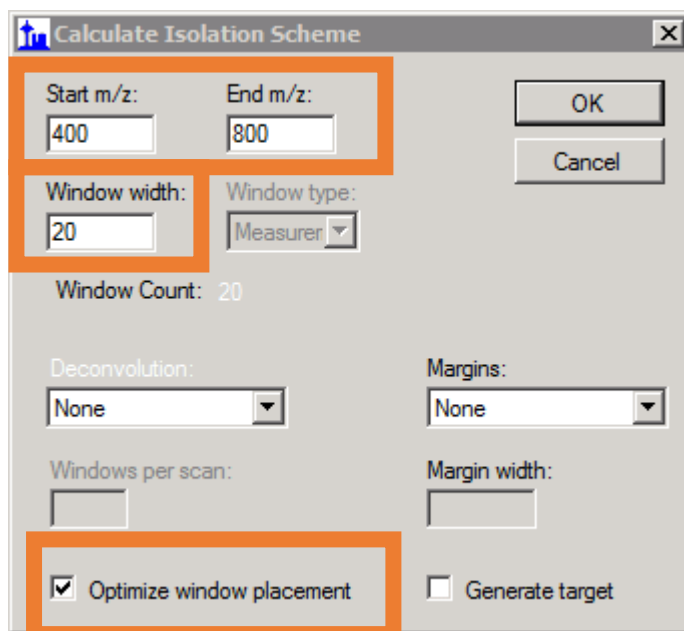
Margins:

Windows per scan:

Specify target

Calculate isolation scheme

400 – 800 because the sample data covers this, normally do 500 - 900



The screenshot shows a dialog box titled "Calculate Isolation Scheme" with the following settings:

- Start m/z: 400
- End m/z: 800
- Window width: 20
- Window type: Measurer
- Window Count: 20
- Deconvolution: None
- Margins: None
- Windows per scan: (empty field)
- Margin width: (empty field)
- Optimize window placement
- Generate target

Buttons for "OK" and "Cancel" are also visible.

Graph isolation scheme

Edit Isolation Scheme

Name:

Use results data isolation targets

Isolation width: *m/z* Deconvolution:

Asymmetric

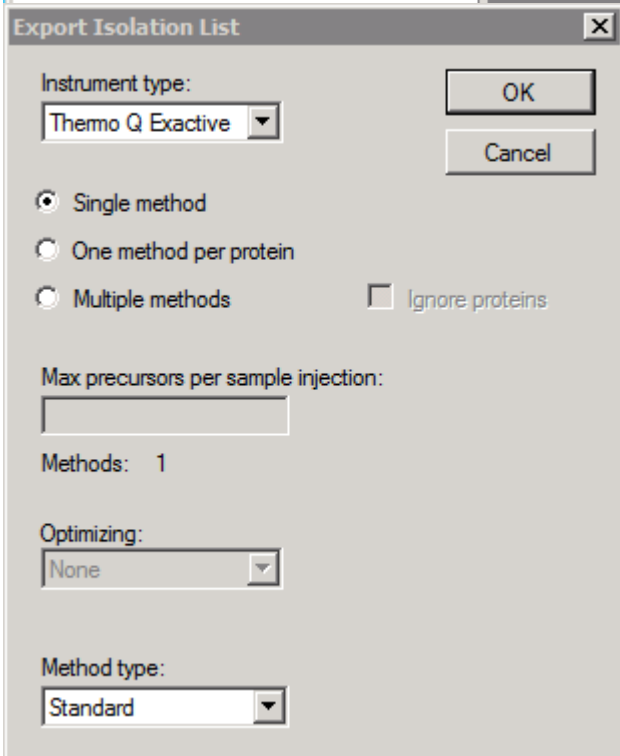
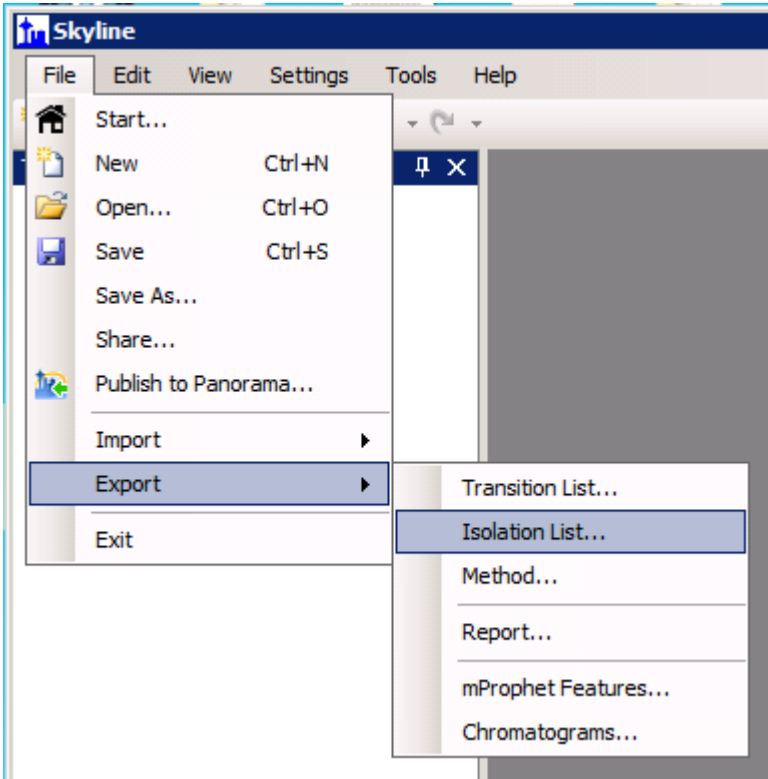
Prespecified isolation windows

Start	End
400.4319	420.4410
420.4410	440.4501
440.4501	460.4592
460.4592	480.4683
480.4683	500.4774
500.4774	520.4865
520.4865	540.4956
540.4956	560.5047
560.5047	580.5138
580.5138	600.5229
600.5229	620.5319
620.5319	640.5410

Deconvolution: Margins:

Windows per scan: Specify target

Export Isolation List



methods / DIAIsolationList.csv

Save Skyline doc to skydocs / DIA.sky
and open QE method editor (Xcalibur
→ Instrument Setup)

Full MS Settings

The screenshot displays the 'DIA.meth - Thermo Xcalibur Instrument Setup' window. The interface is divided into several sections:

- Global Lists:** Includes Lock Masses, Inclusion, Exclusion, Neutral Loss, and Tag Masses.
- Tune Files:** A section for selecting instrument tune files.
- External Hardware:** A section for configuring external hardware.
- Chromatogram:** A section for configuring the chromatogram.
- Scan Groups:** A timeline showing 'Full MS - SIM' and 'DIA' scans from 0 to 80 minutes.
- Experiments:** A list of experiment types including Full MS - SIM, AIF, Full MS / AIF, Full MS / dd-MS² (TopN), Targeted-SIM, Targeted-MS², Targeted-SIM / dd-MS², Full MS / AIF / NL dd-MS², and DIA. A 'Full MS' button is highlighted with a yellow arrow.
- Properties:** A table of method and scan parameters.

Properties of the method

Global Settings	
User Role	Standard
Use lock masses	best
Chrom. peak	15 s

Properties of Full MS — SI

General	
Runtime	0 to 80 min
Polarity	positive
Full MS — SIM	
Resolution	35,000
AGC target	3e6
Maximum IT	55 ms
Scan range	495 to 905 m/z

At the bottom of the window, there are tabs for 'Experiment Setup' and 'Summary', and a status bar showing 'Ready'.

DIA Settings

The screenshot displays the Thermo Xcalibur Instrument Setup software interface for a method named 'DIA.meth'. The interface is divided into several sections:

- Global Lists:** Includes Lock Masses, Inclusion, Exclusion, Neutral Loss, and Tag Masses.
- Tune Files:** A section for selecting instrument tune files.
- External Hardware:** A section for configuring external hardware.
- Chromatogram:** A section for configuring chromatogram parameters.
- Scan Groups:** A timeline showing two scan groups: 'Full MS - SIM' and 'DIA', both running from 0 to 80 minutes.
- Experiments:** A list of experiment types including Full MS - SIM, AIF, Full MS / AIF, Full MS / dd-MS² (TopN), Targeted-SIM, Targeted-MS², Targeted-SIM / dd-MS², Full MS / AIF / NL dd-MS², and DIA.
- Properties:** A detailed configuration panel for the method and DIA settings.

Properties of the method

Global Settings	
User Role	Standard
Use lock mass best	
Chrom. peak	15 s

Time	
Method duration	80.00 min

Properties of DIA

General	
Runtime	0 to 80 min
Polarity	positive
Default charge state	2

DIA	
Resolution	17,500
AGC target	1e6
Maximum IT	auto
Loop count	10
MSX count	1
MSX isochronous ITs	on
Isolation window	20.0 m/z
Fixed first mass	—
NCE / stepped NCE	26
Spectrum data type	Centroid

The Experiments section shows a diagram with two boxes labeled 'Full MS' and 'DIA', each with a yellow arrow pointing down to it, indicating the experimental workflow.

Import Isolation list

CONTEXT

- We are going to start defining our document – the set of peptides, and transitions we would like to extract. Informed by the DDA data.

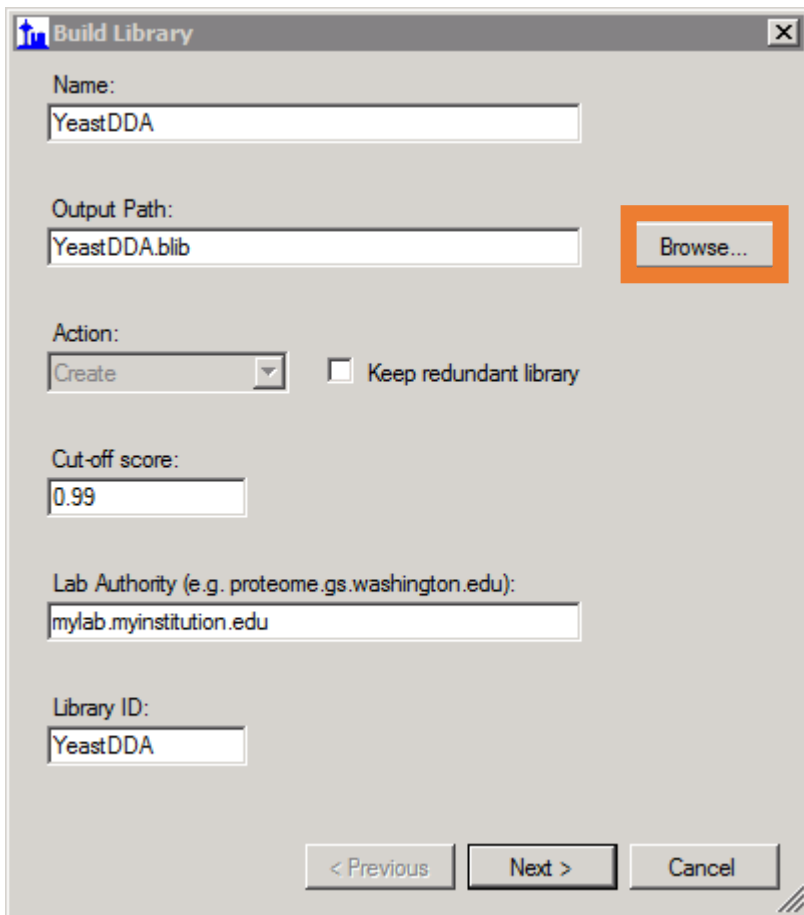
Transition Settings → Filter

The image shows a software dialog box titled "Transition Settings" with a close button (X) in the top right corner. The dialog has four tabs: "Prediction", "Filter", "Library", "Instrument", and "Full-Scan". The "Filter" tab is currently selected. The dialog is divided into several sections:

- Precursor charges:** A text box containing "2, 3".
- Ion charges:** A text box containing "1".
- Ion types:** A text box containing "y, b, p".
- Product ions:** A section containing two dropdown menus: "From:" with "ion 3" selected and "To:" with "last ion - 1" selected.
- Special ions:** A list box with the following items:
 - N-terminal to Proline
 - C-terminal to Glu or Asp
 - iTRAQ-114
 - iTRAQ-115
 - iTRAQ-116
 - iTRAQ-117
 - TMT-126
 - TMT-127LAn "Edit List..." button is located to the right of the list box.
- Use DIA precursor window for exclusion
- Auto-select all matching transitions

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

Settings -> Peptide Settings ->
Library -> Build...



Build Library

Name:
YeastDDA

Output Path:
YeastDDA.blib

Action:
Create

Keep redundant library

Cut-off score:
0.99

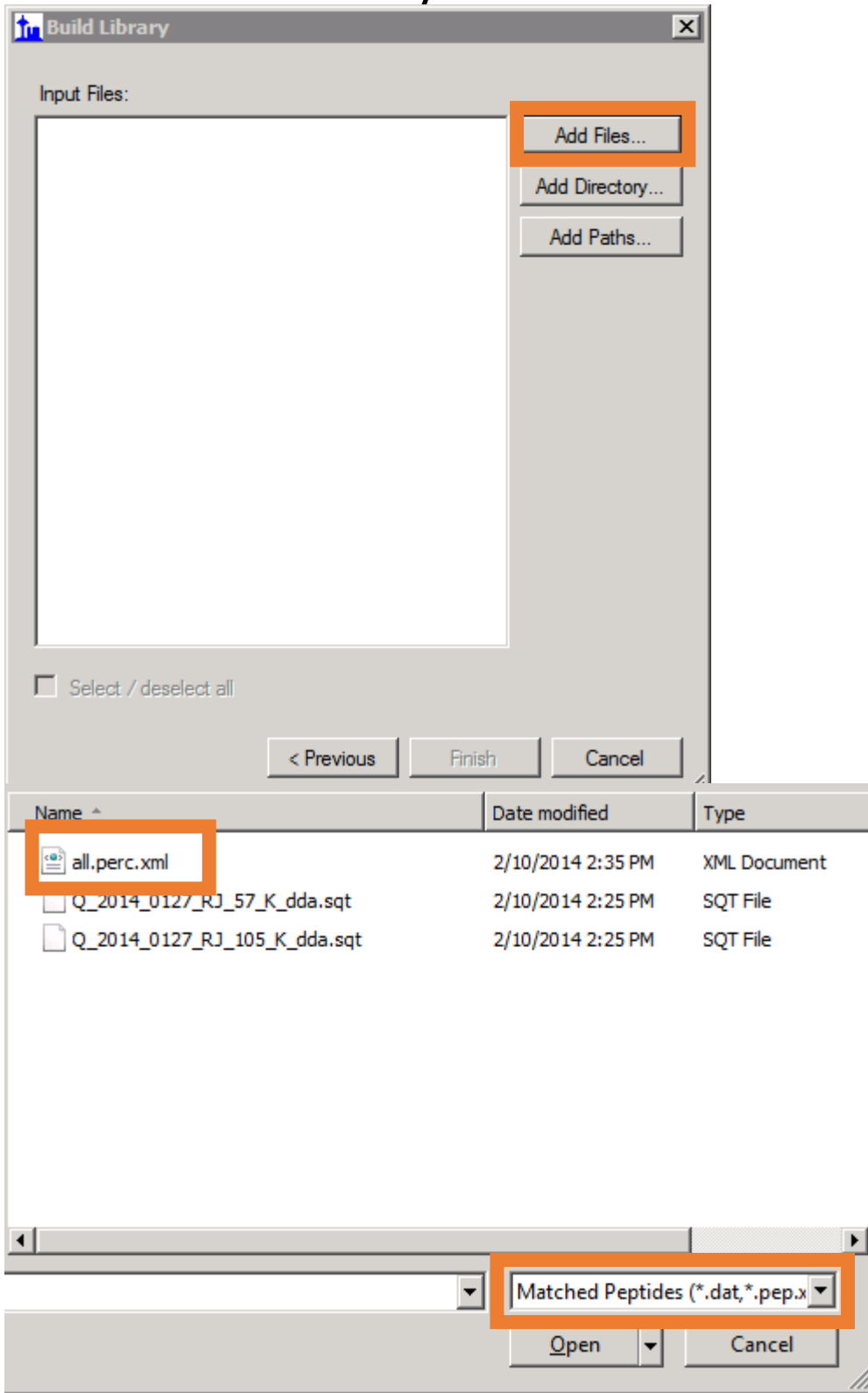
Lab Authority (e.g. proteome.gs.washington.edu):
mylab.myinstitution.edu

Library ID:
YeastDDA

< Previous Next > Cancel

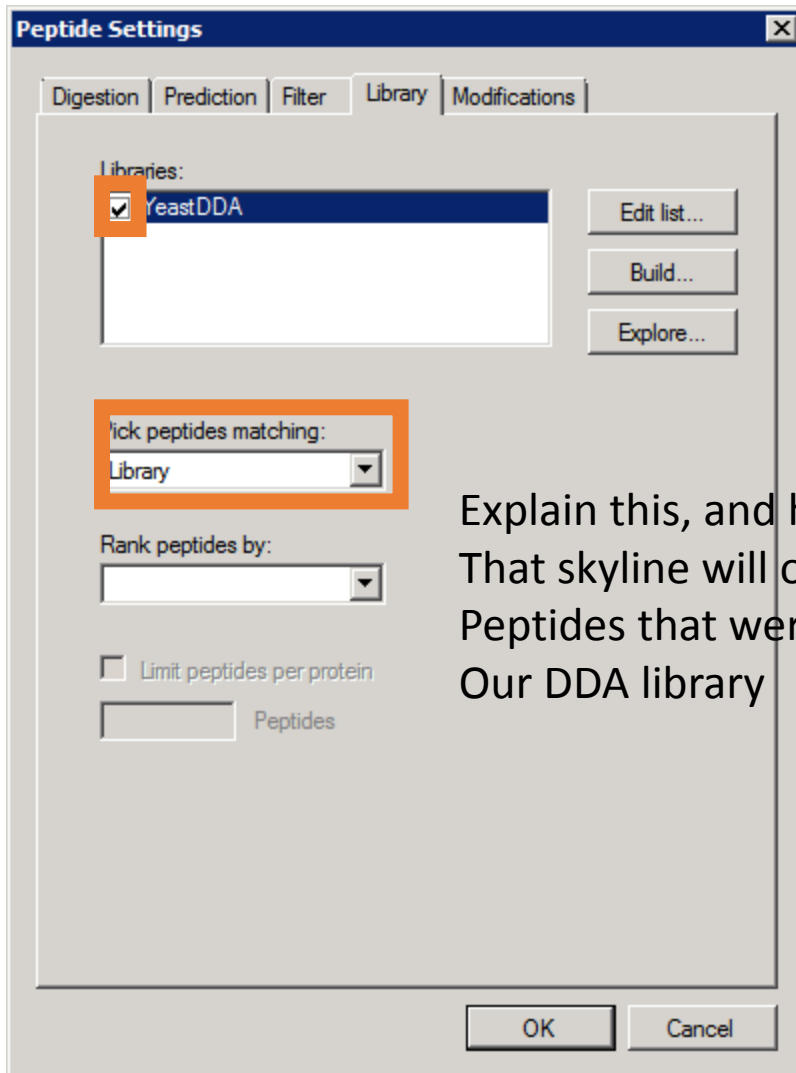
Point to
spectralLibrary/YeastDDA.blib

Build library



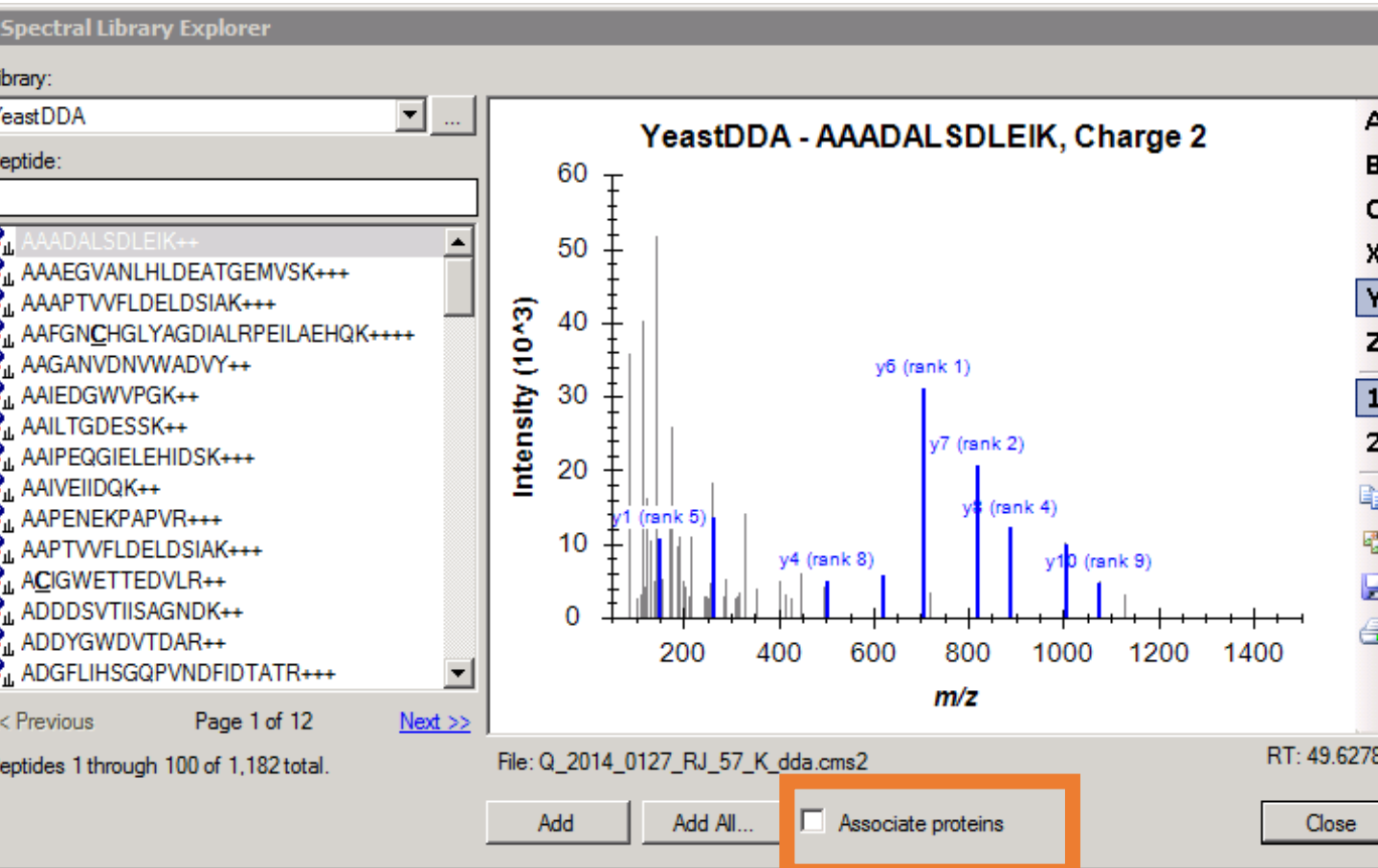
Click and hit all files to show all of the files in the directory

Settings → Peptide Settings → Library



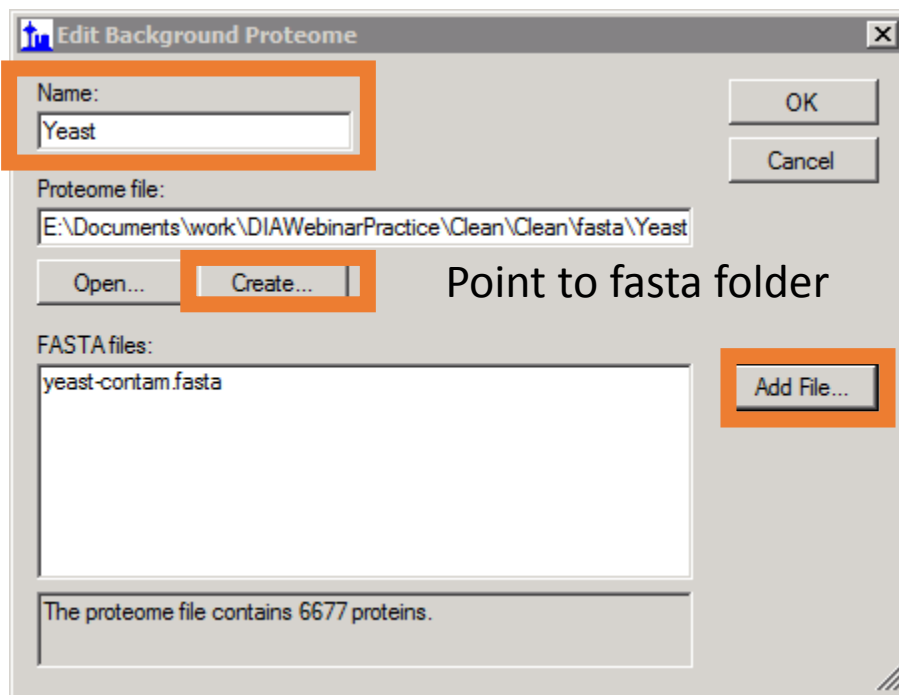
Explain this, and how it means That skyline will only analyze Peptides that were identified in Our DDA library

View → Spectral Libraries



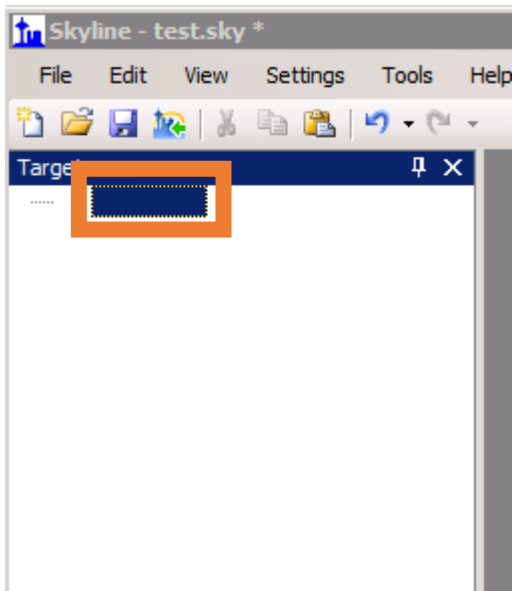
Mention we need to have the background proteome To associate peptides with proteins

Build a background proteome:
Settings → Peptide Settings →
Digestion (change max missed
cleavages to 2)



Click OK → OK to exit out of
peptide settings box

Inserting Proteins in the Main Window



Click here and start typing:

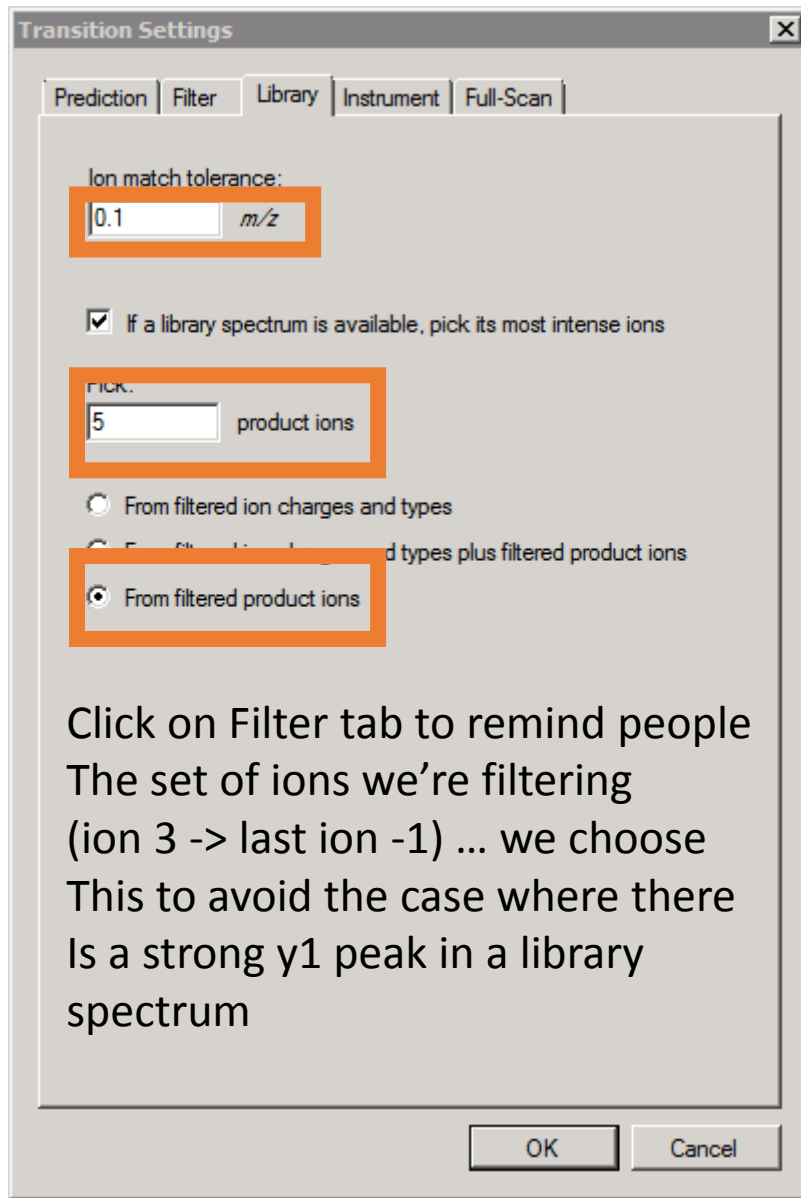
Insert “pyruvate decarboxylase” (YLR044C)

Type “glycolysis” and insert YCR012W

Explain the protein
→ Peptide →
Transition Hierarchy

Show library
spectrum

Settings → Transition Settings → Library



The screenshot shows the 'Transition Settings' dialog box with the 'Library' tab selected. The 'Ion match tolerance' is set to 0.1 m/z. A checkbox is checked for 'If a library spectrum is available, pick its most intense ions'. The 'Pick' value is set to 5 product ions. The radio button 'From filtered product ions' is selected.

Transition Settings

Prediction | Filter | Library | Instrument | Full-Scan

Ion match tolerance:
0.1 m/z

If a library spectrum is available, pick its most intense ions

Pick:
5 product ions

From filtered ion charges and types
 From filtered ion charges and types plus filtered product ions
 From filtered product ions

Click on Filter tab to remind people
The set of ions we're filtering
(ion 3 -> last ion -1) ... we choose
This to avoid the case where there
Is a strong y1 peak in a library
spectrum

OK Cancel

Show the transitions lists with 5 transitions now for a few peptides, then delete all

Inserting Proteins by name

Edit → Insert → Proteins

Pyruvate decarboxylase – works (use YLR044C)

Click on some of the peptides to show the spectra

Show precursors and transitions. (If they don't show up click

View → Library Match)

Try TOR1 and show how it doesn't insert

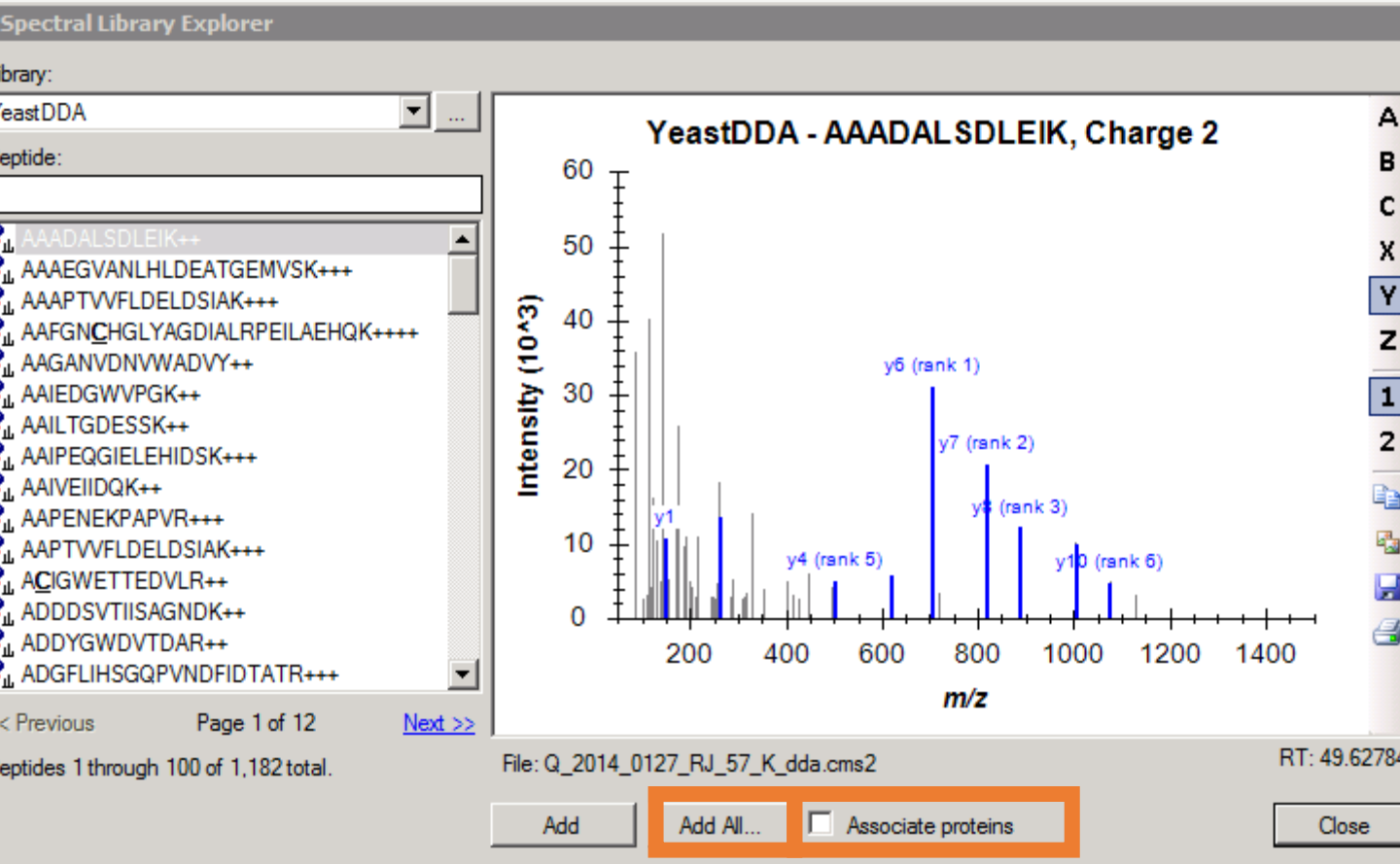
Because there aren't any peptides that were identified in

Our spectral library

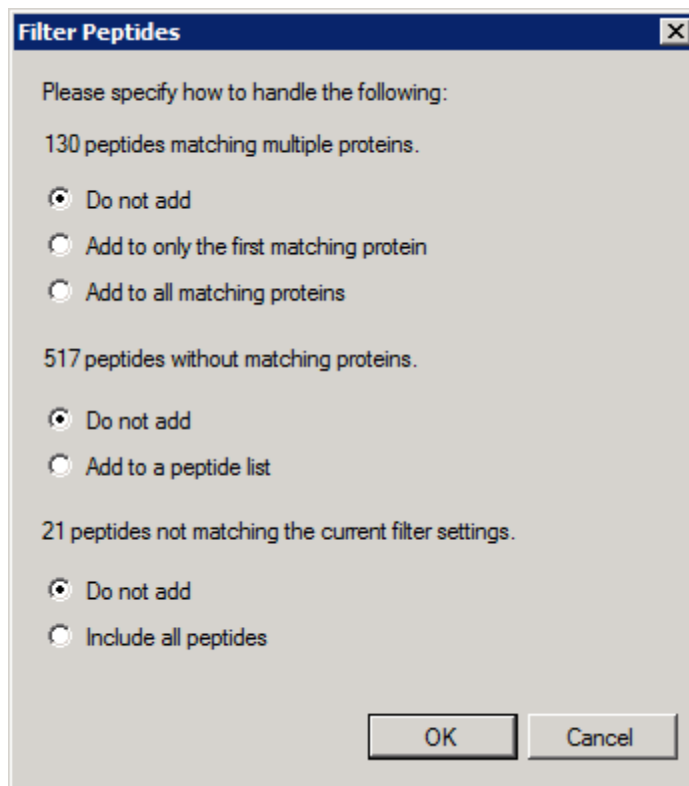
CONTEXT

- We are now going to insert all of the peptides in the DDA library

View → Spectral Libraries



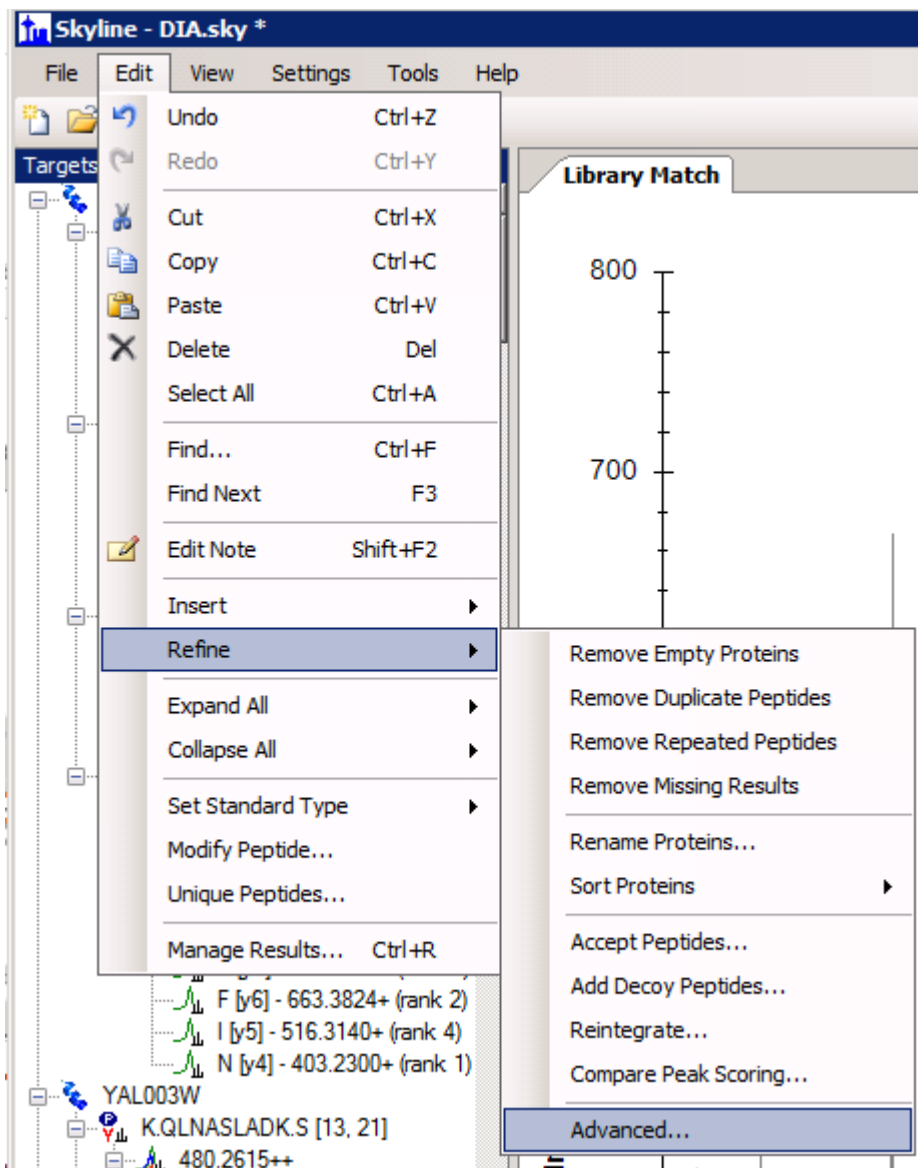
Explain these



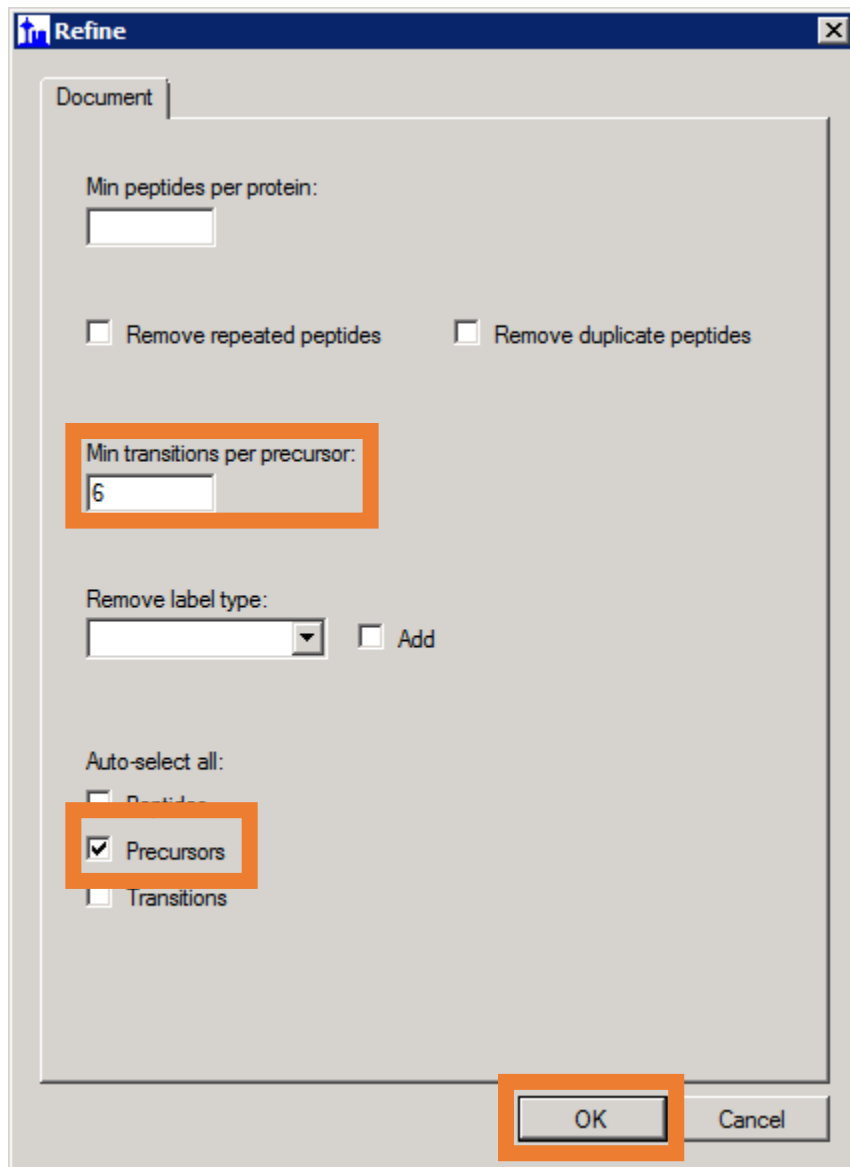
After this, delete trypsin contaminant

Show how some of the peptides have no product ion transitions, or a low number

Ctrl F for peptide SDVIFPILIPT

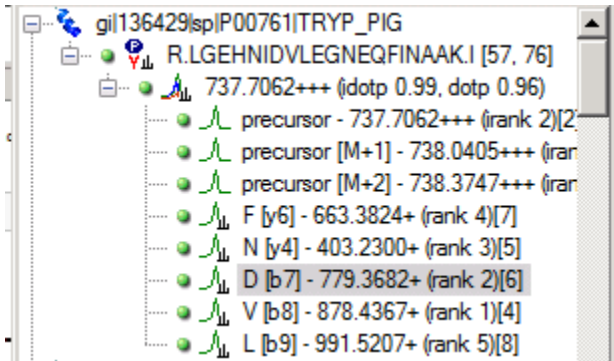


Refine to only keep peptides with 3 or more product transitions

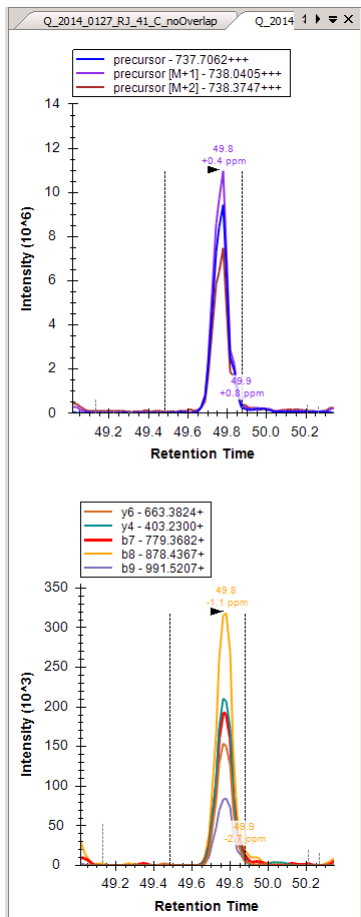


Do ctrl-Z and ctrl-Y to show before and after precursor count

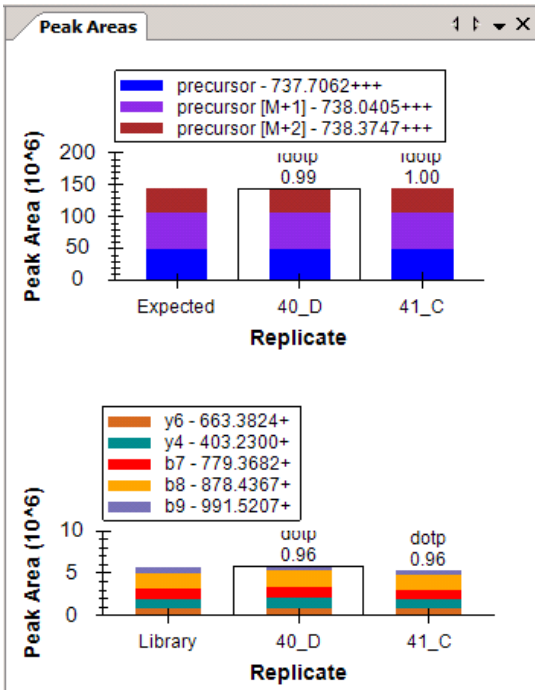
Import Results – 40_D_noOverlap 41_C_noOverlap



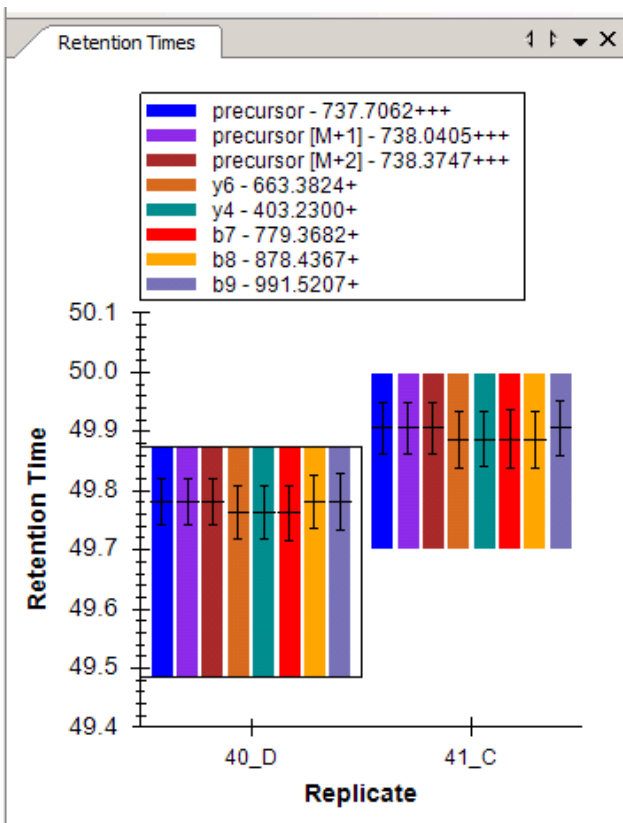
1. **Ctrl + F -> YAL038W**
2. Switch peptides, show graphs changing
3. Split View
4. Auto Zoom
5. Integration Boundaries
6. Show Peptide IDs from Other Run
7. Click on transitions, show highlighting on graph
8. Explain ranking on transitions
9. PPM Error
10. Show spectrum
11. Zoom out to see more of spectrum



Import Results – Peak Area and Retention Time



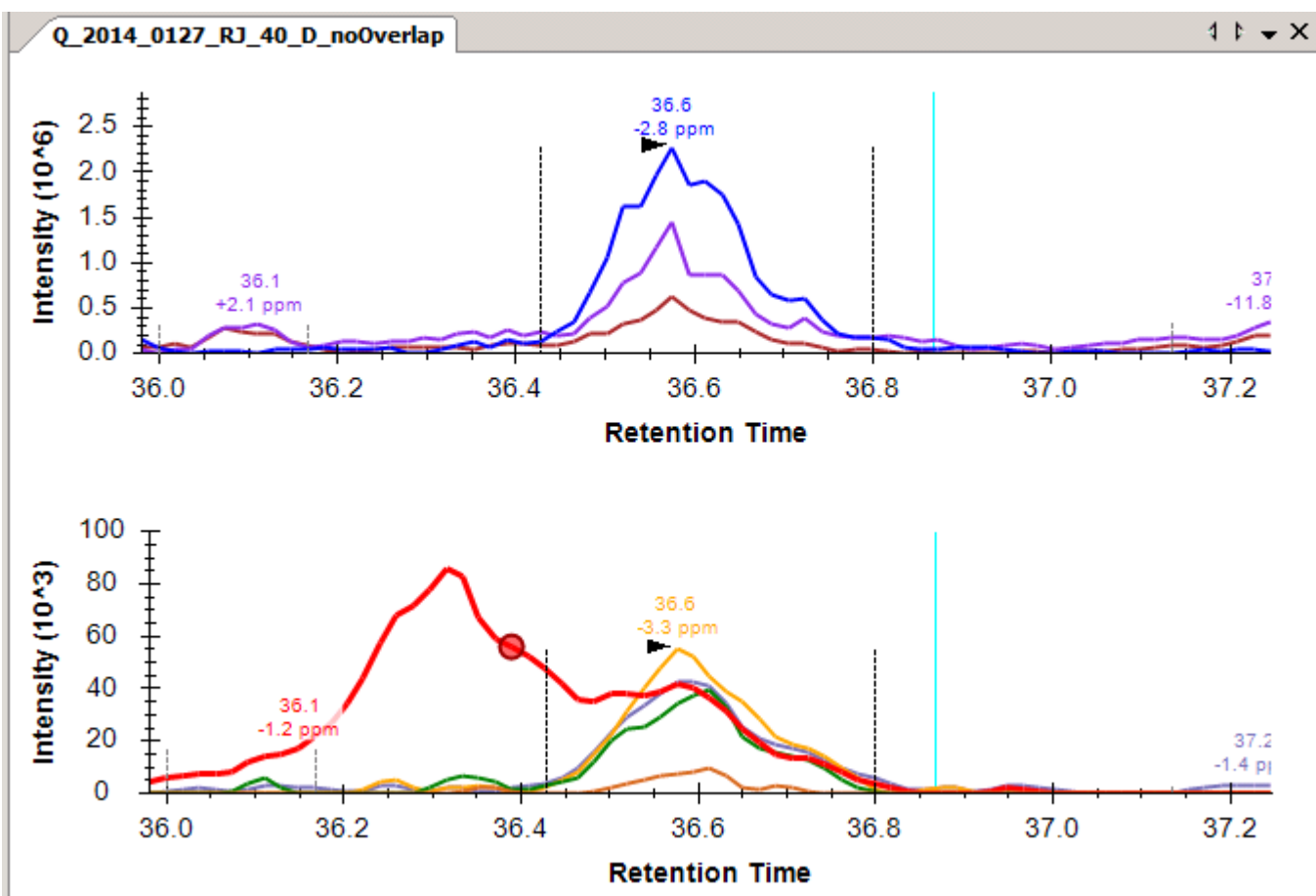
1. Show peak area replicate graph
2. Live graph – demonstrate by adjusting integration boundaries



1. Show retention time replicate graph
 2. Explain the meaning of the bars (center and FWHM of peak)
 3. Click on other peptides to show how these graphs update
1. Move all graphs into document as panes and click View → Arrange Graphs → Tiled

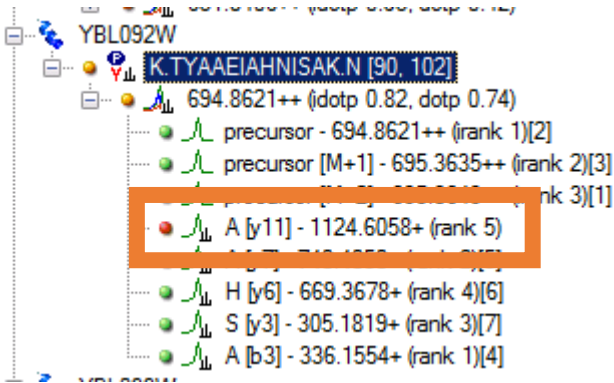
Manual refinement of transitions

1. Find a transition with interference (YAL038W – **SNLAGKPVIC**)
2. Show the spectrum from it
3. Delete the transition

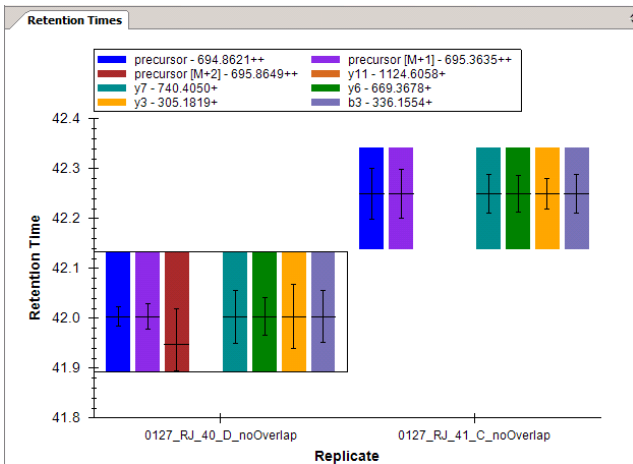


Integrate All Demonstration

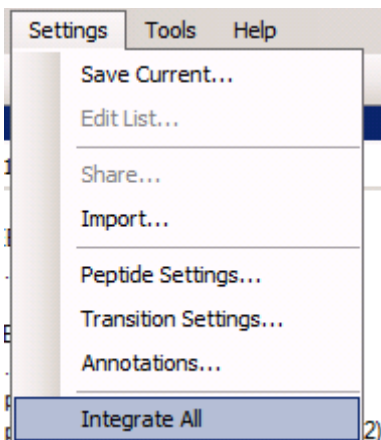
Ctrl -> F -- TYAAEIAHNISAK



y11 is a “misbehaving transition”



It doesn't show up in the retention time graph
And won't be integrated unless....



Settings -> Integrate All

Results Grid Demonstration

View → Results Grid

Click on a protein, then a peptide, then a precursor, then a transition

Click back on the precursor, change integration boundaries, and show resulting change in area on results grid.

Right click in the results grid and click choose columns

Add "Total Area MS1"

Add "Total Area Fragment"

Remove some of the columns on the right

Click around to show how it stays updated

Report Export

- Replicates
 - **Replicate Name**
- Proteins
 - **Protein Name**
- Proteins → Peptides
 - **Peptide Modified Sequence**
- Proteins -> Peptides -> Precursors
 - **Precursor Charge**
- Proteins -> Peptides -> Precursors -> Precursor Results
 - **Total Area MS1**
 - **Total Area Fragment**

Hit Preview

Enter View Name – Test Report

Export Test Report to reports directory

Open in Excel