

# Pinpoint Quick Start Guide

Thermo™ Pinpoint, version 1.0 and later<sup>1</sup>, targeted protein quantification software simplifies the creation of processing methods and helps you do the following:

- Select targeted proteins and proteotypic peptides.
- Determine mass values for monitoring targeted peptides by using:
  - Selected reaction monitoring (SRM) transitions for TSQ™ method building and processing
  - Extracted ion chromatogram (XIC) mass values for charge states and isotopes for targeted peptides
- Test and verify the targeted peptides by using SRM transitions, XIC mass values, or both.
- Refine methods for additional experiments.

Using the Pinpoint application, you can process .raw files from all Thermo Fisher Scientific products, incorporate spectral libraries to accelerate initial method building, and increase confidence in the validity of processed targeted protein data.

This quick start guide outlines the process of creating targeted quantification assays, highlighting and explaining Pinpoint application features.

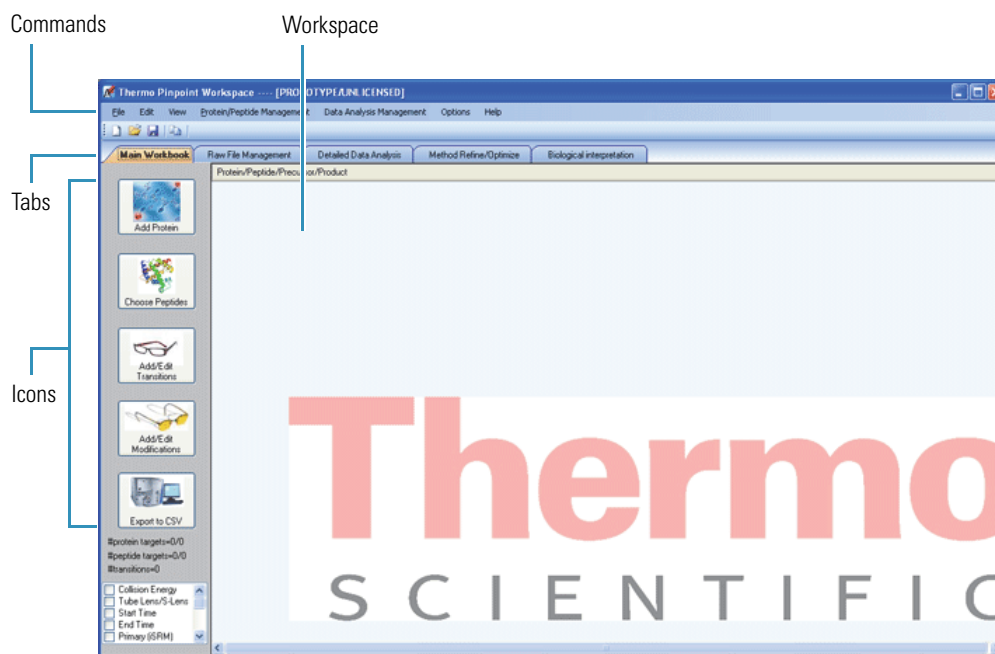
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<sup>1</sup> The application screens might appear differently in any version, but the instructions apply to all versions unless otherwise noted.

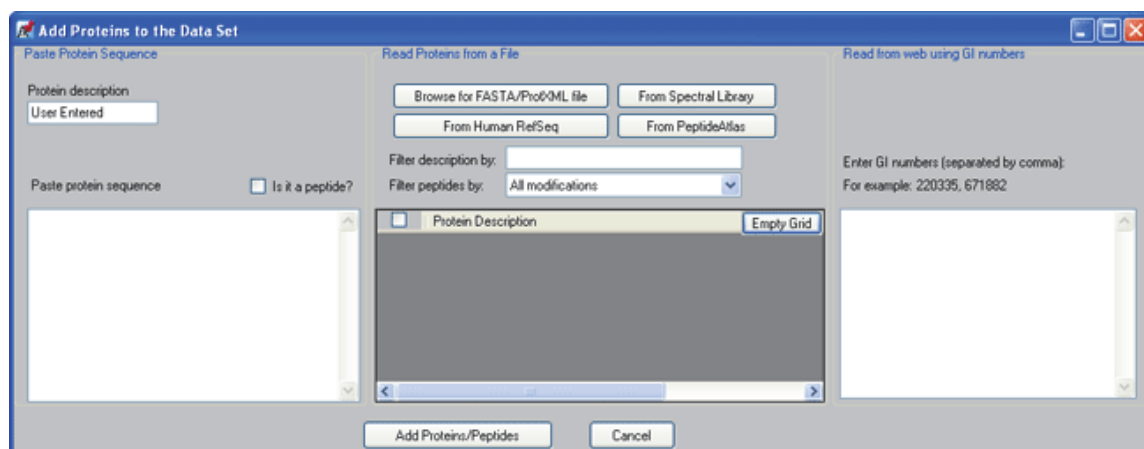
## Pinpoint User Interface



## Step 1: Adding Proteins to the Workbook

### ❖ To add a protein/peptide sequence

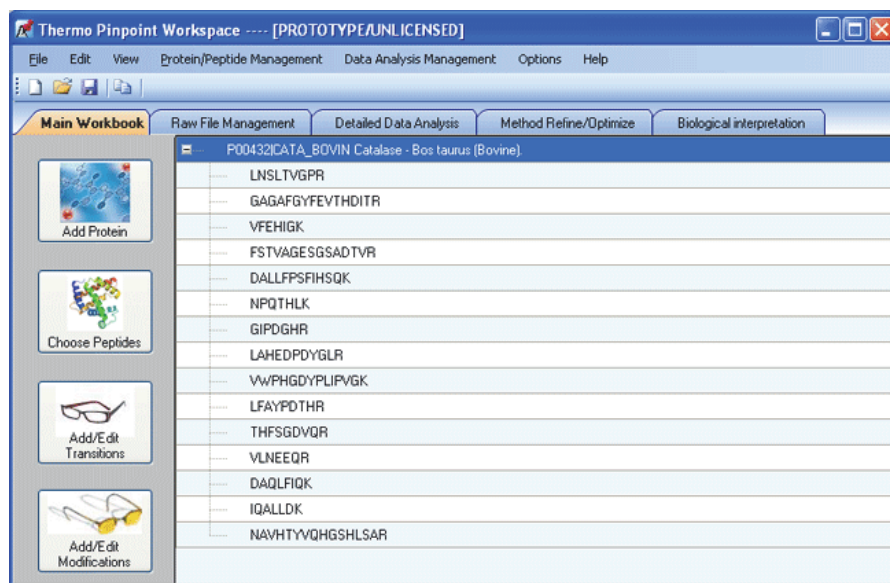
1. In the Main Workbook area, click the **Add Protein** icon, , to open the Add Proteins to the Data Set dialog box.




2. In the Paste Protein Sequence area, do one of the following:
  - In the Protein Description box, type a protein/peptide name.
  - In the Paste Protein Sequence box, type the protein/peptide sequence. If the sequence is a peptide, select the **Is it a peptide?** check box.
  - In Microsoft™ Windows™ Explorer, open a file containing a protein sequence and paste the sequence into the Paste Protein Sequence box. To add information from a file, see the next procedure.

### 3. Click **Add Proteins/Peptides**.

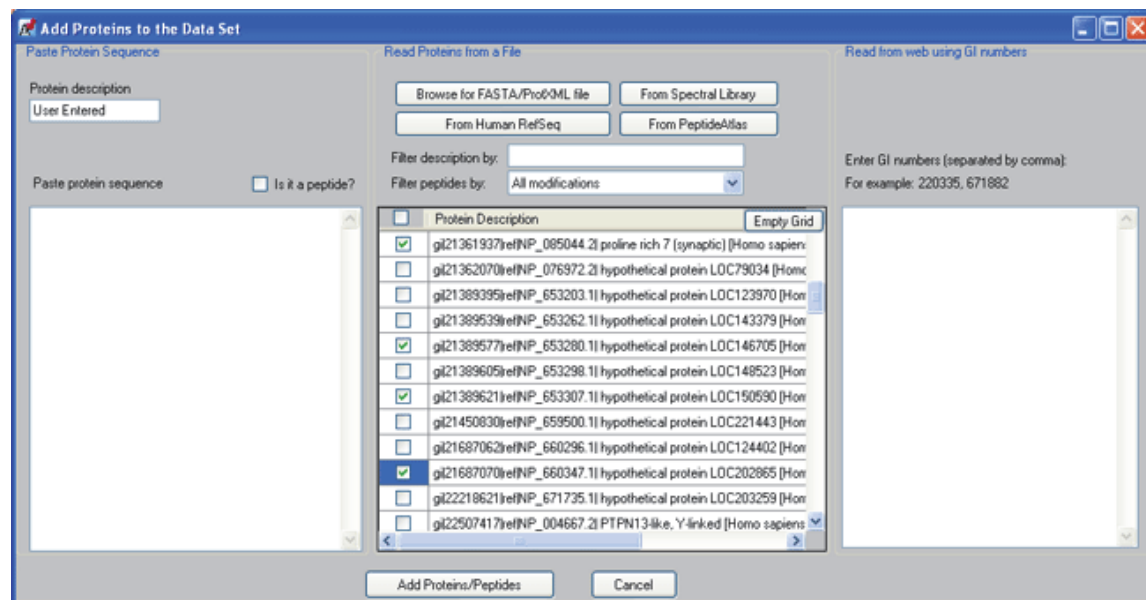
The proteins appear in the Main Workbook area.



#### ❖ To add proteins/peptides from a file

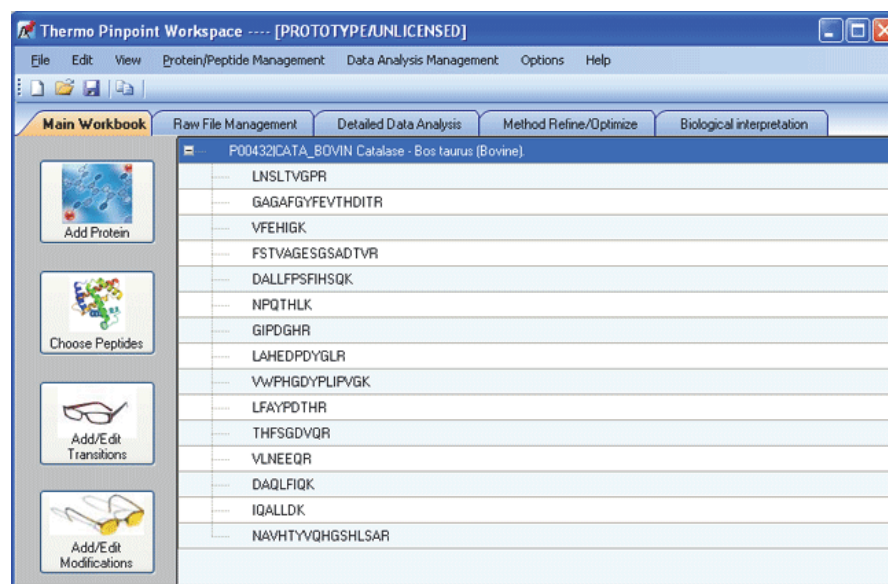
1. In the Main Workbook area, click the **Add Protein** icon, , to open the Add Proteins to the Data Set dialog box.
2. In the Read Proteins from a File area, in the Filter Description By box, type a protein/peptide sequence to select the protein you want from the file.
3. Do one of the following:
  - Click **Browse for FASTA/ProtXML File**.
  - or—
  - Click **From Spectral Library**.
4. Browse to locate and open the file.

The Pinpoint application lists the proteins from the file in the table.




5. (Optional) To select proteins, choose from these options:
  - In the table of proteins imported from the file, do one of the following:
    - Select the check box in front of each target protein that you want to add.
    - To select all proteins in the list, select the Protein Description check box at the top of the list.
  - Use one or both of these filtering options:
    - In the Filter Description By box, enter one or more keywords to select the target proteins.
    - In the Filter Peptides By list, select a peptide filtering option.
6. Click **Add Proteins/Peptides**.

The proteins appear in the Main Workbook area.

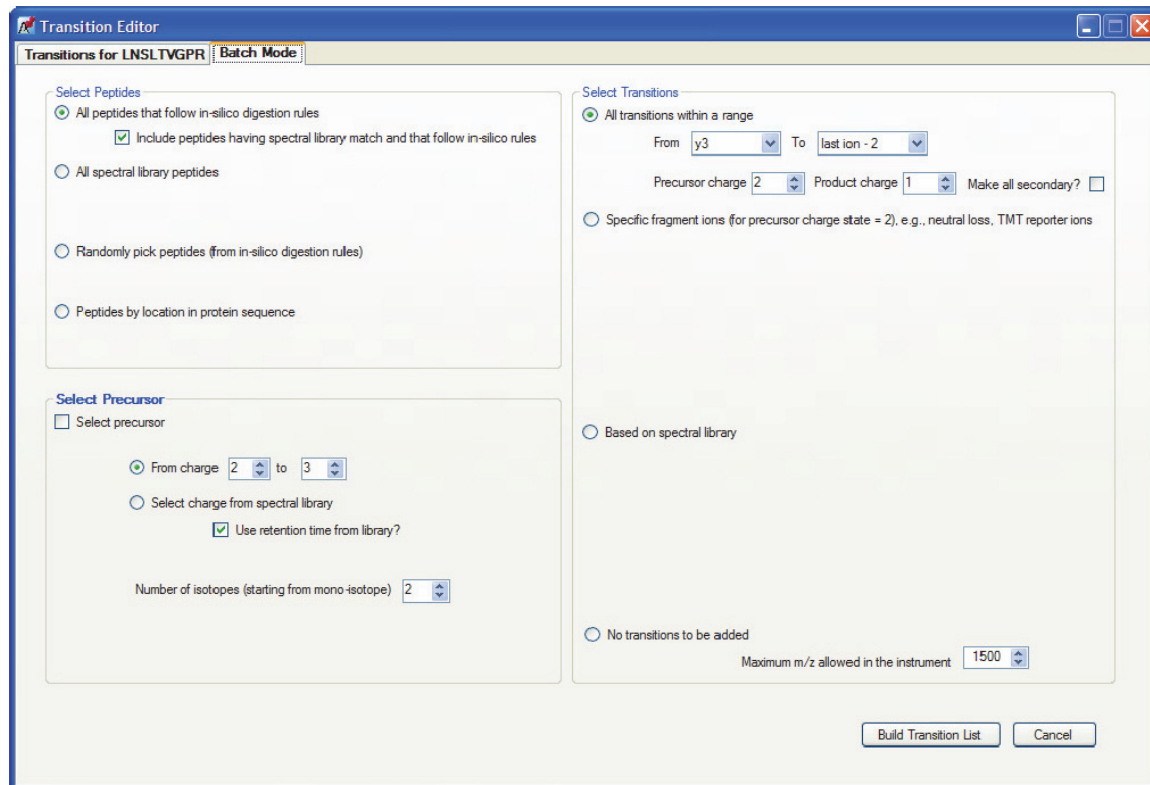


## Step 2: Creating Transitions for Proteins and Peptides

### ❖ To create the transitions for all proteins and peptides in the workbook

1. In the Main Workbook area, automate the transition list creation for all peptides by clicking the **Add/Edit Transitions** icon, .


The Transition Editor dialog box opens.

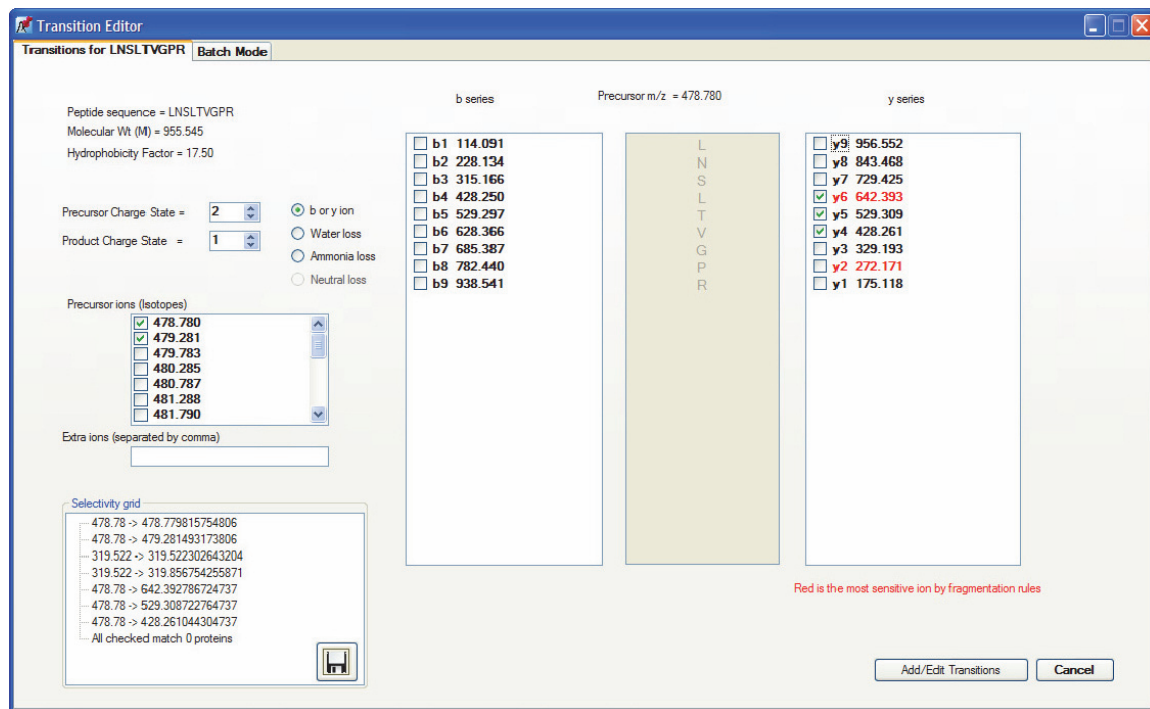


2. Make a selection in the Select Peptides, Select Precursor, and Select Transitions areas.
3. Click **Build Transition List**.

### Step 3: Creating Transitions for Specific Peptides

#### ❖ To create transitions for specific peptides

1. In the Main Workbook area, highlight the peptide.
2. Click the **Add/Edit Transitions** icon, , to open the Transition Editor dialog box.



**Transition Editor**  
Transitions for LNSLTVGPR | Batch Mode

Peptide sequence = LNSLTVGPR  
Molecular Wt (M) = 955.545  
Hydrophobicity Factor = 17.50

Precursor Charge State = 2  
Product Charge State = 1

☒ b or y ion  
☐ Water loss  
☐ Ammonia loss  
☐ Neutral loss

Precursor ions (isotopes):  
☒ 478.780  
☒ 479.281  
☐ 479.783  
☐ 480.285  
☐ 480.787  
☐ 481.288  
☐ 481.790

Extra ions (separated by comma):

Selectivity grid:  
 478.78 -> 478.779815754806  
 478.78 -> 479.281493173806  
 319.522 -> 319.522302643204  
 319.522 -> 319.856754255871  
 478.78 -> 642.392786724737  
 478.78 -> 529.308722764737  
 478.78 -> 428.261044304737  
 All checked match 0 proteins

b series: b1 114.091, b2 228.134, b3 315.166, b4 428.250, b5 529.297, b6 628.366, b7 685.387, b8 782.440, b9 938.541

Precursor m/z = 478.780

L  
N  
S  
L  
T  
V  
G  
P  
R

y series: y9 956.552, y8 843.468, y7 729.425, y6 642.393, y5 529.309, y4 428.261, y3 329.193, y2 272.171, y1 175.118


Red is the most sensitive ion by fragmentation rules

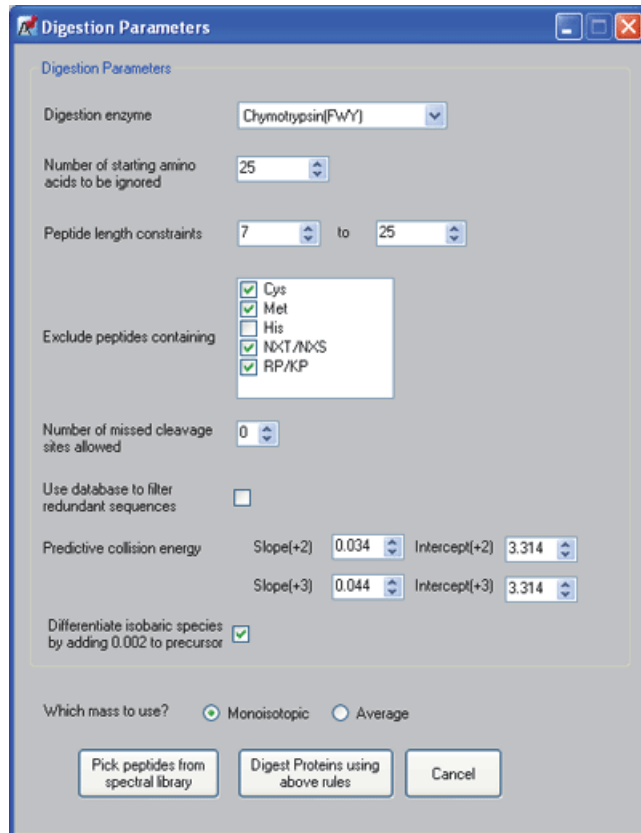
Add/Edit Transitions | Cancel

3. Click the tab with the peptide sequence.
4. Define the transitions to be included in the experimental/processing aspect of the method.
5. Click **Add/Edit Transitions**.

## Step 4: Digesting the Protein(s) into Peptides

### ❖ To specify digestion values for a protein

1. In the Main Workbook area, in the list of proteins, select a protein to digest.
2. Click the **Choose Peptides** icon, , to open the Digestion Parameters dialog box.



**Digestion Parameters**

Digestion enzyme: Chymotrypsin(FWY)

Number of starting amino acids to be ignored: 25

Peptide length constraints: 7 to 25

Exclude peptides containing:

- ☒ Cys
- ☒ Met
- ☐ His
- ☒ NKT/NXS
- ☒ RP/KP

Number of missed cleavage sites allowed: 0

Use database to filter redundant sequences: ☐

Predictive collision energy:

Slope(+2)	0.034	Intercept(+2)	3.314
Slope(+3)	0.044	Intercept(+3)	3.314

Differentiate isobaric species by adding 0.002 to precursor: ☒

Which mass to use? ☒ Monoisotopic ☐ Average

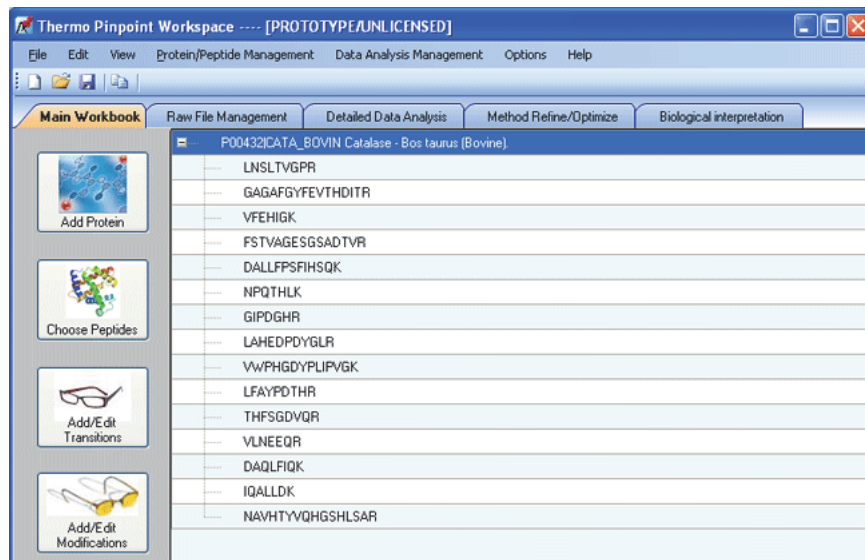
Pick peptides from spectral library    Digest Proteins using above rules    Cancel

3. Do one of the following:
  - Click **Pick Peptides from Spectral Library** and in the Spectral Libraries dialog box that opens, select the **Add Library** option, make other selections, and then click **Apply Library**.

—or—

- Click **Digest Proteins Using Above Rules**.

The peptides are listed in the Main Workbook area below their protein.



Thermo Pinpoint Workspace ---- [PROTOTYPE/UNLICENSED]

File Edit View Protein/Peptide Management Data Analysis Management Options Help


Main Workbook    Raw File Management    Detailed Data Analysis    Method Refine/Optimize    Biological Interpretation

Protein	Peptides
P00432ICATA_BOVIN Catalase - Bos taurus (Bovine)	LNSLTVGPR
	GAGAFGYFEVTHDITR
	VFEHIGK
	FSTVAGESGSADTVR
	DALLFPSFIHSQK
	NPQTHLK
	GIPDGHR
	LAHEDPDYGLR
	VWPHGDYPLIPVGK
	LFAYPDTHR
	THFSGDVQR
	VLNEEQR
	DAQLFIQK
	IQALLDK
	NAVHTYVQHGSLSAR

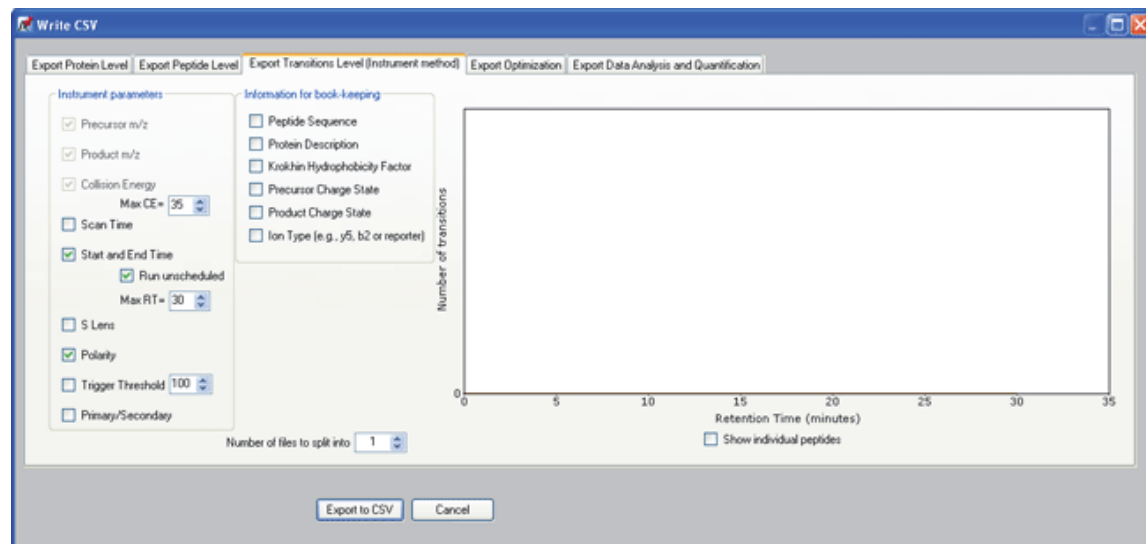
Left sidebar icons: Add Protein, Choose Peptides, Add/Edit Transitions, Add/Edit Modifications

## Step 5: Exporting the Results to a .csv File

### ❖ To export results to a .csv file

1. In the Main Workbook area, select the information you want to save.
2. Click the **Export to CSV** icon, .

The Write CSV dialog box opens to the Export Transitions Level (Instrument method) page.



3. Define the CSV options.
4. Click **Export to CSV**.
5. Browse to select a location for the file.
6. In the Save As box, type a name for the .csv file and click **Save**.
7. In the Main Workbook area, choose **File > Save** and browse to select a location for the workspace information (.vws) file. Use this file when you load the .raw file into the Pinpoint application.

## Step 6: Processing the .csv File on a TSQ Mass Spectrometer

### ❖ To process a .csv file

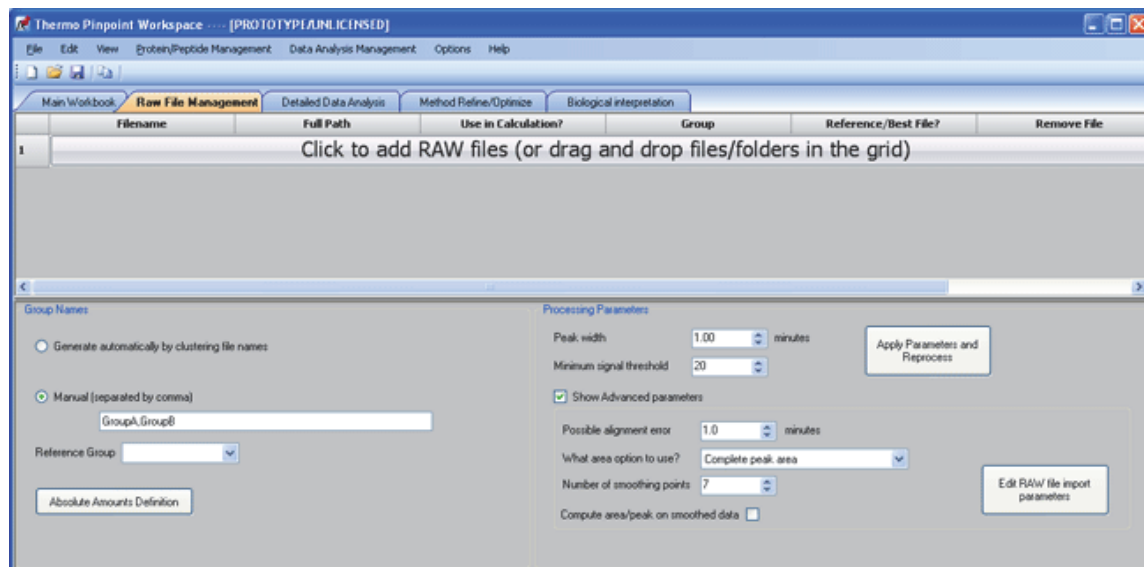
1. Load the .csv file onto the data system computer connected to the mass spectrometer.
2. Process the file.
3. Save the .raw file.



## Step 7: Processing the .raw File

### ❖ To process the .raw file

1. From the Main Workbook area, load the appropriate .vws file containing the targeted proteins, peptides, and targeted quantification transitions used to acquire the .raw file.
2. Click the **Raw File Management** tab to open the Raw File Management page.



3. Click **Click to Add RAW files** and select the data type from the pop-up box. Choose from these options:
  - Data from triple quad experiment
  - Data from trap-based experiment
4. Do one of the following:
  - Browse to open a specific .raw file.

—or—

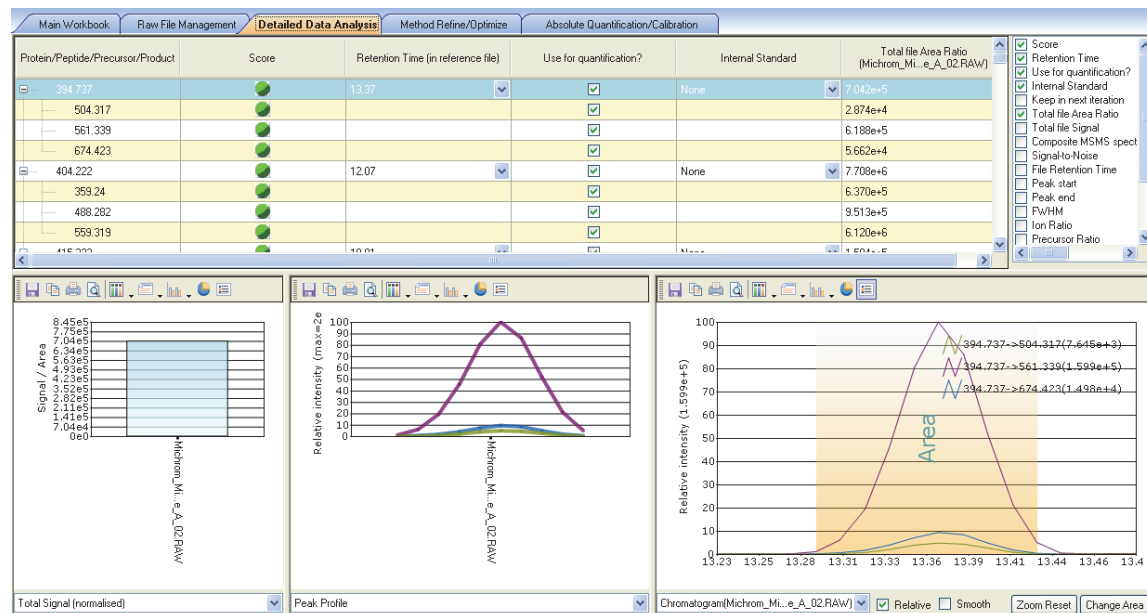
  - Drag files or folders to the Raw File Management page.
5. In the Group Names and Processing Parameters areas, make selections as necessary.
6. Click **Apply Parameters and Reprocess**.

## Step 8: Viewing and Saving the Data Analysis

### ❖ To view data analysis results

1. Click the **Detailed Data Analysis** tab.

The Detailed Data Analysis page displays the .raw file analysis in a results table with graphs.



2. To alter the displays to show the information you want, use the column selections to the right of the table and the icons at the top of each graph.
3. Choose **File > Export to CSV**.
4. Browse to select a location for the file, name the file, and click **Save**.

## Trademarks

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