

Explicit Basic Tutorial

“Explicit” is from the Latin: explain, unfold, extend, set forth, exhibit, disentangle

A brief introduction to program capabilities and functions for new
users of the Explicit software

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The data used in this tutorial come from an analysis of 16S ribosomal RNA gene sequences obtained from many distinct skin sites of healthy humans (Grice EA, et al. (2009) Topographical and Temporal Diversity of the Human Skin Microbiome. Science 324(5931): 1190–1192). To produce a concise tutorial, the data have been reduced from the original dataset and may not represent the findings of the original study.

I. Begin a New Project

An Explicitet project is a single file that contains all of the OTU data, sample names (a.k.a. library names) and metadata that are to be analyzed as a unit. In other words, all data analyzed for one publication are drawn together into a single Explicitet project, independent of how many 454/Miseq runs are involved.

We will begin by creating a project and importing an OTU table. The tutorial example we have selected is based on the Human Skin Microbiome paper published by Grice, et al. This example was picked because it is relatively small and has a nice set of intuitive metadata available.

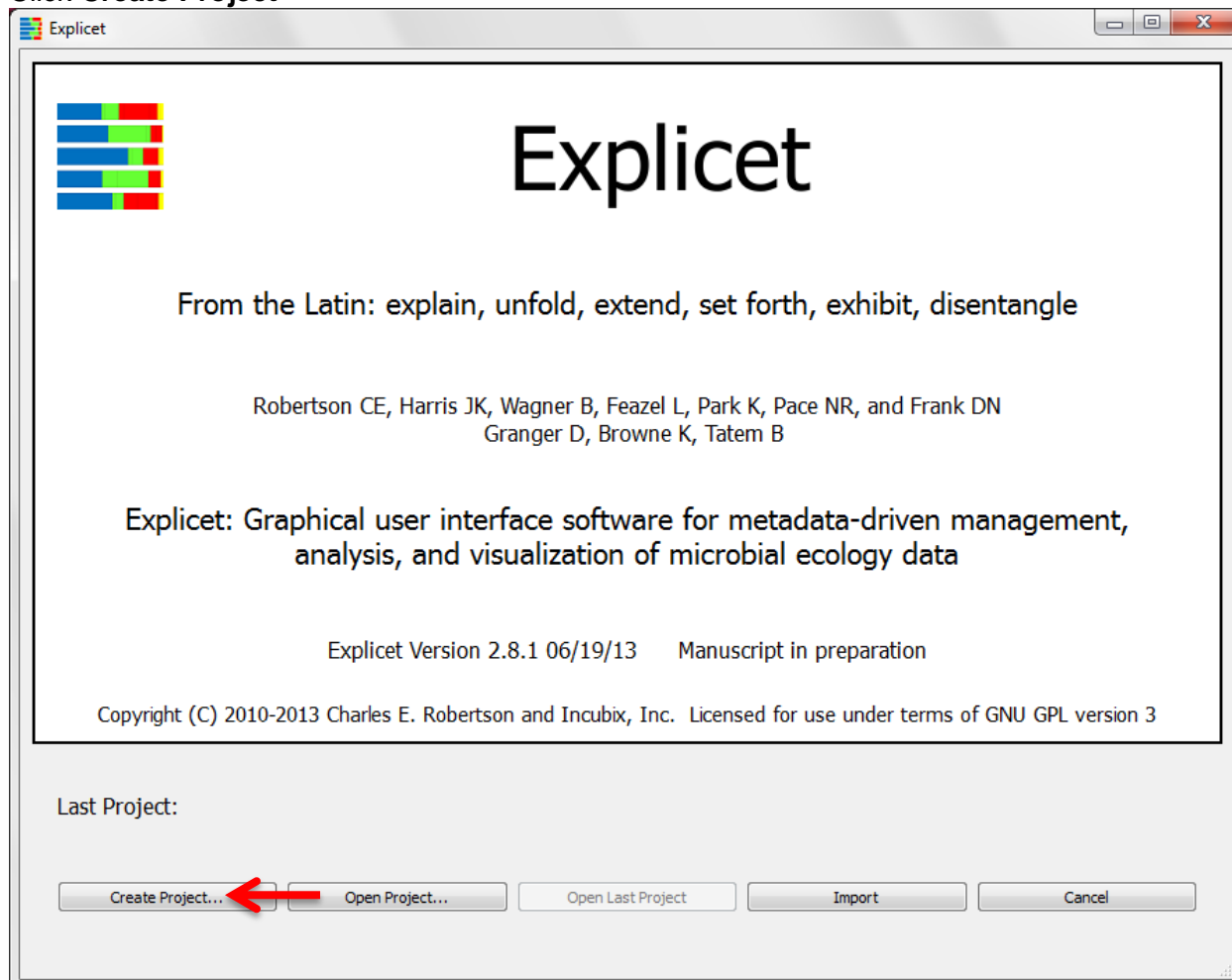
Please do not hesitate to ask questions or make suggestions via our online Explicitet forum. The Explicitet forum link can be found on our web site: www.explicitet.org.

A. Create a New Project

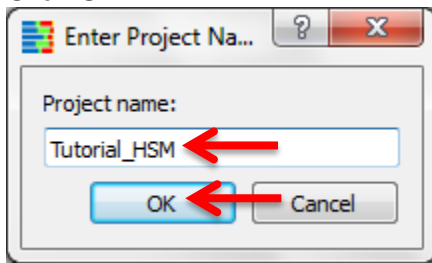
Open Explicitet

A pop-up window will open with several different options

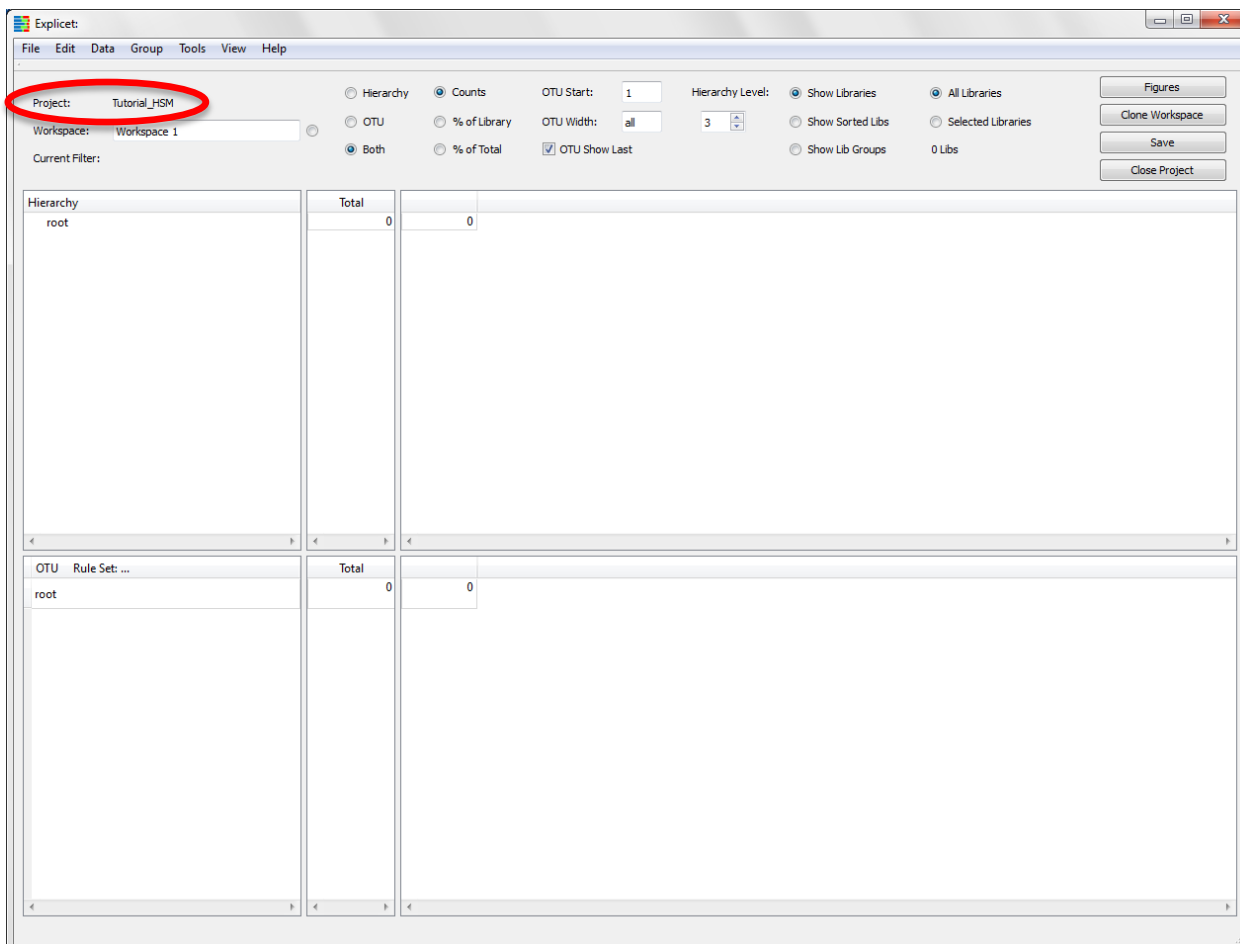
Click **Create Project**



Enter **Project Name**, "Tutorial_HSM", when prompted
Click **OK**



We now have a blank project in Explicit, and the name of the current project is displayed in the upper left corner of the current workspace window.



II. Import OTU Data

The first step in a new project is to import the data that comes out of the 16S pipeline runs into Explicit. In general, OTU tables are the most convenient form of data commonly generated by pipelines. For detailed information on how OTU tables are formatted, please see the Explicit Handbook. In short, OTU tables are a delimited file (tab-separated or comma-separated file) in which the rows are the OTUs, and the columns represent the number of each OTU seen in a given sample.

Explicit supports many other formats for importing the OTU data. For more details on the other OTU import formats, please see the Explicit Handbook. Later, we will discuss more data management tools that allow you to explore and modify subsets of the dataset without disrupting the larger project.

Now we will import the data that will belong to the new project. Once data are imported to a project, they are permanently associated with the project. Additional data can be incrementally imported to the same project. Thus, the Explicit project file can grow as a project evolves.

A. Import the OTU Data

File → Import → File → OTU Table Counts

Select "Tutorial_HSM_OTU_2_Explicit"

Click **Open**

A pop-up window will open

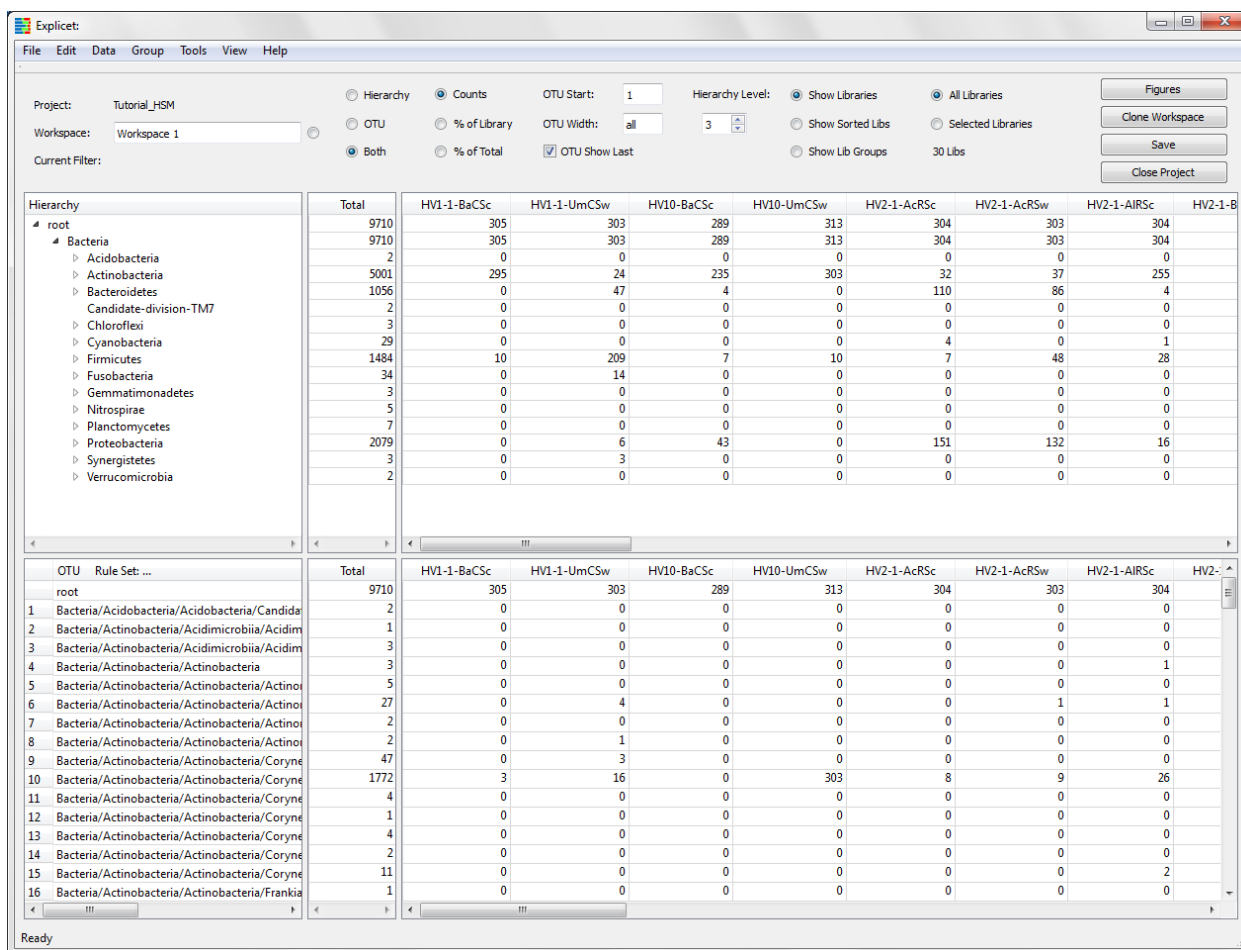
Click **Import**

A dialog box below will open

- On this dialog, Explicit tells the user how it is interpreting the rows and columns in the OTU table. The user needs to verify that Explicit has interpreted the table correctly. Note that in this case, Explicit is telling the user that it is not going to import column 2, "Total", as it will generate that sort of information itself. If Explicit gets it wrong, the user can adjust the interpretation using the provided pull down lists under **Column Type**.

	Column Name	Column Type
1	Taxonomy	OTU Name
2	Total	Do not import this column
3	HV1-1-BaCSc	Library Name and Count
4	HV10-BaCSc	Library Name and Count
5	HV2-1-BaCSc	Library Name and Count
6	HV3-1-BaCSc	Library Name and Count
7	HV4-1-BaCSc	Library Name and Count
8	HV5-BaCSc	Library Name and Count
9	HV6-1-BaCSc	Library Name and Count
10	HV7-BaCSc	Library Name and Count
11	HV8-BaCSc	Library Name and Count
12	HV9-BaCSc	Library Name and Count
13	HV1-1-UmCSw	Library Name and Count
14	HV10-UmCSw	Library Name and Count
15	HV2-1-UmCSw	Library Name and Count

The OTU data now appear in the current workspace window



III. Import Metadata

Now we will import the metadata associated with the OTU data. Metadata refers to information about the sequence data - in this case, a description of the samples and subjects from which the sequence data were generated. In our nomenclature, a “library” represents all of the sequences generated from a single sample (multiple libraries may be generated from a given sample, for example through multiple PCR reactions, but for this tutorial we will assume a one-to-one relationship between libraries and samples). In this study, the metadata for each library includes the anatomical position, microenvironment description, sample acquisition method, and side of the body associated with each skin sample. Just like the OTU data, metadata need be imported only once (unless you choose to add more metadata) - imported metadata are also incorporated into the Explicitet project file. For detailed information on how to format metadata files, please see the Explicitet Handbook. In short, the metadata file is a tab-separated or comma-separated file organized by columns, generally prepared with a spreadsheet package like Microsoft Excel. The first column contains the names of the libraries in the dataset; all subsequent columns are metadata items and their values associated with each library.

A. Import the Metadata

File → Import → Metadata

Select “Tutorial_HSM_Metadata”

Click **Open**

A pop-up window will open

Make sure that the column containing the library name is selected

- Explicitet searches all of the columns in the metadata file looking for the library names that were found when the taxonomy data were imported. In all but rare cases (e.g., when only a small portion of the sample names are present in the imported taxonomy data), Explicitet will find the library column automatically.

Click **Import**

Import Metadata

Select the column which contains the Library name from 5 columns

Column	Sample Data		
1 Lib	HV2-1-AcRSc		
2 Anatomy	antecubital fossa		
3 Symmetry	Right		
4 SampleType	Scrape		
5 Microenvironment	Moist		

☒ Libraries not found ☐ Libraries found

0 Libraries not found in the project for column Lib

All Libraries found

☒ Add missing libraries to the project

Metadata which does not match the Metadata in the project

☒ Check All ☐ Clear All

Metadef Name	Current Metadef Type	New Metadef Type	First Illegal Value	New Lower Bound	New Upper Bound	First New Enumerated Value	Change to new Def
--------------	----------------------	------------------	---------------------	-----------------	-----------------	----------------------------	-------------------

A new pop-up window will open which displays the imported metadata

Click **Done**

Metadata

All Libraries

Selected Libraries

30 Total Libraries

Export

Done

Defined Metadata

	Used	Name
1	30	Anatomy
2	30	Microenvironment
3	30	SampleType
4	30	Symmetry

Add ->

<- Remove

Assigned Metadata

	Library Name	Anatomy	Microenvironmen	SampleType	Symmetry
1	HV1-1-BaCSc	back	Sebaceous	Scrape	Center
2	HV1-1-UmCSw	umbilicus	Moist	Swab	Center
3	HV10-BaCSc	back	Sebaceous	Scrape	Center
4	HV10-UmCSw	umbilicus	Moist	Swab	Center
5	HV2-1-AcRSc	antecubital fossa	Moist	Scrape	Right
6	HV2-1-AcRSw	antecubital fossa	Moist	Swab	Right
7	HV2-1-AIRSc	alar crease	Sebaceous	Scrape	Right
8	HV2-1-BaCSc	back	Sebaceous	Scrape	Center
9	HV2-1-GcCSc	gluteal crease	Moist	Scrape	Center
10	HV2-1-UmCSw	umbilicus	Moist	Swab	Center
11	HV3-1-BaCSc	back	Sebaceous	Scrape	Center
12	HV3-1-RaRSw	retroauricular c...	Sebaceous	Swab	Right
13	HV3-1-UmCSw	umbilicus	Moist	Swab	Center
14	HV4-1-BaCSc	back	Sebaceous	Scrape	Center
15	HV4-1-UmCSw	umbilicus	Moist	Swab	Center
16	HV5-BaCSc	back	Sebaceous	Scrape	Center
17	HV5-UmCSw	umbilicus	Moist	Swab	Center
18	HV6-1-BaCSc	back	Sebaceous	Scrape	Center
19	HV6-1-UmCSw	umbilicus	Moist	Swab	Center

Copy

Paste

Note: Number of rows/columns to paste to must match the number of rows/columns copied. One cell may be copied then pasted to multiple cells.

Metadata Definition

Name:

Type: String that may be more than 8 characters

Upper Bound:

Optional

Lower Bound:

Optional

Status:

Enumerated Values: Optional

Add Value

Values

<New>

Delete Value

Clear Values

Add

Replace

Delete

Clear

For our example dataset, all of the library names were found in the metadata file, as indicated in the left-hand pane: i.e., the number under **Used (30)** matches the total number of libraries shown above the two panes (**30 Total Libraries**).

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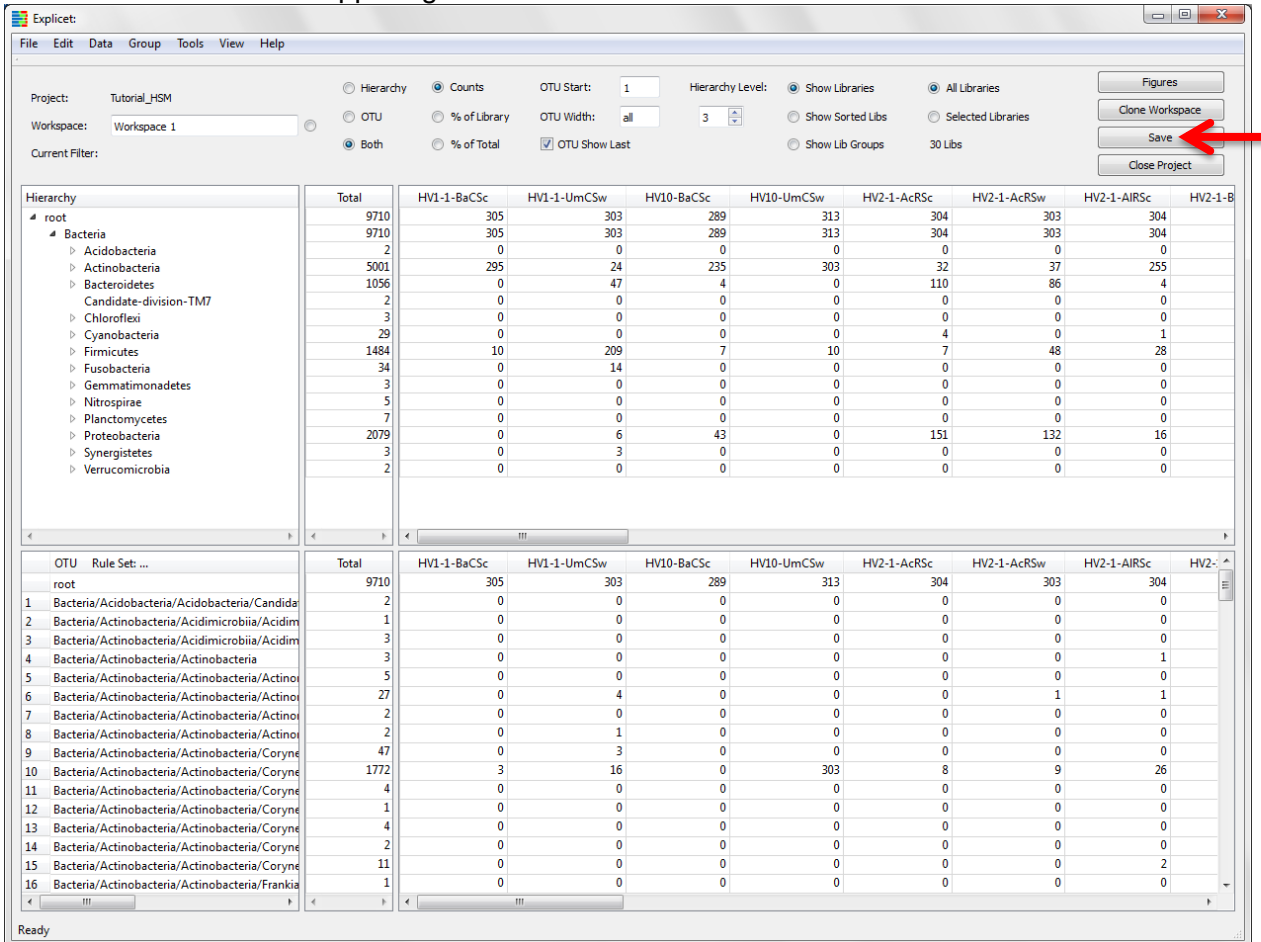
8

IV. Save the Project

Now that all of the data associated with the project are imported, the file should be saved. Explicitet does not auto-save, so remember to save your project frequently!

A. Save the Project

Click the **Save** button in upper right corner of the window



Enter desired project name and location when prompted

- The default file name is the project name with an “_Explicitet_Project” extension.

Click **Save**

All of the imported information is now saved within the project file.

V. Adjust the Display

Now we will adjust the current workspace window display for ease of use (detailed demonstration on next page).

The screenshot shows a settings panel for a workspace. On the left, there are fields for 'Project: Tutorial_HSM', 'Workspace: Workspace 1', and 'Current Filter:'. To the right, there are four main sections labeled A, B, C, and D. Section A contains radio buttons for 'Hierarchy', 'OTU', and 'Both' (selected). Section B contains radio buttons for 'Counts' (selected), '% of Library', and '% of Total'. Section C contains input fields for 'OTU Start: 1' and 'OTU Width: all', and a checkbox for 'OTU Show Last' which is checked. Section D contains a 'Hierarchy Level' dropdown set to '3'. To the right of these sections are three more radio buttons: 'Show Libraries' (selected), 'Show Sorted Libs', and 'Show Lib Groups'. On the far right, there are two radio buttons: 'All Libraries' (selected) and 'Selected Libraries', and a text label '30 Libraries'.

A. Hierarchy, OTU, or Both

Both is the default

This option creates two panes on workspace screen; the upper pane shows the Hierarchy, and the lower pane shows the OTUs. The Hierarchy pane allows exploration of the dataset in a “big tree” hierarchical context, whereas the OTU pane shows a more literal view of the data from the 16S pipeline. The information in the OTU pane is used for input into the statistics and most of the plots (except for pie charts, which are graphical depictions of the Hierarchy pane).

B. Counts, % of Library, % of Total

Select **% of Library** (**Counts** is the default)

While Counts is the default (raw sequence data counts in integers), % of Library tends to be more useful. % of Library is relative abundance, which is important since the total number of Counts received from any library is beyond our control. Using the relative abundance, or % of Library, allows us to fairly compare libraries. Otherwise, the libraries that have a very large number of counts will skew conclusions.

C. OTU displays

These options control the manner in which the taxonomy lines are displayed on the OTU pane.

OTU Start: 1 is the default

This is the position (counting from one) of the first taxonomic category that the user desires to be displayed. In our tutorial example, the taxonomy lines in the OTU pane display will start with Bacteria (Bacteria is the “1st lineage level”).

Set **OTU Width** to 2 (“all” is the default)

This is the number of positions on the line to be displayed. To save space on the screen, now only 2 taxonomic levels will be displayed in the OTU taxonomy line. Taxonomies with more than 2 levels will be shown with an embedded ellipsis; for example, “Bacteria/Actinobacteria/Acidimicrobiia/Acidimicrobiales” becomes “Bacteria/Actinobacteria/.../Acidimicrobiales”.

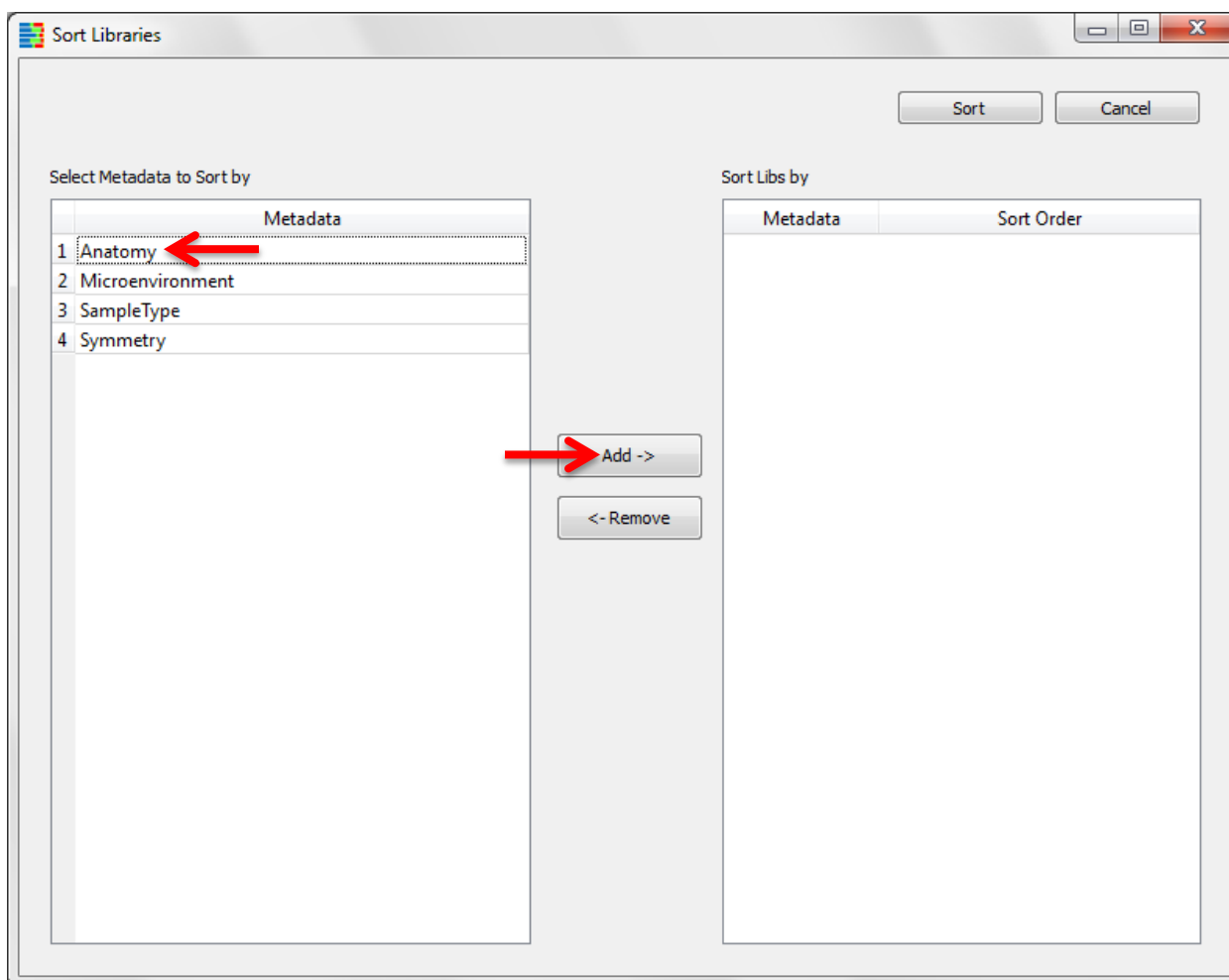
OTU Show Last on is the default

This option appends the last item in the taxonomic line onto a truncated OTU lineage.

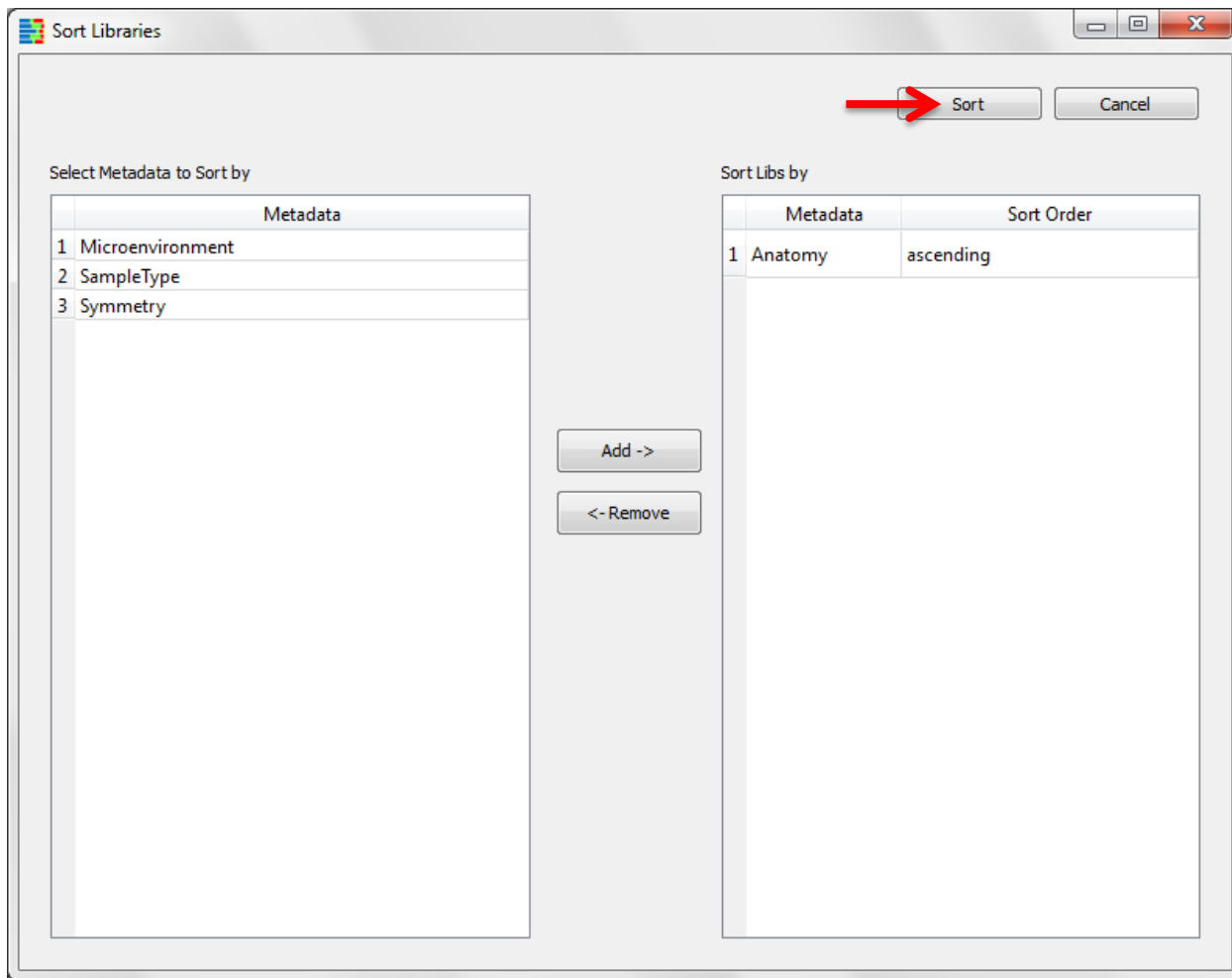
D. Hierarchy Level

Hierarchy Level: 3 is the default

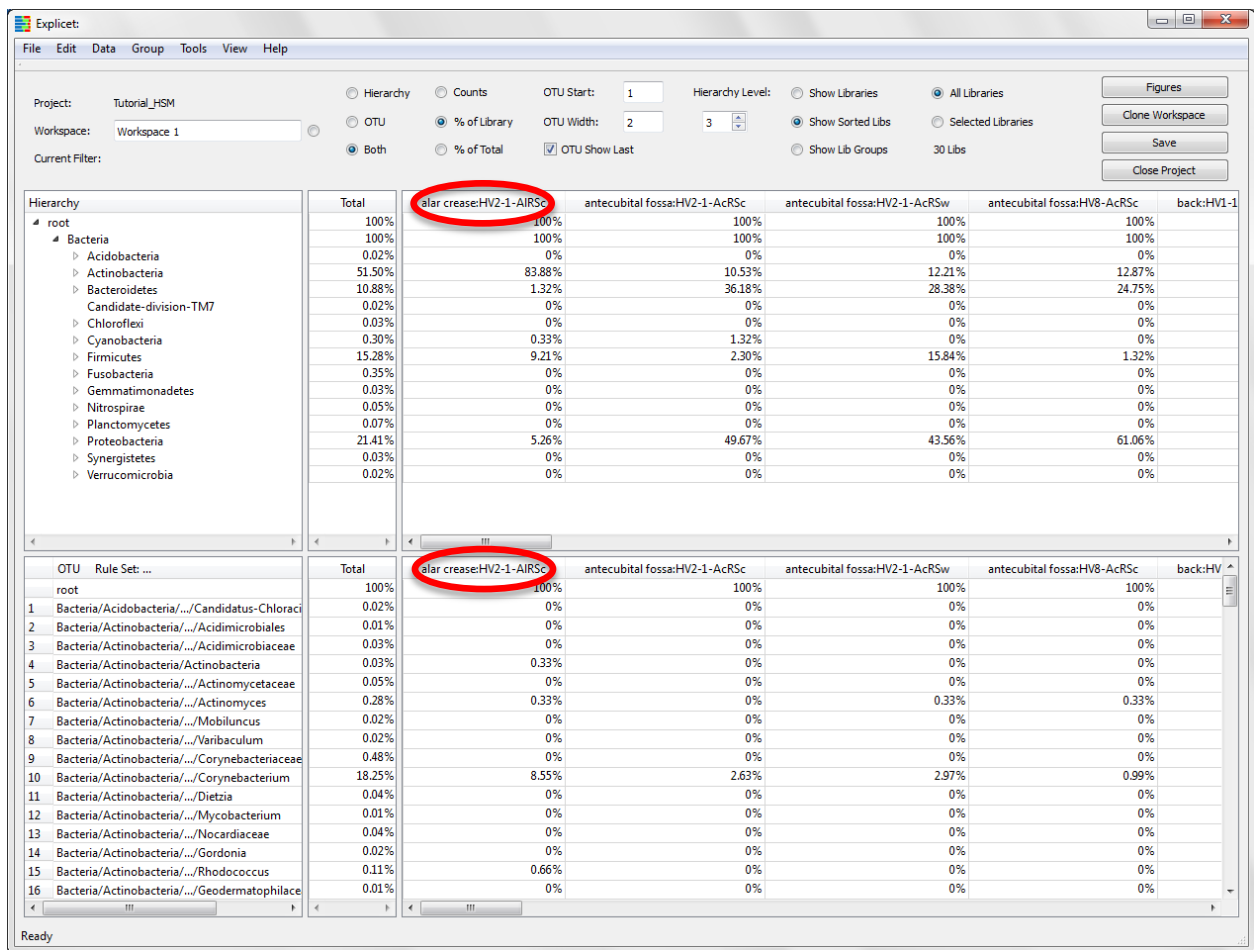
This controls the number of taxonomic categories that will be opened on the hierarchy pane.



Name of metadata descriptor will appear in the right panel
Click **Sort**



Pop-up window will disappear
Both the hierarchy and OTU tables are now sorted by anatomical position



VI. Make an OTU Stacked Bar Chart

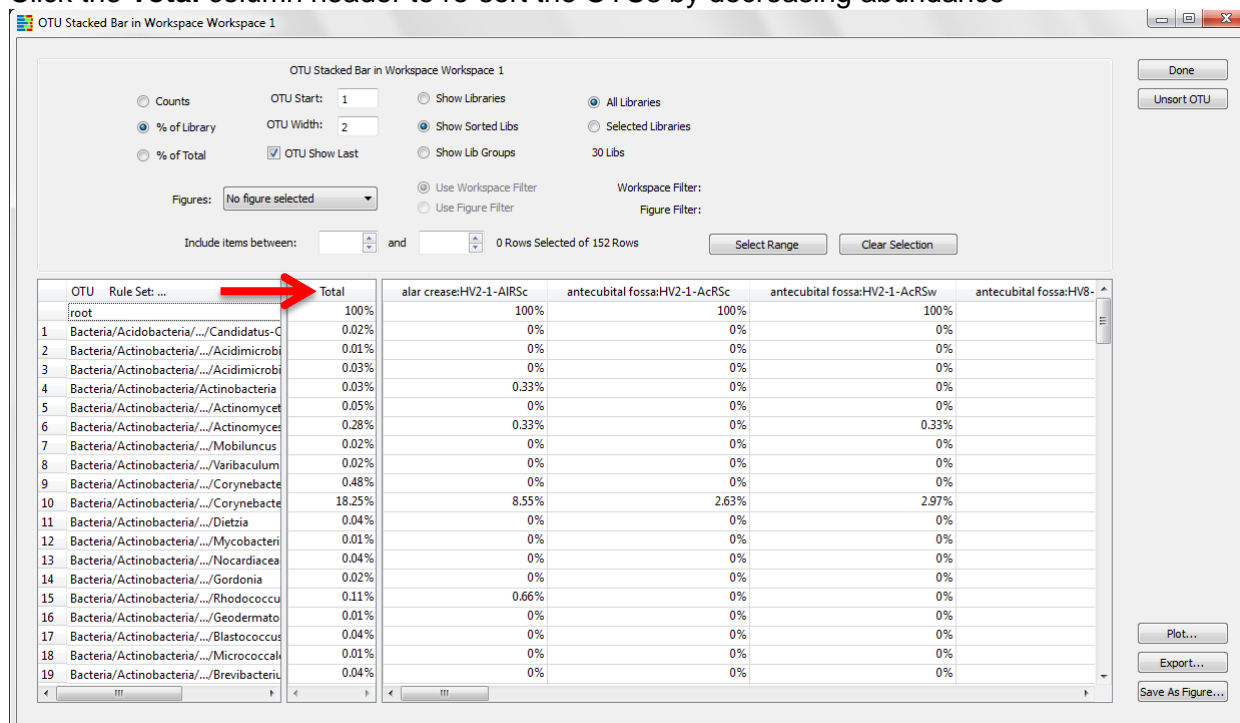
Before diving into a detailed analysis, generating an overview of the dominant organisms that exist in the dataset can be useful. One way to do this is through an OTU stacked bar chart.

A. Create an OTU Stacked Bar Chart of the Top 10 Most Prevalent Taxa

Tools → Plot → OTU Stacked Bar

A new window will appear with the OTU data available in the workspace

Click the **Total** column header to re-sort the OTUs by decreasing abundance



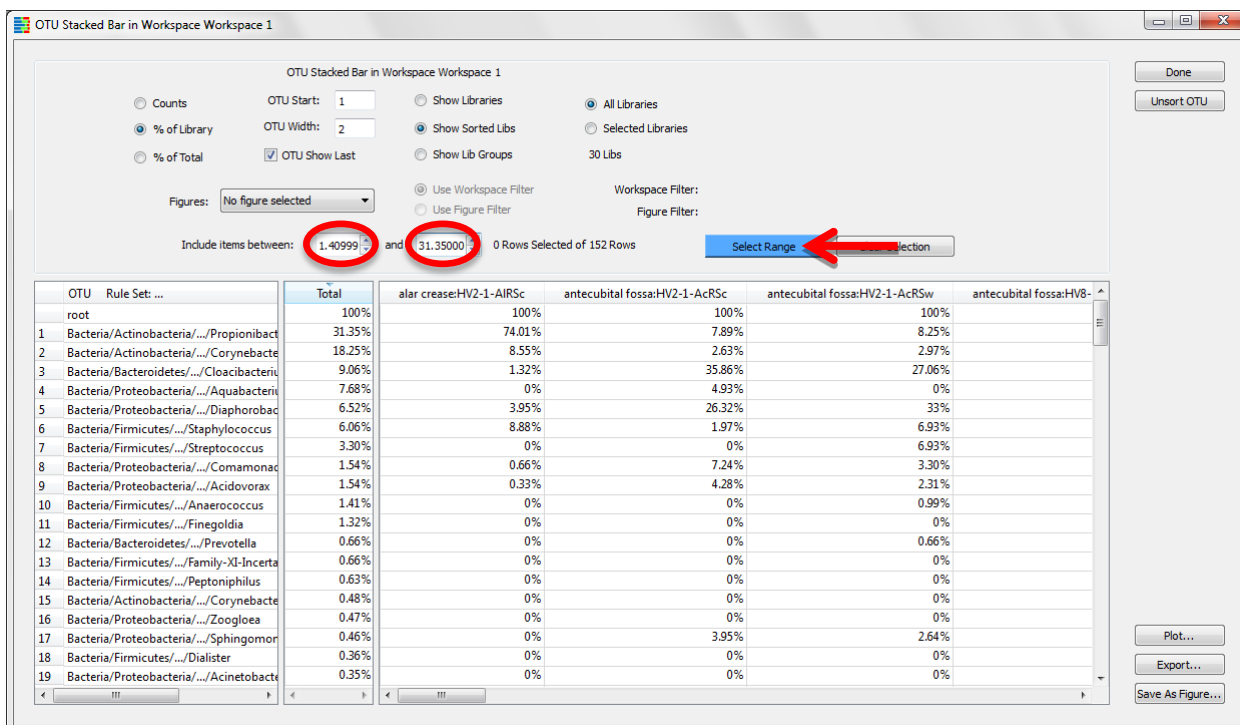
To display only the top 10 taxa in the project, note that the **Total** value of the 1st OTU in the column is 31.35

Note that the **Total** value of the 10th OTU in the column is 1.41

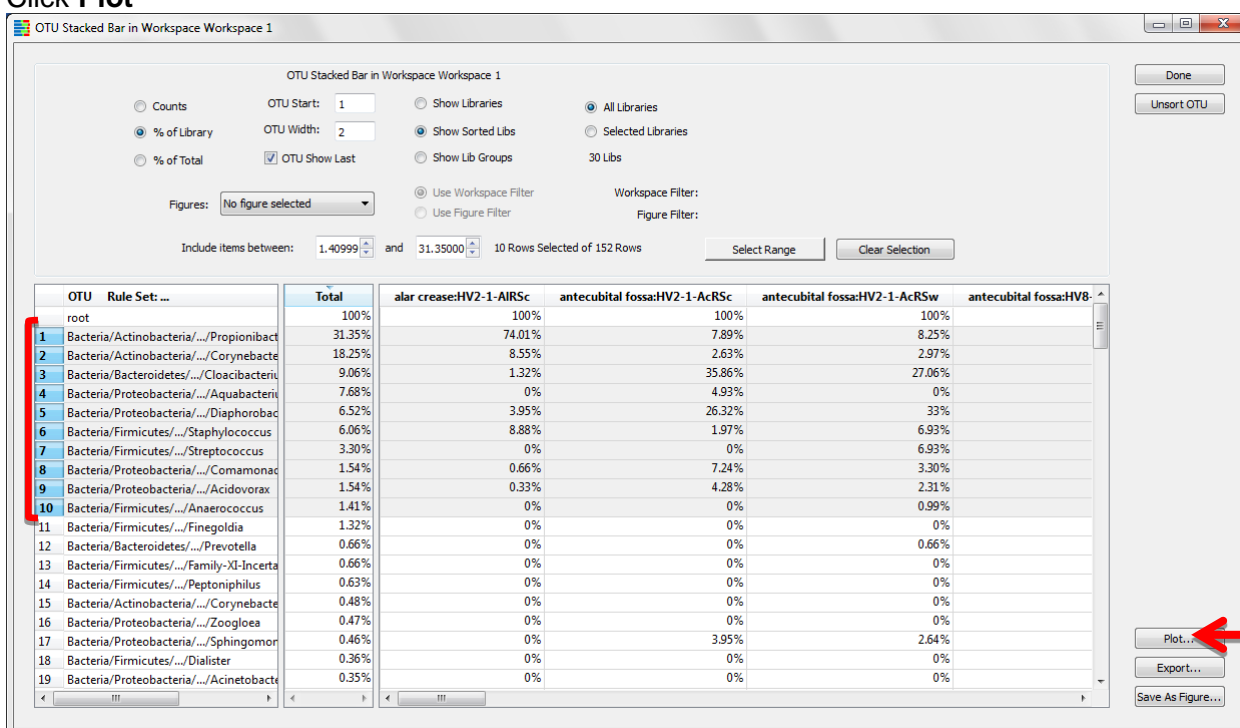
In the **Include items between** field, enter “1.41” into the first box (the lower bounding limit)

In the **Include items between** field, enter “31.35” into the second box (the upper bounding limit)

Click **Select Range**

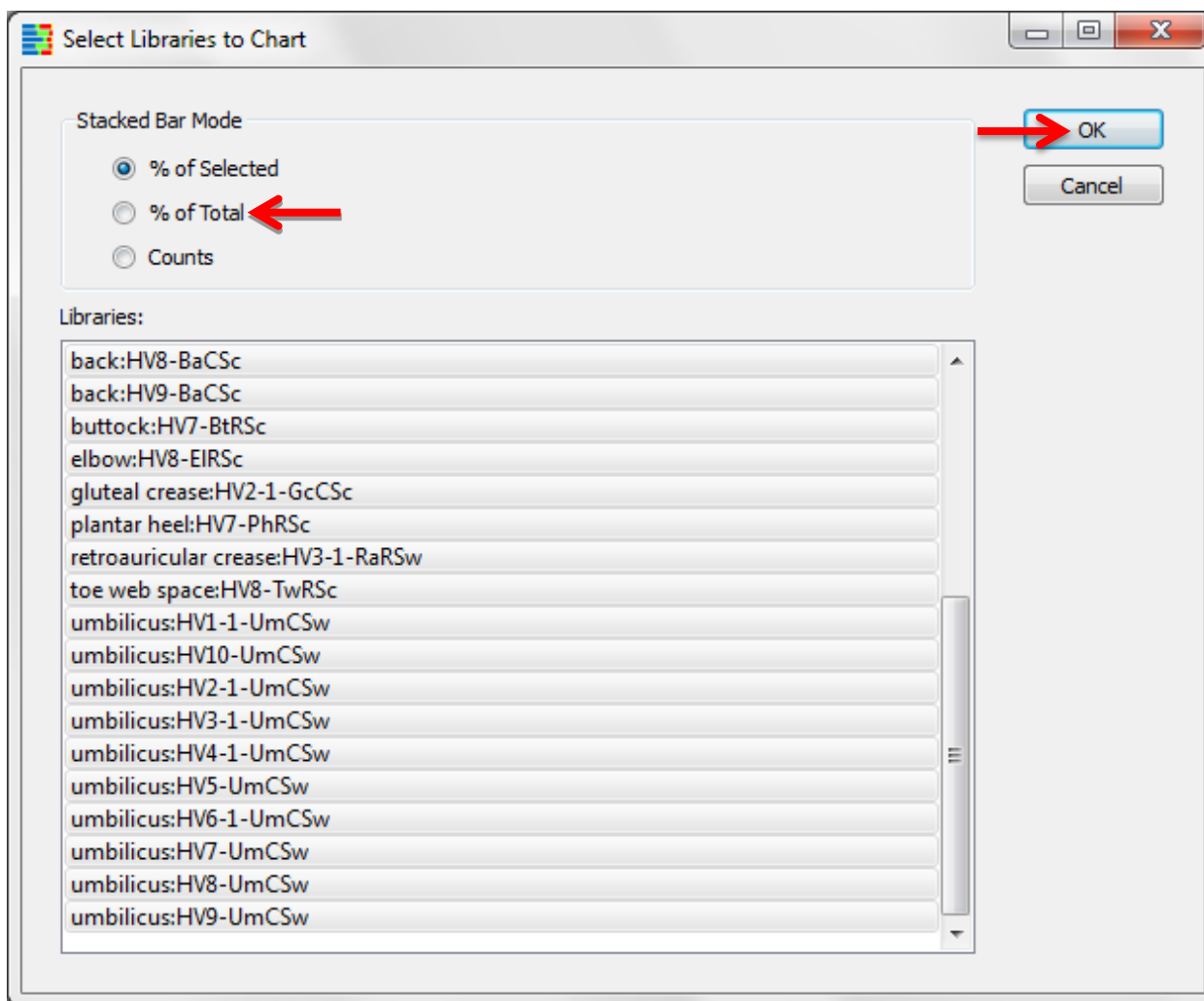


The top 10 OTUs are now highlighted
Click **Plot**

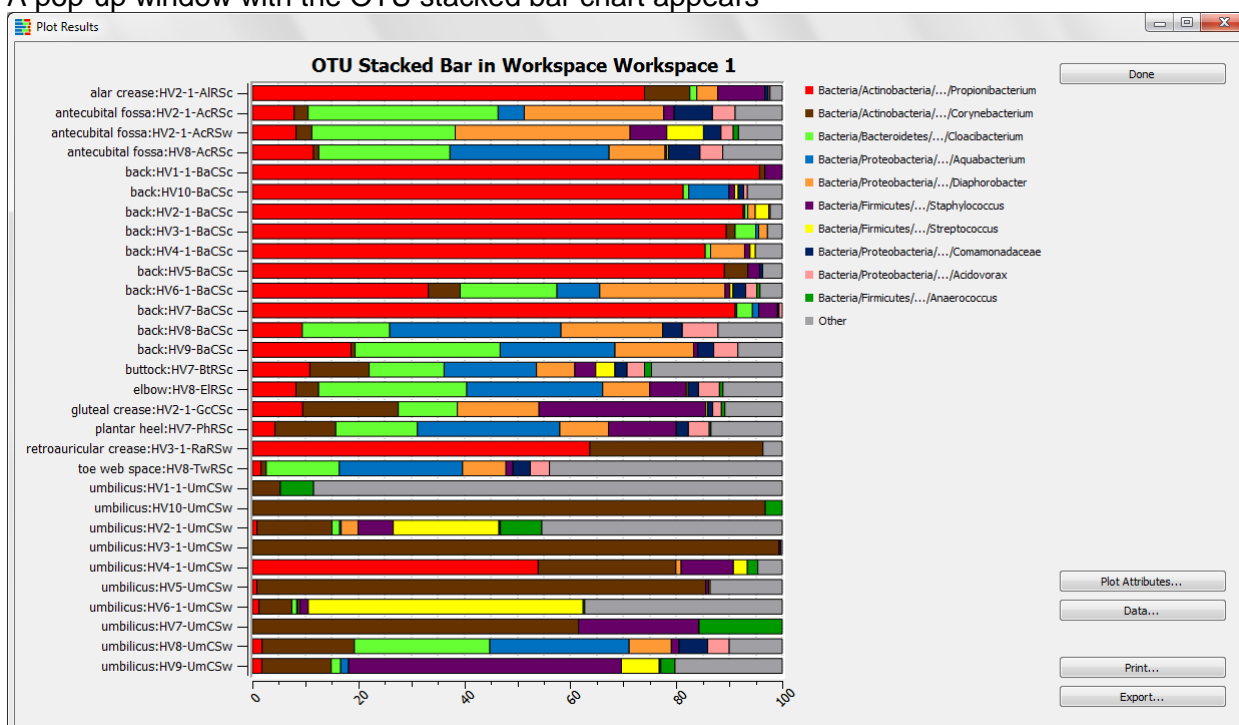


A new window will appear containing stacked bar display options

To create a stacked bar chart which displays a big picture of the project components, select **% of Total**
Click **OK**



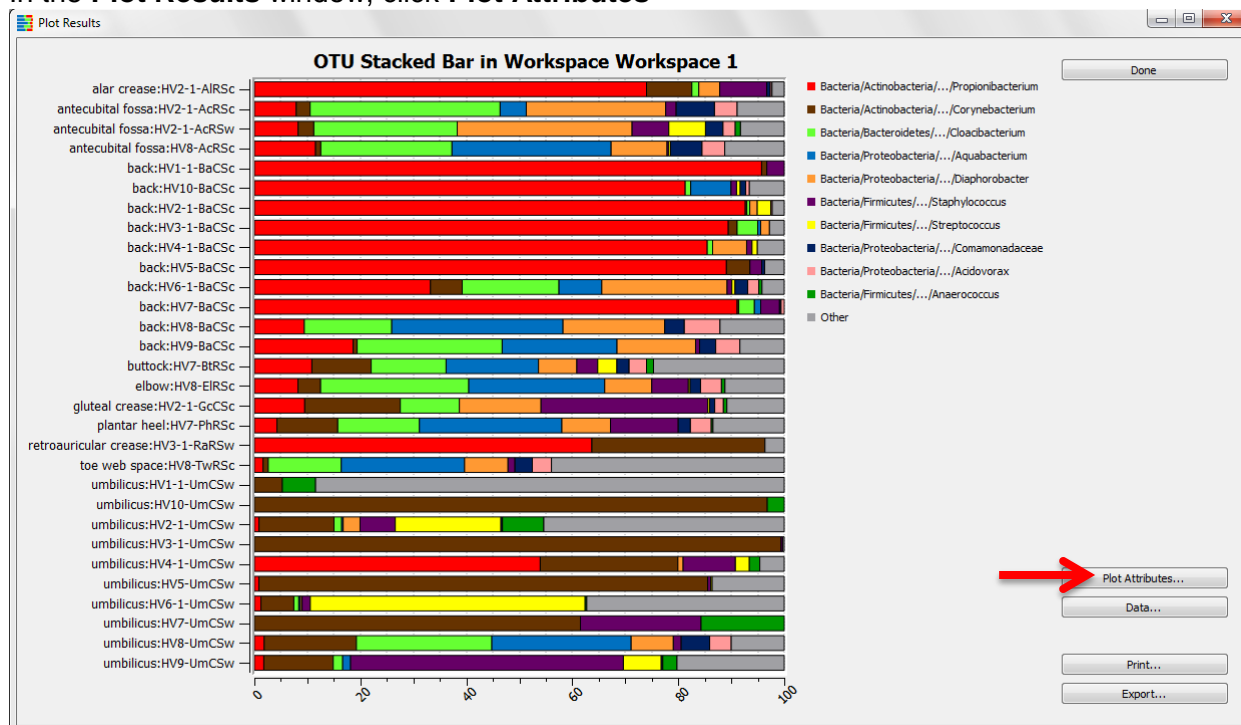
A pop-up window with the OTU stacked bar chart appears



We will now change the default title of the stacked bar chart and add axis labels.

B. Change the Title and Label the Axes

In the **Plot Results** window, click **Plot Attributes**



A pop-up window will appear

The screenshot shows the 'Plot Attributes' window with the 'Titles/Axes' tab selected. The 'Plot' field contains 'OTU Stacked Bar in Workspace Workspace 1'. The 'X Axis' field is empty. The 'Y Axis' field is empty. The 'Show Library Name' checkbox is checked. The 'X Axis' section has 'Autoscale' checked, 'Min Value' set to 0, 'Max Value' set to 100, 'Step Size' set to 0, and 'Label Rotation' set to -45. The 'Y Axis' section has 'Label Rotation' set to 0. The 'Save' and 'Cancel' buttons are on the right.

On the **Titles/Axes** tab, enter “Top 10 Taxa” into the **Plot** field

Enter “OTU % of Total” into the **X Axis** field

Enter “Library Name” into the **Y Axis** field

Click **Save**

Plot Attributes

Titles/Axes Grid Colors Stacked Bar Size

Titles

Plot: Top 10 Taxa

X Axis: OTU % of Total

Y Axis: Library Name

☒ Show Library Name

X Axis

☒ Autoscale

Min Value: 0

Max Value: 100

Step Size: 0

Label Rotation: -45

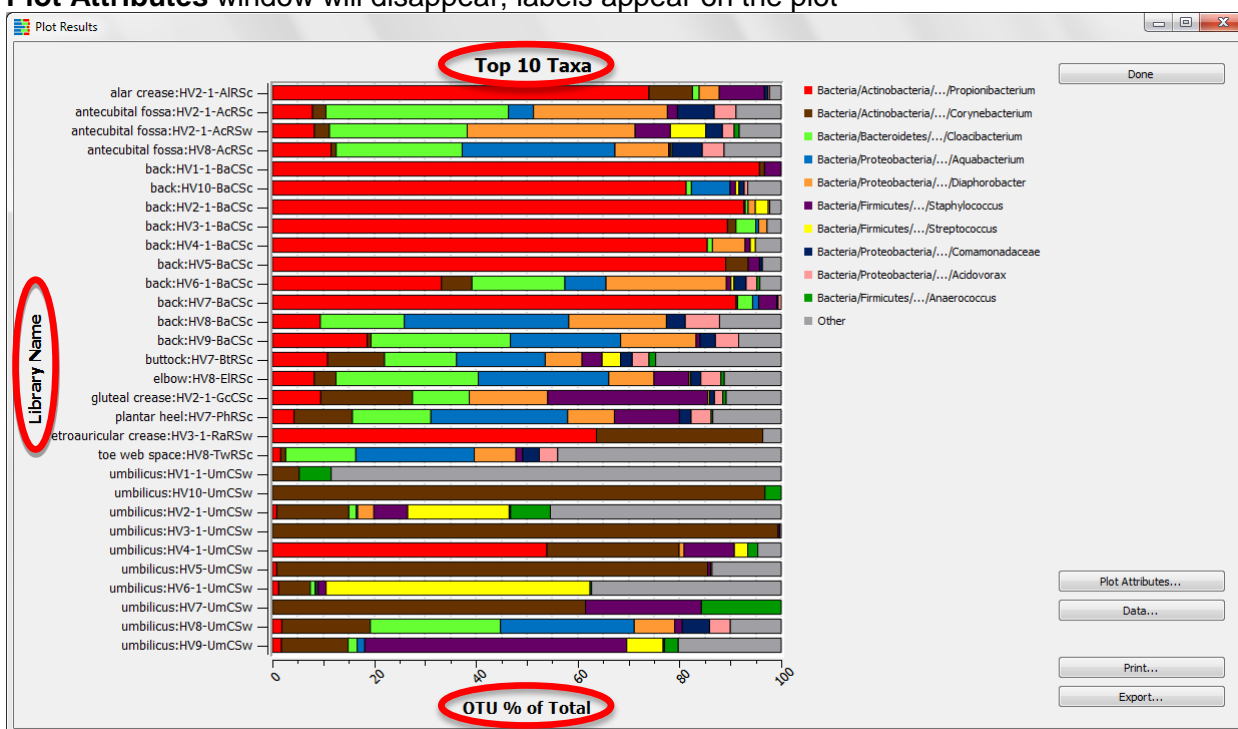
Y Axis

Label Rotation: 0

Save

Cancel

Plot Attributes window will disappear; labels appear on the plot

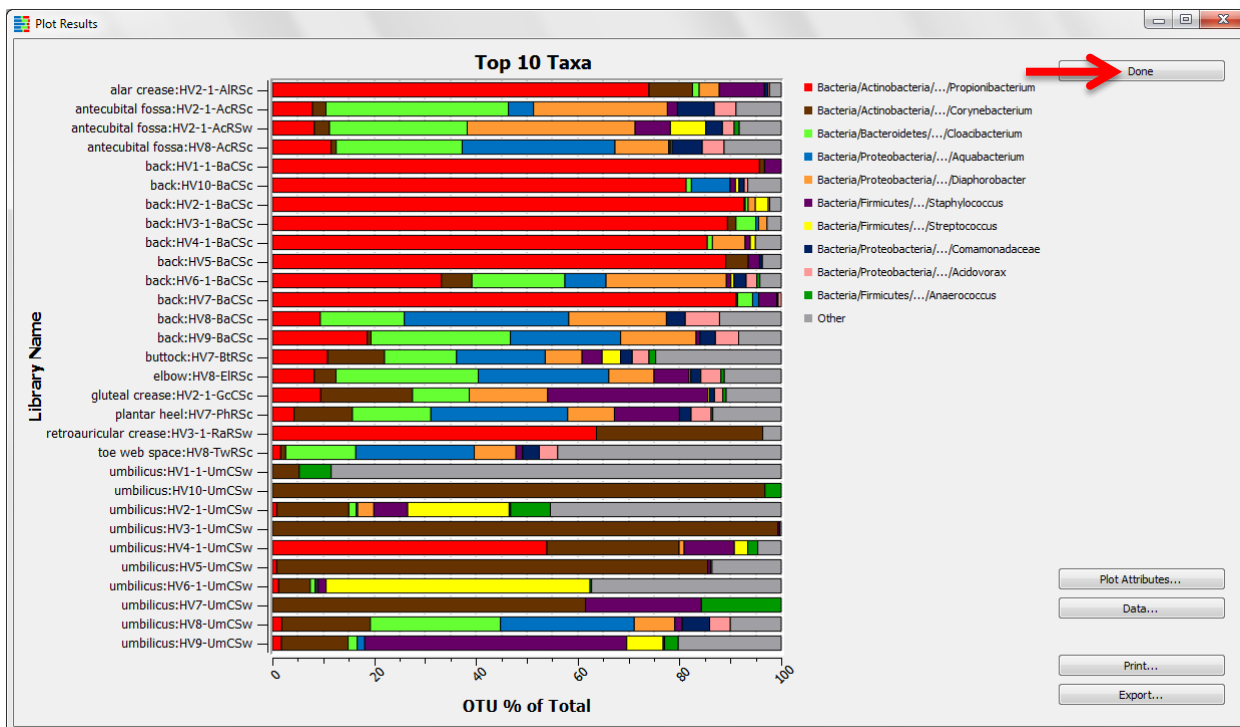


Red and brown appear to be dominant colors in this plot. According to the legend, these colors belong to the “Actinobacteria” phylum. This information may be useful in guiding us toward a hypothesis involving the dominant taxa.

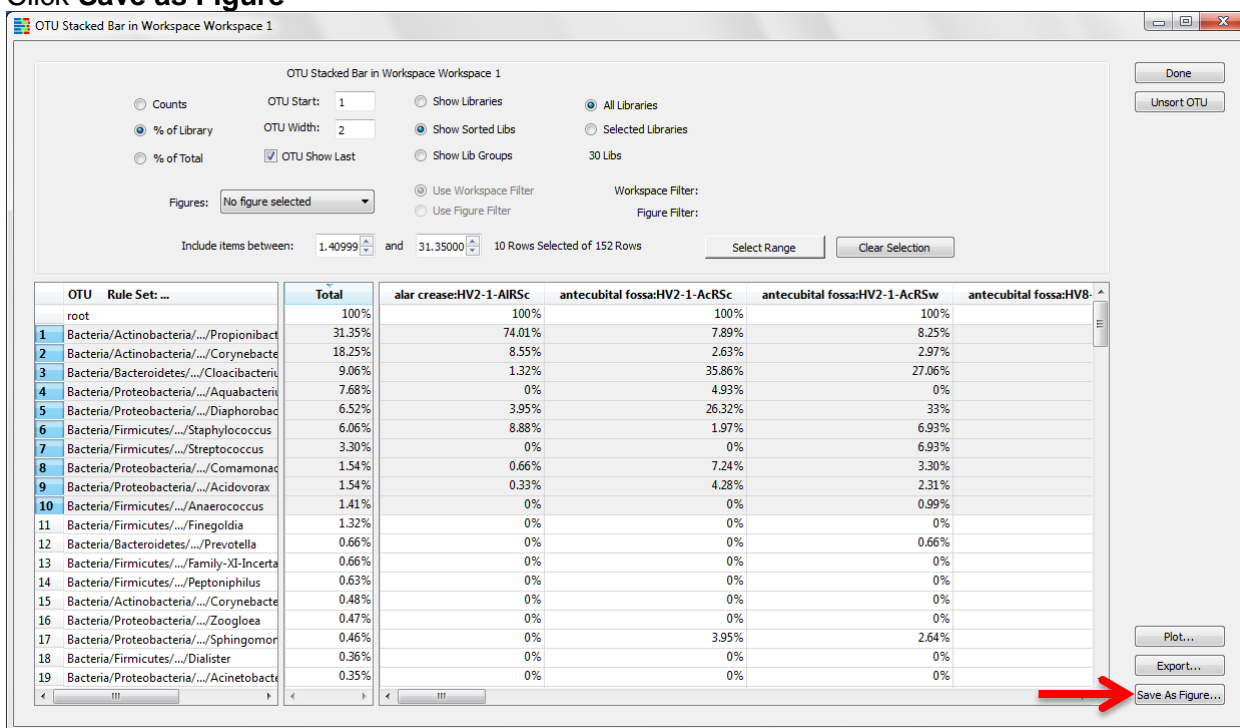
Saving figures in Explicit is easy and convenient. Figures are saved within the larger project, so they stay linked to the data from which they were created and do not create additional files on your computer.

C. Save the OTU Stacked Bar Chart as a Figure

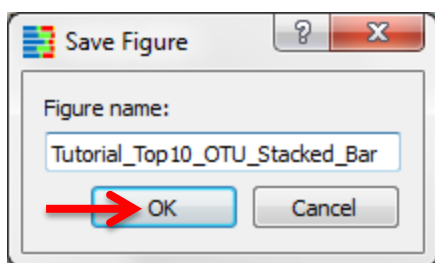
Click **Done** in the stacked bar chart **Plot Results** window



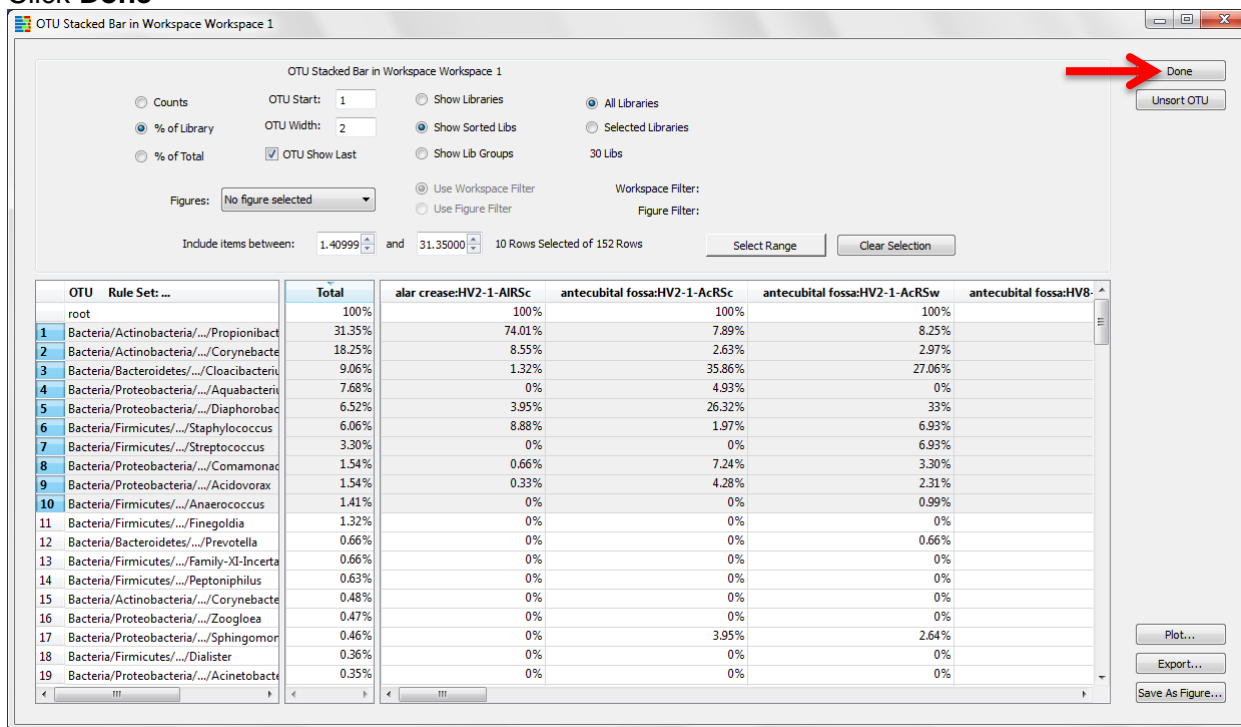
The OTU Stacked Bar setup window is back on the screen
Click **Save as Figure**



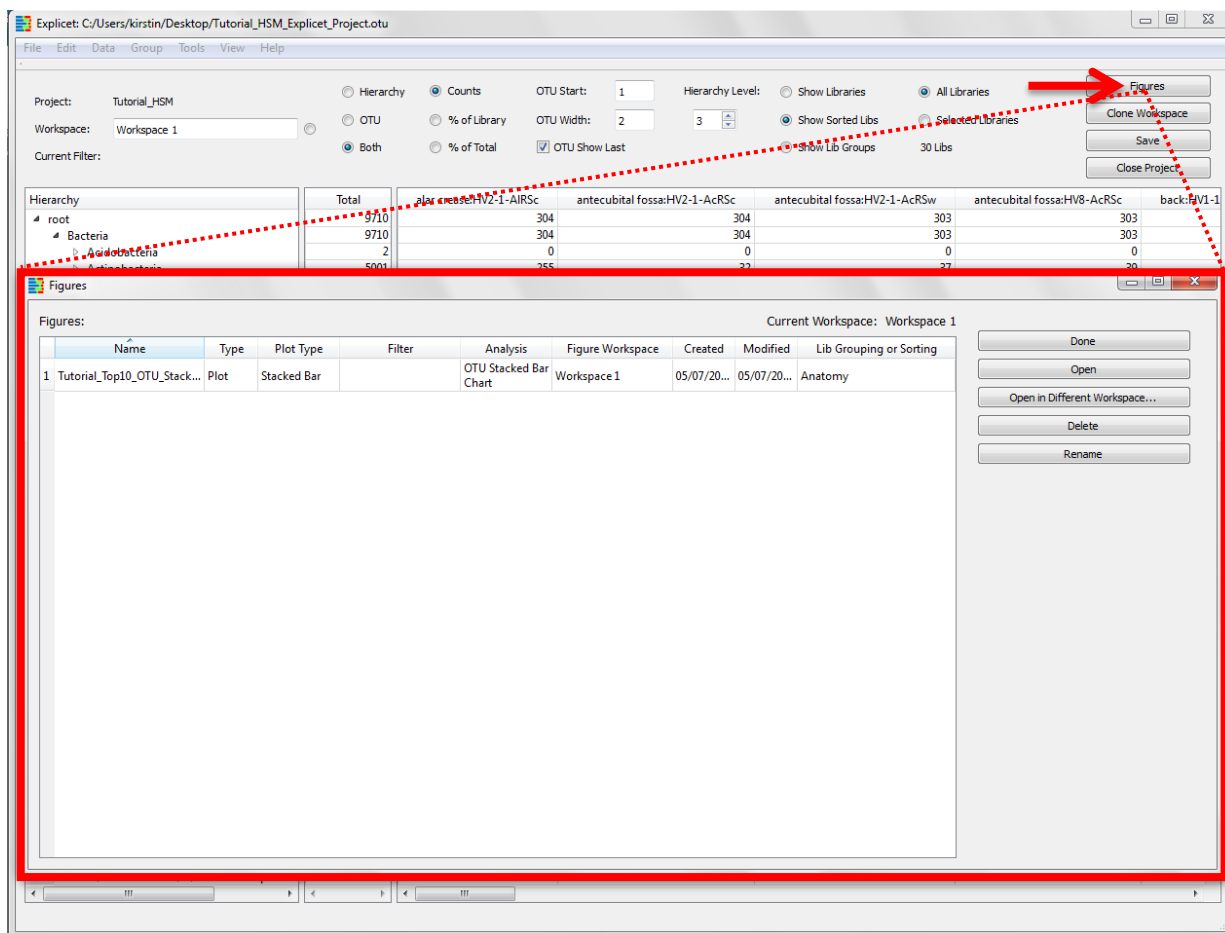
Enter stacked bar chart name in pop-up window
Click **OK**



Click **Done**



Once saved, the stacked bar chart and associated figure data can be recalled at any point by clicking the **Figures** button on the workspace window. This provides a convenient mechanism for editing figures during manuscript preparation. Figures can also be exported in a format suitable for further modification in dedicated drawing software.



VII. Make a Pie Chart

Another useful way to generate an overview of the organisms that exist in the dataset is through a pie chart, which allows graphical depictions of the taxonomic hierarchy.

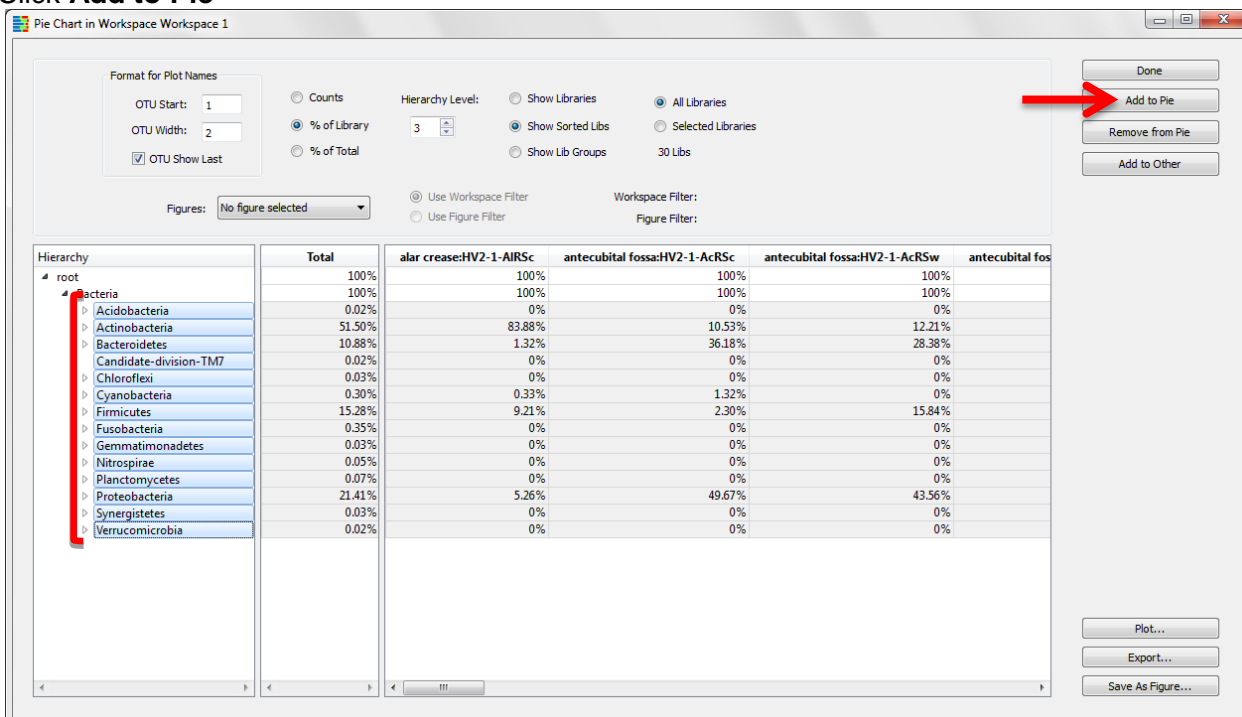
A. Create a Pie Chart of the Project Components

Tools → Plot → Pie Chart

A new window will appear with the hierarchical data available in the workspace

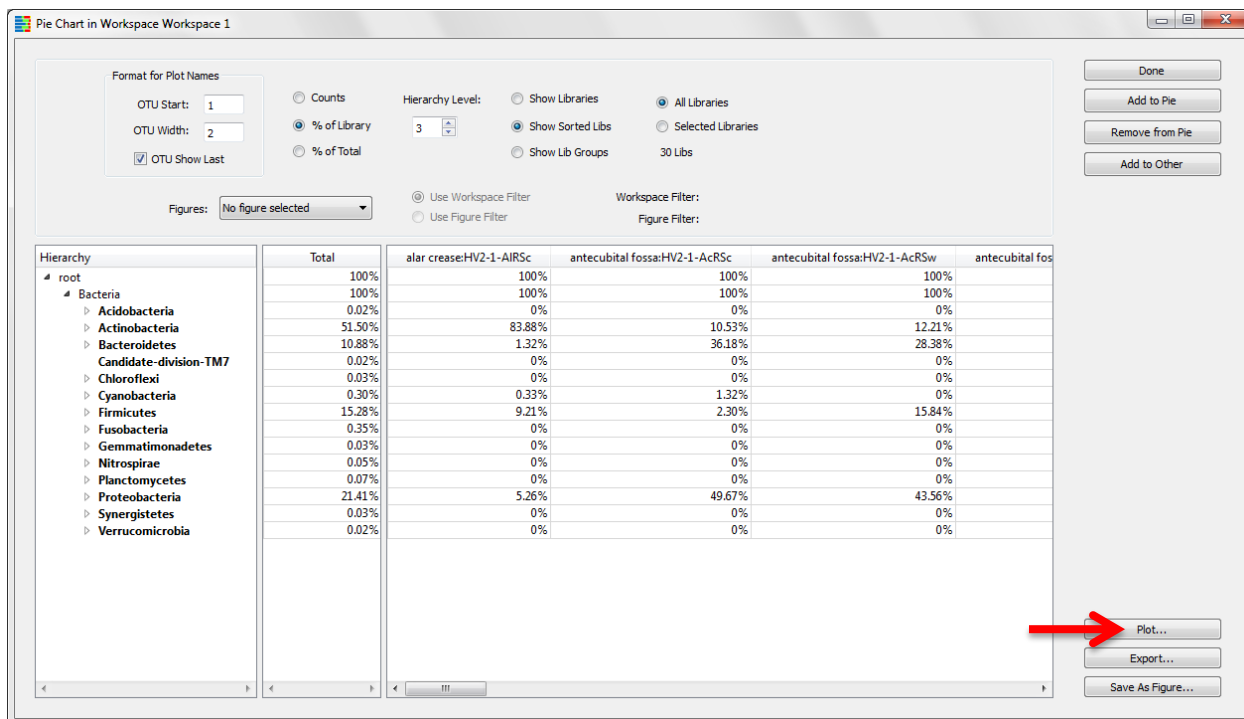
Shift-click all of the phyla in the list

Click **Add to Pie**



The selected phyla which were added to the pie are now bold

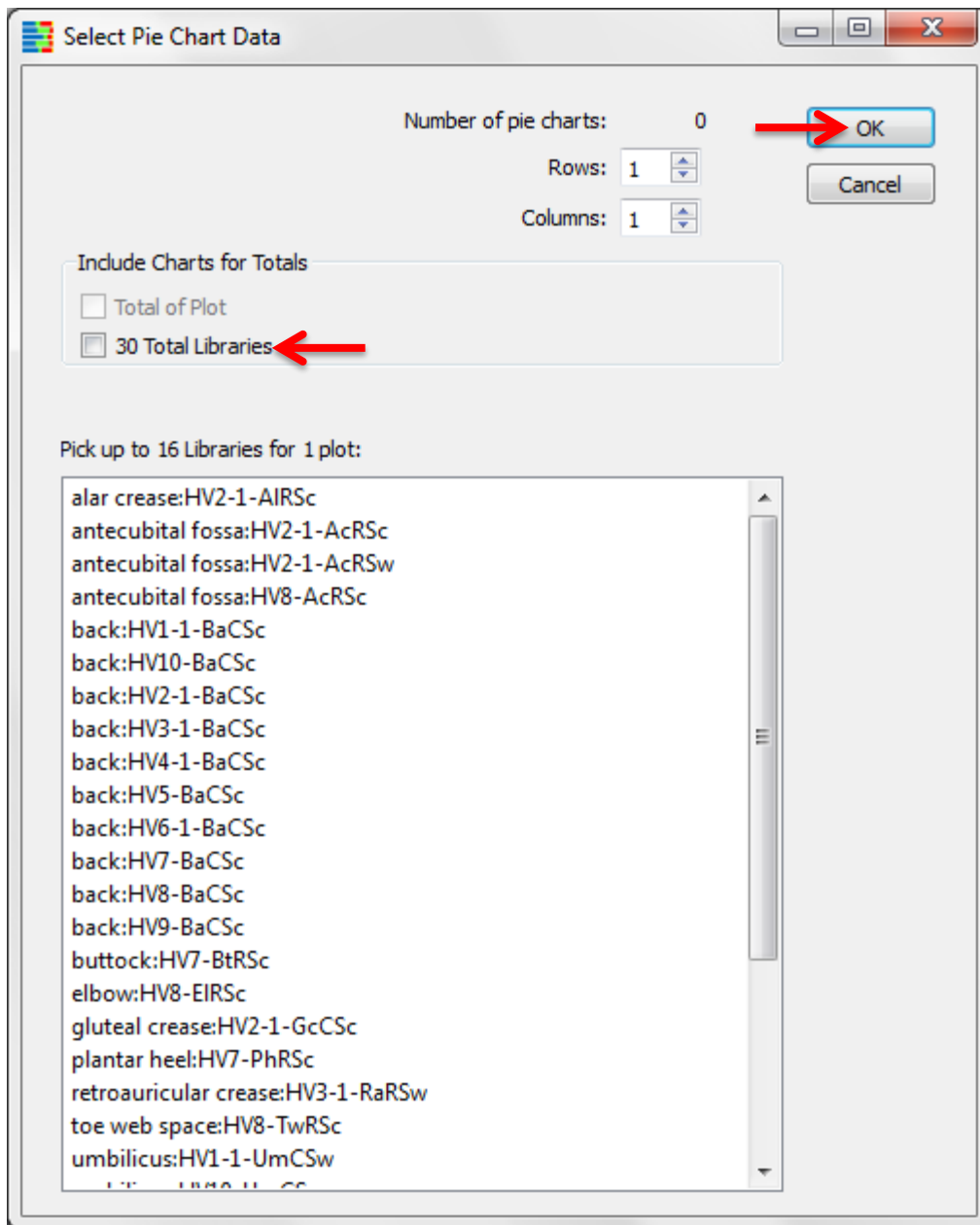
Click **Plot**



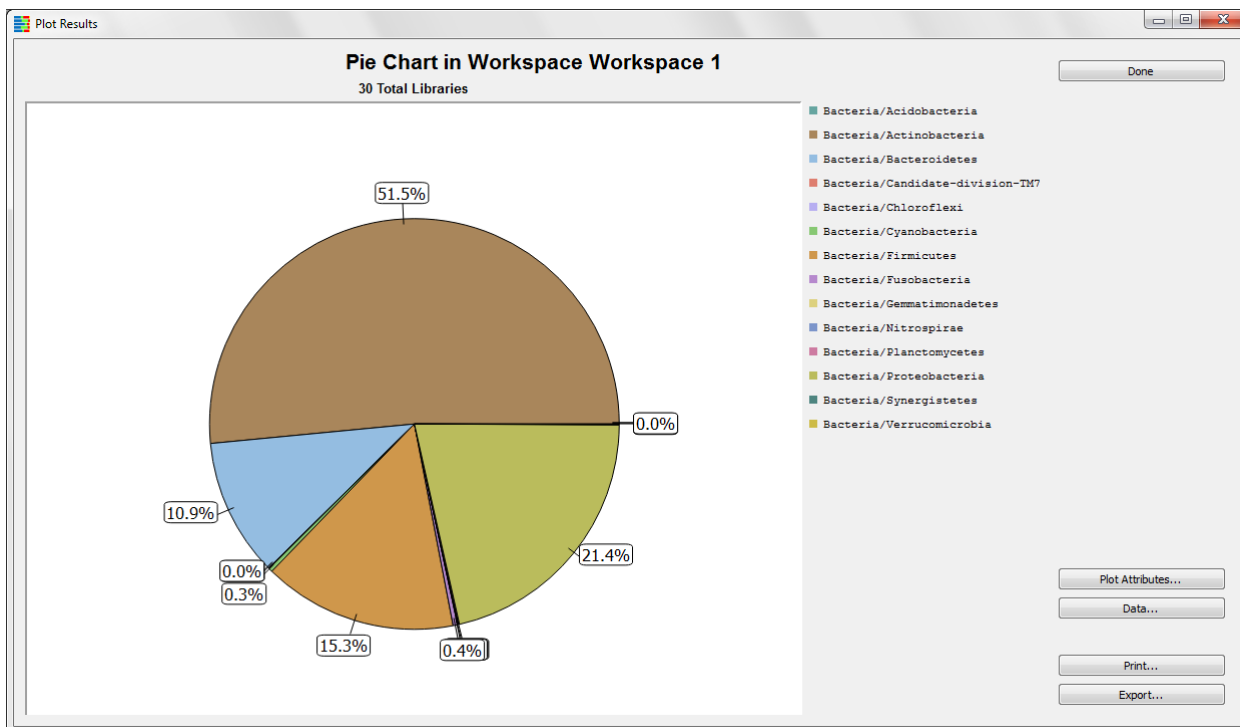
A new window will appear containing pie chart display options

To create only a single pie chart displaying the combined libraries' data, select **30 Total Libraries**

Click **OK**



A pop-up window with the pie chart appears

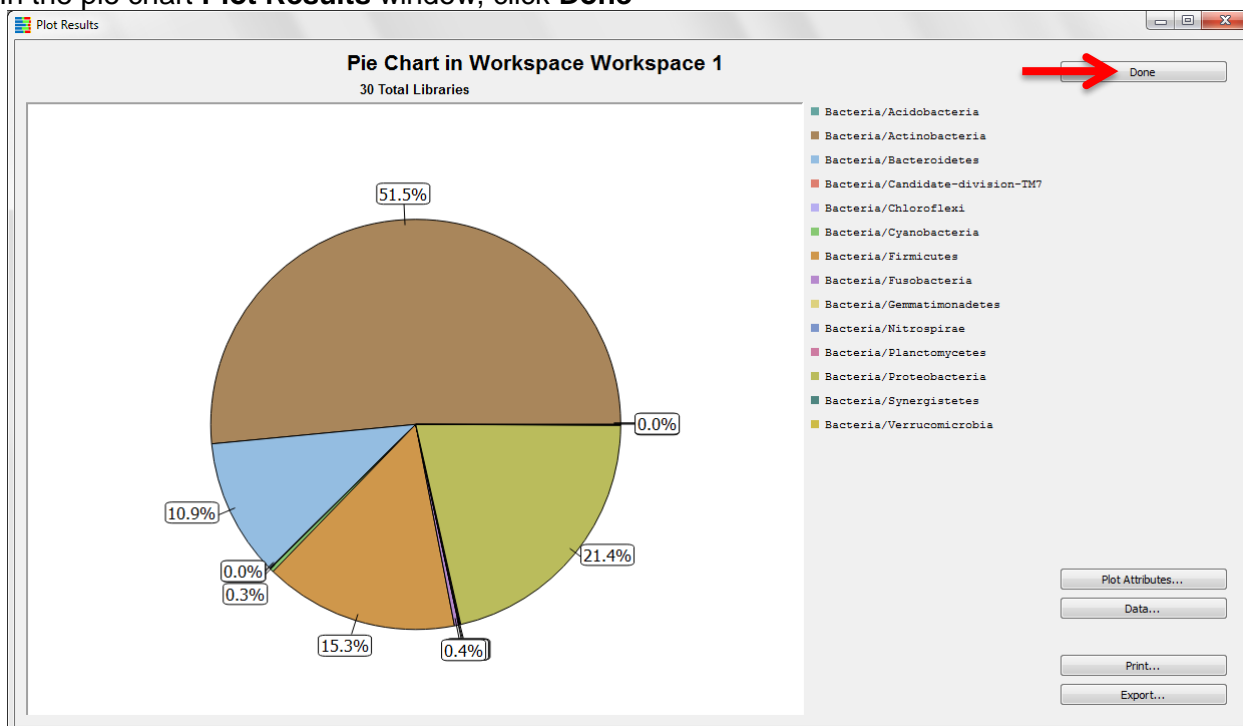


By looking at the pie chart of the phyla, it is clear that the brown wedge, Actinobacteria, is the most prevalent phylum in the data.

Additionally, we can see that the green wedge, Proteobacteria, makes up the second largest portion of the total. To visualize the classes present within the Proteobacteria phylum, we can create pie chart sub-wedges.

B. Make a Pie Chart with Sub-Wedges

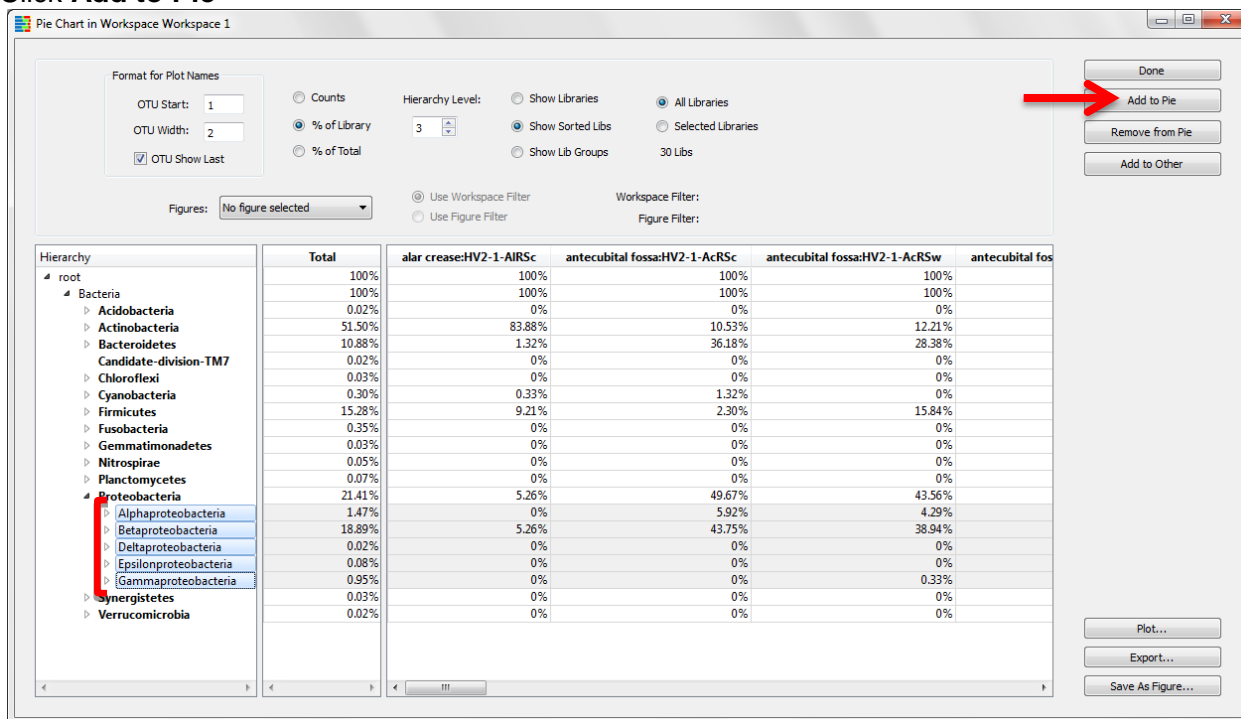
In the pie chart **Plot Results** window, click **Done**



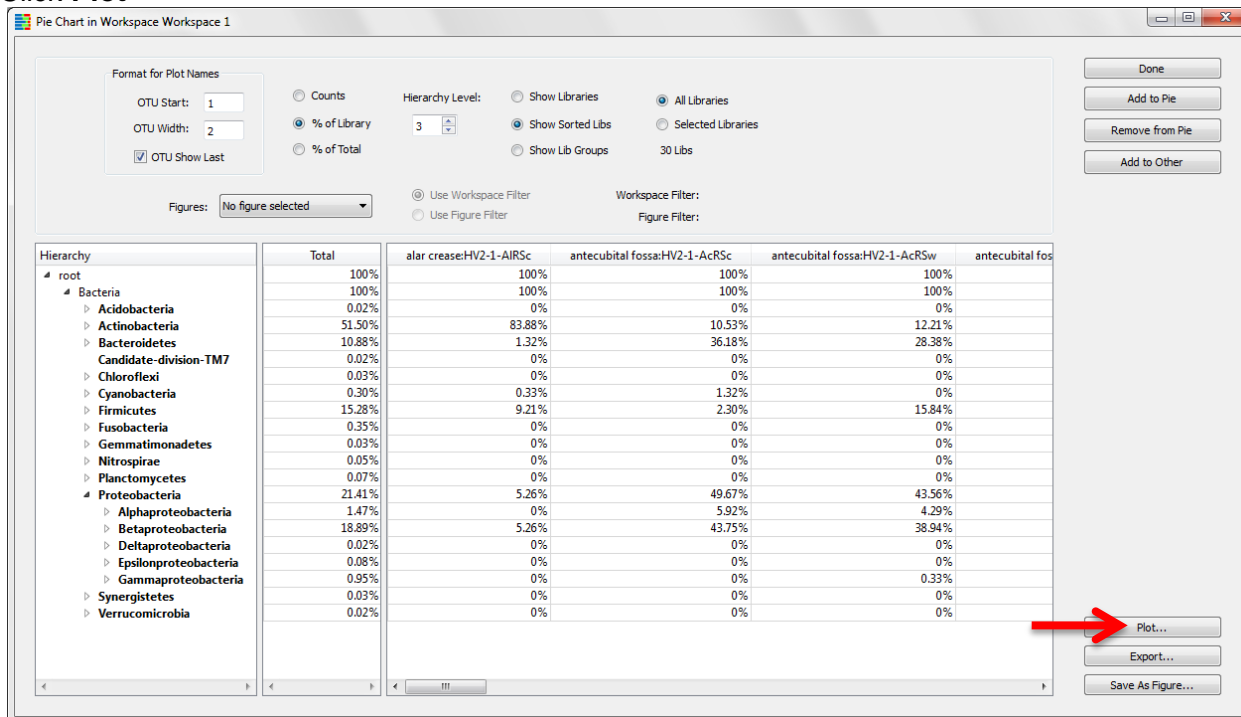
The **Taxonomy Pie Chart** setup window is back on the screen

Use the drop down arrow to the left of “Proteobacteria” to find the classes within the phylum

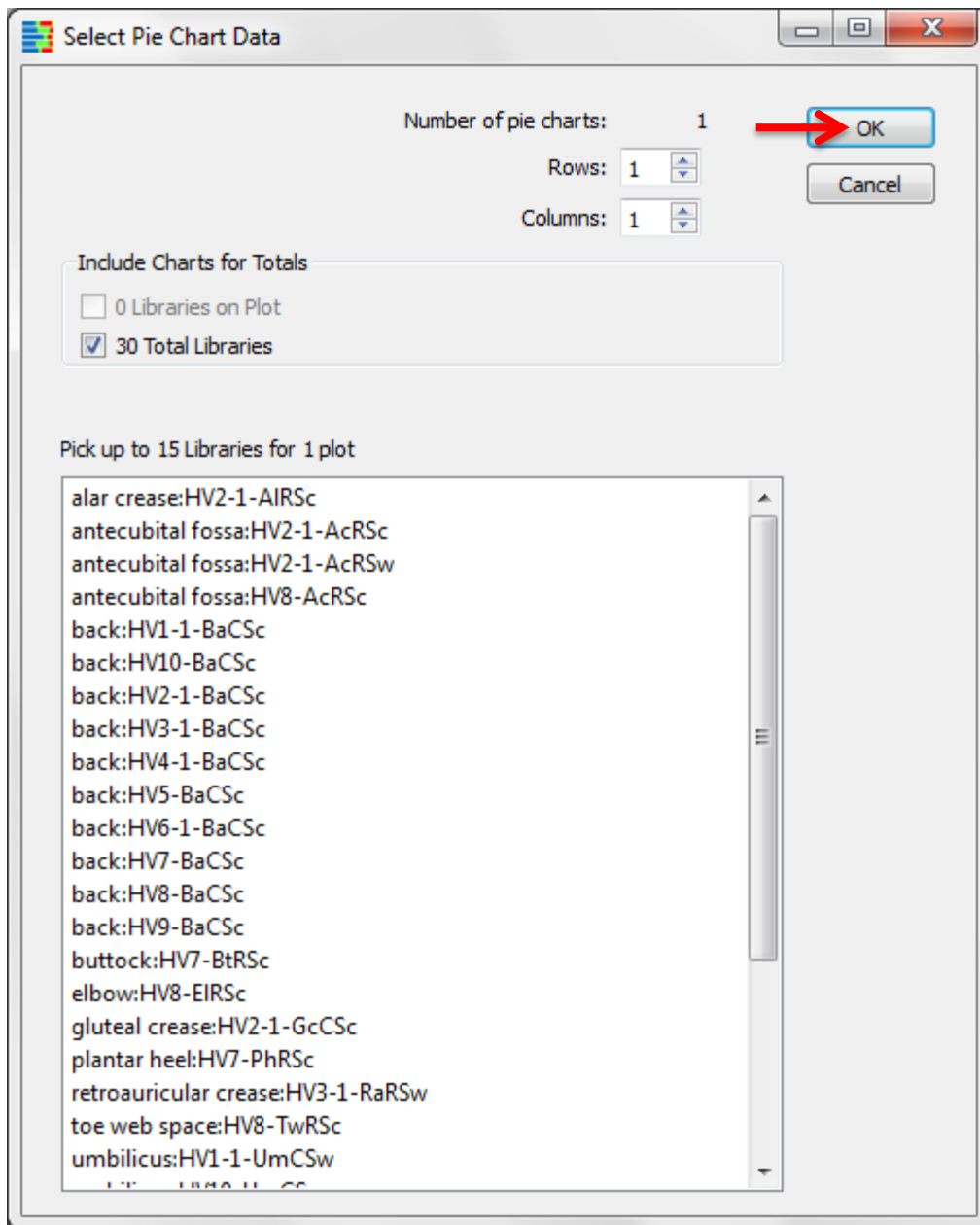
Shift-click all of the classes in the list
Click **Add to Pie**



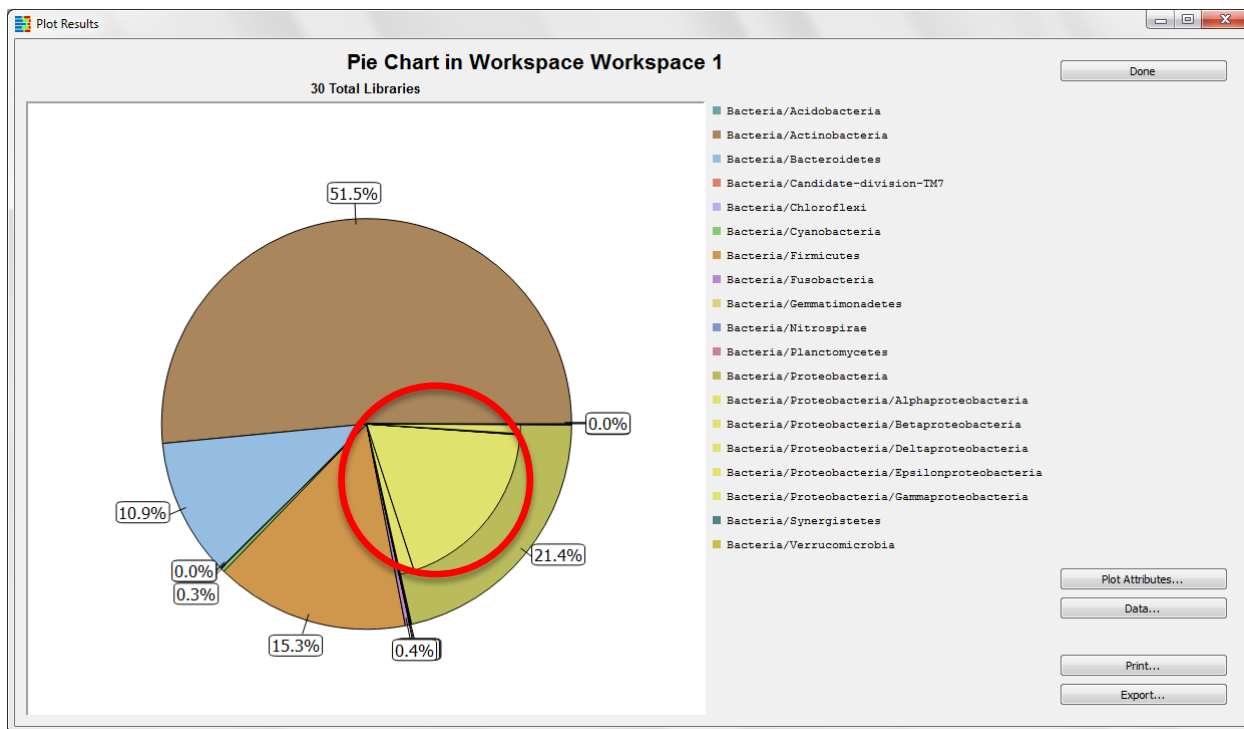
The selected classes that were added to the pie are now bold
Click **Plot**



A new window will appear containing pie chart display options
Again, we will create only a single pie chart displaying the combined libraries' data, so click **OK**



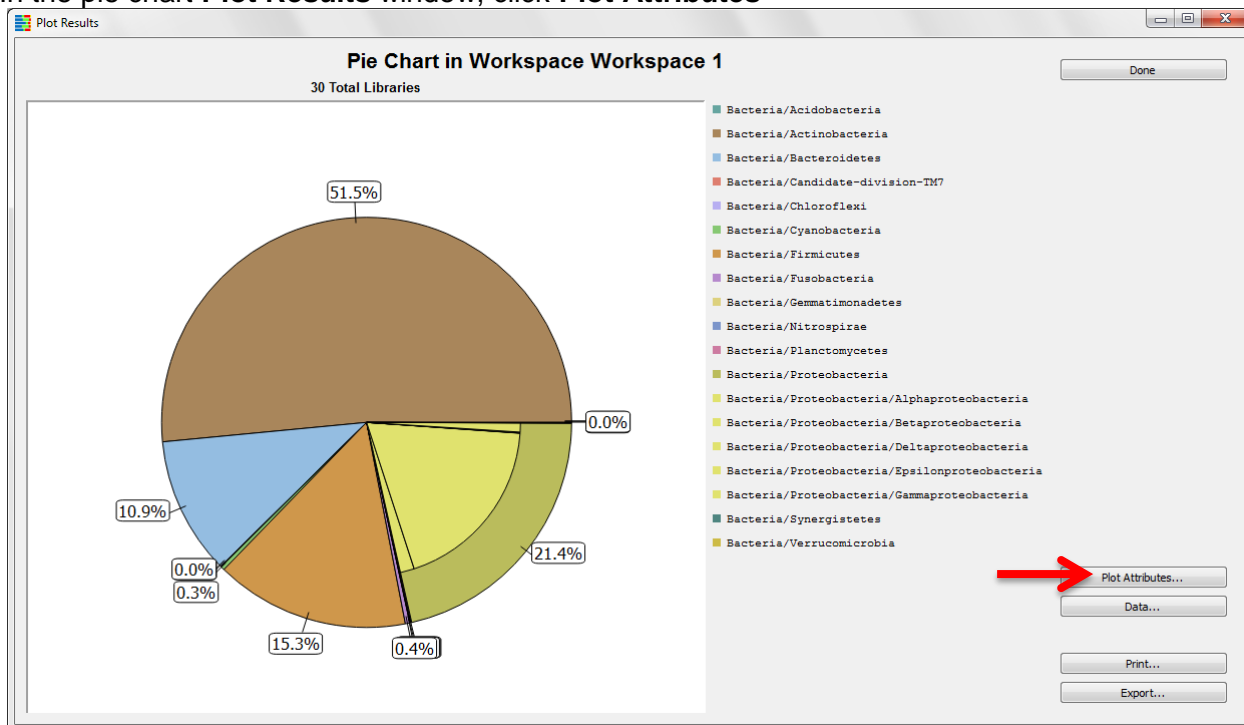
A pop-up window with the pie chart appears. We now see the classes within Proteobacteria represented as sub-wedges.



In order to better differentiate between the different classes, we can change the color of the sub-wedges.

C. Change Wedge Colors in the Pie Chart

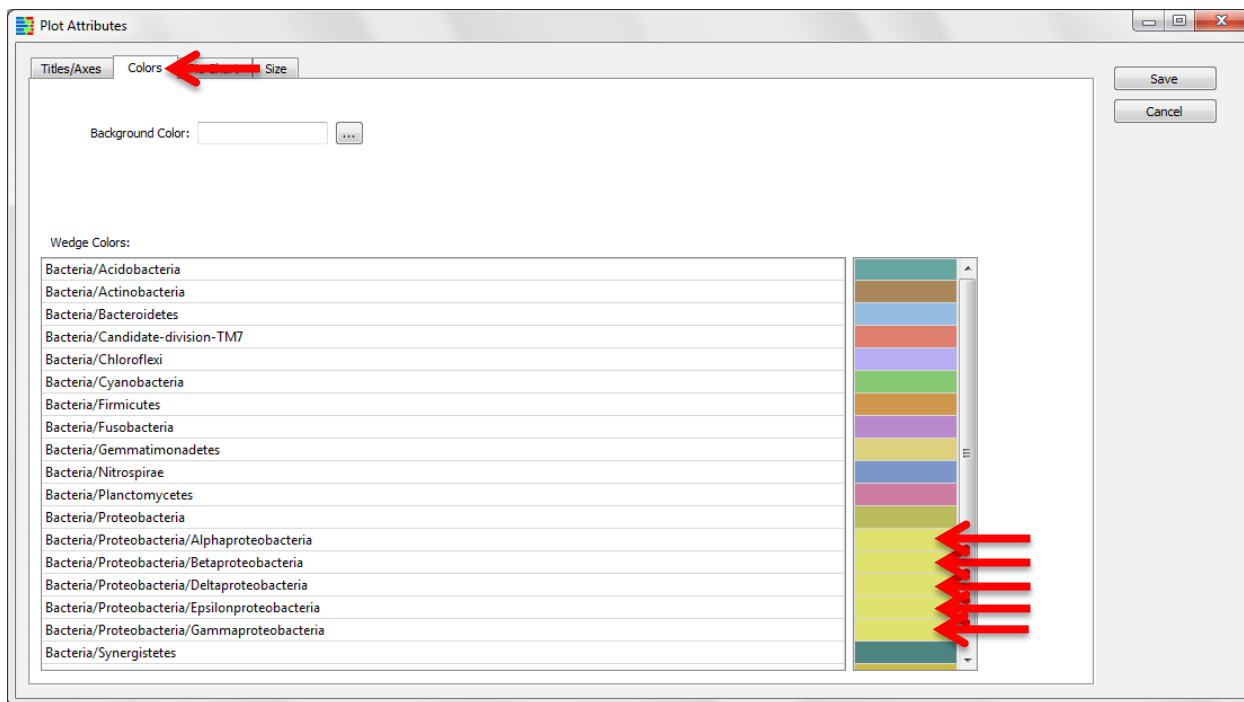
In the pie chart **Plot Results** window, click **Plot Attributes**



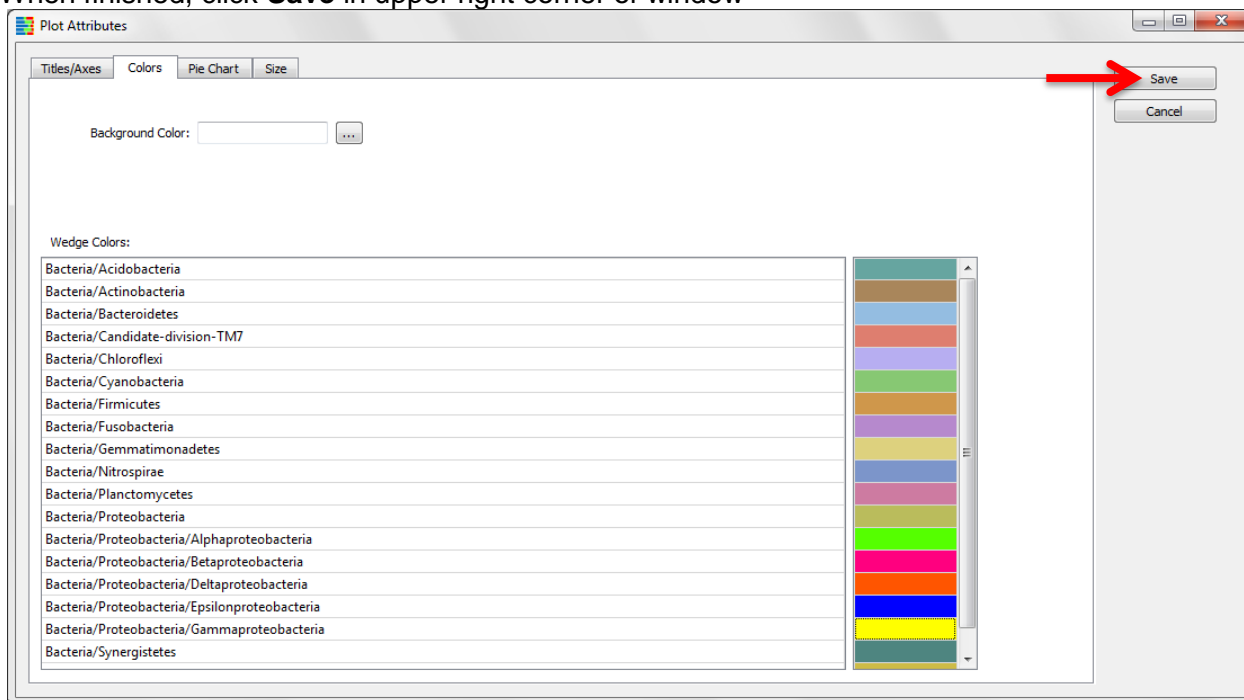
A pop-up window will appear

Click on the **Colors** tab

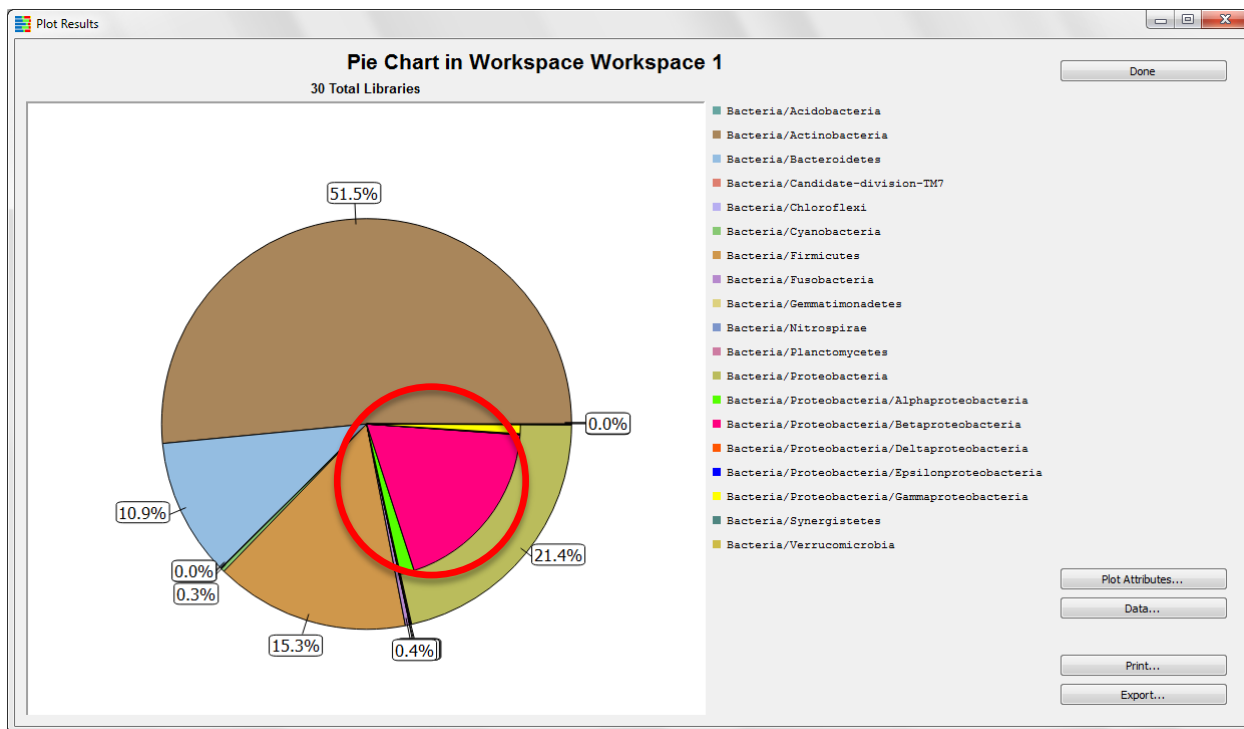
To pick a different wedge color, click on the color, and select a new color from the pop-up display



When finished, click **Save** in upper right corner of window



Plot Attributes window will disappear; changes will be shown on the plot



You may choose to save the pie chart as a figure. To do so, continue as shown earlier in the stacked bar chart example; close the graphics window, and select **Save As Figure** in the **Pie Chart** window.

VIII. Create a Workspace

A workspace is a way for users to make experiments on copies or subsets of their entire data set, while keeping the original data fully intact.

Although the skin is a single organ, it harbors microbial communities that live in a range of physiologically and topographically distinct niches. The back is typically a sebaceous region, whereas the umbilicus is often a moist region of the body. Therefore, these two niches may have different taxa present. We will create a workspace for a mini-experiment to compare data from only these two anatomical positions.

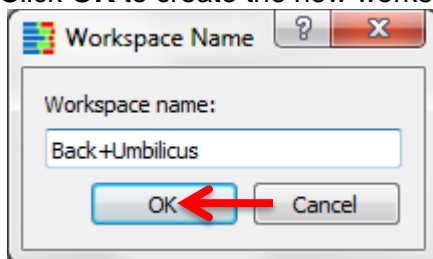
A. Create a New Workspace

File → New → Workspace from Current Workspace

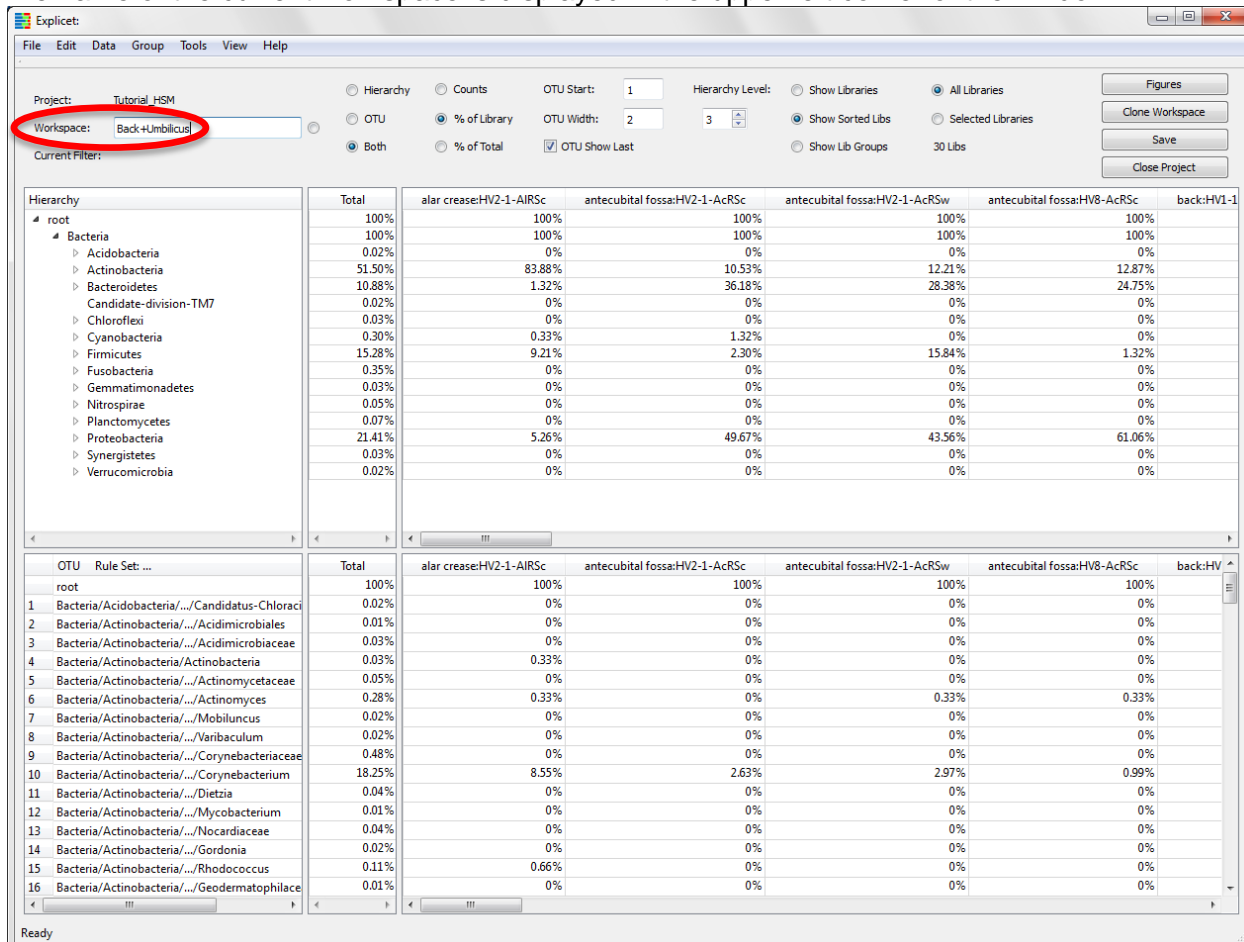
- “from Current Workspace” allows us to copy all of the display changes we’ve already made to the new workspace.

Enter desired workspace name in the pop-up window

Click **OK** to create the new workspace



The name of the current workspace is displayed in the upper left corner of the window



	Total	alar crease:HV2-1-AIRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fossa:HV8-AcRSc	back:HV1-1
root	100%	100%	100%	100%	100%	100%
Bacteria	100%	100%	100%	100%	100%	100%
Acidobacteria	0.02%	0%	0%	0%	0%	0%
Actinobacteria	51.50%	83.88%	10.53%	12.21%	12.87%	12.87%
Bacteroidetes	10.88%	1.32%	36.18%	28.38%	24.75%	24.75%
Candidate-division-TM7	0.02%	0%	0%	0%	0%	0%
Chloroflexi	0.03%	0%	0%	0%	0%	0%
Cyanobacteria	0.30%	0.33%	1.32%	0%	0%	0%
Firmicutes	15.28%	9.21%	2.30%	15.84%	1.32%	1.32%
Fusobacteria	0.35%	0%	0%	0%	0%	0%
Gemmatimonadetes	0.03%	0%	0%	0%	0%	0%
Nitrospirae	0.05%	0%	0%	0%	0%	0%
Planctomycetes	0.07%	0%	0%	0%	0%	0%
Proteobacteria	21.41%	5.26%	49.67%	43.56%	61.06%	61.06%
Synergistetes	0.03%	0%	0%	0%	0%	0%
Verrucomicrobia	0.02%	0%	0%	0%	0%	0%

OTU	Rule Set: ...	Total	alar crease:HV2-1-AIRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fossa:HV8-AcRSc	back:HV1-1
1	Bacteria/Actinobacteria/.../Candidatus-Chloraci	0.02%	0%	0%	0%	0%	0%
2	Bacteria/Actinobacteria/.../Acidimicrobiales	0.01%	0%	0%	0%	0%	0%
3	Bacteria/Actinobacteria/.../Acidimicrobiaceae	0.03%	0%	0%	0%	0%	0%
4	Bacteria/Actinobacteria/Actinobacteria	0.03%	0.33%	0%	0%	0%	0%
5	Bacteria/Actinobacteria/.../Actinomycetaceae	0.05%	0%	0%	0%	0%	0%
6	Bacteria/Actinobacteria/.../Actinomycetes	0.28%	0.33%	0%	0.33%	0.33%	0.33%
7	Bacteria/Actinobacteria/.../Molibuncus	0.02%	0%	0%	0%	0%	0%
8	Bacteria/Actinobacteria/.../Vanibaculum	0.02%	0%	0%	0%	0%	0%
9	Bacteria/Actinobacteria/.../Corynebacteriaceae	0.48%	0%	0%	0%	0%	0%
10	Bacteria/Actinobacteria/.../Corynebacterium	18.25%	8.55%	2.63%	2.97%	0.99%	0.99%
11	Bacteria/Actinobacteria/.../Dietzia	0.04%	0%	0%	0%	0%	0%
12	Bacteria/Actinobacteria/.../Mycobacterium	0.01%	0%	0%	0%	0%	0%
13	Bacteria/Actinobacteria/.../Nocardiaeae	0.04%	0%	0%	0%	0%	0%
14	Bacteria/Actinobacteria/.../Gordonia	0.02%	0%	0%	0%	0%	0%
15	Bacteria/Actinobacteria/.../Rhodococcus	0.11%	0.66%	0%	0%	0%	0%
16	Bacteria/Actinobacteria/.../Geodermatophilaceae	0.01%	0%	0%	0%	0%	0%

IX. Apply a Filter

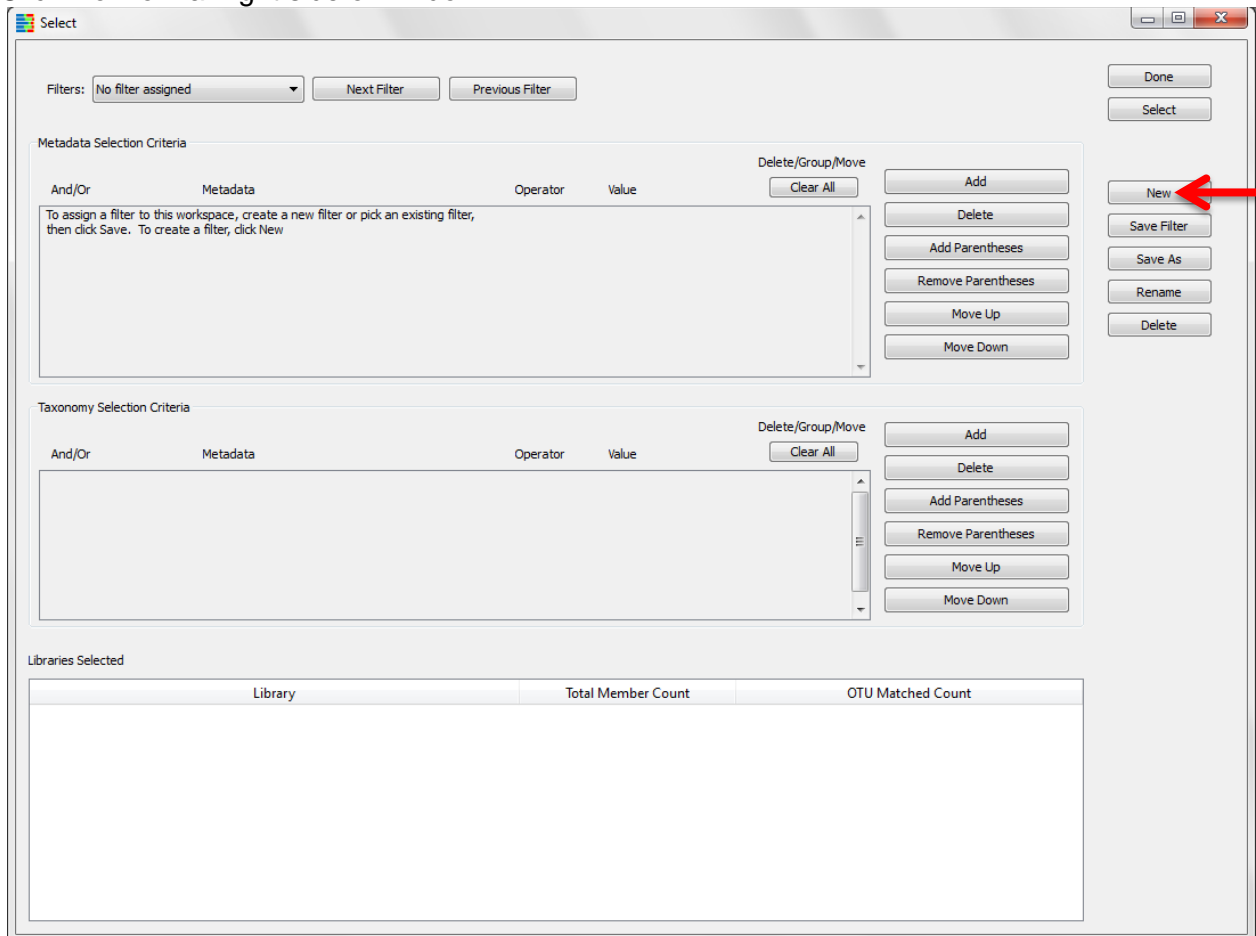
To compare data from only the back and umbilicus, we need to separate these libraries from the other body parts. This is done in Explicitet via “filters”.

A. Create a Filter

Data → Select Libraries

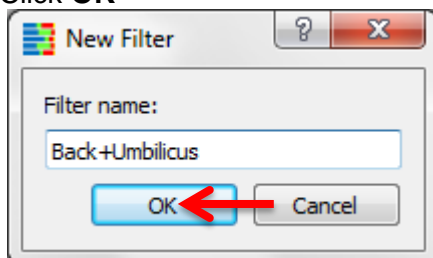
New pop-up window appears for creation of filters

Click **New** on far right side of window



Enter desired filter name in the pop-up window

Click **OK**



The filter name will appear in upper left corner of window

The screenshot shows a window titled "Select" with a toolbar at the top containing a "Filters:" dropdown menu (highlighted with a red circle and showing "Back+Umbilicus"), "Next Filter", and "Previous Filter" buttons. On the right side of the toolbar are "Done" and "Select" buttons.

Below the toolbar are two main sections for defining selection criteria:

- Metadata Selection Criteria:** This section has a table with columns "And/Or", "Metadata", "Operator", and "Value". Below the table is a text prompt: "To add a new criteria for Metadata, click Add". To the right of the table is a "Delete/Group/Move" section with a "Clear All" button and a vertical list of buttons: "Add", "Delete", "Add Parentheses", "Remove Parentheses", "Move Up", and "Move Down".
- Taxonomy Selection Criteria:** This section has a similar table and "Delete/Group/Move" button set. Its text prompt is: "To add a new selection criteria for Taxonomy, click Add".

At the bottom of the window is a section titled "Libraries Selected" containing a table with three columns: "Library", "Total Member Count", and "OTU Matched Count". The table is currently empty.

On the far right of the window, there is a vertical column of buttons: "New", "Save Filter", "Save As", "Rename", and "Delete".

Now that we have created a new filter, we need to set up the parameters to filter by. We will select for all libraries that were sampled from the “back” or “umbilicus” anatomical sites.

B. Set Up the Filter Parameters

Click **Add** in the Metadata Criteria pane

The screenshot shows a window titled "Select" with a filter configuration interface. At the top, there's a "Filters:" dropdown menu currently set to "Back+Umbilicus", with "Next Filter" and "Previous Filter" buttons. Below this are two main sections: "Metadata Selection Criteria" and "Taxonomy Selection Criteria". Each section has a table with columns "And/Or", "Metadata", "Operator", and "Value". The "Metadata" table currently contains the instruction "To add a new criteria for Metadata, click Add". To the right of each table is a "Delete/Group/Move" panel with buttons: "Clear All", "Add", "Delete", "Add Parentheses", "Remove Parentheses", "Move Up", and "Move Down". A red arrow points to the "Add" button in the Metadata section. On the far right, there are buttons for "Done", "Select", "New", "Save Filter", "Save As", "Rename", and "Delete". At the bottom, there's a "Libraries Selected" section with a table with columns "Library", "Total Member Count", and "OTU Matched Count".

Use the first pull-down menu to select "Anatomy" (**Metadata** to filter by)
 Use the second pull-down menu to select "contains" (filter **Operator**)
 Enter "back" into **Value**

Click **Add** in the **Metadata Criteria** pane

The screenshot shows a software window titled "Select" with a filter configuration interface. At the top, there's a "Filters:" dropdown set to "Back+Umbilicus", with "Next Filter" and "Previous Filter" buttons. Below this are two main sections: "Metadata Selection Criteria" and "Taxonomy Selection Criteria".

Metadata Selection Criteria: This section has a table with columns "And/Or", "Metadata", "Operator", and "Value". The first row shows "Anatomy" in the Metadata column, "contains" in the Operator column, and "back" in the Value column. To the right of this table is a "Delete/Group/Move" section with a "Clear All" button and a list of action buttons: "Add", "Delete", "Add Parentheses", "Remove Parentheses", "Move Up", and "Move Down". A red arrow points to the "Add" button. To the far right of the window are buttons for "Done", "Select", "New", "Save Filter", "Save As", "Rename", and "Delete".

Taxonomy Selection Criteria: This section has a similar table structure but is currently empty, displaying the text "To add a new selection criteria for Taxonomy, click Add". It also has a "Delete/Group/Move" section with a "Clear All" button and the same set of action buttons as the Metadata section.

Libraries Selected: At the bottom, there's a table with three columns: "Library", "Total Member Count", and "OTU Matched Count". The table is currently empty.

Use the first pull-down menu to select "Or"
 Use the second pull-down menu to select "Anatomy" (**Metadata** to filter by)
 Use the third pull-down menu to select "contains" (filter **Operator**)
 Enter "umbilicus" into **Value**

Select

Filters: Back+Umbilicus Next Filter Previous Filter

Done
Select

Metadata Selection Criteria

And/Or	Metadata	Operator	Value	Delete/Group/Move
	Anatomy	contains	back	<input type="checkbox"/>
or	Library	equals		<input type="checkbox"/>

Clear All Add Delete Add Parentheses Remove Parentheses Move Up Move Down

Taxonomy Selection Criteria

And/Or	Metadata	Operator	Value	Delete/Group/Move
To add a new selection criteria for Taxonomy, click Add				

Clear All Add Delete Add Parentheses Remove Parentheses Move Up Move Down

Libraries Selected

Library	Total Member Count	OTU Matched Count

To apply filter, click **Select** in upper right corner of window
 Click **Save Filter** on far right side of window to keep the filter

Filters: Back+Umbilicus

Next Filter

Previous Filter

Done

Select

Metadata Selection Criteria

And/Or	Metadata	Operator	Value	Delete/Group/Move
	Anatomy	contains	back	<input type="checkbox"/>
or	Anatomy	contains	umbilicus	<input type="checkbox"/>

Clear All

Add

Delete

Add Parentheses

Remove Parentheses

Move Up

Move Down

Taxonomy Selection Criteria

And/Or	Metadata	Operator	Value	Delete/Group/Move
To add a new selection criteria for Taxonomy, click Add				

Clear All

Add

Delete

Add Parentheses

Remove Parentheses

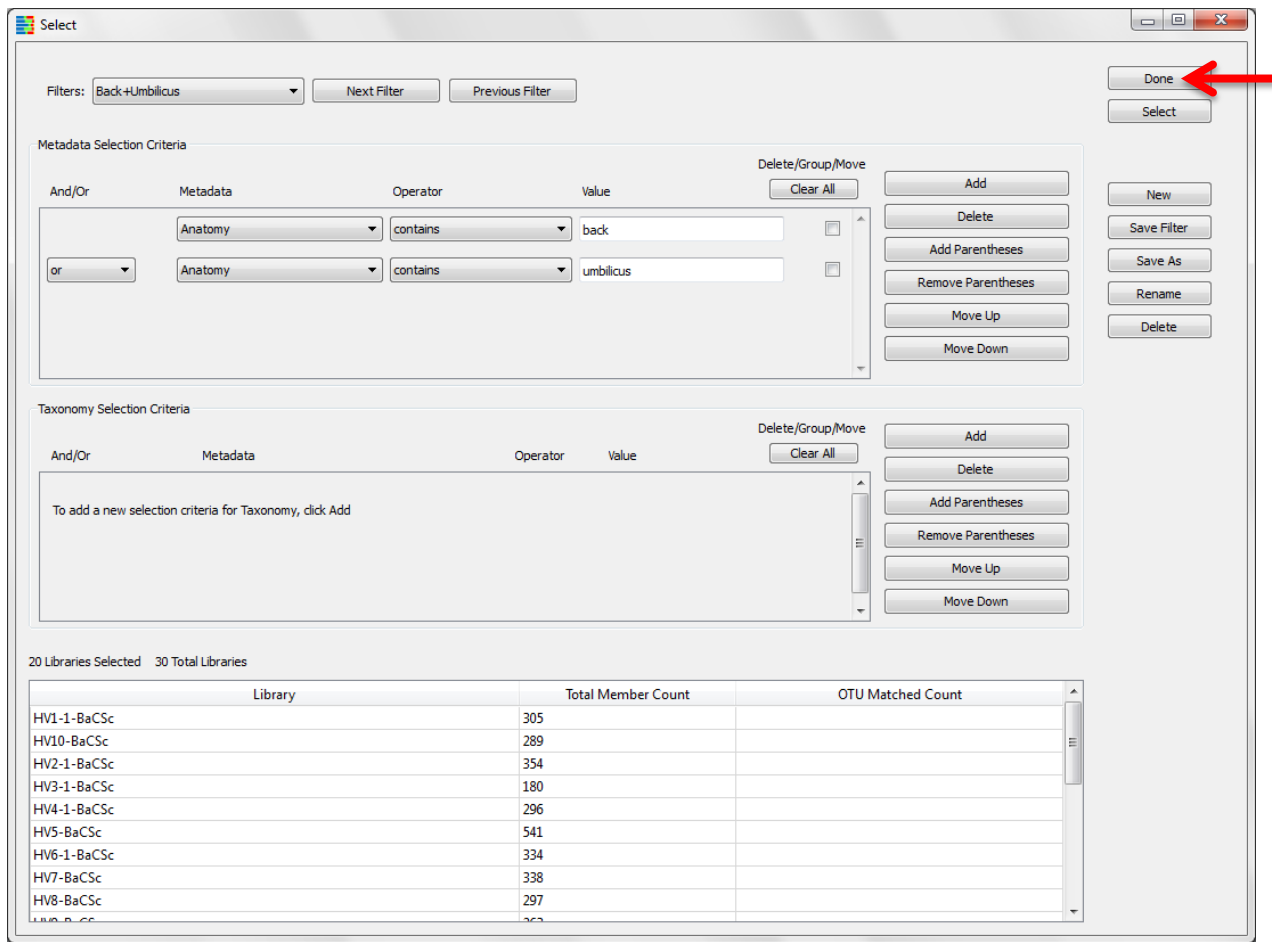
Move Up

Move Down

Libraries Selected

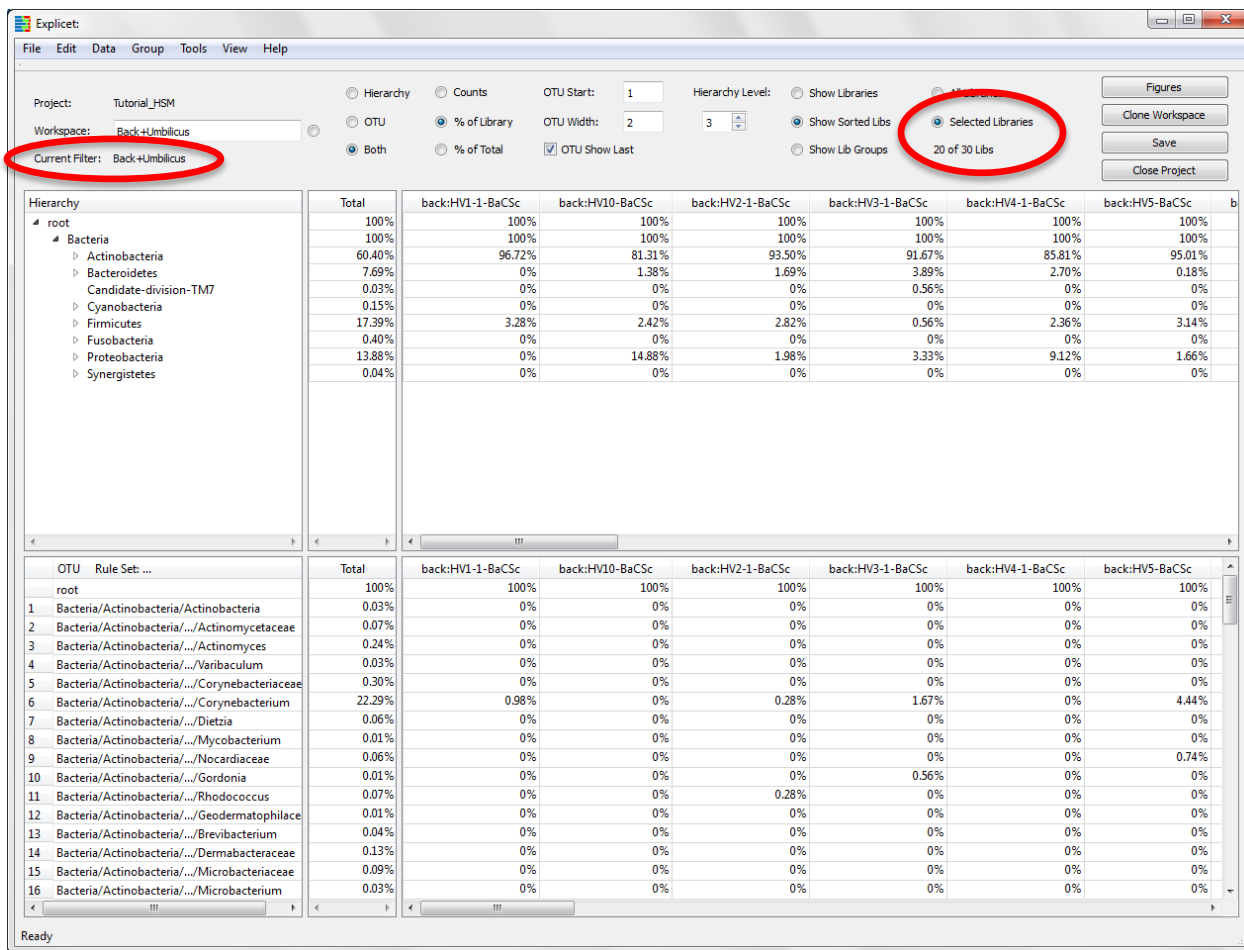
Library	Total Member Count	OTU Matched Count

Click **Done** in upper right corner of window



Pop-up window will disappear

On the current workspace window, **Selected Libraries** is now selected, and the name of the **Current Filter** is displayed in the upper left corner of the window. The workspace window now only displays libraries from the 20 back and umbilicus samples.



X. Beta Diversity (Morisita-Horn)

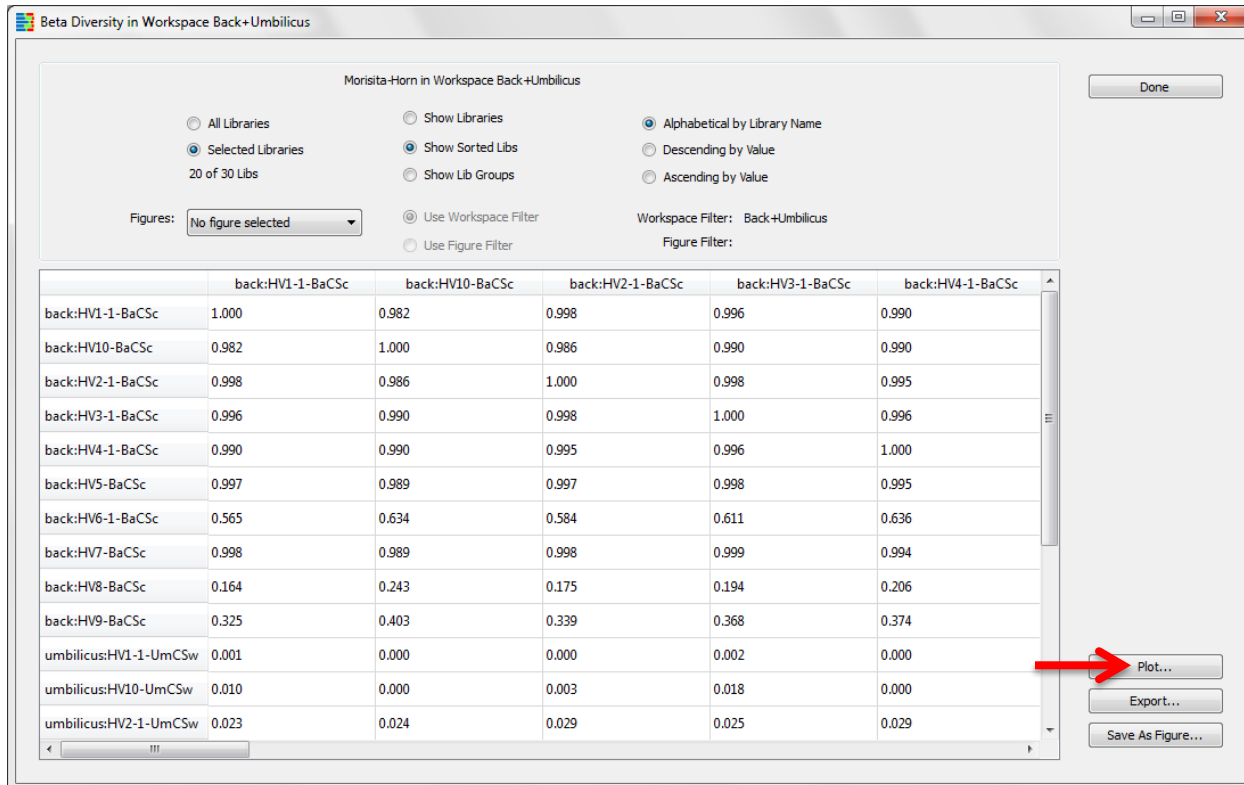
By viewing our libraries in a Morisita-Horn heatmap, we can estimate the similarity of the microbial communities present in the samples at these two anatomical positions. Morisita-Horn is an often used metric that can give insight into how similar or how different sets of samples are from each other by looking at the patterns of all of the different OTUs at the same time.

A. Create a Morisita-Horn Heatmap

Tools → Analyze → Beta Diversity → Morisita-Horn

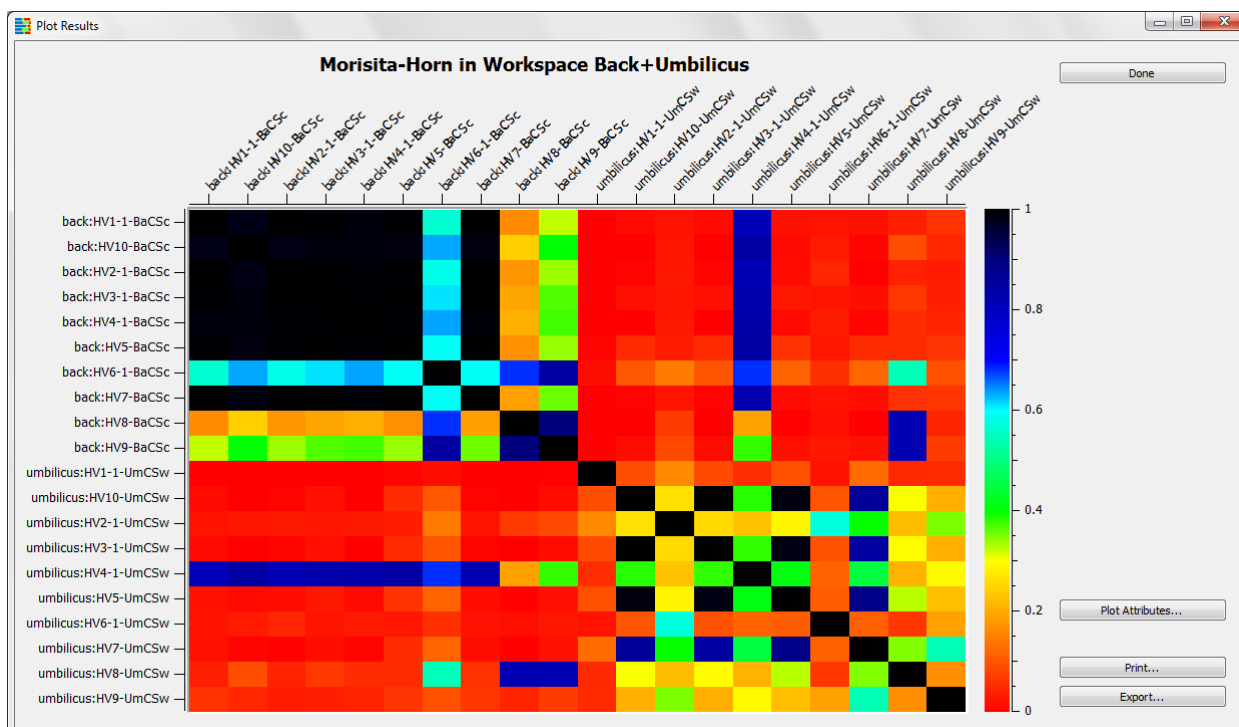
A new window will appear with a table of the sequence variant counts

Click **Plot**



A new window will appear containing the heatmap of Morisita-Horn sequence variant counts

Note: In our workspace, we have **Selected Libraries** selected, so the heatmap will only display results from our libraries of interest (only those libraries sampled from the back or umbilicus).



Anatomical positions with Morisita-Horn values near 1 (implying the samples' constituent taxonomy patterns are very *similar*) appear black. Anatomical positions with Morisita-Horn values near 0 (implying the samples' constituent taxonomy patterns are very *different*) appear red. Based on this data, the back is more similar across subjects than the umbilicus. Plot attributes allow control of plot characteristics and color usage as described earlier.

You may choose to save the Morisita-Horn heatmap as a figure. To do so, continue as shown earlier in the stacked bar chart example; close the graphics window, and select **Save As Figure** in the **OTU Heatmap** window.

XI. Alpha Diversity

The alpha diversity statistics computed by Explicitet are generally shown in one of two ways: either as a single value calculated at the size of the smallest library (known as the rarefaction point) or as multiple values plotted as collector's curves for each library. Collector's curves are the classic way to evaluate the impact of increasing sample size (i.e., more sequencing) on the information content of the dataset. All collector's curves in Explicitet are computed with rarefaction, meaning all libraries are resampled to allow fair comparison between libraries of greatly different size. The higher the resolution of the calculations (large number of bootstrap iterations, large number of steps), the slower the computations will proceed. It is recommended that users start with the defaults and then increase as needed to get the curves to smooth out. Very large bootstrap iterations and a large number of steps may result in a run of multiple days... So, start small and work up.

The alpha diversity metrics are often quick, reliable ways to determine if samples in a dataset are sequenced adequately. Since we have a workspace set up to run mini-experiments on a subset of our data, we should make sure that the data is representative. We need to make sure that enough sequences were generated from the back and umbilicus samples to be considered representative of the anatomical position for a subject. We can test this by running an alpha diversity test called Good's Coverage.

A. Run a Good's Coverage Test

Tools → **Analyze** → **Alpha Diversity**

New pop-up window appears

To create curves, deselect **Single statistic at Rarefaction point only**

Click **Bootstrap**

When **Bootstrap** is finished running, click **Plot**

Alpha Diversity in Workspace Back+Umbilicus

☐ All Libraries ☒ Selected Libraries 20 of 30 Libs

Libraries: 20 Min Size: 180 Max Size: 541 Avg Size: 334 ☐ Show Libraries

Bootstrap Size: 25 1st Sigma: 255 2nd Sigma: 177 3rd Sigma: 98 ☒ Show Sorted Libs

Cutoff Size: 98 # Libs Inc: 20 # Libs Exc: 0 ☐ Show Lib Groups

Steps: 10 ☒ Use Min Lib Size Min Inc Lib Size: 180 Step Size: 18

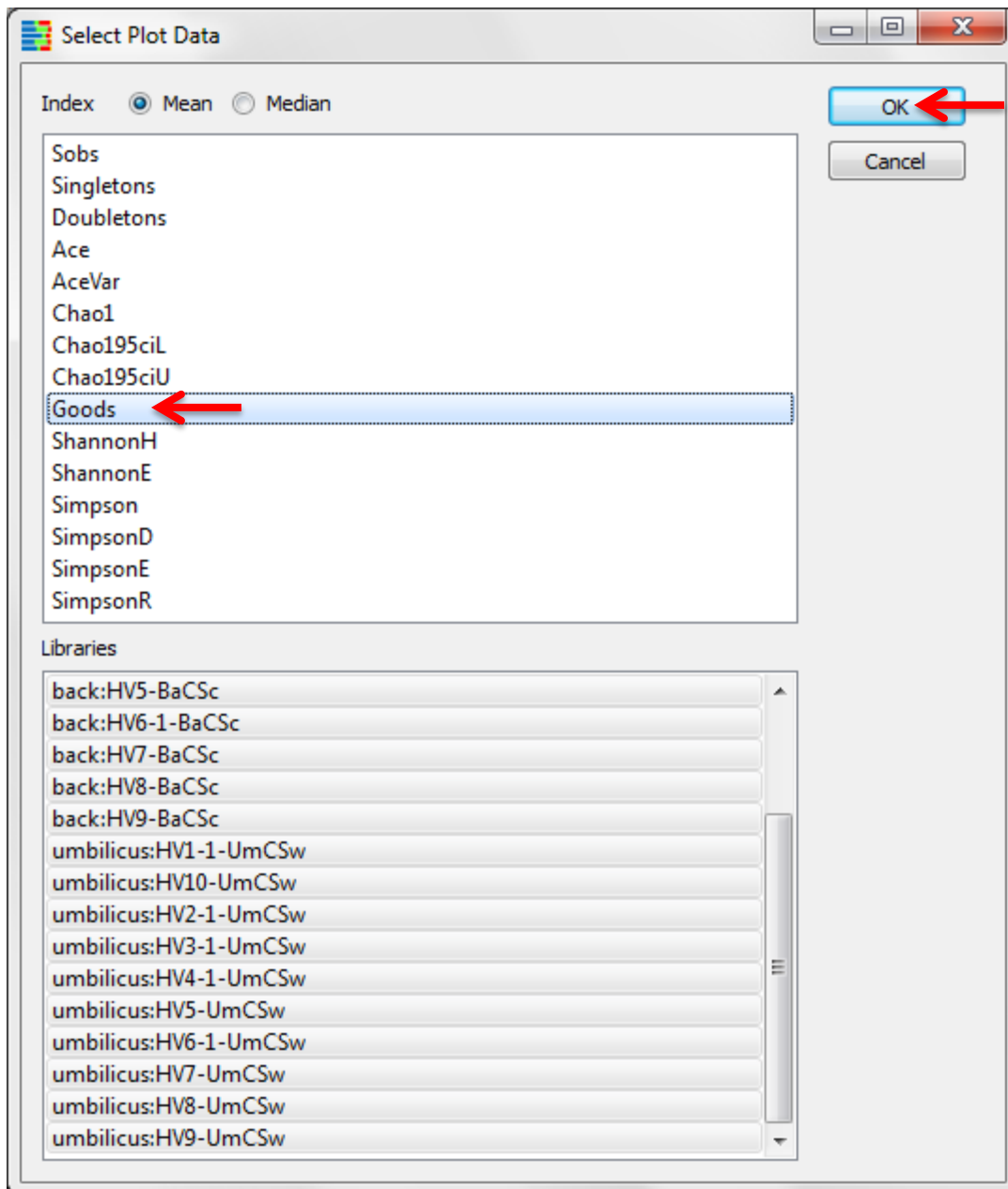
☐ Single statistic at Rarefaction point only

Figures: No figure selected ☒ Use Workspace Filter Workspace Filter: Back+Umbilicus
☐ Use Figure Filter Figure Filter:

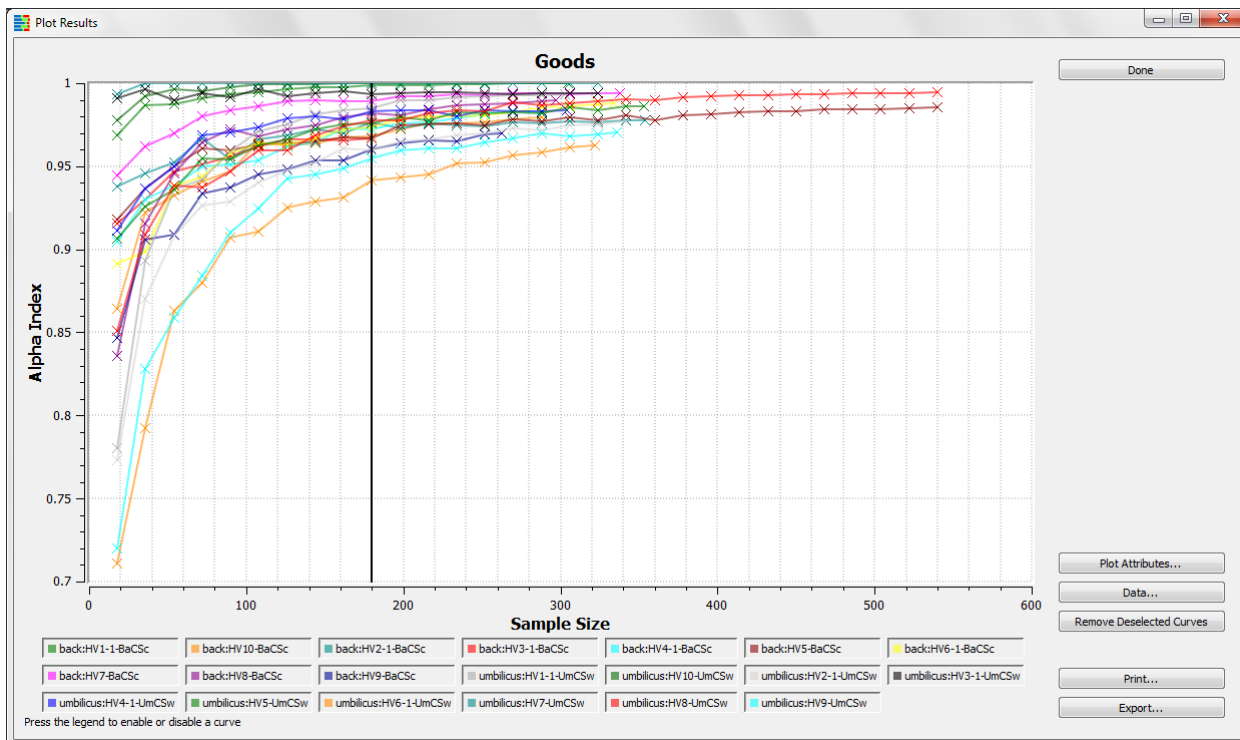
	Sobs Mean	Sobs Median	Sobs 2.5%	Sobs 97.5%	Singletons Mean	Singletons Median	Sing
back:HV1-1-BaCSc : 18	1.840	2.000	1.000	3.000	0.560	1.000	0.000
back:HV1-1-BaCSc : 36	1.840	2.000	1.000	3.000	0.480	0.000	0.000
back:HV1-1-BaCSc : 54	2.400	2.000	2.000	3.000	0.680	1.000	0.000
back:HV1-1-BaCSc : 72	2.600	3.000	1.000	3.000	0.640	1.000	0.000
back:HV1-1-BaCSc : 90	2.600	3.000	2.000	3.000	0.600	0.000	0.000
back:HV1-1-BaCSc : 108	2.720	3.000	1.000	3.000	0.560	1.000	0.000
back:HV1-1-BaCSc : 126	2.680	3.000	2.000	3.000	0.480	0.000	0.000
back:HV1-1-BaCSc : 144	2.760	3.000	2.000	3.000	0.320	0.000	0.000
back:HV1-1-BaCSc : 162	2.800	3.000	2.000	3.000	0.360	0.000	0.000
back:HV1-1-BaCSc : 180	2.920	3.000	2.000	3.000	0.200	0.000	0.000
back:HV1-1-BaCSc : 198	2.760	3.000	2.000	3.000	0.200	0.000	0.000

Done
 Bootstrap
 Plot...
 Export...
 Save As Figure...

A new pop-up window appears which lists the various alpha diversity tests
 Select **Goods**
 Click **OK**



A new pop-up window appears showing the Good's Coverage plot



Since the curves on the plot generally reach asymptotes, we conclude that both sites were sampled reasonably well to be considered representative of the anatomical positions.

You may choose to save your Good's Coverage plot as a figure. To do so, continue as shown earlier in the stacked bar chart example; close the graphics window, and select **Save As Figure** in the **Alpha Diversity** window.

XII. Two-Part Test

Now that we know our data are representative, we will continue with another statistical test. A Two-Part statistical test can identify taxa that differ between two groups. We will use the Two-Part test to compare sequence counts between the back and umbilicus. The Two-Part Test is a combined statistic that examines both the proportion of the samples that contain a given OTU and the median relative abundance of the OTU across two categories. Because microbiome data often are non-normally distributed, parametric tests such as the familiar t-test may not be appropriate. Consequently, we use a non-parametric Wilcoxon test to examine percent abundance data. For more information on the Two-Part Test, please see: Wagner BD, Robertson CE, Harris JK (2011) Application of Two-Part Statistics for Comparison of Sequence Variant Counts. *PLoS ONE* 6(5): e20296.

A. Run a Two-Part Test

Tools → **Analyze** → **Two-Part**

A new pop-up window appears

In order to compare the back data against the umbilicus data, we need to set up individual filters for each anatomical position. To do so, we will proceed as discussed earlier in “To create a filter...”.

Click **Setup Filters**

Two-Part in Workspace Back+Umbilicus: Back+Umbilicus vs Back+Umbilicus

Category 1 Filter: Back+Umbilicus 20 of 20 Libs OTU Start: 1 ☐ All Libraries ☒ Alphabetical by OTU Name

Category 2 Filter: Back+Umbilicus 20 of 20 Libs OTU Width: 2 ☒ Selected Libraries ☐ Descending by PValue

P Threshold: 0 ☒ OTU Show Last 20 of 30 Libs ☐ Ascending by PValue

Figures: No figure selected ☒ Use Workspace Filter ☐ Use Figure Filter Workspace Filter: Back+Umbilicus Figure Filter:

OTU	Collisions: Libraries that are in both Category 1 and Category 2
HV1-1-BaCSc	
HV10-BaCSc	
HV2-1-BaCSc	
HV3-1-BaCSc	
HV4-1-BaCSc	
HV5-BaCSc	
HV6-1-BaCSc	
HV7-BaCSc	
HV8-BaCSc	
HV9-BaCSc	
HV1-1-UmCSw	
HV10-UmCSw	
HV2-1-UmCSw	
HV3-1-UmCSw	
HV4-1-UmCSw	
HV5-UmCSw	
HV6-1-UmCSw	
HV7-UmCSw	
HV8-UmCSw	
HV9-UmCSw	

Done Calculate Setup Filters

Plot... Export... Save As Figure...

New pop-up window appears for creation of filters

Click **New** on far right side of window

Filters: Back+Umbilicus Next Filter Previous Filter

Done
Select

Metadata Selection Criteria

And/Or	Metadata	Operator	Value	Delete/Group/Move
	Anatomy	contains	back	<input type="checkbox"/>
or	Anatomy	contains	umbilicus	<input type="checkbox"/>

Clear All Add Delete Add Parentheses Remove Parentheses Move Up Move Down

Taxonomy Selection Criteria

To add a new selection criteria for Taxonomy, click Add

Clear All Add Delete Add Parentheses Remove Parentheses Move Up Move Down

20 Libs Selected of 20 Two Part Libs Selected 30 Total Libraries

Library	Total Member Count	OTU Matched Count
HV1-1-BaCSc	305	
HV10-BaCSc	289	
HV2-1-BaCSc	354	
HV3-1-BaCSc	180	
HV4-1-BaCSc	296	
HV5-BaCSc	541	
HV6-1-BaCSc	334	
HV7-BaCSc	338	
HV8-BaCSc	297	

New

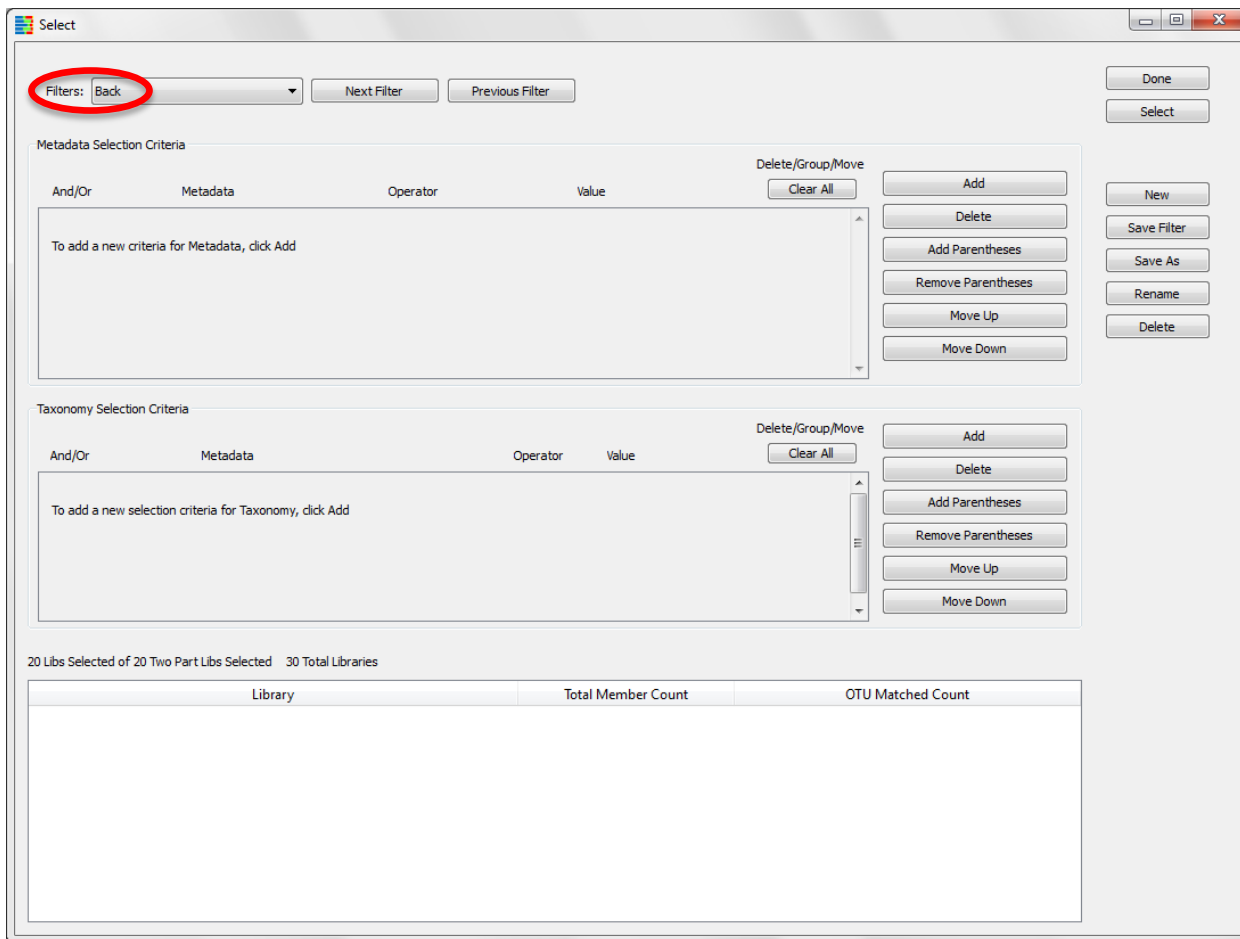
Enter desired filter name in the pop-up window
Click **OK**

New Filter

Filter name:
Back

OK Cancel

The filter name will appear in upper left corner of window



Now that we have created a new filter, we need to set up the parameters to filter by. We will select for all libraries which were sampled from the “back”.

B. Set Up Filter Parameters

Click **Add** in the **Metadata Criteria** pane

The screenshot shows a window titled "Select" with a toolbar at the top containing "Filters:", "Back", "Next Filter", "Previous Filter", "Done", and "Select".

Metadata Selection Criteria

And/Or	Metadata	Operator	Value	Delete/Group/Move
				Clear All
To add a new criteria for Metadata, click Add				

Buttons for Metadata criteria: Add (highlighted with a red arrow), Delete, Add Parentheses, Remove Parentheses, Move Up, Move Down.

Taxonomy Selection Criteria

And/Or	Metadata	Operator	Value	Delete/Group/Move
				Clear All
To add a new selection criteria for Taxonomy, click Add				

Buttons for Taxonomy criteria: Add, Delete, Add Parentheses, Remove Parentheses, Move Up, Move Down.

Summary: 20 Libs Selected of 20 Two Part Libs Selected 30 Total Libraries

Library	Total Member Count	OTU Matched Count

Use the first pull-down menu to select "Anatomy" (**Metadata** to filter by)
 Use the second pull-down menu to select "contains" (filter **Operator**)

Enter “back” into Value

The screenshot shows a window titled "Select" with a toolbar at the top containing "Filters: Back", "Next Filter", and "Previous Filter". Below this are two main sections: "Metadata Selection Criteria" and "Taxonomy Selection Criteria".

Metadata Selection Criteria: This section has columns for "And/Or", "Metadata", "Operator", and "Value". The "Metadata" dropdown is set to "Library", the "Operator" dropdown is set to "equals", and the "Value" text box is empty. Three red arrows point to these three fields respectively. To the right of these fields are buttons for "Delete/Group/Move" (with a "Clear All" sub-button), "Add", "Delete", "Add Parentheses", "Remove Parentheses", "Move Up", and "Move Down".

Taxonomy Selection Criteria: This section has similar columns for "And/Or", "Metadata", "Operator", and "Value". It currently contains the text "To add a new selection criteria for Taxonomy, click Add". It also has "Delete/Group/Move" buttons and the same set of action buttons as the Metadata section.

At the bottom, a status bar shows "20 Libs Selected of 20 Two Part Libs Selected 30 Total Libraries". Below this is a table with three columns: "Library", "Total Member Count", and "OTU Matched Count". The table body is currently empty.

To apply filter, click **Select** in upper right corner of window

Click **Save Filter** on far right side of window to keep the filter

The screenshot shows a window titled "Select" with a toolbar at the top containing "Filters: Back", "Next Filter", and "Previous Filter". Below this are two sections: "Metadata Selection Criteria" and "Taxonomy Selection Criteria".

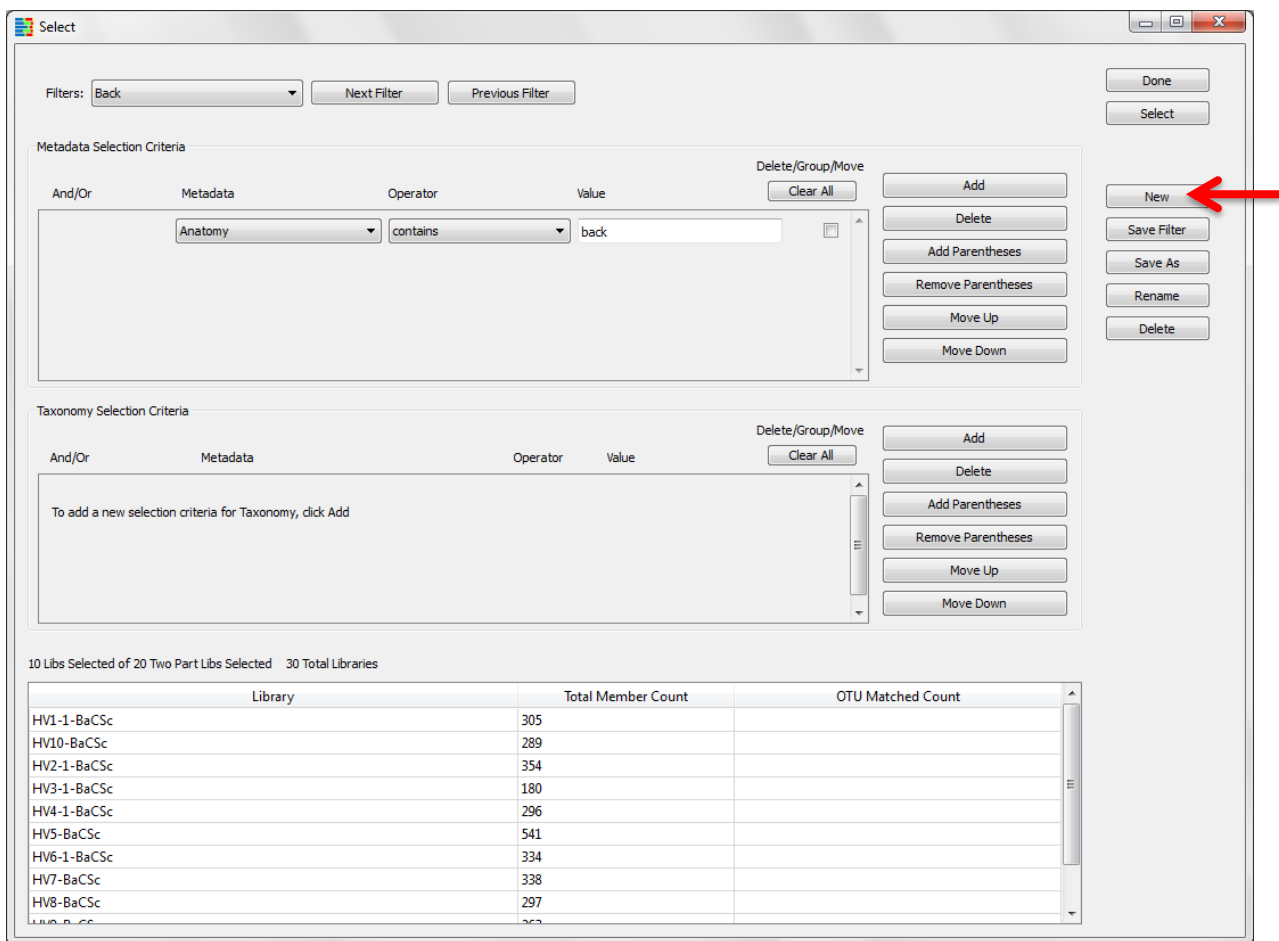
Metadata Selection Criteria: This section has a table with columns "And/Or", "Metadata", "Operator", and "Value". It contains one row with "Anatomy" in the Metadata column, "contains" in the Operator column, and "back" in the Value column. To the right of the table are buttons for "Delete/Group/Move" (with a "Clear All" sub-button), "Add", "Delete", "Add Parentheses", "Remove Parentheses", "Move Up", and "Move Down".

Taxonomy Selection Criteria: This section has a similar table structure but is currently empty, with a message "To add a new selection criteria for Taxonomy, click Add". It also has "Delete/Group/Move" and "Add", "Delete", "Add Parentheses", "Remove Parentheses", "Move Up", and "Move Down" buttons.

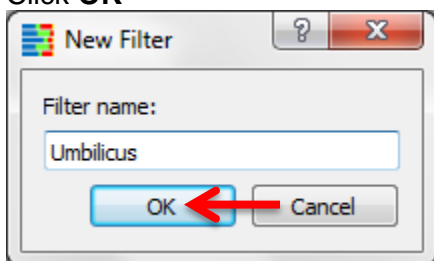
At the bottom, a status bar shows "20 Libs Selected of 20 Two Part Libs Selected 30 Total Libraries". Below this is a table with three columns: "Library", "Total Member Count", and "OTU Matched Count".

On the far right side of the window, there is a vertical stack of buttons: "Done", "Select", "New", "Save Filter", "Save As", "Rename", and "Delete". Two red arrows point to the "Select" and "Save Filter" buttons.

Now we will create a separate filter for the umbilicus
Click **New** on far right side of window



Enter desired filter name in the pop-up window
Click **OK**



The filter name will appear in upper left corner of window.

Click **Add** in the **Metadata Criteria** pane

The screenshot shows a window titled "Select" with a toolbar at the top containing "Filters:", a dropdown menu showing "Umbilicus", and buttons for "Next Filter" and "Previous Filter". On the right side of the toolbar are "Done" and "Select" buttons.

The main area is divided into two sections: "Metadata Selection Criteria" and "Taxonomy Selection Criteria". Each section has a table with columns: "And/Or", "Metadata", "Operator", "Value", and "Delete/Group/Move".

In the "Metadata Selection Criteria" section, the table is empty except for a message: "To add a new criteria for Metadata, click Add". The "Delete/Group/Move" column contains buttons: "Clear All", "Add", "Delete", "Add Parentheses", "Remove Parentheses", "Move Up", and "Move Down". A red arrow points to the "Add" button.

The "Taxonomy Selection Criteria" section has a similar structure with a message: "To add a new selection criteria for Taxonomy, click Add".

At the bottom, there is a status bar showing "10 Libs Selected of 20 Two Part Libs Selected 30 Total Libraries". Below this is a table with three columns: "Library", "Total Member Count", and "OTU Matched Count". The table is currently empty.

Use the first pull-down menu to select "Anatomy" (**Metadata** to filter by)
Use the second pull-down menu to select "contains" (filter **Operator**)

Enter “umbilicus” into **Value**

The screenshot shows a window titled "Select" with a filter criteria interface. At the top, a "Filters:" dropdown is set to "Umbilicus", with "Next Filter" and "Previous Filter" buttons. Below this is the "Metadata Selection Criteria" section, which has a table with columns "And/Or", "Metadata", "Operator", and "Value". The "Metadata" column contains "Library", the "Operator" column contains "equals", and the "Value" column is empty. Three red arrows point to these three columns. To the right of the table are buttons for "Delete/Group/Move" (Clear All), "Add", "Delete", "Add Parentheses", "Remove Parentheses", "Move Up", and "Move Down". Below the Metadata section is the "Taxonomy Selection Criteria" section, which is currently empty and contains the text "To add a new selection criteria for Taxonomy, click Add". To the right of this section are similar buttons for "Add", "Delete", "Add Parentheses", "Remove Parentheses", "Move Up", and "Move Down". At the bottom of the window, a status bar shows "10 Libs Selected of 20 Two Part Libs Selected 30 Total Libraries". Below the status bar is a table with three columns: "Library", "Total Member Count", and "OTU Matched Count". The table is currently empty.

Filters: Umbilicus Next Filter Previous Filter

Metadata Selection Criteria

And/Or	Metadata	Operator	Value
	Library	equals	

Delete/Group/Move: Clear All, Add, Delete, Add Parentheses, Remove Parentheses, Move Up, Move Down

Taxonomy Selection Criteria

To add a new selection criteria for Taxonomy, click Add

10 Libs Selected of 20 Two Part Libs Selected 30 Total Libraries

Library	Total Member Count	OTU Matched Count
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To apply filter, click **Select** in upper right corner of window

Click **Save Filter** on far right side of window to keep the filter

The screenshot shows a software window titled "Select". At the top, there is a "Filters:" dropdown menu currently set to "Umbilicus", with "Next Filter" and "Previous Filter" buttons. Below this is the "Metadata Selection Criteria" section, which contains a table with columns "And/Or", "Metadata", "Operator", and "Value". The table has one row with "Anatomy" in the Metadata column, "contains" in the Operator column, and "umbilicus" in the Value column. To the right of the table are buttons for "Delete/Group/Move" (with a "Clear All" sub-button), "Add", "Delete", "Add Parentheses", "Remove Parentheses", "Move Up", and "Move Down". Below the metadata section is the "Taxonomy Selection Criteria" section, which has a similar layout but is currently empty, with a message "To add a new selection criteria for Taxonomy, click Add". At the bottom of the window, a status bar indicates "10 Libs Selected of 20 Two Part Libs Selected 30 Total Libraries". Below the status bar is a table with three columns: "Library", "Total Member Count", and "OTU Matched Count". On the far right side of the window, there is a vertical column of buttons: "Done", "Select", "New", "Save Filter", "Save As", "Rename", and "Delete". Two red arrows point to the "Select" and "Save Filter" buttons.

And/Or	Metadata	Operator	Value
	Anatomy	contains	umbilicus

Library	Total Member Count	OTU Matched Count
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Click **Done** to return to the Two-Part test setup window

Filters: Umbilicus Next Filter Previous Filter

Metadata Selection Criteria

And/Or	Metadata	Operator	Value	Delete/Group/Move
	Anatomy	contains	umbilicus	<input type="checkbox"/>

Taxonomy Selection Criteria

And/Or	Metadata	Operator	Value	Delete/Group/Move
To add a new selection criteria for Taxonomy, click Add				

10 Libs Selected of 20 Two Part Libs Selected 30 Total Libraries

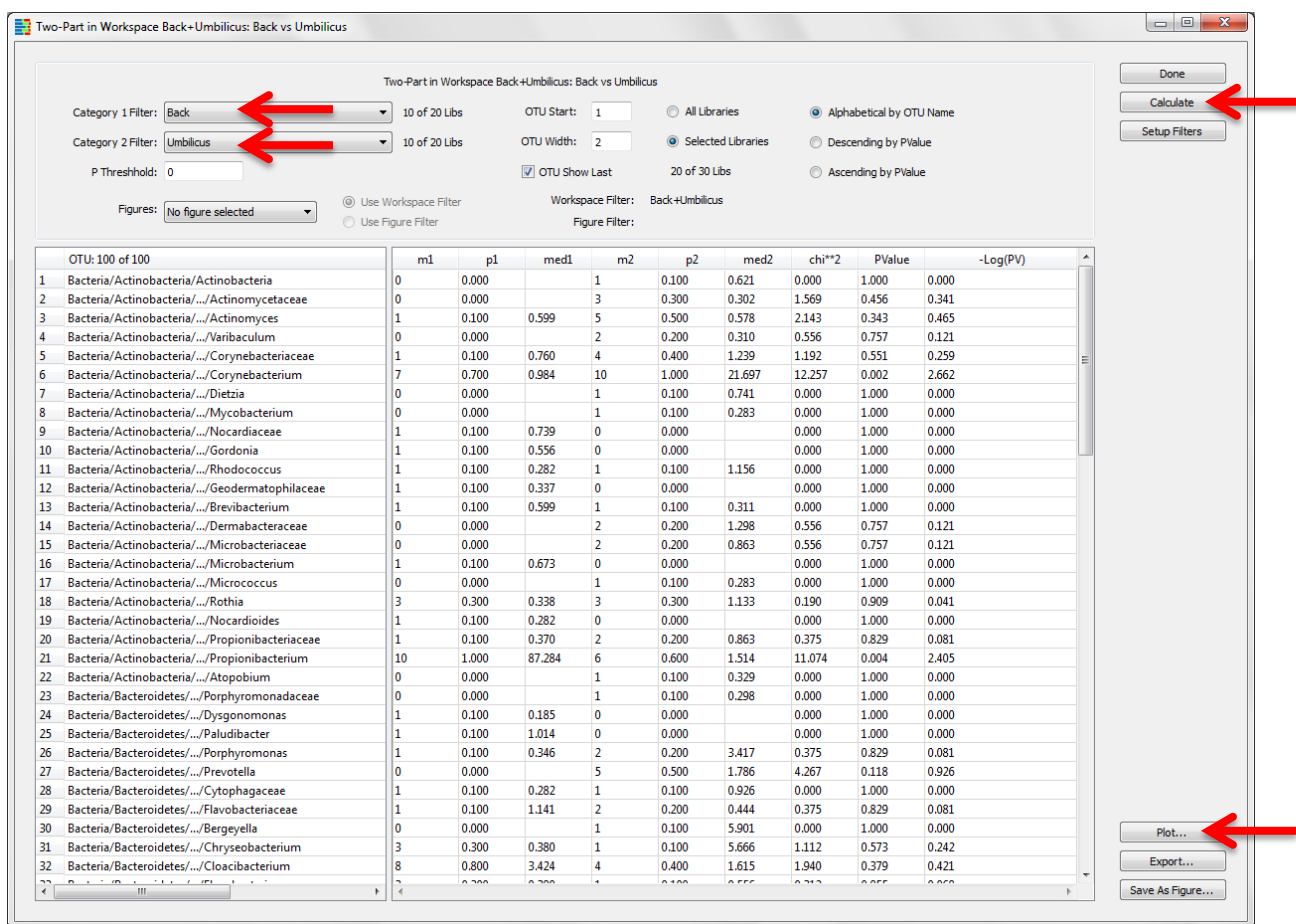
Library	Total Member Count	OTU Matched Count
HV1-1-UmCSw	303	
HV10-UmCSw	313	
HV2-1-UmCSw	346	
HV3-1-UmCSw	331	
HV4-1-UmCSw	304	
HV5-UmCSw	353	
HV6-1-UmCSw	322	
HV7-UmCSw	325	
HV8-UmCSw	540	

Select “Back” for the **Category 1 Filter**

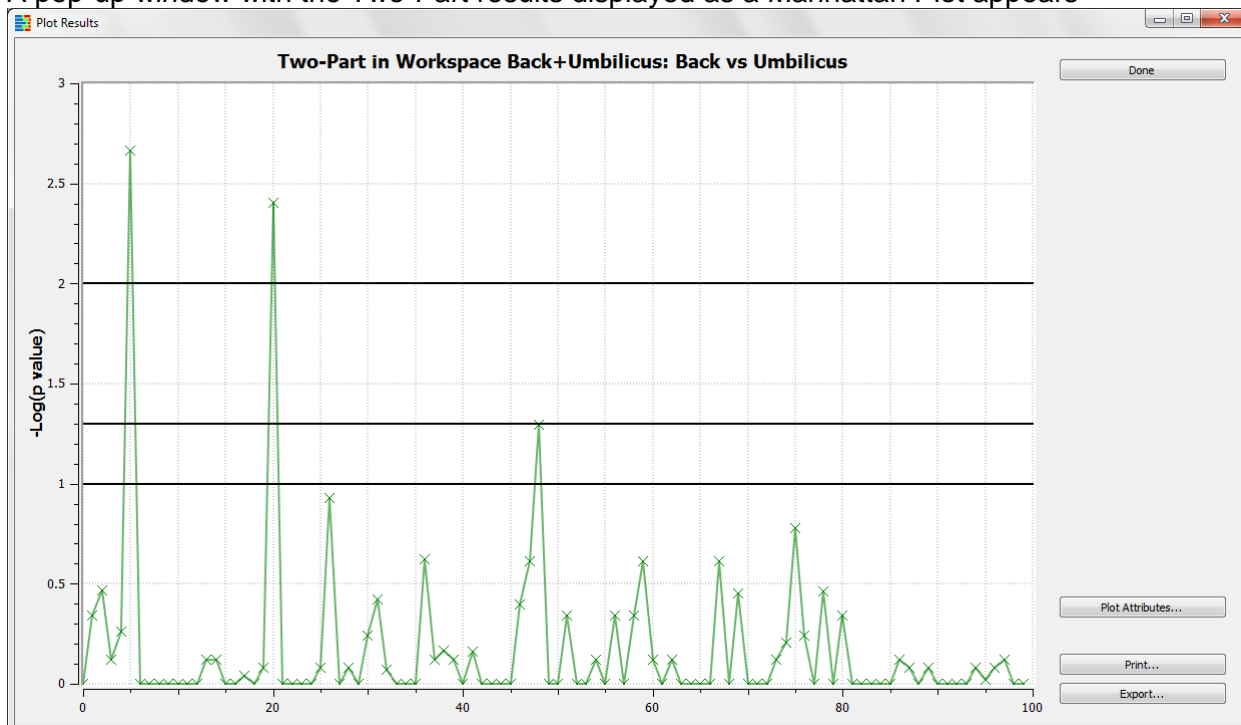
Select “Umbilicus” for the **Category 2 Filter**

Click **Calculate**

Click **Plot**



A pop-up window with the Two-Part results displayed as a Manhattan Plot appears



The Manhattan Plot displays logarithmically transformed p-values, with higher peaks representing lower (more significant) p-values. The horizontal lines represent p-values of 0.10, 0.05, and 0.01. Inclusion of the p=0.10

line is intended to highlight taxa that are approaching significance in an analysis. The x-axis represents the alphabetical position, by number, of each OTU name in the Two-Part setup dialog above.

In the Manhattan Plot, the first significant peak (position 6) corresponds to *Corynebacterium*, which have a higher proportion and relative abundance in the umbilicus samples. The second peak (position 21) represents *Propionibacterium* that is present at a higher proportion and relative abundance in the back samples. The third peak that approaches significance (position 49) represents *Anaerococcus*. This taxon is not seen in many of the libraries generated from back samples, and thus is present at higher proportion and relative abundance in the umbilicus samples.

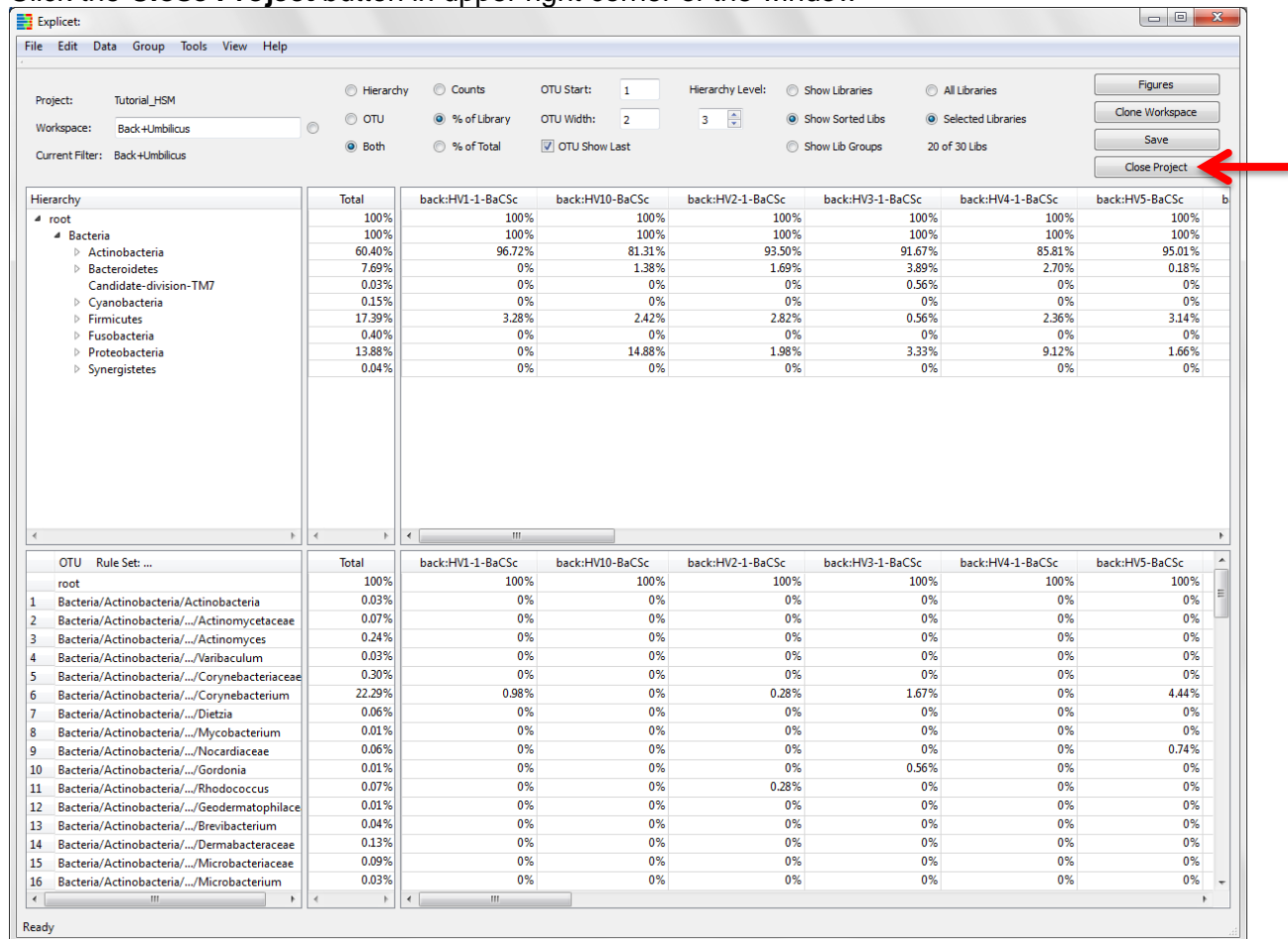
Data can be exported from the **Plot Results** window as tab delimited text using the export button (available in all graphics windows). The data incorporated for each taxon in the Two-Part statistic are summarized for each category. The number of samples with sequences belonging to an OTU within each category is designated “m”, proportion of positive libraries in a category “p”, and median relative abundance “med”.

You may choose to save the Two-Part test as a figure. To do so, continue as shown earlier in the stacked bar chart example; close the graphics window, and select **Save As Figure** in the **Two-Part** window.

This tutorial has provided a quick overview of how to use Explicit. For more complete information on Explicit capabilities, please see the Explicit Handbook. We will now save our changes and close the project.

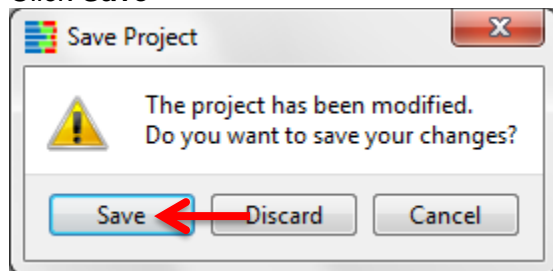
XIII. Close the Project

Click the **Close Project** button in upper right corner of the window



A pop-up window will open

Click **Save**



The Explicitet window will close, and all of the OTU data, metadata, and figures are now saved within the project file.

Thus ends a basic overview of some functions contained in Explicitet. Please do not hesitate to ask questions or make suggestions via our online Explicitet forum. The Explicitet forum link can be found on our web site: www.explicitet.org.