

# **PERCEPTRON** *v 1.0.0.0*

A Next Generation Top-Down Proteoform  
Identification and Characterization Platform

## **USER MANUAL**

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<http://birl.lums.edu.pk/>

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## 3 Introduction to PERCEPTRON

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This chapter introduces the user to the PERCEPTRON application and describes its basic features.

### 3.1. About PERCEPTRON

PERCEPTRON is a freely available web-based proteoform identification pipeline for Top-Down Proteomics (TDP). Top-down proteomics is an emerging experimental protocol for analysis of intact proteoforms. PERCEPTRON search pipeline brings together algorithms for: (i) intact mass tuning, (ii) *de novo* peptide sequence tag extraction, (iii) *in silico* spectral comparison, (iv) identification of post-translational modifications as well as truncated proteins, and (v) a novel composite scoring scheme for candidate protein scoring. PERCEPTRON achieves high performance by leveraging NVIDIA GPU technology coupled with Microsoft ASP.NET and ANGULAR frameworks. The search results obtained include a list of proteins, their scores and details on the matching information. This information can be visualized as well as downloaded. Overall, PERCEPTRON is aimed at filling the crucial void of **open-source and open-architecture** protein identification software for TDP data, employing state-of-the-art algorithms.

### 3.2. Features

The salient features of the pipeline are summarized below:

- **Graphical User Interface** - A set of rich and intuitive graphical user interface has been developed for setting up the search parameters as well as for integrating the main components of the engine.
- **Whole Protein Molecular Weight Estimation** - The protein identification begins with the tuning of precursor protein's monoisotopic MW (MS1) as guided by its fragmentation spectra (MS2). Relative abundances and mass/charge ( $m/z$ ) ratios are used to calculate the consensus MW which is then employed in the search and scoring process.
- **Peptide Sequence Tag Extractor** - Peptide sequence tag ladders (PST) are extracted from the spectra by enumerating successive peaks having MW differences equal to an amino acid and within the user specified mass tolerance. Protein database is then filtered for proteins reporting these PSTs. The length of PST ladders, cumulative mass off-sets and relative abundances are used in calculating the PST scores.
- ***In silico* fragmentation** – *In silico* fragments of candidate proteins are generated by the user selected fragmentation techniques. *In vitro* and *in silico* spectral comparisons are performed and scored.

- **Post-translational Modification (PTM) Search** - Support for predicting typical PTMs has been provided in the tool. Users can select and search variable and fixed PTMs of their choice along with blind-PTMs by simply selecting them from the GUI.
- **Multifactorial Composite Scoring System** - A multifactorial candidate protein scoring scheme incorporating the aforementioned algorithms has been developed. User customization of the parameters and weights in the scoring function is admitted via a GUI.
- **Single and Batch Search** – PERCEPTRON provides support for search in single as well as batch modes. Towards an automated processing of multiple spectral data files, a batch processing mode allows for the selection of multiple files from the folder by clicking the attach file button. The experimental spectra, search parameters and results are automatically stored in the project directory for further processing and visualization.

## 4 Hardware and Software

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### 4.1. Hardware

PERCEPTRON requires

### 4.2. Software

### 4.3. Testing

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## 5 Video Tutorials

Video tutorials have been provided for: (i) using PERCEPTRON, (ii) search in single and batch modes, (iii) performing search for MGF and text files, and (iv) interpreting results. The videos are available as a playlist at: [https://www.youtube.com/playlist?list=PLaNVq-kFOnOZ\\_7b-iL59M\\_CeV06JxEXmA](https://www.youtube.com/playlist?list=PLaNVq-kFOnOZ_7b-iL59M_CeV06JxEXmA)

## 6 Getting Started with PERCEPTRON

PERCEPTRON manual, samples and issues database is freely available (under the MIT open license) at (<https://perceptron.lums.edu.pk/>)

To log in, click on the link provided above.

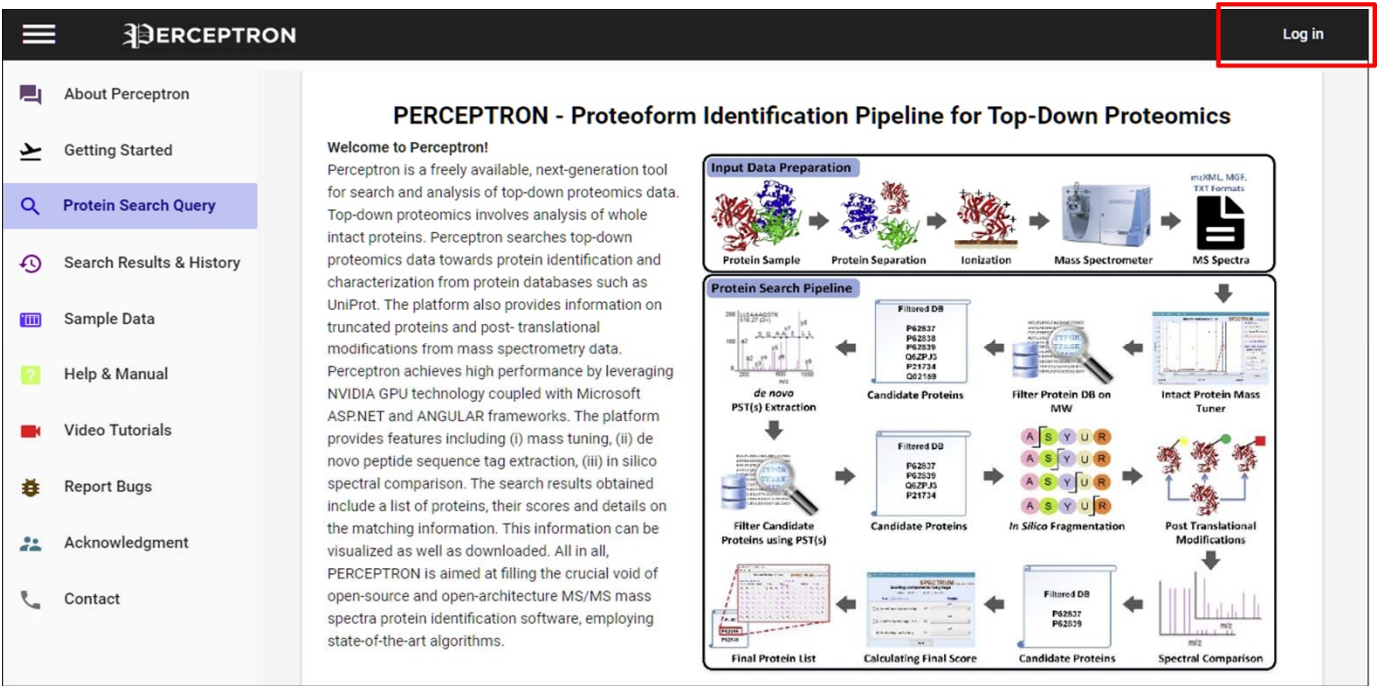
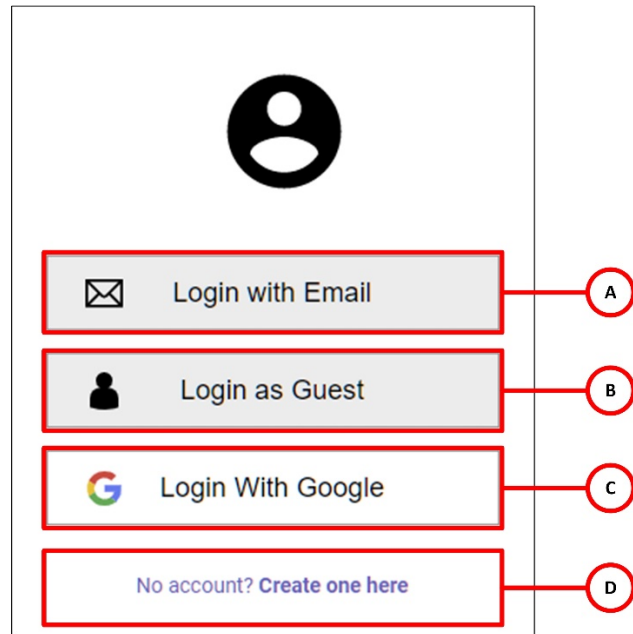


Figure 1. PERCEPTRON Homepage

Click the ‘log in’ button on the top right. A window will appear. Enter details to proceed.



*Figure 2. PERCEPTRON Login options*

- A. User can login with their email account
- B. Enables user to login as a guest, perform search in PERCEPTRON but the results will not be saved
- C. User can only login via their g-mail account
- D. If the user does not have any account, they can make a new one here in order to login.



## 7 GUI description:

This chapter presents the interface overview for user facilitation.

### 7.1 Window 1: PERCEPTON Tool for Top-down Proteomics

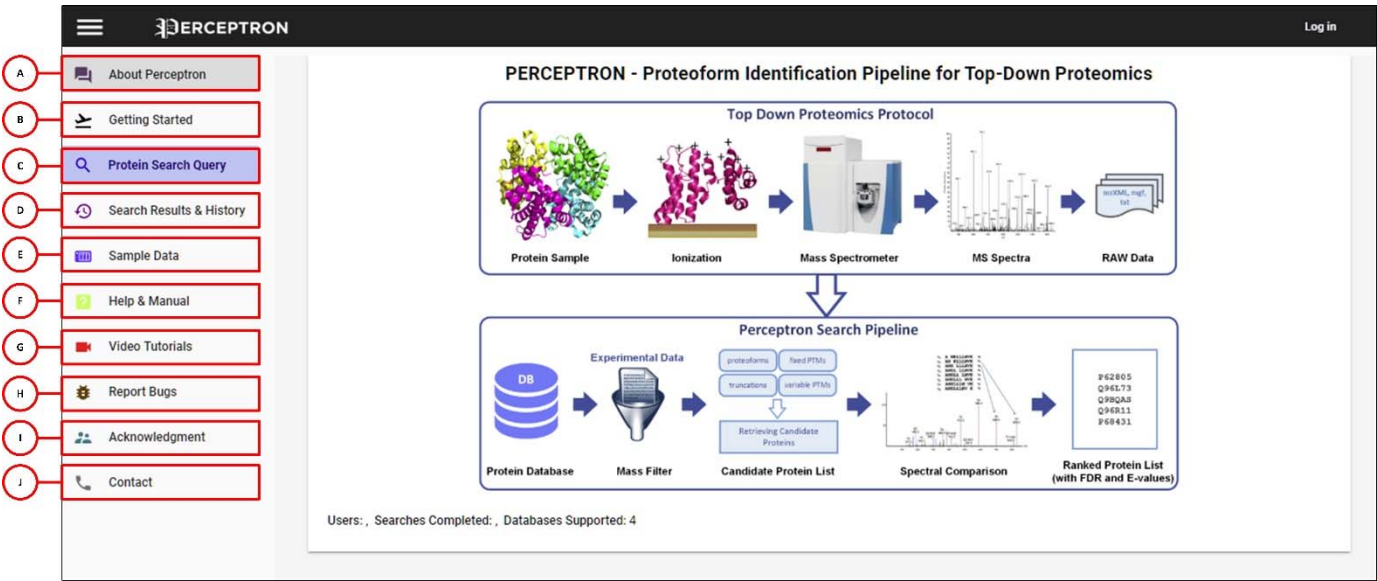


Figure 3. PERCEPTON - Overview of User Interface

- A. About PERCEPTON: A next-generation top-down proteoform search and identification platform
- B. Getting Started: Quick guide to proteoform search and identification using PERCEPTON
- C. Protein Search Query: Job submission - Search top-down proteomics data files to identify and characterize proteoforms
- D. Search Results & History: View search results and history
- E. Sample Data: Sample top-down mass spectrometry data for use with PERCEPTON
- F. Help & Manual: Get assistance with using PERCEPTON and download manual
- G. Video Tutorials: View step-by-step video tutorials demonstrating usage of PERCEPTON
- H. Report Bugs: Report problems and issues here
- I. Acknowledgement: PERCEPTON project team members
- J. Contact: Contact us for further information

In order to start protein search, click on the ‘Protein Search Query’ tab and the following window will appear:

## 7.2 Window 2: Protein Search Query

### Basic Parameters:

The screenshot shows the 'Submit Protein Identification Query' form in the PERCEPTRON application. The form is divided into several sections. On the left is a sidebar with navigation links: About Perceptron, Getting Started, Protein Search Query (highlighted), Search Results & History, Sample Data, Help & Manual, Video Tutorials, Report Bugs, Acknowledgment, and Contact. The main form area is titled 'Submit Protein Identification Query' and contains a 'Basic Parameters' section with a link to 'Load Default Parameters'. This section includes a text input for 'Protein Search Title' (labeled A), a text input for 'Intact Protein Mass' (labeled B), a file upload button for 'Upload Mass-Spectrometry Data File(s):' (labeled C), a dropdown for 'Select Database' (labeled D), an email input for 'Enter your E-mail Address \*' (labeled E), and a dropdown for 'Recieve Top' (labeled F) next to a 'Results' button. Below the 'Basic Parameters' section are four expandable sections: 'Set Experimental Parameters', 'Set De Novo Sequencing Parameters', 'Set Protein Modifications Parameters', and 'Set Scoring Components Weight'. At the bottom right are 'Reset' and 'Submit' buttons. The footer contains 'Contact Us' and '(c) 2019 Biomedical Informatics Research Laboratory (BIRL)'.

Figure 4. PERCEPTRON - Overview of Basic Parameters

- A. In order to start the protein search, user has to enter the ‘Protein Search Title’ first (for example: MyProject)
- B. User can enter intact protein mass (MS1)
- C. Browse and upload protein database (.fasta)
- D. Browse and upload experimental data (.mzXML/ .MGF/ .txt) for Single mode; Peak-list files for Batch mode
- E. Enter your email address. You will be notified about the compilation of your results via email
- F. Select the number of candidate protein hits to be received in results

## Set Experimental Parameters:

The screenshot shows the PERCEPTRON web interface. The sidebar on the left contains links: About Perceptron, Getting Started, Protein Search Query (highlighted), Search Results & History, Sample Data, Help & Manual, Video Tutorials, Report Bugs, Acknowledgment, and Contact. The main header shows 'Hi guest' and a user icon. The main content area is titled 'Submit Protein Identification Query'. It includes a 'Basic Parameters' section with fields for 'Protein Search Title', 'Intact Protein Mass' (Da), 'Upload Mass-Spectrometry Data File(s)' (0 Files Selected), 'Select Database', 'Enter your E-mail Address \*', 'Recieve Top', and 'Results'. Below this is the 'Set Experimental Parameters' section, which is expanded. It contains several sub-sections: 'Mass Mode' (radio buttons for MH+ and M(Neutral)), 'Intact Mass Tolerance' (Da), 'Peptide Tolerance' (ppm), 'Filter Database using MS1' (checkbox), 'Tune Intact Protein Mass' (checkbox), 'Select Fragmentation Type' (dropdown), 'Handle Truncated Proteoforms' (checkbox), 'Neutral Mass Loss' (Da), and 'Select Corresponding Special Ions' (dropdown). Red lines connect labels A through I to specific fields: A to Mass Mode, B to Intact Mass Tolerance, C to Peptide Tolerance, D to Select Fragmentation Type, E to Filter Database using MS1, F to Handle Truncated Proteoforms, G to Neutral Mass Loss, H to Select Corresponding Special Ions, and I to Tune Intact Protein Mass. At the bottom right are 'Reset' and 'Submit' buttons.

Figure 5. PERCEPTRON - Overview of Experimental Parameters

- A. Select Mass Mode. MS data can only be provided in either m/z form with  $z = 1$  or neutral masses
- B. Set the tolerance value for Protein Mass and select its unit
- C. Select the tolerance value for Peptide and select its unit
- D. Select the 'Fragmentation type' from drop down menu
- E. User can filter database by checking the option 'Filter Database using MS1'
- F. Check 'Handle Truncated Proteoforms' to allow search for truncated proteoforms
- G. Provide the value of Neutral loss, if any
- H. Choose the corresponding special ions for the type of fragmentation selected (i.e. a', b', y', z'', a\*, b\*, y\*, z' ions)
- I. Check the option 'Tune Intact Protein Mass' to allow for tuning of MS1 using MS2 data

Set *De Novo* Sequencing Parameters:

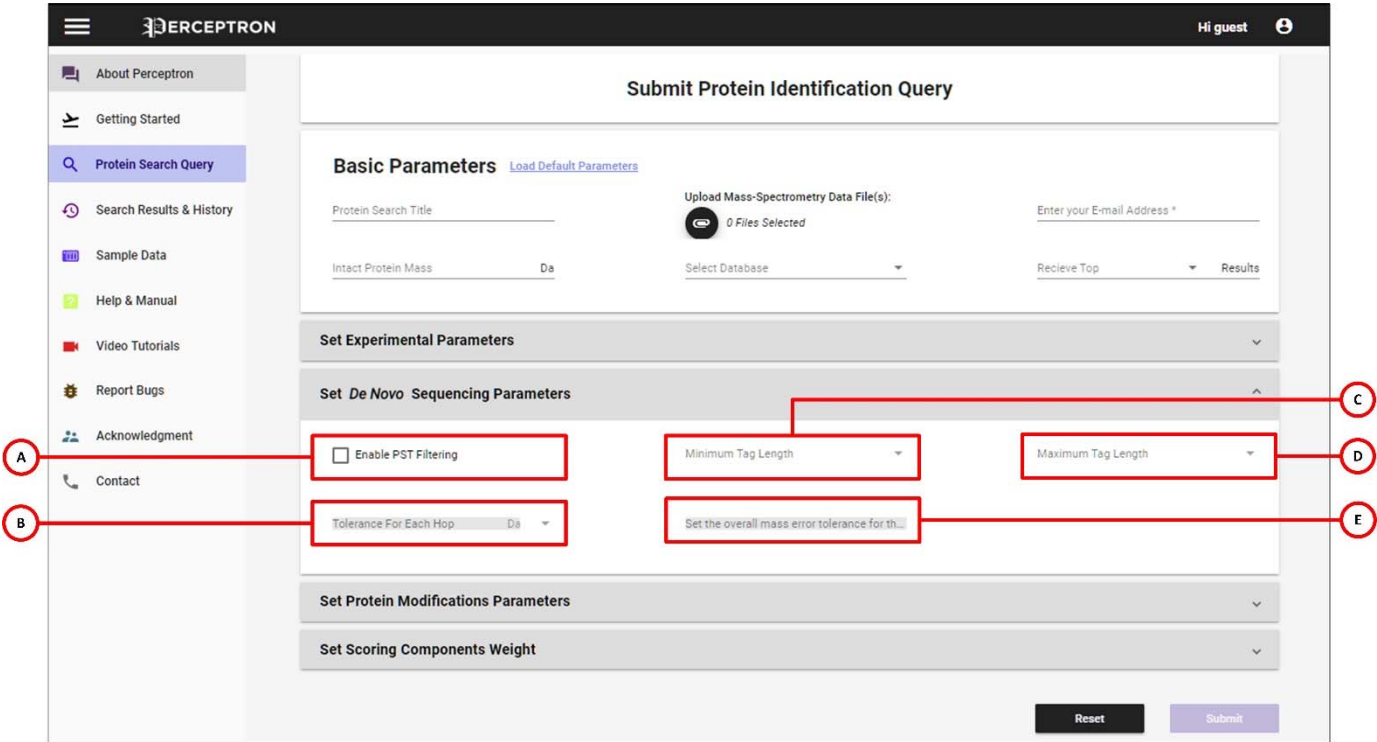


Figure 6. PERCEPTRON - Overview of *De Novo* Sequencing Parameters

- A. Check ‘Enable PST Filtering’ to filter PSTs
- B. Set the ‘Tolerance for each Hop’
- C. Tags will be filtered above the minimum length of PST selected from the drop down menu by the user
- D. Tags will be filtered below the maximum length of PST selected from the drop down menu by the user
- E. Overall mass error tolerance shows error margin for the whole PST

## Set Protein Modifications Parameters:

The screenshot displays the PERCEPTRON web application interface. The main section is titled 'Submit Protein Identification Query'. Below this, there are several parameter settings sections. The 'Set Protein Modifications Parameters' section is highlighted with a red box and labeled with letters A through G. This section includes a 'Terminal Modification' dropdown (A), a 'Blind-PTM Search' checkbox (B), a 'PTM Tolerance' dropdown (C), a 'Methionine Chemical Modifications' dropdown (D), a 'Variable Modifications' list (E), a 'Fixed Modifications' list (G), and a 'Cysteine Chemical Modifications' dropdown (F). The 'List of Modifications' is shown on the left, and the 'Set Scoring Components Weight' section is at the bottom. The interface also includes a sidebar with navigation links and a top header with the PERCEPTRON logo and user information.

Figure 7. PERCEPTRON - Overview of Protein Modifications Parameters

- A. Allows user to select specified terminal modifications. PERCEPTRON handles four cases: 1) None – No modification, 2) NME – N terminal methionine excision, 3) NME\_ACETYLTATION – N terminal acetylation with initiator methionine removed, and 4) M\_ACETYLTATION – N terminal methionine acetylation
- B. Allows user to perform Blind-PTM search and find unknown modifications
- C. Set the tolerance value for Post Translational Modification (PTM)
- D. Allows the user to select instrument specific modification on Methionine
- E. User can opt for required Variable ‘Post translation Modifications’ from the list of modifications
- F. Allows the user to select instrument specific modification on Cysteine
- G. Similarly, various ‘Fixed Modifications’ are also selected from the list

Set Scoring Components Weight:

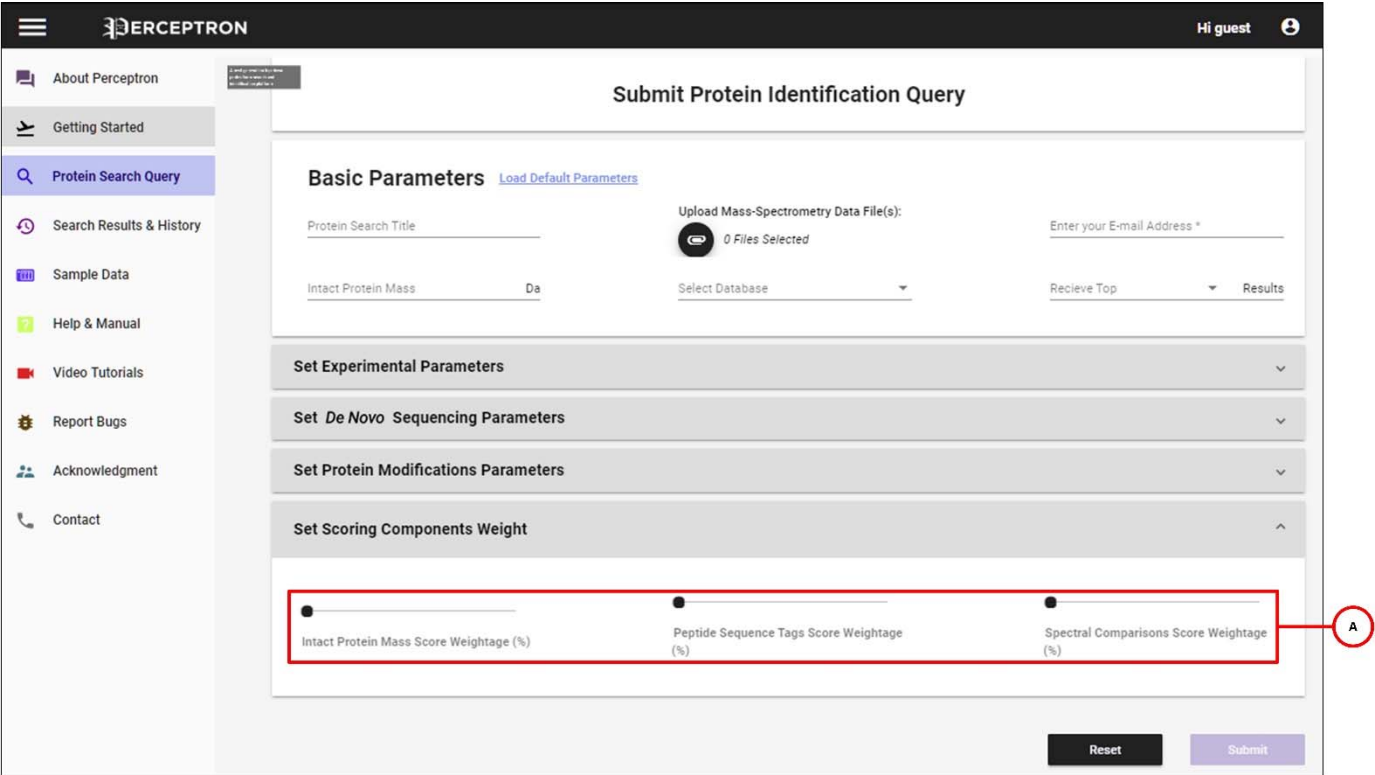


Figure 8. PERCEPTRON - Overview of Scoring Component Weight

- A. Set the respective weights of the Scoring Components from by shifting the slider left or right accordingly

### 7.3 Window 3: Summary and Detailed Results View

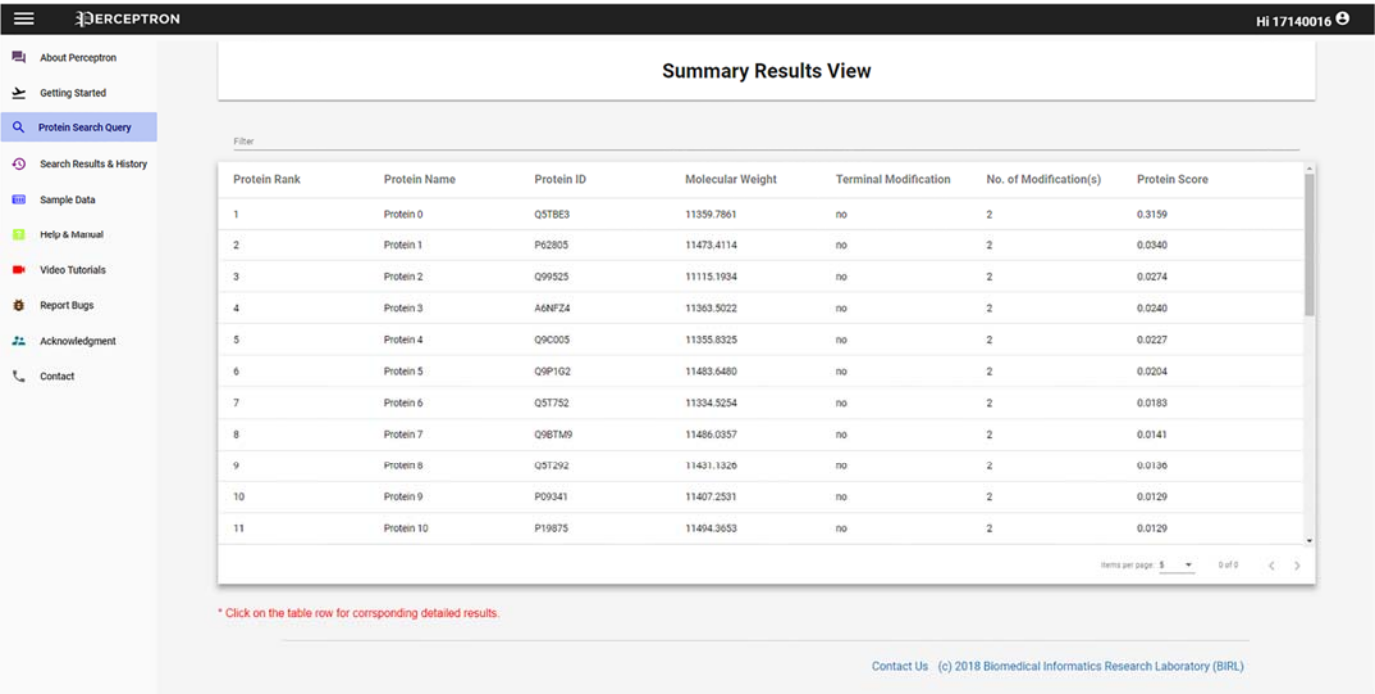


Figure 9. Summary Results window showing candidate proteins

Resultant proteins along with their protein ID, molecular weight and protein score according to the uploaded and selected data are represented in the list. Click on any protein to see the ‘Detailed Result View’ of the protein. Click on the ‘protein ID’ to go to the detailed UniProt view of the protein.

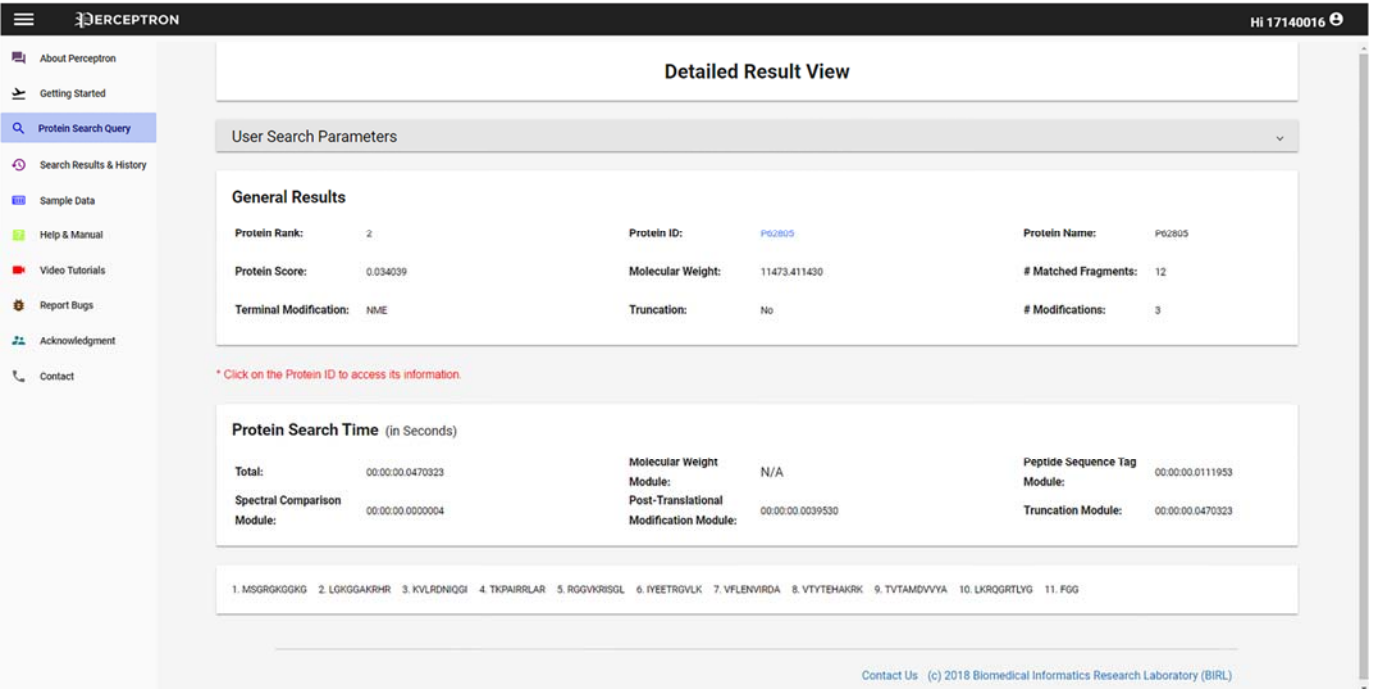


Figure 10. Detailed Results window showing candidate proteins

## 8 Search

### 8.1 File Formats

PERCEPTRON provides support for plain text files (data in columns containing mass to charge ratios ( $m/z$ ) and relative intensities), eXtensible Markup Language (XML) files with  $m/z$  and relative abundances (mzXML)<sup>1</sup>, Mass Spectrometry Markup Language (mzML)<sup>2,3</sup> and Mascot Generic Format (MGF)<sup>4</sup> data formats in both single and batch file processing modes.

#### 8.1.1 Raw to mzXML File Format Conversion

User can convert raw data files to mzXML file format by using MS-Convert<sup>5</sup>.

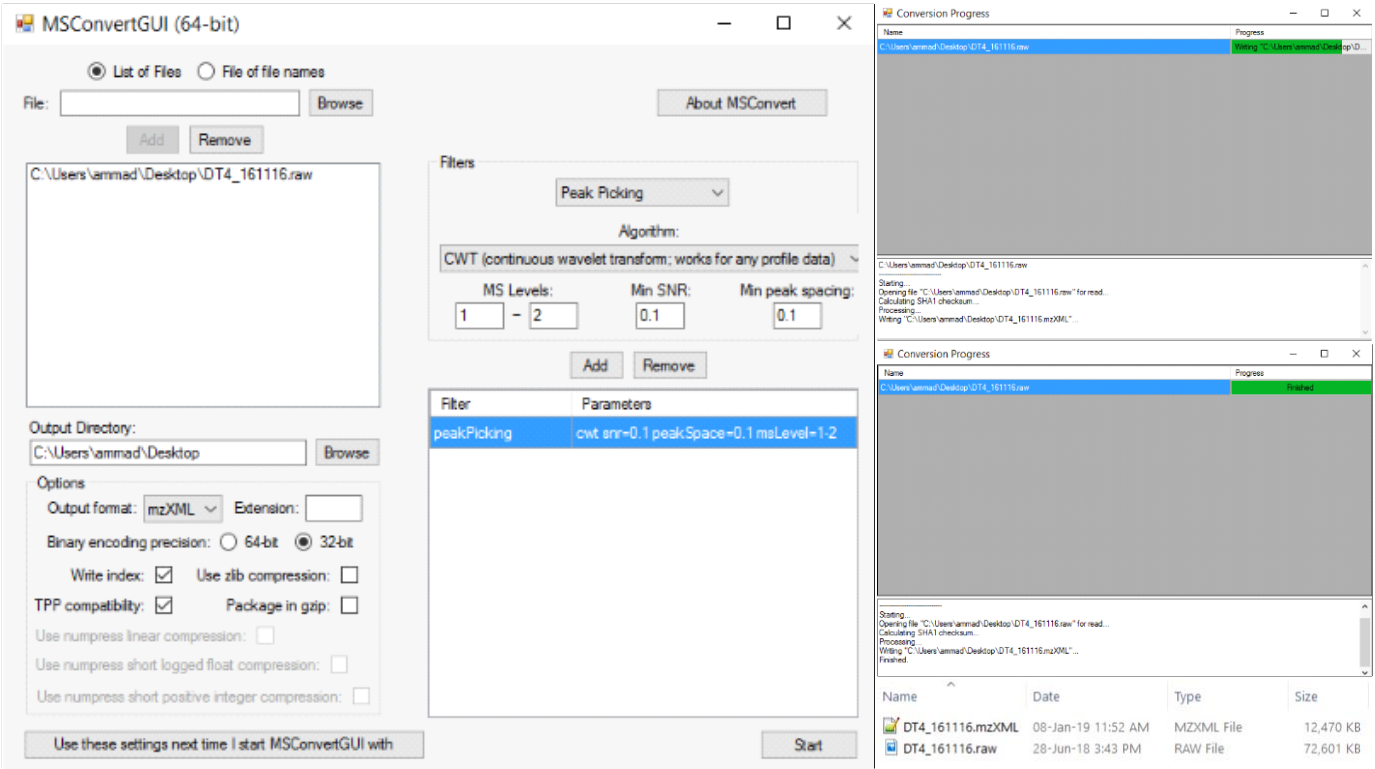


Figure 11. Conversion of raw to mzXML

#### 8.1.2 Raw to mzML File Format Conversion

Raw data files can be converted to mzML file format by using MS-Convert<sup>5</sup>.



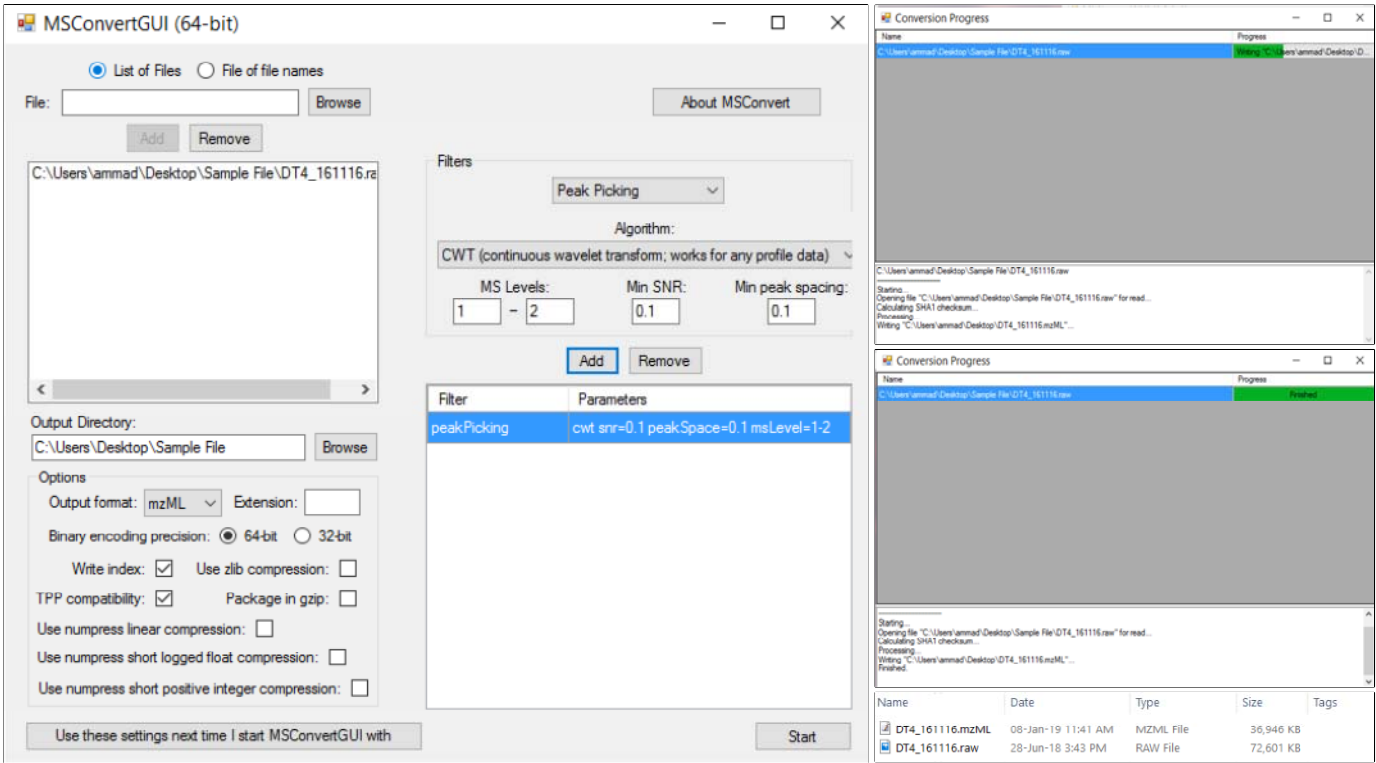


Figure 12. Conversion of raw to mzML

8.1.3 MzXML to MGF File Format Conversion

User can convert mzXML files to MGF using MS-Decov<sup>6</sup>.

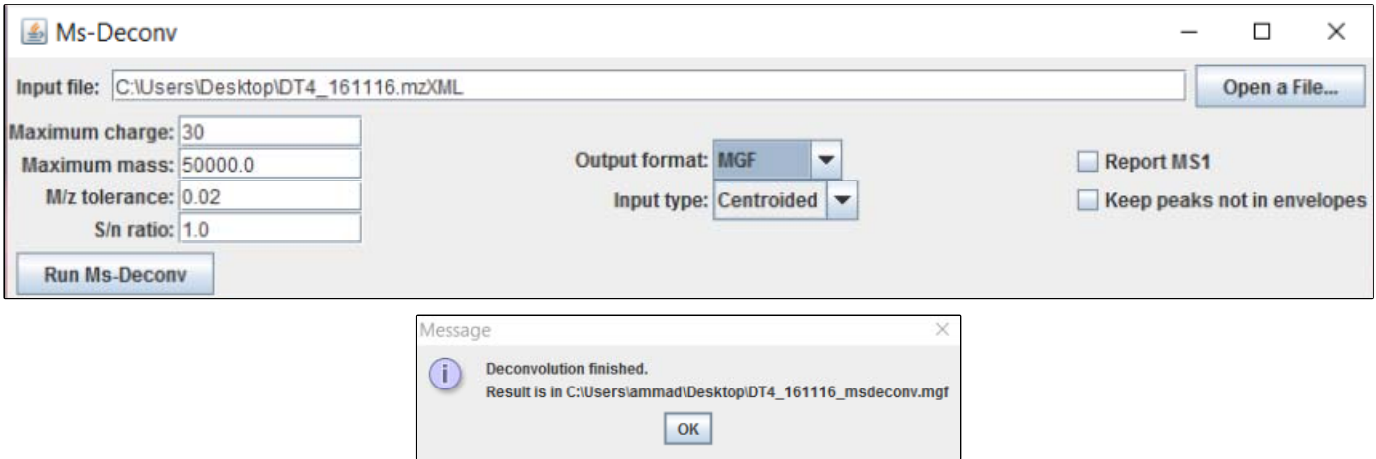


Figure 13. Conversion of mzXML to MGF

8.1.4 MGF to Flat Text File Format Conversion

PERCEPTRON converts MGF files to flat text (peak list) using built-in custom file reader.

## 8.2 Parameters

PERCEPTRON works when all parameters are set. Two kind of parameters can be used by the user which includes: (i) Default Parameters, and (ii) Selected parameters.

### How to load default Parameters?

To submit the job using default parameters, select ‘Load Default Parameters’ option in front of Basic Parameters.

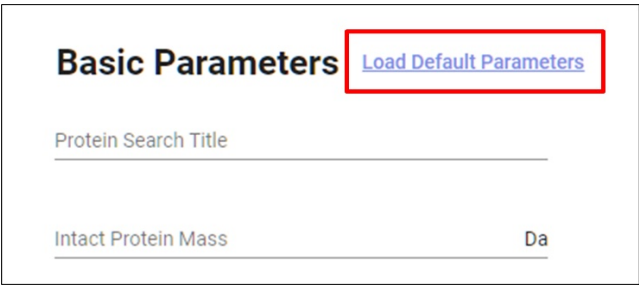


Figure 14. Load Default Parameters

## 8.3 Databases

SwissProt database is included in PERCEPTRON by default. User can take any protein sequence from other databases (such as Uniprot) in .fasta format.

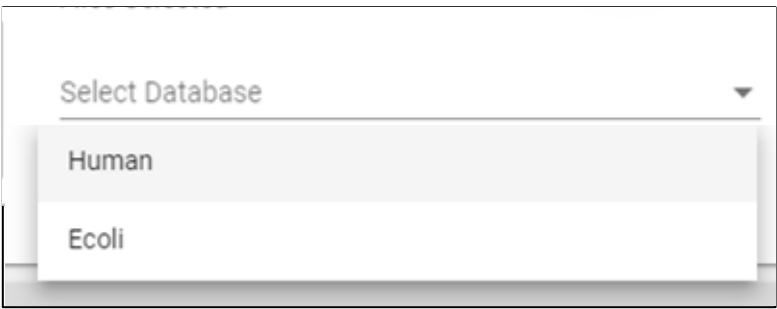


Figure 15. Selecting Protein Database

## 8.4 Modes

The search modes are auto-selected based on the number of files that the user inputs. If one file is given as input, PERCEPTRON runs single search mode whereas if multiple files are given as input, the mode switches to batch mode.

- (i) Single Search Mode
- (ii) Batch Mode

Batch mode takes more processing time as it deals with larger data. The experimental spectra, search parameters and results are automatically stored in the project directory for further processing and visualization.

## 9 References

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