Peptide Mapping Demo Workflows

Biologics Explorer Software Guidelines

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Peptide Mapping Demo Workflows

CONTENTS OF THIS GUIDE

Part A

Overview of the Peptide Mapping Demo Workflows

A

Overview of the Applications for Peptide Mapping Demo Workflows

- The Peptide Mapping demo workflows contain examples of how to analyze enzymatically digested biotherapeutic molecules, and include:
  - Sequence coverage and confirmation
  - Glycopeptide analysis
  - Post-translational modification (PTM) analysis
  - Disulfide-bond (DSB) analysis
  - Sequence variant analysis (SVA)

- The Pepmap demo workflows **_Simple**, **_Extended**, **_Comparative** and **_SVA** create common peak boundaries across all replicate samples.
- The Pepmap demo workflow **_BatchProcessing** analyses different molecules as individual replicates with no shared peak boundaries.

Overview of the Peptide Mapping Demo Workflows

Pepmap_Simple_Demo:

- A Peptide Mapping workflow for routine characterization, with identification and quantification of common modifications and glycosylations.

Pepmap_Extended_Demo:

- A Peptide Mapping workflow that has more search nodes to maximize sequence coverage and identification of less common modifications.

Pepmap_SVA_Demo:

- A version of the Pepmap_Extended workflow that has more search nodes for identification of potential sequence variants.

Pepmap_Comparative_Demo:

- A version of the Pepmap_Extended workflow that has activity nodes to complete a statistical comparison between sample sets.

Pepmap_ReviewSnapshots_Demo:

- A workflow to open or review saved results.

Pepmap_BatchProcessing_Demo :

- A version of the Pepmap_Extended workflow that analyzes each multiple data files, one sample at a time.

Part B

Information About Peptide Mapping Demo Workflows

B

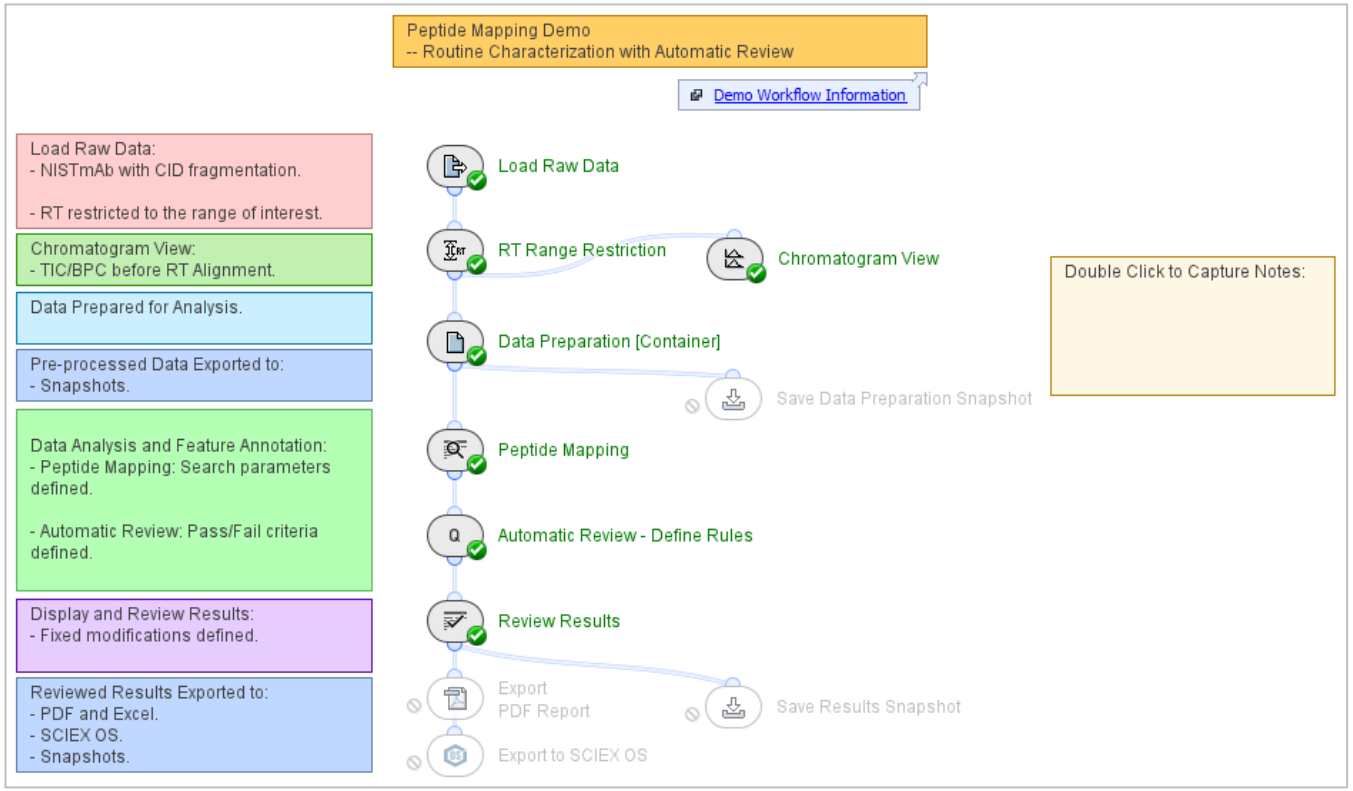
Simple Peptide Mapping

Demo Workflow Information

B1

Overview and Application: Pepmap_Simple_Demo

- This workflow uses data that was acquired with CID fragmentation for a routine analysis of a non-complex biotherapeutic molecule.
- The search parameters in the *Peptide Mapping* activity node are optimized to identify peptides, common post-translational modifications, and glycosylation.
- For more information about how to use Biologics Explorer software, refer to the *Biologics Explorer Quick Guide* and *Peptide Mapping Template Workflow Guidelines*.



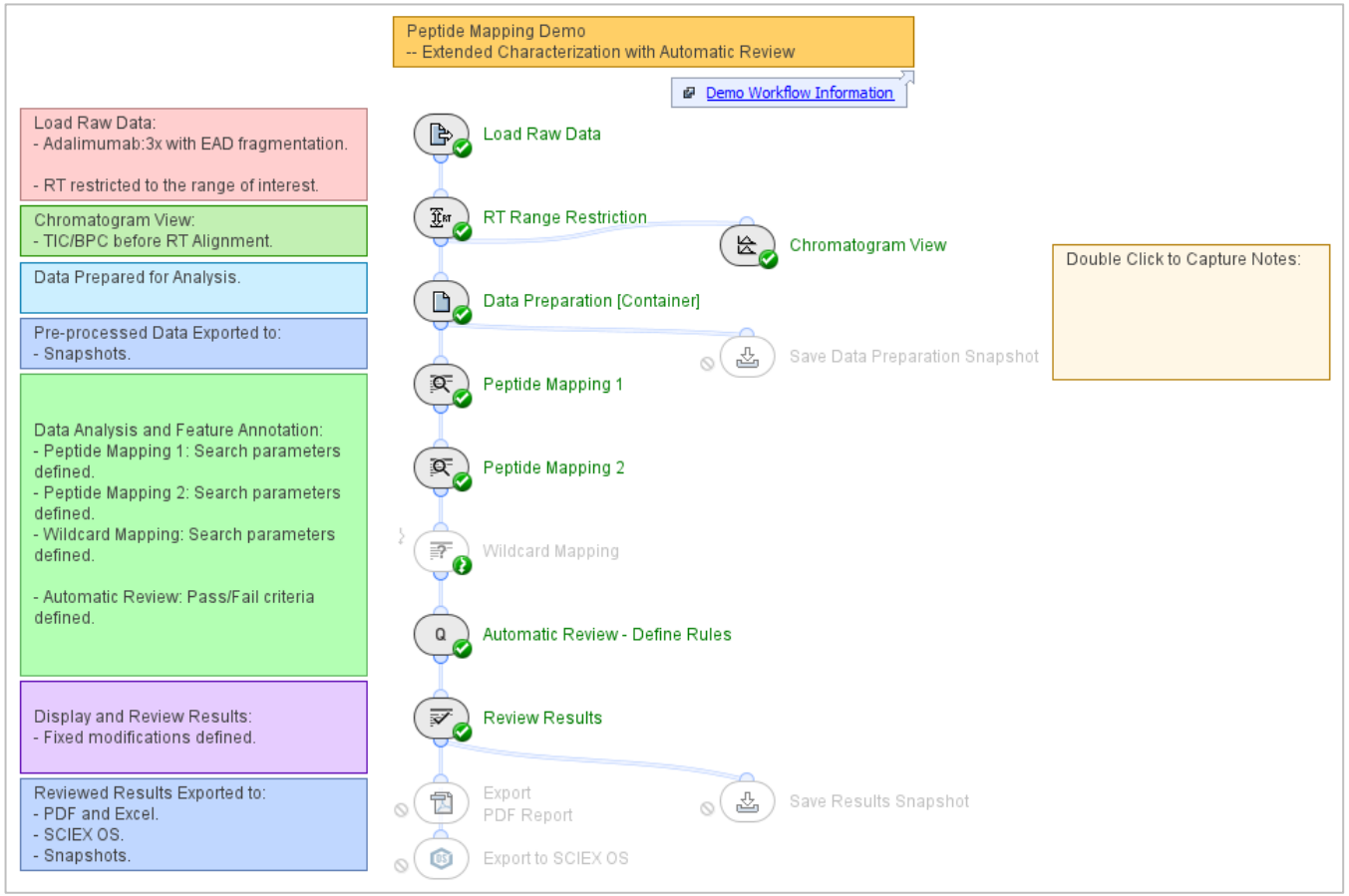
Extended Characterization

Demo Workflow Information

B2

Overview and Application: Pepmap_Extended_Demo

- This workflow uses data that was acquired with EAD fragmentation for a comprehensive Peptide Mapping analysis of three replicates of a biotherapeutic molecule.
- The search parameters in the two *Peptide Mapping* activity nodes are optimized to extend the search space and increase identifications, but keep false positives to a minimum.
- For more information about how to use Biologics Explorer software, refer to the *Biologics Explorer Quick Guide* and *Peptide Mapping Template Workflow Guidelines*.



Stepwise Peptide Mapping

- This workflow uses up to three consecutive search nodes to extend the search space but minimize false positives:

Peptide Mapping 1

– Identifies the most likely peptides and modifications.

Peptide Mapping 2

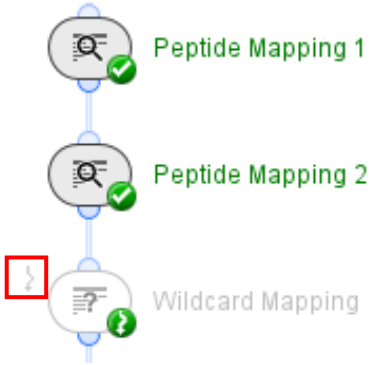
– Identifies less common modifications.

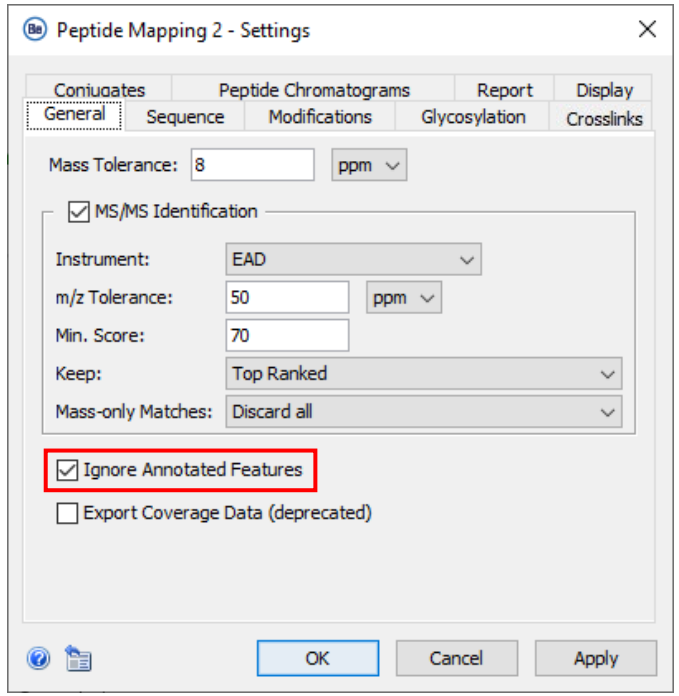
– **Ignore Annotated Features**: Makes sure that only unannotated features from the previous search are considered.

Wildcard Mapping

– Identifies unexpected modifications.

- Deactivate the **Bypass** icon to use *Wildcard Mapping*.
- Add identified modifications to a *Peptide Mapping* activity node.
- Activate the **Bypass** icon when *Wildcard Mapping* is not required.





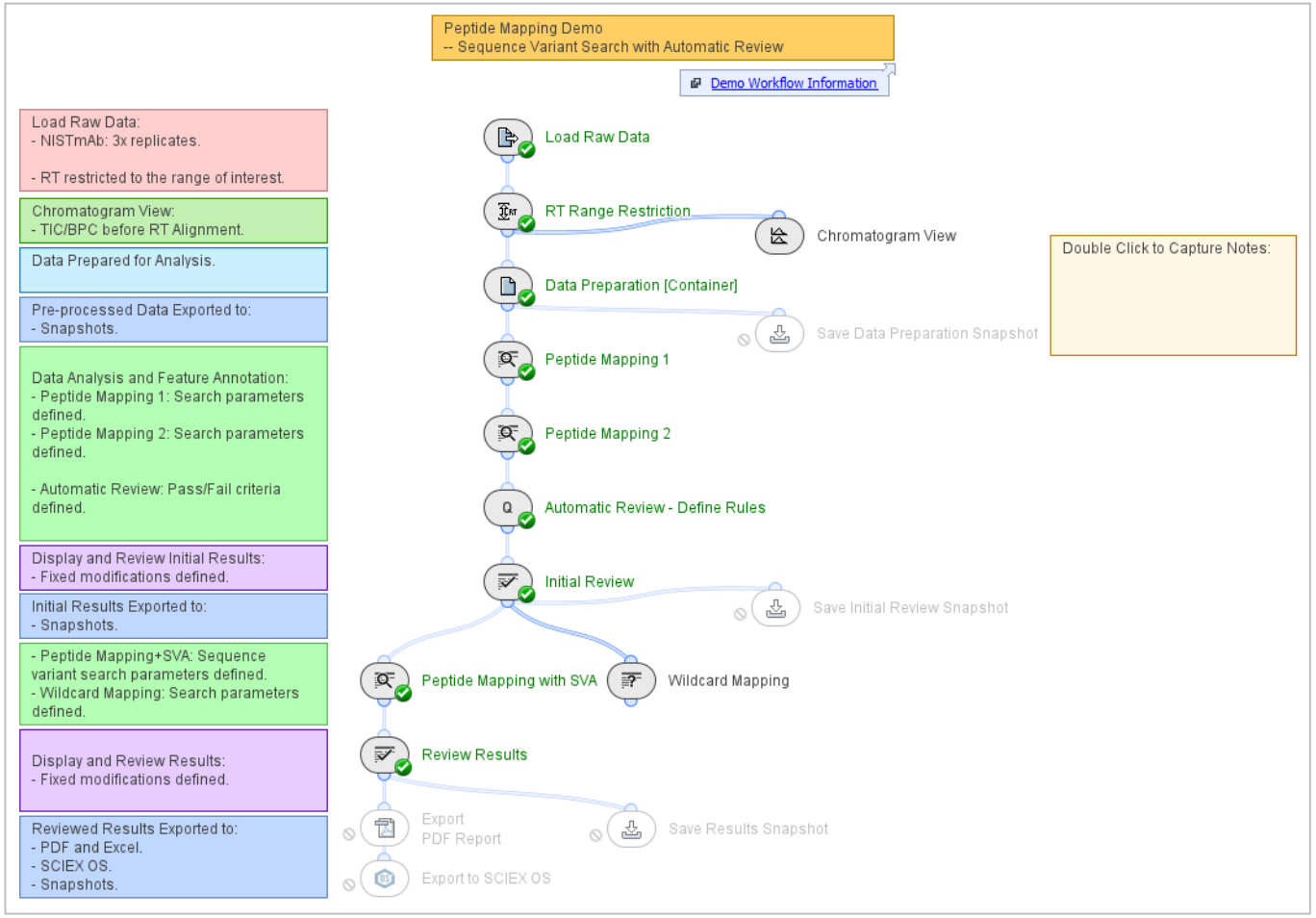
Sequence Variant Search

Demo Workflow Information

B3

Overview and Application: Pepmap_SVA_Demo

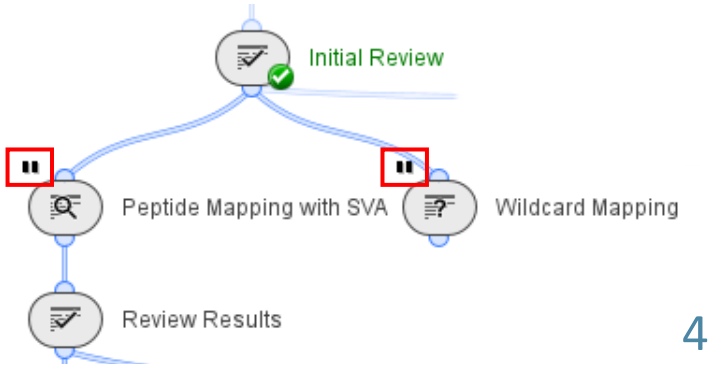
- This workflow is optimized to identify potential sequence variants.
  - Note: High quality MS and MS/MS data is required for confident identification of sequence variants. Instrument acquisition should be optimized for SVA.
- Two consecutive *Peptide Mapping* activity nodes identify the non-variant peptides and remove them from the search space.
- The *Peptide Mapping with SVA* activity node searches for sequence variants.
- Possible SVA identifications can be compared with the results from the *Wildcard Mapping* activity node for verification.
- For more information about how to use Biologics Explorer software, refer to the *Biologics Explorer Quick Guide* and *Peptide Mapping Template Workflow Guidelines*.



SVA Identification Strategy

To detect potential sequence variants:

1. Use *Peptide Mapping 1* and *Peptide Mapping 2* to complete a typical analysis for non-variant peptides.
   - Refine the *Peptide Mapping* settings for the molecule under investigation.
     - Note: For more information about stepwise Peptide Mapping, refer to the section: **B:** 2.Extended Peptide Mapping Demo Workflow.
   - Identified peptides are removed from the search space, which decreases false positives in Stage 3.
2. Complete an initial review of the data.
   - Click the **Save** icon, and then click **Save and Reload**.
   - Features from the rejected annotations are searched again with the *Peptide Mapping with SVA* activity node.



3. Deactivate the **Pause** icons on the *Peptide Mapping with SVA* and *Wildcard Mapping* activity nodes, and then click the **Play** icon to run them.
   - To increase the number of possible identifications, decrease the **Min. Score** in *Peptide Mapping with SVA* and *Wildcard Mapping*. A lower **Min. Score** increases the false positives for review. Manually review identifications that are close to the **Min. Score** threshold to reject incorrect annotations
4. Compare identifications in *Peptide Mapping with SVA* and *Wildcard Mapping*.
5. Review, and then **Accept** and **Reject** entries in the **Peptide Table** as required.

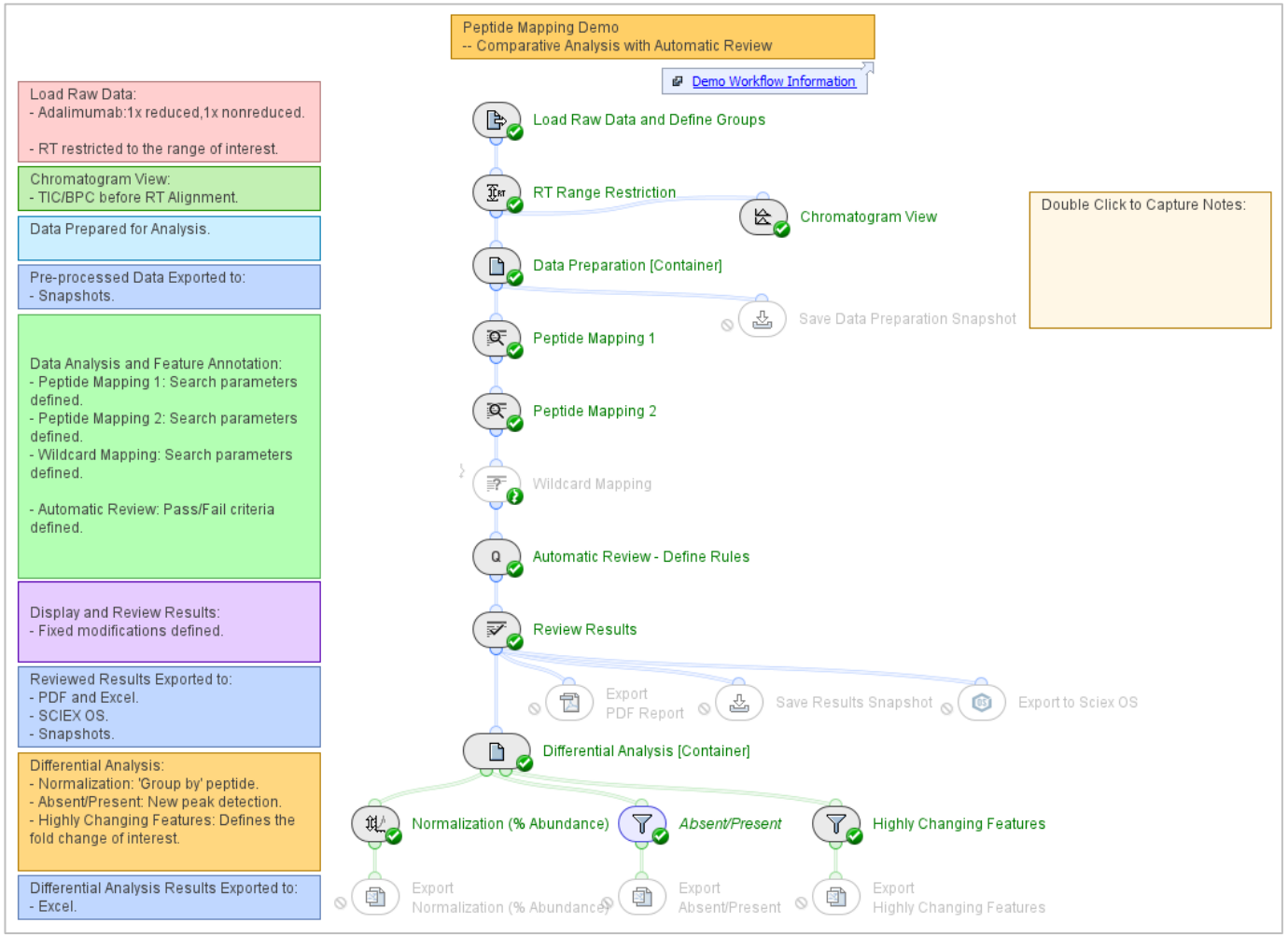
Comparative Analysis

Demo Workflow Information

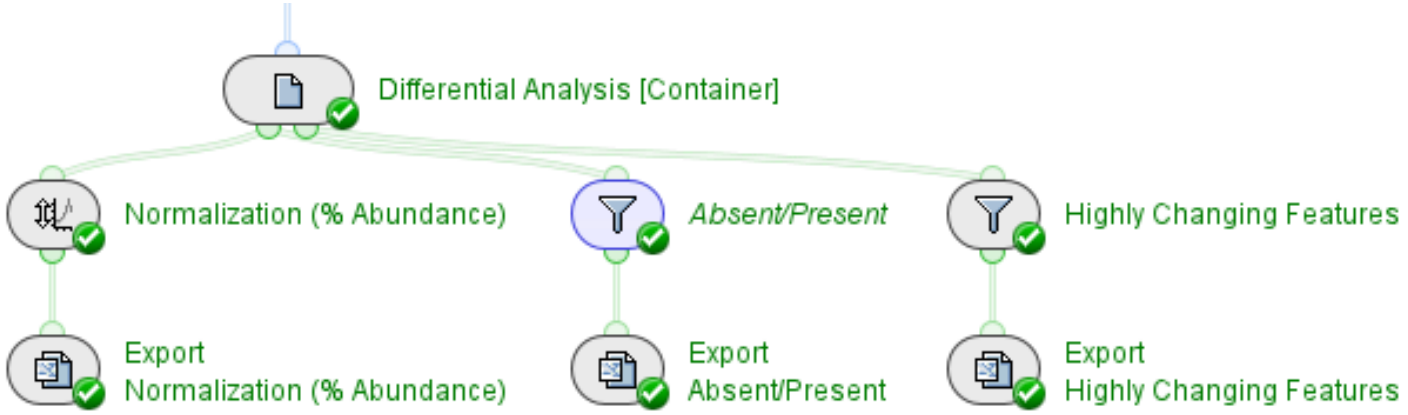
B4

Overview and Application: Pepmap_Comparative_Demo

- This workflow compares data from a reduced and nonreduced biotherapeutic molecule to identify crosslinkers.
- The search parameters in the two *Peptide Mapping* activity nodes are optimized to identify reduced and nonreduced peptides with PTMs and glycosylation.
- The *Automatic Review* activity node adds a flag to unexpected crosslinked peptides.
- The *Differential Analysis [Container]* prepares the data for comparisons to be made.
- For more information about how to use Biologics Explorer software, refer to the *Biologics Explorer Quick Guide* and *Peptide Mapping Template Workflow Guidelines*.



Differential Analysis



The statistical activity nodes identify features that are different between the two sample groups that are compared in the workflow.

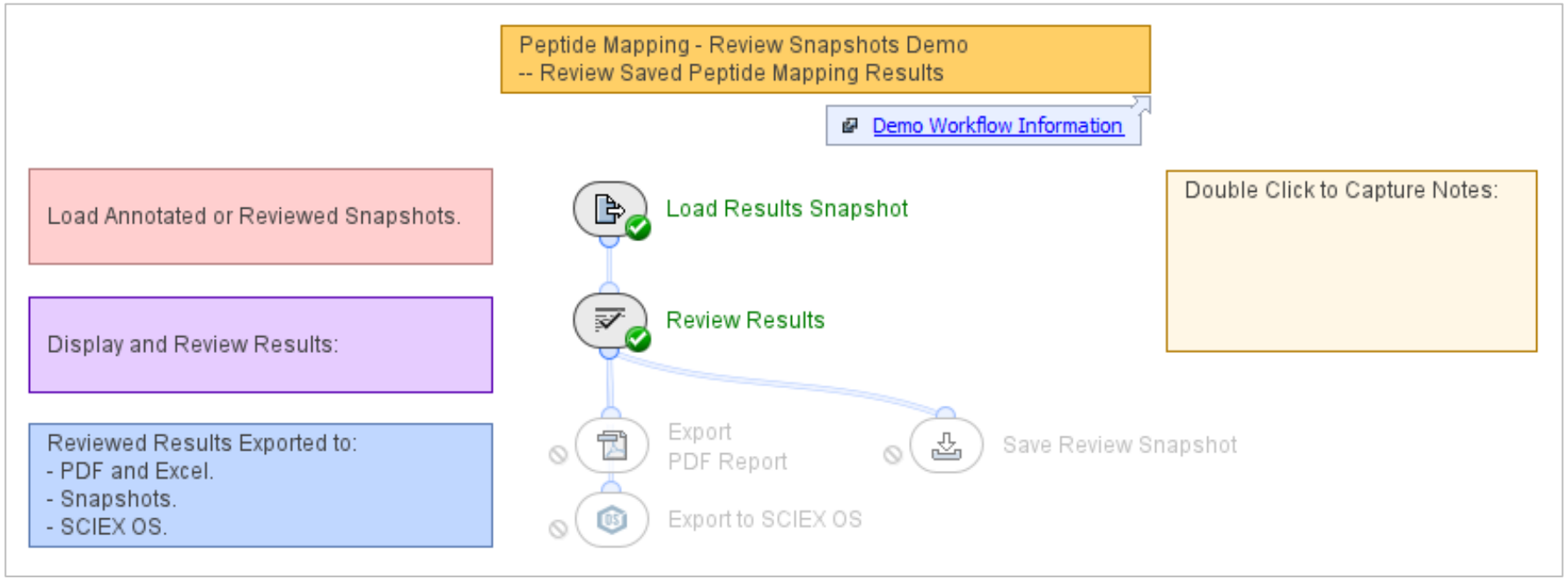
- The activity nodes connected with green lines contain statistical tools that can be used to compare two datasets.
- The workflow reports:
  - The relative (%) abundance of peptides in each dataset.
  - The peptides that are absent in one sample set, but present in the other.
  - The peptides that have a specified fold-change difference between sample sets.

Review Snapshots

Demo Workflow Information

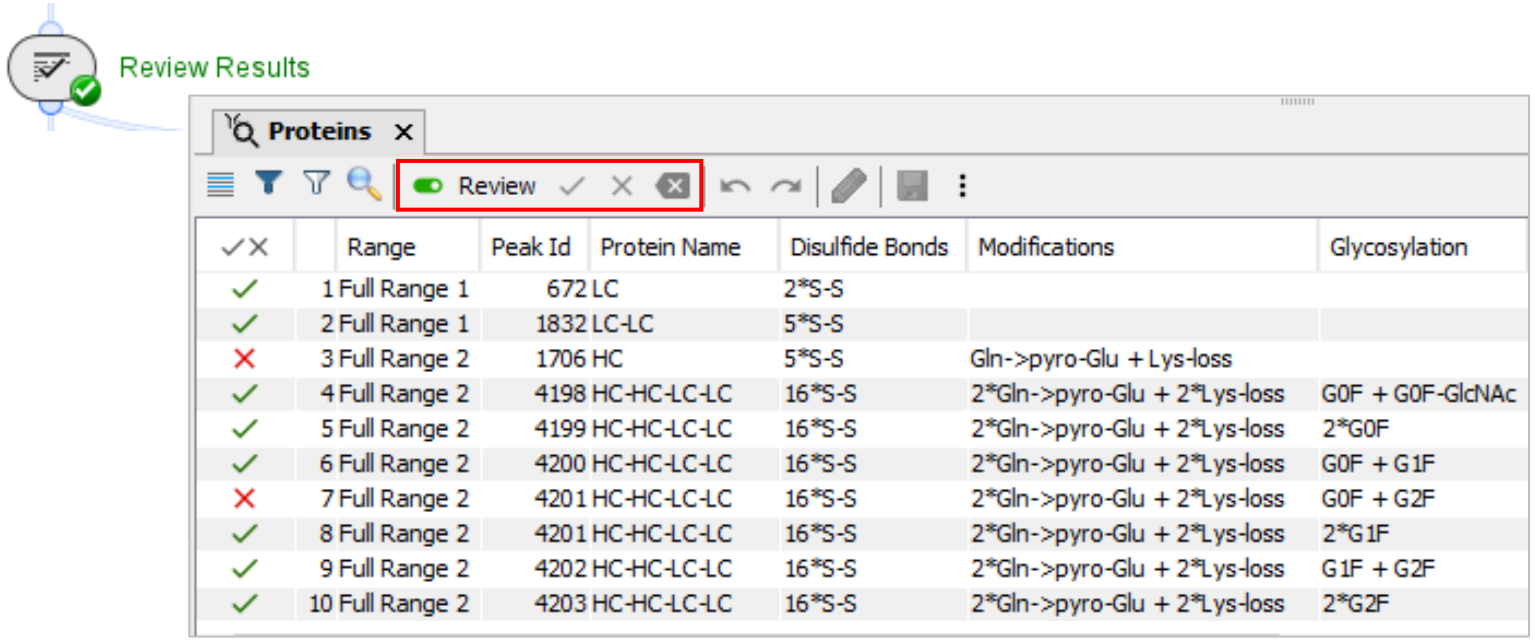
B5

Overview and Application: Pepmap_ReviewSnapshots_Demo



- This workflow uses saved Snapshots to show results that have peptide annotations.
- For more information about how to use Biologics Explorer software, refer to the *Biologics Explorer Quick Guide* and *Peptide Mapping Template Workflow Guidelines*.

Review Saved Results



✓✗		Range	Peak Id	Protein Name	Disulfide Bonds	Modifications	Glycosylation
✓	1	Full Range 1	672	LC	2*S-S		
✓	2	Full Range 1	1832	LC-LC	5*S-S		
✗	3	Full Range 2	1706	HC	5*S-S	Gln->pyro-Glu + Lys-loss	
✓	4	Full Range 2	4198	HC-HC-LC-LC	16*S-S	2*Gln->pyro-Glu + 2*Lys-loss	G0F + G0F-GlcNAc
✓	5	Full Range 2	4199	HC-HC-LC-LC	16*S-S	2*Gln->pyro-Glu + 2*Lys-loss	2*G0F
✓	6	Full Range 2	4200	HC-HC-LC-LC	16*S-S	2*Gln->pyro-Glu + 2*Lys-loss	G0F + G1F
✗	7	Full Range 2	4201	HC-HC-LC-LC	16*S-S	2*Gln->pyro-Glu + 2*Lys-loss	G0F + G2F
✓	8	Full Range 2	4201	HC-HC-LC-LC	16*S-S	2*Gln->pyro-Glu + 2*Lys-loss	2*G1F
✓	9	Full Range 2	4202	HC-HC-LC-LC	16*S-S	2*Gln->pyro-Glu + 2*Lys-loss	G1F + G2F
✓	10	Full Range 2	4203	HC-HC-LC-LC	16*S-S	2*Gln->pyro-Glu + 2*Lys-loss	2*G2F

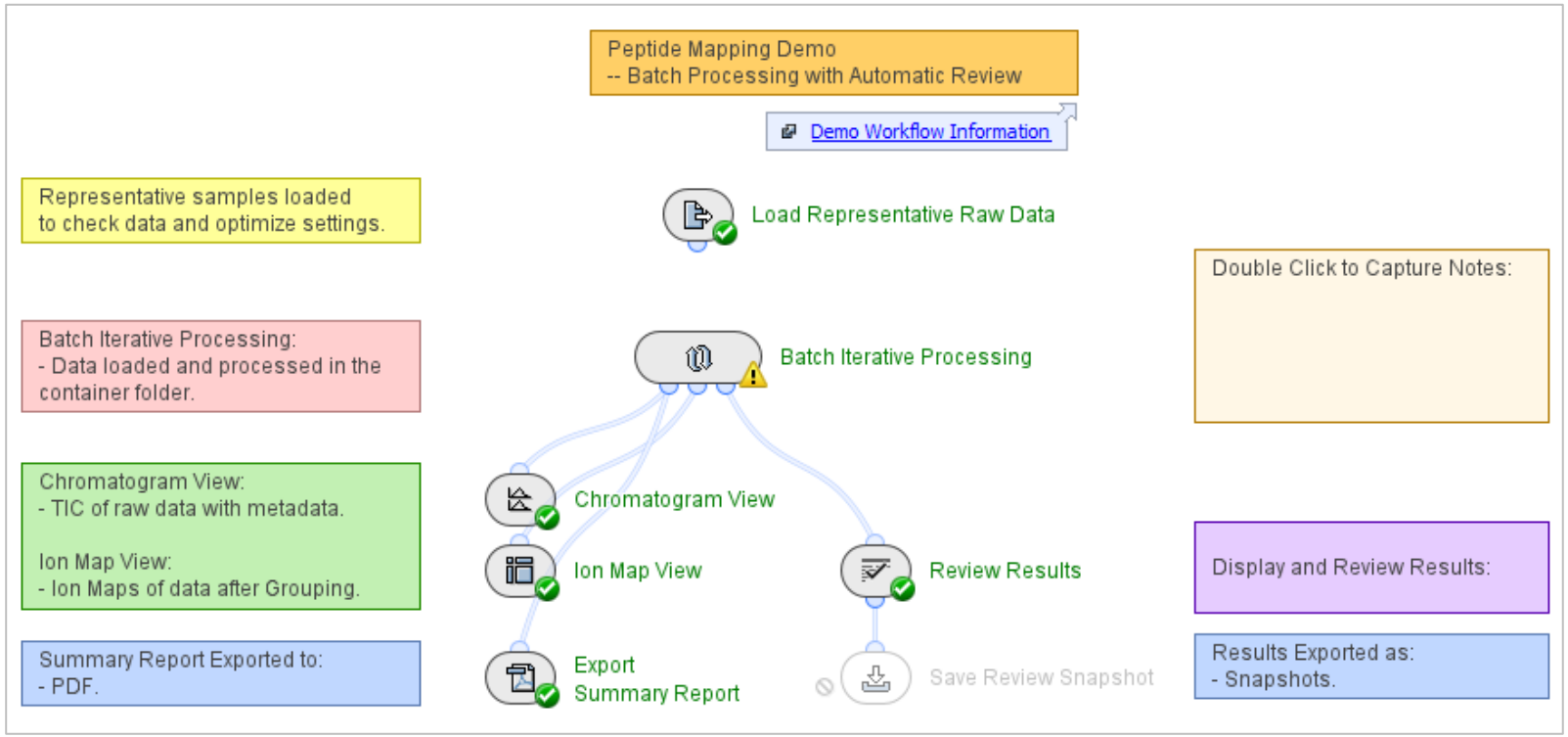
- The *Review Results* activity node opens a copy of the previous analysis.
  - Any previously accepted or rejected peptides have the applicable entry in the **Flags** column.
  - Another stage of review is then possible.
  - The reviewed sbf files and a new report can be saved.

Peptide Mapping Batch Processing

Demo Workflow Information

B6

Overview and Application: Pepmap_BatchProcessing_Demo



- This workflow uses sequence information from metadata that was imported into the workflow to analyze multiple samples independently.
- For more information about how to use Biologics Explorer software, refer to the *Biologics Explorer Quick Guide* and *Peptide Mapping Template Workflow Guidelines*.

How to Use the Batch Processing Workflow

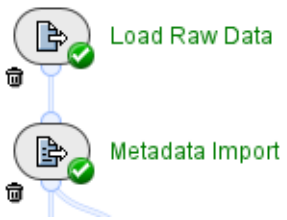
Use this workflow to analyze multiple samples independently.

- The samples do not require consistent chromatography.
- The samples can have different protein sequences.

All samples and their associated metadata are analyzed in the *Batch Iterative Processing* container.

- Intermediate results for each sample are not saved when the *Batch Iterative Processing* container is used for data analysis.
- To optimize workflow parameters, deactivate the **Trash** icon for activity nodes in the *Batch Iterative Processing* container, and use a single representative sample.
- To save memory when large numbers of samples are analyzed, activate the **Trash** icon for activity nodes in the *Batch Iterative Processing* container.

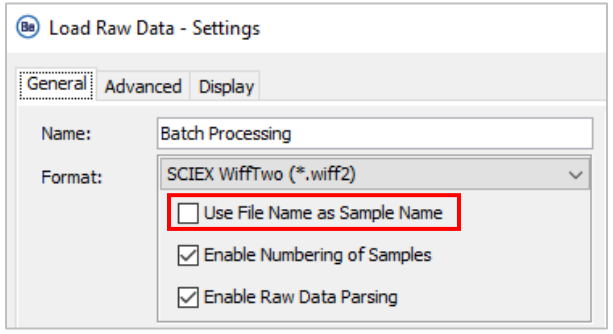
Load Raw Data: Experiment Names and Metadata

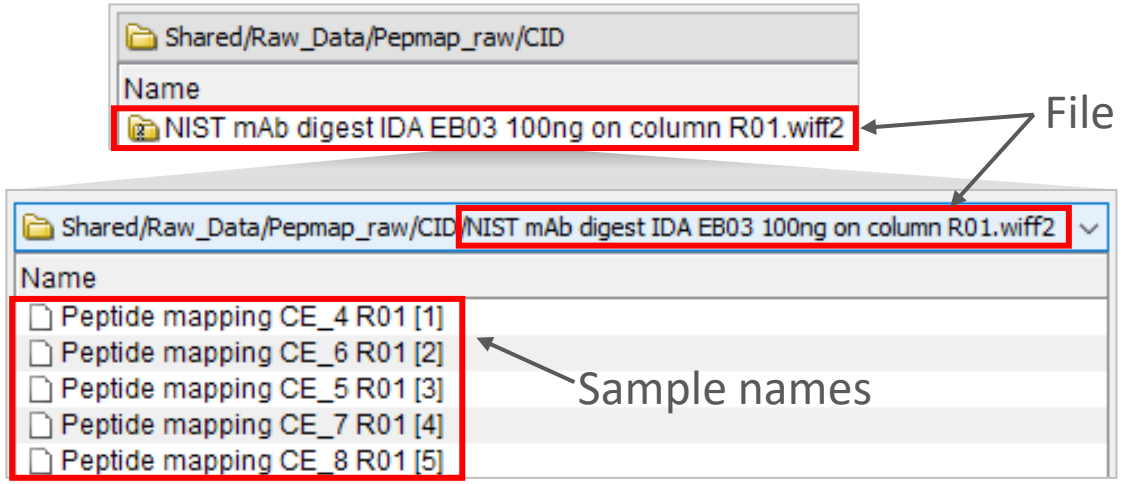


To analyze replicate samples from different acquisition files:

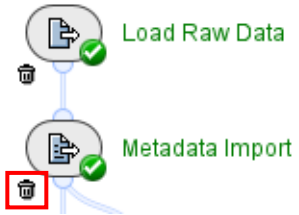
1. Select **Use File Name as Sample Name** in *Load Raw Data*.
2. Use the **File Name** (name of the wiff or wiff2 container file) in the **Experiment** column of the txt file for *Metadata Import*.

- To analyze multiple samples from a single acquisition file:
  1. Do not select **Use File Name as Sample Name** in *Load Raw Data*.
  2. Use the **Sample Name** in the **Experiment** column of the txt file for *Metadata Import*.





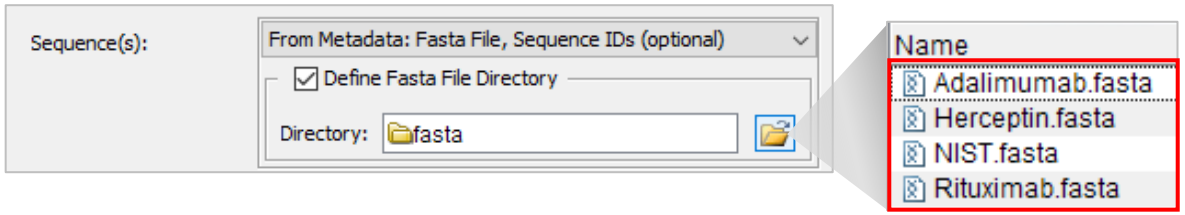
Metadata Import



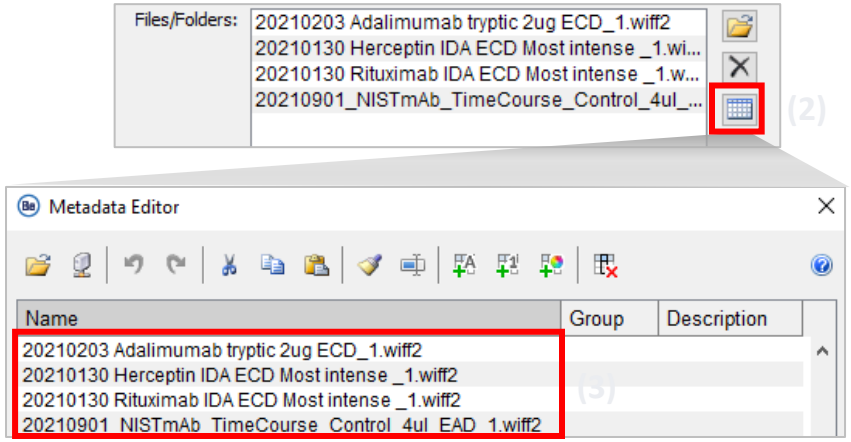
- To analyze multiple samples with the same sequence:
  - Deactivate the **Trash** icon, and then activate the **Bypass** icon for *Metadata Import*.
  - On the **Sequences** tab in *Peptide Mapping*, select **From Text** or **From Fasta File**.
- To analyze multiple samples with different sequences:
  - Use *Metadata Import* to select the FASTA file (protein sequence) that will be used for identification in the *Peptide Mapping* activity nodes.

- Upload a txt file with *Metadata Import* that links each sample to the correct FASTA file.
  - The name in the **Experiment** column must be the same as in the **Experiment** table in *Load Raw Data*.
  - The name in the **Fasta File** column must be the same as the name of the FASTA file that is in the specified **Fasta File Directory**, including the file extension (fasta or txt).

	A	B
1	Experiment	Fasta File
2	20210203 Adalimumab tryptic 2ug ECD_1	Adalimumab.fasta
3	20210130 Herceptin IDA ECD Most intense _1	Herceptin.fasta
4	20210130 Rituximab IDA ECD Most intense _1	Rituximab.fasta
5	20210901_NISTmAb_TimeCourse_Control_4ul_EAD_1	NIST.fasta



Metadata Import: How to Create the Metadata File



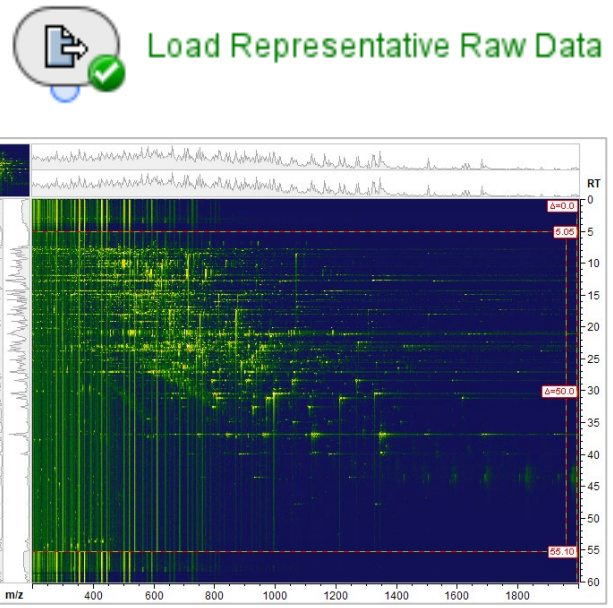
Note: Any metadata added in the *Load Raw Data* **Metadata Editor** table must be completed for all rows (all samples).

Experiment	Fasta File
20210203 Adalimumab tryptic 2ug ECD_1	Adalimumab.fasta
20210130 Herceptin IDA ECD Most intense _1	Herceptin.fasta
20210130 Rituximab IDA ECD Most intense _1	Rituximab.fasta
20210901_NISTmAb_TimeCourse_Control_4ul_EAD_1	NIST.fasta

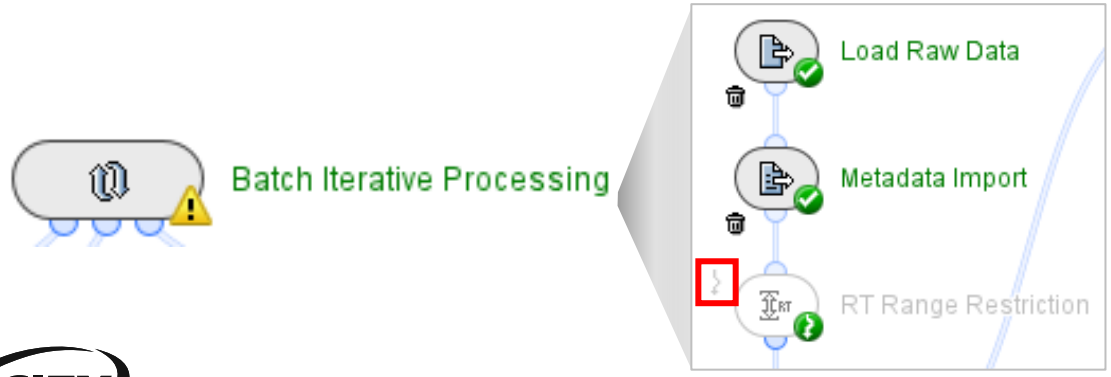
(4)

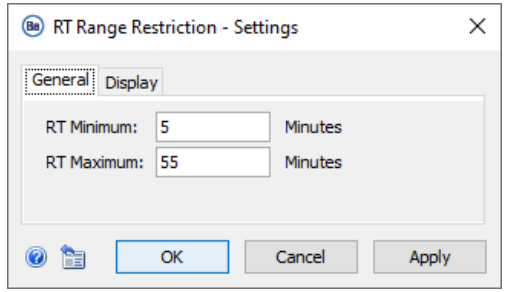
- To create the metadata file in Excel or Notepad:
  1. Select the samples for batch processing in *Load Raw Data*.
  2. Open the **Metadata Editor** table.
  3. Select all of the entries in the **Metadata Editor** table, and then select copy.
  4. Paste the entries into the **Experiment** column of the metadata txt file.
  5. Delete ".wiff" or ".wiff2" from the end of each name. (Tip: Use the Replace command in Excel or Notepad.)
  6. Type the applicable FASTA file name in each row in the **Fasta File** column.
  7. Save the file in txt format, and then upload the file in the *Metadata Import* activity node.

Restrict the RT Range



- Use the *Load Representative Raw Data* activity node to review a small number of representative samples outside of the *Batch Iterative Processing* container.
  - To identify the RT ranges where there is meaningful data, open (double-click) *Load Representative Raw Data* after the data is loaded.
- If the RT ranges are consistent across all samples, then deactivate the **Bypass** icon and enter **RT Minimum** and **RT Maximum** values in the *RT Range Restriction* activity node in the *Batch Iterative Processing* container.

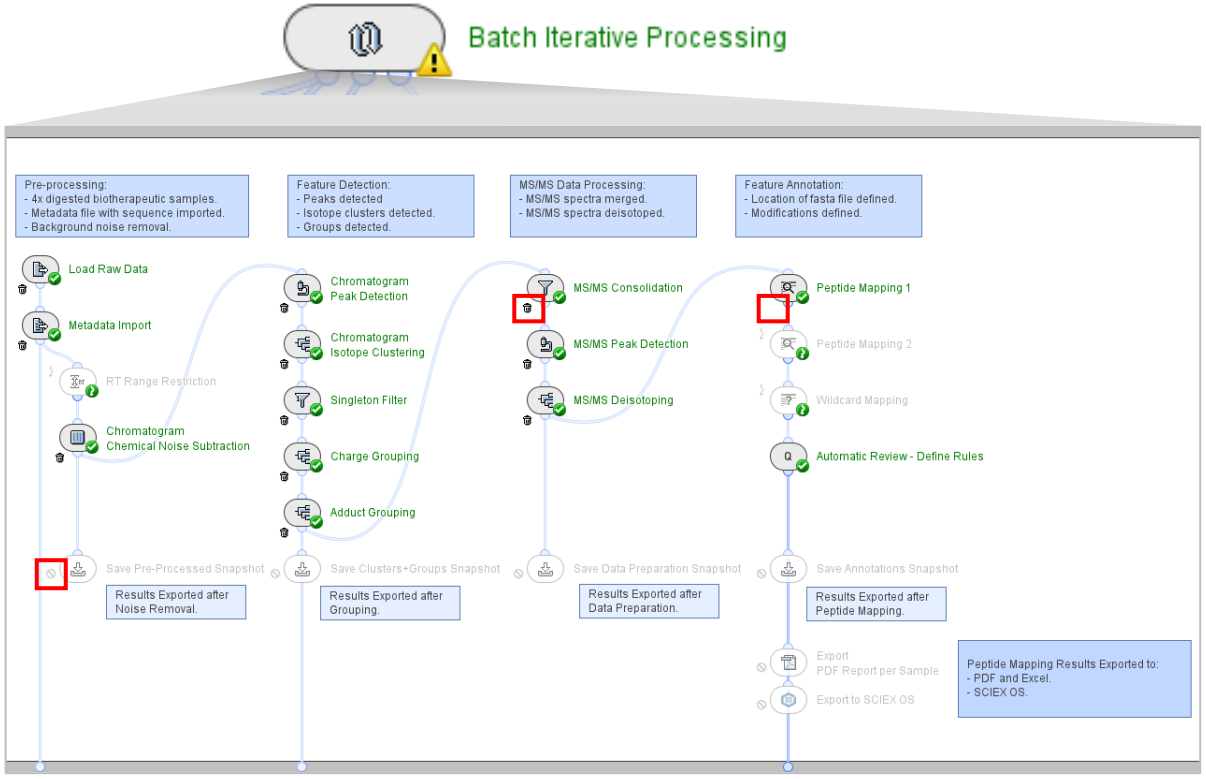




Note: If the fields are blank, or if *RT Range Restriction* has the **Bypass** icon activated, then the full RT range is used.

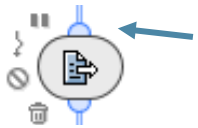
Batch Iterative Processing Container

- The *Batch Iterative Processing* container is not the same as other Biologics Explorer software containers.
  - Only intermediate results from the last sample to be processed can be opened from the activity nodes in the *Batch Iterative Processing* container.



Note: If activity nodes in the container have the **Bypass** icon activated, then the container shows a yellow warning symbol.

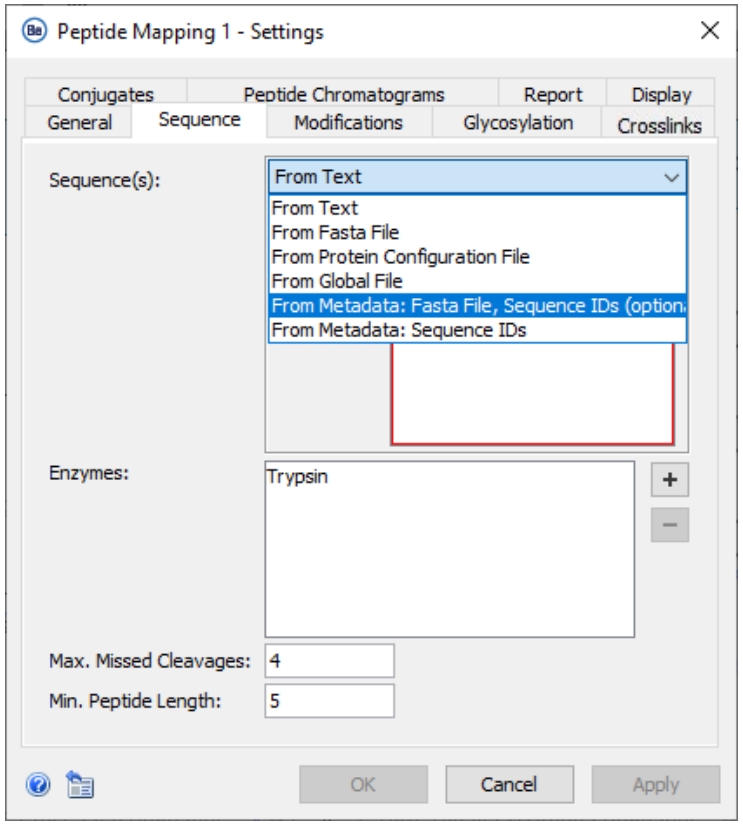
- To open the intermediate results of an activity node, deactivate the **Trash** icon before the workflow is started.
- Do not activate the **Trash** icon for activities that are used in the PDF Report.



Activity nodes in the *Batch Iterative Processing* container do not have a **Run** or **Reset** icon.

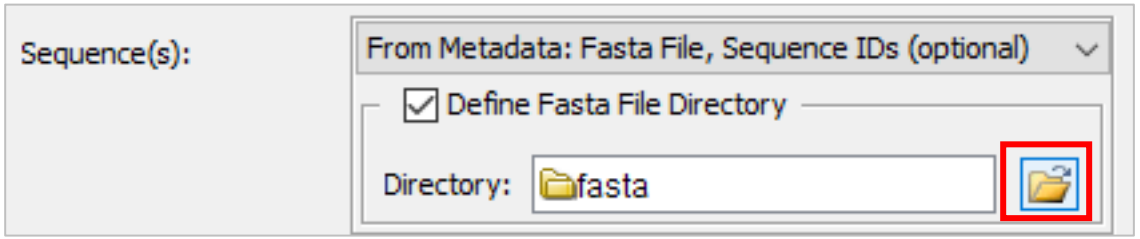
- Activity nodes in the *Batch Iterative Processing* container cannot be run individually.
  - To use a *Save Snapshot* or *Export* activity node in the *Batch Iterative Processing* container, deactivate the **Block** icon before the workflow is started.

Peptide Mapping: Sequences



Sequence tab:

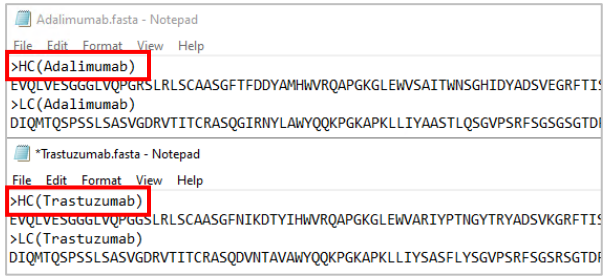
- **Sequence(s)**:
  - If all samples have the same sequence, then select **From Text** and type the sequence, or **From Fasta File** and select the applicable file.
  - If different samples require different sequences, then select **From Metadata: Fasta File, Sequence IDs (optional)**, and then browse to the location of the folder that contains all of the applicable FASTA files.



  - For more information, refer to the next page: *Review Results*: Protein Name in FASTA Files.
- **Enzymes**:
  - Adjust enzyme specificity, maximum number of missed cleavages, and minimum peptide length as required.

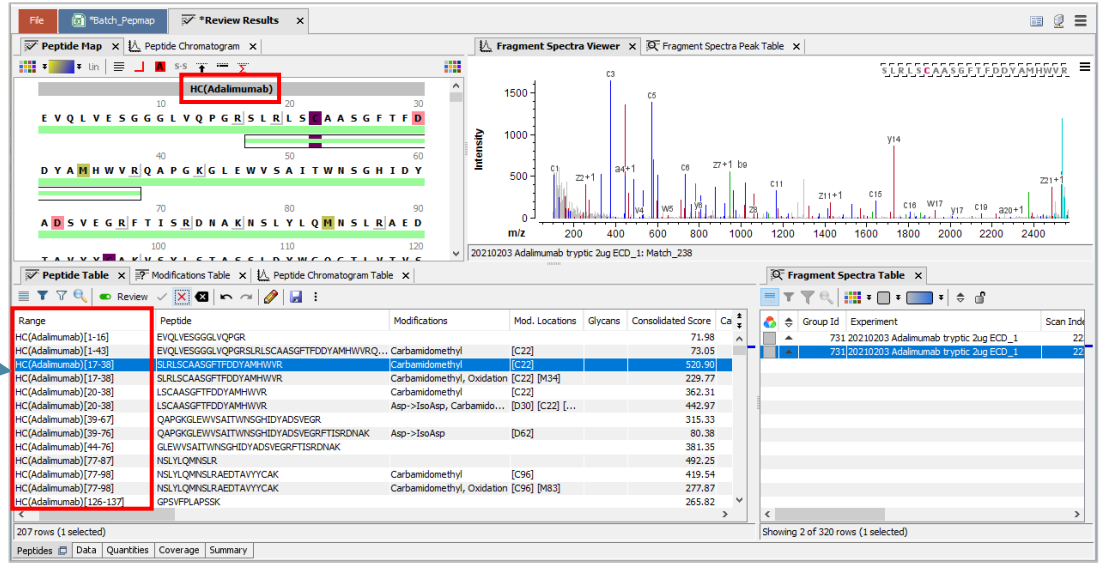
Review Results: Protein Name in FASTA Files

If the protein sequence names <u>are unique</u> across the FASTA files used for identification:

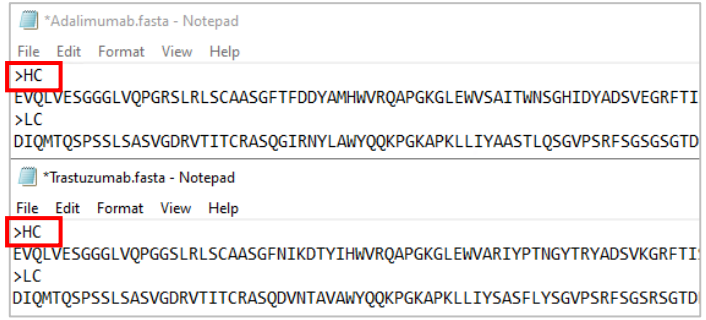


– The protein sequence name in *Review Results* is the same as the name in the FASTA file.

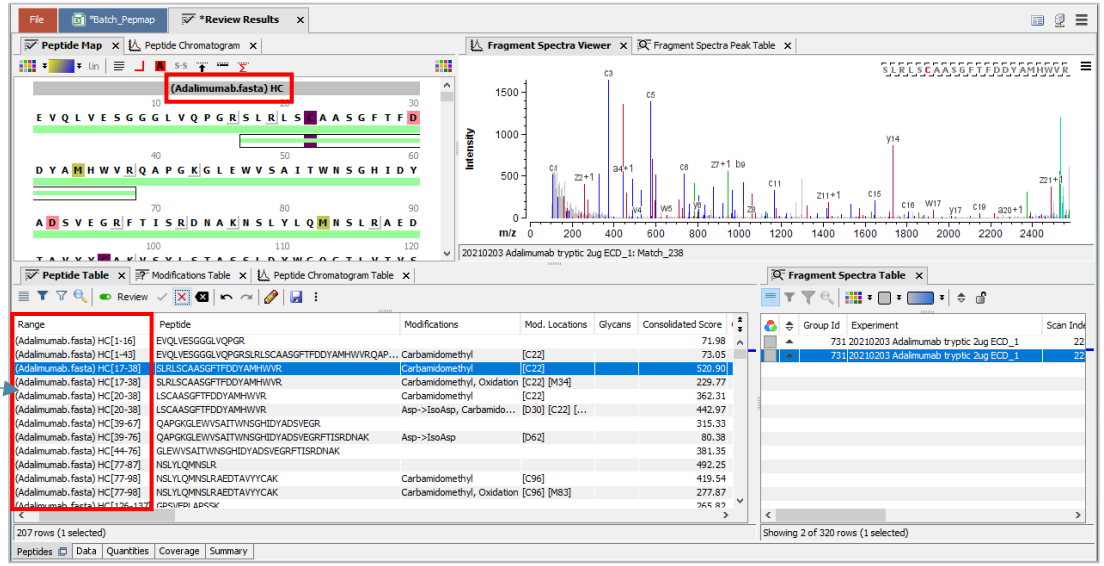
Note: If the names are too long, then some table columns in the PDF report might be missing.



- If the protein sequence names <u>are not unique</u> across the FASTA files used for identification:



– The protein sequence name in *Review Results* includes the FASTA file name.



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