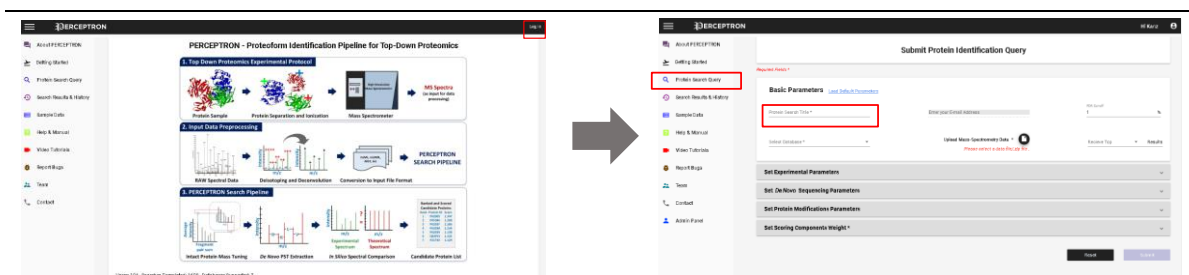
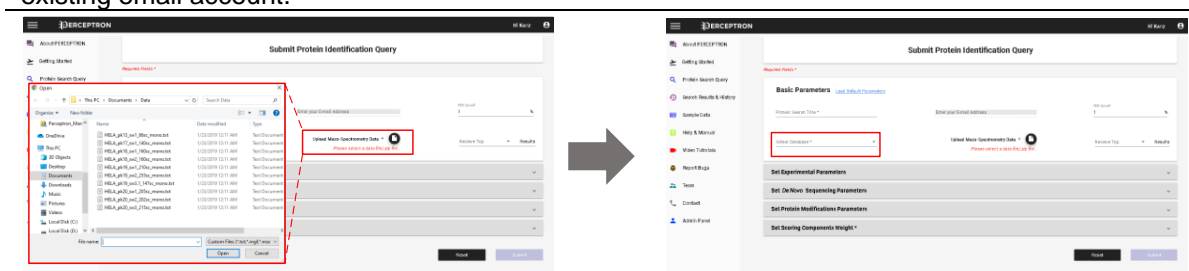


Step-by-Step Guidelines for using PERCEPTRON



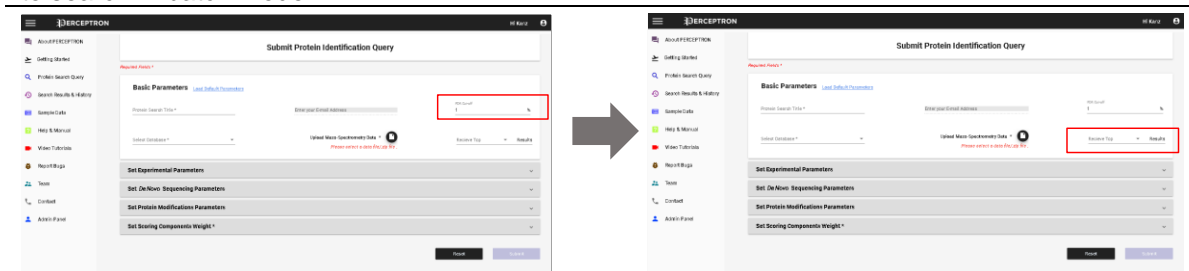
Step 1: Login PERCEPTRON as a guest (without user account) or register for PERCEPTRON user account to login using an existing email account.

Step 2: Go to the 'Protein Search Query' in the menu on left and enter 'Protein Search Title' to start proteoform search.



Step 3: Browse and upload Data file(s). Upload one file (.mzXML/.mzML/.MGF/.txt) to perform single file search or add a zipped folder with multiple peak-lists (zipped .txt files) to search in batch mode.

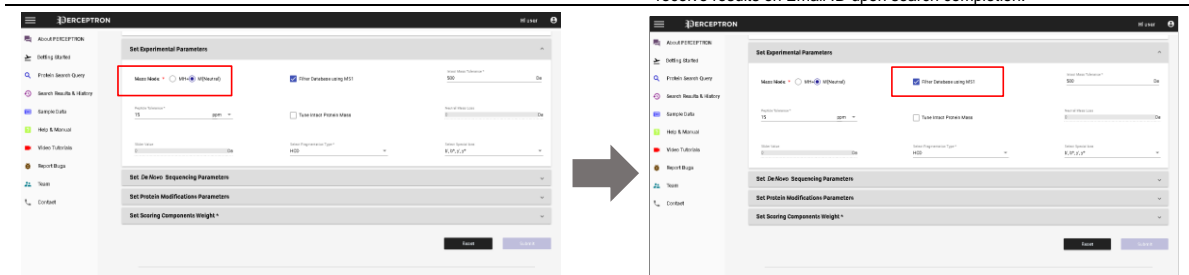
Step 4: Select a Database to search from (e.g. Human).



Step 5: Enter a False Discovery Rate (FDR) percentage to shortlist identified proteoforms at desired FDR cut off.

Step 6: Select the number of proteoforms identified to be received via email.

Note: Users logging in via Guest Account can provide email address to receive results on Email ID upon search completion.



Step 7: Select the Mass Mode. MS data can only be provided in either m/z form with $z = 1$ (MH^+) or neutral masses ($M(Neutral)$).

Step 8: Click 'Filter Database using MS1' to filter protein database on the basis of precursor ion mass (MS1).

DECEPTION v1.0.0

Set Experimental Parameters

Mass Mode: ☒ MS1 ☐ MS2 ☐ MS3

Other Database using MS1: ☒

Intact Mass Tolerance: 500 Da

Search Results & History

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Set De Novo Sequencing Parameters

Set Protein Modifications Parameters

Set Scoring Components Weight *

Save Cancel

Control ID: 40201-Bonobon-IntactMass-Tolerance (MS1)

Step 9: Enter the desired 'Intact Mass Tolerance' and select its unit (Da or ppm).

DECEPTION v1.0.0

Set Experimental Parameters

Mass Mode: ☒ MS1 ☐ MS2 ☐ MS3

Other Database using MS1: ☒

Peptide Mass Tolerance: 10 ppm

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Set De Novo Sequencing Parameters

Set Protein Modifications Parameters

Set Scoring Components Weight *

Save Cancel

Control ID: 40201-Bonobon-IntactMass-Tolerance (MS1)

Step 10: Enter your desired 'Peptide Mass Tolerance' and select its unit (Da or ppm).

DECEPTION v1.0.0

Set Experimental Parameters

Mass Mode: ☒ MS1 ☐ MS2 ☐ MS3

Other Database using MS1: ☒

Intact Mass Tolerance: 500 Da

Peptide Mass Tolerance: 10 ppm

Tune Intact Protein Mass: ☒

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Set De Novo Sequencing Parameters

Set Protein Modifications Parameters

Set Scoring Components Weight *

Save Cancel

Control ID: 40201-Bonobon-IntactMass-Tolerance (MS1)

Step 11: Select 'Tune Intact Protein Mass' to allow for tuning of MS1 using MS2 data.

DECEPTION v1.0.0

Set Experimental Parameters

Mass Mode: ☒ MS1 ☐ MS2 ☐ MS3

Other Database using MS1: ☒

Intact Mass Tolerance: 500 Da

Peptide Mass Tolerance: 10 ppm

Tune Intact Protein Mass: ☒ Range: 1 to 10000

Search Results & History

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Set De Novo Sequencing Parameters

Set Protein Modifications Parameters

Set Scoring Components Weight *

Save Cancel

Control ID: 40201-Bonobon-IntactMass-Tolerance (MS1)

Step 12: Select the 'slider value' to provide a range for tuning MS1 while using 'Tune Intact Protein Mass' option.

DECEPTION v1.0.0

Set Experimental Parameters

Mass Mode: ☒ MS1 ☐ MS2 ☐ MS3

Other Database using MS1: ☒

Intact Mass Tolerance: 500 Da

Peptide Mass Tolerance: 10 ppm

Tune Intact Protein Mass: ☒ Range: 1 to 10000

Fragmentation Type: b/y

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Set De Novo Sequencing Parameters

Set Protein Modifications Parameters

Set Scoring Components Weight *

Save Cancel

Control ID: 40201-Bonobon-IntactMass-Tolerance (MS1)

Step 13: Select the fragmentation type from the drop-down menu.

DECEPTION v1.0.0

Set Experimental Parameters

Mass Mode: ☒ MS1 ☐ MS2 ☐ MS3

Other Database using MS1: ☒

Intact Mass Tolerance: 500 Da

Peptide Mass Tolerance: 10 ppm

Tune Intact Protein Mass: ☒ Range: 1 to 10000

Fragmentation Type: b/y

Special Ions: a, b, y, z

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Set Protein Modifications Parameters

Set Scoring Components Weight *

Save Cancel

Control ID: 40201-Bonobon-IntactMass-Tolerance (MS1)

Step 14: Choose the corresponding special ions for the type of fragmentation method selected (i. e. a', b', y', z", a*, b*, y*, z' ions).

DECEPTION v1.0.0

Set Experimental Parameters

Mass Mode: ☒ MS1 ☐ MS2 ☐ MS3

Other Database using MS1: ☒

Intact Mass Tolerance: 500 Da

Peptide Mass Tolerance: 10 ppm

Tune Intact Protein Mass: ☒ Range: 1 to 10000

Fragmentation Type: b/y

Special Ions: a, b, y, z

Neutral Loss: 42

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Set Protein Modifications Parameters

Set Scoring Components Weight *

Save Cancel

Control ID: 40201-Bonobon-IntactMass-Tolerance (MS1)

Step 15: Provide the value of Neutral loss, if any.

DECEPTION v1.0.0

Set Experimental Parameters

Mass Mode: ☒ MS1 ☐ MS2 ☐ MS3

Other Database using MS1: ☒

Intact Mass Tolerance: 500 Da

Peptide Mass Tolerance: 10 ppm

Tune Intact Protein Mass: ☒ Range: 1 to 10000

Fragmentation Type: b/y

Special Ions: a, b, y, z

Neutral Loss: 42

Enable PST Filtering: ☒

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Set De Novo Sequencing Parameters

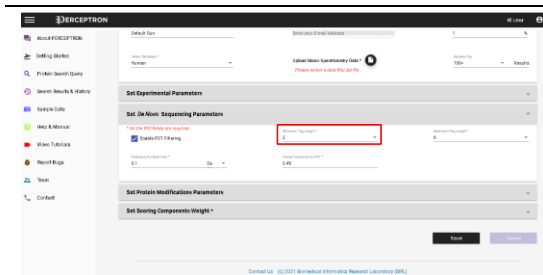
Set Protein Modifications Parameters

Set Scoring Components Weight *

Save Cancel

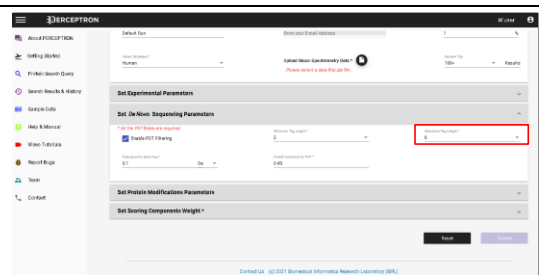
Control ID: 40201-Bonobon-IntactMass-Tolerance (MS1)

Step 16: Select 'Enable PST Filtering' to allow for peptide sequence tag (PST)-based search.

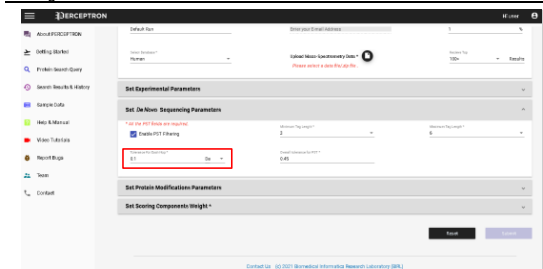


Step 17: Select your desired minimum PST tag length from the drop-down menu.

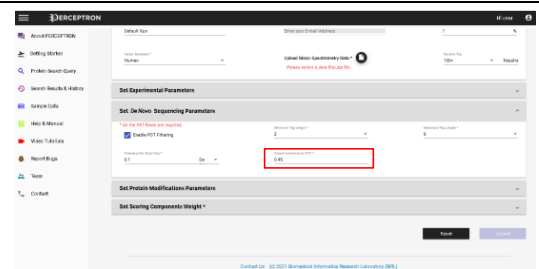
Note: Input value for Minimum and Maximum Tag length should be different. Minimum Tag Length should be smaller than Maximum Tag Length.



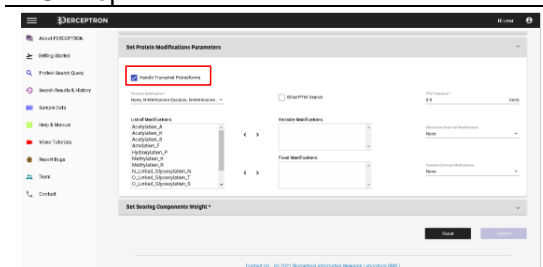
Step 18: Select your desired maximum PST tag length from the drop-down menu.



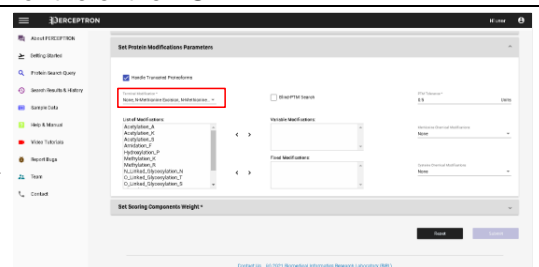
Step 19: Set the desired tolerance for each PST hop.



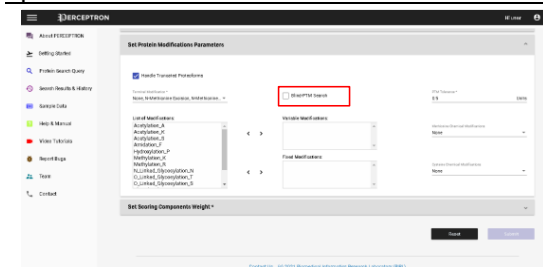
Step 20: Set the overall mass error tolerance for the entire PST.



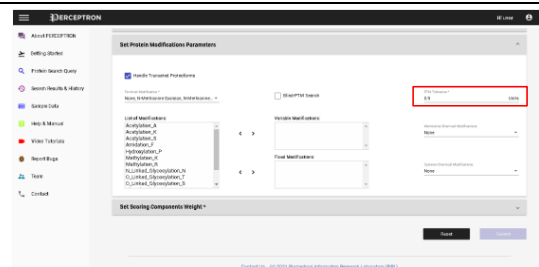
Step 21: Check 'Handle Truncated Proteoforms' to allow search for truncated proteoforms.



Step 22: Select 'Terminal modifications' to be included in the search.

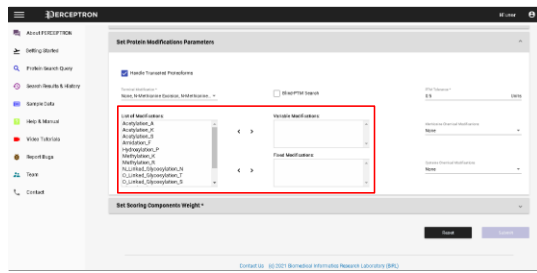


Step 23: To allow search for unknown post-translational modifications (PTMs), search for Blind PTMs instead of variable or fixed modifications.

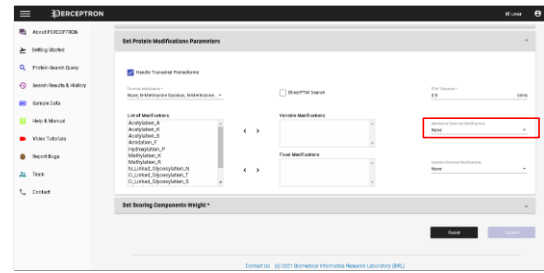


Step 24: Set the 'PTM tolerance'.

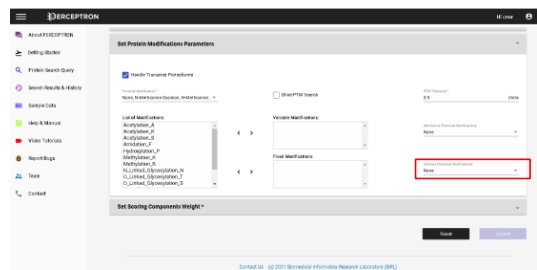
Note: If 'Blind-PTM Search' is selected, there is no need to set PTM tolerance.



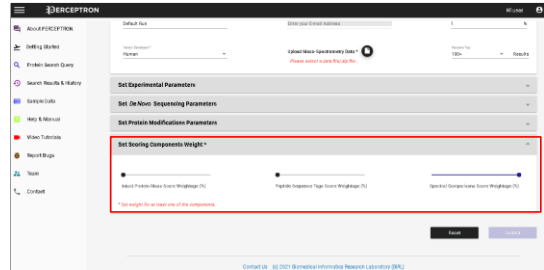
Step 25: Select 'Fixed' or 'Variable' post-translational modifications (PTMs) from the List of Modifications.



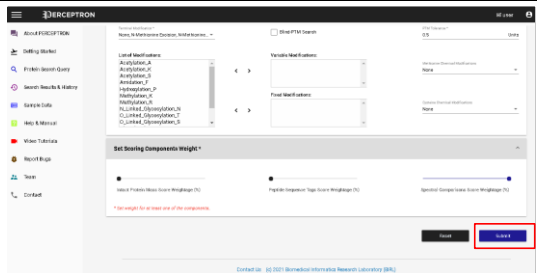
Step 26: Select the 'Methionine Chemical Modifications' from the drop-down menu.



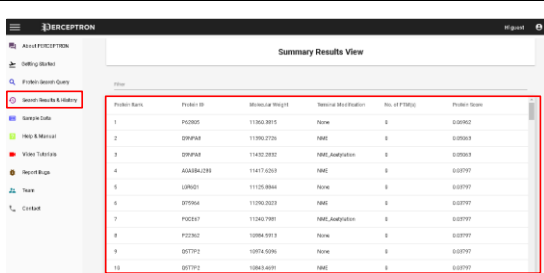
Step 27: Select the 'Cysteine Chemical Modifications' from the drop-down menu.



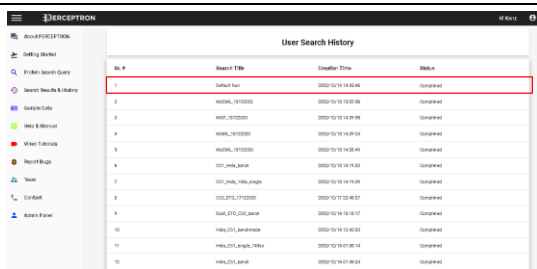
Step 28: Set the weights of the scoring components by moving the sliders.



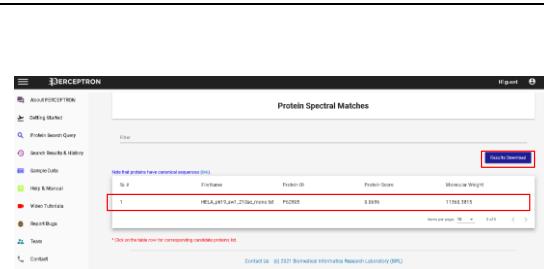
Step 29: Submit the Protein Query Search by clicking on 'Submit'.



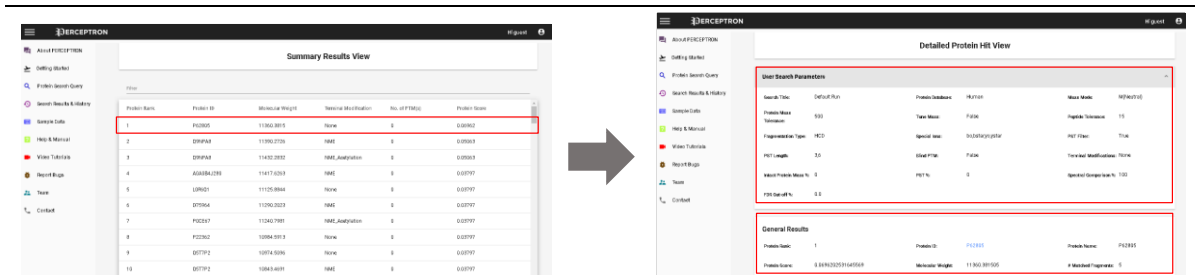
Step 30: Click on 'Search Results & History' to see the search results.



Step 31: Click on any protein to go to 'Protein Spectral Matches' by clicking the 'Search Title.'



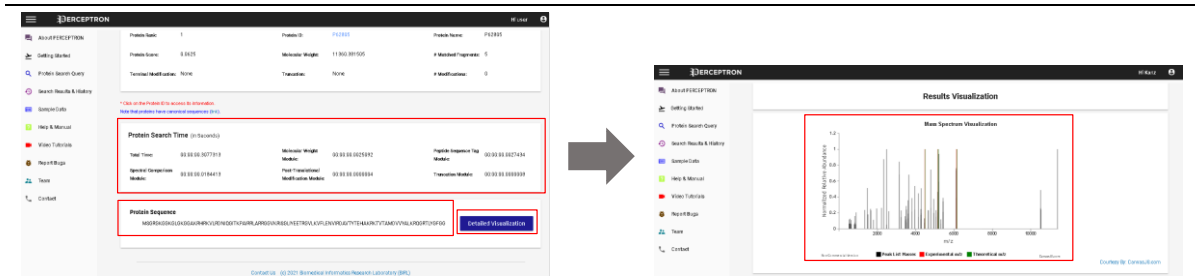
Step 32: Click on 'Results Download' option provided on top right to download results. The results along with search parameters will be downloaded as a .zip folder.



Step 33: In 'Summary Results View,' user can click on any 'Protein ID' to go to its 'Detailed Protein Hit View'

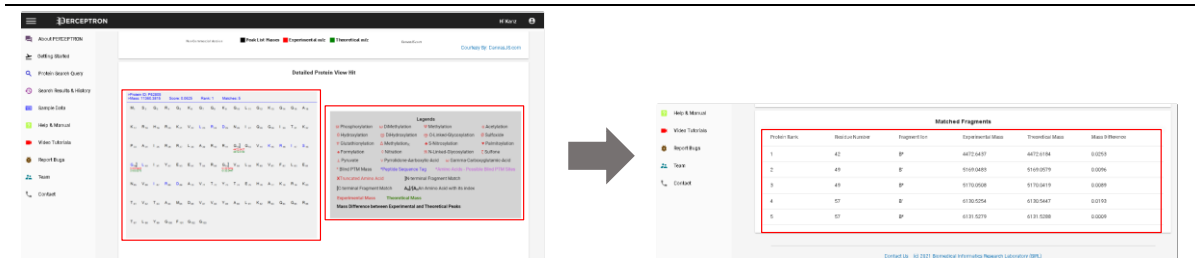
Note: 'Detailed Visualization' feature is not provided for proteoform search in batch mode. For batch mode, users can only download results as a .zip folder using the 'Results Download' option.

Step 34: In 'Detailed Result View' user can find search parameters used while submitting protein search query. User can view details of the selected proteoform in 'General Results.'



Step 35: User can see total time taken for processing protein query in 'Protein Search Time' panel below 'General Results.' Additionally, these runtimes have been split for highlighting time taken by each algorithmic component. 'Protein Sequence' panel shows the sequence of selected proteoform. User can visualize search results in detail by clicking 'Detailed Visualization' button provided on bottom right.

Step 36: User can find 'Mass Spectrum Visualization' on top which shows Experimental Spectrum superimposed with Theoretical Spectrum highlighting peaks matched during spectral comparison. User can hover the cursor over to find peak list masses.



Step 37: User can find 'Detailed Protein Hit View' which shows complete information of the selected proteoform. 'Legends' (on right) is a key to help user understand 'Detailed Protein Hit View.'

Step 38: User can find details of fragments matched in 'Matched Fragments' panel.