Using the Open-Source MALDI TOF-MS IDBac Pipeline for Analysis of Microbial Protein and Specialized Metabolite Data

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Abstract

In order to visualize the relationship between bacterial phylogeny and specialized metabolite production of bacterial colonies growing on nutrient agar, we developed IDBac—a low-cost and high-throughput matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) bioinformatics pipeline. IDBac software is designed for non-experts, is freely available, and capable of analyzing a few to thousands of bacterial colonies. Here, we present procedures for the preparation of bacterial colonies for MALDI-TOF MS analysis, MS instrument operation, and data processing and visualization in IDBac. In particular, we instruct users how to cluster bacteria into dendrograms based on protein MS fingerprints and interactively create Metabolite Association Networks (MANs) from specialized metabolite data.

SUMMARY

IDBac is an open-source mass spectrometry-based bioinformatics pipeline that integrates data from both intact protein and specialized metabolite spectra, collected on cell material scraped from bacterial colonies. The pipeline allows researchers to rapidly organize hundreds to thousands of bacterial colonies into putative taxonomic groups, and further differentiate them based on specialized metabolite production.

Keywords

Mass spectrometry; microbiology; natural products; specialized metabolites; MALDI-TOF MS; bioinformatics

INTRODUCTION

A major barrier to researchers who study bacterial function is the ability to quickly and simultaneously assess the taxonomic identity of a microorganism and its capacity to produce

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The authors have nothing to disclose.

specialized metabolites. This has prevented significant advances in understanding the relationship between bacterial phylogeny and specialized metabolite production in the majority of bacteria isolated from the environment. Although MS-based methods that use protein fingerprints to group and identify bacteria are well described[1–4], these studies have generally been performed on small groups of isolates, in a species-specific manner. Importantly, information on specialized metabolite production, a major driver of microbial function in the environment, has remained unincorporated in these studies. Silva et al.[5] recently provided a comprehensive history detailing the underuse of MALDI-TOF MS to analyze specialized metabolites and the shortage of software to relieve current bioinformatics bottlenecks. In order to address these shortcomings, we created IDBac, a bioinformatics pipeline that integrates both linear and reflectron modes of MALDI-TOF MS[6]. This allows users to rapidly visualize and differentiate bacterial isolates based on both protein and specialized metabolite MS fingerprints, respectively.

IDBac is cost-effective, high-throughput, and designed for the lay user. It is freely available (chasemc.github.io/IDBac), and only requires access to a MALDI-TOF mass spectrometer (reflectron mode will be required for specialized metabolite analysis). Sample preparation relies on the simple “extended direct transfer” method[7,8] and data are collected with consecutive linear and reflectron acquisitions on a single MALDI-target spot. With IDBac, it is possible to analyze the putative phylogeny and specialized metabolite production of hundreds of colonies in under four hours, including sample preparation, data acquisition, and data visualization. This presents a significant time and cost advantage over traditional methods of identifying bacteria (such as gene sequencing), and analyzing metabolic output (liquid chromatography–mass spectrometry [LCMS] and similar chromatographic methods).

Using data obtained in linear mode analysis, IDBac employs hierarchical clustering to represent the relatedness of protein spectra. Since the spectra mostly represent ionized ribosomal proteins, they provide a representation of the phylogenetic diversity present in a sample. In addition, IDBac incorporates reflectron mode data to display specialized metabolite fingerprints as Metabolite Association Networks (MANs). MANs are bipartite networks that allow for easy visualization of shared and unique metabolite production between bacterial isolates. The IDBac platform allows researchers to analyze both protein and specialized metabolite data in tandem but also individually if only one data-type is acquired. Importantly, IDBac processes raw data from Bruker and Xiamen instruments, as well as txt, tab, csv, mzXML, and mzML. This eliminates the need for manual conversion and formatting of data sets, and significantly reduces the risk of user error or mishandling of MS data.

PROTOCOL

1. Preparation of MALDI matrix

1.1. Prepare 10 mg/mL MALDI-grade, and/or recrystallized α-cyano-4-hydroxycinnamic acid (CHCA) in MS-grade solvents: 50% acetonitrile (ACN), 47.5% water (H_2O), 2.5% trifluoroacetic acid (TFA). Example: 100 μL solution = 50 μL ACN + 47.5 μL H_2O + 2.5 μL TFA + 1 mg CHCA

1.1.1. Prepare at least 1 µL of matrix solution per MALDI plate spot and vortex or sonicate until in solution (approximately 5 min sonication or no visible solids).

CAUTION: TFA is a strong acid that should be handled in a chemical fume hood while wearing proper personal protective equipment, as it can damage skin, eyes, and airways with contact or inhalation.

NOTE: CHCA is hygroscopic and light-sensitive and should be stored in amber vials in a desiccator. There are many MALDI matrix options available. CHCA is most common for protein profiling of bacteria, but also works for specialized metabolite analysis. Matrix selection depends on individual user/experiment needs.

2. Preparation of MALDI target plates

NOTE: See Sauer et al.[7], for more details.

2.1. Rinse MALDI plate with methanol (HPLC-grade or higher) and wipe dry with soft paper wipes. Do not use abrasive brushes when cleaning target plates, as this can permanently damage the surface of the target plate.

2.2 Assign protein and specialized metabolite calibrant spots. Organize calibration spots evenly across the sample population, to account for MALDI-plate-irregularities and instrument drift over time. Assign an appropriate number of media/matrix-blank spots for the study; these spots will contain only media and matrix, or only matrix.

2.3. Using a sterile toothpick, transfer a small portion of a bacterial colony to the appropriate spot on the MALDI plate. Spread the bacterial colony evenly over the spot. The spot should appear as flat as possible.

NOTE: It will be easier to flatten bacterial colonies that are more mucoid/amorphous. For more rigid/solid colonies, avoid leaving visible clusters of cell mass on the MALDI spot (Figure 1).

2.4. Prepare a matrix/media control by using a sterile toothpick to transfer a minimal amount of agar/media onto the appropriate spot(s) on the MALDI plate.

2.5. Overlay 1 µL of 70% mass spectrometry grade formic acid onto each sample spot, including the matrix control spots. Allow acid to air dry completely in a chemical fume hood (approximately 5 min).

CAUTION: Formic acid is a caustic chemical and should be handled in chemical fume hoods. It can damage airways if inhaled.

2.6. Add 1 µL of the prepared MALDI matrix solution to each sample spot, as well as to the matrix/media control spots. Allow matrix solution to air dry completely (approximately 5 min).

NOTE: It is possible to store the plate in a desiccator, in the dark, until it can be analyzed on a MALDI-TOF mass spectrometer. Allowable storage times may vary depending on sample stability.

2.7. Add 0.5–1.0 μL calibrant to the assigned calibration spots, followed by 1 μL MALDI matrix solution. Pipette the resulting solution up and down to mix. Allow all spots to air dry completely prior to introduction into the MALDI-TOF mass spectrometer.

NOTE: The protein and specialized metabolite calibrants should be added within 30 min of MALDI analysis, as both are susceptible to degradation.

3. Data acquisition

NOTE: The general parameters for data acquisition are listed in Table 1.

3.1. Following the protocols specific to the instrument being used, set up both protein and specialized metabolite calibrations.

3.2. Test a few separate target spots to determine the optimal laser power and detector gain to use when acquiring spectra (this will vary day-to-day and by instrument).

NOTE: Figure 2A and Figure 3A show optimal spectra, while Figure 2D and Figure 3D are examples of poor-quality spectra.

3.4. Acquire spectra, saving protein spectra into one folder and specialized metabolite spectra into a second, separate folder.

4. Cleaning the MALDI target plate (adapted from Sauer et al.[7])

4.1. Remove the MALDI target plate from its holder and rinse with acetone.

4.2. Wash with a non-abrasive liquid soap to remove trace proteins and lipids, and soft paper wipes/soft-bristled toothbrush.

4.3. Rinse with de-ionized water for approximately 2 min to completely remove soap.

4.4. Sonicate the target plate in water (HPLC grade or higher) for 5 min.

4.5. Rinse the target plate with water (HPLC grade or higher).

4.6. Rinse the target plate with methanol (HPLC grade or higher).

5. Installing the IDBac Software

5.1. Download the IDBac software.

NOTE: Permanent, versioned backups are also available for download (see the Table of Materials).

5.2. Double-click the downloaded “Install_IDBac.exe” to initiate the installer and follow the on-screen instructions.

6. Starting with Raw Data

NOTE: Detailed explanations and instructions of each data processing step are embedded within IDBac, however the main analyses and interactive inputs are described below.

6.1. Double-click the IDBac desktop shortcut to launch IDBac. IDBac will open on the **Introduction** tab by default.

6.2. Use the **Check for Updates** button to ensure that the most current version of IDBac is being used (requires internet access). If a newer version is available, IDBac will automatically download and install the update, after which IDBac will request to be restarted.

6.3. Click on the **Starting with Raw Data** tab and choose from the menu the type of data to be used with IDBac; continue by following the in-app instructions.

6.4. When setting-up the conversion and processing of data files, input a descriptive name for the experiment where prompted (see Figure 4). Experiments will later be displayed alphabetically, so a helpful strategy is to start experiment names with a group-attribute (e.g., “bacillus-trials_experiment-1”; “bacillus-trials_experiment-2”).

7. Work with previous experiments

7.1. After converting files and processing them with IDBac, or anytime one wishes to reanalyze an experiment, navigate to the **Work with previous experiments** page and **Select an experiment to work with** (Figure 5).

7.2. (Optional) Add information about samples using the menu **Click here to modify the selected experiment**. Input information into the auto-populated spreadsheet and press **Save** (Figure 6). This option allows the user to color-code data during analyses.

7.3. (Optional) Transfer all, or a subset of samples to a new or another experiment by clicking **Transfer samples from previous experiments to new/other experiments** and following the provided instructions (Figure 7).

7.4. When ready to begin analysis, ensure the experiment to work with is selected. Select either **Protein Data Analysis** or **Small Molecule Data Analysis**.

8. Setting up protein data analysis and creating mirror plots

8.1. If analyzing protein data, first navigate to the **Protein Data Analysis** page. Choose peak-picking settings and evaluate protein spectra of samples via the displayed mirror plots (Figure 8).

NOTE: In the mirror plots, a red peak signifies the presence of that peak only in the top spectrum, while blue peaks represent those occurring in both spectra.

8.2. Adjust the percentage of replicates in which a peak must be present in order for it to be included for analyses (e.g., if the threshold is set to 70% and a peak occurs in at least 7 out of 10 replicates, it will be included).

8.3. Using the mirror plots as visual guidance, adjust the signal to noise cutoff that retains the most "genuine" peaks and the least noise, noting that more replicates and a higher "percentage peak presence" value will allow selection of a lower signal to noise cutoff.

8.4. Specify the lower and upper *m/z* cutoffs, dictating the range of mass values within each spectrum to be used in further analyses by IDBac.

9. Clustering samples using protein data

9.1. Within the **Protein Data Analysis** page, select the **Dendrogram** tab. This allows for grouping samples into a dendrogram according to user-selected distance measures and clustering algorithms.

9.2. Click **Select Samples** on the menu and follow the instructions to select samples to include in the analyses. Only samples that contain protein spectra will be displayed within the **Available Samples** box (Figure 9).

9.3. Use the default values or, under **Choose Clustering Settings**, select the desired **Distance** and **Clustering** algorithms to be applied to the generation of the dendrogram.

9.4. Select **Presence/Absence** as input. Alternatively, if confident about the peak heights of the samples (e.g., after performing a study to assess variability of peak intensity), select **Intensities** as input.

NOTE: At the time of publication, IDBac provides flexibility in settings for clustering, relying on users to choose the appropriate combinations. If unfamiliar with these options, it is suggested to pair either A: "cosine" distance, and "average (UPGMA)" clustering; or B: "Euclidean" distance, and "Ward.D2" clustering.

9.5. To display bootstrap values on the dendrogram, enter a number between 2 and 1000 under **Bootstraps**.

9.6. When reporting results, copy the text within the **Suggestions for Reporting Protein Analysis** paragraph. This provides the user-defined settings that generated the specific dendrogram.

10. Customizing the protein dendrogram

10.1. To begin customizing the dendrogram, open the **Adjust the dendrogram** menu (Figure 10).

10.2. To color the dendrogram's lines and/or labels select the appropriate button: **Click to modify lines** or **Click to modify labels** and select the desired options.

10.3. To plot information from the spreadsheet next to the dendrogram (see step 7.2), select the button **Incorporate info about samples**. This will open a panel where a category (column in the spreadsheet) will self-populate based on the entered values (Figure 11).

11. Insert samples from a separate experiment into the dendrogram

11.1. To insert samples from another experiment, select the menu button **Insert Samples from Another Experiment**. Follow the directions in the newly-opened panel (Figure 12).

12. Analyzing specialized metabolite data and metabolite association networks (MANs)

12.1. Proceed to the **Metabolite Association Network (Small-Molecule)** page. This page allows for data visualization by principle components analysis (PCA) and MANs, which use bipartite networks to display the correlation of small molecule *m/z* values with samples.

12.2. If a protein dendrogram was created (section 9), it will be displayed on this page as well. Click-and-drag on the dendrogram to highlight select samples of interest to be analyzed. If no samples are highlighted or no protein dendrogram was created, a MAN of a either a random subset or all samples will appear, respectfully (Figure 13).

12.3. To subtract a matrix/media blank in the MAN, open the menu **Select a Sample to Subtract** and choose the appropriate sample to use as a blank.

12.4. Open the menu **Show/Hide MAN Settings** to select the desired values for percentage of peak presence in replicates, signal to noise, and upper and lower mass cutoffs, as was done for protein spectra in Section 9. Use the small molecule mirror plots to guide the selection of these settings.

12.5. Select “Download Current Network Data” to save the data of the MAN that is currently displayed. These data can be used in network analysis software other than IDBac.

12.6. For reporting results, copy the text within the **Suggestions for Reporting MAN Analysis** paragraph. This provides the user-defined settings used to generate the created MAN.

13. Sharing data

13.1. Each IDBac “experiment” is saved as a single SQLite database. It contains the converted mzML raw spectra, detected peaks, and all user-input information about samples. Therefore, to share an IDBac experiment simply share the SQlite file that has the same name as the experiment (the file location is displayed on the **Working with Previous Experiments** page).

REPRESENTATIVE RESULTS

We analyzed six strains of *Micromonospora chokoriensis* and two strains of *Bacillus subtilis*, which were previously characterized[6], using data available at DOI: 10.5281/zenodo.2574096. Following directions in the **Starting with Raw Data** tab, we selected the option **Click here to convert Bruker files** and followed the IDBac-provided instructions for each dataset (Figure 14).

After the automated conversion and preprocessing/peak-peaking steps were completed, we proceeded to create a new combined IDBac experiment by transferring samples from the two experiments into a single experiment containing both *Bacillus* and *Micromonosopora* samples (Figure 15). The resulting analysis involved comparing protein spectra using mirror plots, as pictured in Figure 16, which was useful for evaluating spectra quality and adjusting peak-picking settings. Figure 17 displays a screenshot of the protein clustering results with default settings selected. The dendrogram was colored by adjusting the threshold on the plot (appears as a dotted line). Of note is the clear separation between genera, with both *M. chokoriensis* and *B. subtilis* isolates clustering separately.

Figure 18, Figure 19, and Figure 20 highlight the ability to generate MANs of user-selected regions by clicking and dragging across the protein dendrogram. With this we were able to rapidly create MANs to compare only the *B. subtilis* strains (Figure 18), only the *M. chokoriensis* strains (Figure 19), and all the strains simultaneously (Figure 20). The primary function of these networks is to provide researchers with a broad overview of the degree of specialized metabolite overlap between bacteria. With these data in hand, researchers now have the capacity to make informed decisions from only a small amount of material scraped from a bacterial colony.

DISCUSSION

The IDBac protocol details bacterial protein and specialized metabolite data acquisition and analysis of up to 384 bacterial isolates in 4 h by a single researcher. With IDBac there is no need to extract DNA from bacterial isolates or generate specialized metabolite extracts from liquid fermentation broths and analyze them using chromatographic methods. Instead, protein and specialized metabolite data are gathered by simply spreading material from bacterial colonies directly onto a MALDI target plate. This greatly reduces the time and cost associated with alternative techniques such as 16S rRNA gene sequencing and LCMS[9].

It is important to add a matrix blank and calibration spots to the MALDI plate, and we recommend using an appropriate number of replicates to ensure reproducibility and statistical confidence. The numbers of replicates will be experiment-dependent. For example, if a user intends to differentiate thousands of colonies from a collection of environmental diversity plates, fewer replicates may be necessary (our lab collects three technical replicates per colony). Alternatively, if a user wishes to create a custom database of strains from specific bacterial taxa to rapidly determine sub-species classifications of unknown isolates, then more replicates are appropriate (our lab collects eight biological replicates per strain).

IDBac is a tool for rapidly differentiating highly-related bacterial isolates based on putative taxonomic information and specialized metabolite production. It can complement or serve as a precursor to orthogonal methods such as in-depth genetic analyses, studies involving metabolite production and function, or characterization of specialized metabolite structure by Nuclear Magnetic Resonance spectroscopy and/or LC-MS/MS.

Specialized metabolite production (IDBac MANs) is highly susceptible to bacterial growth conditions, especially using different media, which is a potential limitation of the method. However these traits may be exploited by the user, as IDBac can readily generate MANs showing the differences in specialized metabolite production under a variety of growth conditions. It is important to note that while specialized metabolite fingerprints may vary by growth condition, we have shown that protein fingerprints remain relatively stable (see Clark et al.[6]). When dealing with environmental diversity plates, we recommend purifying bacterial isolates prior to analysis in order to reduce possible contributions from neighboring bacterial cross-talk.

Finally, the lack of a searchable public database of protein MS fingerprints is a major shortcoming in the use of this method to classify unknown environmental bacteria. We created IDBac with this in mind, and included automated conversion of data into a community-accepted open-source format (mzML)[10–12] and designed the software to allow searching, sharing, and creation of custom databases. We are in the process of creating a large public database (>10,000 fully characterized strains), which will allow for the classification of some isolates to the species-level, including links to GenBank accession numbers when available.

IDBac is open source and the code is available for anyone to customize their data analysis and visualization needs. We recommend that users consult an extensive body of literature (Sauer et al.[7], Silva et al.[5]) to help support and design their experimental goals. We host a forum for discussion at: https://groups.google.com/forum/#!forum/idbac and a means to report issues with the software at: https://github.com/chasemc/IDBacApp/issues.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

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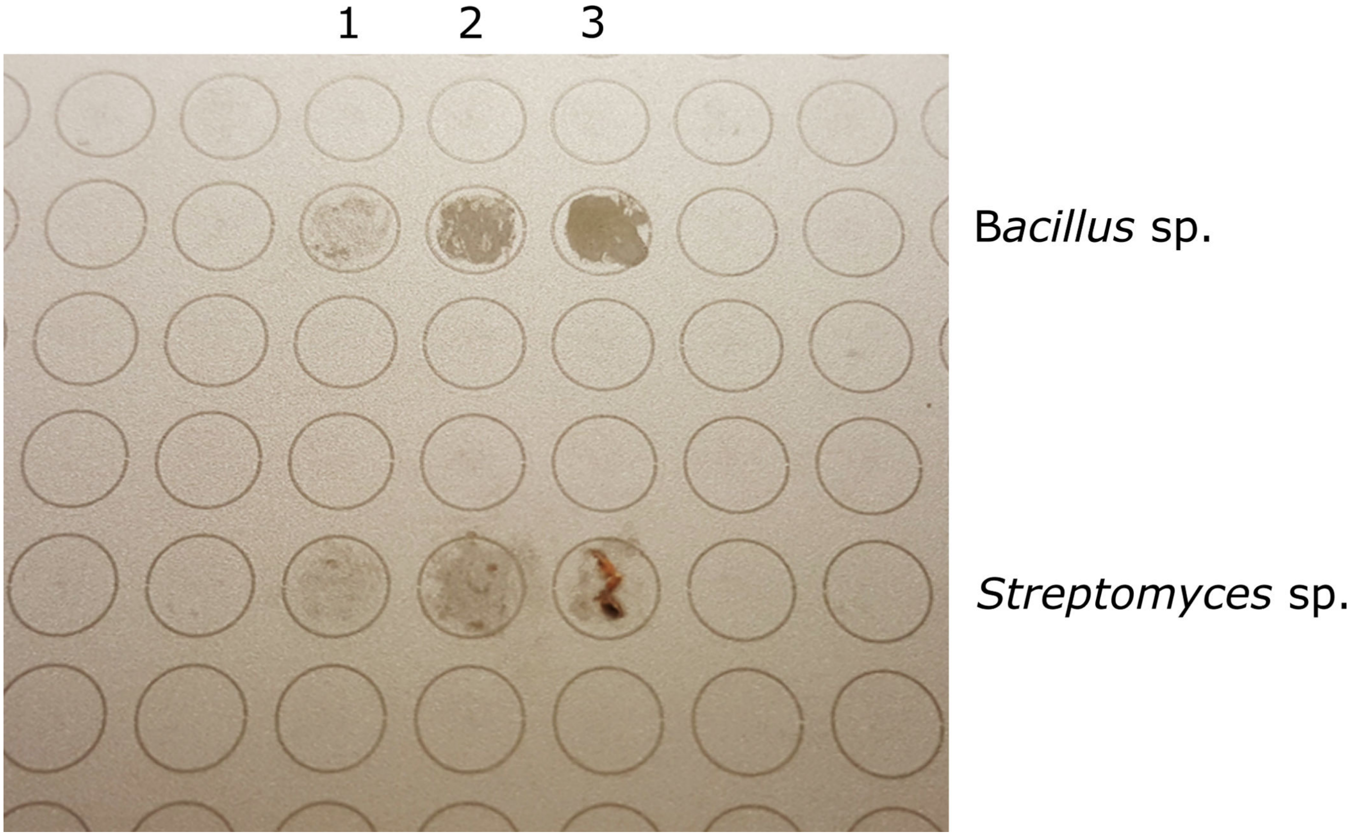


Figure 1: MALDI-target plate showing two different isolates before adding formic acid and MALDI matrix (top 3 spots – *Bacillus* sp.; bottom 3 spots – *Streptomyces* sp.).

For both, column **3** represents excess sample; column **2** represents the appropriate amount of sample; column **1** represents insufficient sample for MALDI analysis.

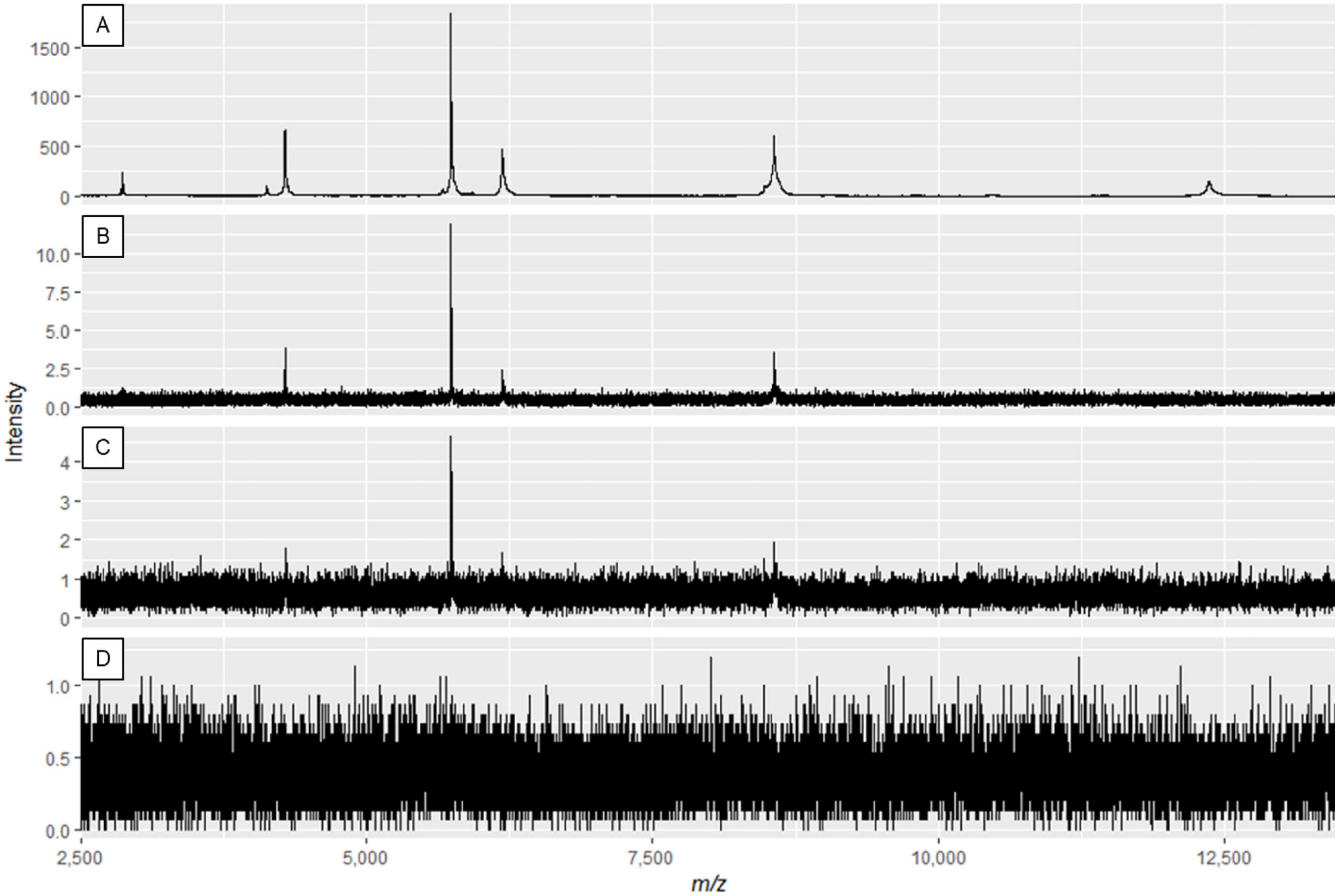


Figure 2: Example protein spectra displaying the effect of modifying laser power and detector gain.

Spectra quality is best in panel **A**, and decreases until insufficient spectra quality in panels **C** and **D**. While the spectrum in panel **B** may result in useable peaks, panel **A** displays optimal data.

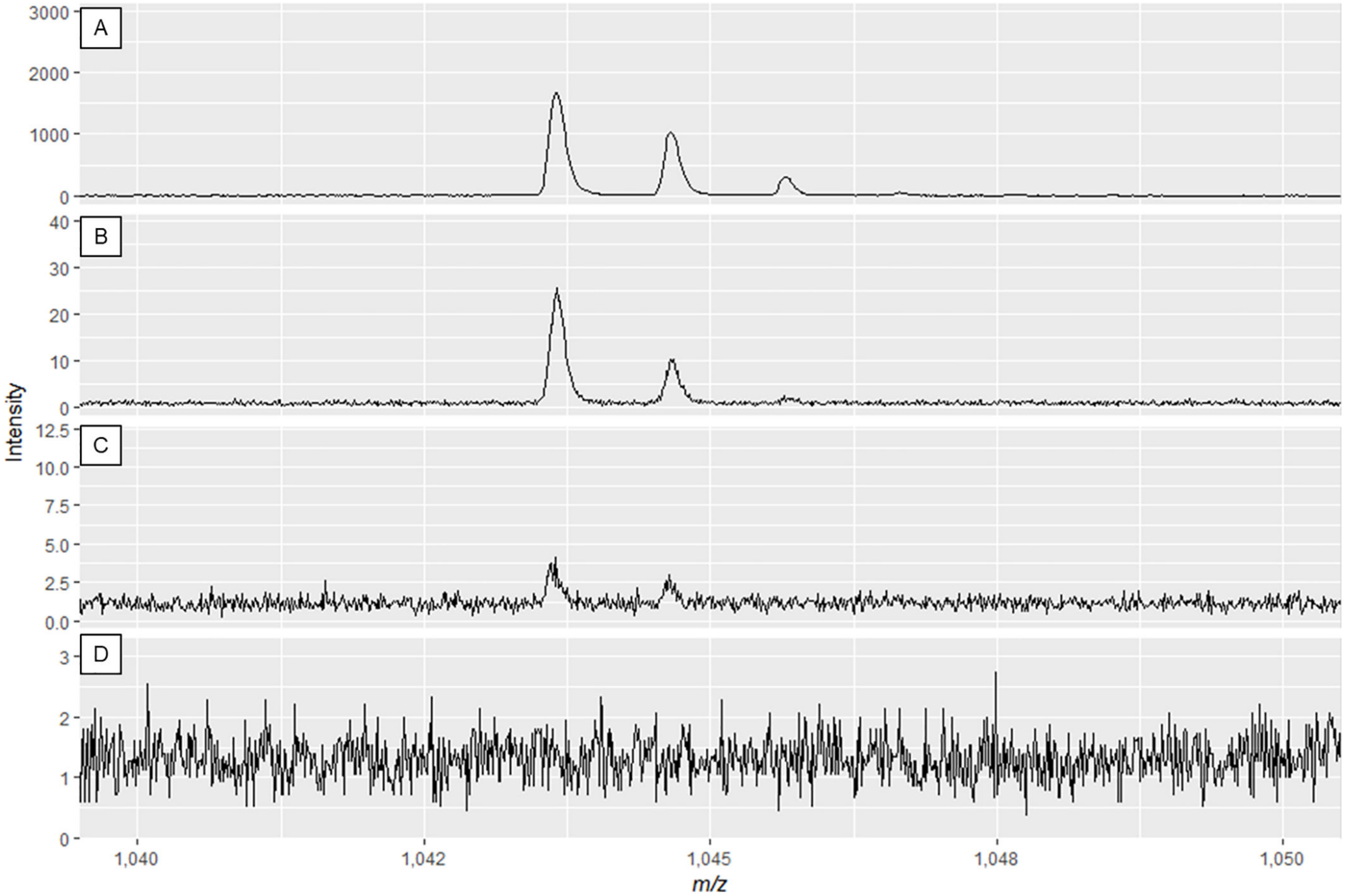
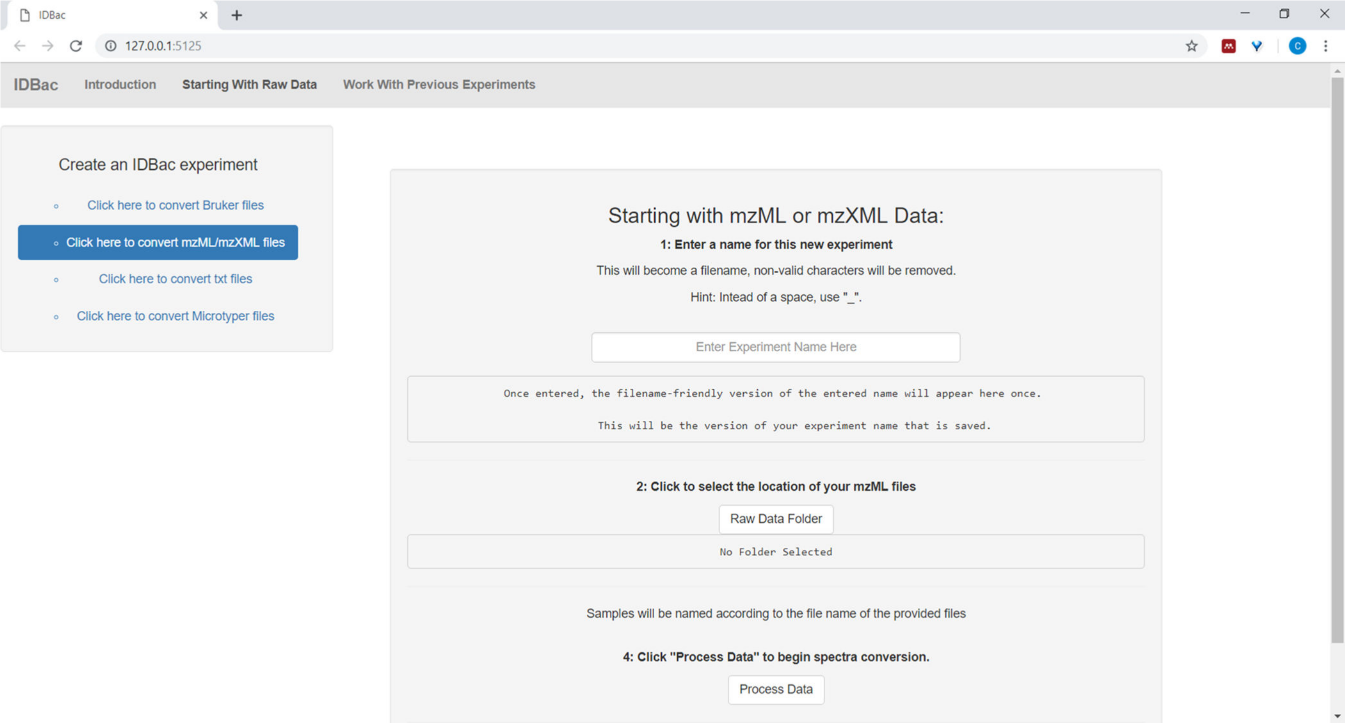


Figure 3: Example specialized metabolite spectra displaying the effect of modifying laser power and detector gain.

Spectra quality is best in panel **A** and decreases until insufficient spectra quality in panels **C** and **D**. While the spectrum in panel **B** may result in useable peaks, panel **A** displays optimal data.



Create an IDBac experiment

- Click here to convert Bruker files
- Click here to convert mzML/mzXML files
- Click here to convert txt files
- Click here to convert Microtyper files

Starting with mzML or mzXML Data:

1: Enter a name for this new experiment

This will become a filename, non-valid characters will be removed.

Hint: Intead of a space, use "_".

Enter Experiment Name Here

```
Once entered, the filename-friendly version of the entered name will appear here once.

This will be the version of your experiment name that is saved.
```

2: Click to select the location of your mzML files

Raw Data Folder

```
No Folder Selected
```

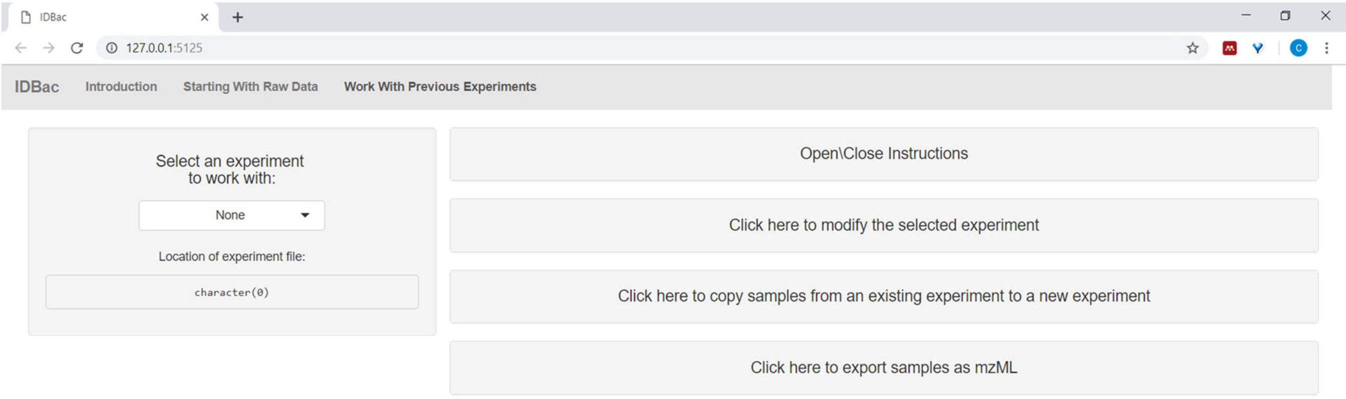
Samples will be named according to the file name of the provided files

4: Click "Process Data" to begin spectra conversion.

Process Data

Figure 4: IDBac data conversion and preprocessing step.

IDBac converts raw spectra into the open mzML format and stores mzML, peak lists, and sample information in a database for each experiment.

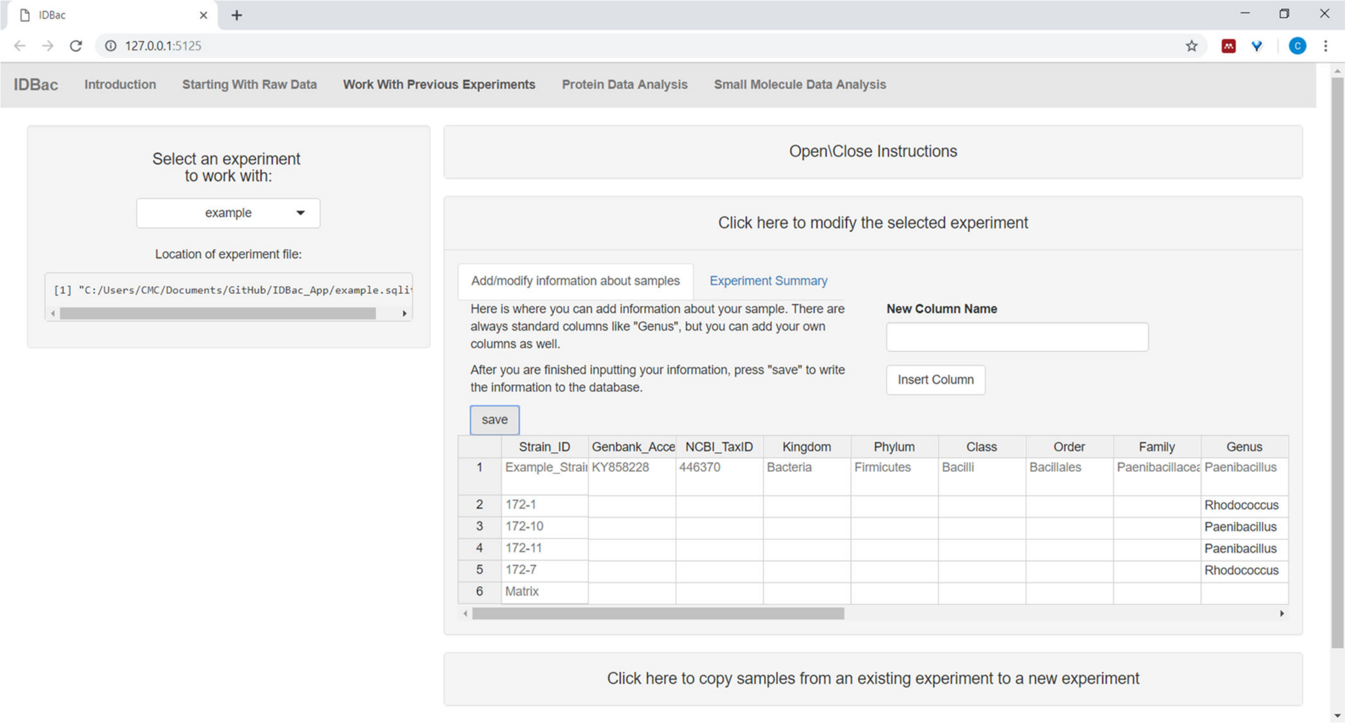

IDBac
127.0.0.1:5125
IDBac
Introduction
Starting With Raw Data
Work With Previous Experiments
Open\Close Instructions
Click here to modify the selected experiment
Click here to copy samples from an existing experiment to a new experiment
Click here to export samples as mzML

Select an experiment to work with: None

Location of experiment file: character(0)

Figure 5: “Work with Previous Experiments” page.

Use IDBac’s “Work with Previous Experiments” page to select an experiment to analyze or modify.


IDBac
127.0.0.1:5125
IDBac
Introduction
Starting With Raw Data
Work With Previous Experiments
Protein Data Analysis
Small Molecule Data Analysis
Select an experiment to work with:
example
Location of experiment file:
Open\Close Instructions
Click here to modify the selected experiment
Add/modify information about samples
Experiment Summary
New Column Name
Insert Column
save
Click here to copy samples from an existing experiment to a new experiment

```
[1] "C:/Users/CMC/Documents/GitHub/IDBac_App/example.sqli
```

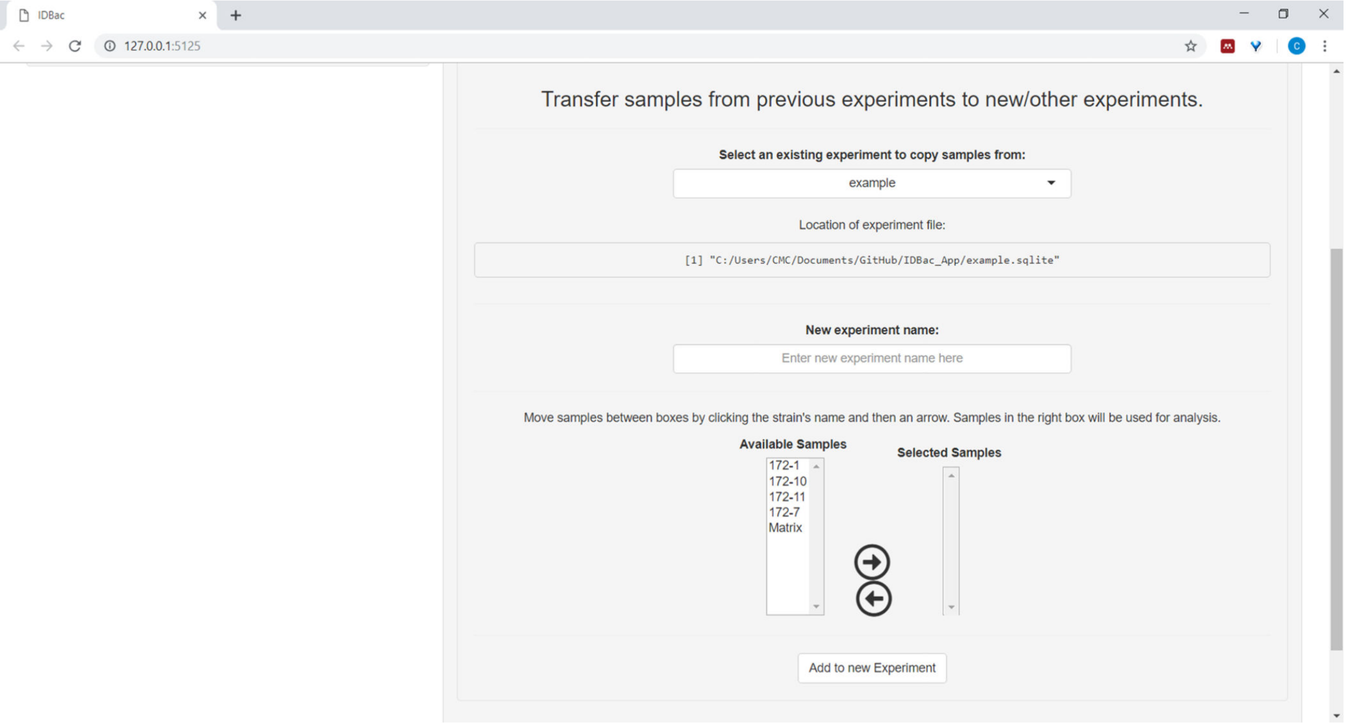
Here is where you can add information about your sample. There are always standard columns like "Genus", but you can add your own columns as well.

After you are finished inputting your information, press "save" to write the information to the database.

	Strain_ID	Genbank_Acce	NCBI_TaxID	Kingdom	Phylum	Class	Order	Family	Genus
1	Example_Strai	KY858228	446370	Bacteria	Firmicutes	Bacilli	Bacillales	Paenibacillace	Paenibacillus
2	172-1								Rhodococcus
3	172-10								Paenibacillus
4	172-11								Paenibacillus
5	172-7								Rhodococcus
6	Matrix								

Figure 6: Input sample information.

Within the “Work with Previous Experiments” page users can input information about samples such as taxonomic identity, collection location, isolation conditions, etc.



Transfer samples from previous experiments to new/other experiments.

Select an existing experiment to copy samples from: example

Location of experiment file:

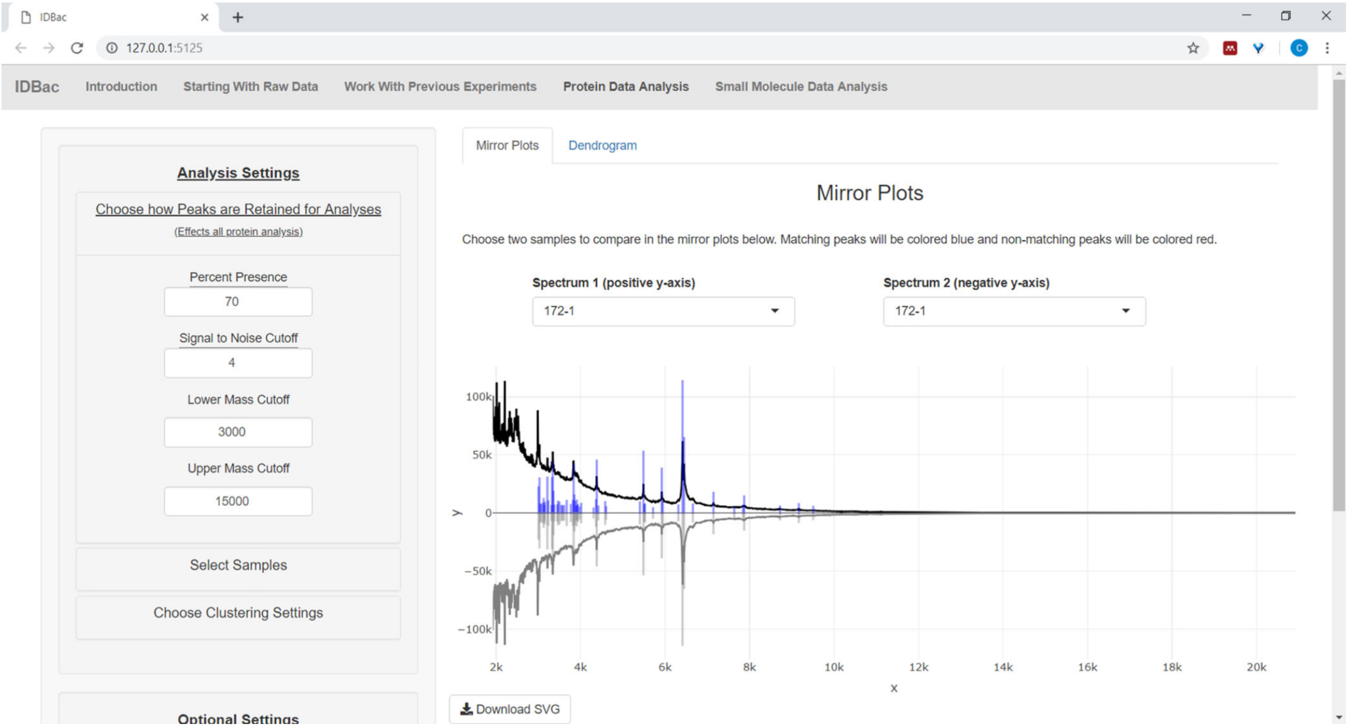
```
[1] "C:/Users/CMC/Documents/GitHub/IDBac_App/example.sqlite"
```

New experiment name: Enter new experiment name here

Move samples between boxes by clicking the strain's name and then an arrow. Samples in the right box will be used for analysis.

Figure 7: Transfer data.

The “Work with Previous Experiments” page contains the option to transfer data between existing experiments and to new experiments.



Analysis Settings

Choose how Peaks are Retained for Analyses

(Effects all protein analysis)

Percent Presence: 70

Signal to Noise Cutoff: 4

Lower Mass Cutoff: 3000

Upper Mass Cutoff: 15000

Mirror Plots

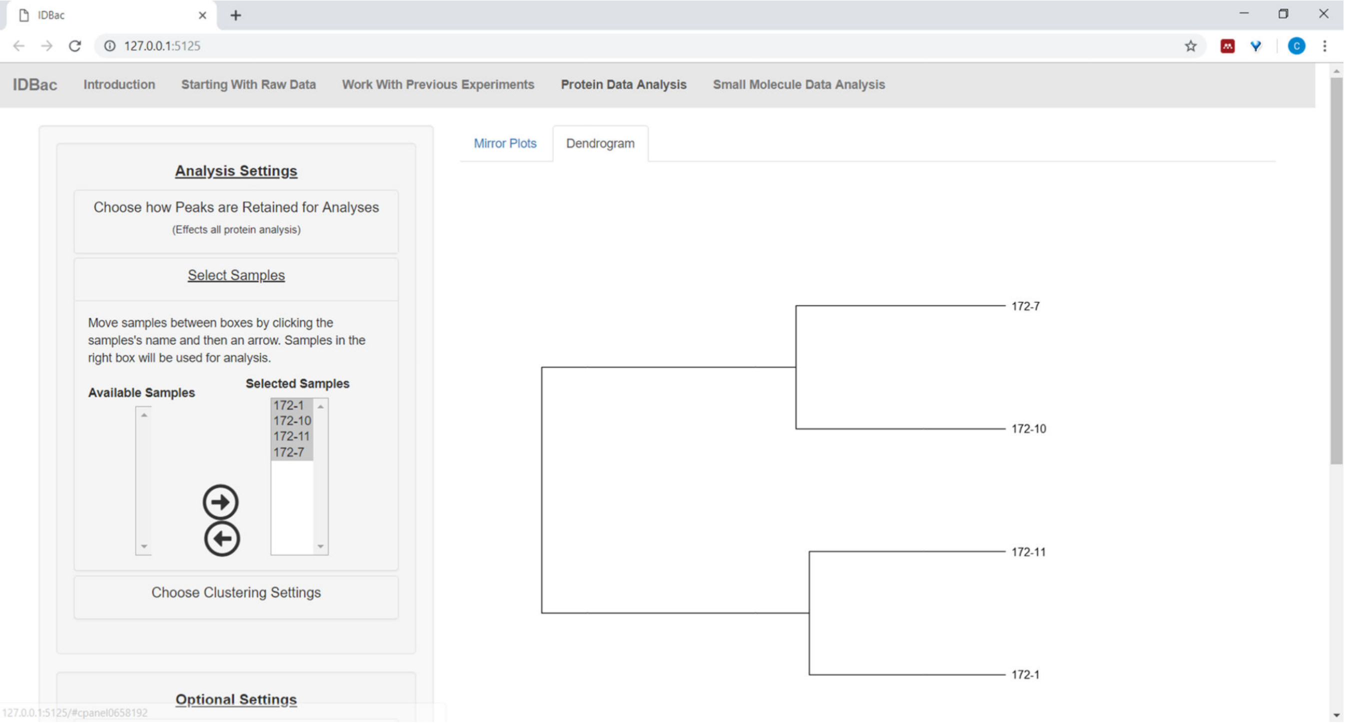
Choose two samples to compare in the mirror plots below. Matching peaks will be colored blue and non-matching peaks will be colored red.

Spectrum 1 (positive y-axis): 172-1

Spectrum 2 (negative y-axis): 172-1

Figure 8: Choose how peaks are retained for analysis.

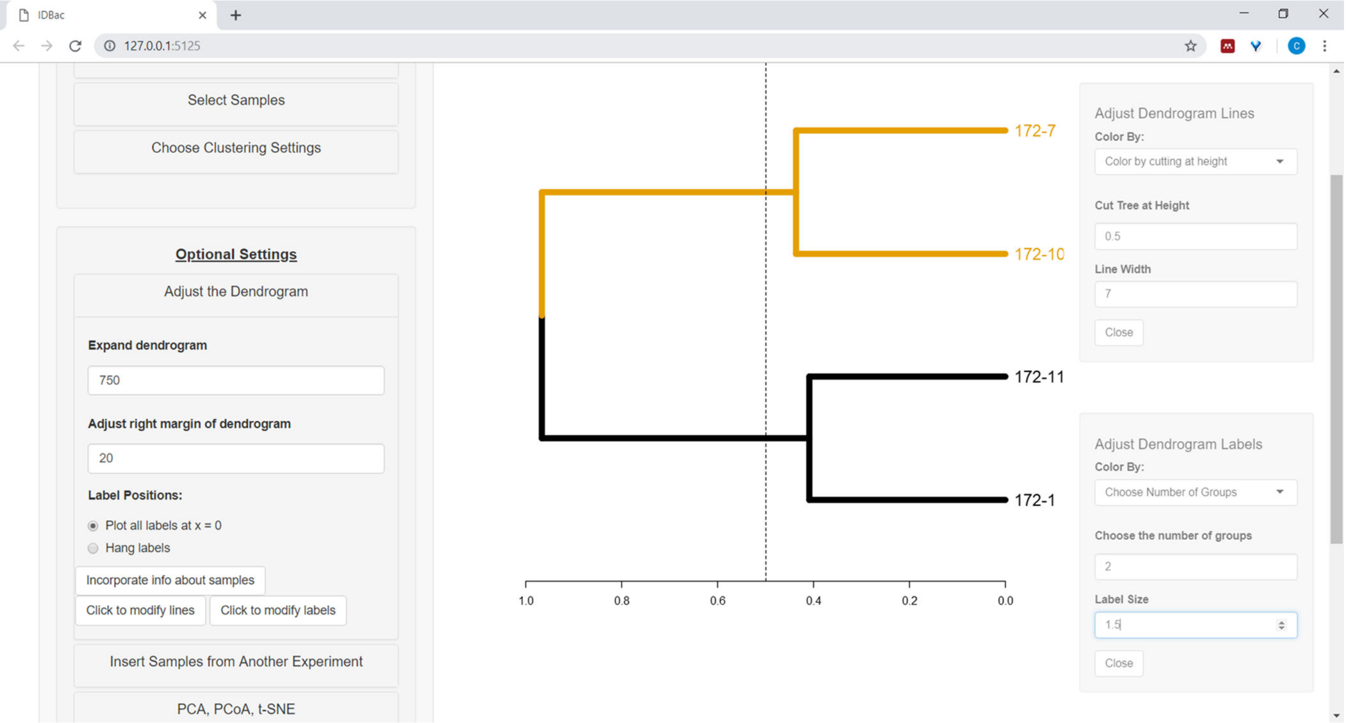
After selecting an experiment to analyze, visiting the “Protein Data Analysis” page and subsequently opening the “Choose how Peaks are Retained for Analysis” menu allows users to choose settings like signal-to-noise ratio for retaining peaks. The displayed mirror plot (or dendrogram) will automatically update to reflect the chosen settings.



Move samples between boxes by clicking the samples's name and then an arrow. Samples in the right box will be used for analysis.

Figure 9:

Select samples from the chosen experiment to include within the displayed dendrogram.



Expand dendrogram: 750

Adjust right margin of dendrogram: 20

Label Positions:

● Plot all labels at x = 0

○ Hang labels

Adjust Dendrogram Lines

Color By: Color by cutting at height

Cut Tree at Height: 0.5

Line Width: 7

Adjust Dendrogram Labels

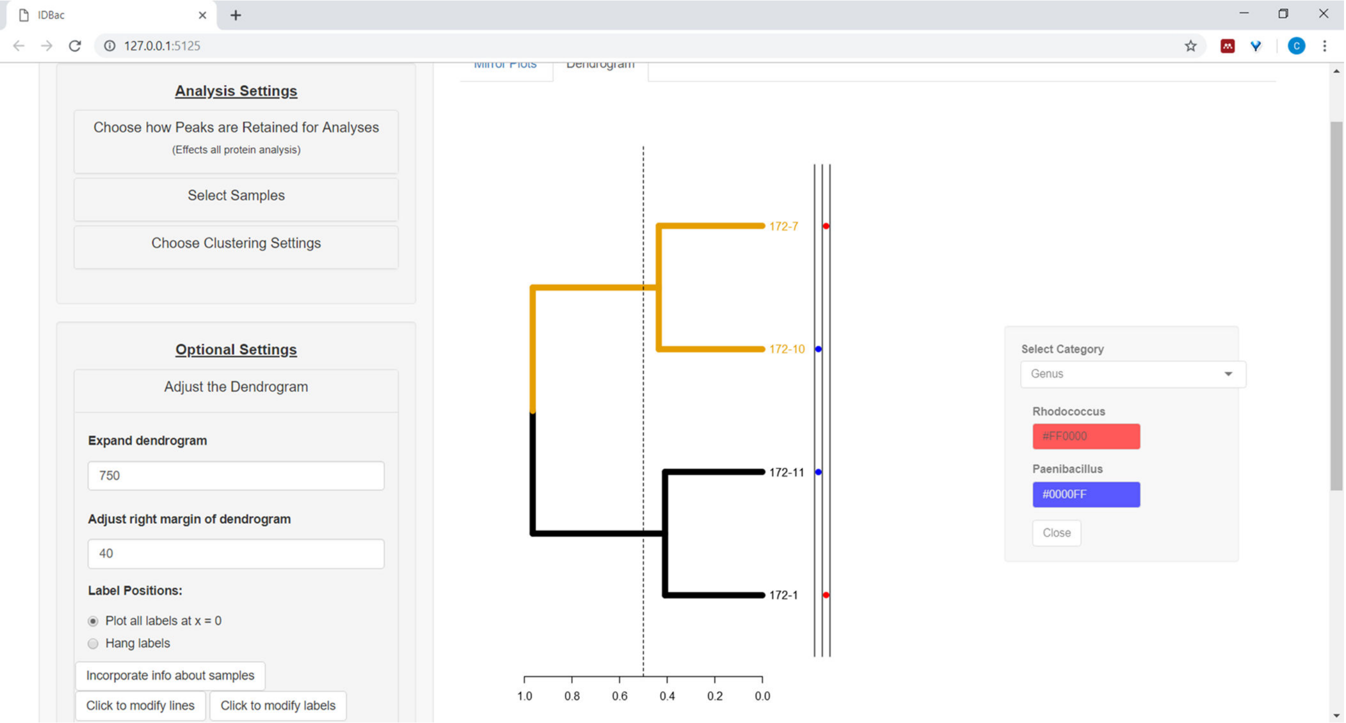
Color By: Choose Number of Groups

Choose the number of groups: 2

Label Size: 1.5

Figure 10: Adjust the dendrogram.

IDBac provides a few options for modifying how the dendrogram looks, these may be found within the menu “Adjust the Dendrogram”. This includes coloring branches and labels by k-means, or by “cutting” the dendrogram at a user-provided height.



Analysis Settings

Choose how Peaks are Retained for Analyses
(Effects all protein analysis)

Select Samples

Choose Clustering Settings

Optional Settings

Adjust the Dendrogram

Expand dendrogram: 750

Adjust right margin of dendrogram: 40

Label Positions:
● Plot all labels at x = 0
○ Hang labels

Incorporate info about samples

Click to modify lines | Click to modify labels

Select Category: Genus

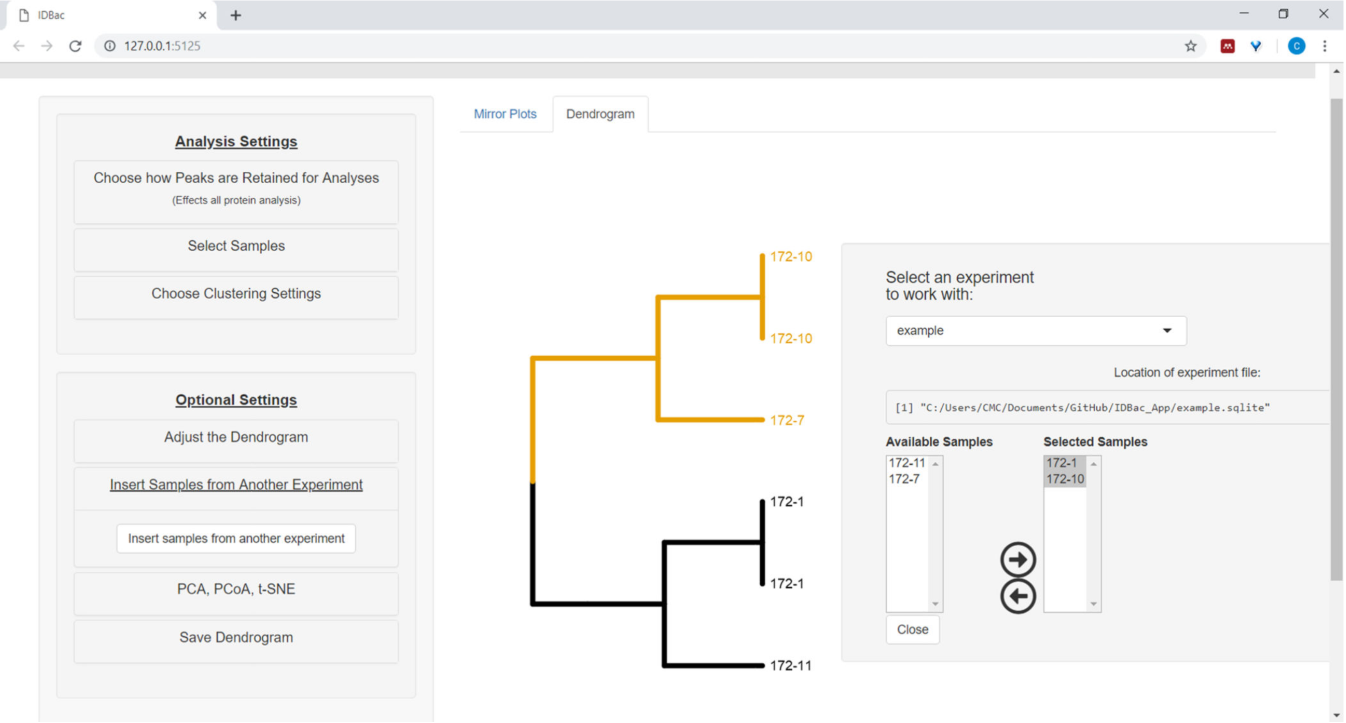
Rhodococcus: #FF0000

Paenibacillus: #0000FF

Close

Figure 11: Incorporate info about samples.

Within the “Adjust the Dendrogram” menu is the option “Incorporate info about samples”. Selecting this will allow plotting information about samples next to the dendrogram. Sample information is input within the “Work with Previous Experiments” page.



Select an experiment to work with: example

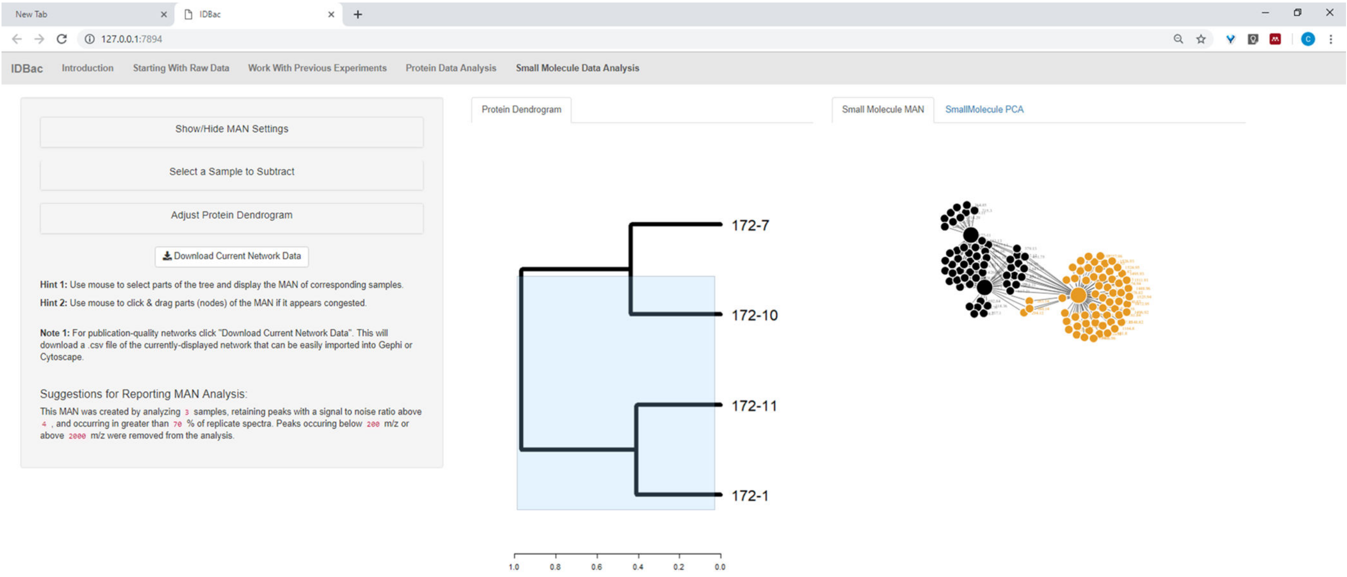
Location of experiment file:

```
[1] "C:/Users/CMC/Documents/GitHub/IDBac_App/example.sqlite"
```

Available Samples	Selected Samples
172-11	172-1
172-7	172-10

Figure 12: Insert Samples from Another Experiment menu.

Sometimes it is helpful to compare samples from another experiment. Use the “Insert Samples from Another Experiment” menu to choose samples to include within the currently-displayed dendrogram.



Hint 1: Use mouse to select parts of the tree and display the MAN of corresponding samples.

Hint 2: Use mouse to click & drag parts (nodes) of the MAN if it appears congested.

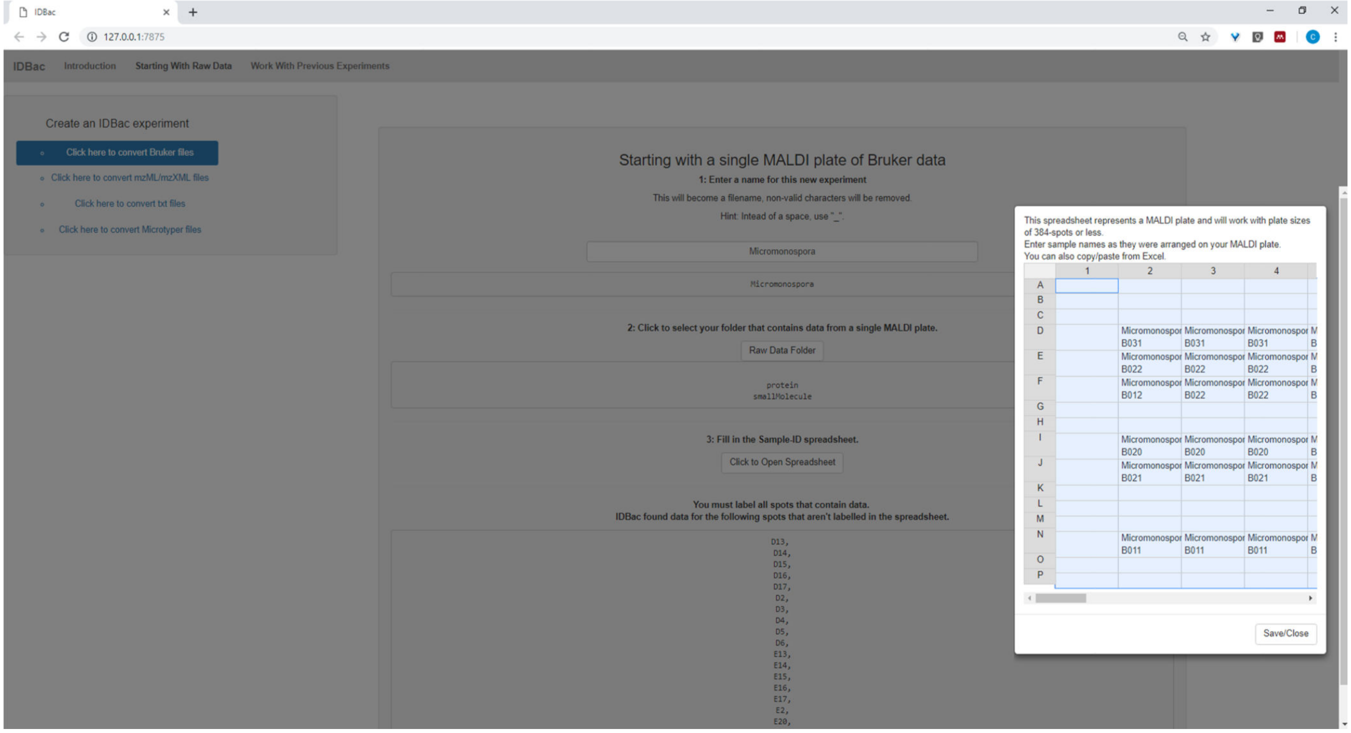
Note 1: For publication-quality networks click "Download Current Network Data". This will download a .csv file of the currently-displayed network that can be easily imported into Gephi or Cytoscape.

Suggestions for Reporting MAN Analysis:

This MAN was created by analyzing `3` samples, retaining peaks with a signal to noise ratio above `4` , and occurring in greater than `70` % of replicate spectra. Peaks occuring below `200` m/z or above `2000` m/z were removed from the analysis.

Figure 13: Small Molecule Data Analysis” page.

If a dendrogram was created from protein spectra, it will be displayed within the “Small Molecule Data Analysis” page. This page will also display Metabolite Associate Networks (MANs) and Principle Components Analysis (PCA) for small molecule data.


IDBac
127.0.0.1:7875
IDBac
Introduction
Starting With Raw Data
Work With Previous Experiments
Create an IDBac experiment
Click here to convert Bruker files
Click here to convert mzML/mzXML files
Click here to convert txt files
Click here to convert Microtyper files
Raw Data Folder
Click to Open Spreadsheet
Save/Close

Starting with a single MALDI plate of Bruker data

1: Enter a name for this new experiment

This will become a filename, non-valid characters will be removed.

Hint: Intead of a space, use "_".

Micromonospora

```
Micromonospora
```

2: Click to select your folder that contains data from a single MALDI plate.

```
protein
smallMolecule
```

3: Fill in the Sample-ID spreadsheet.

You must label all spots that contain data.
IDBac found data for the following spots that aren't labelled in the spreadsheet.

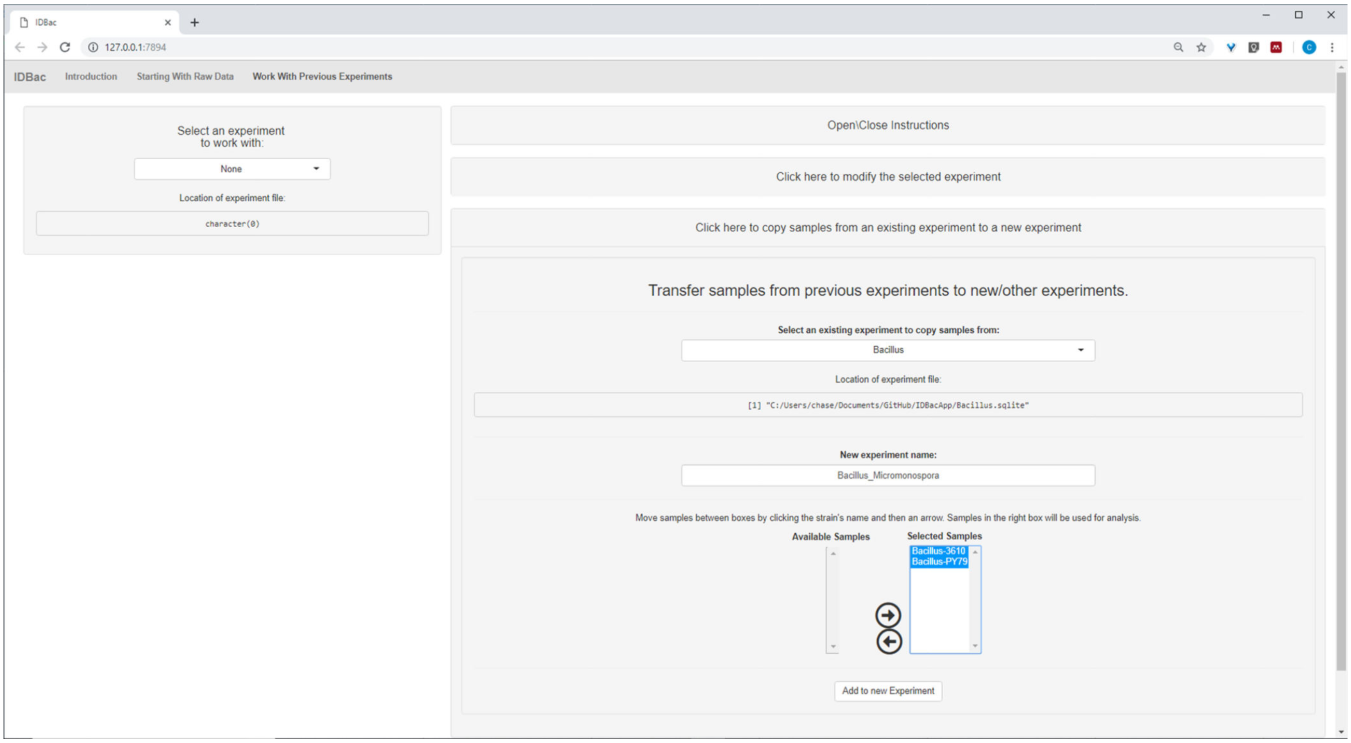
```
D13,
D14,
D15,
D16,
D17,
D2,
D3,
D4,
D5,
D6,
E13,
E14,
E15,
E16,
E17,
E2,
E20,
```

This spreadsheet represents a MALDI plate and will work with plate sizes of 384-spots or less.
Enter sample names as they were arranged on your MALDI plate.
You can also copy/paste from Excel.

	1	2	3	4	
A					
B					
C					
D		Micromonospor B031	Micromonospor B031	Micromonospor B031	M B
E		Micromonospor B022	Micromonospor B022	Micromonospor B022	M B
F		Micromonospor B012	Micromonospor B022	Micromonospor B022	M B
G					
H					
I		Micromonospor B020	Micromonospor B020	Micromonospor B020	M B
J		Micromonospor B021	Micromonospor B021	Micromonospor B021	M B
K					
L					
M					
N		Micromonospor B011	Micromonospor B011	Micromonospor B011	M B
O					
P					

Figure 14: Spectra processing.

Downloaded Bruker autoFlex spectra were converted and processed using IDBac.


IDBac
127.0.0.1:7894
IDBac
Introduction
Starting With Raw Data
Work With Previous Experiments
Open\Close Instructions
Click here to modify the selected experiment
Click here to copy samples from an existing experiment to a new experiment
Add to new Experiment

Select an experiment to work with:

None

Location of experiment file:

```
character(0)
```

Transfer samples from previous experiments to new/other experiments.

Select an existing experiment to copy samples from:

Bacillus

Location of experiment file:

```
[1] "C:/Users/chase/Documents/GitHub/IDBacApp/Bacillus.sqlite"
```

New experiment name:

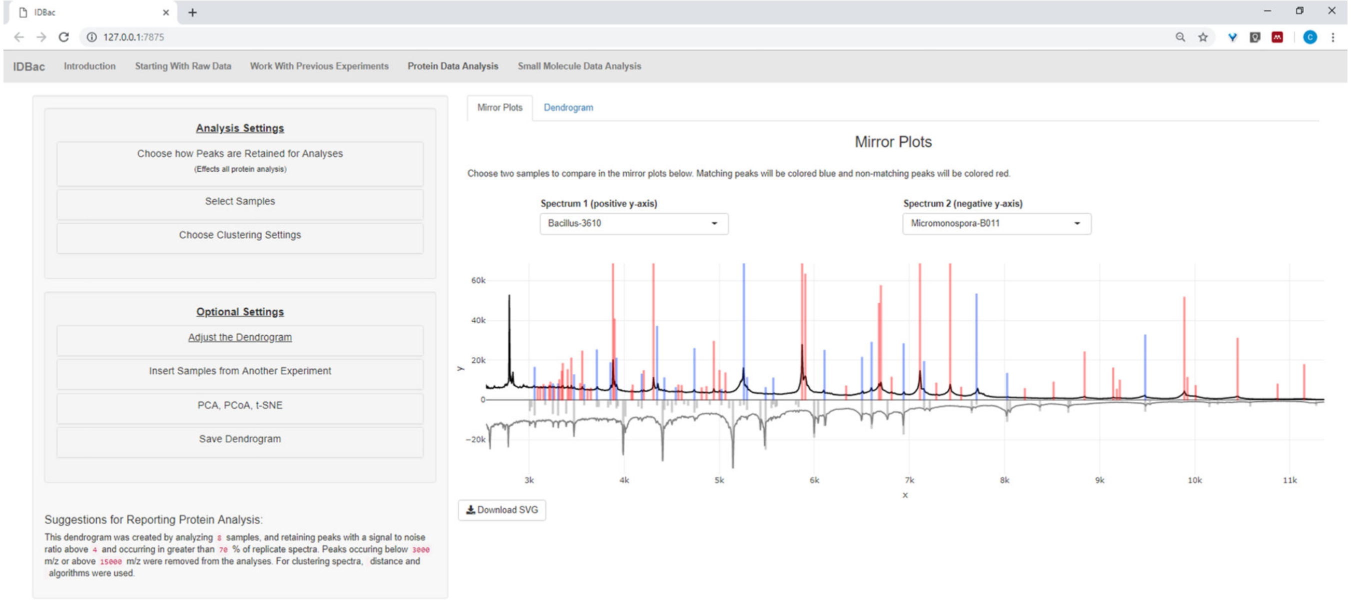
Bacillus_Micromonospora

Move samples between boxes by clicking the strain's name and then an arrow. Samples in the right box will be used for analysis.

Available Samples	**Selected Samples**
	Bacillus-3610 Bacillus-PY79

Figure 15: Combined IDBac experiment.

Because the *Micromonospora* and *Bacillus* spectra were collected on different MALDI target plates, the two experiments were subsequently combined into a single experiment- “Bacillus_Micromonsopora”. This was done within the “Work with Previous Experiments” tab, following directions within the menu “Transfer samples from previous experiments to new/other experiments”.



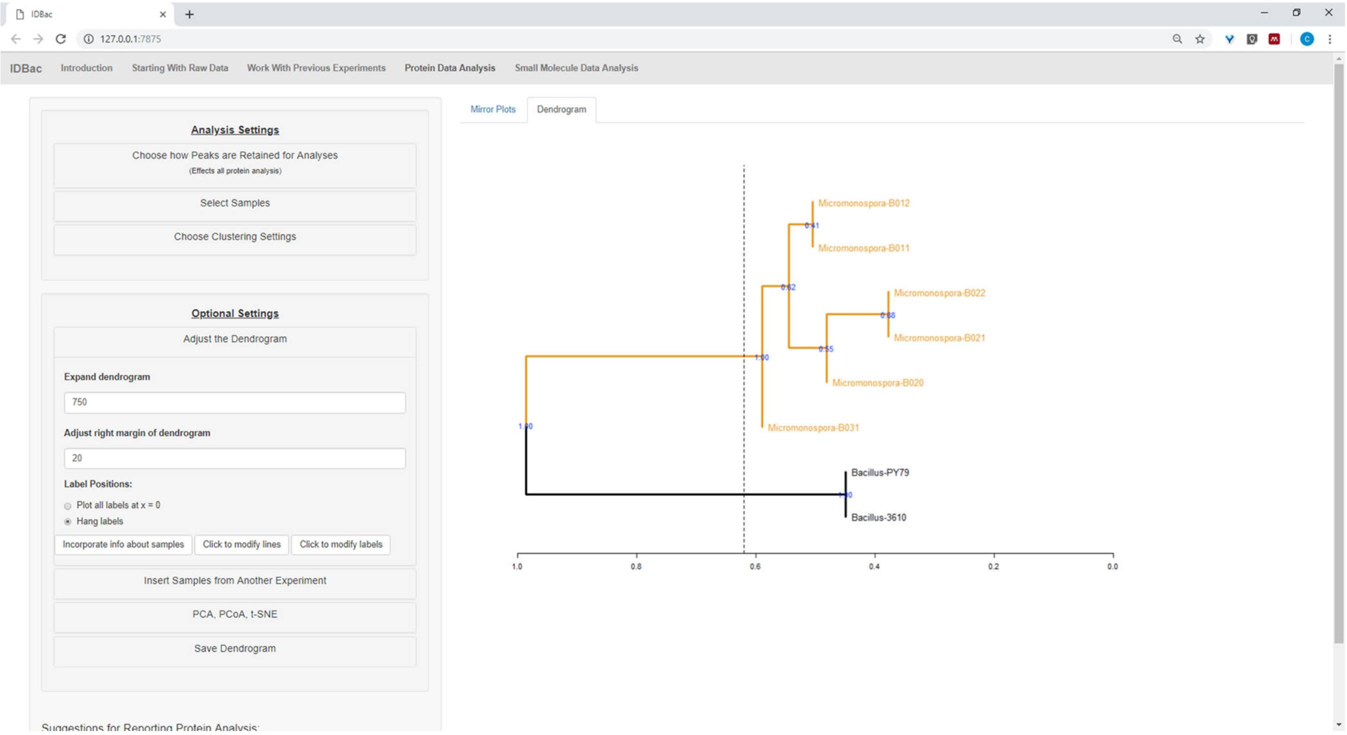
Choose two samples to compare in the mirror plots below. Matching peaks will be colored blue and non-matching peaks will be colored red.

Suggestions for Reporting Protein Analysis:

This dendrogram was created by analyzing 8 samples, and retaining peaks with a signal to noise ratio above 4 and occurring in greater than 70 % of replicate spectra. Peaks occuring below 3000 m/z or above 15000 m/z were removed from the analyses. For clustering spectra, distance and algorithms were used.

Figure 16: Comparison.

Micromonspora and *Bacillus* spectra were compared using the mirror plots within the “Protein Data Analysis” page. Ultimately, default peak settings were chosen.



Expand dendrogram

750

Adjust right margin of dendrogram

20

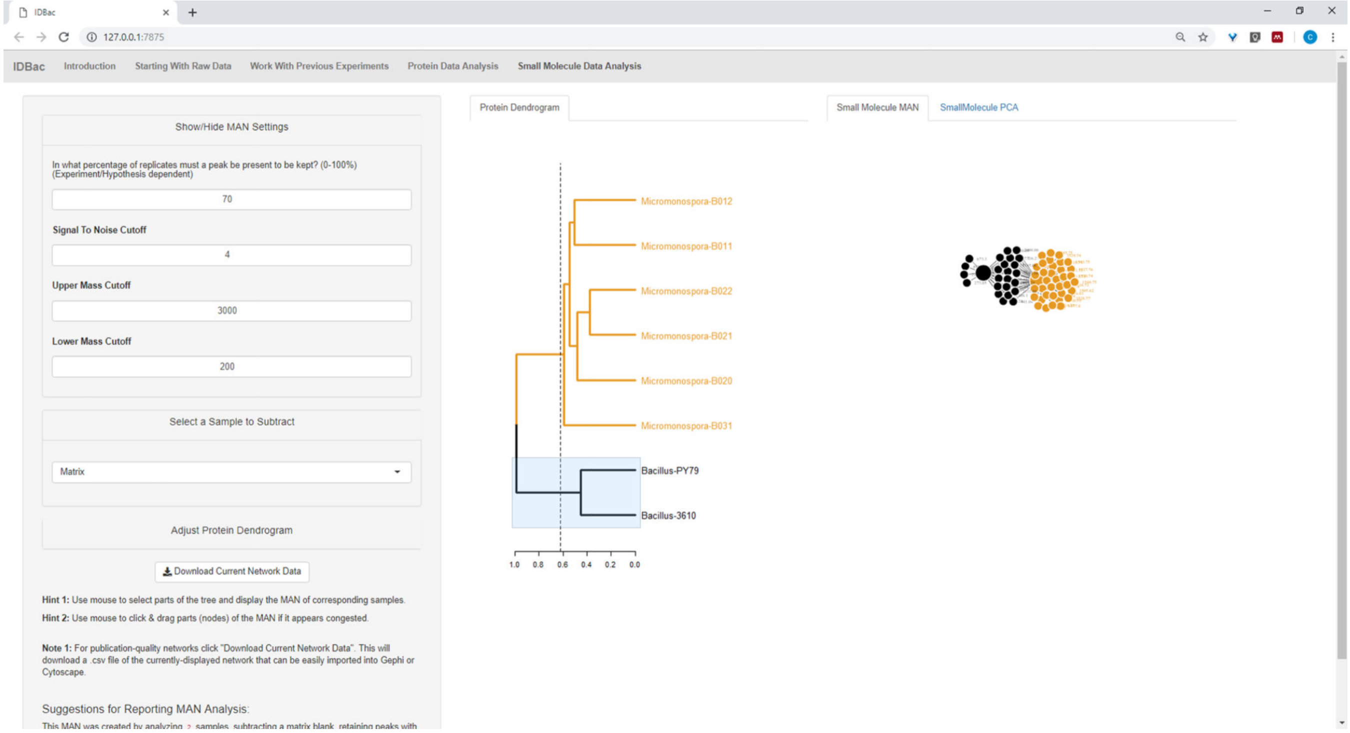
Label Positions:

○ Plot all labels at x = 0

● Hang labels

Figure 17: Hierarchical clustering.

Hierarchical clustering, using default settings, correctly grouped *Bacillus* and *Micromonospora* isolates. The dendrogram was colored by "cutting" the dendrogram at an arbitrary height (displayed as a dashed-line) and 100 bootstraps used to show confidence in branching.



In what percentage of replicates must a peak be present to be kept? (0-100%) (Experiment/Hypothesis dependent)

70

Signal To Noise Cutoff

4

Upper Mass Cutoff

3000

Lower Mass Cutoff

200

Matrix

Hint 1: Use mouse to select parts of the tree and display the MAN of corresponding samples.

Hint 2: Use mouse to click & drag parts (nodes) of the MAN if it appears congested.

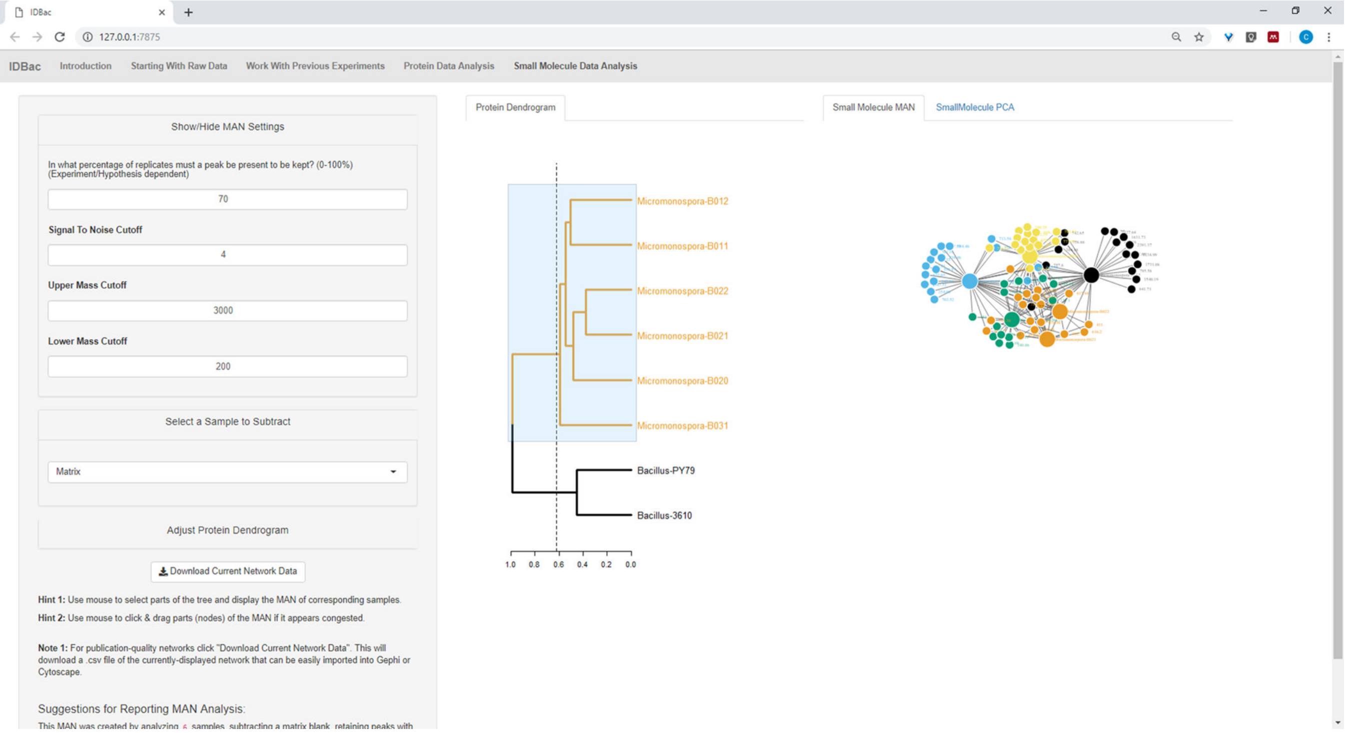
Note 1: For publication-quality networks click "Download Current Network Data". This will download a .csv file of the currently-displayed network that can be easily imported into Gephi or Cytoscape.

Suggestions for Reporting MAN Analysis:

This MAN was created by analyzing 2 samples, subtracting a matrix blank, retaining peaks with

Figure 18:

MAN created by selecting the *Bacillus* sp. strains from the protein dendrogram showed differential production of specialized metabolites.



Show/Hide MAN Settings

In what percentage of replicates must a peak be present to be kept? (0-100%) (Experiment/Hypothesis dependent)

70

Signal To Noise Cutoff

4

Upper Mass Cutoff

3000

Lower Mass Cutoff

200

Select a Sample to Subtract

Matrix

Adjust Protein Dendrogram

Download Current Network Data

Hint 1: Use mouse to select parts of the tree and display the MAN of corresponding samples.

Hint 2: Use mouse to click & drag parts (nodes) of the MAN if it appears congested.

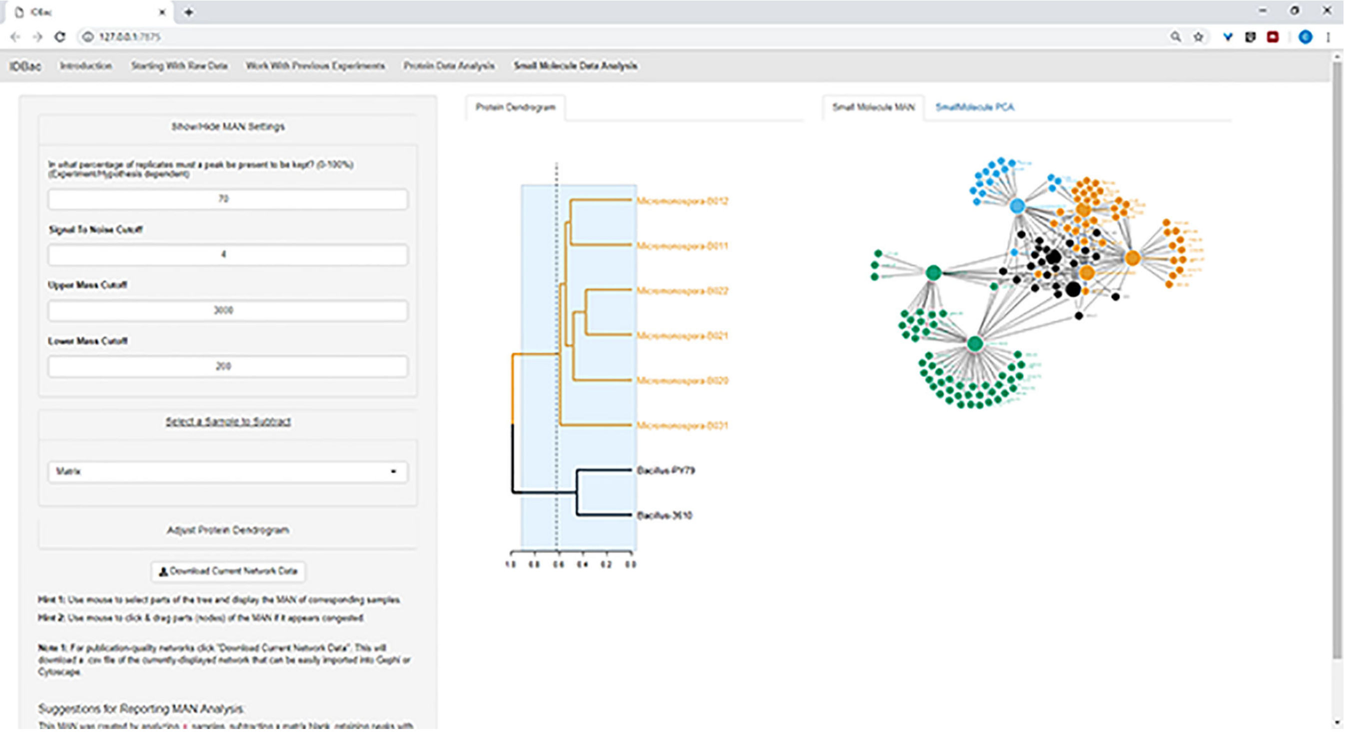
Note 1: For publication-quality networks click "Download Current Network Data". This will download a .csv file of the currently-displayed network that can be easily imported into Gephi or Cytoscape.

Suggestions for Reporting MAN Analysis:

This MAN was created by analyzing 6 samples, subtracting a matrix blank, retaining peaks with

Figure 19:

MAN created by selecting the six *Micromonospora* sp. strains from the protein dendrogram showed differential production of specialized metabolites.



In what percentage of replicates must a peak be present to be kept? (0-100%) (Experiment/Hypothesis dependent)

70

Signal To Noise Cutoff

4

Upper Mass Cutoff

3000

Lower Mass Cutoff

200

Matrix

Hint 1: Use mouse to select parts of the tree and display the MAN of corresponding samples.

Hint 2: Use mouse to click & drag parts (nodes) of the MAN if it appears congested.

Note 1: For publication-quality networks click "Download Current Network Data". This will download a .csv file of the currently-displayed network that can be easily imported into Gephi or Cytoscape.

Suggestions for Reporting MAN Analysis:

This MAN was created by analyzing x samples, subtracting a matrix blank, retaining peaks with

Figure 20:

MAN of *Bacillus* sp. and *Micromonospora* sp. strains showing a differential production of specialized metabolites.

Parameter	**Protein**	**Specialized Metabolite**
Mass Start	1920	60
Mass End	21000	2700
Mass Deflection	1900	50
Shots	500	1000
Frequency	2000	2000
Laser Size	Large	Medium
MaxStdDev (ppm)	300	30