Top/Middle-Down Protein Sequencing Demo Workflows

Biologics Explorer Software Guidelines

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Top/Middle-Down Protein Sequencing Demo Workflows

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Part A

Overview of the Protein Sequencing Demo Workflows

A

Overview of the Applications for Protein Sequencing Demo Workflows

- The Protein Sequencing demo workflows contain examples of how to analyze top-down or middle-down protein sequencing data of:
  - Antibody subunits. For example, Fd', Fc/2, and LC after IdeS digestion and reduction of disulfide bonds.
  - Antibody chains. For example, HC and LC after reduction of disulfide bonds.
  - Proteins with masses to a maximum of approximately 50 kDa.
- Data quality for protein sequencing can be increased if multiple consistent technical replicates are analyzed at the same time.
  - If the overall signal intensity is good, with a satisfactory signal-to-noise ratio, then a single sample can be sufficient for analysis.
- The **Top_Middle-Down_ProteinSequencing_Demo** workflow analyzes each antibody subunit or chain separately.
- The **Top_Middle-Down_ReviewSnapshots_Demo** reviews all of the results together.

Overview of the Protein Sequencing Demo Workflows

Top_Middle-Down_ProteinSequencing_Demo:

- A workflow for top-down characterization of small proteins or middle-down characterization of reduced and IdeS digested biotherapeutic molecules.

Top_Middle-Down_ReviewSnapshots_Demo :

- A workflow to review results of all subunits and show total sequence coverage from different fragmentation types.

Part B

Information About Protein Sequencing Demo Workflows

B

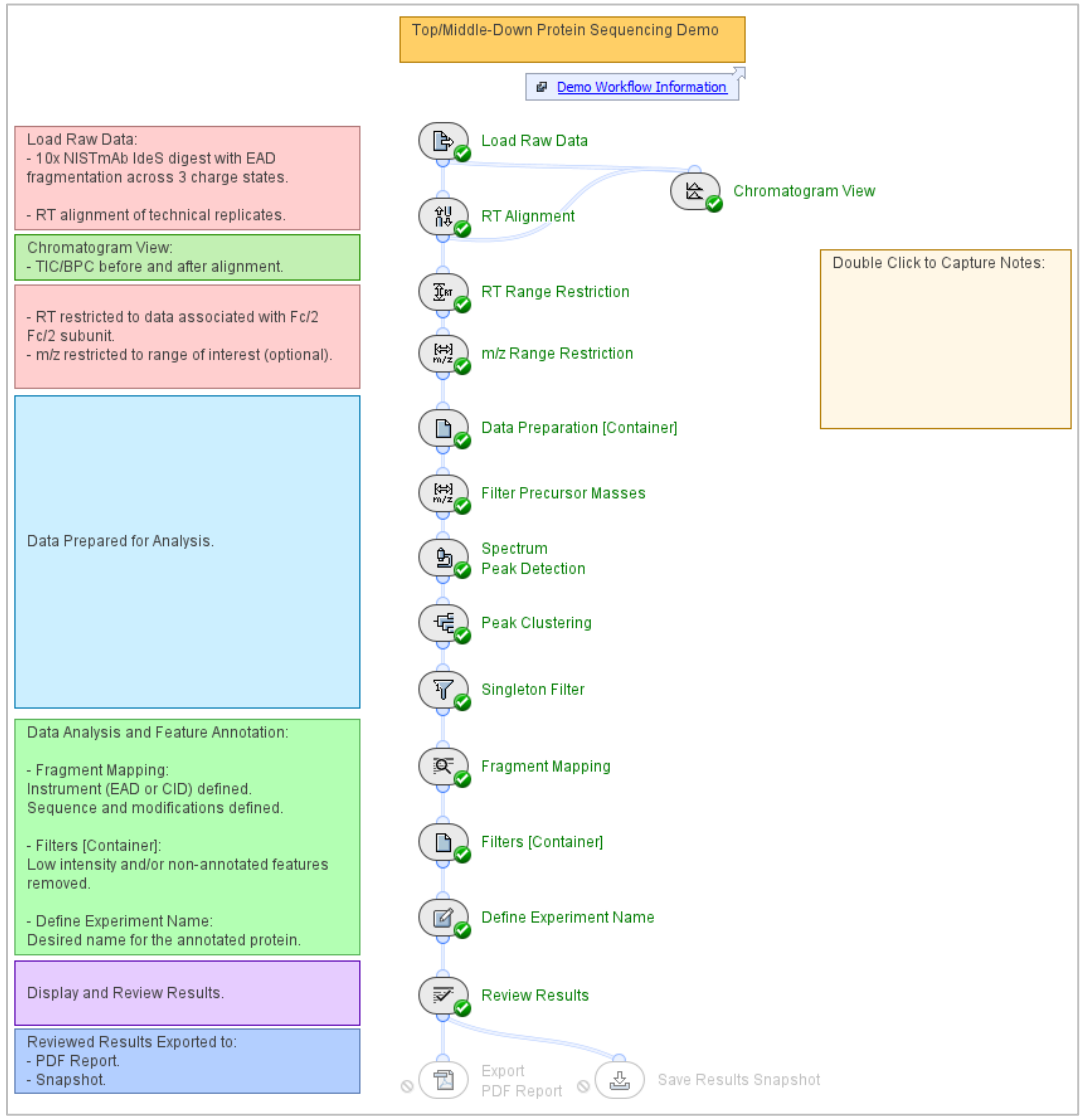
Protein Sequencing

Demo Workflow Information

B1

Overview and Application: Top_Middle-Down_ProteinSequencing_Demo

- This workflow uses data from 10 replicate injections of a biotherapeutic molecule after reduction and IdeS digestion.
- Each subunit (LC, Fd', Fc/2) is analyzed separately.
- MS/MS fragmentation data from the 3 selected charge states is summed together, and then averaged for each subunit.
- The search parameters in the *Fragment Mapping* activity node are optimized to identify MS/MS fragments, with post-translational modifications, and glycosylation at fixed positions.
- For more information about how to use Biologics Explorer software, refer to the *Biologics Explorer Quick Guide* and *Top/Middle-Down Protein Sequencing Template Workflow Guidelines*.

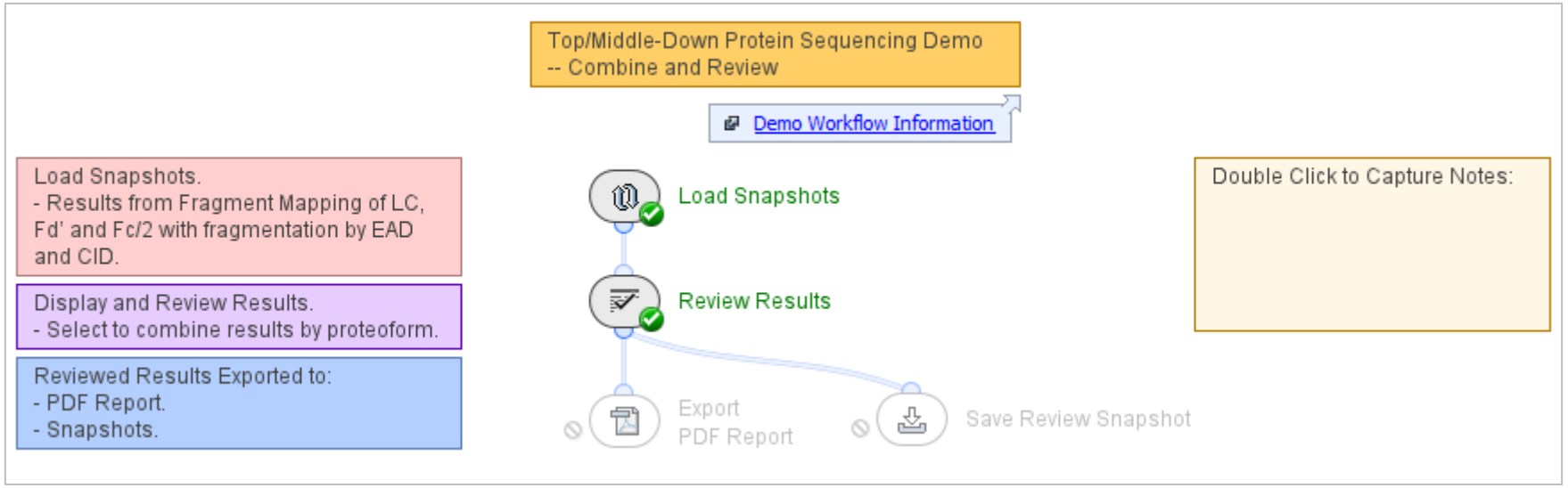


Protein Sequencing Review

Demo Workflow Information

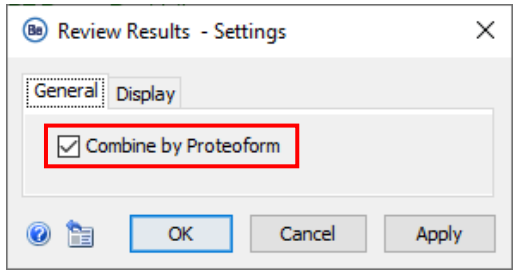
B2

Overview and Application: Top_Middle-Down_ReviewSnapshots_Demo



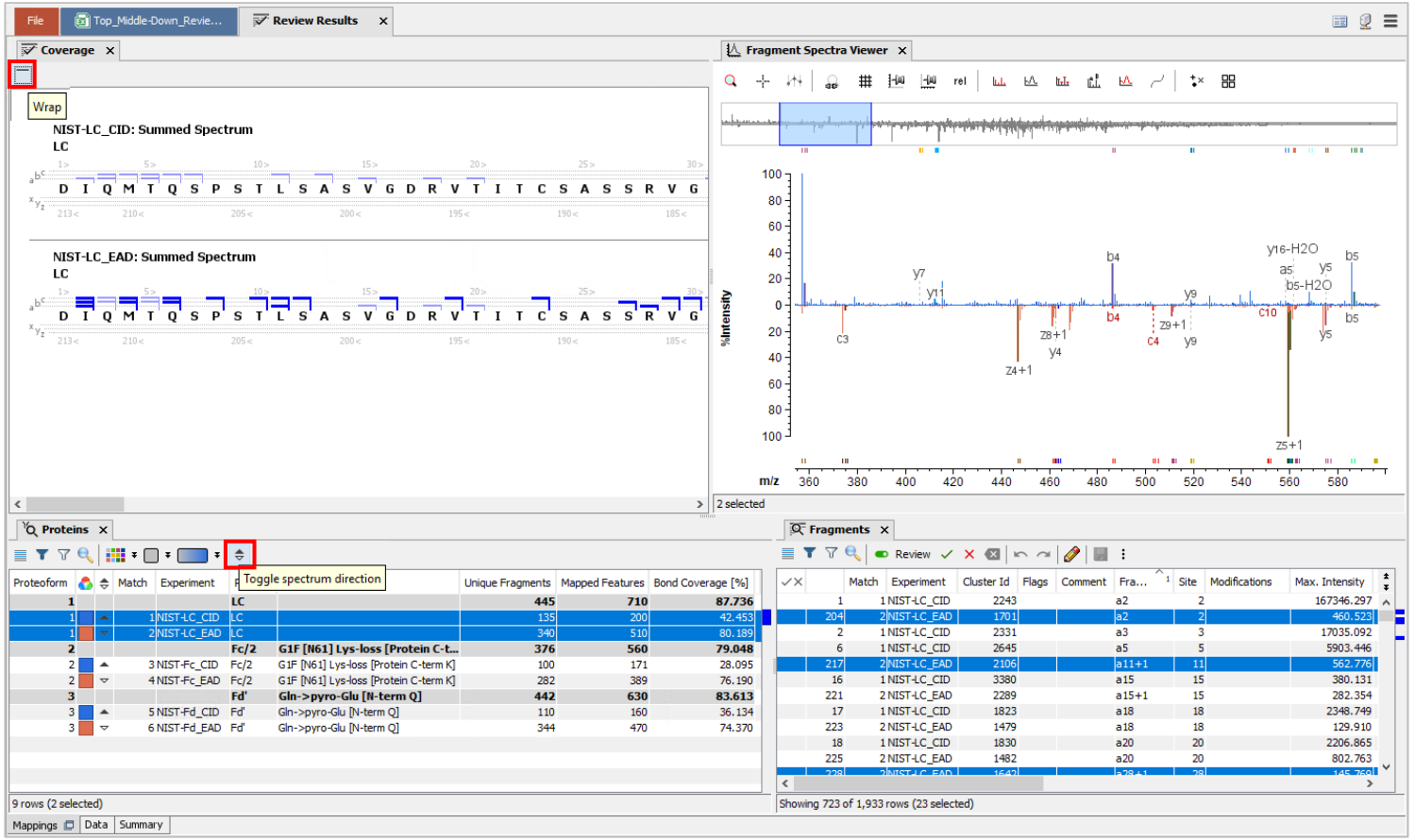
- This workflow uses Snapshot files of results from middle-down analysis of a biotherapeutic molecule after reduction and IdeS digestion. Each subunit was fragmented with EAD and CID across 3 charge states.
- The sequence coverage for each subunit (LC, Fd', Fc/2) is the combination of EAD and CID fragment ions.
- For more information about how to use Biologics Explorer software, refer to the *Biologics Explorer Quick Guide* and *Top/Middle-Down Protein Sequencing Template Workflow Guidelines*.

Review Results



- **Combine by Proteoform** shows the combined sequence coverage of data acquired with EAD and CID fragmentation for each subunit (LC, Fd', Fc/2).

- Use the **Wrap** icon to compare **Coverage** for different sequences
- Use the **Toggle spectrum direction** icon to create a mirror plot in the **Fragment Spectra Viewer**.
- To change **Labels**, right-click the **Fragment Spectra Viewer**, and then and select **Settings**.
  - To increase the number of labeled peaks that are shown, select **Label Features: Offset**.
- Annotations that are selected in **Coverage**, **Fragment Spectra Viewer** and **Fragment Table** are synchronized.



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