

Explicitet Basic Tutorial

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

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

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I. Begin a New Project

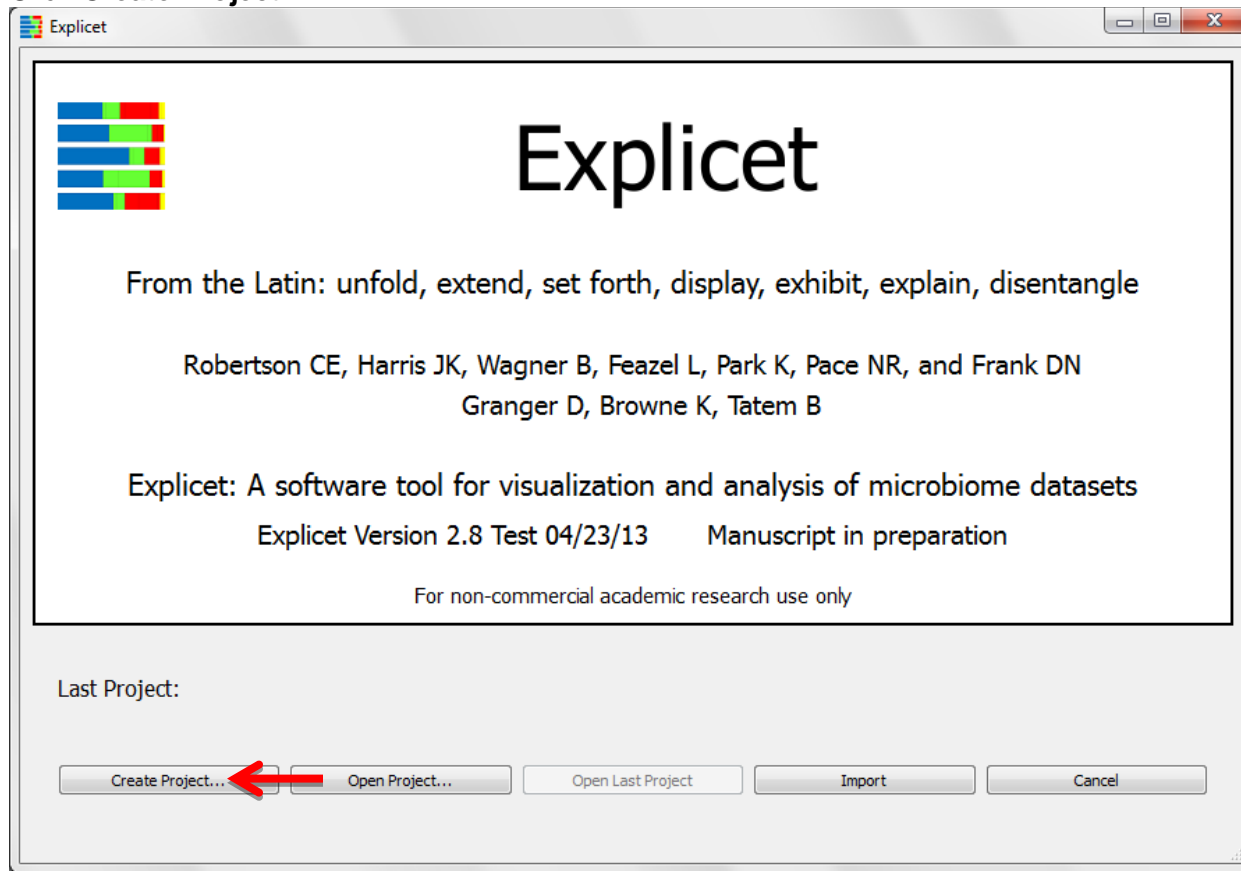
Projects typically contain data from one dataset. Later, we will discuss more data management tools that allow you to explore and modify subsets of the dataset without disrupting the larger project.

A. Create a New Project

Open Explicit

A pop-up window will open with several different options

Click **Create Project**



We now have a blank project in Explicit.

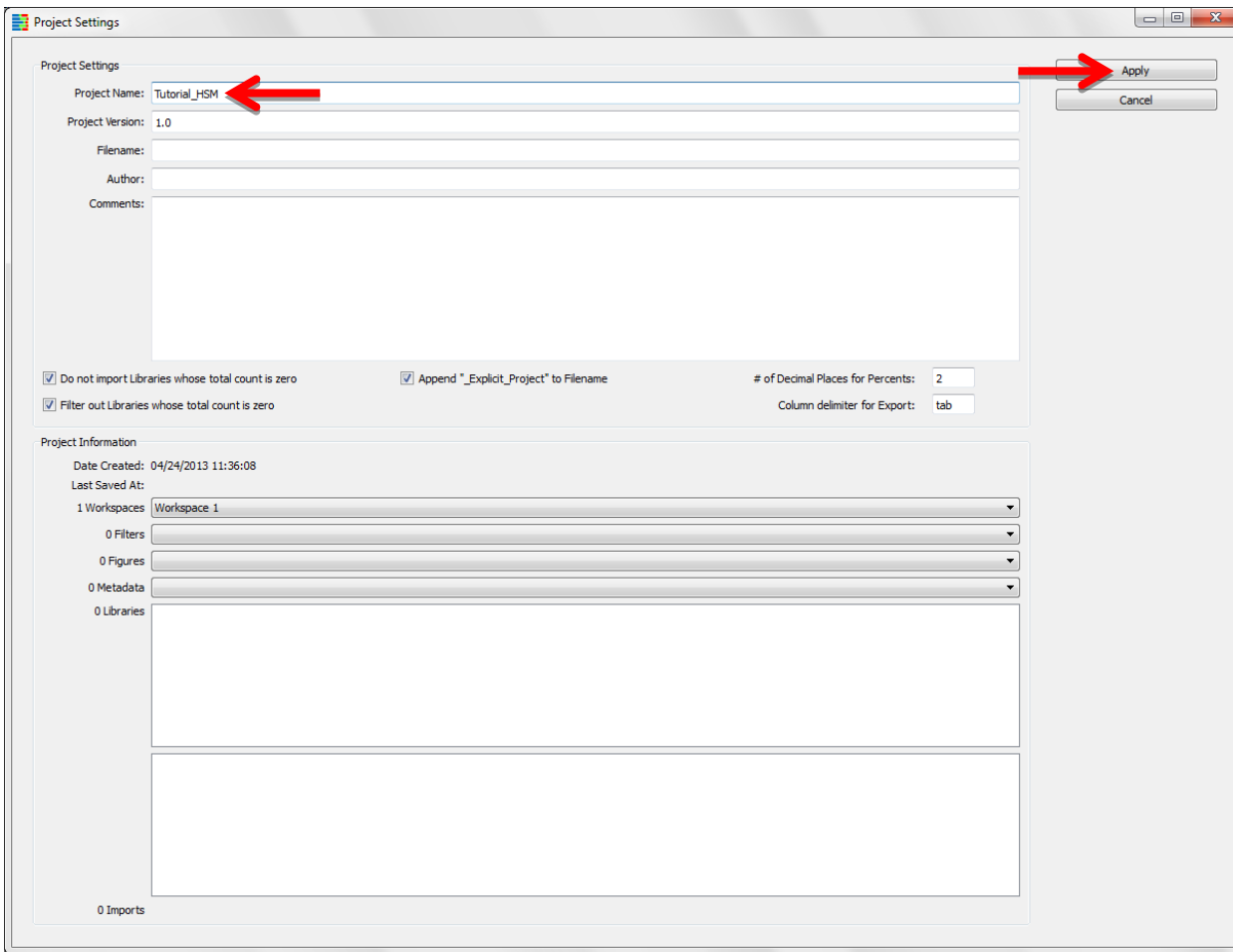
B. Name the Project

Edit → **Project Settings**

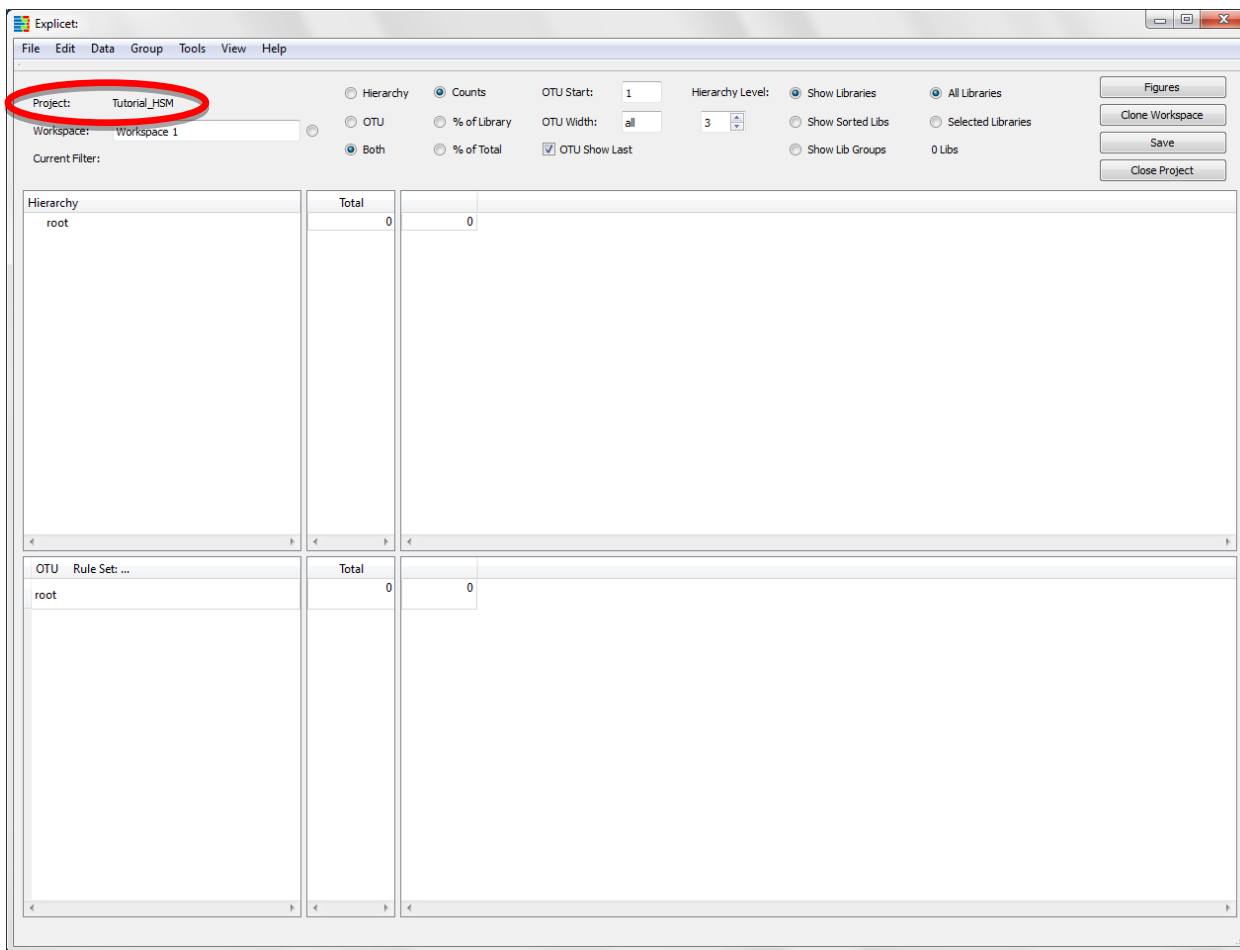
A pop-up window will open

Enter desired project name in the **Project Name** box at the top of the window

Click **Apply**



The name of the current project is displayed in the upper left corner of the main window.



Note: The **Project Settings** window is helpful for both adding comments about the project and viewing a simplified inventory of all the project components.

II. Import Data

Next, we need to 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 imported to the same project. Thus, the Explicit project file can grow as a project evolves.

A. Import the OTU Data

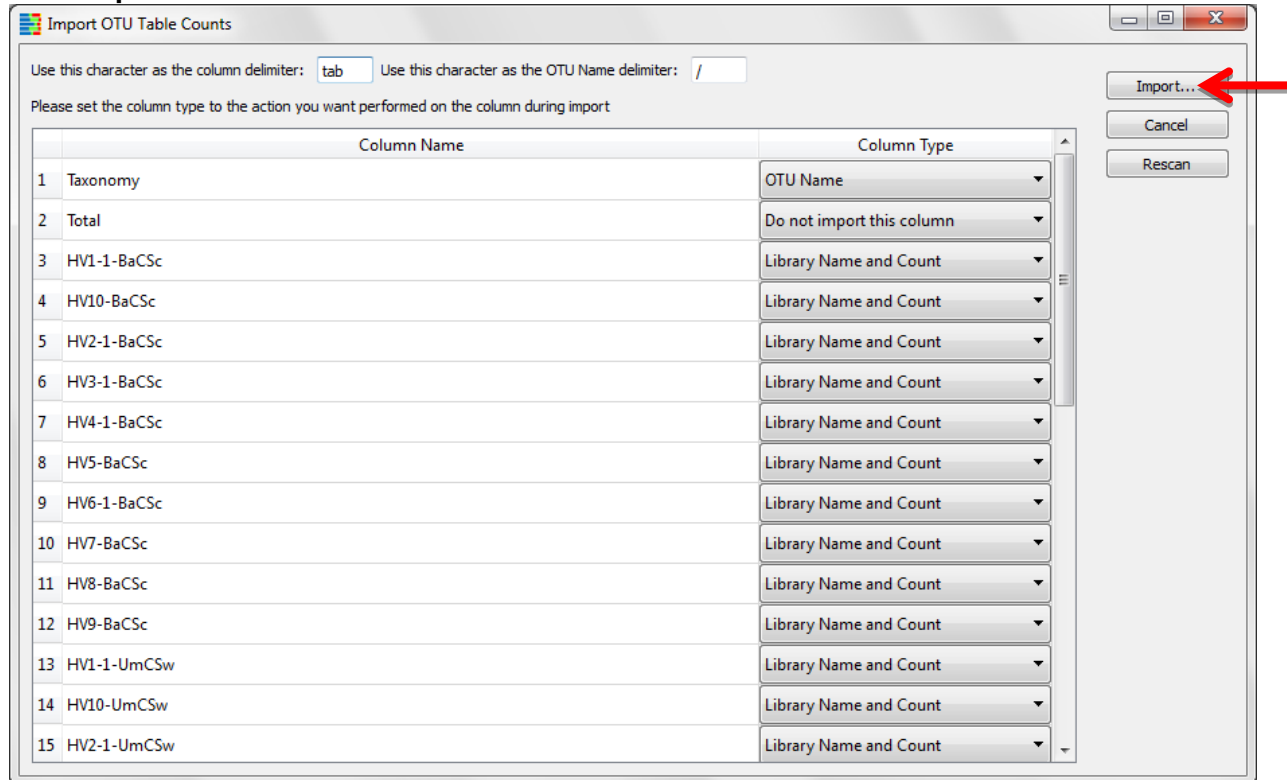
File → **Import** → **File** → **OTU Table Counts**

Select "Tutorial_HSM_OTU_2_Explicit"

Click **Open**

A pop-up window will open

Click **Import**



The OTU data now appear in the main Explicit window.

Explicit: Tutorial_HSM

Workspace: Workspace 1

Current Filter: Both

OTU Start: 1, Hierarchy Level: Show Libraries, All Libraries

OTU Width: all, 3

Show Sorted Libs, Selected Libraries, Show Lib Groups, 30 Libs

Buttons: Figures, Clone Workspace, Save, Close Project

Hierarchy	Total	HV1-1-BaCSc	HV1-1-UmCSw	HV10-BaCSc	HV10-UmCSw	HV2-1-AcRSc	HV2-1-AcRSw	HV2-1-AIRSc	HV2-1-B
root	9710	305	303	289	313	304	303	304	
Bacteria	9710	305	303	289	313	304	303	304	
Acidobacteria	2	0	0	0	0	0	0	0	
Actinobacteria	5001	295	24	235	303	32	37	255	
Bacteroidetes	1056	0	47	4	0	110	86	4	
Candidate-division-TM7	2	0	0	0	0	0	0	0	
Chloroflexi	3	0	0	0	0	0	0	0	
Cyanobacteria	29	0	0	0	0	4	0	1	
Firmicutes	1484	10	209	7	10	7	48	28	
Fusobacteria	34	0	14	0	0	0	0	0	
Gemmatimonadetes	3	0	0	0	0	0	0	0	
Nitrospirae	5	0	0	0	0	0	0	0	
Planctomycetes	7	0	0	0	0	0	0	0	
Proteobacteria	2079	0	6	43	0	151	132	16	
Synergistetes	3	0	3	0	0	0	0	0	
Verrucomicrobia	2	0	0	0	0	0	0	0	

OTU	Rule Set: ...	Total	HV1-1-BaCSc	HV1-1-UmCSw	HV10-BaCSc	HV10-UmCSw	HV2-1-AcRSc	HV2-1-AcRSw	HV2-1-AIRSc	HV2-1-B
root		9710	305	303	289	313	304	303	304	
1	Bacteria/Actinobacteria/Actinobacteria/Candida	2	0	0	0	0	0	0	0	
2	Bacteria/Actinobacteria/Actinobacteria/Actinomyces	1	0	0	0	0	0	0	0	
3	Bacteria/Actinobacteria/Actinobacteria/Actinomyces	3	0	0	0	0	0	0	0	
4	Bacteria/Actinobacteria/Actinobacteria	3	0	0	0	0	0	0	1	
5	Bacteria/Actinobacteria/Actinobacteria/Actinomyces	5	0	0	0	0	0	0	0	
6	Bacteria/Actinobacteria/Actinobacteria/Actinomyces	27	0	4	0	0	0	1	1	
7	Bacteria/Actinobacteria/Actinobacteria/Actinomyces	2	0	0	0	0	0	0	0	
8	Bacteria/Actinobacteria/Actinobacteria/Actinomyces	2	0	1	0	0	0	0	0	
9	Bacteria/Actinobacteria/Actinobacteria/Corynebacterium	47	0	3	0	0	0	0	0	
10	Bacteria/Actinobacteria/Actinobacteria/Corynebacterium	1772	3	16	0	303	8	9	26	
11	Bacteria/Actinobacteria/Actinobacteria/Corynebacterium	4	0	0	0	0	0	0	0	
12	Bacteria/Actinobacteria/Actinobacteria/Corynebacterium	1	0	0	0	0	0	0	0	
13	Bacteria/Actinobacteria/Actinobacteria/Corynebacterium	4	0	0	0	0	0	0	0	
14	Bacteria/Actinobacteria/Actinobacteria/Corynebacterium	2	0	0	0	0	0	0	0	
15	Bacteria/Actinobacteria/Actinobacteria/Corynebacterium	11	0	0	0	0	0	0	2	
16	Bacteria/Actinobacteria/Actinobacteria/Frankia	1	0	0	0	0	0	0	0	

Explicit can import taxonomy data in numerous formats as detailed in the Explicit Handbook.

This data comes 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.

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”

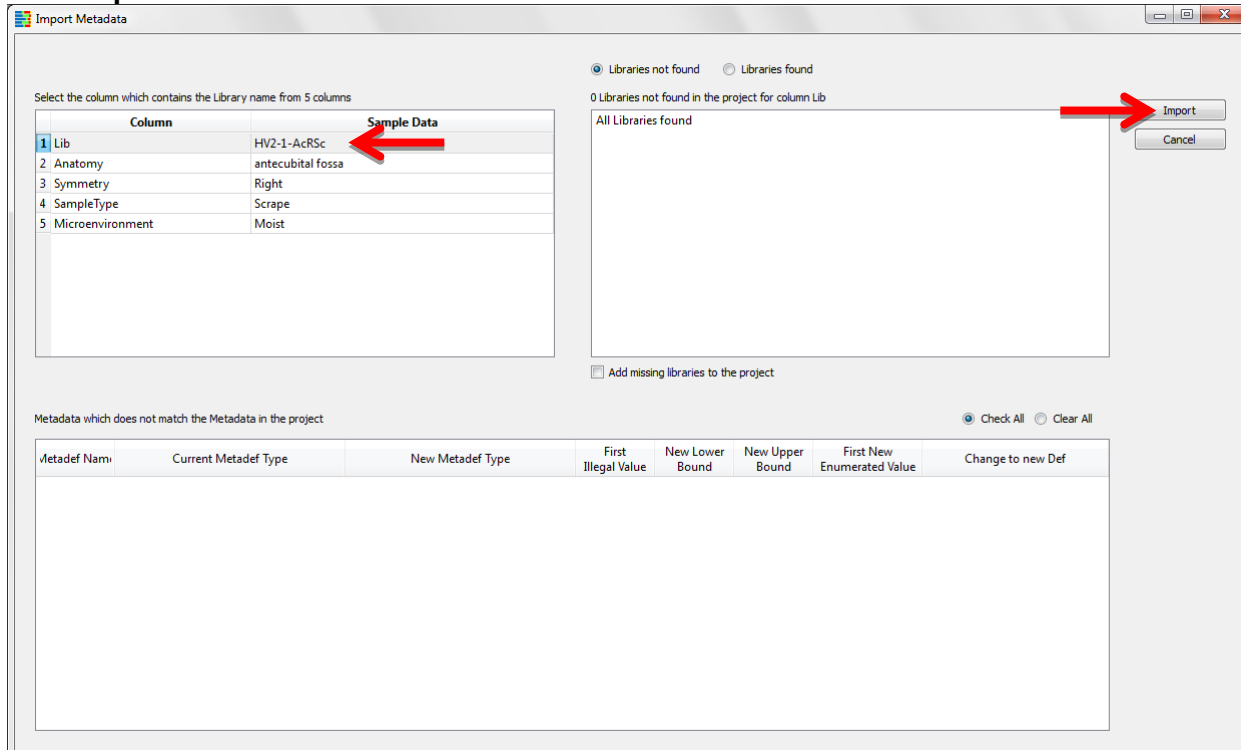
Click **Open**

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**



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

Enumerated Values: Optional

Name:

Type: String that may be more than 8 characters

Upper Bound: Optional

Lower Bound: Optional

Status:

Values

<New>

Add Value Delete Value Clear Values

Add Replace Delete Clear

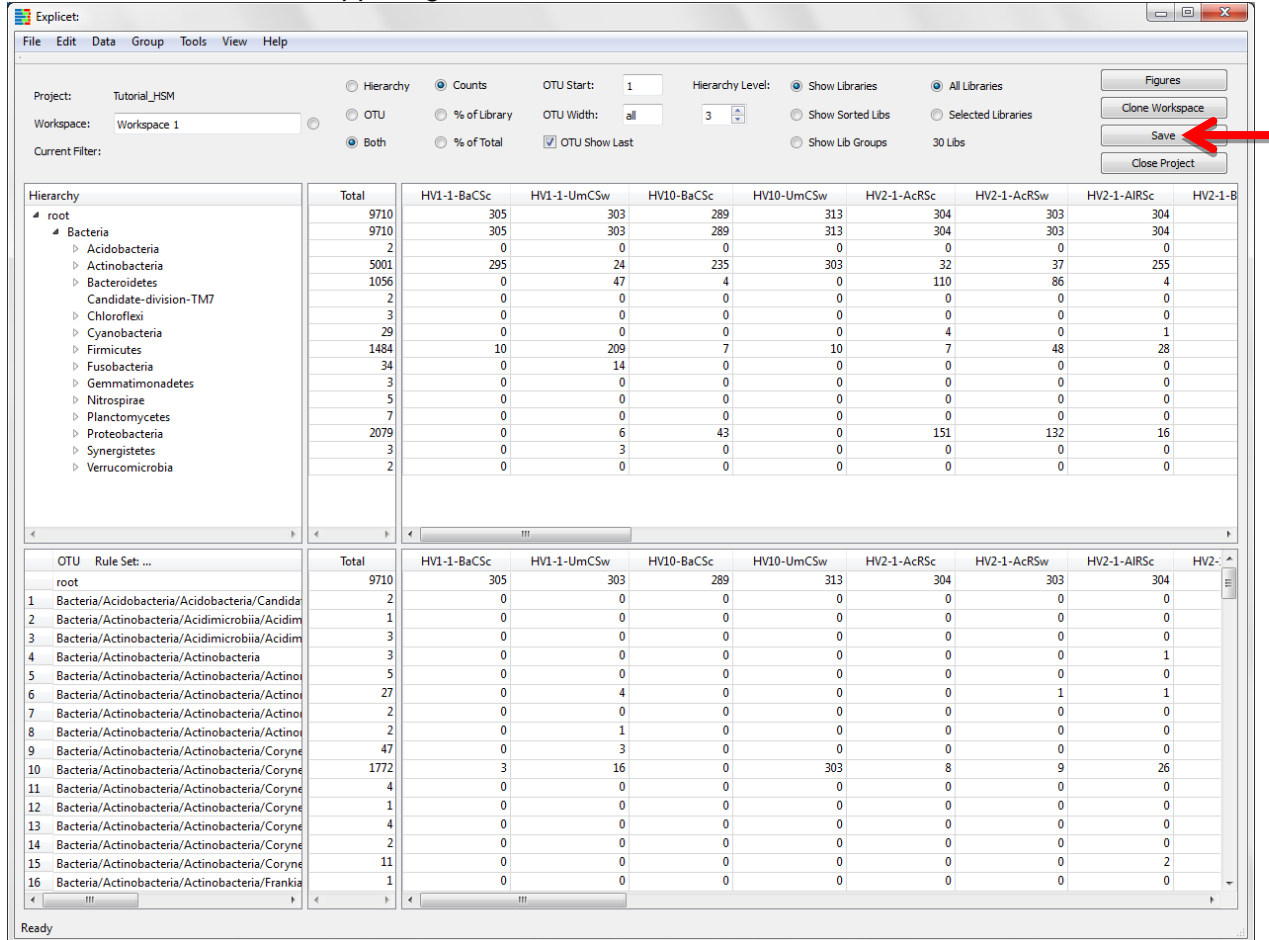
For our example dataset, all of the library names were found in the metadata file, as indicated in the left-hand pane: i.e., the number under **Used (30)** matches the total number of libraries shown above the two panes (**30 Total Libraries**).

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



The screenshot shows the Explicitet software window. The 'Project' field is set to 'Tutorial_HSM' and the 'Workspace' is 'Workspace 1'. The 'Counts' radio button is selected under the 'Hierarchy' section. The 'Save' button in the top right corner is highlighted with a red arrow. Below the menu bar, there are several configuration options including 'OTU Start', 'Hierarchy Level', and 'OTU Width'. The main area displays a taxonomic hierarchy on the left and a data table with columns for 'Total' and various OTU counts (HV1-1-BaCSc, HV1-1-UmCSw, HV10-BaCSc, HV10-UmCSw, HV2-1-AcRSc, HV2-1-AcRSw, HV2-1-AIRSc, HV2-1-B).

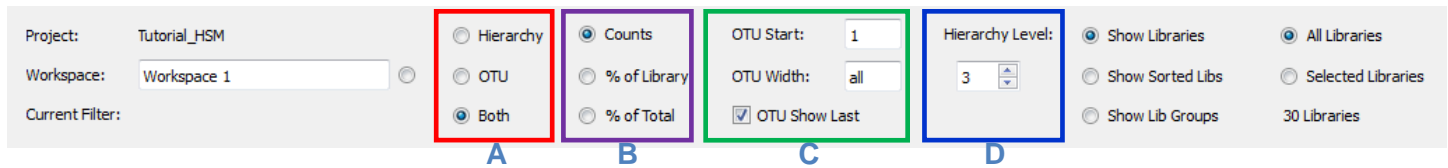
Enter desired project name and location when prompted (the default file name is the project name with an “_Explicit_Project” extension)

Click **Save**

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

V. Adjust the Display

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



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.

B. Counts, % of Library, % of Total

Counts is the default

This option shows the raw sequence data counts (integers).

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 “1”st 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.

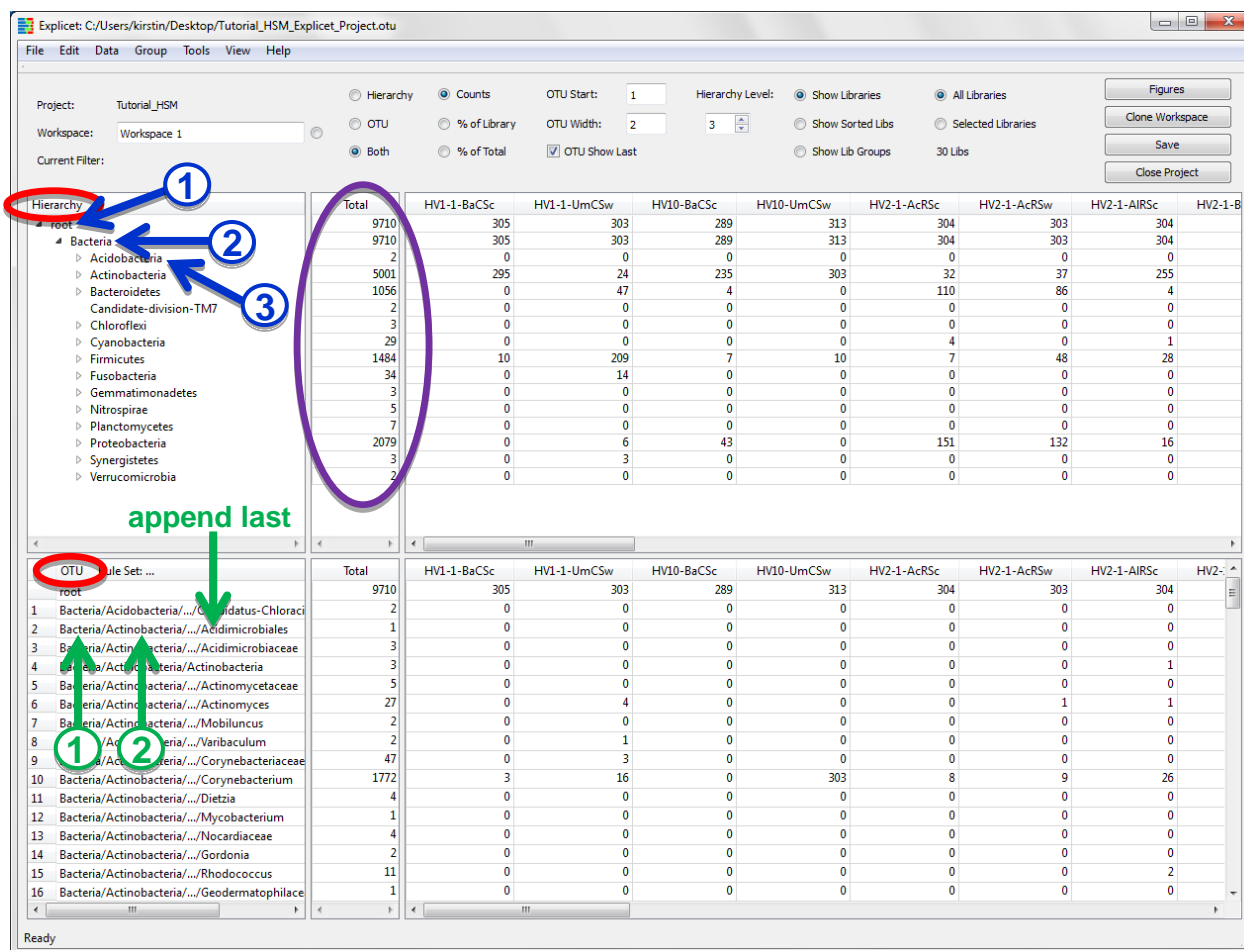
OTU Show Last *on* is the default

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.



Since libraries are often cryptically named, it's nice to add a readable metadata tag in the view so that we have some context for the libraries we are viewing. To do this, we will sort the libraries in the view based on anatomical position.

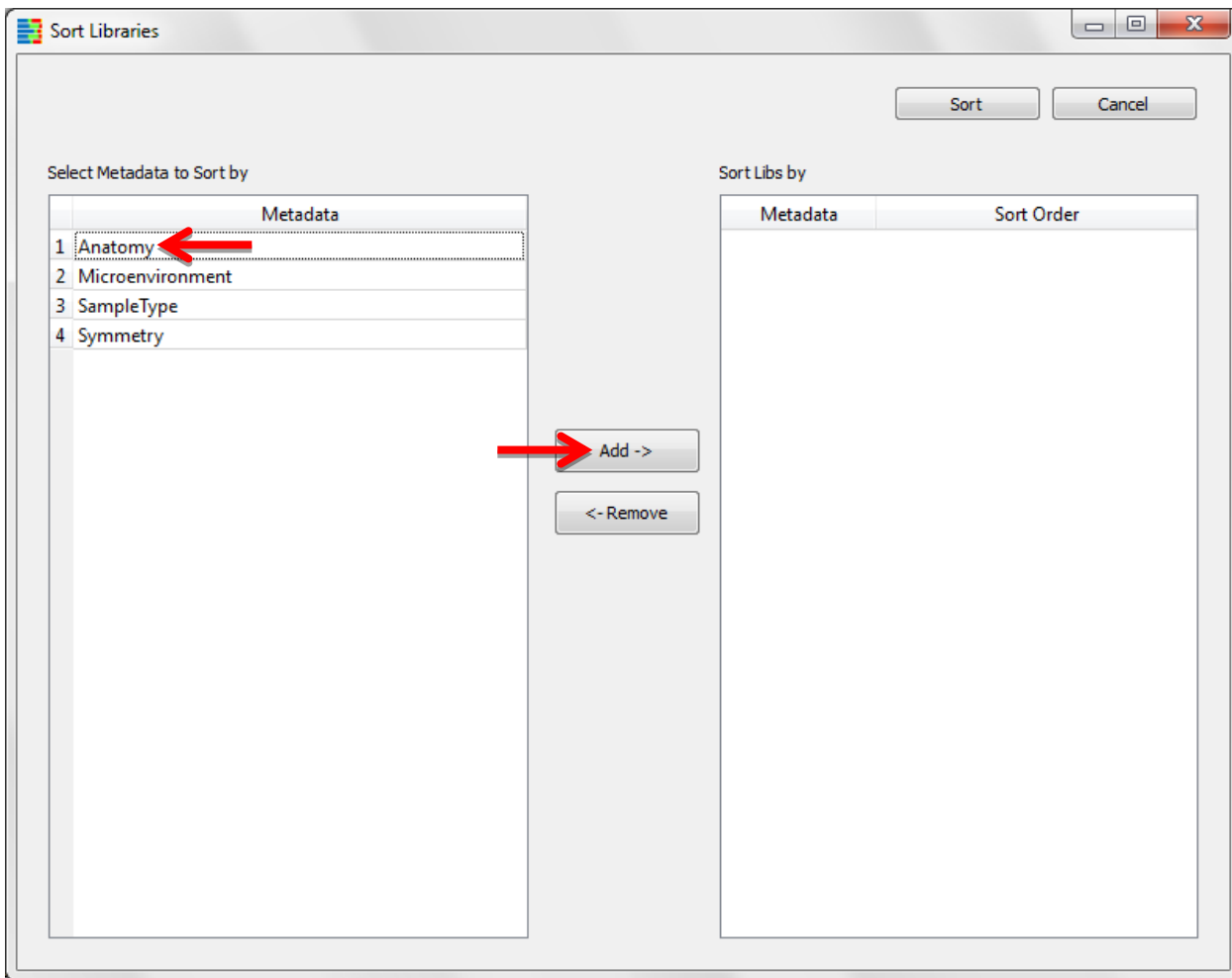
E. Sort Libraries Based on a Metadata Tag (Anatomical Position)

View → Sort Libraries

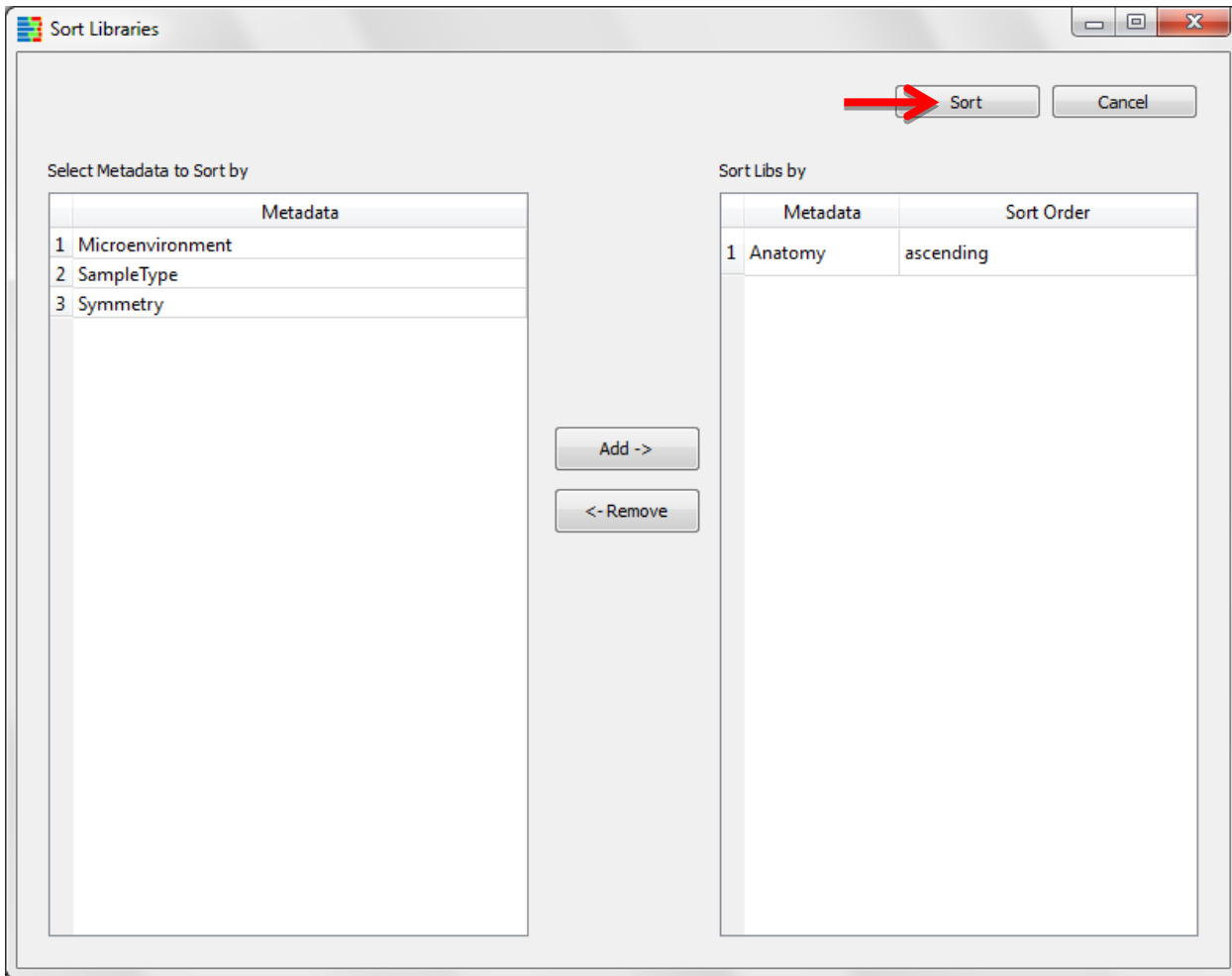
A pop-up window will open

In left panel, select **Anatomy**

Click **Add** button between panels



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



Pop-up window will disappear
On the main project window, select **Show Sorted Libs**

Explicit: C:/Users/kirstin/Desktop/Tutorial_HSM_Explicit_Project.otu

File Edit Data Group Tools View Help

Project: Tutorial_HSM
 Workspace: Workspace 1
 Current Filter: Both

Hierarchy Level: Show Libraries All Libraries
 Show Sorted Libs Selected Libraries
 Show Lib Groups 30 Libs

OTU Start: 1 OTU Width: 2

OTU Show Last

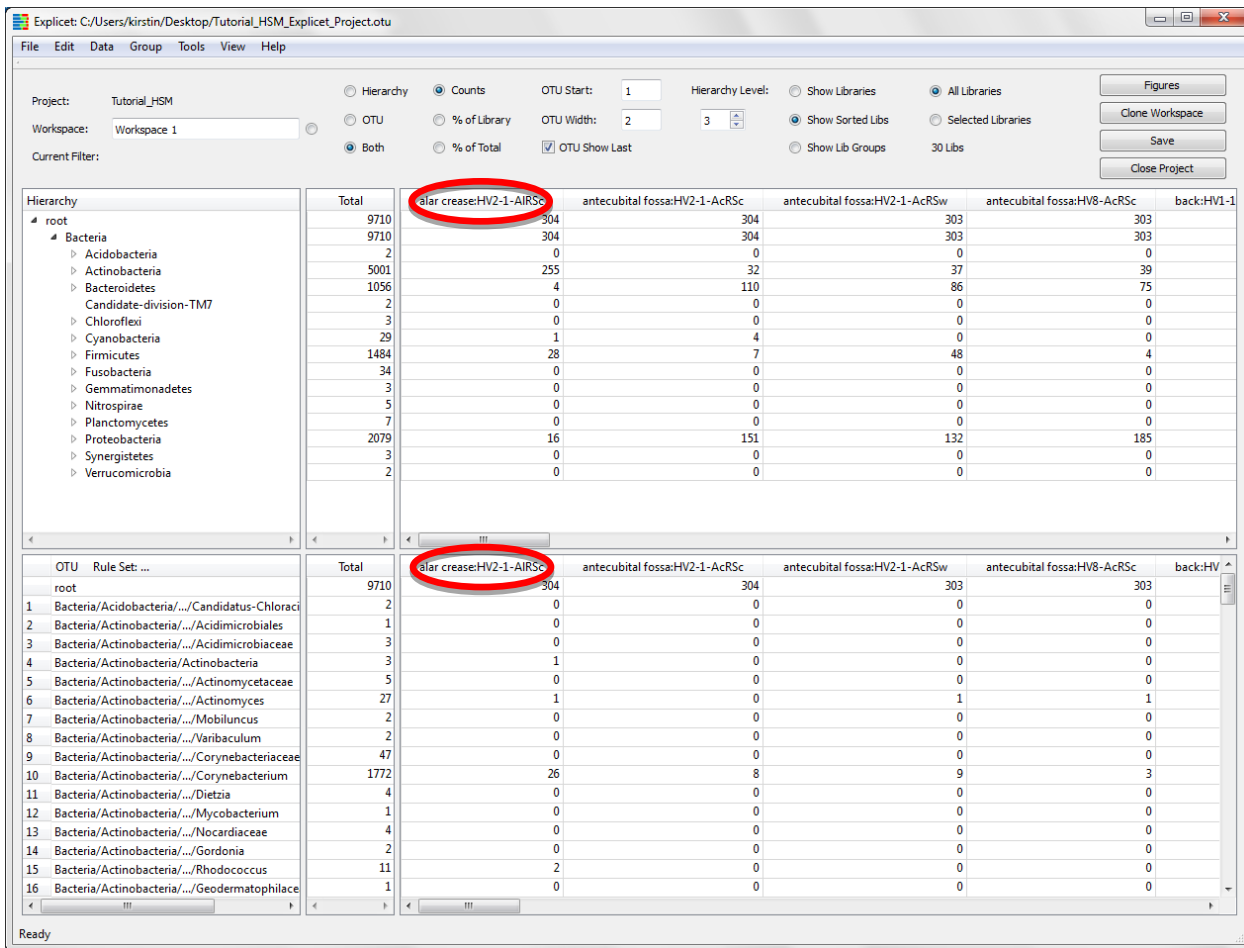
Buttons: Figures, Clone Workspace, Save, Close Project

Hierarchy	Total	HV1-1-BaCSc	HV1-1-UmCSw	HV10-BaCSc	HV10-UmCSw	HV2-1-AcRSc	HV2-1-AcRSw	HV2-1-AIRSc	HV2-1-B
root	9710	305	303	289	313	304	303	304	
▲ Bacteria	9710	305	303	289	313	304	303	304	
▶ Acidobacteria	2	0	0	0	0	0	0	0	
▶ Actinobacteria	5001	295	24	235	303	32	37	255	
▶ Bacteroidetes	1056	0	47	4	0	110	86	4	
▶ Candidate-division-TM7	2	0	0	0	0	0	0	0	
▶ Chloroflexi	3	0	0	0	0	0	0	0	
▶ Cyanobacteria	29	0	0	0	0	4	0	1	
▶ Firmicutes	1484	10	209	7	10	7	48	28	
▶ Fusobacteria	34	0	14	0	0	0	0	0	
▶ Gemmatimonadetes	3	0	0	0	0	0	0	0	
▶ Nitrospirae	5	0	0	0	0	0	0	0	
▶ Planctomycetes	7	0	0	0	0	0	0	0	
▶ Proteobacteria	2079	0	6	43	0	151	132	16	
▶ Synergistetes	3	0	3	0	0	0	0	0	
▶ Verrucomicrobia	2	0	0	0	0	0	0	0	

OTU	Rule Set: ...	Total	HV1-1-BaCSc	HV1-1-UmCSw	HV10-BaCSc	HV10-UmCSw	HV2-1-AcRSc	HV2-1-AcRSw	HV2-1-AIRSc	HV2-1-B
root		9710	305	303	289	313	304	303	304	
1	Bacteria/Acidobacteria/.../Candidatus-Chloraci	2	0	0	0	0	0	0	0	
2	Bacteria/Actinobacteria/.../Acidimicrobiales	1	0	0	0	0	0	0	0	
3	Bacteria/Actinobacteria/.../Acidimicrobiaceae	3	0	0	0	0	0	0	0	
4	Bacteria/Actinobacteria/Actinobacteria	3	0	0	0	0	0	0	1	
5	Bacteria/Actinobacteria/.../Actinomycetaceae	5	0	0	0	0	0	0	0	
6	Bacteria/Actinobacteria/.../Actinomycetes	27	0	4	0	0	0	1	1	
7	Bacteria/Actinobacteria/.../Mobiluncus	2	0	0	0	0	0	0	0	
8	Bacteria/Actinobacteria/.../Varibaculum	2	0	1	0	0	0	0	0	
9	Bacteria/Actinobacteria/.../Corynebacteriaceae	47	0	3	0	0	0	0	0	
10	Bacteria/Actinobacteria/.../Corynebacterium	1772	3	16	0	303	8	9	26	
11	Bacteria/Actinobacteria/.../Dietzia	4	0	0	0	0	0	0	0	
12	Bacteria/Actinobacteria/.../Mycobacterium	1	0	0	0	0	0	0	0	
13	Bacteria/Actinobacteria/.../Nocardiaceae	4	0	0	0	0	0	0	0	
14	Bacteria/Actinobacteria/.../Gordonia	2	0	0	0	0	0	0	0	
15	Bacteria/Actinobacteria/.../Rhodococcus	11	0	0	0	0	0	0	2	
16	Bacteria/Actinobacteria/.../Geodermatophilaceae	1	0	0	0	0	0	0	0	

Ready

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

OTU Stacked Bar in Workspace Workspace 1

OTU Stacked Bar in Workspace Workspace 1

Counts OTU Start: 1 Show Libraries All Libraries

% of Library OTU Width: 2 Show Sorted Libs Selected Libraries

% of Total OTU Show Last Show Lib Groups 30 Libs

Figures: No figure selected Use Workspace Filter Workspace Filter:

Use Figure Filter Figure Filter:

Include items between: and 0 Rows Selected of 152 Rows

OTU	Rule Set ...	Total	alar crease:HV2-1-AIRSc	antecubital fossa:HV2-1-AcrSc	antecubital fossa:HV2-1-AcrSw	antecubital fossa:HV8-
root		9710	304	304	303	
1	Bacteria/Acidobacteria/.../Candidatus-G	2	0	0	0	
2	Bacteria/Actinobacteria/.../Acidimicrobi	1	0	0	0	
3	Bacteria/Actinobacteria/.../Acidimicrobi	3	0	0	0	
4	Bacteria/Actinobacteria/Actinobacteria	3	1	0	0	
5	Bacteria/Actinobacteria/.../Actinomycet	5	0	0	0	
6	Bacteria/Actinobacteria/.../Actinomycet	27	1	0	1	
7	Bacteria/Actinobacteria/.../Mobiluncus	2	0	0	0	
8	Bacteria/Actinobacteria/.../Varibaculum	2	0	0	0	
9	Bacteria/Actinobacteria/.../Corynebacte	47	0	0	0	
10	Bacteria/Actinobacteria/.../Corynebacte	1772	26	8	9	
11	Bacteria/Actinobacteria/.../Dietzia	4	0	0	0	
12	Bacteria/Actinobacteria/.../Mycobacteri	1	0	0	0	
13	Bacteria/Actinobacteria/.../Nocardiacea	4	0	0	0	
14	Bacteria/Actinobacteria/.../Gordonia	2	0	0	0	
15	Bacteria/Actinobacteria/.../Rhodococcu	11	2	0	0	
16	Bacteria/Actinobacteria/.../Geodermato	1	0	0	0	
17	Bacteria/Actinobacteria/.../Blastococcus	4	0	0	0	
18	Bacteria/Actinobacteria/.../Micrococcal	1	0	0	0	
19	Bacteria/Actinobacteria/.../Brevibacteri	4	0	0	0	

To display only the top 10 taxa in the project, note that the **Total** value of the 1st OTU in the column is 3044

Note that the **Total** value of the 10th OTU in the column is 137

In the **Include items between** field, enter "137" into the first box (the lower bounding limit)

In the **Include items between** field, enter "3044" into the second box (the upper bounding limit)

Click **Select Range**

OTU Stacked Bar in Workspace Workspace 1

OTU Stacked Bar in Workspace Workspace 1

Counts
 % of Library
 % of Total

OTU Start: 1
 OTU Width: 2

Show Libraries
 Show Sorted Libs
 Show Lib Groups

All Libraries
 Selected Libraries
 30 Libs

Figures: No figure selected

Use Workspace Filter
 Use Figure Filter

Workspace Filter:
 Figure Filter:

Include items between: 137 and 3044 10 Rows Selected of 152 Rows

Select Range

OTU	Rule Set: ...	Total	alar crease:HV2-1-AIRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fossa:HV8
root		9710	304	304	303	
1	Bacteria/Actinobacteria/.../Propionibact	3044	225	24	25	
2	Bacteria/Actinobacteria/.../Corynebacte	1772	26	8	9	
3	Bacteria/Bacteroidetes/.../Cloacibacteri	880	4	109	82	
4	Bacteria/Proteobacteria/.../Aquabacteri	746	0	15	0	
5	Bacteria/Proteobacteria/.../Diaphorobac	633	12	80	100	
6	Bacteria/Firmicutes/.../Staphylococcus	588	27	6	21	
7	Bacteria/Firmicutes/.../Streptococcus	320	0	0	21	
8	Bacteria/Proteobacteria/.../Comamonad	150	2	22	10	
9	Bacteria/Proteobacteria/.../Acidovorax	150	1	13	7	
10	Bacteria/Firmicutes/.../Anaerococcus	137	0	0	3	
11	Bacteria/Firmicutes/.../Finegoldia	128	0	0	0	
12	Bacteria/Bacteroidetes/.../Prevotella	64	0	0	2	
13	Bacteria/Firmicutes/.../Family-XI-Incerta	64	0	0	0	
14	Bacteria/Firmicutes/.../Peptoniphilus	61	0	0	0	
15	Bacteria/Actinobacteria/.../Corynebacte	47	0	0	0	
16	Bacteria/Proteobacteria/.../Zoogloea	46	0	0	0	
17	Bacteria/Proteobacteria/.../Sphingomor	45	0	12	8	
18	Bacteria/Firmicutes/.../Dialister	35	0	0	0	
19	Bacteria/Proteobacteria/.../Acinetobact	34	0	0	0	

Done
Unsort OTU
Plot...
Export...
Save As Figure...

The top 10 OTUs are now highlighted.

Click **Plot**

OTU Stacked Bar in Workspace Workspace 1

OTU Stacked Bar in Workspace Workspace 1

Counts
 % of Library
 % of Total

OTU Start: 1
 OTU Width: 2

Show Libraries
 Show Sorted Libs
 Show Lib Groups

All Libraries
 Selected Libraries
 30 Libs

Figures: No figure selected

Use Workspace Filter
 Use Figure Filter

Workspace Filter:
 Figure Filter:

Include items between: 137 and 3044 10 Rows Selected of 152 Rows

Select Range Clear Selection

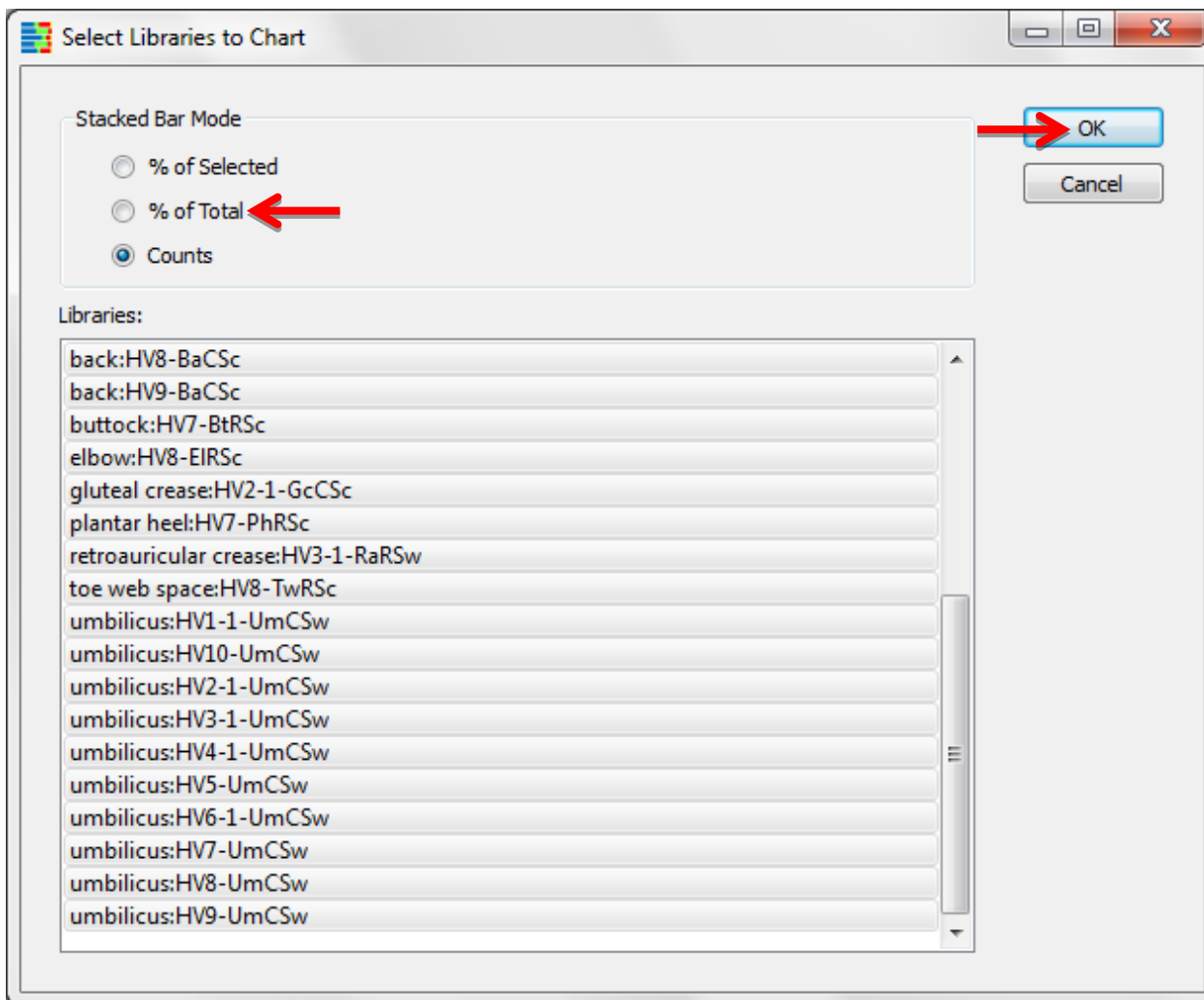
OTU	Rule Set: ...	Total	alar crease:HV2-1-AIRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fossa:HV8
root		9710	304	304	303	
1	Bacteria/Actinobacteria/.../Propionibact	3044	225	24	25	
2	Bacteria/Actinobacteria/.../Corynebacte	1772	26	8	9	
3	Bacteria/Bacteroidetes/.../Cloacibacteri	880	4	109	82	
4	Bacteria/Proteobacteria/.../Aquabacteri	746	0	15	0	
5	Bacteria/Proteobacteria/.../Diaphorobac	633	12	80	100	
6	Bacteria/Firmicutes/.../Staphylococcus	588	27	6	21	
7	Bacteria/Firmicutes/.../Streptococcus	320	0	0	21	
8	Bacteria/Proteobacteria/.../Comamonad	150	2	22	10	
9	Bacteria/Proteobacteria/.../Acidovorax	150	1	13	7	
10	Bacteria/Firmicutes/.../Anaerococcus	137	0	0	3	
11	Bacteria/Firmicutes/.../Finegoldia	128	0	0	0	
12	Bacteria/Bacteroidetes/.../Prevotella	64	0	0	2	
13	Bacteria/Firmicutes/.../Family-XI-Incerta	64	0	0	0	
14	Bacteria/Firmicutes/.../Peptoniphilus	61	0	0	0	
15	Bacteria/Actinobacteria/.../Corynebacte	47	0	0	0	
16	Bacteria/Proteobacteria/.../Zoogloea	46	0	0	0	
17	Bacteria/Proteobacteria/.../Sphingomor	45	0	12	8	
18	Bacteria/Firmicutes/.../Dialister	35	0	0	0	
19	Bacteria/Proteobacteria/.../Acinetobact	34	0	0	0	

Done
Unsort OTU
Plot...
Export...
Save As Figure...

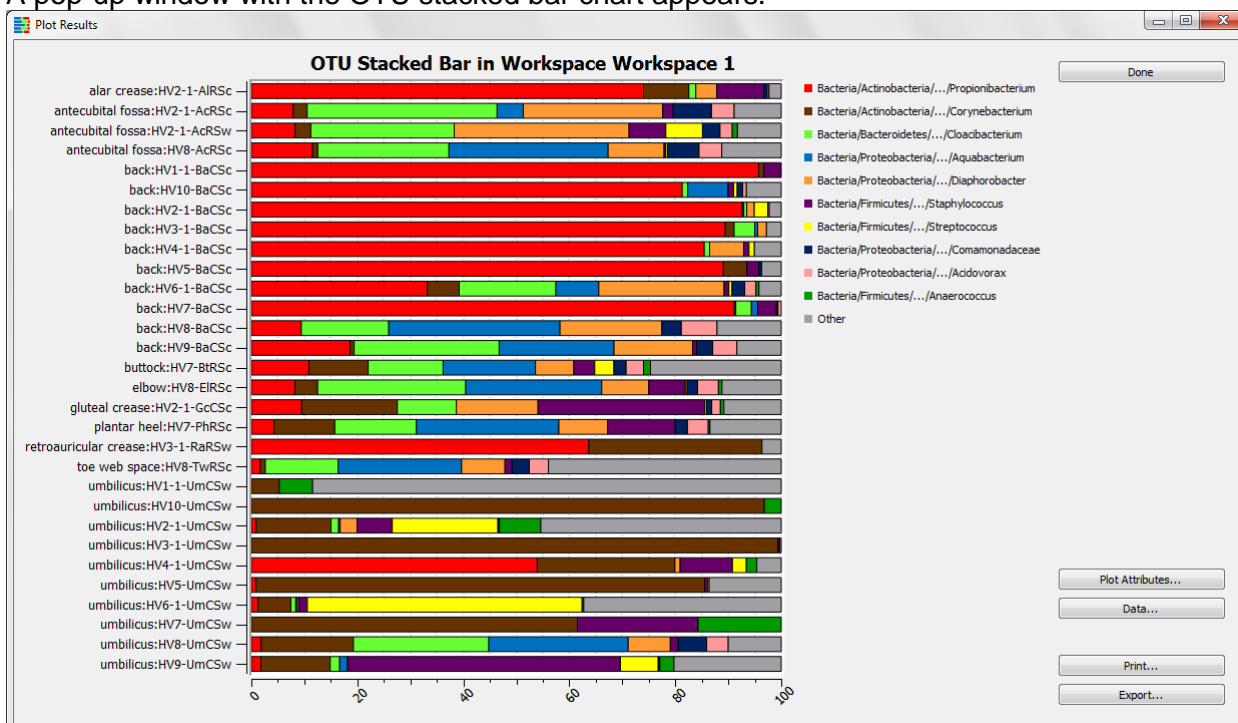
A new window will appear containing stacked bar display options

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

Click **OK**



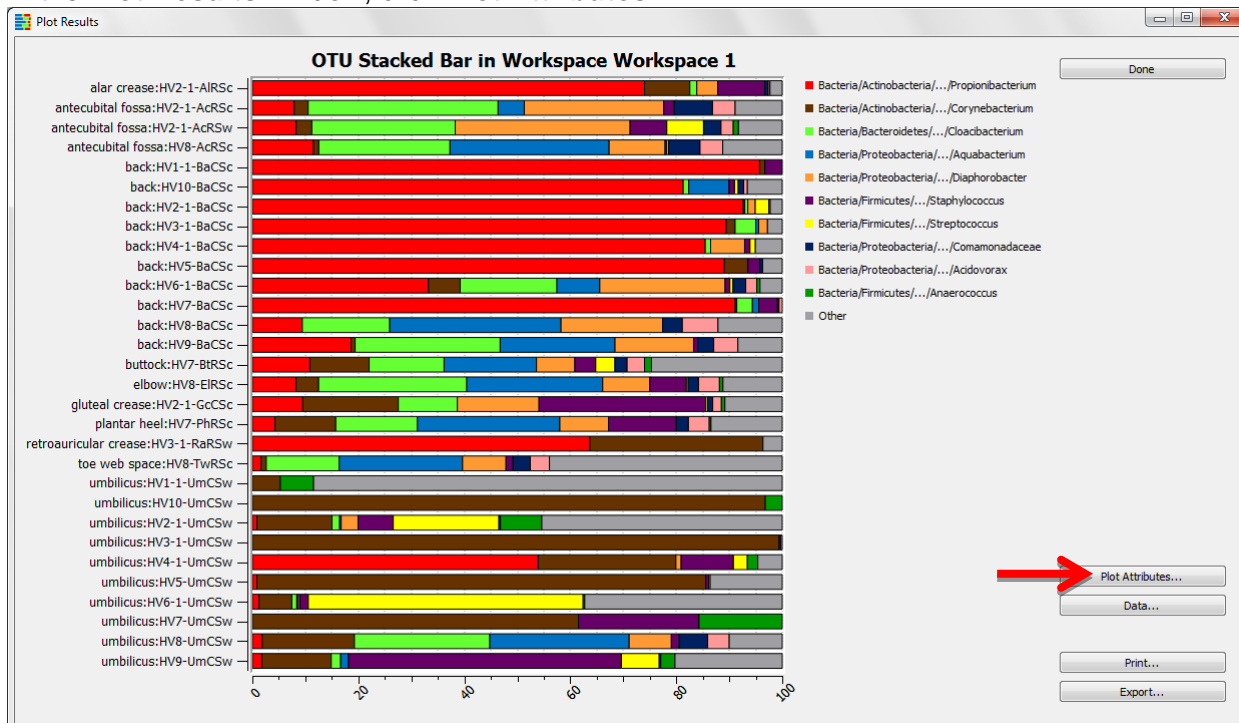
A pop-up window with the OTU stacked bar chart appears.



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

B. Change the Title and Label the Axes

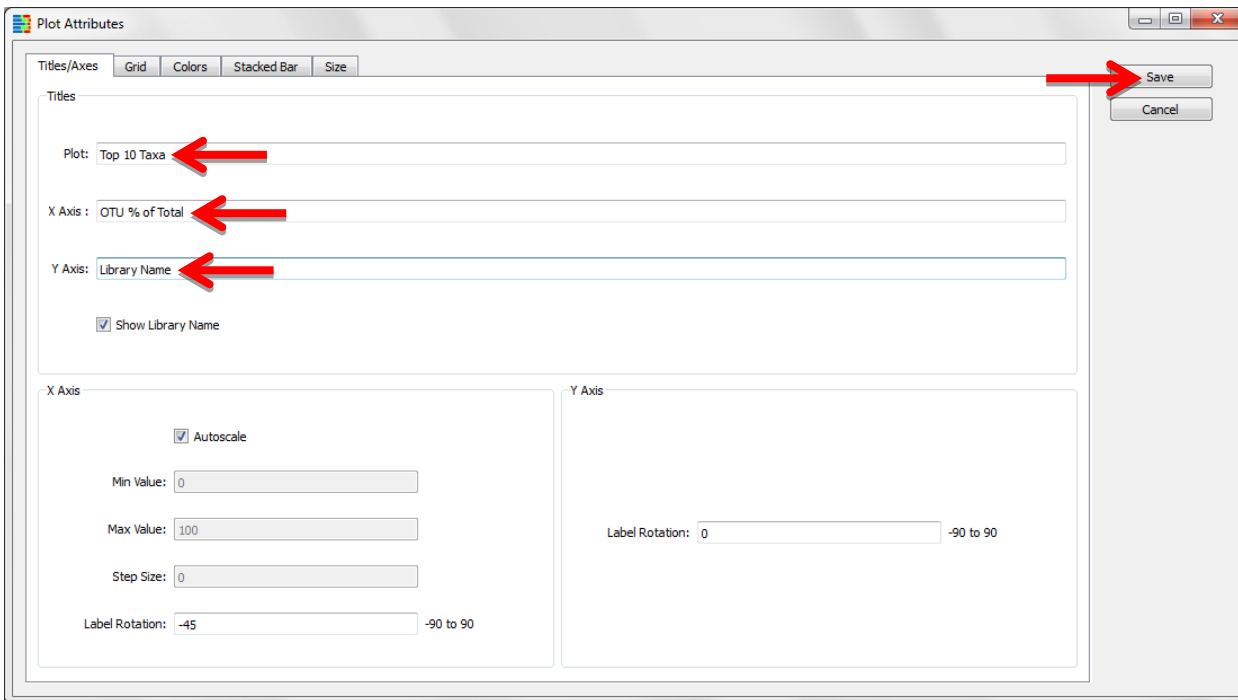
In the **Plot Results** window, click **Plot Attributes**



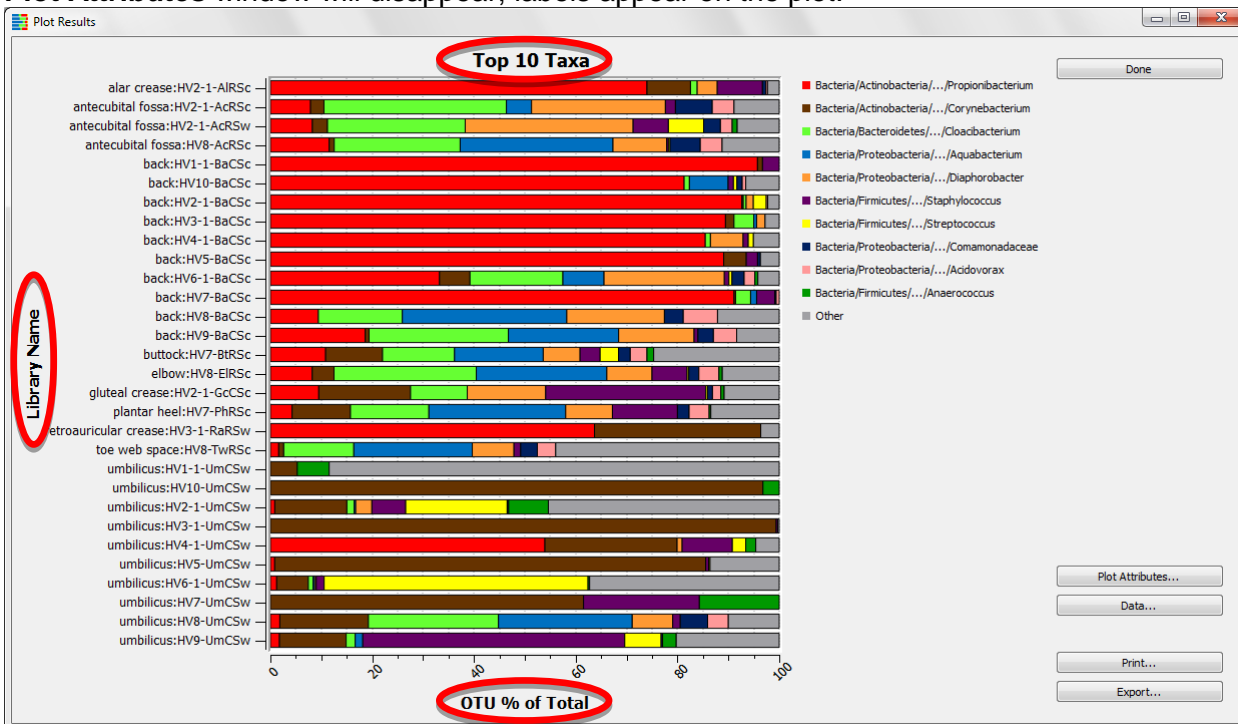
A pop-up window will appear.

The screenshot shows the "Plot Attributes" dialog box with the "Titles/Axes" tab selected. The "Plot" field contains "OTU Stacked Bar in Workspace Workspace 1". The "X Axis" and "Y Axis" fields are empty. The "X Axis" section has "Autoscale" checked, with "Min Value" at 0, "Max Value" at 100, "Step Size" at 0, and "Label Rotation" at -45. The "Y Axis" section has "Label Rotation" at 0. There are "Save" and "Cancel" buttons on the right side.

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 window will disappear; labels appear on the plot.

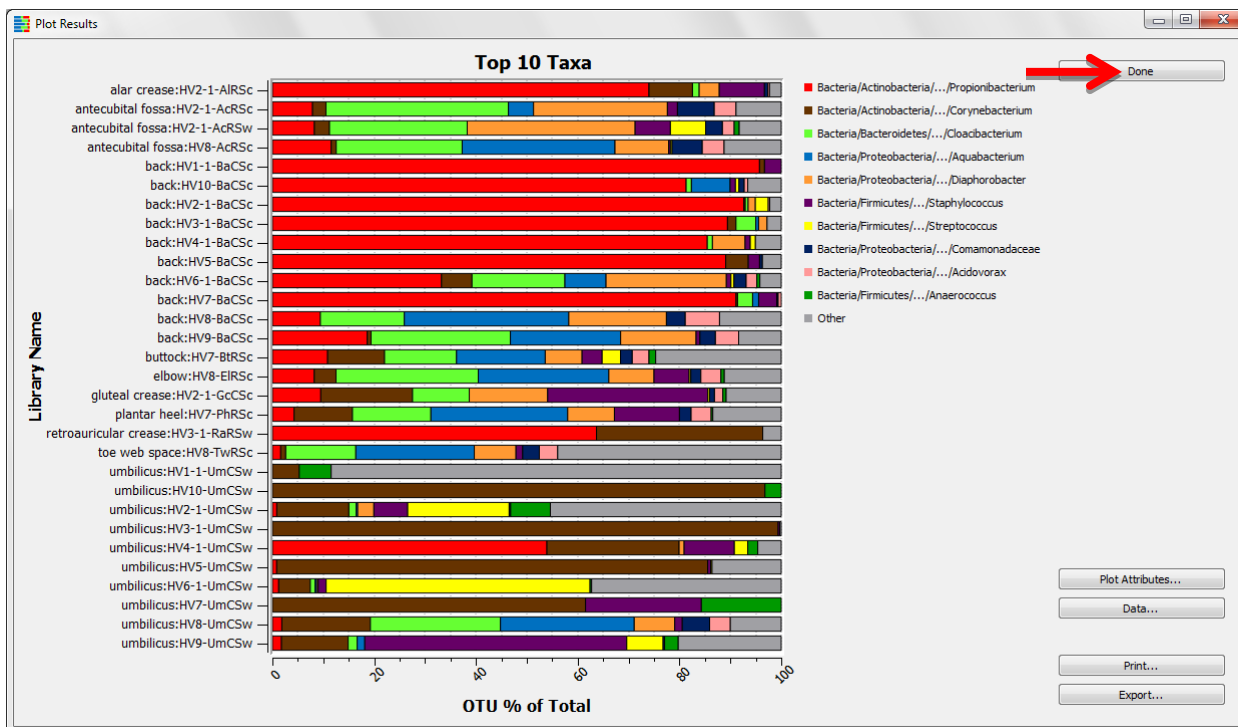


Red and brown appear to be dominant colors in this plot. According to the legend, these colors belong to the “Actinobacteria” phylum. This information may be useful in guiding us toward a hypothesis involving the dominant taxa.

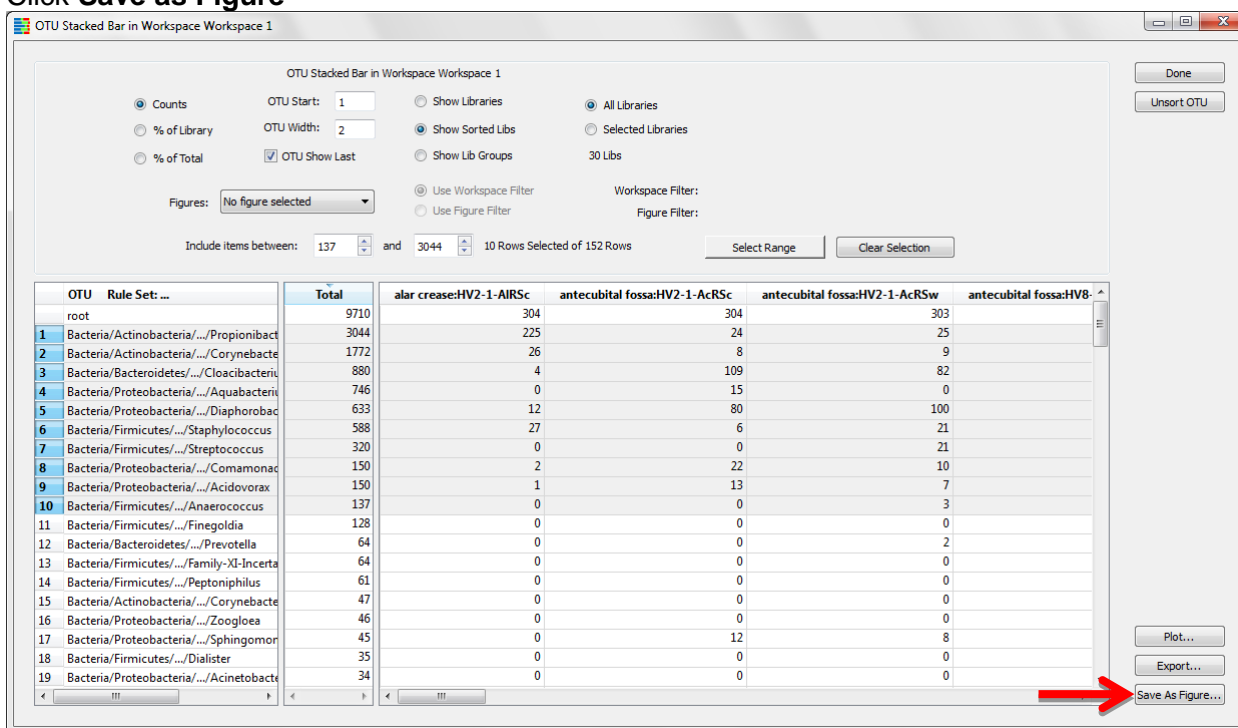
Saving figures in Explicit is easy and convenient. Figures are saved within the larger project, so they stay linked to the data from which they were created and do not create additional files on your computer.

C. Save the OTU Stacked Bar Chart as a Figure

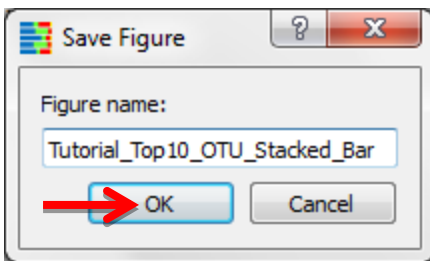
Click **Done** in the stacked bar chart **Plot Results** window



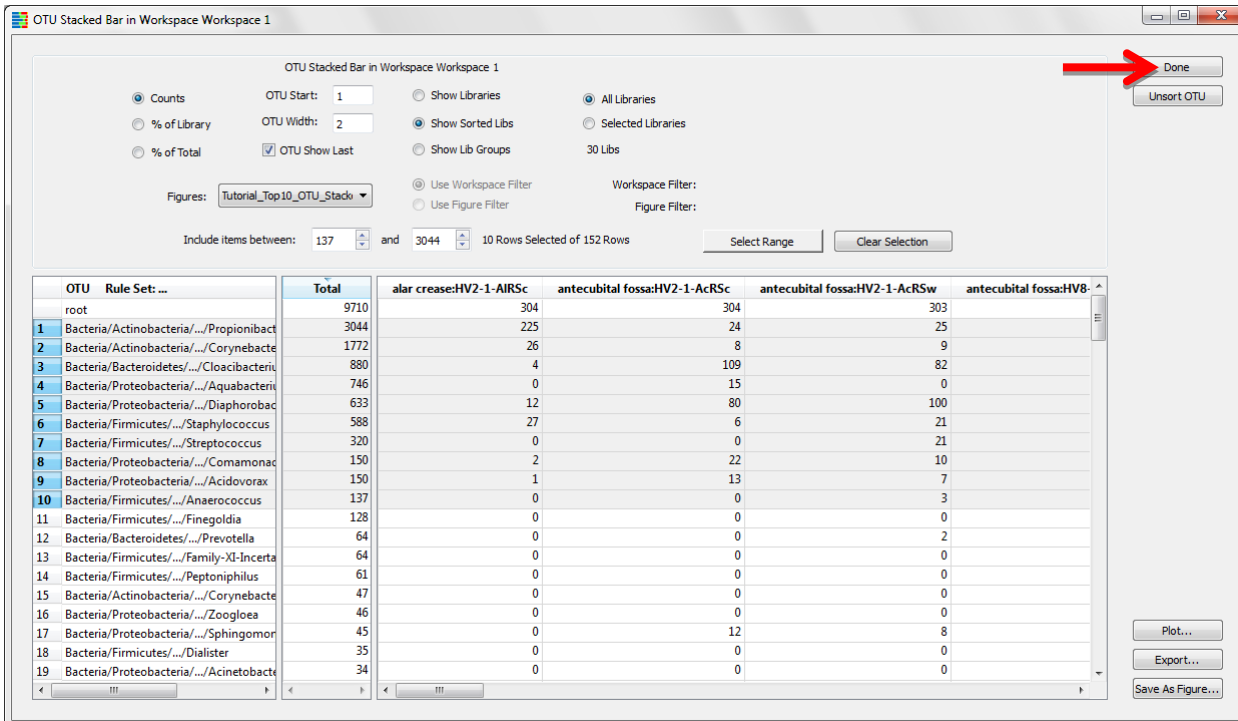
The OTU Stacked Bar setup window is back on the screen
 Click **Save as Figure**



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 main project 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.

Explicit: C:/Users/kirstin/Desktop/Tutorial_HSM_Explicit_Project.otu

File Edit Data Group Tools View Help

Project: Tutorial_HSM
 Workspace: Workspace 1
 Current Filter:

Hierarchy Counts OTU Start: 1 Hierarchy Level: Show Libraries All Libraries
 OTU % of Library OTU Width: 2 Show Sorted Libs Selected Libraries
 Both % of Total OTU Show Last Show Lib Groups 30 Libs

Buttons: **Figures**, Clone Workspace, Save, Close Project

Hierarchy	Total	alar cresens:HV2-1-AIRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fossa:HV8-AcRSc	back:HV1-1
root	9710	304	304	303	303	
Bacteria	9710	304	304	303	303	
Actinobacteria	2	0	0	0	0	
Actinobacteria	5001	355	37	37	30	

Figures

Current Workspace: Workspace 1

Figures:	Name	Type	Plot Type	Filter	Analysis	Figure Workspace	Created	Modified	Lib Grouping or Sorting
1	Tutorial_Top10_OTU_Stack...	Plot	Stacked Bar		OTU Stacked Bar Chart	Workspace 1	05/07/20...	05/07/20...	Anatomy

Buttons: Done, Open, Open in Different Workspace..., Delete, Rename

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 displays the distribution of OTUs in the project.

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**

Format for Plot Names

OTU Start: 1
OTU Width: 2
 OTU Show Last

Counts
 Counts
 % of Library
 % of Total

Hierarchy Level: 3
 Show Libraries
 Show Sorted Libs
 Show Lib Groups

All Libraries
 All Libraries
 Selected Libraries
30 Libs

Use Workspace Filter
 Use Workspace Filter
 Use Figure Filter

Workspace Filter: Figure Filter:

Figures: No figure selected

Hierarchy	Total	alar crease:HV2-1-AIRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRsw	antecubital fos
root	9710	304	304	303	
Bacteria	9710	304	304	303	
Acidobacteria	2	0	0	0	
Actinobacteria	5001	255	32	37	
Bacteroidetes	1056	4	110	86	
Candidate-division-TM7	2	0	0	0	
Chloroflexi	3	0	0	0	
Cyanobacteria	29	1	4	0	
Firmicutes	1484	28	7	48	
Fusobacteria	34	0	0	0	
Gemmatimonadetes	3	0	0	0	
Nitrospirae	5	0	0	0	
Planctomycetes	7	0	0	0	
Proteobacteria	2079	16	151	132	
Synergistetes	3	0	0	0	
Verrucomicrobia	2	0	0	0	

Done
Add to Pie
Remove from Pie
Add to Other

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

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

Click **Plot**

Pie Chart in Workspace Workspace 1

Format for Plot Names

OTU Start: 1
OTU Width: 2
 OTU Show Last

Counts
 % of Library
 % of Total

Hierarchy Level: 3

Show Libraries
 Show Sorted Libs
 Show Lib Groups

All Libraries
 Selected Libraries
30 Libs

Workspace Filter:
 Use Workspace Filter
 Use Figure Filter

Figure Filter:

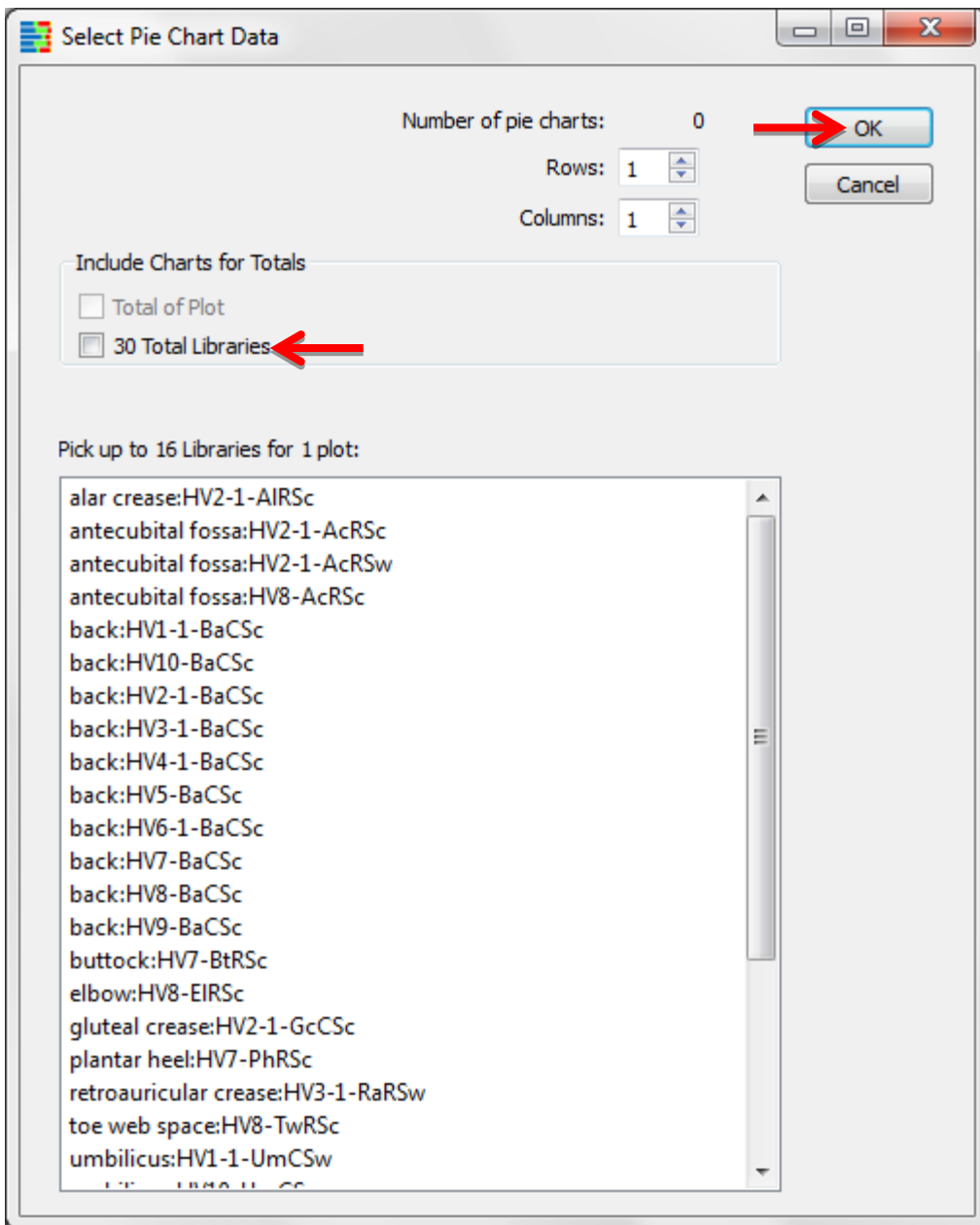
Figures: No figure selected

Done
Add to Pie
Remove from Pie
Add to Other

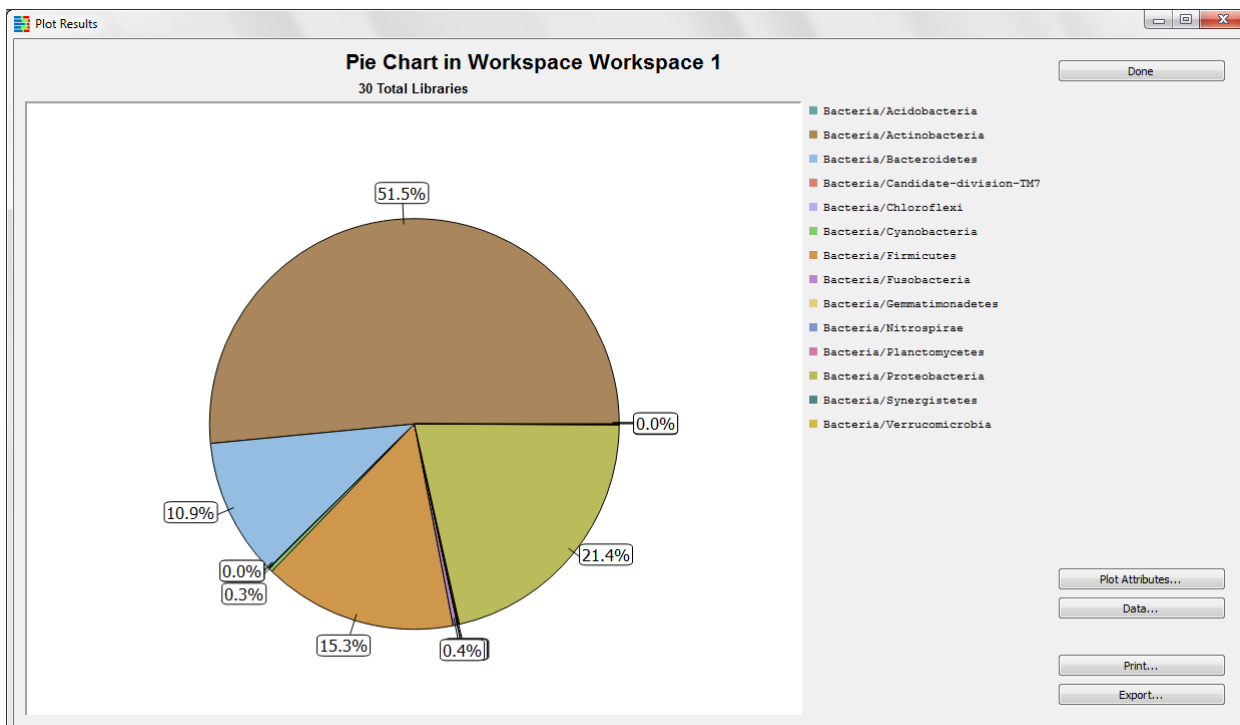
Hierarchy	Total	alar crease:HV2-1-AIRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fossa:HV2-1-AcRSc
root	9710	304	304	303	303
Bacteria	9710	304	304	303	303
Acidobacteria	2	0	0	0	0
Actinobacteria	5001	255	32	37	37
Bacteroidetes	1056	4	110	86	86
Candidate-division-TM7	2	0	0	0	0
Chloroflexi	3	0	0	0	0
Cyanobacteria	29	1	4	0	0
Firmicutes	1484	28	7	48	48
Fusobacteria	34	0	0	0	0
Gemmatimonadetes	3	0	0	0	0
Nitrospirae	5	0	0	0	0
Planctomycetes	7	0	0	0	0
Proteobacteria	2079	16	151	132	132
Synergistetes	3	0	0	0	0
Verrucomicrobia	2	0	0	0	0

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

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.

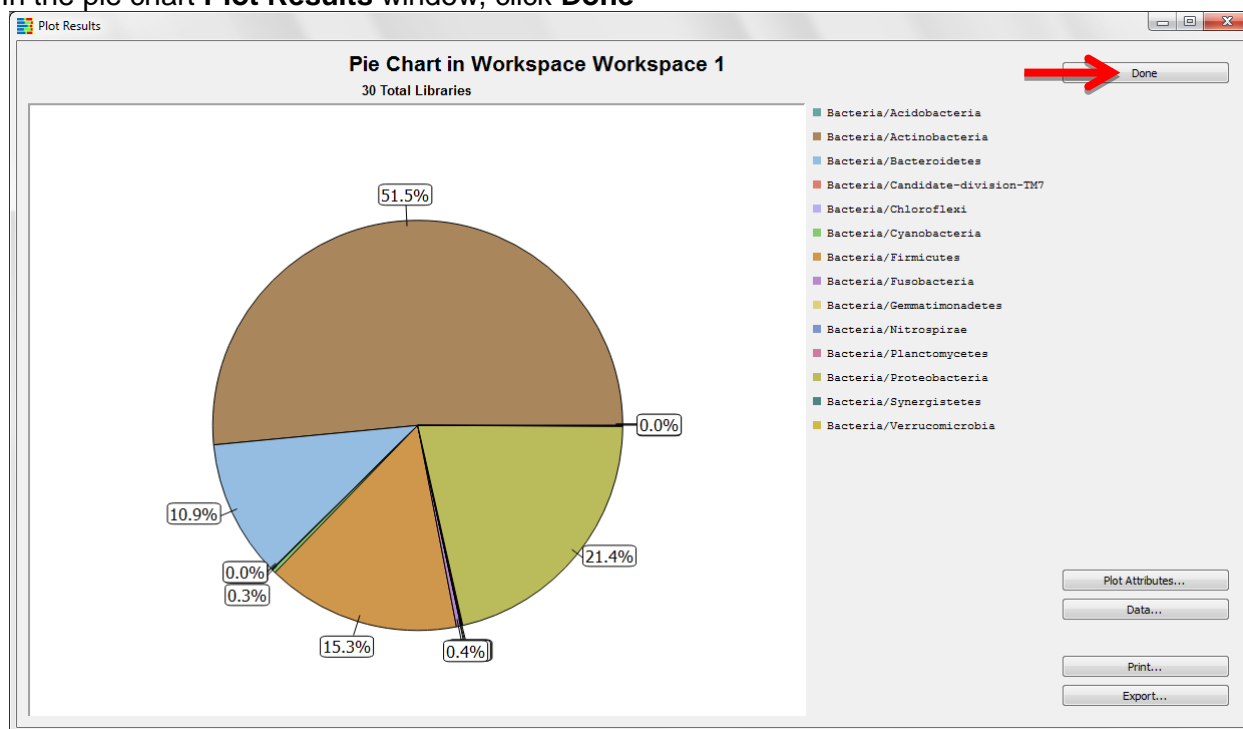


By looking at the pie chart of the phyla, it is clear that the brown wedge, Actinobacteria, is the most prevalent phylum in the data.

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

Format for Plot Names

OTU Start: 1
OTU Width: 2
 OTU Show Last

Counts
 % of Library
 % of Total

Hierarchy Level: Show Libraries All Libraries Selected Libraries

Show Sorted Libs
 Show Lib Groups 30 Libs

Figures: No figure selected

Use Workspace Filter
 Use Figure Filter

Workspace Filter: Figure Filter:

Hierarchy

- root
- Bacteria
- Acidobacteria
- Actinobacteria
- Bacteroidetes
- Candidate-division-TM7
- Chloroflexi
- Cyanobacteria
- Firmicutes
- Fusobacteria
- Gemmatimonadetes
- Nitrospirae
- Planctomycetes
- Proteobacteria**
- Alphaproteobacteria
- Betaproteobacteria
- Deltaproteobacteria
- Epsilonproteobacteria
- Gammaproteobacteria
- Synergistetes
- Verrucomicrobia

Total	alar crease:HV2-1-AIRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fos
9710	304	304	303	
9710	304	304	303	
2	0	0	0	
5001	255	32	37	
1056	4	110	86	
2	0	0	0	
3	0	0	0	
29	1	4	0	
1484	28	7	48	
34	0	0	0	
3	0	0	0	
5	0	0	0	
7	0	0	0	
2079	16	151	132	
143	0	18	13	
1834	16	133	118	
2	0	0	0	
8	0	0	0	
92	0	0	1	
3	0	0	0	
2	0	0	0	

Done
Add to Pie
Remove from Pie
Add to Other

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

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

Format for Plot Names

OTU Start: 1
OTU Width: 2
 OTU Show Last

Counts
 % of Library
 % of Total

Hierarchy Level: Show Libraries All Libraries Selected Libraries

Show Sorted Libs
 Show Lib Groups 30 Libs

Figures: No figure selected

Use Workspace Filter
 Use Figure Filter

Workspace Filter: Figure Filter:

Hierarchy

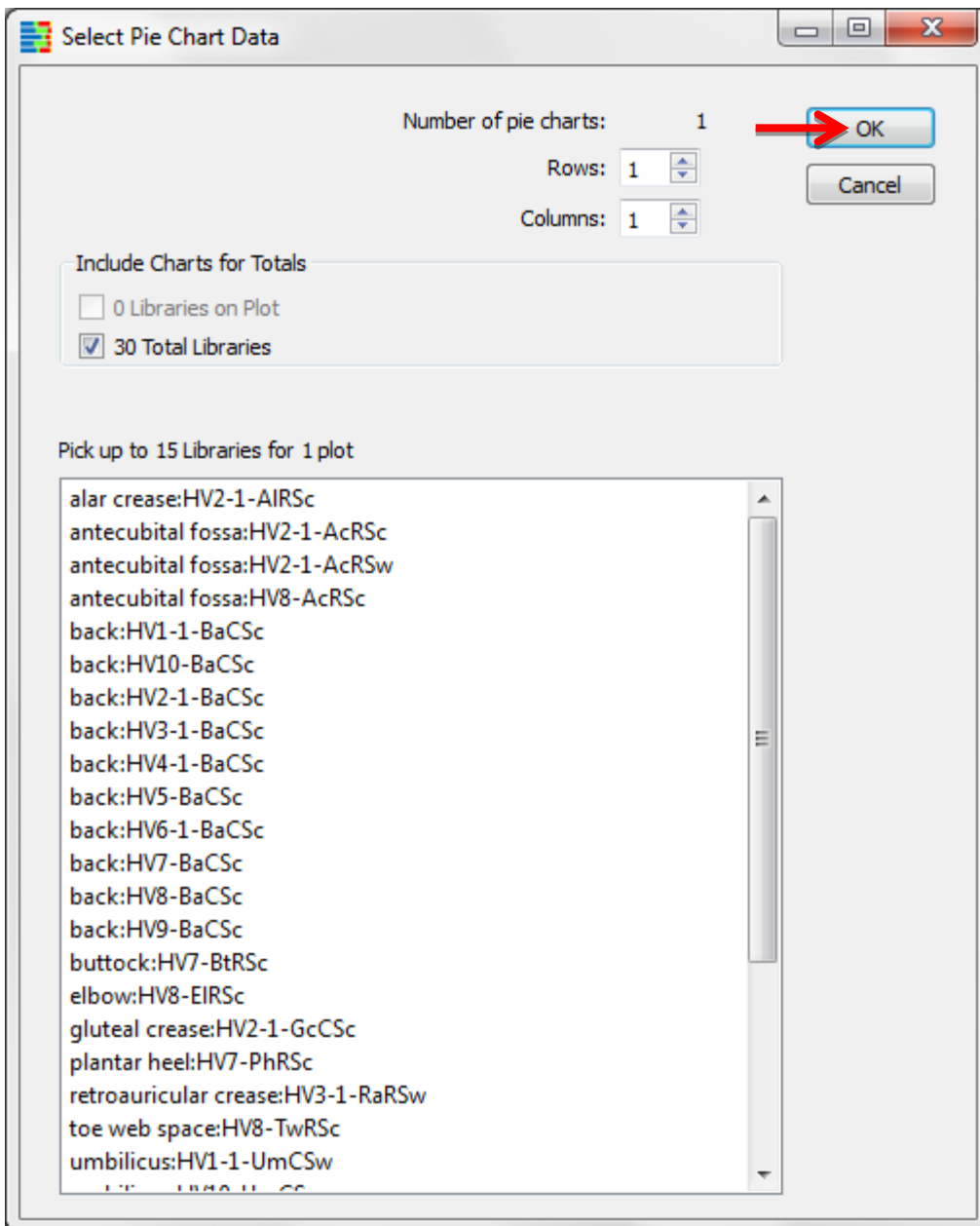
- root
- Bacteria
- Acidobacteria
- Actinobacteria
- Bacteroidetes
- Candidate-division-TM7
- Chloroflexi
- Cyanobacteria
- Firmicutes
- Fusobacteria
- Gemmatimonadetes
- Nitrospirae
- Planctomycetes
- Proteobacteria**
- Alphaproteobacteria
- Betaproteobacteria
- Deltaproteobacteria
- Epsilonproteobacteria
- Gammaproteobacteria
- Synergistetes
- Verrucomicrobia

Total	alar crease:HV2-1-AIRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fos
9710	304	304	303	
9710	304	304	303	
2	0	0	0	
5001	255	32	37	
1056	4	110	86	
2	0	0	0	
3	0	0	0	
29	1	4	0	
1484	28	7	48	
34	0	0	0	
3	0	0	0	
5	0	0	0	
7	0	0	0	
2079	16	151	132	
143	0	18	13	
1834	16	133	118	
2	0	0	0	
8	0	0	0	
92	0	0	1	
3	0	0	0	
2	0	0	0	

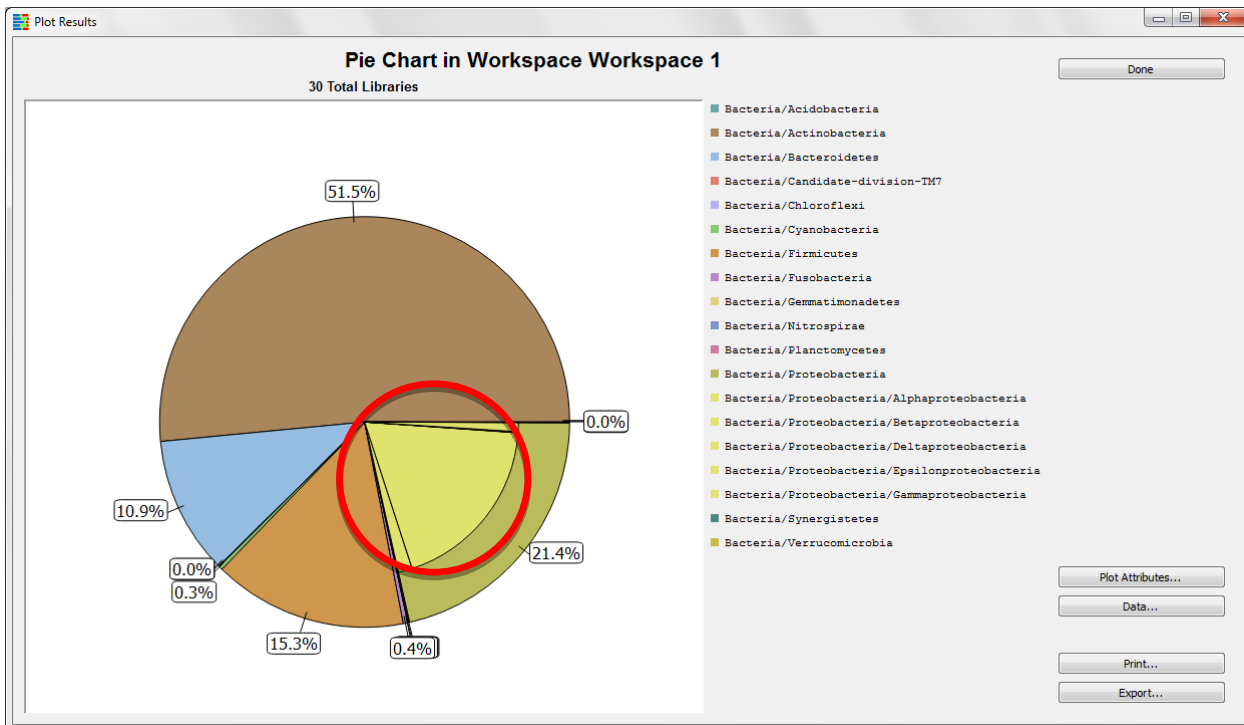
Done
Add to Pie
Remove from Pie
Add to Other

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

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



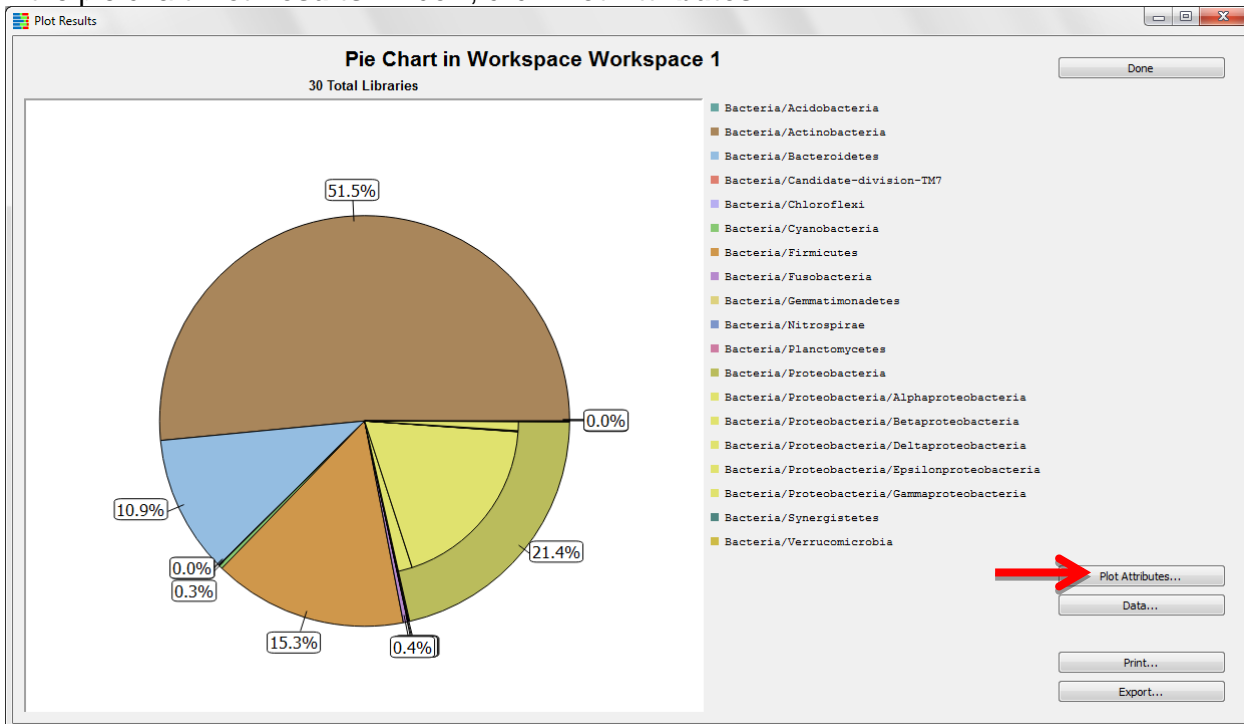
A pop-up window with the pie chart appears. We now see the classes within Proteobacteria represented as sub-wedges.



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

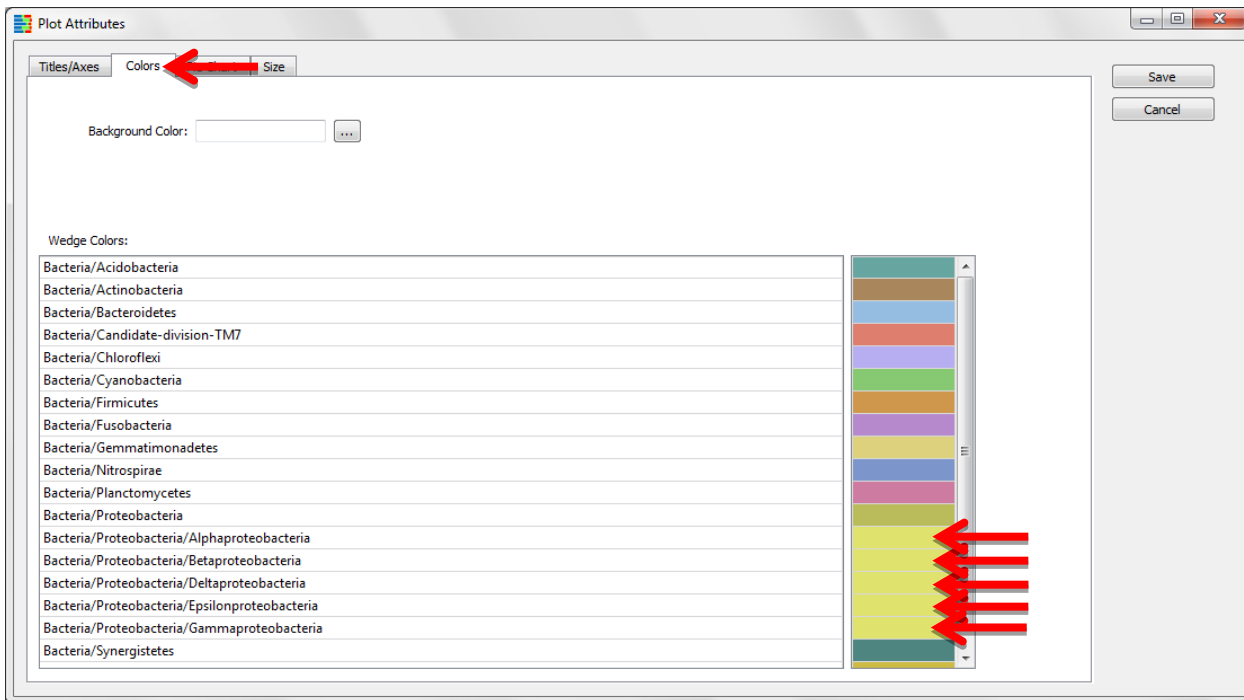
In the pie chart **Plot Results** window, click **Plot Attributes**



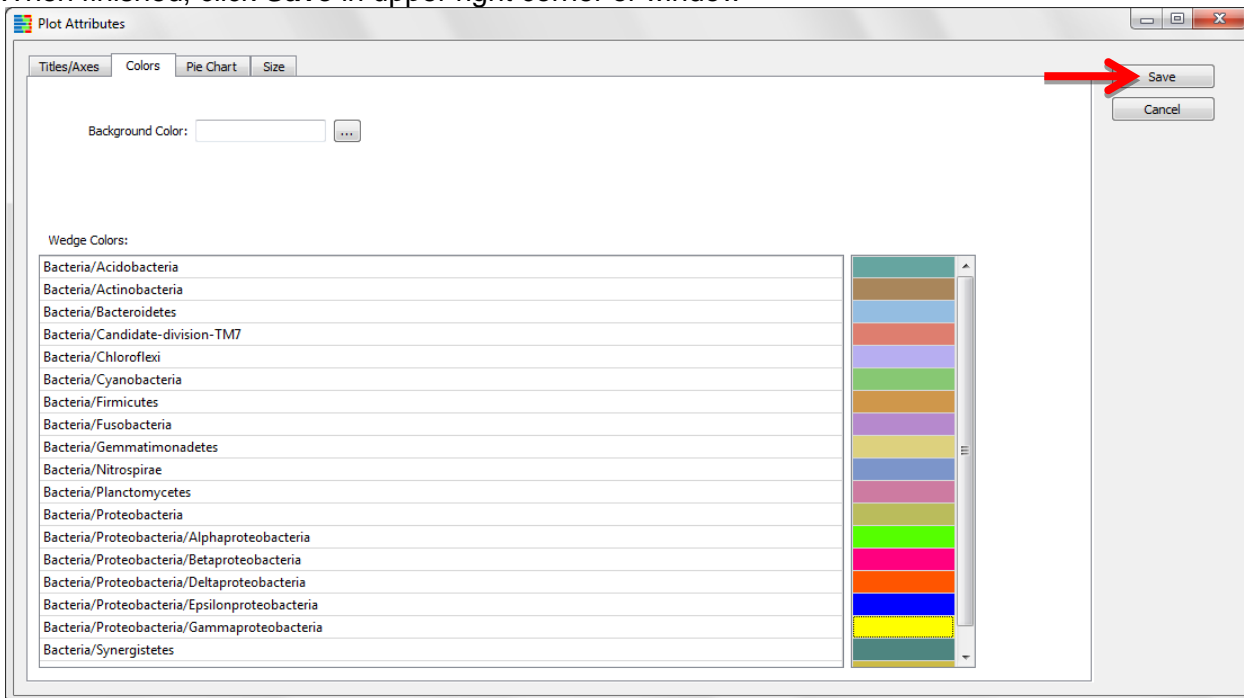
A pop-up window will appear

Click on the **Colors** tab

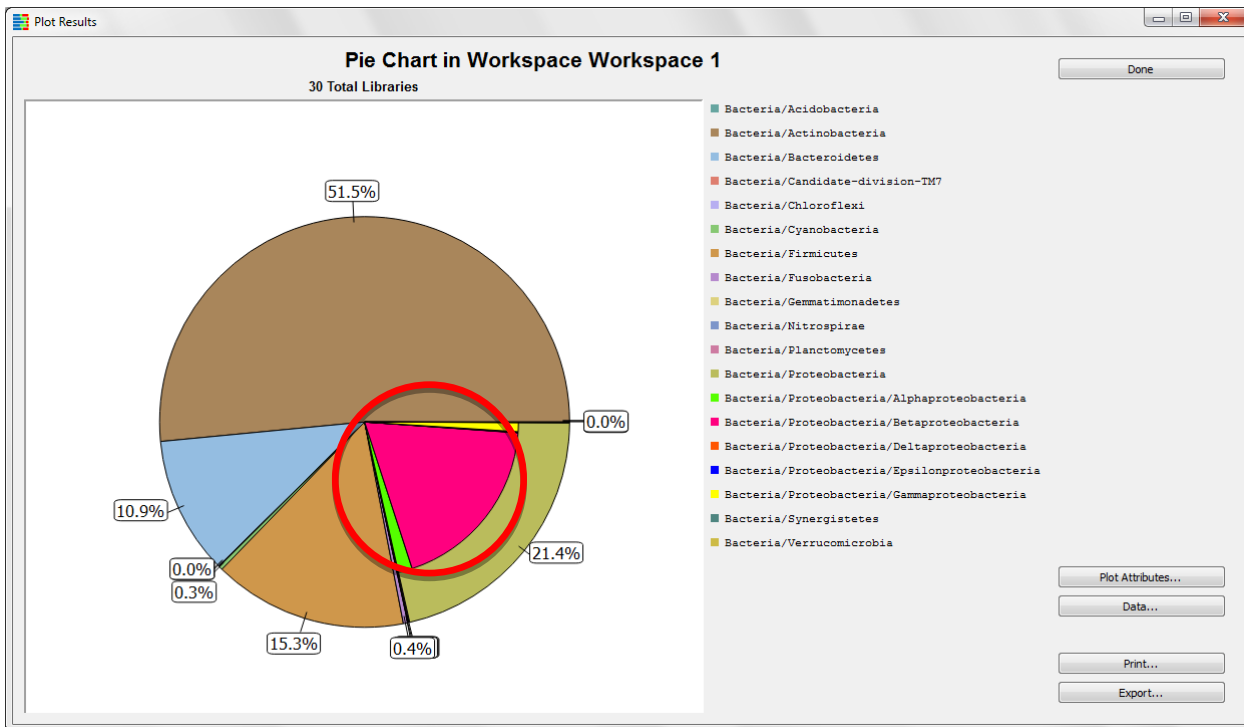
To pick a different wedge color, click on the color, and select a new color from the pop-up display



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.

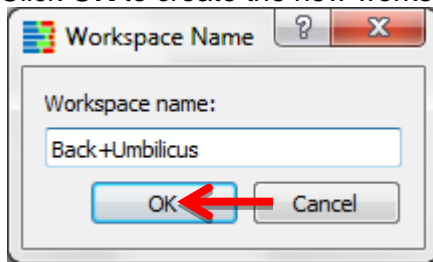
Although the skin is a single organ, it harbors microbial communities that live in a range of physiologically and topographically distinct niches. The back is typically a sebaceous region, whereas the umbilicus is often a moist region of the body. Therefore, these two niches may have different taxa present.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

A. Create a New Workspace

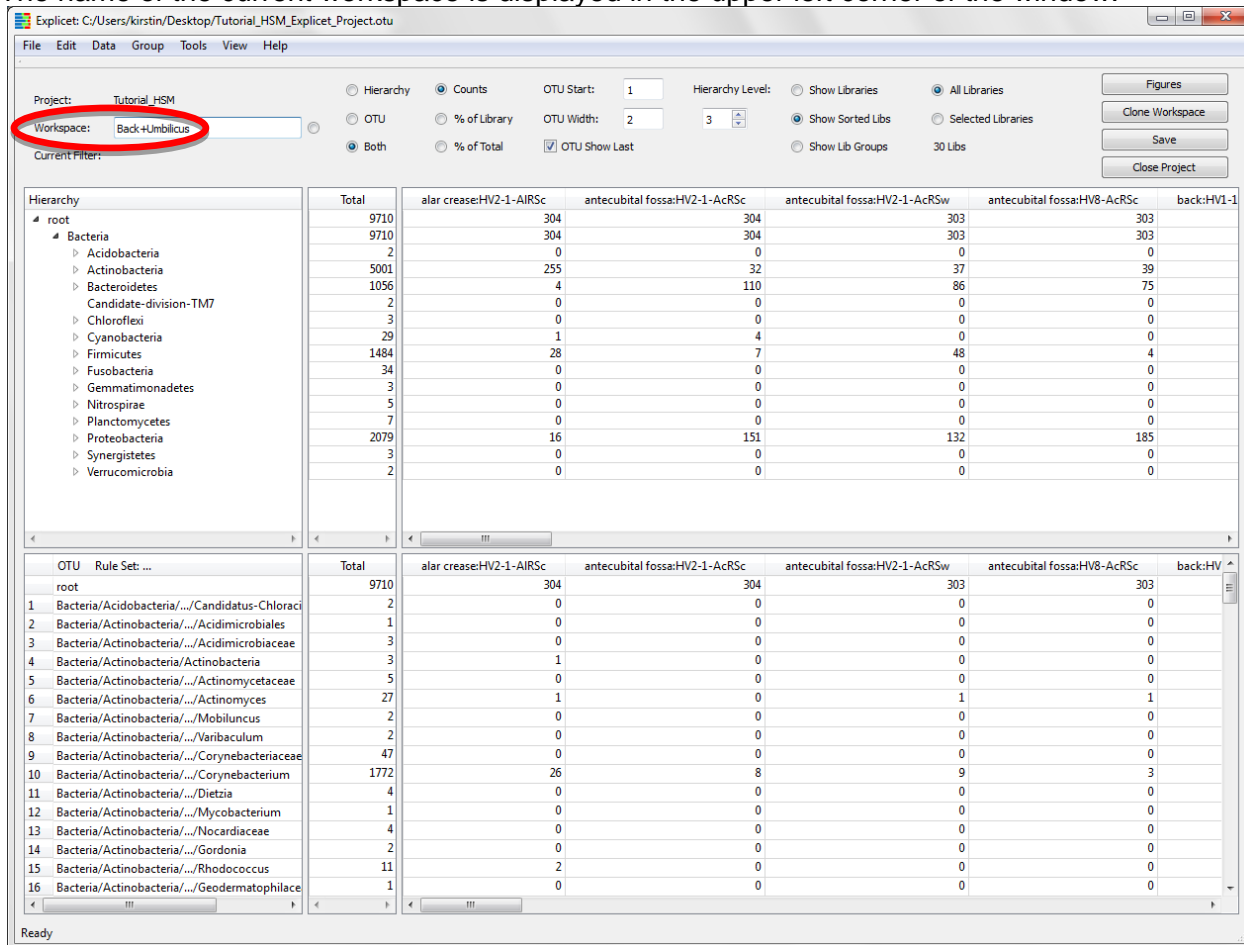
File → **New** → **Workspace from Current Workspace** (“from Current Workspace” allows us to copy all of the display changes we’ve already made to the new workspace)

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.



Project: Tutorial_HSM
Workspace: Back+Umbilicus
Current Filter:

Hierarchy	Total	alar crease:HV2-1-AIRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fossa:HV8-AcRSc	back:HV1-1
root	9710	304	304	303	303	
Bacteria	9710	304	304	303	303	
Acidobacteria	2	0	0	0	0	
Actinobacteria	5001	255	32	37	39	
Bacteroidetes	1056	4	110	86	75	
Candidate-division-TM7	2	0	0	0	0	
Chloroflexi	3	0	0	0	0	
Cyanobacteria	29	1	4	0	0	
Firmicutes	1484	28	7	48	4	
Fusobacteria	34	0	0	0	0	
Gemmatimonadetes	3	0	0	0	0	
Nitrospirae	5	0	0	0	0	
Nitrospirae	7	0	0	0	0	
Planctomycetes	7	0	0	0	0	
Proteobacteria	2079	16	151	132	185	
Synergistetes	3	0	0	0	0	
Verrucomicrobia	2	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	2	0	0	0	0	
2	Bacteria/Actinobacteria/.../Acidimicrobiales	1	0	0	0	0	
3	Bacteria/Actinobacteria/.../Acidimicrobiaceae	3	0	0	0	0	
4	Bacteria/Actinobacteria/Actinobacteria	3	1	0	0	0	
5	Bacteria/Actinobacteria/.../Actinomycetaceae	5	0	0	0	0	
6	Bacteria/Actinobacteria/.../Actinomycetes	27	1	0	1	1	
7	Bacteria/Actinobacteria/.../Mobiluncus	2	0	0	0	0	
8	Bacteria/Actinobacteria/.../Varibaculum	2	0	0	0	0	
9	Bacteria/Actinobacteria/.../Corynebacteriaceae	47	0	0	0	0	
10	Bacteria/Actinobacteria/.../Corynebacterium	1772	26	8	9	3	
11	Bacteria/Actinobacteria/.../Dietzia	4	0	0	0	0	
12	Bacteria/Actinobacteria/.../Mycobacterium	1	0	0	0	0	
13	Bacteria/Actinobacteria/.../Nocardiaceae	4	0	0	0	0	
14	Bacteria/Actinobacteria/.../Gordonia	2	0	0	0	0	
15	Bacteria/Actinobacteria/.../Rhodococcus	11	2	0	0	0	
16	Bacteria/Actinobacteria/.../Geodermatophilaceae	1	0	0	0	0	

IX. Apply a Filter

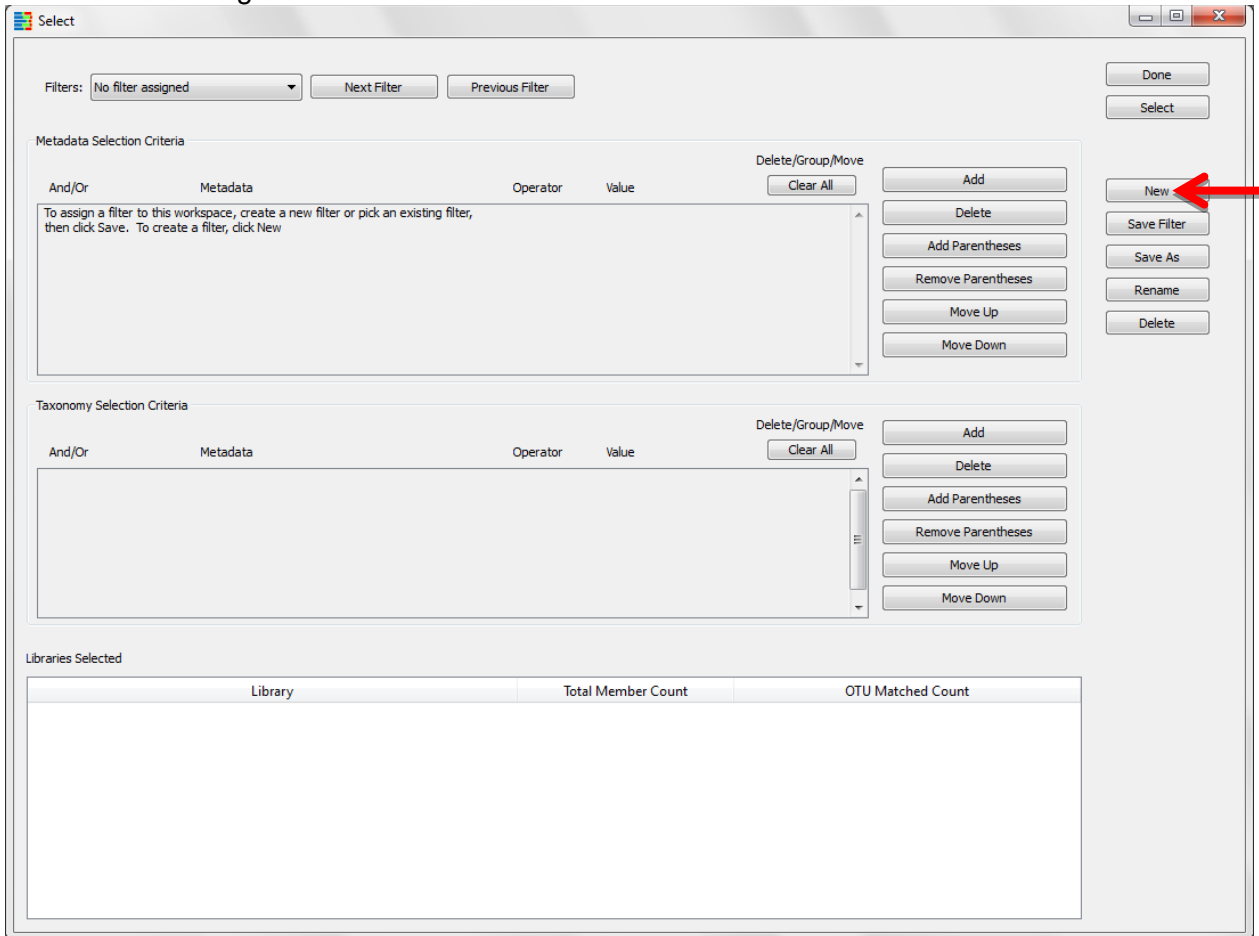
To compare data from only the back and umbilicus, we need to separate these libraries from the other body parts. This is done in Explicit via “filters”.

A. Create a Filter

Data → Select Libraries

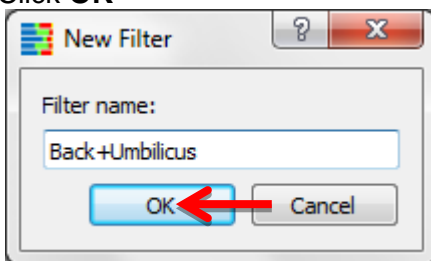
New pop-up window appears for creation of filters

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

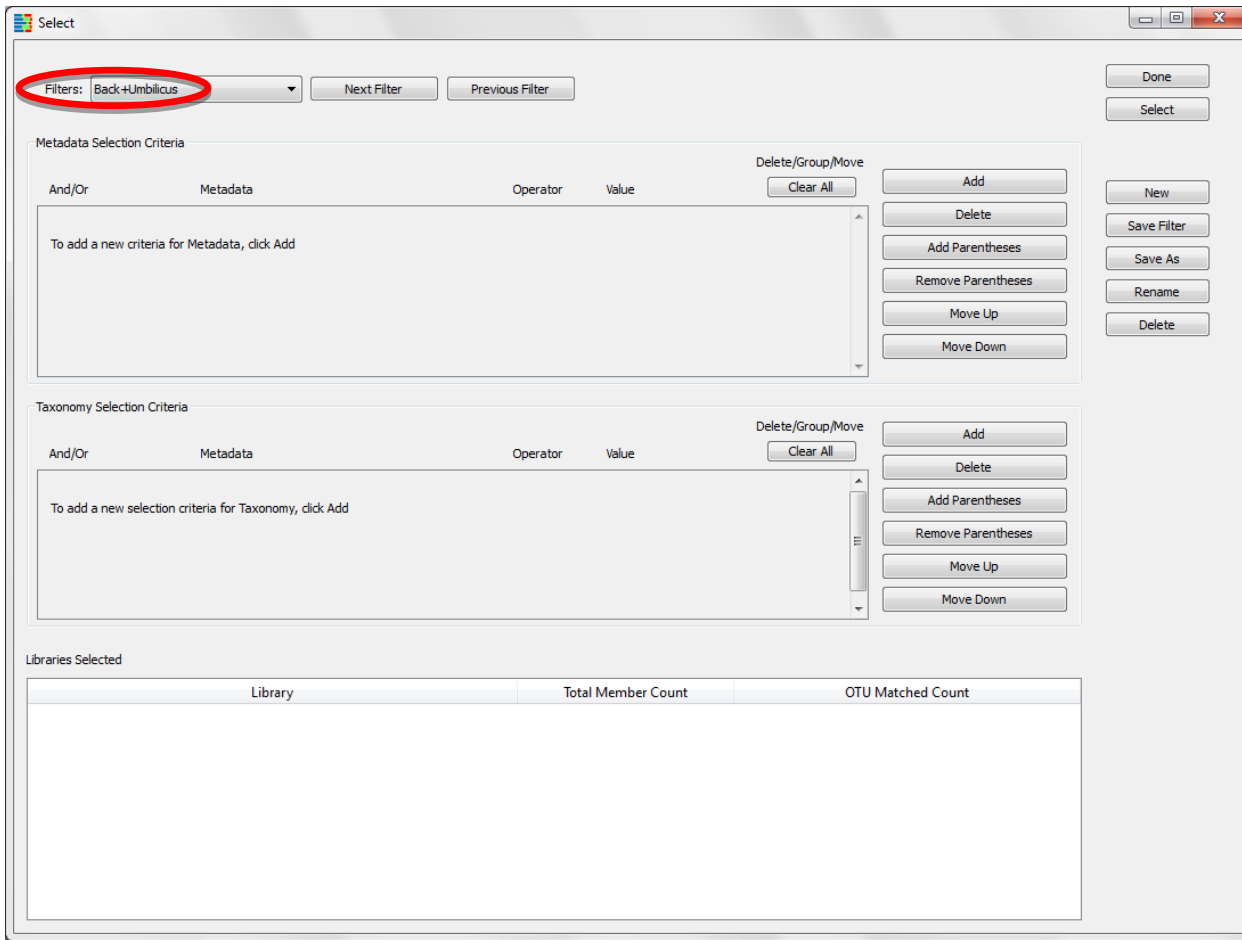


Enter desired filter name in the pop-up window

Click **OK**



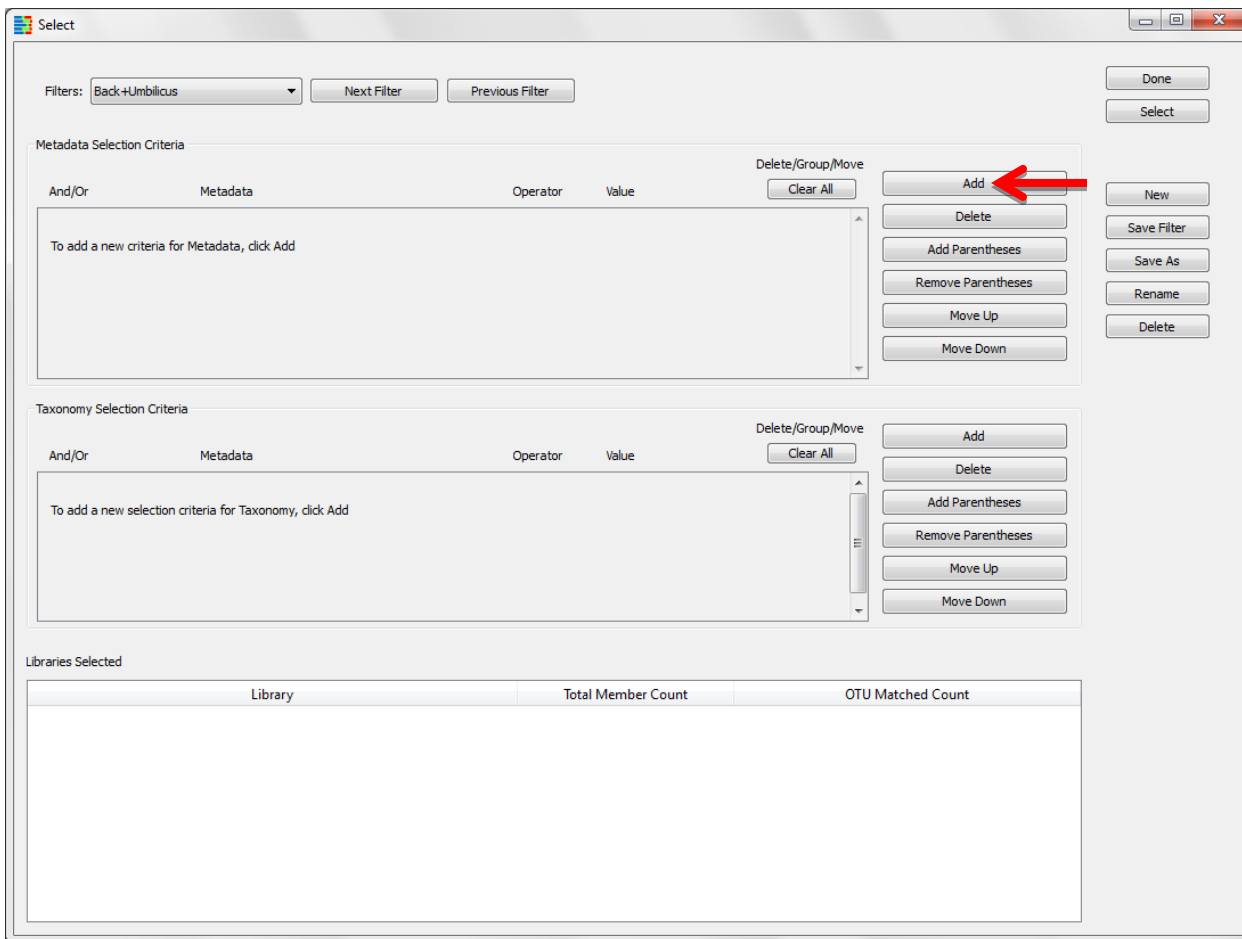
The filter name will appear in upper left corner of window.



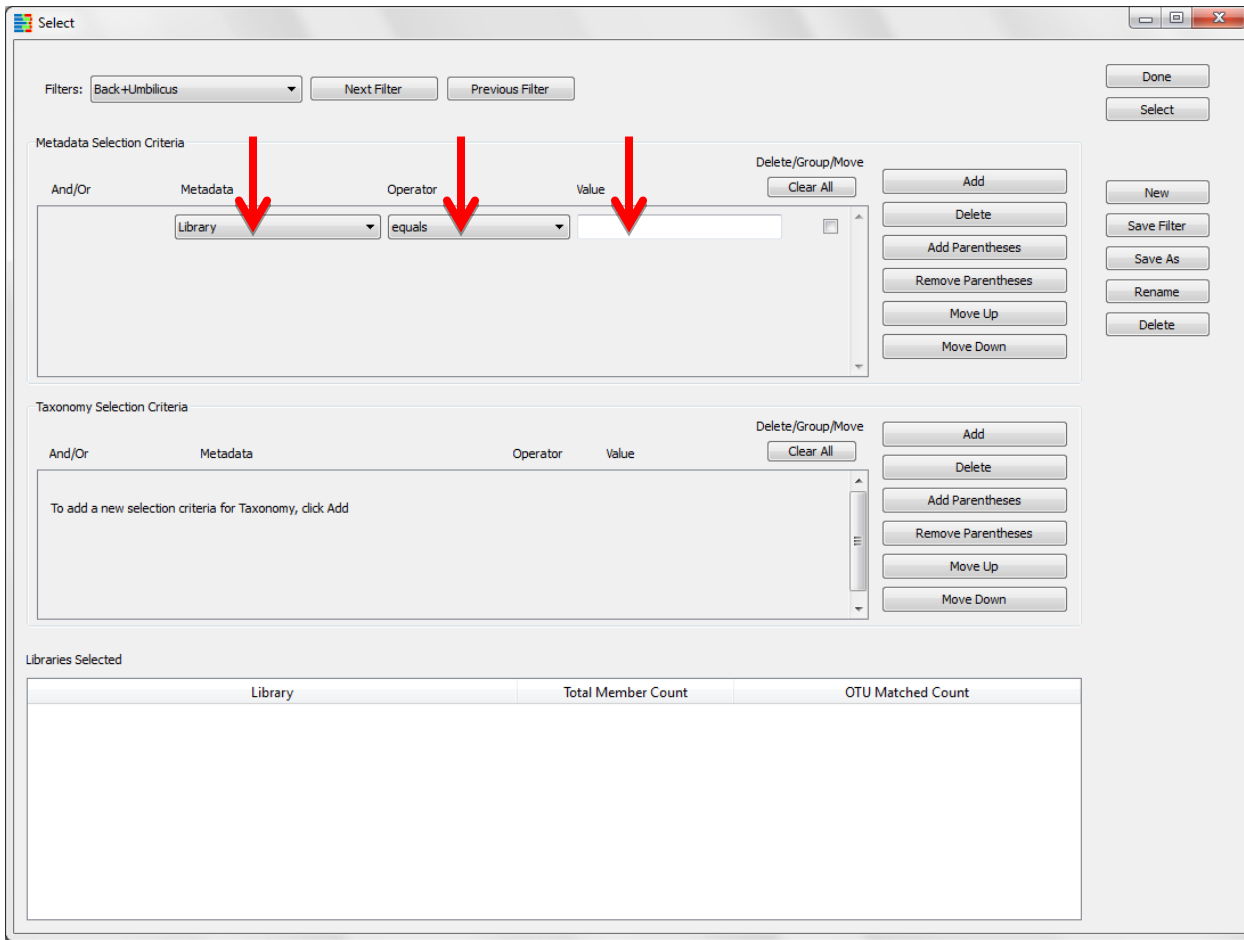
Now that we have created a new filter, we need to set up the parameters to filter by. We will select for all libraries that were sampled from the “back” or “umbilicus” anatomical sites.

B. Set Up the Filter Parameters

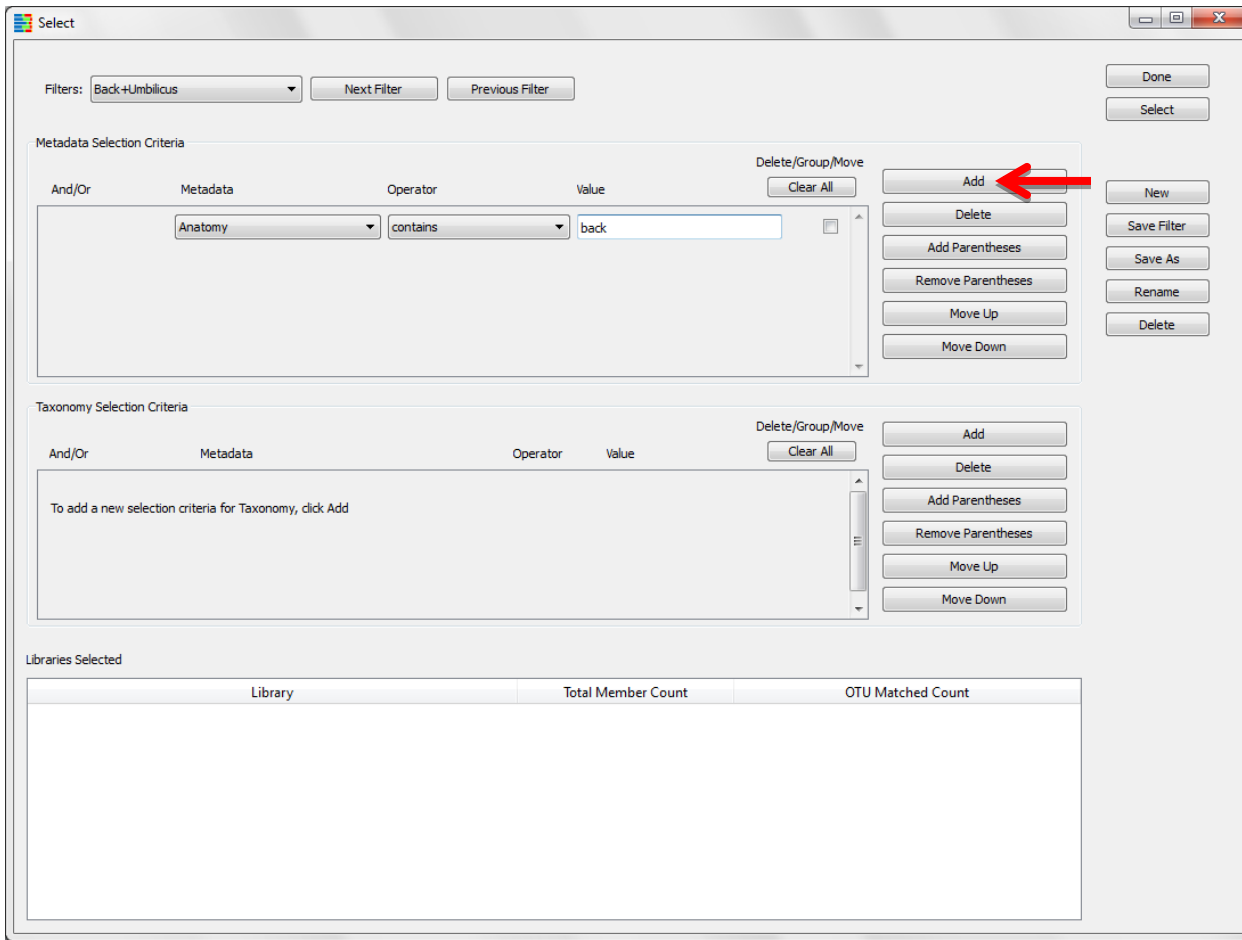
Click **Add** in the Metadata Criteria pane



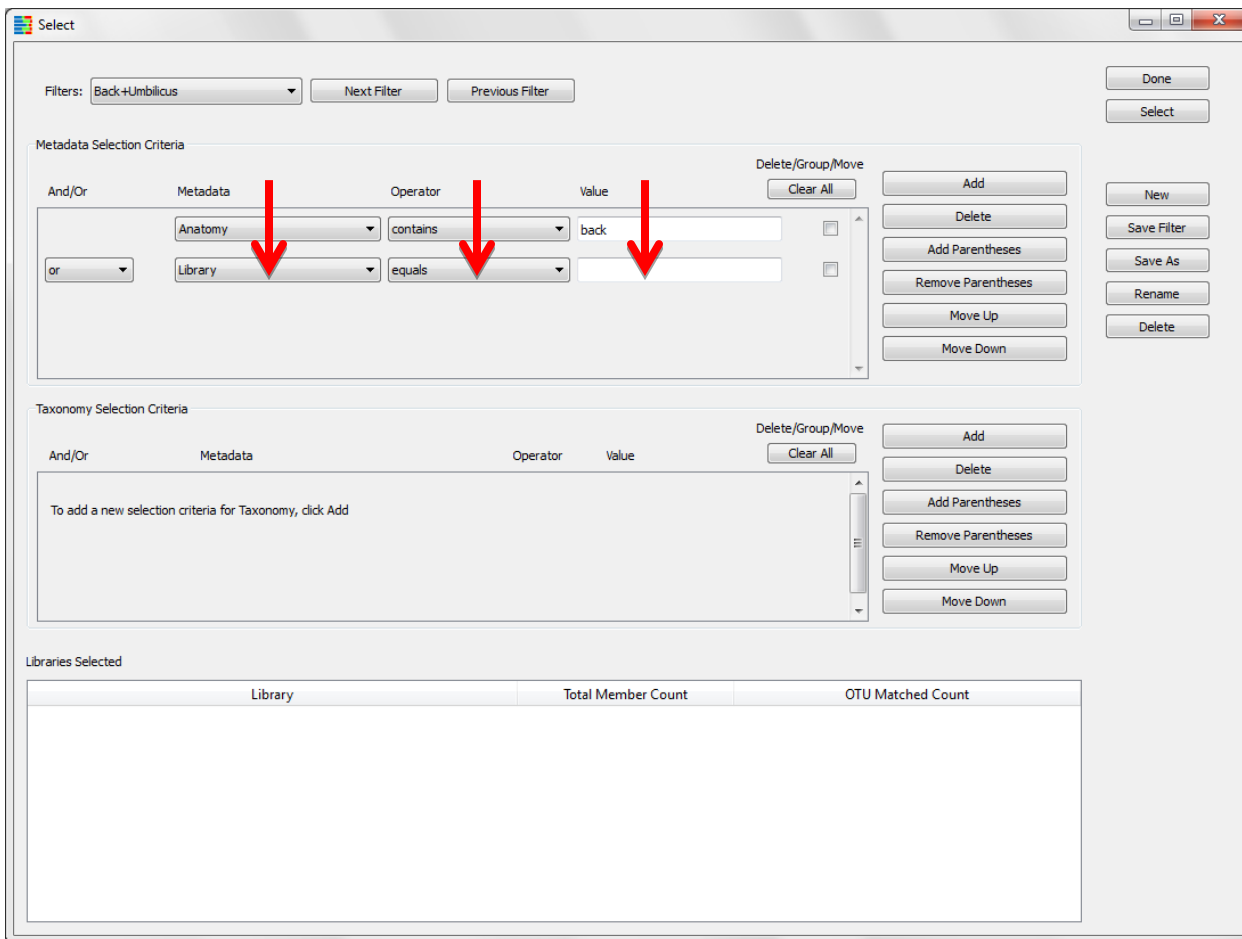
Use the first pull-down menu to select “Anatomy” (**Metadata** to filter by)
Use the second pull-down menu to select “contains” (filter **Operator**)
Enter “back” into **Value**



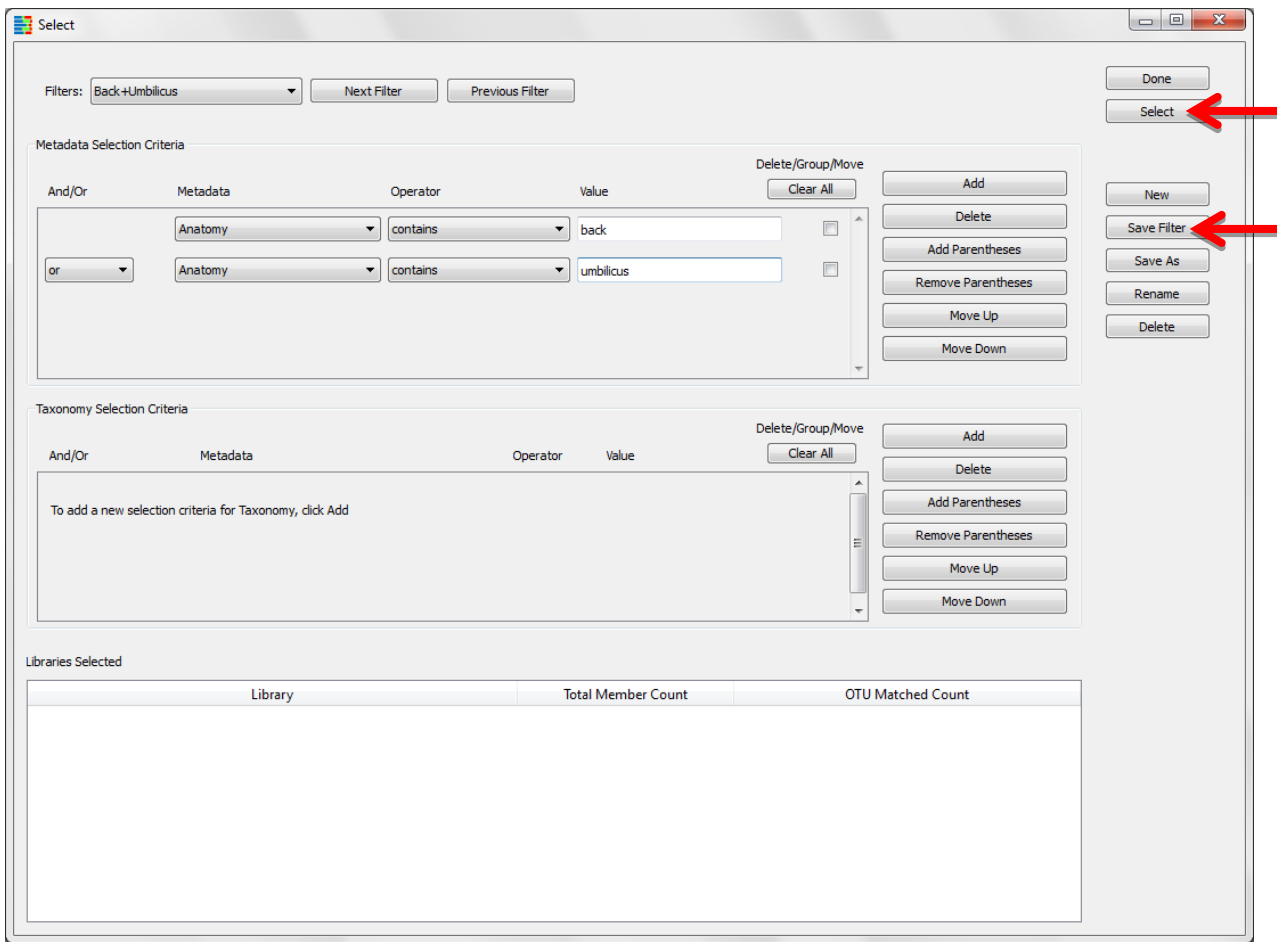
Click **Add** in the **Metadata Criteria** pane



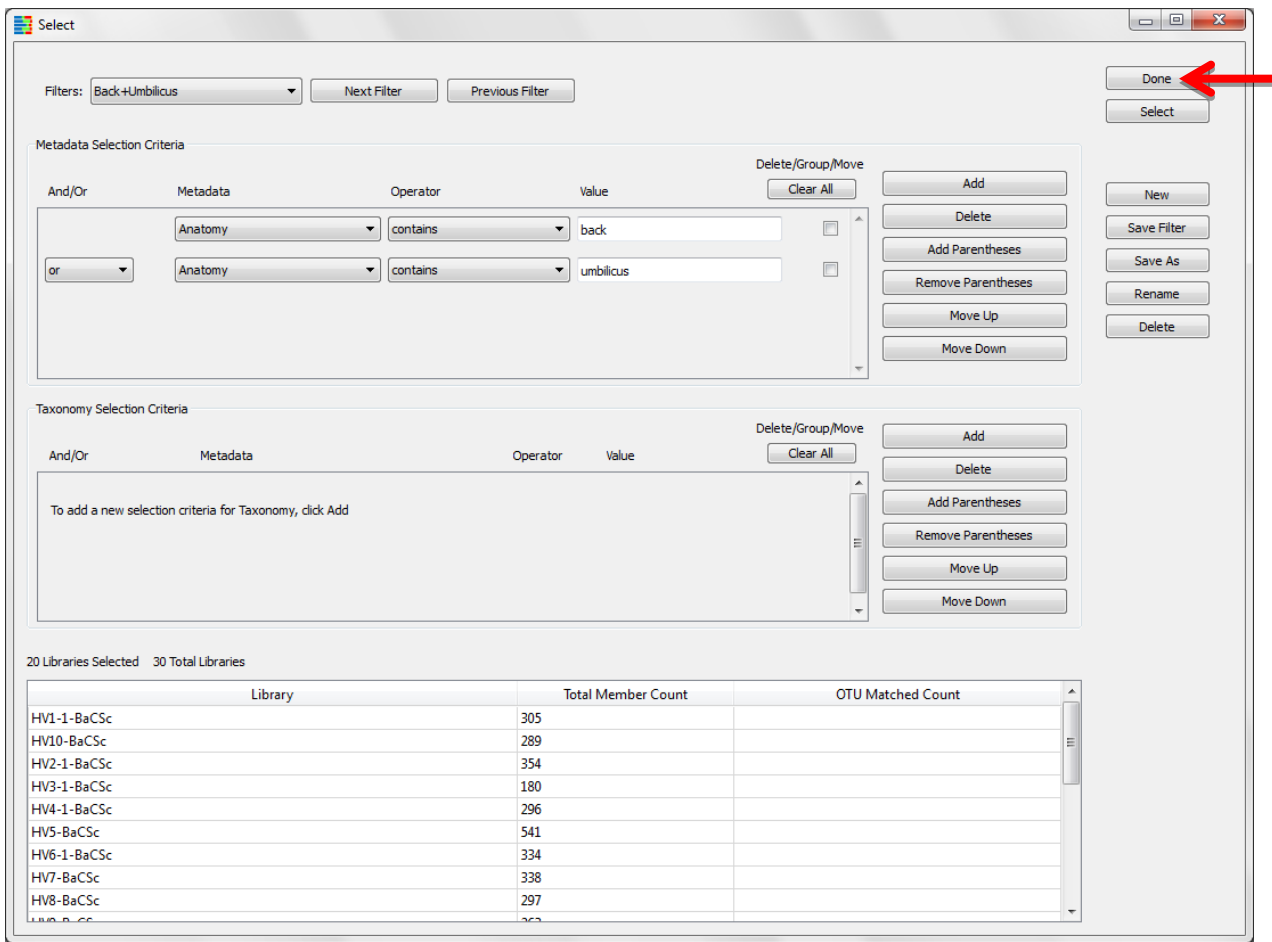
Use the first pull-down menu to select "Or"
 Use the second pull-down menu to select "Anatomy" (**Metadata** to filter by)
 Use the third pull-down menu to select "contains" (filter **Operator**)
 Enter "umbilicus" into **Value**



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



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



Pop-up window will disappear
On the main workspace window, select **Selected Libraries**

Explicit: C:/Users/kirstin/Desktop/Tutorial_HSM_Explicit_Project.otu

File Edit Data Group Tools View Help

Project: Tutorial_HSM
 Workspace: Back+Umbilicus
 Current Filter: Back+Umbilicus

Hierarchy Counts OTU Start: 1 Hierarchy Level: Show Libraries All Libraries
 OTU % of Library OTU Width: 2 3 Show Sorted Libs Selected Libraries
 Both % of Total OTU Show Last Show Lib Groups 30 Libs

Buttons: Figures, Clone Workspace, Save, Close Project

Hierarchy	Total	alar crease:HV2-1-AIRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fossa:HV8-AcRSc	back:HV1-1
root	9710	304	304	303	303	
Bacteria	9710	304	304	303	303	
Acidobacteria	2	0	0	0	0	
Actinobacteria	5001	255	32	37	39	
Bacteroidetes	1056	4	110	86	75	
Candidate-division-TM7	2	0	0	0	0	
Chloroflexi	3	0	0	0	0	
Cyanobacteria	29	1	4	0	0	
Firmicutes	1484	28	7	48	4	
Fusobacteria	34	0	0	0	0	
Gemmatimonadetes	3	0	0	0	0	
Nitrospirae	5	0	0	0	0	
Planctomycetes	7	0	0	0	0	
Proteobacteria	2079	16	151	132	185	
Synergistetes	3	0	0	0	0	
Verrucomicrobia	2	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
root		9710	304	304	303	303	
1	Bacteria/Acidobacteria/.../Candidatus-Chloraci	2	0	0	0	0	
2	Bacteria/Actinobacteria/.../Acidimicrobiales	1	0	0	0	0	
3	Bacteria/Actinobacteria/.../Acidimicrobiaceae	3	0	0	0	0	
4	Bacteria/Actinobacteria/Actinobacteria	3	1	0	0	0	
5	Bacteria/Actinobacteria/.../Actinomycetaceae	5	0	0	0	0	
6	Bacteria/Actinobacteria/.../Actinomycetes	27	1	0	1	1	
7	Bacteria/Actinobacteria/.../Mobiluncus	2	0	0	0	0	
8	Bacteria/Actinobacteria/.../Varibaculum	2	0	0	0	0	
9	Bacteria/Actinobacteria/.../Corynebacteriaceae	47	0	0	0	0	
10	Bacteria/Actinobacteria/.../Corynebacterium	1772	26	8	9	3	
11	Bacteria/Actinobacteria/.../Dietzia	4	0	0	0	0	
12	Bacteria/Actinobacteria/.../Mycobacterium	1	0	0	0	0	
13	Bacteria/Actinobacteria/.../Nocardiaceae	4	0	0	0	0	
14	Bacteria/Actinobacteria/.../Gordonia	2	0	0	0	0	
15	Bacteria/Actinobacteria/.../Rhodococcus	11	2	0	0	0	
16	Bacteria/Actinobacteria/.../Geodermatophilaceae	1	0	0	0	0	

Ready

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.

Explicit: C:/Users/kirstin/Desktop/Tutorial_HSM_Explicit_Project.otu

File Edit Data Group Tools View Help

Project: Tutorial_HSM

Workpace: Back+Umbilicus

Current Filter: Back+Umbilicus

Hierarchy Counts OTU Start: 1 Hierarchy Level: Show Libraries All Libraries
 OTU % of Library OTU Width: 2 Show Sorted Libs Selected Libraries
 Both % of Total OTU Show Last Show Lib Groups 20 of 30 Libs

Figures
Clone Workspace
Save
Close Project

Hierarchy	Total	back:HV1-1-BaCSc	back:HV10-BaCSc	back:HV2-1-BaCSc	back:HV3-1-BaCSc	back:HV4-1-BaCSc	back:HV5-BaCSc
root	6670	305	289	354	180	296	541
▲ Bacteria	6670	305	289	354	180	296	541
▶ Actinobacteria	4029	295	235	331	165	254	514
▶ Bacteroidetes	513	0	4	6	7	8	1
▶ Candidate-division-TM7	2	0	0	0	1	0	0
▶ Cyanobacteria	10	0	0	0	0	0	0
▶ Firmicutes	1160	10	7	10	1	7	17
▶ Fusobacteria	27	0	0	0	0	0	0
▶ Proteobacteria	926	0	43	7	6	27	9
▶ Synergistetes	3	0	0	0	0	0	0

OTU	Rule Set: ...	Total	back:HV1-1-BaCSc	back:HV10-BaCSc	back:HV2-1-BaCSc	back:HV3-1-BaCSc	back:HV4-1-BaCSc	back:HV5-BaCSc
root		6670	305	289	354	180	296	541
1	Bacteria/Actinobacteria/Actinobacteria	2	0	0	0	0	0	0
2	Bacteria/Actinobacteria/./Actinomycetaceae	5	0	0	0	0	0	0
3	Bacteria/Actinobacteria/./Actinomycetes	16	0	0	0	0	0	0
4	Bacteria/Actinobacteria/./Varibaculum	2	0	0	0	0	0	0
5	Bacteria/Actinobacteria/./Corynebacteriaceae	20	0	0	0	0	0	0
6	Bacteria/Actinobacteria/./Corynebacterium	1487	3	0	1	3	0	24
7	Bacteria/Actinobacteria/./Dietzia	4	0	0	0	0	0	0
8	Bacteria/Actinobacteria/./Mycobacterium	1	0	0	0	0	0	0
9	Bacteria/Actinobacteria/./Nocardiaceae	4	0	0	0	0	0	4
10	Bacteria/Actinobacteria/./Gordonia	1	0	0	0	1	0	0
11	Bacteria/Actinobacteria/./Rhodococcus	5	0	0	1	0	0	0
12	Bacteria/Actinobacteria/./Geodermatophilaceae	1	0	0	0	0	0	0
13	Bacteria/Actinobacteria/./Brevibacterium	3	0	0	0	0	0	0
14	Bacteria/Actinobacteria/./Dermabacteraceae	9	0	0	0	0	0	0
15	Bacteria/Actinobacteria/./Microbacteriaceae	6	0	0	0	0	0	0
16	Bacteria/Actinobacteria/./Microbacterium	2	0	0	0	0	0	0

Ready

X. Beta Diversity (Morisita-Horn)

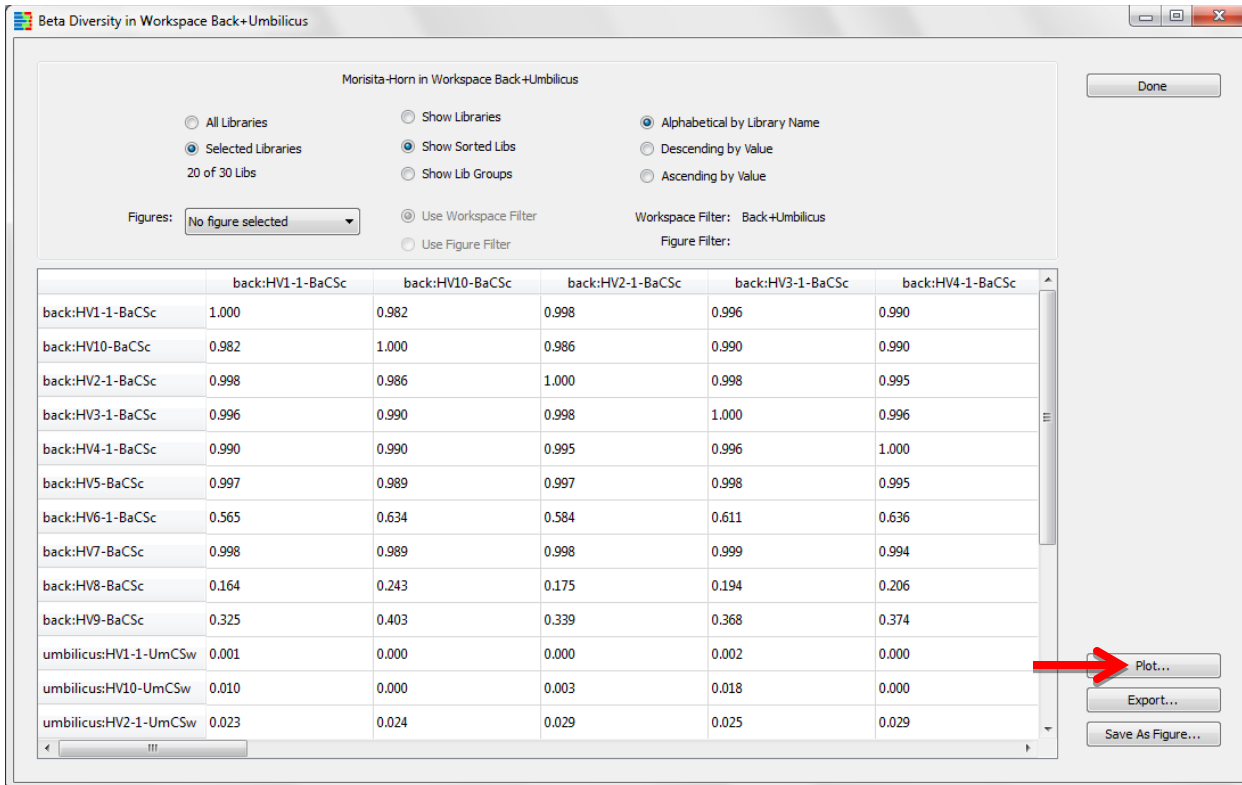
By viewing our libraries in a Morisita-Horn heatmap, we can estimate the similarity of the microbial communities present in the samples at these two anatomical positions.

A. Create a Morisita-Horn Heatmap

Tools → Analyze → Beta Diversity → Morisita-Horn

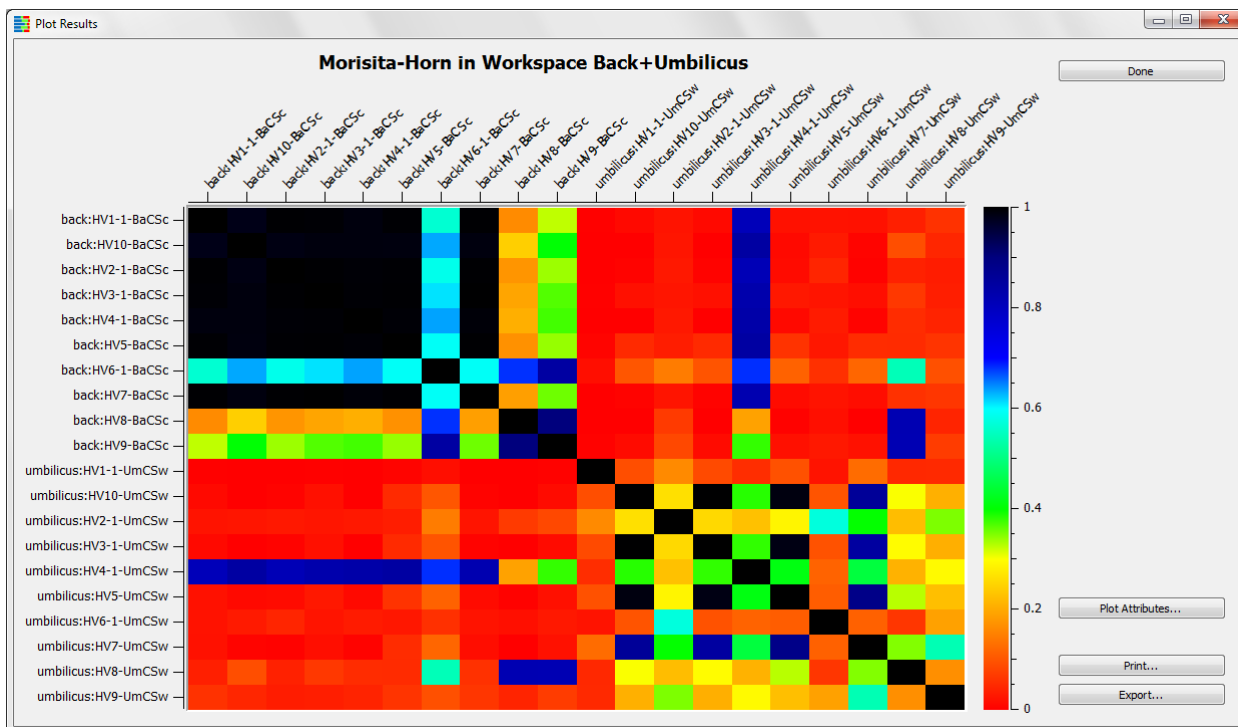
A new window will appear with a table of the sequence variant counts

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 identical OTU counts appear black, while anatomical positions with very different OTU counts appear red. Based on this data, the umbilicus is more similar across subjects than the back. Interestingly, a subset of back libraries has high similarity with 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

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**

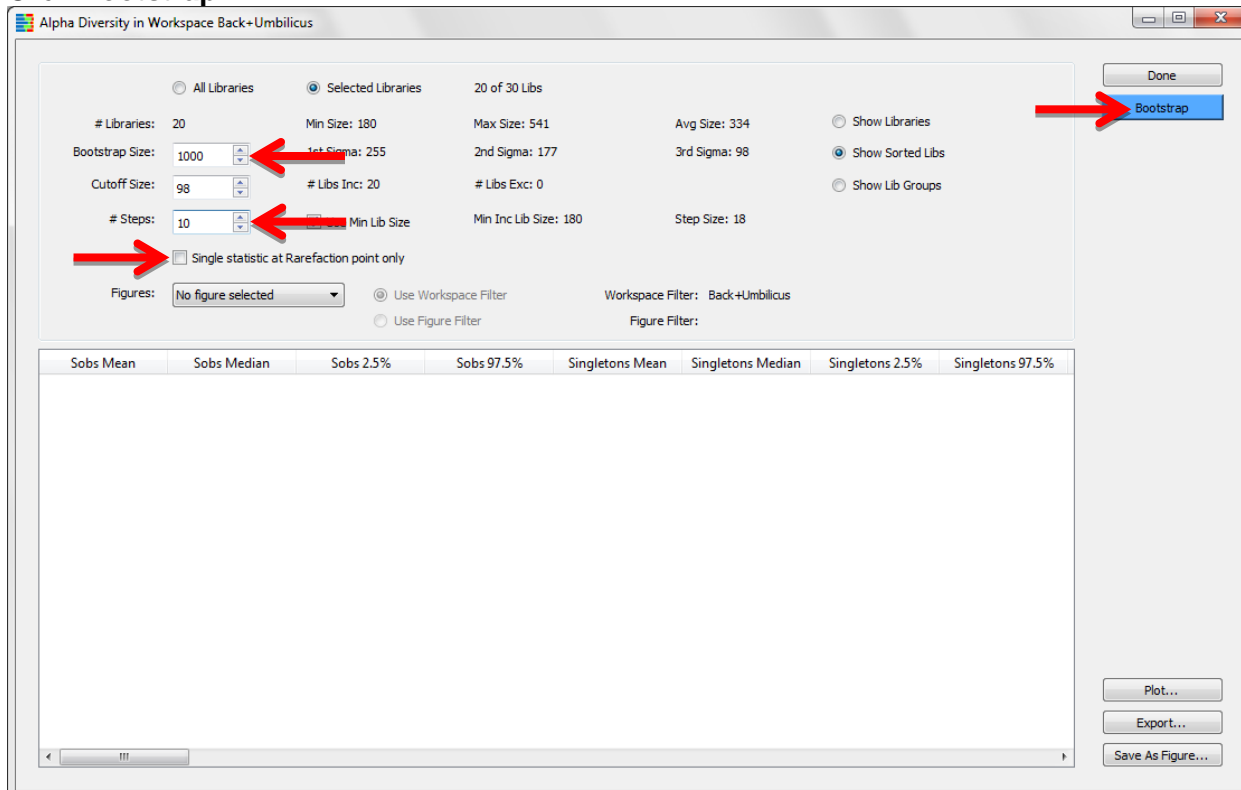
New pop-up window appears

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

Change # **Steps** to 10

To create smooth curves, change **Bootstrap Size** to 1000

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: 1000
 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

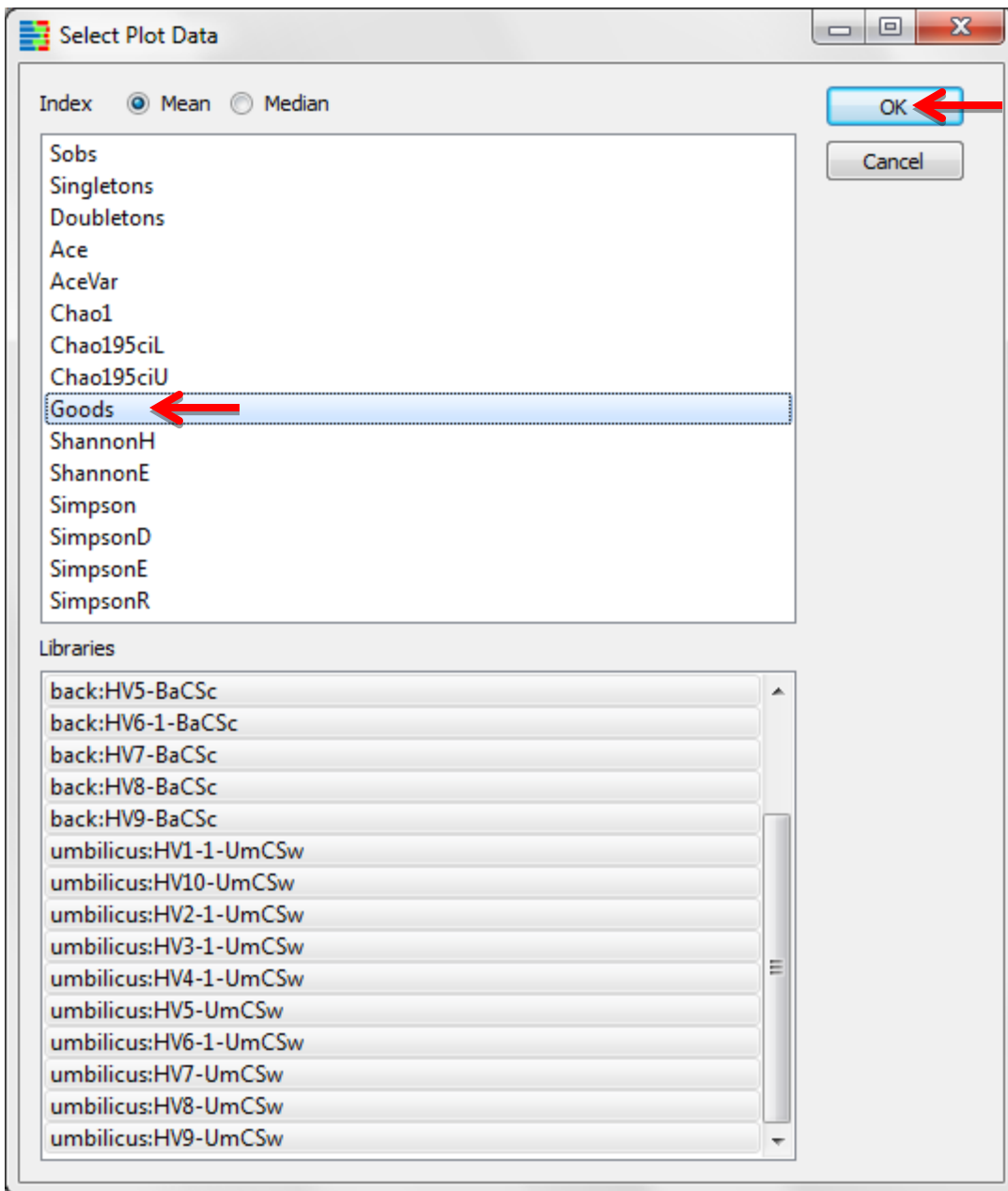
Single statistic at Rarefaction point only

Figures:
 Use Workspace Filter
 Workspace Filter: Back+Umbilicus

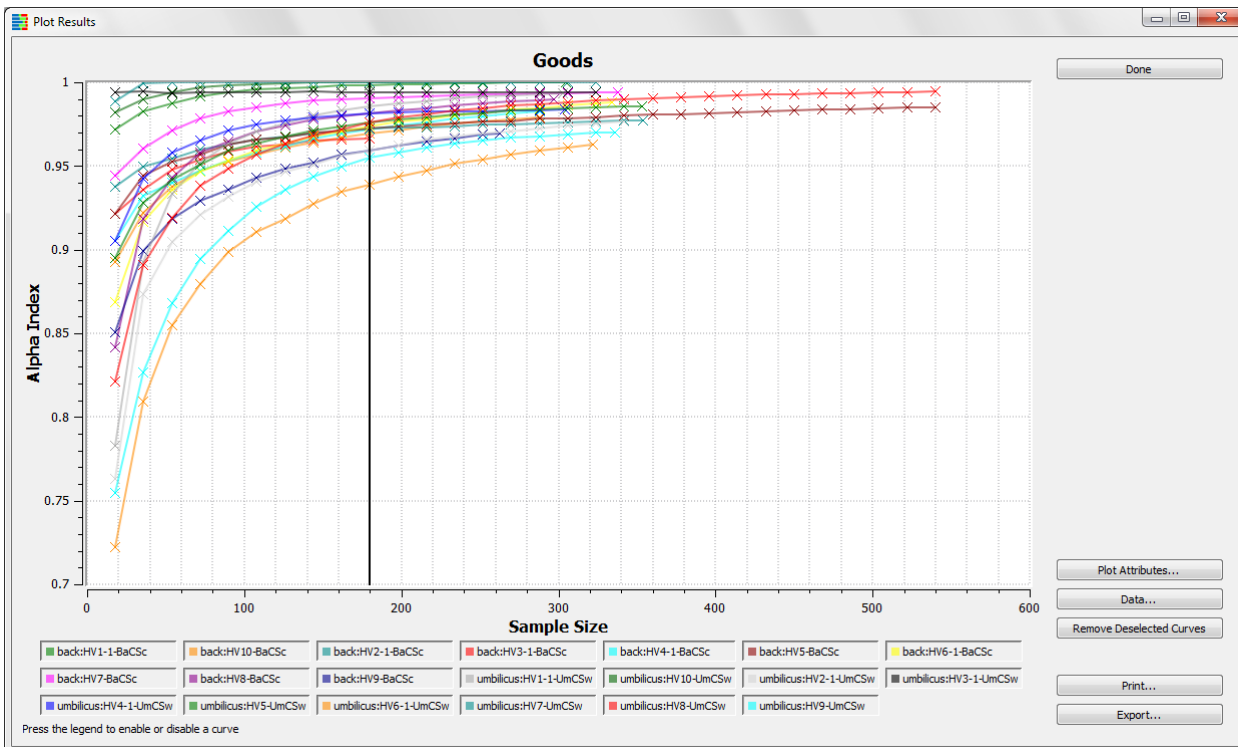
Use Figure Filter
 Figure Filter:

	Sobs Mean	Sobs Median	Sobs 2.5%	Sobs 97.5%	Singletons Mean	Singletons Median	Sing
back:HV1-1-BaCSc : 18	1.624	2.000	1.000	3.000	0.503	0.000	0.000
back:HV1-1-BaCSc : 36	2.010	2.000	1.000	3.000	0.617	1.000	0.000
back:HV1-1-BaCSc : 54	2.297	2.000	1.000	3.000	0.675	1.000	0.000
back:HV1-1-BaCSc : 72	2.417	2.000	1.000	3.000	0.588	0.000	0.000
back:HV1-1-BaCSc : 90	2.551	3.000	2.000	3.000	0.552	0.000	0.000
back:HV1-1-BaCSc : 108	2.645	3.000	2.000	3.000	0.477	0.000	0.000
back:HV1-1-BaCSc : 126	2.735	3.000	2.000	3.000	0.436	0.000	0.000
back:HV1-1-BaCSc : 144	2.771	3.000	2.000	3.000	0.399	0.000	0.000
back:HV1-1-BaCSc : 162	2.839	3.000	2.000	3.000	0.334	0.000	0.000
back:HV1-1-BaCSc : 180	2.852	3.000	2.000	3.000	0.302	0.000	0.000
back:HV1-1-BaCSc : 198	2.908	3.000	2.000	3.000	0.249	0.000	0.000

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 **Two-Part** 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

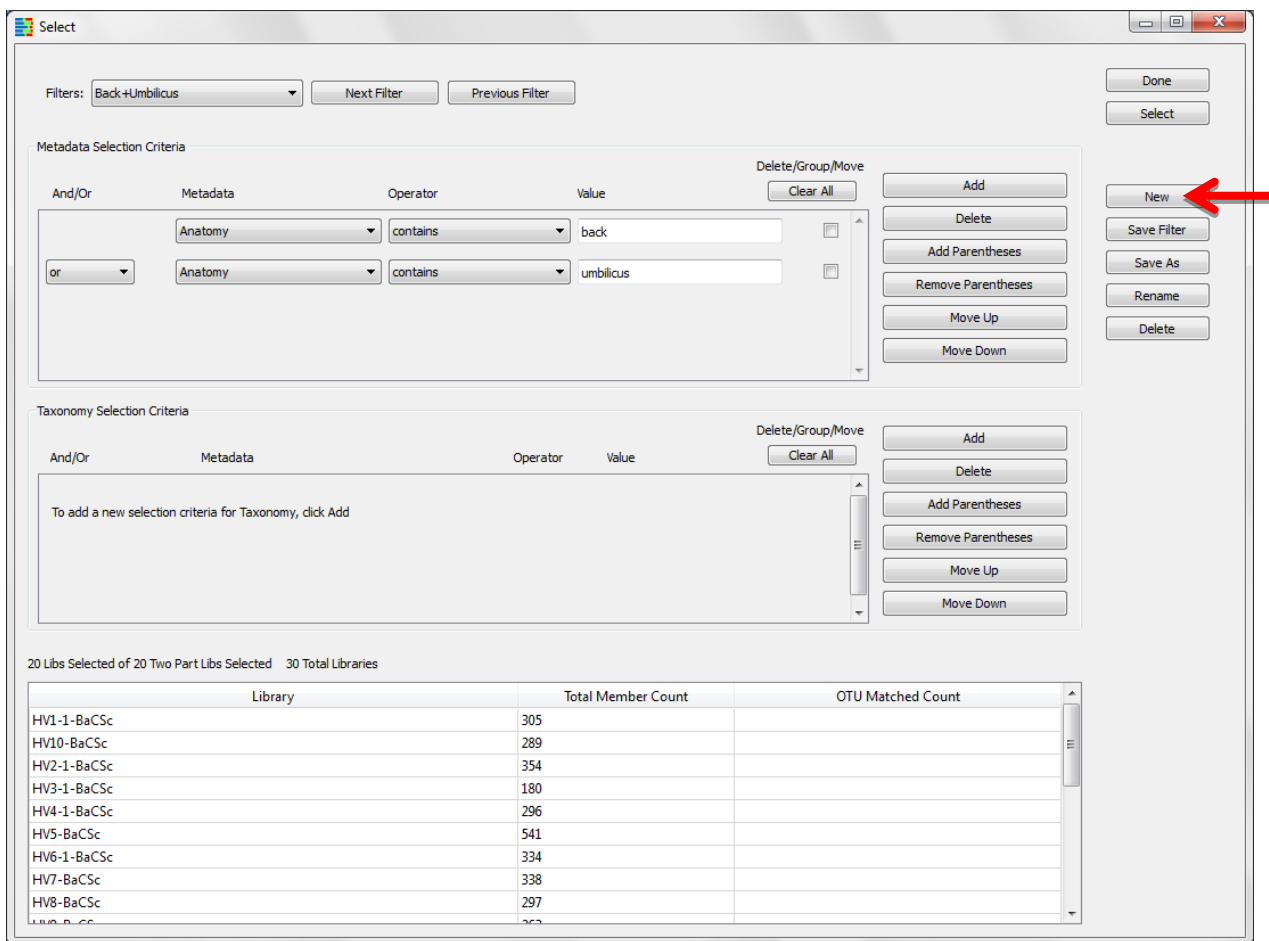
Figures: No figure selected Use Workspace Filter Use Figure Filter Workspace Filter: Back+Umbilicus Figure Filter:

Collisions: Libraries that are in both Category 1 and Category 2

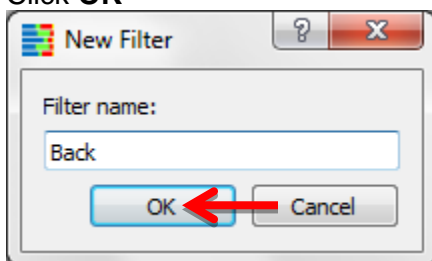
HV1-1-BaCSc
HV10-BaCSc
HV2-1-BaCSc
HV3-1-BaCSc
HV4-1-BaCSc
HV5-BaCSc
HV6-1-BaCSc
HV7-BaCSc
HV8-BaCSc
HV9-BaCSc
HV1-1-UmCSw
HV10-UmCSw
HV2-1-UmCSw
HV3-1-UmCSw
HV4-1-UmCSw
HV5-UmCSw
HV6-1-UmCSw
HV7-UmCSw
HV8-UmCSw
HV9-UmCSw

New pop-up window appears for creation of filters

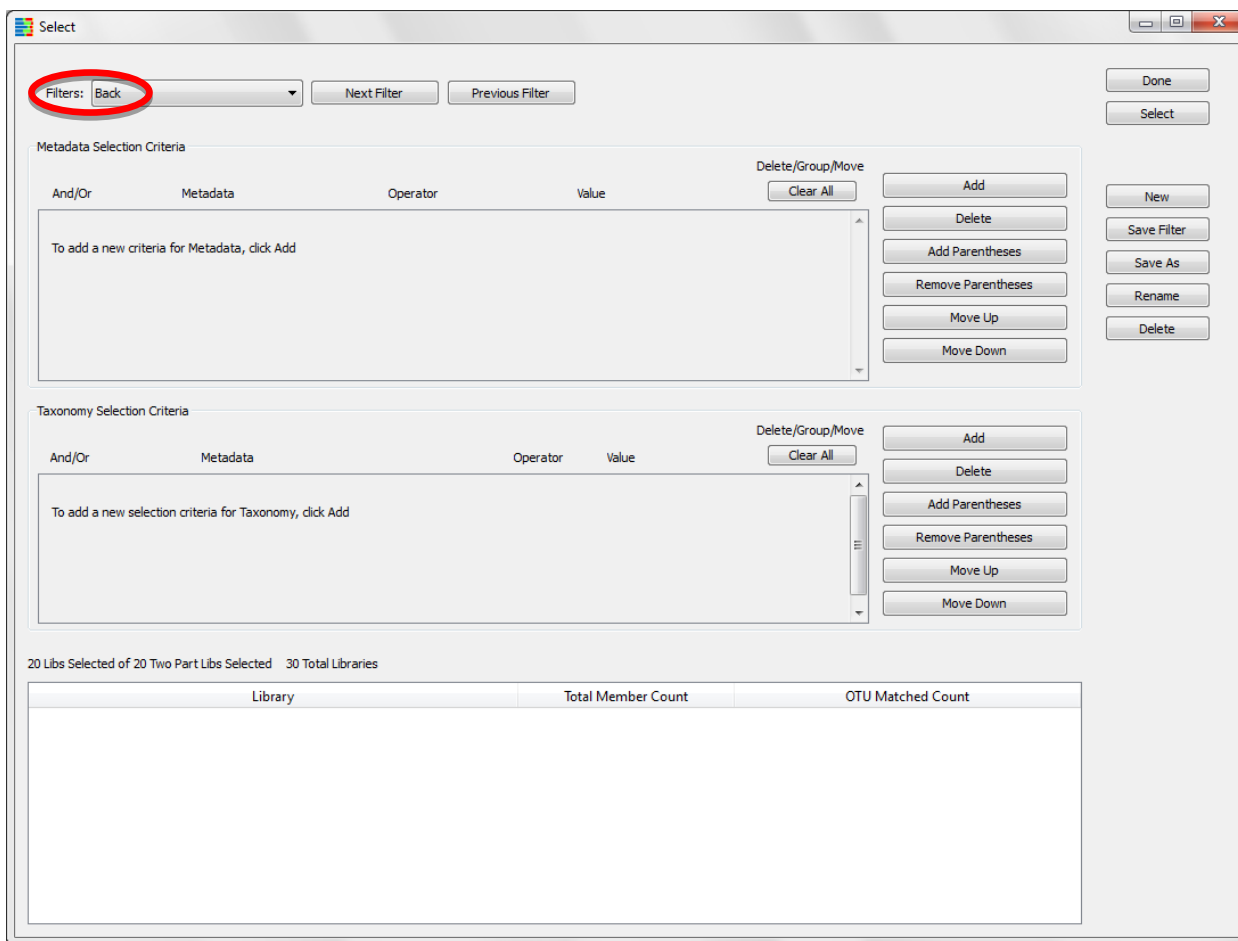
Click **New** on far right side of window



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



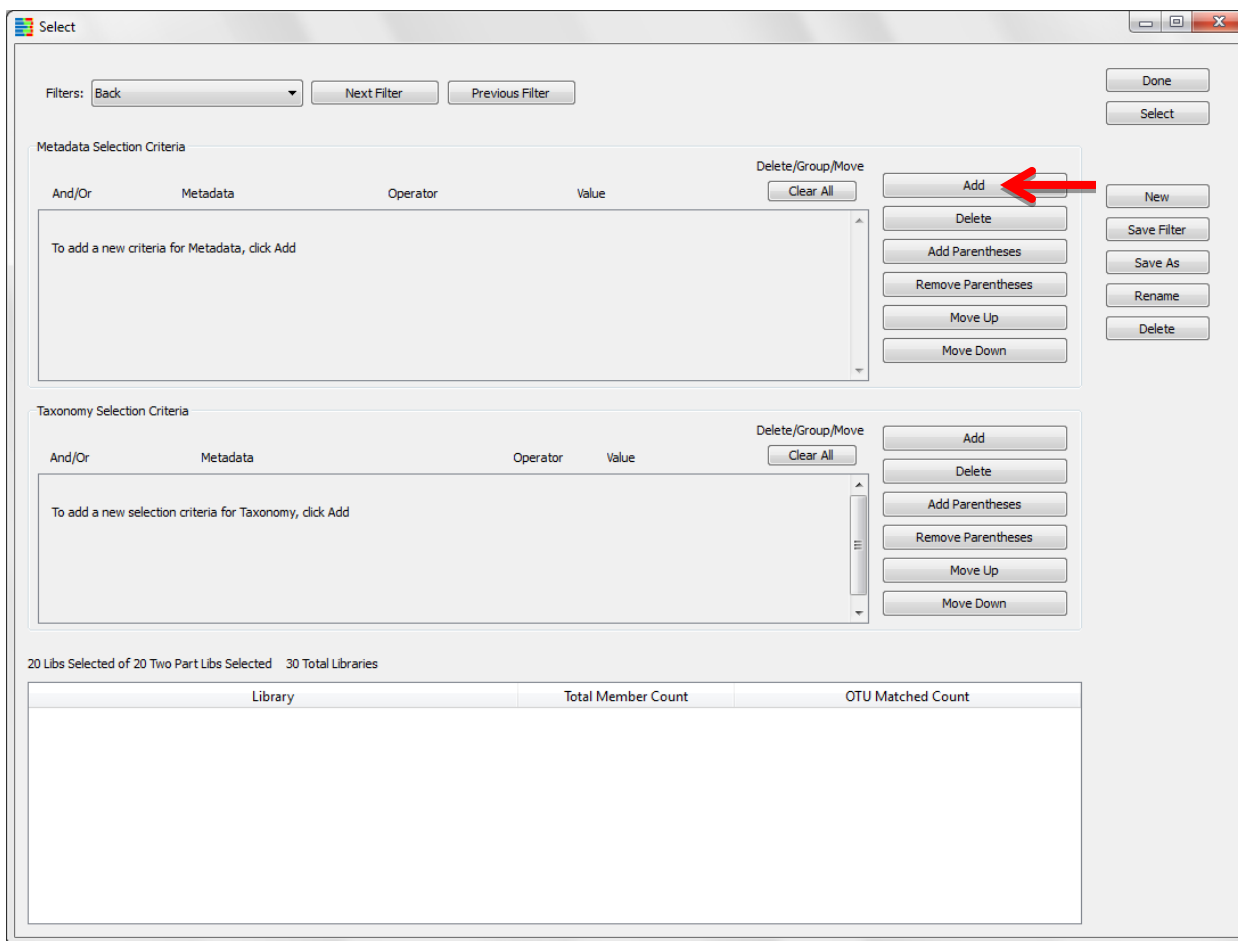
The filter name will appear in upper left corner of window.



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



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 filter configuration interface. At the top, there are buttons for "Back", "Next Filter", and "Previous Filter". The "Filters:" dropdown is set to "Back".

Metadata Selection Criteria

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

Buttons for Metadata Selection Criteria: 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				

Buttons for Taxonomy Selection Criteria: 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
---------	--------------------	-------------------

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" with a filter configuration interface. At the top, there are buttons for "Back", "Next Filter", and "Previous Filter". The "Filters:" dropdown is set to "Back". On the right side, there are buttons for "Done", "Select", "New", "Save Filter", "Save As", "Rename", and "Delete". Two red arrows point to the "Select" and "Save Filter" buttons.

The main area is divided into two sections: "Metadata Selection Criteria" and "Taxonomy Selection Criteria".

Metadata Selection Criteria:

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

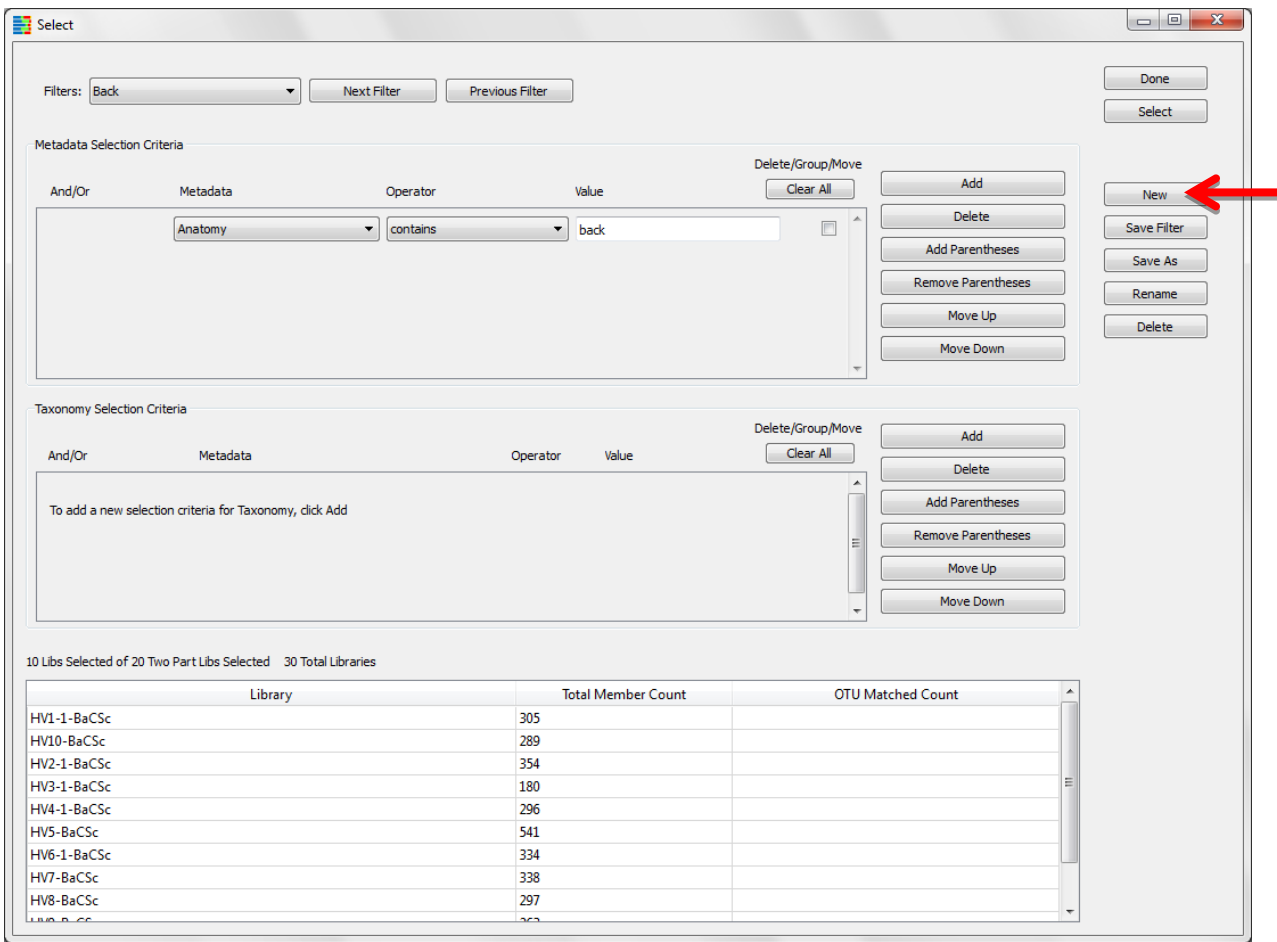
Taxonomy Selection Criteria:

To add a new selection criteria for Taxonomy, click Add

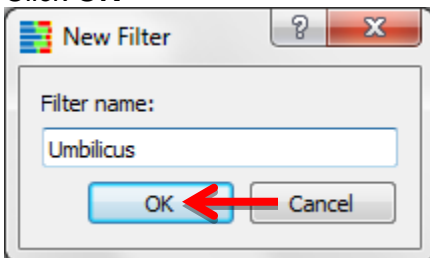
20 Libs Selected of 20 Two Part Libs Selected 30 Total Libraries

Library	Total Member Count	OTU Matched Count
---------	--------------------	-------------------

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.

The screenshot shows the 'Select' application window. At the top left, a dropdown menu labeled 'Filters:' contains the text 'Umbilicus', which is circled in red. To its right are 'Next Filter' and 'Previous Filter' buttons. On the right side of the window, there are buttons for 'Done', 'Select', 'New', 'Save Filter', 'Save As', 'Rename', and 'Delete'. The main area is divided into two sections: 'Metadata Selection Criteria' and 'Taxonomy Selection Criteria'. Each section has a table with columns for 'And/Or', 'Metadata', 'Operator', and 'Value'. Below each table is a text prompt: 'To add a new criteria for Metadata, click Add' and 'To add a new selection criteria for Taxonomy, click Add'. To the right of each table is a 'Delete/Group/Move' section with a 'Clear All' button and a vertical stack of buttons: 'Add', 'Delete', 'Add Parentheses', 'Remove Parentheses', 'Move Up', and 'Move Down'. At the bottom left, a status bar reads '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.

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 “umbilicus”.

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

The screenshot shows the 'Select' application window. At the top, there is a 'Filters' section with a dropdown menu set to 'Umbilicus' and buttons for 'Next Filter' and 'Previous Filter'. Below this are two main panes: 'Metadata Selection Criteria' and 'Taxonomy Selection Criteria'. Each pane has a table with columns for 'And/Or', 'Metadata', 'Operator', and 'Value'. The 'Metadata Selection Criteria' pane is currently empty, with a message 'To add a new criteria for Metadata, click Add'. To the right of each table is a 'Delete/Group/Move' column with buttons for 'Clear All', 'Add', 'Delete', 'Add Parentheses', 'Remove Parentheses', 'Move Up', and 'Move Down'. A red arrow points to the 'Add' button in the 'Metadata Selection Criteria' pane. On the far right of the window, there is a vertical column of buttons: 'Done', 'Select', 'New', 'Save Filter', 'Save As', 'Rename', and 'Delete'. At the bottom of the window, there is a status bar showing '10 Libs Selected of 20 Two Part Libs Selected 30 Total Libraries' and a table with columns for '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 "umbilicus" into Value

Filters: Umbilicus Next Filter Previous Filter

Done
Select

Metadata Selection Criteria

And/Or Metadata Operator Value Delete/Group/Move

Library equals 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

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

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

The screenshot shows a software window titled "Select". At the top, there are buttons for "Next Filter" and "Previous Filter", and a "Filters:" dropdown menu currently set to "Umbilicus". Below this is the "Metadata Selection Criteria" section, which contains a table with columns for "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 this table are buttons for "Delete/Group/Move" (including "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 its right are similar "Delete/Group/Move" and "Add", "Delete", "Add Parentheses", "Remove Parentheses", "Move Up", and "Move Down" buttons. At the bottom of the window, there is a status bar that reads "10 Libs Selected of 20 Two Part Libs Selected 30 Total Libraries" and 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.

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	Clear All

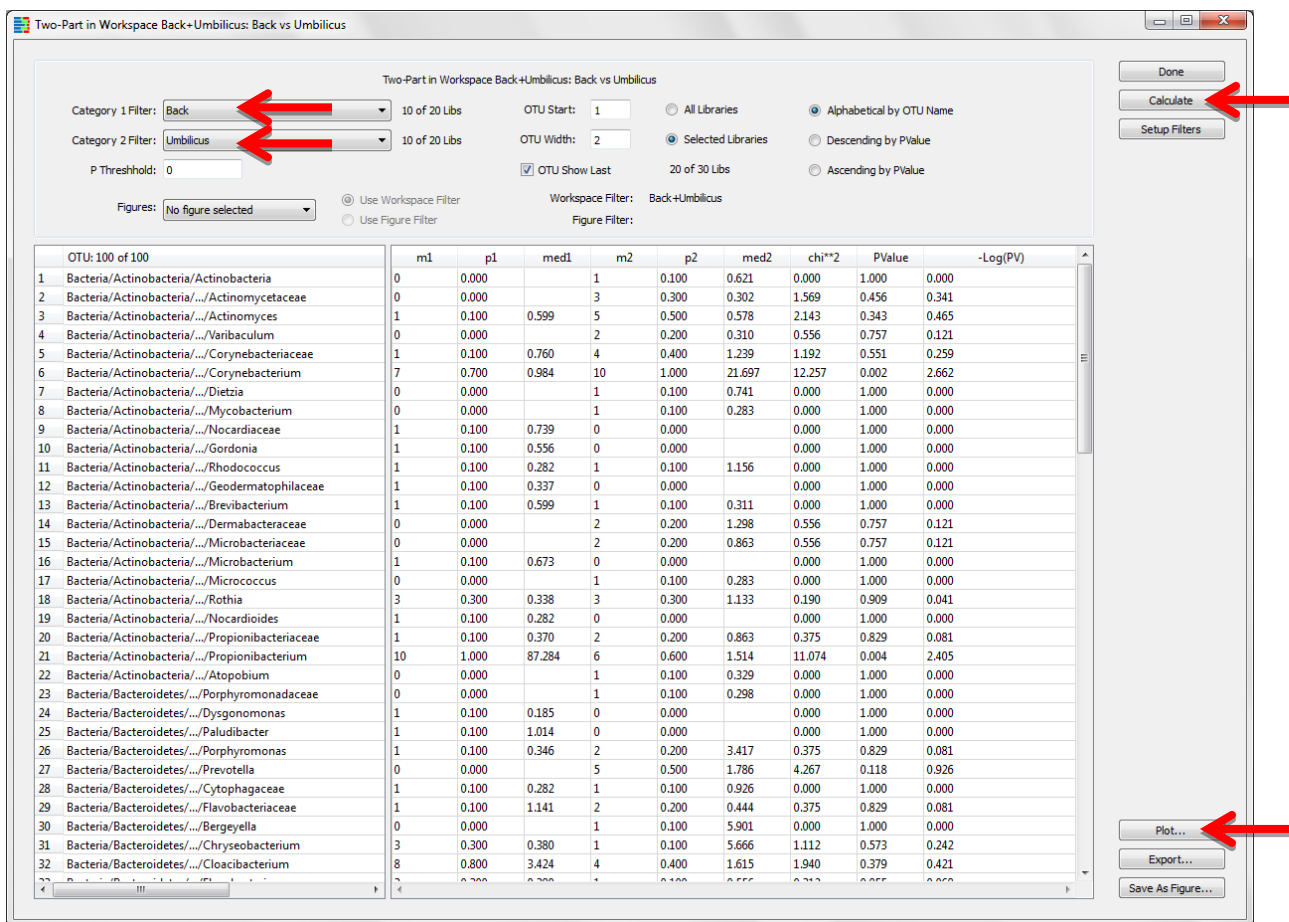
Taxonomy Selection Criteria

To add a new selection criteria for Taxonomy, click Add

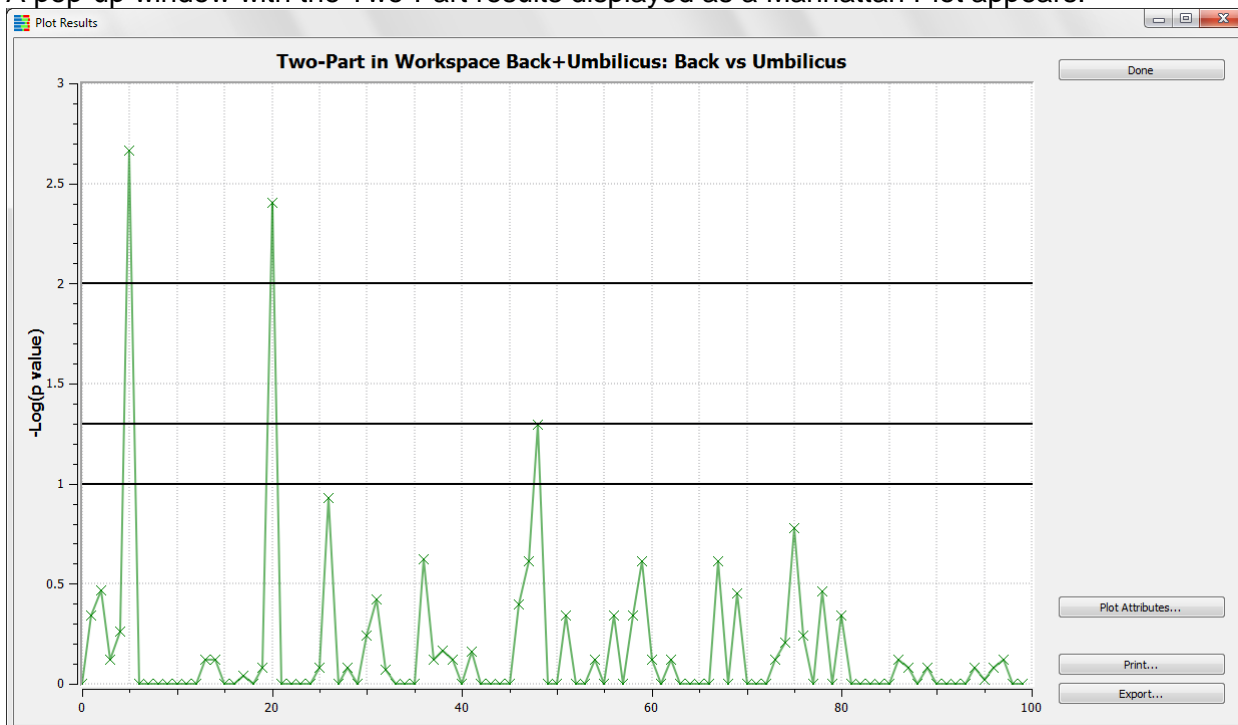
10 Libs Selected of 20 Two Part Libs Selected 30 Total Libraries

Library	Total Member Count	OTU Matched Count
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	
HV9-1-UmCSw	326	

- 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.

In the example above, 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.

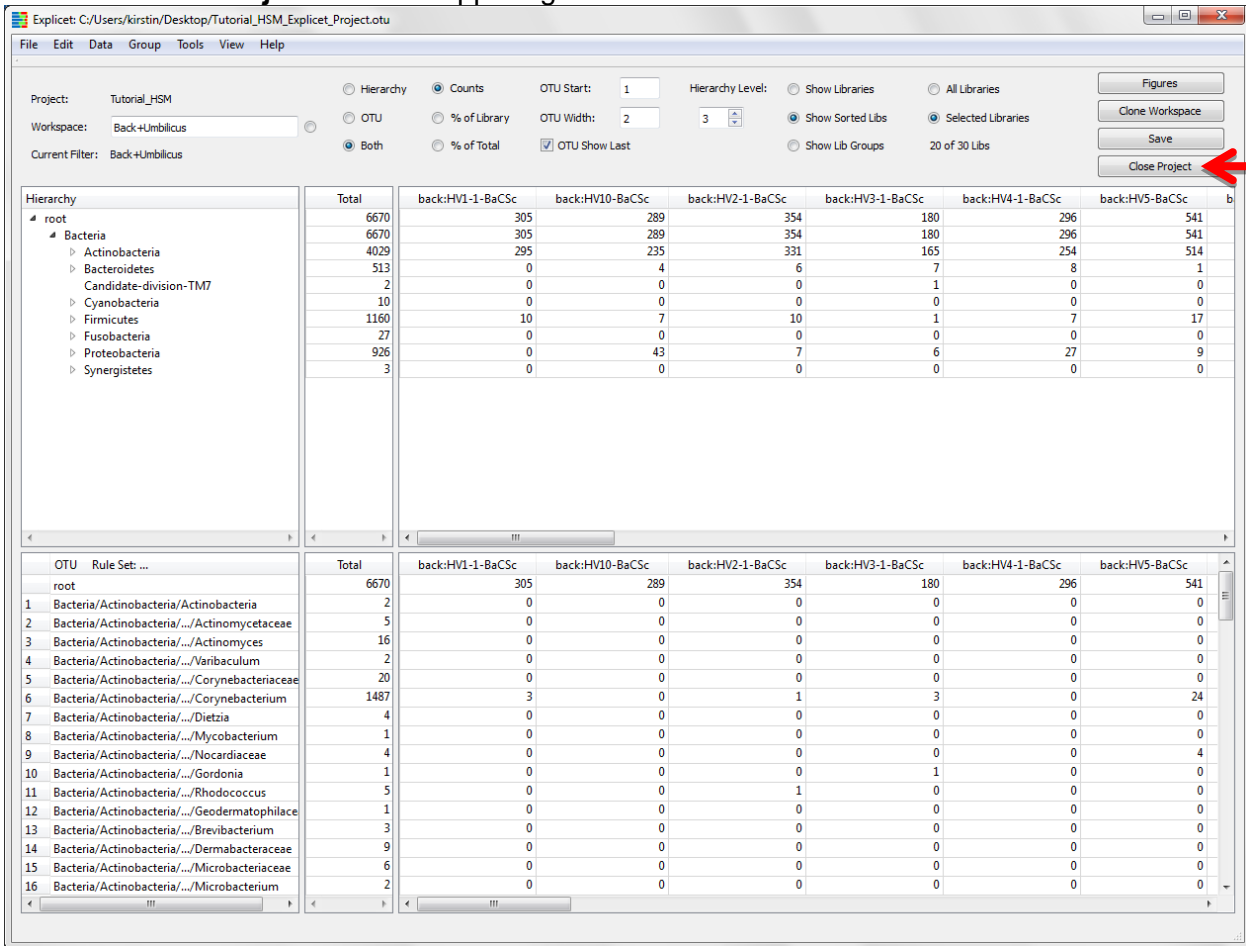
Data can be exported from the “Plot Results” window as tab delimited text using the export button (available in all graphics windows). The data incorporated for each taxon in the Two-Part statistic are summarized for each category. The number of samples with sequences belonging to an OTU within each 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.

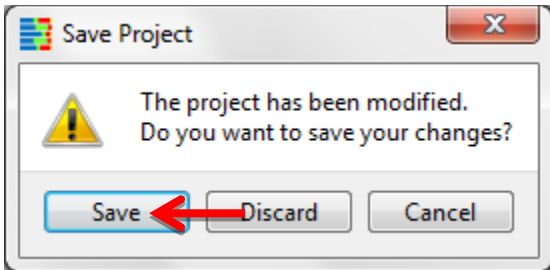
XIII. Close the Project

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



A pop-up window will open

Click **Save**



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