

Tutorial for the PNNL Biodiversity Library Skyline Plugin

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This tutorial is to help users install and use the PNNL Biodiversity Library plugin with Skyline.

Troubleshooting help will follow at the end of the tutorial. We note that this plugin does not work with Windows XP and must be used with Skyline version 3.1.1.7490 or greater. For information about the Skyline program, including how to download, please visit

<https://skyline.gs.washington.edu/labkey/project/home/begin.view?>

Further information about the Biodiversity Library available at:

<http://omics.pnl.gov/project-data/biodiversity-library>

Program Goal

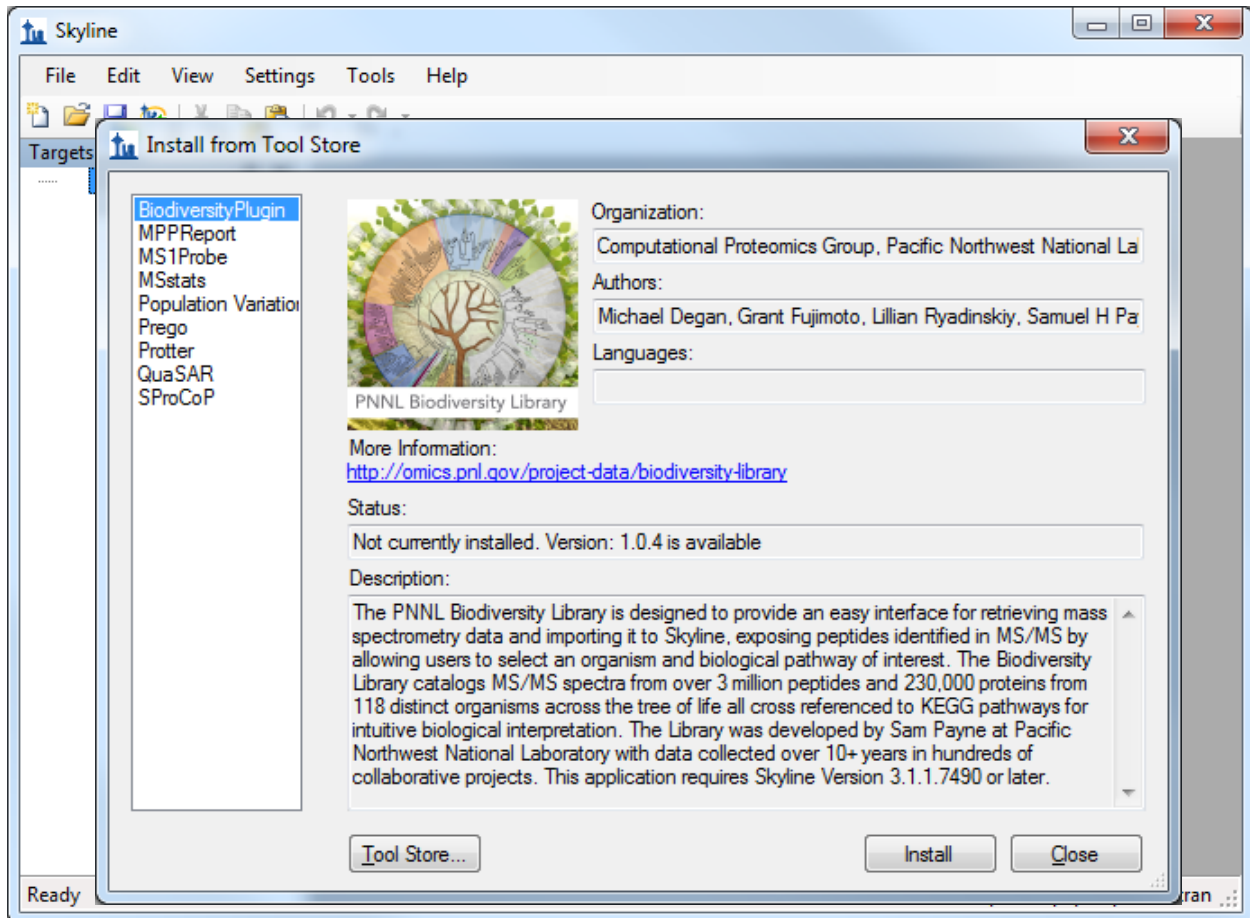
The goal of the plug-in is to help users browse the large spectral libraries from the PNNL Biodiversity Library and import relevant data into Skyline. Additional functionality allows users to import their own data into the plugin for easy navigation and loading into Skyline.

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Installation:

The PNNL Biodiversity Library Plugin for Skyline can be installed through the Skyline tool store interface.
Tools -> Tool Store -> PNNL Biodiversity Library



Running the Plugin:

Overview

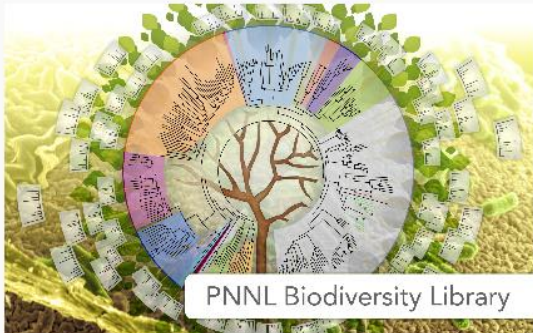
Organism Chooser

Pathway Chooser

Pathway Visualization

Review and Export

Upload New Data



PNNL Biodiversity Library

The PNNL Biodiversity Library is designed to provide an easy interface for retrieving mass spectrometry data. This data can be exported into Skyline to assist in SRM assay design or DIA data analysis. The tool exposes peptides identified in MS/MS by allowing users to select an organism and biological pathway of interest. In total the Biodiversity Library catalogs MS/MS spectra from 3 million peptides and 230,000 proteins from 118 distinct organisms across the tree of life. All proteins are cross referenced to KEGG pathways for intuitive biological interpretation. The Library was developed by Sam Payne at [Pacific Northwest National Laboratory](#) with data collected over 10+ years in hundreds of collaborative projects.

The wizard helps users browse data using the following steps. Users can upload new data into the plugin by clicking "Upload New Data" on the left hand side at the bottom of the page.

- 1) Select an Organism.
- 2) Select pathways of interest.
- 3) View pathways and curate proteins.
- 4) Review and export data to Skyline.

PNNL Biodiversity Plugin Version: 1.0.4
Database Source: [Biodiversity Library v2.1.230](#) Thursday, March 31, 2016
Funding: US DOE, [Biological and Environmental Research](#)
Contact: [Samuel Payne](#)

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The plug-in is a wizard that walks users through the process of importing data into Skyline.

Select an Organism:

You are able to select your desired organisms in one of two ways, either through the Taxonomy Explorer or by directly searching for the organism by name.

The Taxonomy Explorer organizes organisms through scientific naming convention, i.e. Kingdom, Phylum, Class and then individual strain of the organism. Human data is additionally broken down by tissue.

The Organism search (right panel) allows for users to type in a name and auto-filters the dataset to matching organisms.

The screenshot displays the PNNL Biodiversity Library web application. On the left is a vertical sidebar with buttons: Overview, Organism Chooser (highlighted in dark blue), Pathway Chooser, Pathway Visualization, Review and Export, and Upload New Data. The main content area is divided into three sections. The top section, '1) Select an Organism.', provides instructions on how to retrieve data. Below this are two panels: 'Taxonomy Explorer' on the left and 'Organism search' on the right. The Taxonomy Explorer shows a hierarchical tree of taxonomic levels, with 'Mycobacterium tuberculosis H37Rv' highlighted by a red box. The Organism search panel shows a list of organisms, including 'Acidiphilium cryptum', 'Actinosynnema mirum', and 'Mycobacterium tuberculosis H37Rv'. At the bottom right are '< Previous' and 'Next >' buttons.

1) Select an Organism.
Data in the Biodiversity Library is organized by the organism. To retrieve data, please select an organism of interest, either through the Phylogeny explorer or through the Organism search box. If you want to analyze data from multiple organisms, please export the data one organism at a time. If you would like to add or customize an organism, click "Upload New Data" on the left hand side at the bottom of the page. Organisms that have been customized are shown in red.

Taxonomy Explorer

- Archaea
- Bacteria
 - Acidobacteria
 - Actinobacteria
 - Actinosynnema
 - Arthrobacter
 - Brachybacterium
 - Cellulomonas
 - Cryptobacterium
 - Gardnerella
 - Kineococcus
 - Mycobacterium
 - Mycobacterium tuberculosis H37Rv**
 - Nakamurella
 - Nocardiopsis
 - Pseudonocardia
 - Saccharomonospora
 - Sanguibacter
 - Slackia
 - Stackebrandtia
 - Thermobispora
 - Xylanimonas
 - Alphaproteobacteria
 - Bacteroidetes
 - Betaproteobacteria
 - Chlorobi

Organism search

- Acidiphilium cryptum
- Actinosynnema mirum
- Anabaena variabilis
- Anaeromyxobacter dehalogenans 2CP-C
- Anaplasma phagocytophilum HZ
- Arthrobacter sp. FB24
- Bacillus anthracis Ames
- Bacillus anthracis Sterne
- Bacillus subtilis subsp. subtilis 168
- Bacteroides fragilis 638R
- Bacteroides thetaiotaomicron
- Bartonella henselae Houston-1
- Borrelia burgdorferi B31
- Brachybacterium faecium
- Burkholderia mallei ATCC 23344
- Candidatus Pelagibacter ubique
- Caulobacter crescentus CB15
- Cellulomonas flavigena
- Cenarchaeum symbiosum
- Chloracidobacterium thermophilum
- Chlorobium tepidum
- Chloroflexus aurantiacus
- Cryptobacterium curtum
- Cyanothece sp. ATCC 51142
- Cyanothece sp. PCC 7424
- Cyanothece sp. PCC 7425
- Cyanothece sp. PCC 7822
- Cyanothece sp. PCC 8801
- Dehalococcoides mccartyi 195

< Previous Next >

Select Pathways of Interest:

After you have selected an organism, you then choose the KEGG pathways you are interested in investigating. The coverage metric is dynamically calculated for each Organism/Pathway combination and shows the percentage of proteins in a pathway for which the Library contains proteomic data. You can select as many pathways as you desire.

The screenshot shows the 'PNNL Biodiversity Library' application window. On the left is a vertical navigation menu with buttons: 'Overview', 'Organism Chooser', 'Pathway Chooser' (highlighted in dark blue), 'Pathway Visualization', 'Review and Export', and 'Upload New Data'. The main area is titled '2) Select pathways of interest.' and contains instructions: 'Pathway definitions and membership are defined by KEGG. Navigate to pathways and click the checkbox. Multiple pathways can be selected. If you decide to use your own data, you can do so by clicking "Upload New Data" on the bottom left.'

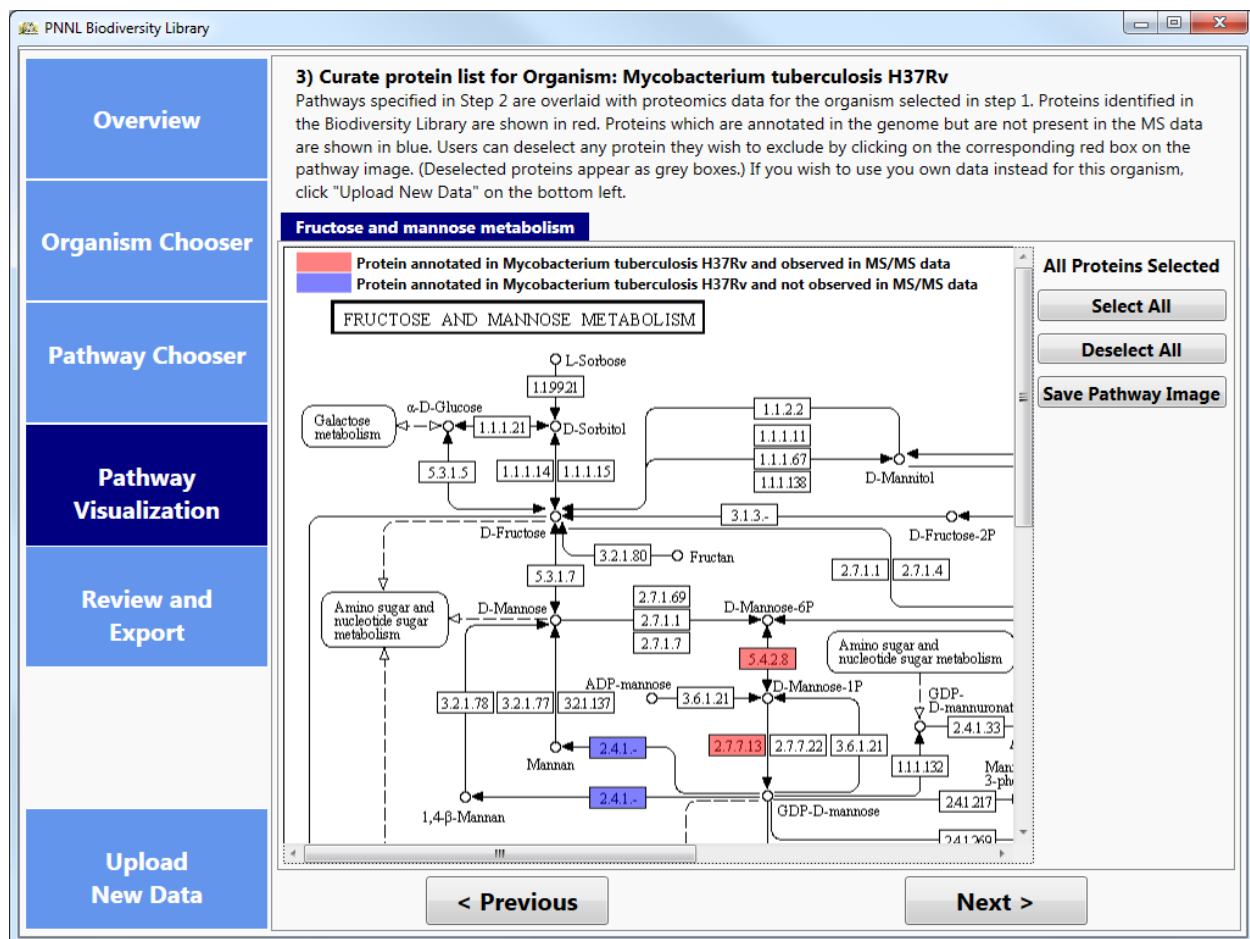
The main content is divided into two panels: 'Kegg Pathways' and 'Pathways Selected'. The 'Kegg Pathways' panel shows a tree view of metabolic pathways. Under 'Metabolism', 'Carbohydrate metabolism' is expanded, listing various pathways with their MSMS coverage percentages. The 'Fructose and mannose metabolism' pathway is selected with a checked checkbox and highlighted by a red rectangle. The 'Pathways Selected' panel on the right currently lists 'Fructose and mannose metabolism'. At the bottom of the window are 'Previous' and 'Next' navigation buttons.

Pathway	Coverage in MSMS
Glycolysis / Gluconeogenesis	66.67%
Citrate cycle (TCA cycle)	75.00%
Pentose phosphate pathway	55.56%
Pentose and glucuronate interconversions	60.00%
Fructose and mannose metabolism	60.00%
Galactose metabolism	75.00%
Ascorbate and aldarate metabolism	75.00%
Starch and sucrose metabolism	68.00%
Amino sugar and nucleotide sugar metabolism	65.38%
Pyruvate metabolism	53.85%
Glyoxylate and dicarboxylate metabolism	68.75%
Propanoate metabolism	56.25%
Butanoate metabolism	85.71%
C5-Branched dibasic acid metabolism	60.00%
Inositol phosphate metabolism	42.86%

Select proteins of interest through KEGG Pathway maps:

The images for the pathways you selected will be created dynamically, highlighting all the KEGG orthologs for the organism in the pathway. If that protein has been observed in the spectral library, it will be highlighted initially as red; unobserved proteins are highlighted in blue. From here, you can select the proteins which are of interest to you in the pathway in two ways, either by clicking on individual boxes or by clicking the select/deselect all buttons on the right hand side of the screen. If an ortholog does become deselected it will then be highlighted as grey to show that the information will not be pushed to the next step of the process.

Additionally, you can save the pathway image by clicking the button on the right-hand side of the screen, below the Deselect All button. This will save the image for the pathway along with your current selection of orthologs as a .png file with a resolution of 300 dpi.



Review and export:

On the Review and Export tab, the application will display the total number of genes selected from all of your Organism/Pathway combinations as well as an individual breakdown of the proteins. If there are Organism/Pathway combinations you are no longer interested in, or individual proteins which you do not wish to export to Skyline, you can uncheck the box for the row(s).

Overview

Organism Chooser

Pathway Chooser

Pathway Visualization

Review and Export

Upload New Data

4) Review and export data to Skyline.

From this final tab, a table of genes for each organism and pathway are shown for review. By hitting the export button, all relevant data will be transferred to Skyline. This includes a FASTA file for all selected proteins, as well as the identified peptides in a bibliospec library.


Organism	Pathway	# of Genes	Export?
Mycobacterium tuberculosis H37R	Fructose and mannose metabolism	10	<input checked="" type="checkbox"/>

Organism: Mycobacterium tuberculosis H37Rv
Pathway: Fructose and mannose metabolism
Genes (10)

Export?	Accession	Protein name
<input checked="" type="checkbox"/>	O53634	gca: GDP-mannose 4,6-dehydratase
<input checked="" type="checkbox"/>	P9WN21	glpX: fructose 1,6-bisphosphatase
<input checked="" type="checkbox"/>	P9WG43	tpi: triosephosphate isomerase
<input checked="" type="checkbox"/>	P71790	gmdA: GDP-D-mannose dehydratase GmdA
<input checked="" type="checkbox"/>	P71791	epiA: nucleotide-sugar epimerase EpiA
<input checked="" type="checkbox"/>	P9WKD7	rpiB: ribose-5-phosphate isomerase B
<input checked="" type="checkbox"/>	O05898	manA: mannose-6-phosphate isomerase
<input checked="" type="checkbox"/>	O86374	pmmA: phosphomannomutase PmmA
<input checked="" type="checkbox"/>	L7N6A5	manB: D-alpha-D-mannose-1-phosphate guanylyltransferase ManB
<input checked="" type="checkbox"/>	O53360	pmmB: phosphomannomutase PmmB

<< Select another Organism

< Previous

 Confirm to Export

Export data to Skyline:

Once you are satisfied with your selection, you can then either select another organism and repeat the wizard steps, or export the data to Skyline.

Once you confirm the export to skyline, the application will create a FASTA for the proteins you indicated and download the Spectral Library files for the organisms of interest from the Biodiversity Library's Repository on MassIVE (<ftp://massive.ucsd.edu/MSV000079053/library/>). Both of these files (FASTA and Bibliospec library) are then imported to Skyline.

The screenshot shows the 'PNNL Biodiversity Library' application window. On the left is a vertical sidebar with five buttons: 'Overview', 'Organism Chooser', 'Pathway Chooser', 'Pathway Visualization', and 'Review and Export' (which is highlighted in dark blue). The main content area is titled '4) Review and export data to Skyline.' and contains the following information:

From this final tab, a table of genes for each organism and pathway are shown for review. By hitting the export button, all relevant data will be transferred to Skyline. This includes a FASTA file for all selected proteins, as well as the identified peptides in a bibliospec library.

Organism	Pathway	# of Genes	Export?
Mycobacterium tuberculosis H37Rv	Fructose and mannose metabolism	10	<input checked="" type="checkbox"/>

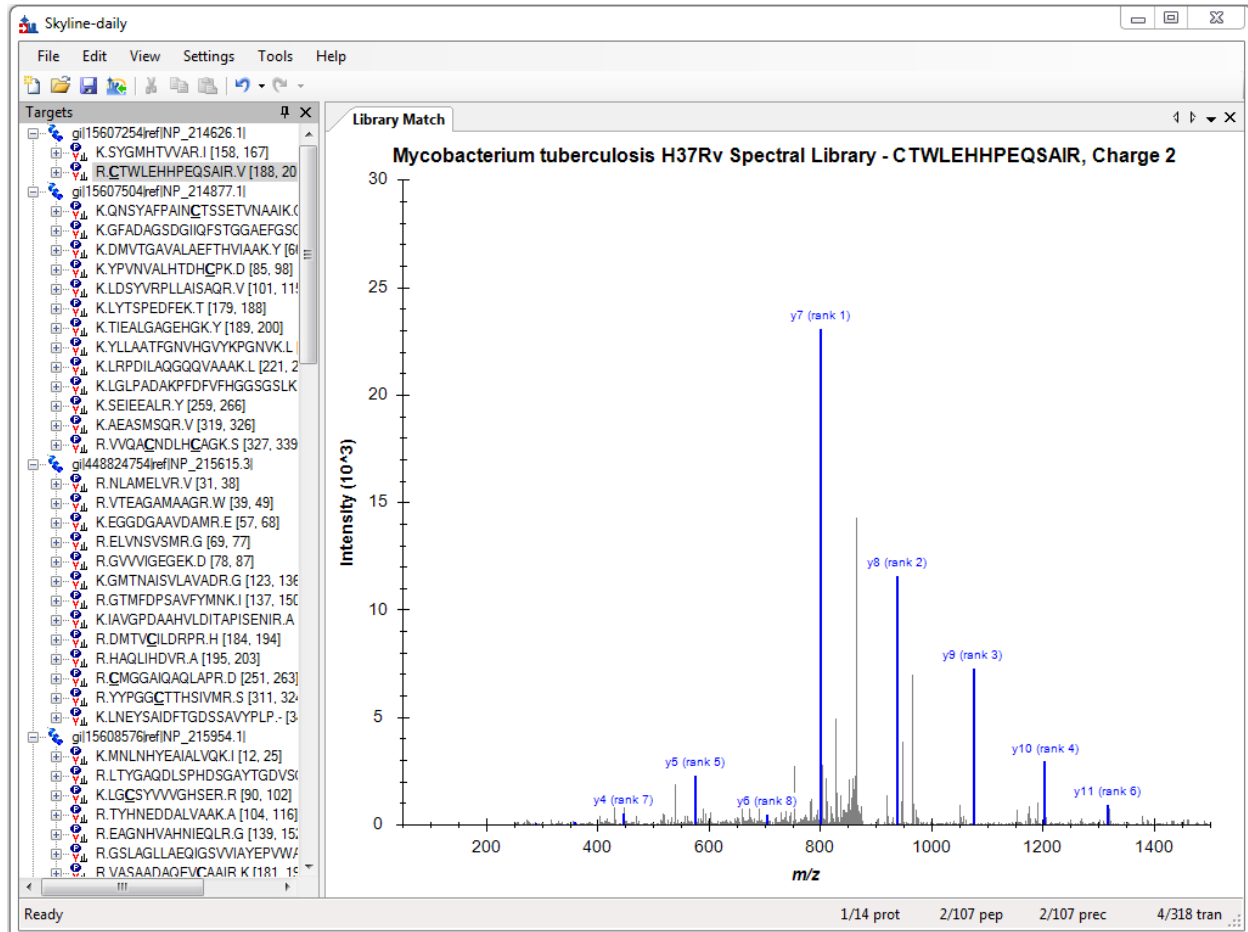
Organism: **Mycobacterium tuberculosis H37Rv**
Pathway: **Fructose and mannose metabolism**
Genes (10)

Export?	Accession	Protein name
<input checked="" type="checkbox"/>	O53634	gca: GDP-mannose 4,6-dehydratase
<input checked="" type="checkbox"/>	P9WN21	glpX: fructose 1,6-bisphosphatase
<input checked="" type="checkbox"/>	P9WG43	tpi: triosephosphate isomerase
<input checked="" type="checkbox"/>	P71790	gmdA: GDP-D-mannose dehydratase GmdA
<input checked="" type="checkbox"/>	P71791	epiA: nucleotide-sugar epimerase EpiA
<input checked="" type="checkbox"/>	P9WKD7	rpiB: ribose-5-phosphate isomerase B
<input checked="" type="checkbox"/>	O05898	manA: mannose-6-phosphate isomerase
<input checked="" type="checkbox"/>	O86374	pmmA: phosphomannomutase PmmA
<input checked="" type="checkbox"/>	L7N6A5	manB: D-alpha-D-mannose-1-phosphate guanylyltransferase ManB
<input checked="" type="checkbox"/>	O53360	pmmB: phosphomannomutase PmmB

At the bottom of the window, there are three buttons: '<< Select another Organism' (highlighted with a red box), '< Previous', and 'Confirm to Export' (highlighted with a red box and containing a skyline icon).

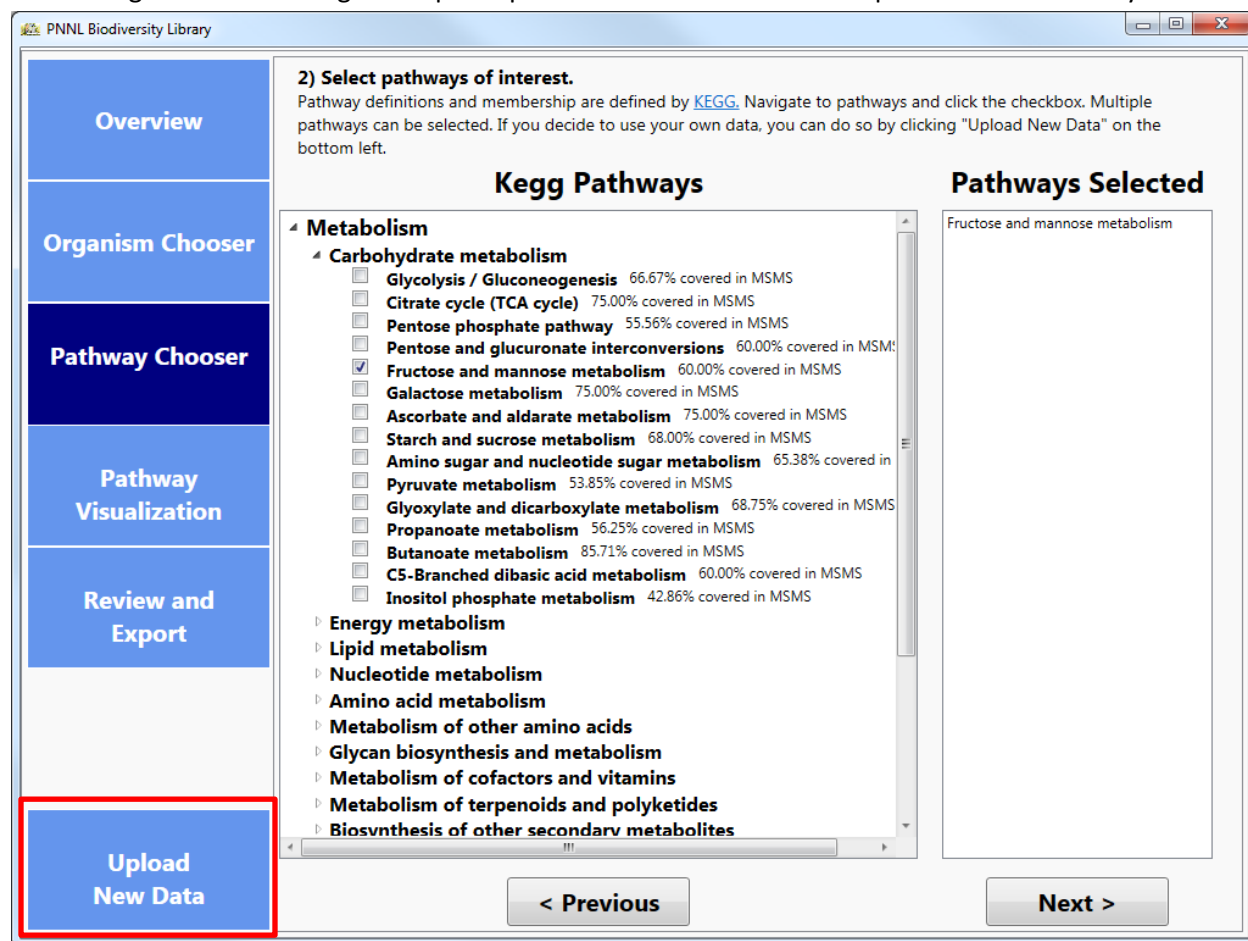
View spectral data in Skyline:

The plugin will automatically propagate the information downloaded into Skyline, where it uses your peptide settings to create peptides for proteins in the FASTA and uses the Spectral Library downloaded through the plugin to perform library matching.



Customizing the plugin with your data:

The Biodiversity Plugin enables users to update the MS/MS data to include personal data and use the same pathway oriented interface to load their data directly into Skyline. Users start customizing their data by selecting the **Upload New Data** button at the bottom left of the window. This starts a new wizard to guide users through the upload process. Users can choose to upload new data at any time.



Input Mass Spec Data:

The screenshot shows a software window titled "Customize Data for Biodiversity Library". On the left is a vertical sidebar with four buttons: "Welcome", "Input MS/MS Data" (which is highlighted in dark blue), "Customizing Options", and "Review and Confirm". Below these buttons is a small help icon. The main area of the window is titled "1. Input Mass Spectrometry Data". It contains the following text: "To load data into the plugin, we require the annotated spectrum (in a spectrum library). Libraries must be in the Bibliospec (.blib) format. Details and assistance on generating a blib can be found under the help button." Below this text is a label ".blib File Location" followed by a text input field and a magnifying glass icon. At the bottom of the window are two buttons: "< Previous" and "Next >".

Only one file is required, a [Bibliospec formatted](#) spectral library. Please consult the [online documentation](#) for details about building a Bibliospec library. To build a library from mzIdentML results, and example command line is:

```
~> BlibBuild.exe -c 0.9999 E:\path\to\PSM_results.mzid E:\path\to\Library.blib
```

It is then necessary to filter the Blib file to contain ONE and only one PSM per peptide. Therefore, you must filter the above library file, for example:

```
~> BlibFilter.exe -b 1 E:\path\to\Library.blib E:\path\to\FilteredLibrary.blib
```

Customizing Options:

There are three options for updating organism data:

- Replace the existing organism entirely with custom data.
- Supplement an existing organism with additional data.
- Add a new organism that does not yet exist in the current database.

The screenshot shows a software window titled "Customize Data for Biodiversity Library". On the left is a vertical sidebar with four buttons: "Welcome", "Input MS/MS Data", "Customizing Options" (which is highlighted in dark blue), and "Review and Confirm". The main area of the window is titled "2. Set customizing options". Below the title, there is a paragraph of text: "Your personal data can be added to the plugin in there different ways. Click an option and then choose an organism from the selection box below. Details and assistance can be found under the help button. Organisms that have already been customized will appear in red." Below this text are three buttons: "Replace", "Supplement", and "Add New". These three buttons are enclosed in a red rectangular box. To the right of each button is a short description: "Replace an organism's data entirely with custom data.", "Supplement an existing organism with custom data.", and "Add a new organism that does not yet exist in the current database." respectively. Below these buttons is a large empty rectangular box with a red 'X' icon in the top right corner. At the bottom of the window are two buttons: "< Previous" and "Next >".

2. Set customizing options

Your personal data can be added to the plugin in there different ways. Click an option and then choose an organism from the selection box below. Details and assistance can be found under the help button. Organisms that have already been customized will appear in red.

Replace Replace an organism's data entirely with custom data.

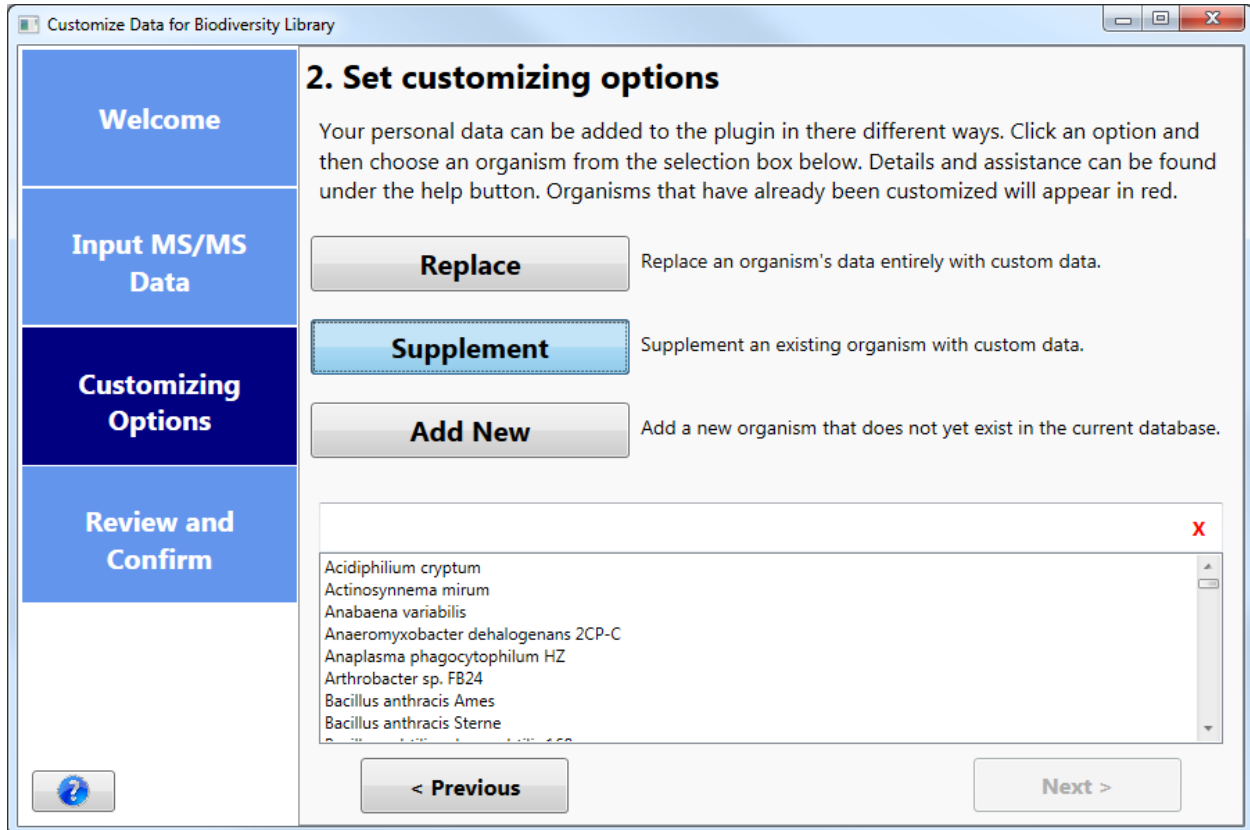
Supplement Supplement an existing organism with custom data.

Add New Add a new organism that does not yet exist in the current database.

< Previous Next >

Selecting an Organism:

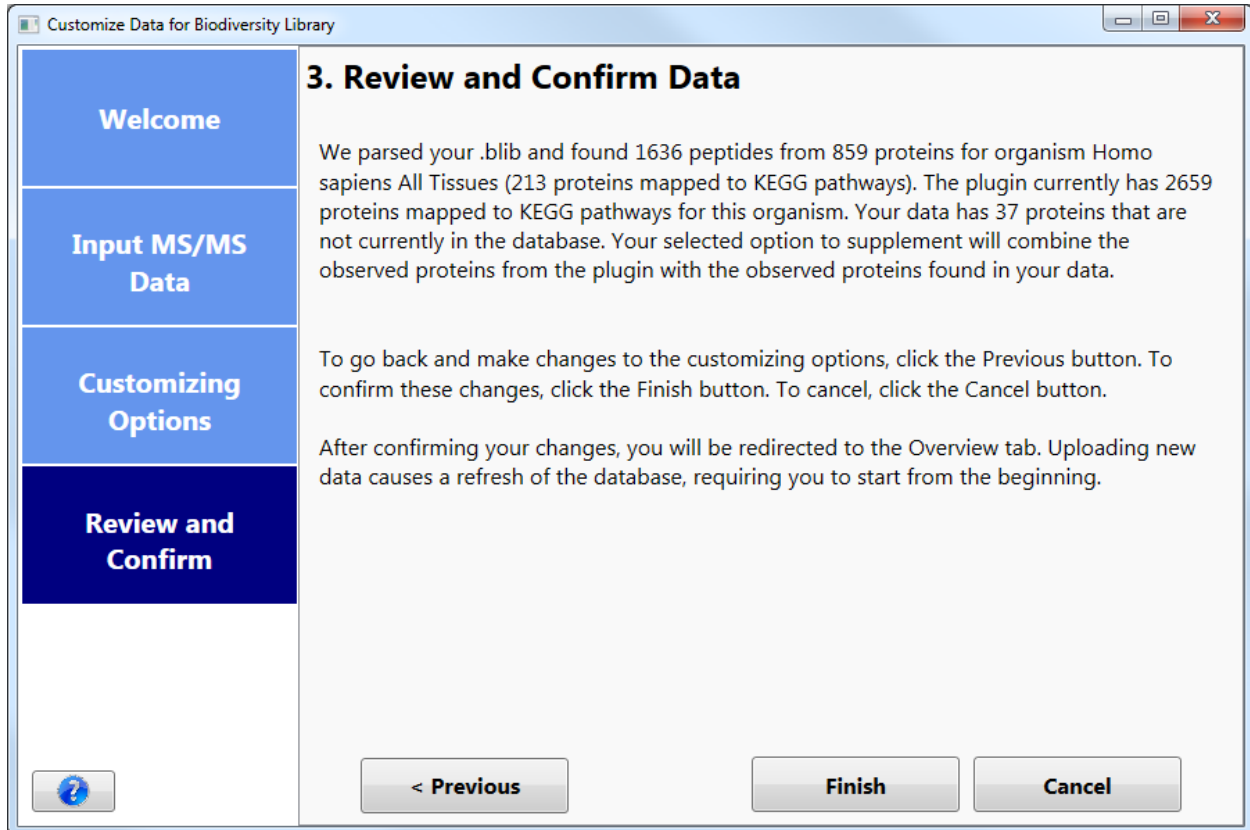
Once the customizing option has been selected, the list box at the bottom of the window will populate with organisms to search and choose from.



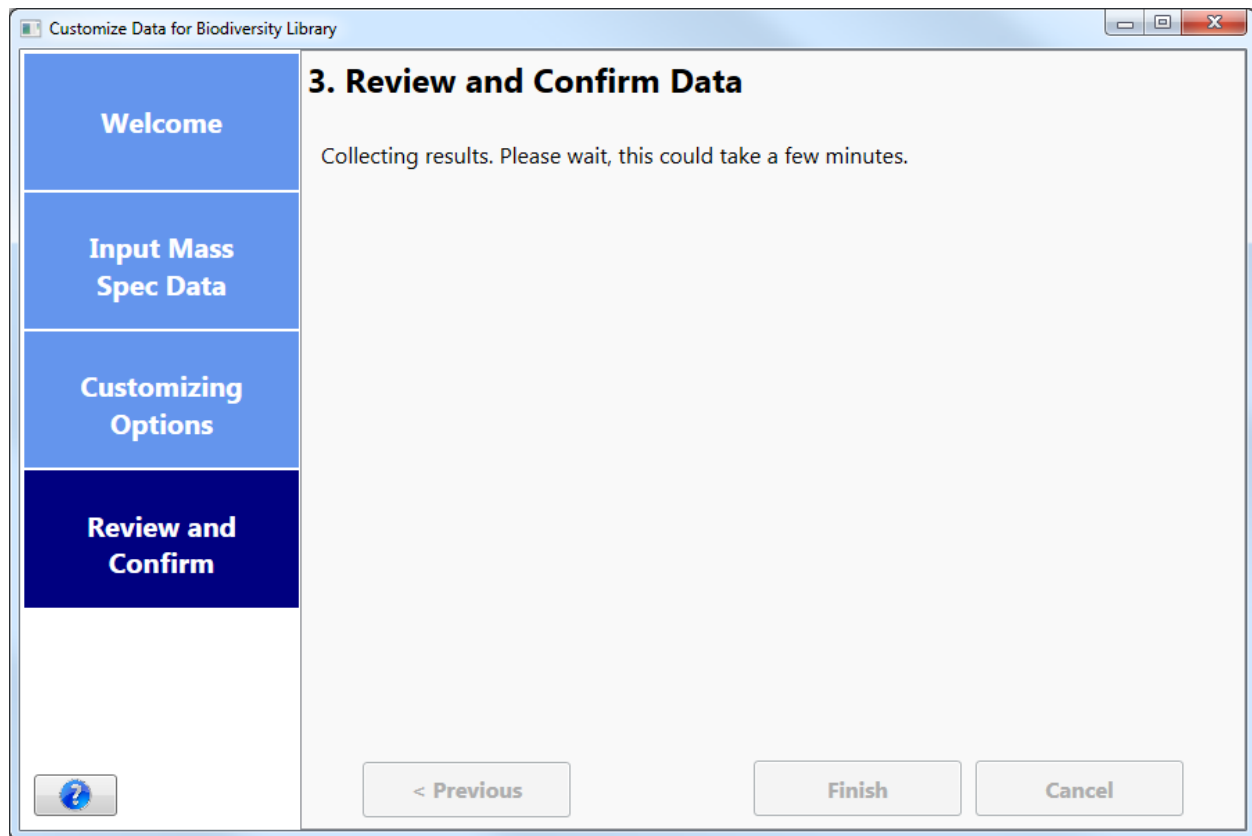
Once you have selected an organism, the next button will enable. Once you click the **Next** button, the wizard will begin collecting the results. Users will be navigated to the Review and Confirm tab where they will wait for their results to appear.

Reviewing and Confirming:

Once the results have been collected, a message will appear prompting you to confirm the results and finish the process by clicking the **Finish** button, cancel the process by clicking the **Cancel** button, or go back and modify your customizing options by clicking the **Previous** button.



Please note: Adding a new organism can take longer than Replacing or Supplementing. If the process takes longer than expected, a message (shown below) will appear, allowing you to wait there until the results are collected.



For your convenience, any organism that was customized by you will appear in red. Unmodified organisms will remain black.

PNNL Biodiversity Library

Overview

Organism Chooser

Pathway Chooser

Pathway Visualization

Review and Export

Upload New Data

1) Select an Organism.

Data in the Biodiversity Library is organized by the organism. To retrieve data, please select an organism of interest, either through the Phylogeny explorer or through the Organism search box. If you want to analyze data from multiple organisms, please export the data one organism at a time. If you would like to add or customize an organism, click "Upload New Data" on the left hand side at the bottom of the page. Organisms that have been customized are shown in red.

Phylogeny Explorer

Eukaryota

Animalia

Homo sapiens

Homo sapiens Adult Adrenal Gland
Homo sapiens Adult B cell
Homo sapiens Adult CD4 T Cell
Homo sapiens Adult CD8 T Cell
Homo sapiens Adult Colon
Homo sapiens Adult Esophagus
Homo sapiens Adult Frontal Cortex
Homo sapiens Adult Gallbladder
Homo sapiens Adult Heart
Homo sapiens Adult Kidney
Homo sapiens Adult Liver
Homo sapiens Adult Lung
Homo sapiens Adult Monocytes
Homo sapiens Adult NK Cells
Homo sapiens Adult Ovary
Homo sapiens Adult Pancreas
Homo sapiens Adult Platelets
Homo sapiens Adult Prostate
Homo sapiens Adult Rectum
Homo sapiens Adult Retina
Homo sapiens Adult Spinal Cord
Homo sapiens Adult testis
Homo sapiens Adult Urinary Bladder
Homo sapiens All Tissues
Homo sapiens Fetal Brain
Homo sapiens Fetal Gut
Homo sapiens Fetal Heart
Homo sapiens Fetal Liver
Homo sapiens Fetal Ovary
Homo sapiens Fetal Placenta
Homo sapiens Fetal Testis
Kineococcus radiotolerans
Kosmotoga olearia
Lactobacillus crispatus
Lactobacillus gasseri ATCC 33323
Methanosarcina barkeri Fusaro
Methanospirillum hungatei
Mycobacterium tuberculosis H37Rv
Nakamurella multipartita
Nocardiopsis dassonvillei
Novosphingobium aromaticivorans
Pelobacter carbinolicus
Prevotella ruminicola

Organism search

Homo sapiens Adult Ovary
Homo sapiens Adult Pancreas
Homo sapiens Adult Platelets
Homo sapiens Adult Prostate
Homo sapiens Adult Rectum
Homo sapiens Adult Retina
Homo sapiens Adult Spinal Cord
Homo sapiens Adult testis
Homo sapiens Adult Urinary Bladder
Homo sapiens All Tissues
Homo sapiens Fetal Brain
Homo sapiens Fetal Gut
Homo sapiens Fetal Heart
Homo sapiens Fetal Liver
Homo sapiens Fetal Ovary
Homo sapiens Fetal Placenta
Homo sapiens Fetal Testis
Kineococcus radiotolerans
Kosmotoga olearia
Lactobacillus crispatus
Lactobacillus gasseri ATCC 33323
Methanosarcina barkeri Fusaro
Methanospirillum hungatei
Mycobacterium tuberculosis H37Rv
Nakamurella multipartita
Nocardiopsis dassonvillei
Novosphingobium aromaticivorans
Pelobacter carbinolicus
Prevotella ruminicola

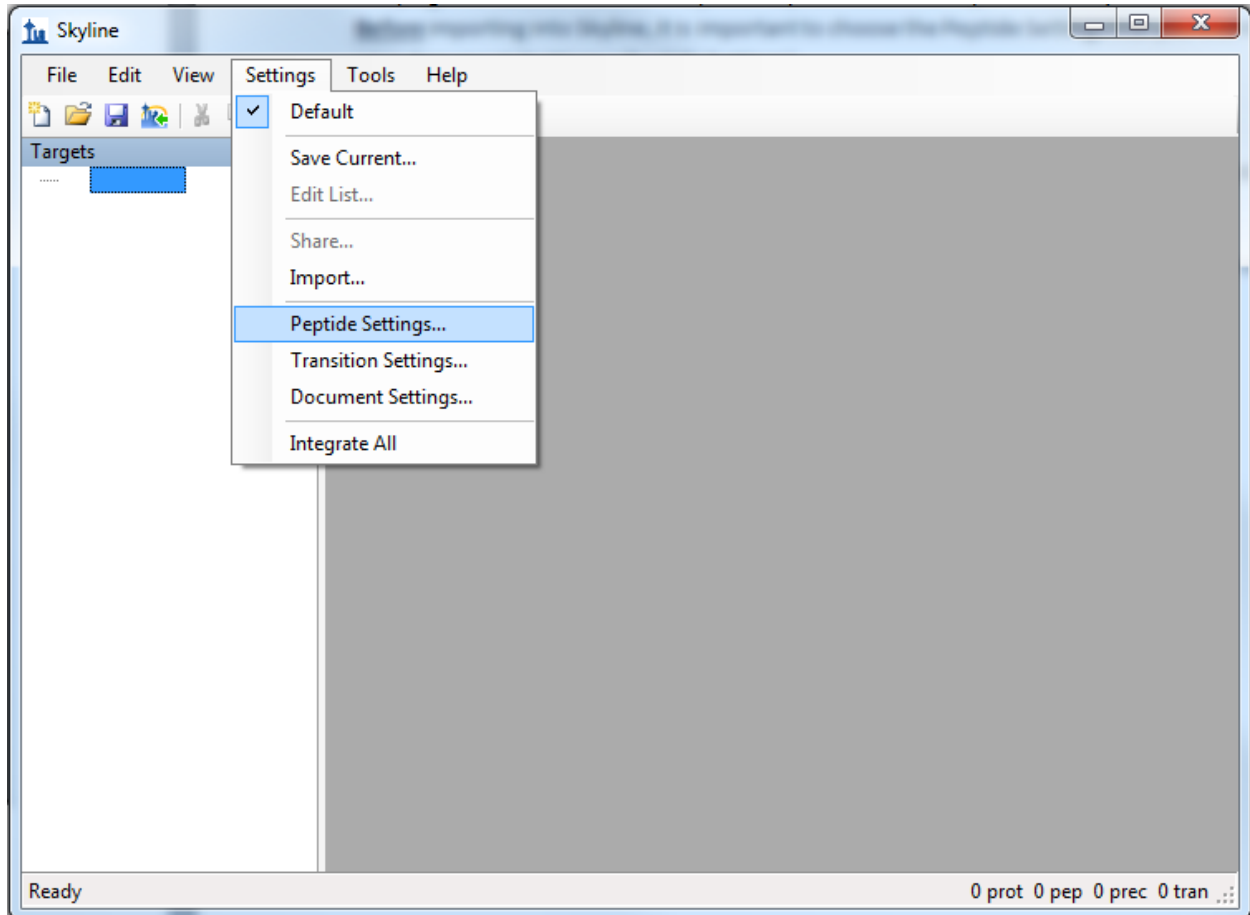
< Previous

Next >

Settings in Skyline

The plug-in creates a FASTA of the proteins you choose and imports that sequence file into Skyline.

Before importing into Skyline, it is important to choose the Peptide Settings that you wish to use for your document (Settings > Peptide Settings).



After the FASTA has been imported, users will not be able to make changes in Digestion, Filter or Modifications tabs.

Additionally, it is recommended to set Transition Settings before importing the FASTA. Be sure to select precursor charge states (in the Filter Tab).

Troubleshooting

- The application is taking an unusually long time while generating a FASTA from my selection of proteins.
 - Depending on your Skyline settings, Skyline could be seeing that there are proteins incoming from this FASTA which do not contain peptides according to the current filter settings. While this window is open from Skyline, our application is waiting for a response. To allow the information to be fully exported, select “Keep” so that the proteins will export and the .blib file(s) which download afterwards will provide a library match and populate the peptides for these new proteins.
- No proteins appeared as red in the KEGG pathway maps.
 - This could be due to two possibilities:
 - The organism contains proteins which should be observed in MS/MS space on this pathway, but were not observed in experimental results. These ortholog boxes will still be highlighted as blue.
 - The organism does not contain proteins which should be observed in MS/MS space on this pathway. In this case, no ortholog boxes will be highlighted on the image at all.
- Spectral Library doesn’t download.
 - Due to the server based location of the Spectral libraries, our application does require a stable internet connection to acquire these files. If you have confirmed that your internet connection is stable, the issue is most likely due to the repository on MassIVE being inaccessible. Please contact either ccms-web@proteomics.ucsd.edu or ccms-web@cs.ucsd.edu for further assistance with their FTP connection.
- No proteins were downloaded from NCBI.
 - Due to the application creating FASTA information dynamically based on protein selection, our application does require a stable internet connection to acquire these files from NCBI at runtime. If you have confirmed that your internet connection is stable, the issue is most likely due to an issue with NCBI’s FTP connection. Please send an email directly to info@ncbi.nlm.nih.gov for further assistance with their FTP connection.
- My favorite protein was not found or exported to Skyline.
 - There are a number of reasons why a specific protein was not pushed into skyline.
 - Skyline peptide settings (missed cleavages)
 - Protein not observed in spectral library
 - To add custom observed data, see the *Customizing the plugin with your data* section of the tutorial
 - Protein is not functionally classified by KEGG
 - To manually insert new protein:
 - File -> Insert -> FASTA and copy your FASTA snippet in (Skyline will automatically populate the protein with peptides based on your digestion settings)
 - Edit -> Insert -> Proteins and fill out the table.
 - To manually insert a peptide go to Edit -> Insert -> Peptides and fill out the table.
- No proteins were imported into Skyline
 - This could be due to two possibilities:
 - The plugin was unable to obtain FASTA information for all proteins selected for export. NCBI or your local computer may be experiencing connectivity issues.

- No proteins were selected for export from the plugin. The Spectral Library for the organism(s) examined still should export, but a message will display informing you that no proteins were selected and thus no FASTA was created
- Error Messages:
 - When the Biodiversity Plugin encounters an error, the user is prompted with the option to send an error report. We highly encourage all users who encounter problems to send the error report along with a description of what lead up to the failure. These messages allow our developers to emulate the failure state so the problem can be diagnosed and fixed. We will try to address issues as soon as possible.
 - My version of Skyline is out of date.
 - Our application requires Skyline to be version 3.1.1.7490 or later. To update to the most recent version, please go to [Skyline Daily's webpage](#) to download the latest version of Skyline.
 - Unable to establish connection to NCBI to acquire FASTA for your organisms.
 - The plugin queries NCBI in real time and requires a stable internet connection. NCBI or your local computer may be experiencing connectivity issues.
 - MASSIVE Server Unreachable
 - The plugin downloads the spectral library files from the UCSD MASSIVE FTP server and requires a stable internet connection. MASSIVE or your local computer may be experiencing connectivity issues.
- How do I add my data to the Biodiversity Library?
 - The Biodiversity Library is curated by Samuel Payne at Pacific Northwest National Laboratory. For more information about your data being visible in the Library, please contact Samuel.Payne@pnnl.gov.
- Observed protein count is zero when customizing data.
 - This could be due to two possibilities:
 - The organism you selected to update doesn't match the organism that was used in your results
 - The identifiers in your data are not Uniprot identifiers (e.g. RefSeq, Genbank, GI numbers)
 - The organism you selected does not use Uniprot as identifiers in KEGG