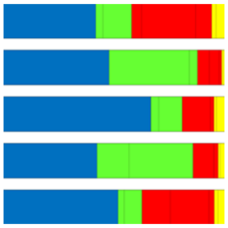

Explicet Basic Tutorial

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

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

v2.10.5_20140721

Table of Contents

Note: Clicking on any level of the table of contents will take you directly to that section.

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 Explicet 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 Explicet 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 Explicet forum. The Explicet forum link can be found on our web site: www.explicet.org

A. Create a New Project

Open Explicet

A pop-up window will open with several different options

Click **Create Project**

From the Latin: explain, unfold, extend, set forth, exhibit, disentangle

Robertson CE, Harris JK, Wagner BD, Granger D, Browne K,
Tatem B, Feazel LM, Park K, Pace NR, and Frank DN.

Explicet: Graphical user interface software for metadata-driven
management, analysis, and visualization of microbiome data.

Bioinformatics (2013) 29 (23): 3100-3101.

Explicet Version 2.10.5 07/21/14

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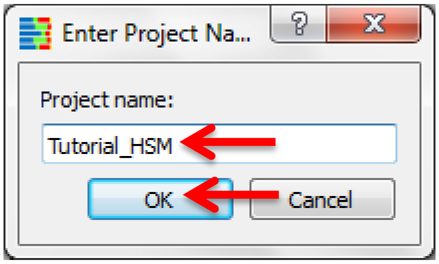
Last Project:

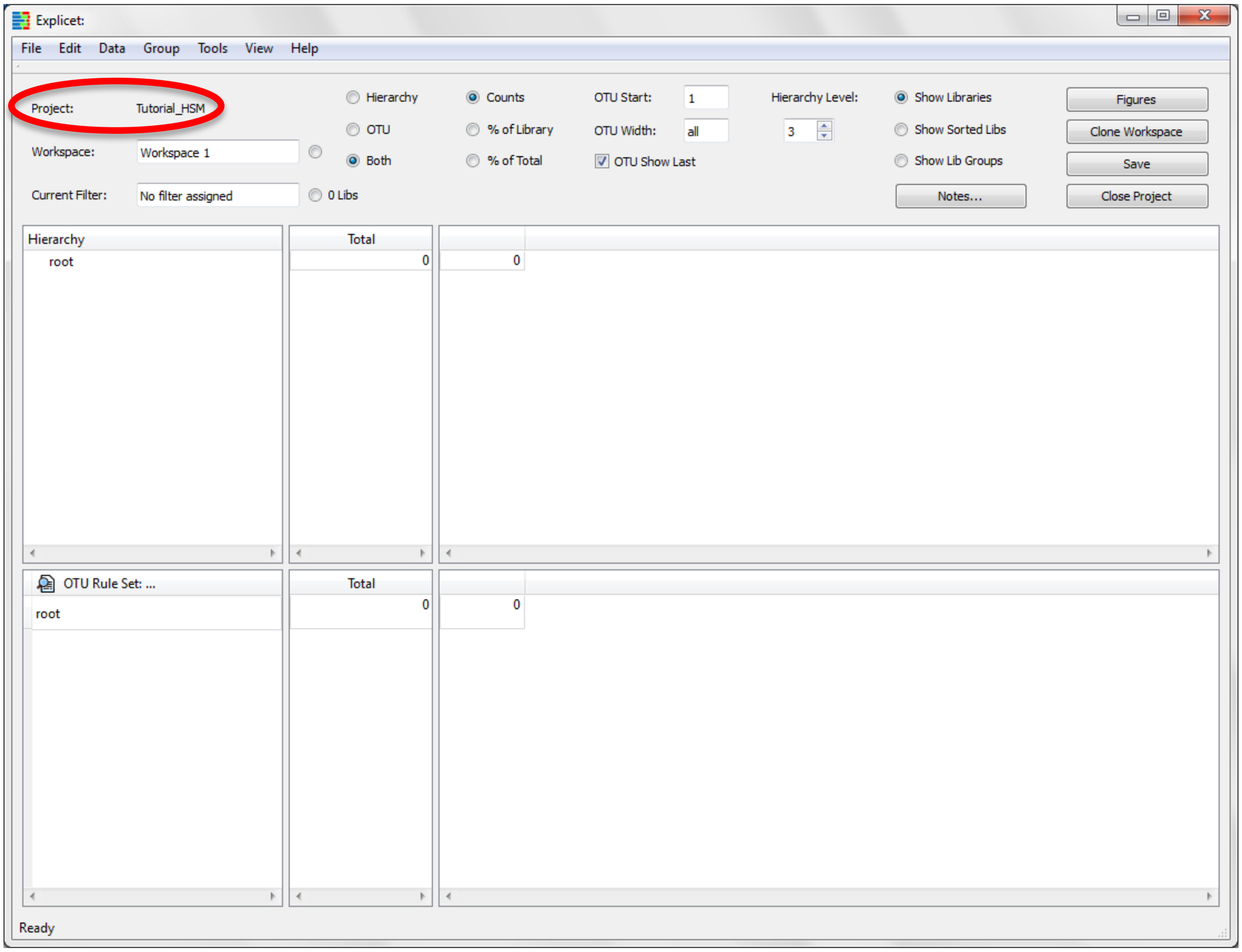
Enter **Project Name**, “Tutorial_HSM”, when prompted

Click **OK**



Project name: Tutorial_HSM

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



Project: Tutorial_HSM

Workspace: Workspace 1 ○

Current Filter: No filter assigned ○ 0 Libs

○ Hierarchy ○ OTU ● Both

● Counts ○ % of Library ○ % of Total

OTU Start: 1

OTU Width: all

☑ OTU Show Last

Hierarchy Level: 3

● Show Libraries ○ Show Sorted Libs ○ Show Lib Groups

Hierarchy	Total	
root	0	0

OTU Rule Set: ...	Total	
root	0	0

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 Explicet. 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 Explicet 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.

Explicet supports many other formats for importing the OTU data. For more details on the other OTU import formats, please see the Explicet 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 Explicet project file can grow as a project evolves.

A. Import the OTU Data

File → Import → File → OTU Table Counts

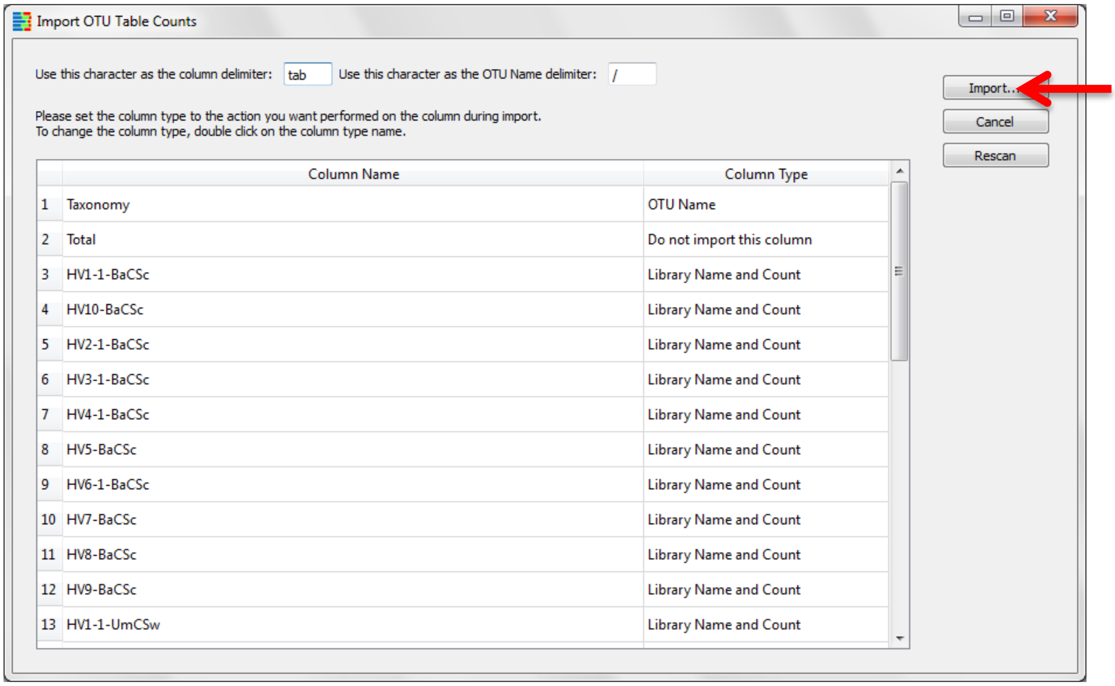
Select “Tutorial_HSM_OTU_2_Explicet”

Click **Open**

A dialog box below will open

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

Click **Import**

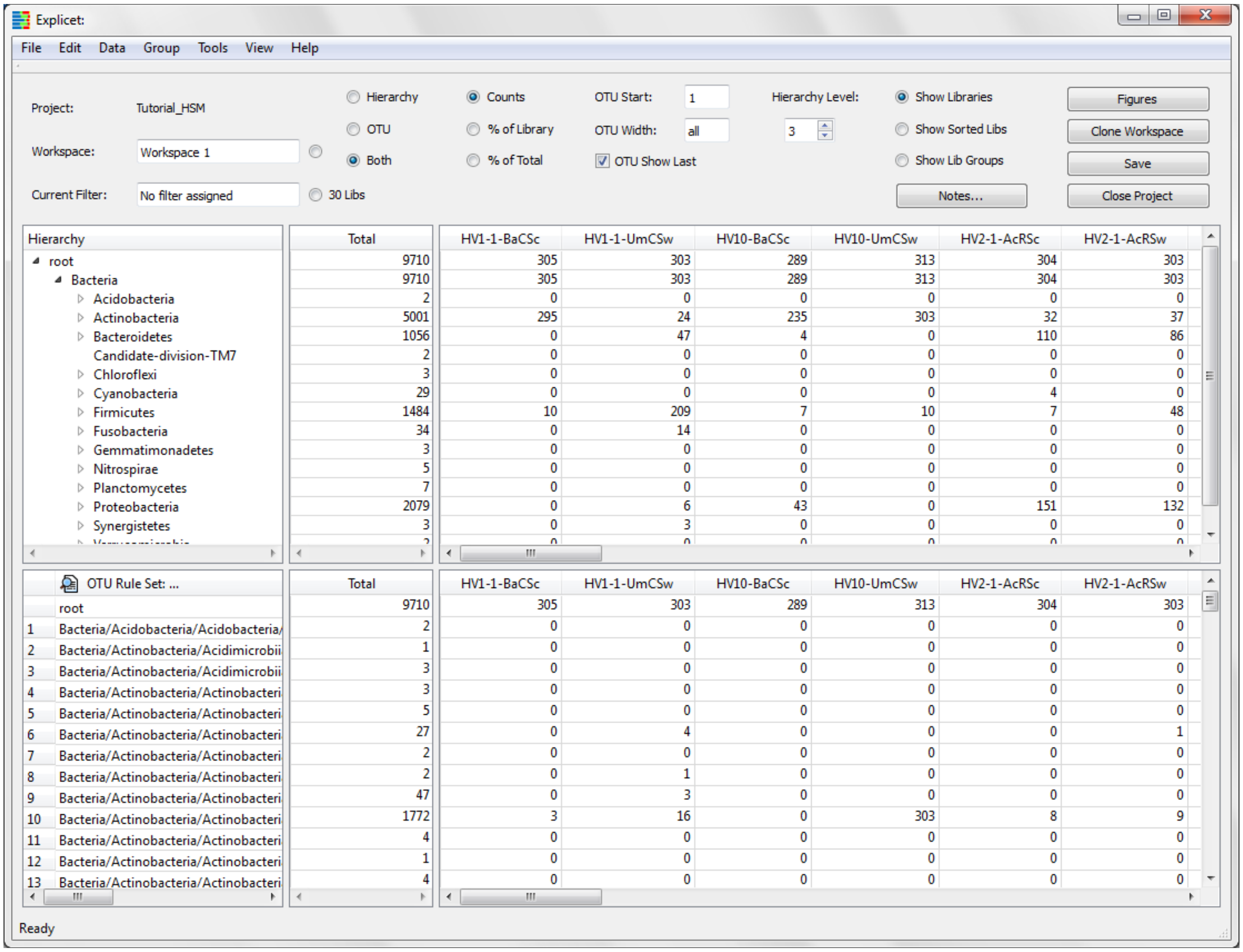


Use this character as the column delimiter: tab Use this character as the OTU Name delimiter: /

Please set the column type to the action you want performed on the column during import.
To change the column type, double click on the column type name.

	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

The OTU data now appear in the current workspace window



Project: Tutorial_HSM

Workspace: Workspace 1

Current Filter: No filter assigned

○ 30 Libs

○ Hierarchy
○ OTU
● Both

● Counts
○ % of Library
○ % of Total

OTU Start: 1
OTU Width: all
☑ OTU Show Last

Hierarchy Level: 3

● Show Libraries
○ Show Sorted Libs
○ Show Lib Groups

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

	OTU Rule Set: …	Total	HV1-1-BaCSc	HV1-1-UmCSw	HV10-BaCSc	HV10-UmCSw	HV2-1-AcRSc	HV2-1-AcRSw
	root	9710	305	303	289	313	304	303
1	Bacteria/Acidobacteria/Acidobacteria/	2	0	0	0	0	0	0
2	Bacteria/Actinobacteria/Acidimicrobii	1	0	0	0	0	0	0
3	Bacteria/Actinobacteria/Acidimicrobii	3	0	0	0	0	0	0
4	Bacteria/Actinobacteria/Actinobacteri	3	0	0	0	0	0	0
5	Bacteria/Actinobacteria/Actinobacteri	5	0	0	0	0	0	0
6	Bacteria/Actinobacteria/Actinobacteri	27	0	4	0	0	0	1
7	Bacteria/Actinobacteria/Actinobacteri	2	0	0	0	0	0	0
8	Bacteria/Actinobacteria/Actinobacteri	2	0	1	0	0	0	0
9	Bacteria/Actinobacteria/Actinobacteri	47	0	3	0	0	0	0
10	Bacteria/Actinobacteria/Actinobacteri	1772	3	16	0	303	8	9
11	Bacteria/Actinobacteria/Actinobacteri	4	0	0	0	0	0	0
12	Bacteria/Actinobacteria/Actinobacteri	1	0	0	0	0	0	0
13	Bacteria/Actinobacteria/Actinobacteri	4	0	0	0	0	0	0

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 Explicet project file. For detailed information on how to format metadata files, please see the Explicet 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”

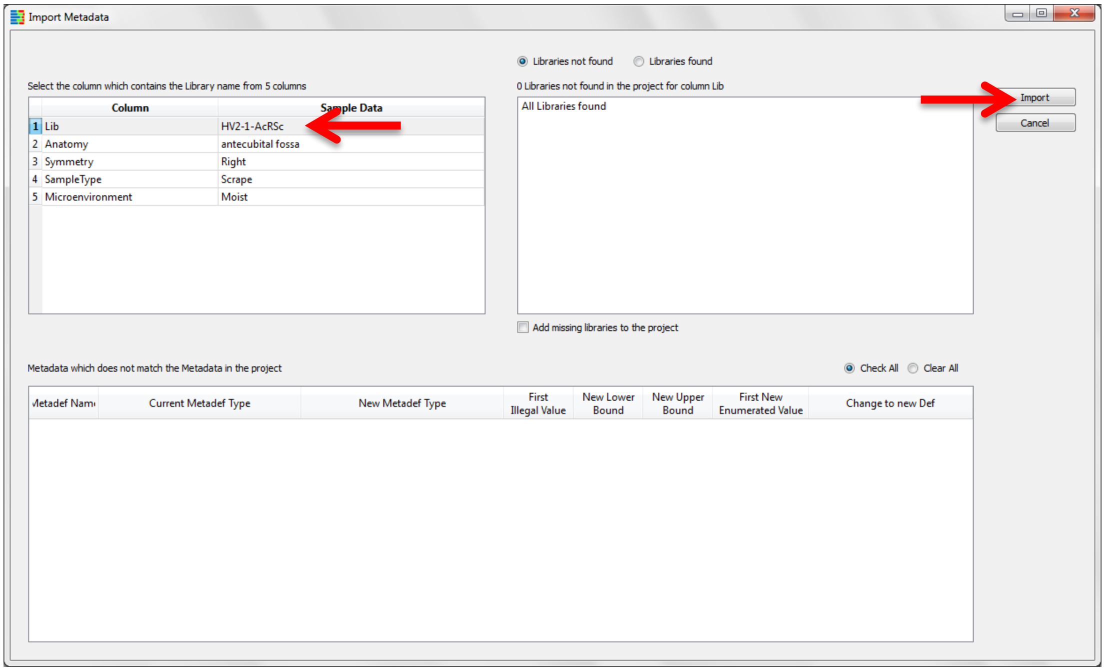
Click **Open**

A pop-up window will open

Make sure that the column containing the library name is selected

- Explicet 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), Explicet will find the library column automatically.

Click **Import**



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

0 Libraries not found in the project for column Lib

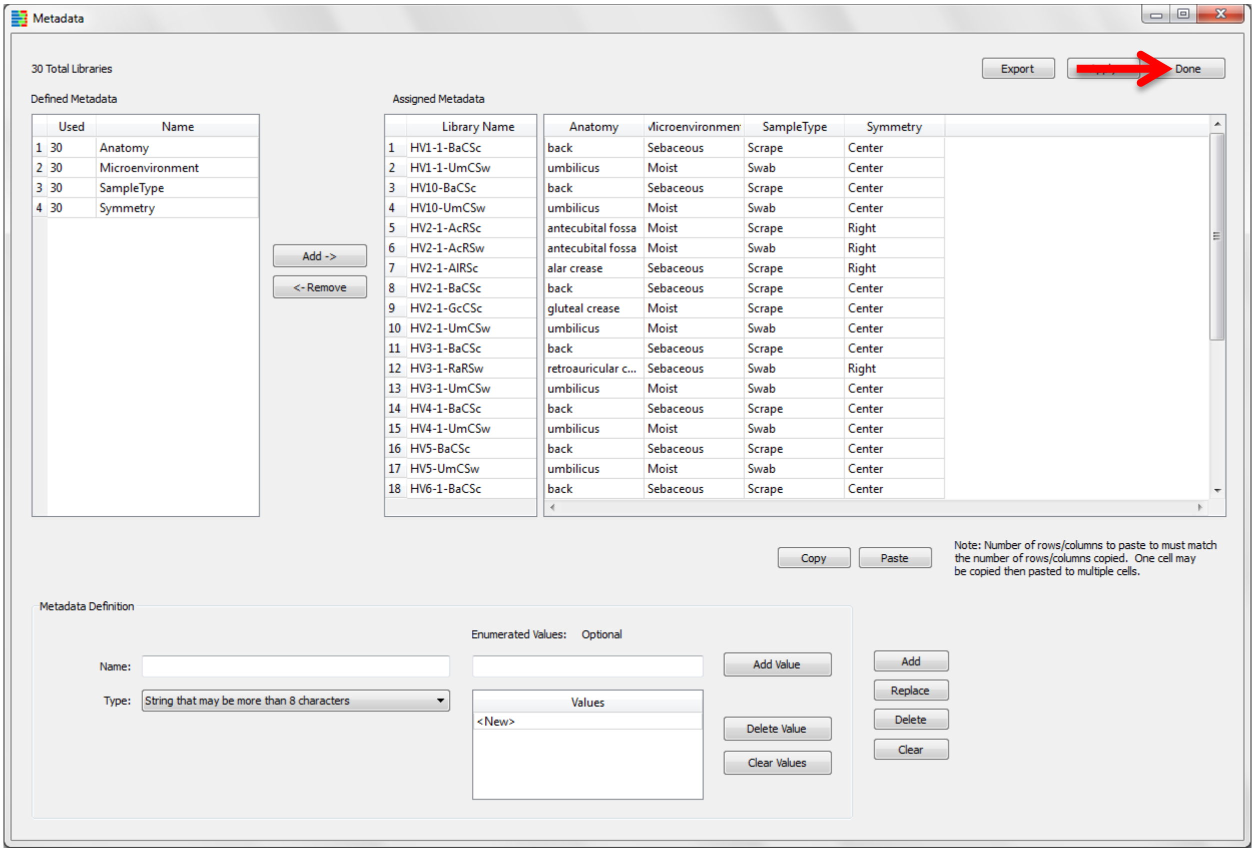
All Libraries found

Metadata which does not match the Metadata in the project

Metadef Nam	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**



30 Total Libraries

Defined Metadata

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

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-AlRSc	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

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

Enumerated Values: Optional

Values

<New>

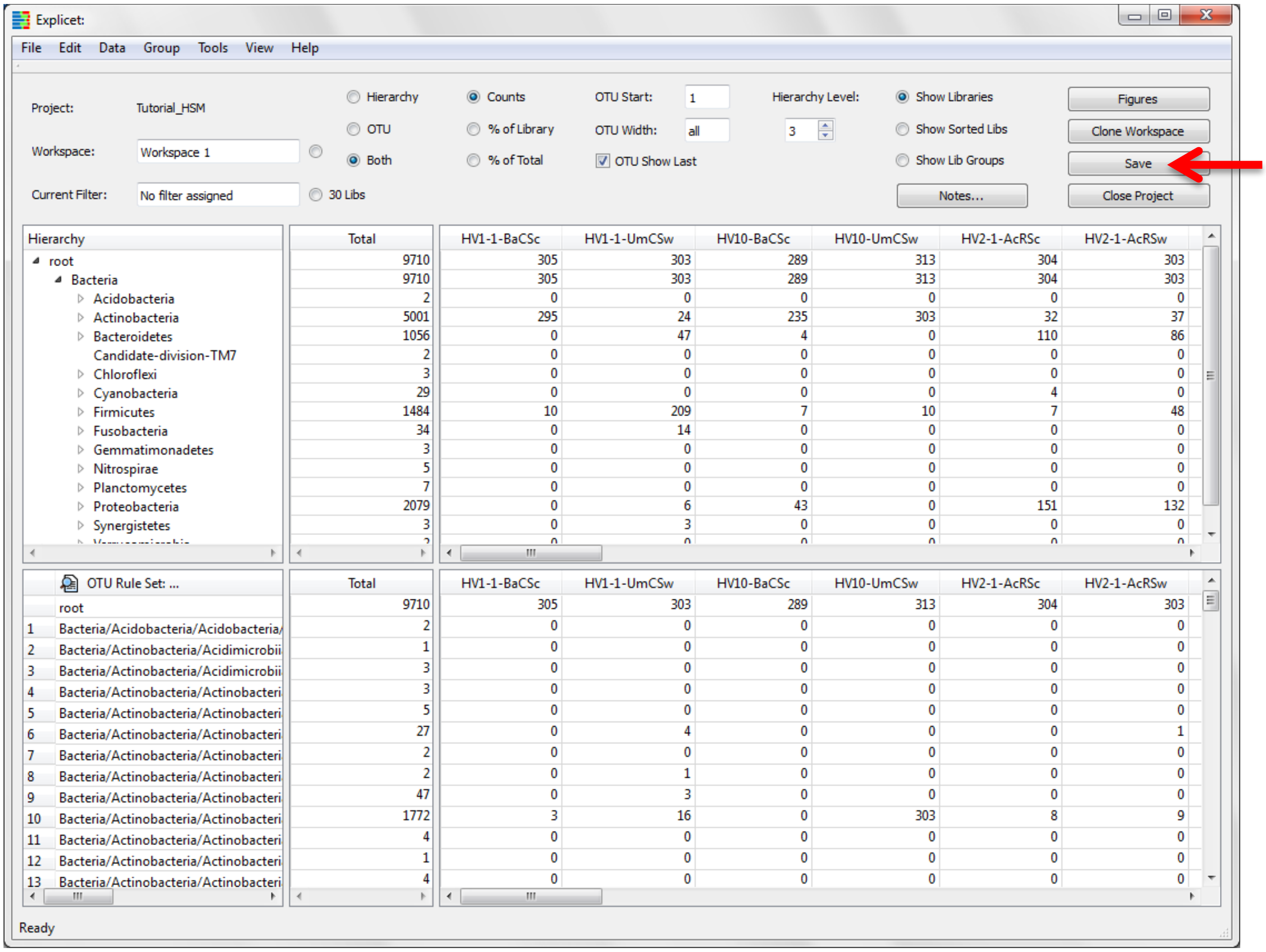
For our example dataset, all of the library names were found in the metadata file, as indicated in the left-hand pane: i.e., the number under **Used** (**30**) matches the total number of libraries shown above the two panes (**30 Total Libraries**).

IV. Save the Project

Now that all of the data associated with the project are imported, the file should be saved. Explicet 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



Project: Tutorial_HSM

Workspace: Workspace 1

Current Filter: No filter assigned

○ 30 Libs

○ Hierarchy
○ OTU
● Both

● Counts
○ % of Library
○ % of Total

OTU Start: 1
OTU Width: all
☑ OTU Show Last

Hierarchy Level: 3

● Show Libraries
○ Show Sorted Libs
○ Show Lib Groups

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

	OTU Rule Set: ...	Total	HV1-1-BaCSc	HV1-1-UmCSw	HV10-BaCSc	HV10-UmCSw	HV2-1-AcRSc	HV2-1-AcRSw
	root	9710	305	303	289	313	304	303
1	Bacteria/Acidobacteria/Acidobacteria/	2	0	0	0	0	0	0
2	Bacteria/Actinobacteria/Acidimicrobii	1	0	0	0	0	0	0
3	Bacteria/Actinobacteria/Acidimicrobii	3	0	0	0	0	0	0
4	Bacteria/Actinobacteria/Actinobacteri	3	0	0	0	0	0	0
5	Bacteria/Actinobacteria/Actinobacteri	5	0	0	0	0	0	0
6	Bacteria/Actinobacteria/Actinobacteri	27	0	4	0	0	0	1
7	Bacteria/Actinobacteria/Actinobacteri	2	0	0	0	0	0	0
8	Bacteria/Actinobacteria/Actinobacteri	2	0	1	0	0	0	0
9	Bacteria/Actinobacteria/Actinobacteri	47	0	3	0	0	0	0
10	Bacteria/Actinobacteria/Actinobacteri	1772	3	16	0	303	8	9
11	Bacteria/Actinobacteria/Actinobacteri	4	0	0	0	0	0	0
12	Bacteria/Actinobacteria/Actinobacteri	1	0	0	0	0	0	0
13	Bacteria/Actinobacteria/Actinobacteri	4	0	0	0	0	0	0

Enter desired project name and location when prompted

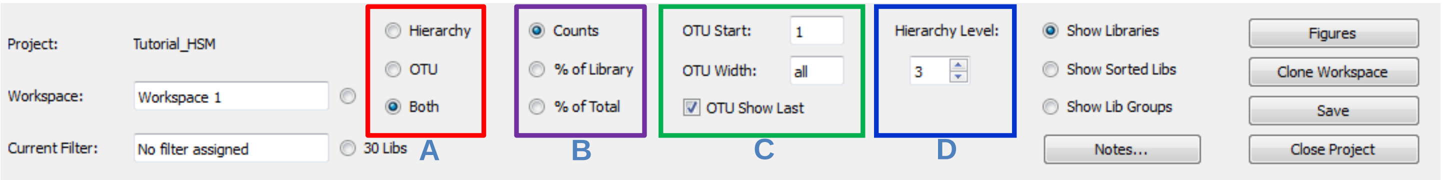
- The default file name is the project name with an "_Explicet_Project" extension.

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).


Project: Tutorial_HSM
Workspace: Workspace 1
Current Filter: No filter assigned
○ 30 Libs
○ Hierarchy
○ OTU
● Both
● Counts
○ % of Library
○ % of Total
OTU Start: 1
OTU Width: all
☑ OTU Show Last
Hierarchy Level: 3
● Show Libraries
○ Show Sorted Libs
○ Show Lib Groups

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 “1”st lineage level).

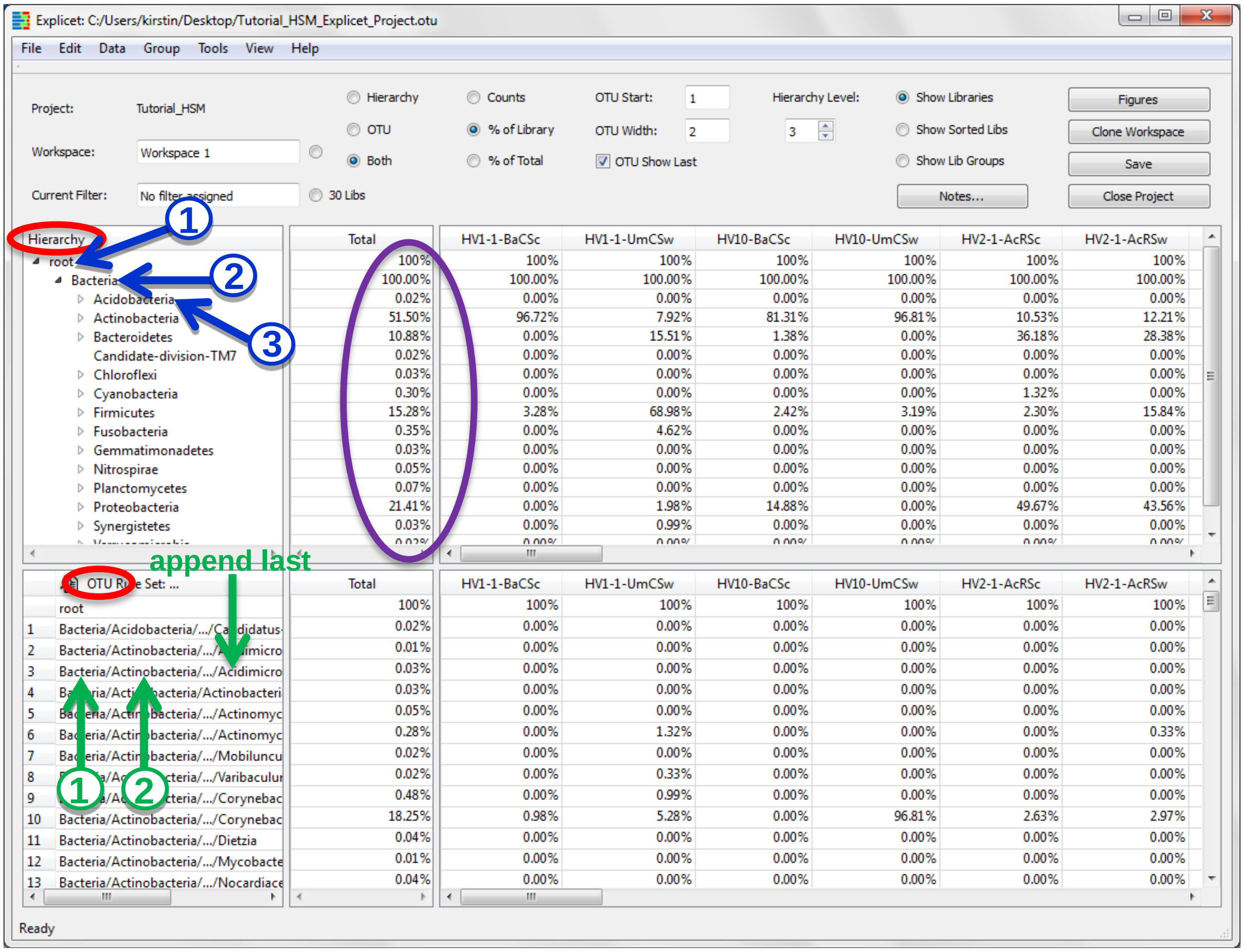
Set **OTU Width** to 2 (“all” is the default)
This is the number of positions on the line to be displayed. To save space on the screen, now only 2 taxonomic levels will be displayed in the OTU taxonomy line. Taxonomies with more than 2 levels will be shown with an embedded ellipsis; for example, “Bacteria/Actinobacteria/Acidimicrobiia/Acidimicrobiales”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



Project: Tutorial_HSM
Workspace: Workspace 1
Current Filter: No filter assigned
○ 30 Libs
○ Hierarchy
○ OTU
● Both
○ Counts
● % of Library
○ % of Total
OTU Start: 1
OTU Width: 2
☑ OTU Show Last
Hierarchy Level: 3
● Show Libraries
○ Show Sorted Libs
○ Show Lib Groups

Hierarchy	Total	HV1-1-BaCSc	HV1-1-UmCSw	HV10-BaCSc	HV10-UmCSw	HV2-1-AcRSc	HV2-1-AcRSw
root	100%	100%	100%	100%	100%	100%	100%
Bacteria	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
Acidobacteria	0.02%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Actinobacteria	51.50%	96.72%	7.92%	81.31%	96.81%	10.53%	12.21%
Bacteroidetes	10.88%	0.00%	15.51%	1.38%	0.00%	36.18%	28.38%
Candidate-division-TM7	0.02%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Chloroflexi	0.03%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Cyanobacteria	0.30%	0.00%	0.00%	0.00%	0.00%	1.32%	0.00%
Firmicutes	15.28%	3.28%	68.98%	2.42%	3.19%	2.30%	15.84%
Fusobacteria	0.35%	0.00%	4.62%	0.00%	0.00%	0.00%	0.00%
Gemmatimonadetes	0.03%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Nitrospirae	0.05%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Planctomycetes	0.07%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Proteobacteria	21.41%	0.00%	1.98%	14.88%	0.00%	49.67%	43.56%
Synergistetes	0.03%	0.00%	0.99%	0.00%	0.00%	0.00%	0.00%

	OTU Rule Set: ...	Total	HV1-1-BaCSc	HV1-1-UmCSw	HV10-BaCSc	HV10-UmCSw	HV2-1-AcRSc	HV2-1-AcRSw
	root	100%	100%	100%	100%	100%	100%	100%
1	Bacteria/Acidobacteria/.../Candidatus-	0.02%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
2	Bacteria/Actinobacteria/.../Acidimicro	0.01%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
3	Bacteria/Actinobacteria/.../Acidimicro	0.03%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
4	Bacteria/Actinobacteria/Actinobacteri	0.03%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
5	Bacteria/Actinobacteria/.../Actinomyc	0.05%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
6	Bacteria/Actinobacteria/.../Actinomyc	0.28%	0.00%	1.32%	0.00%	0.00%	0.00%	0.33%
7	Bacteria/Actinobacteria/.../Mobiluncu	0.02%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
8	Bacteria/Actinobacteria/.../Varibaculu	0.02%	0.00%	0.33%	0.00%	0.00%	0.00%	0.00%
9	Bacteria/Actinobacteria/.../Corynebac	0.48%	0.00%	0.99%	0.00%	0.00%	0.00%	0.00%
10	Bacteria/Actinobacteria/.../Corynebac	18.25%	0.98%	5.28%	0.00%	96.81%	2.63%	2.97%
11	Bacteria/Actinobacteria/.../Dietzia	0.04%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
12	Bacteria/Actinobacteria/.../Mycobacte	0.01%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
13	Bacteria/Actinobacteria/.../Nocardiace	0.04%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

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.

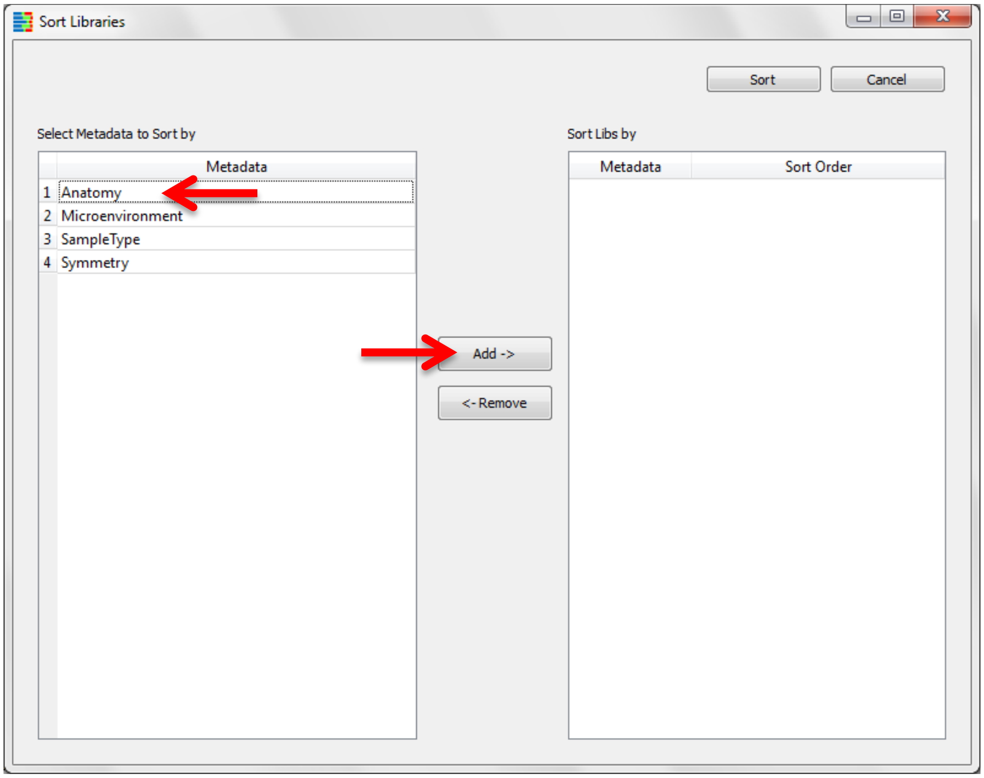
E. Sort Libraries Based on a Metadata Tag (Anatomical Position)

View → Sort Libraries

A pop-up window will open

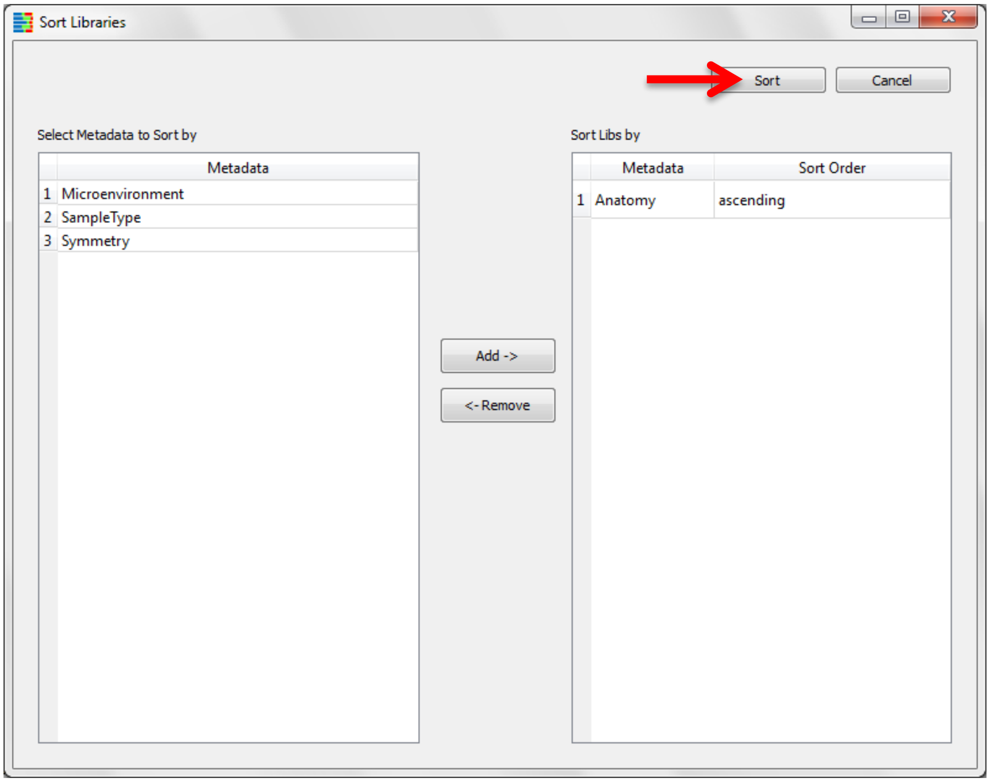
In left panel, select **Anatomy**

Click **Add** button between panels



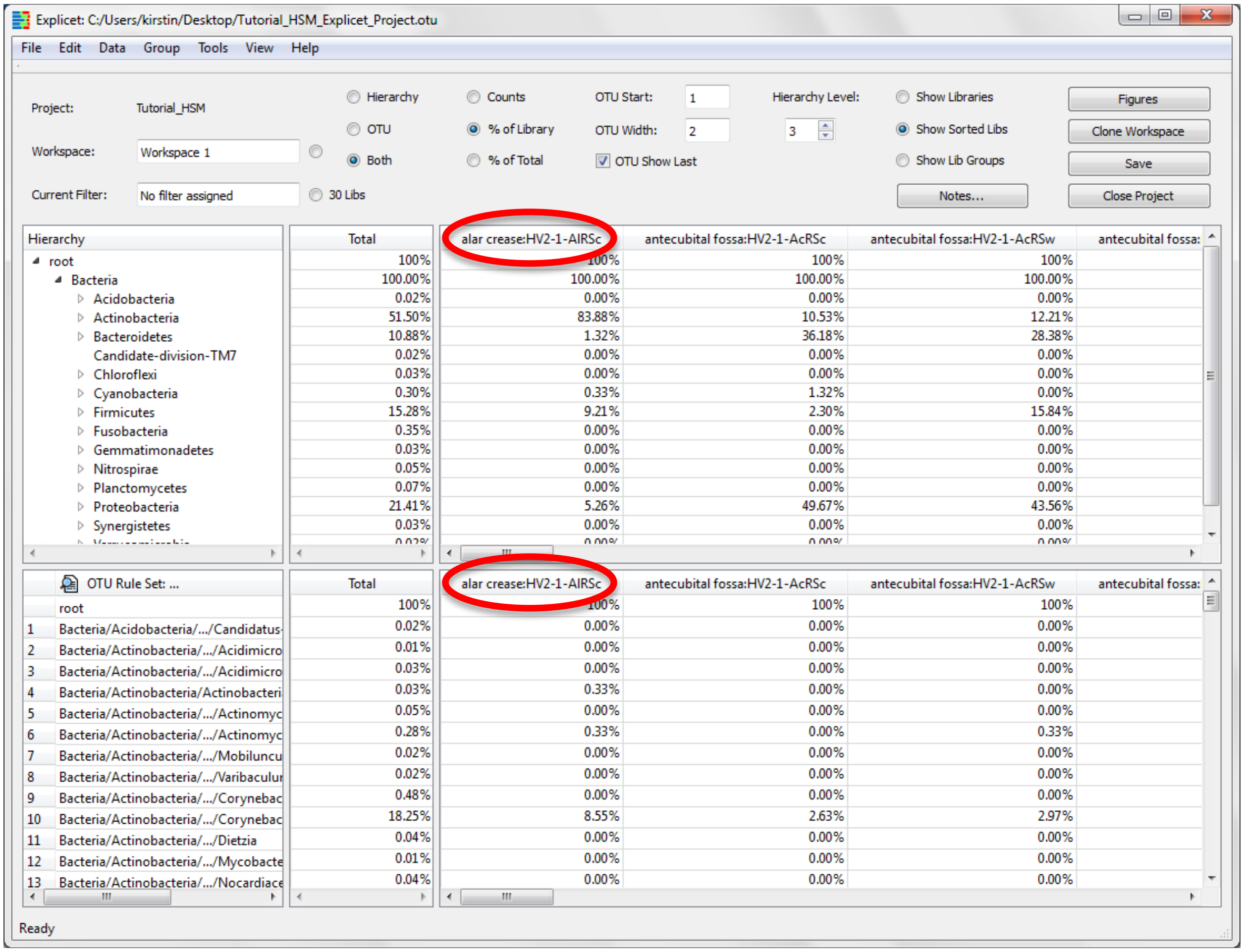
Name of metadata descriptor will appear in the right panel

Click **Sort**



Pop-up window will disappear

Both the hierarchy and OTU tables are now sorted by anatomical position



Project: Tutorial_HSM
Workspace: Workspace 1 ○
Current Filter: No filter assigned ○ 30 Libs

○ Hierarchy
○ OTU
● Both

○ Counts
● % of Library
○ % of Total

OTU Start: 1
OTU Width: 2
☑ OTU Show Last

Hierarchy Level: 3

○ Show Libraries
● Show Sorted Libs
○ Show Lib Groups

Hierarchy	Total	alar crease:HV2-1-AlRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fossa:
root	100%	100%	100%	100%	
Bacteria	100.00%	100.00%	100.00%	100.00%	
Acidobacteria	0.02%	0.00%	0.00%	0.00%	
Actinobacteria	51.50%	83.88%	10.53%	12.21%	
Bacteroidetes	10.88%	1.32%	36.18%	28.38%	
Candidate-division-TM7	0.02%	0.00%	0.00%	0.00%	
Chloroflexi	0.03%	0.00%	0.00%	0.00%	
Cyanobacteria	0.30%	0.33%	1.32%	0.00%	
Firmicutes	15.28%	9.21%	2.30%	15.84%	
Fusobacteria	0.35%	0.00%	0.00%	0.00%	
Gemmatimonadetes	0.03%	0.00%	0.00%	0.00%	
Nitrospirae	0.05%	0.00%	0.00%	0.00%	
Planctomycetes	0.07%	0.00%	0.00%	0.00%	
Proteobacteria	21.41%	5.26%	49.67%	43.56%	
Synergistetes	0.03%	0.00%	0.00%	0.00%	

	OTU Rule Set: ...	Total	alar crease:HV2-1-AlRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fossa:
	root	100%	100%	100%	100%	
1	Bacteria/Acidobacteria/.../Candidatus-	0.02%	0.00%	0.00%	0.00%	
2	Bacteria/Actinobacteria/.../Acidimicro	0.01%	0.00%	0.00%	0.00%	
3	Bacteria/Actinobacteria/.../Acidimicro	0.03%	0.00%	0.00%	0.00%	
4	Bacteria/Actinobacteria/Actinobacteri	0.03%	0.33%	0.00%	0.00%	
5	Bacteria/Actinobacteria/.../Actinomyc	0.05%	0.00%	0.00%	0.00%	
6	Bacteria/Actinobacteria/.../Actinomyc	0.28%	0.33%	0.00%	0.33%	
7	Bacteria/Actinobacteria/.../Mobiluncu	0.02%	0.00%	0.00%	0.00%	
8	Bacteria/Actinobacteria/.../Varibaculu	0.02%	0.00%	0.00%	0.00%	
9	Bacteria/Actinobacteria/.../Corynebac	0.48%	0.00%	0.00%	0.00%	
10	Bacteria/Actinobacteria/.../Corynebac	18.25%	8.55%	2.63%	2.97%	
11	Bacteria/Actinobacteria/.../Dietzia	0.04%	0.00%	0.00%	0.00%	
12	Bacteria/Actinobacteria/.../Mycobacte	0.01%	0.00%	0.00%	0.00%	
13	Bacteria/Actinobacteria/.../Nocardiace	0.04%	0.00%	0.00%	0.00%	

VI. Make an OTU Stacked Bar Chart

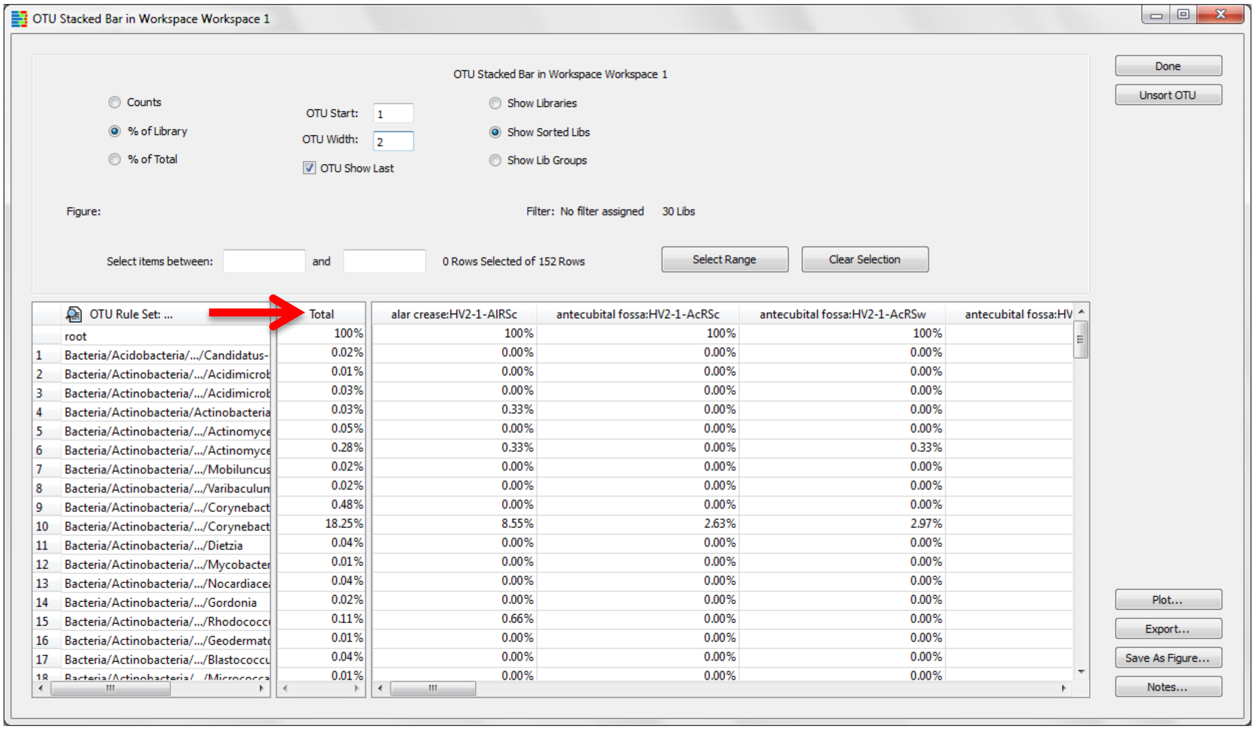
Before diving into a detailed analysis, generating an overview of the dominant organisms that exist in the dataset can be useful. One way to do this is through an OTU stacked bar chart.

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



○ Counts
● % of Library
○ % of Total

OTU Start: 1
OTU Width: 2
☑ OTU Show Last

○ Show Libraries
● Show Sorted Libs
○ Show Lib Groups

Figure:

Filter: No filter assigned 30 Libs

Select 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:HV
	root	100%	100%	100%	100%	
1	Bacteria/Acidobacteria/.../Candidatus-	0.02%	0.00%	0.00%	0.00%	
2	Bacteria/Actinobacteria/.../Acidimicrob	0.01%	0.00%	0.00%	0.00%	
3	Bacteria/Actinobacteria/.../Acidimicrob	0.03%	0.00%	0.00%	0.00%	
4	Bacteria/Actinobacteria/Actinobacteria	0.03%	0.33%	0.00%	0.00%	
5	Bacteria/Actinobacteria/.../Actinomyce	0.05%	0.00%	0.00%	0.00%	
6	Bacteria/Actinobacteria/.../Actinomyce	0.28%	0.33%	0.00%	0.33%	
7	Bacteria/Actinobacteria/.../Mobiluncus	0.02%	0.00%	0.00%	0.00%	
8	Bacteria/Actinobacteria/.../Varibaculum	0.02%	0.00%	0.00%	0.00%	
9	Bacteria/Actinobacteria/.../Corynebact	0.48%	0.00%	0.00%	0.00%	
10	Bacteria/Actinobacteria/.../Corynebact	18.25%	8.55%	2.63%	2.97%	
11	Bacteria/Actinobacteria/.../Dietzia	0.04%	0.00%	0.00%	0.00%	
12	Bacteria/Actinobacteria/.../Mycobacter	0.01%	0.00%	0.00%	0.00%	
13	Bacteria/Actinobacteria/.../Nocardiace	0.04%	0.00%	0.00%	0.00%	
14	Bacteria/Actinobacteria/.../Gordonia	0.02%	0.00%	0.00%	0.00%	
15	Bacteria/Actinobacteria/.../Rhodococc	0.11%	0.66%	0.00%	0.00%	
16	Bacteria/Actinobacteria/.../Geodermat	0.01%	0.00%	0.00%	0.00%	
17	Bacteria/Actinobacteria/.../Blastococcu	0.04%	0.00%	0.00%	0.00%	
18	Bacteria/Actinobacteria/.../Micrococca	0.01%	0.00%	0.00%	0.00%	

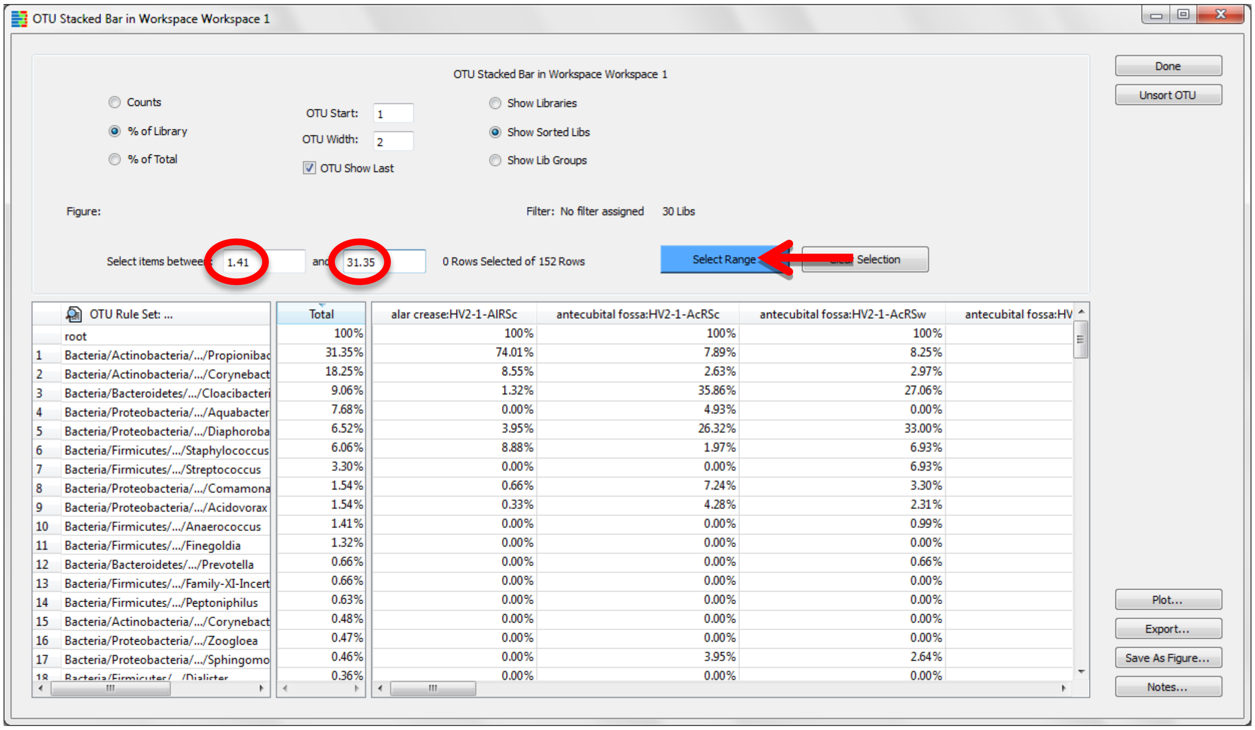
To display only the top 10 taxa in the project, note that the **Total** value of the 1st OTU in the column is 31.35

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**



OTU Stacked Bar in Workspace Workspace 1

○ Counts
● % of Library
○ % of Total

OTU Start: 1
OTU Width: 2
☑ OTU Show Last

○ Show Libraries
● Show Sorted Libs
○ Show Lib Groups

Figure:

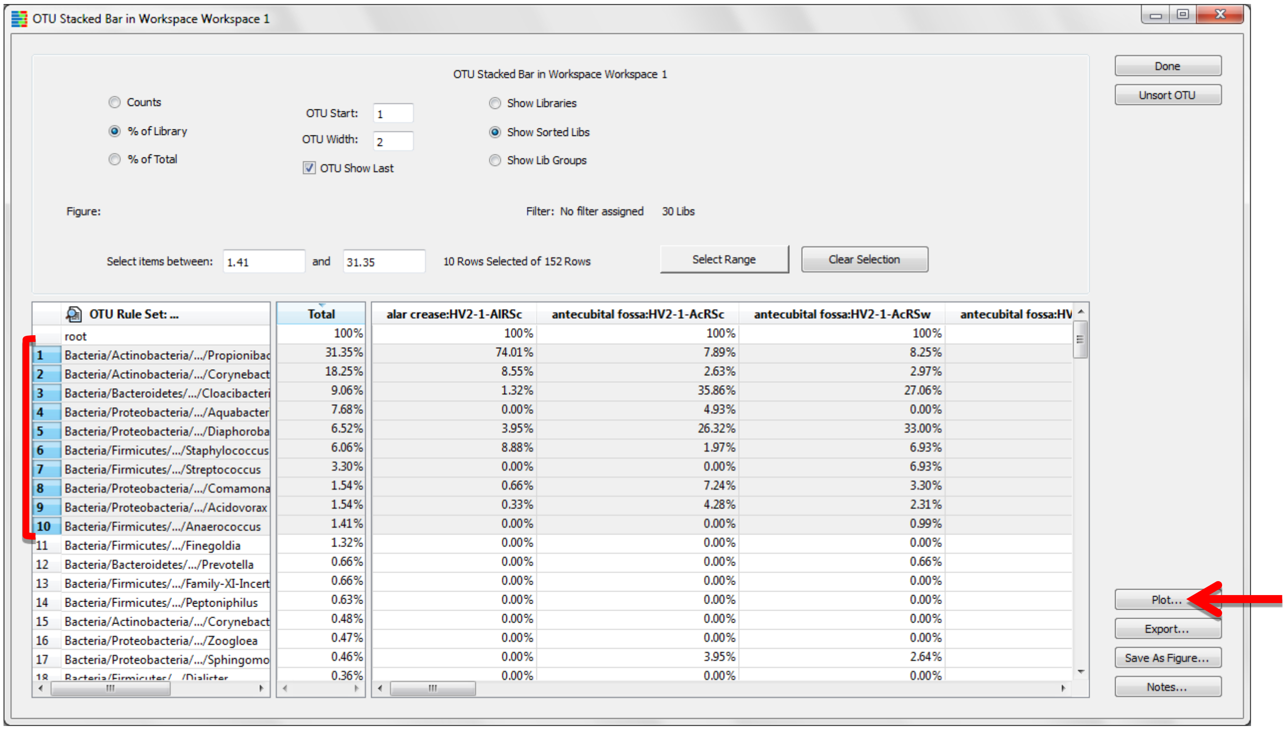
Filter: No filter assigned 30 Libs

Select items between: 1.41 and 31.35 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:HV
	root	100%	100%	100%	100%	
1	Bacteria/Actinobacteria/.../Propionibac	31.35%	74.01%	7.89%	8.25%	
2	Bacteria/Actinobacteria/.../Corynebact	18.25%	8.55%	2.63%	2.97%	
3	Bacteria/Bacteroidetes/.../Cloacibacteri	9.06%	1.32%	35.86%	27.06%	
4	Bacteria/Proteobacteria/.../Aquabacter	7.68%	0.00%	4.93%	0.00%	
5	Bacteria/Proteobacteria/.../Diaphoroba	6.52%	3.95%	26.32%	33.00%	
6	Bacteria/Firmicutes/.../Staphylococcus	6.06%	8.88%	1.97%	6.93%	
7	Bacteria/Firmicutes/.../Streptococcus	3.30%	0.00%	0.00%	6.93%	
8	Bacteria/Proteobacteria/.../Comamona	1.54%	0.66%	7.24%	3.30%	
9	Bacteria/Proteobacteria/.../Acidovorax	1.54%	0.33%	4.28%	2.31%	
10	Bacteria/Firmicutes/.../Anaerococcus	1.41%	0.00%	0.00%	0.99%	
11	Bacteria/Firmicutes/.../Finegoldia	1.32%	0.00%	0.00%	0.00%	
12	Bacteria/Bacteroidetes/.../Prevotella	0.66%	0.00%	0.00%	0.66%	
13	Bacteria/Firmicutes/.../Family-XI-Incert	0.66%	0.00%	0.00%	0.00%	
14	Bacteria/Firmicutes/.../Peptoniphilus	0.63%	0.00%	0.00%	0.00%	
15	Bacteria/Actinobacteria/.../Corynebact	0.48%	0.00%	0.00%	0.00%	
16	Bacteria/Proteobacteria/.../Zoogloea	0.47%	0.00%	0.00%	0.00%	
17	Bacteria/Proteobacteria/.../Sphingomo	0.46%	0.00%	3.95%	2.64%	
18	Bacteria/Firmicutes/.../Dialister	0.36%	0.00%	0.00%	0.00%	

The top 10 OTUs are now highlighted

Click **Plot**



OTU Stacked Bar in Workspace Workspace 1

○ Counts
● % of Library
○ % of Total

OTU Start: 1
OTU Width: 2
☑ OTU Show Last

○ Show Libraries
● Show Sorted Libs
○ Show Lib Groups

Figure:

Filter: No filter assigned 30 Libs

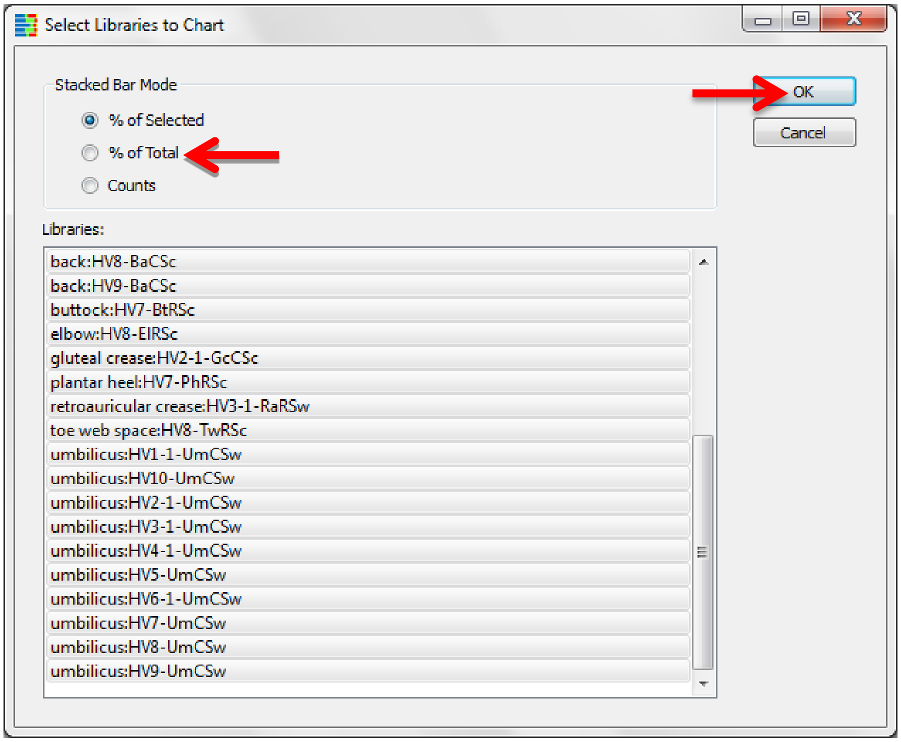
Select items between: 1.41 and 31.35 10 Rows Selected of 152 Rows

	OTU Rule Set: ...	Total	alar crease:HV2-1-AIRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fossa:HV
	root	100%	100%	100%	100%	
1	Bacteria/Actinobacteria/.../Propionibac	31.35%	74.01%	7.89%	8.25%	
2	Bacteria/Actinobacteria/.../Corynebact	18.25%	8.55%	2.63%	2.97%	
3	Bacteria/Bacteroidetes/.../Cloacibacteri	9.06%	1.32%	35.86%	27.06%	
4	Bacteria/Proteobacteria/.../Aquabacter	7.68%	0.00%	4.93%	0.00%	
5	Bacteria/Proteobacteria/.../Diaphoroba	6.52%	3.95%	26.32%	33.00%	
6	Bacteria/Firmicutes/.../Staphylococcus	6.06%	8.88%	1.97%	6.93%	
7	Bacteria/Firmicutes/.../Streptococcus	3.30%	0.00%	0.00%	6.93%	
8	Bacteria/Proteobacteria/.../Comamona	1.54%	0.66%	7.24%	3.30%	
9	Bacteria/Proteobacteria/.../Acidovorax	1.54%	0.33%	4.28%	2.31%	
10	Bacteria/Firmicutes/.../Anaerococcus	1.41%	0.00%	0.00%	0.99%	
11	Bacteria/Firmicutes/.../Finegoldia	1.32%	0.00%	0.00%	0.00%	
12	Bacteria/Bacteroidetes/.../Prevotella	0.66%	0.00%	0.00%	0.66%	
13	Bacteria/Firmicutes/.../Family-XI-Incert	0.66%	0.00%	0.00%	0.00%	
14	Bacteria/Firmicutes/.../Peptoniphilus	0.63%	0.00%	0.00%	0.00%	
15	Bacteria/Actinobacteria/.../Corynebact	0.48%	0.00%	0.00%	0.00%	
16	Bacteria/Proteobacteria/.../Zoogloea	0.47%	0.00%	0.00%	0.00%	
17	Bacteria/Proteobacteria/.../Sphingomo	0.46%	0.00%	3.95%	2.64%	
18	Bacteria/Firmicutes/.../Dialister	0.36%	0.00%	0.00%	0.00%	

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**



Select Libraries to Chart

Stacked Bar Mode

● % of Selected

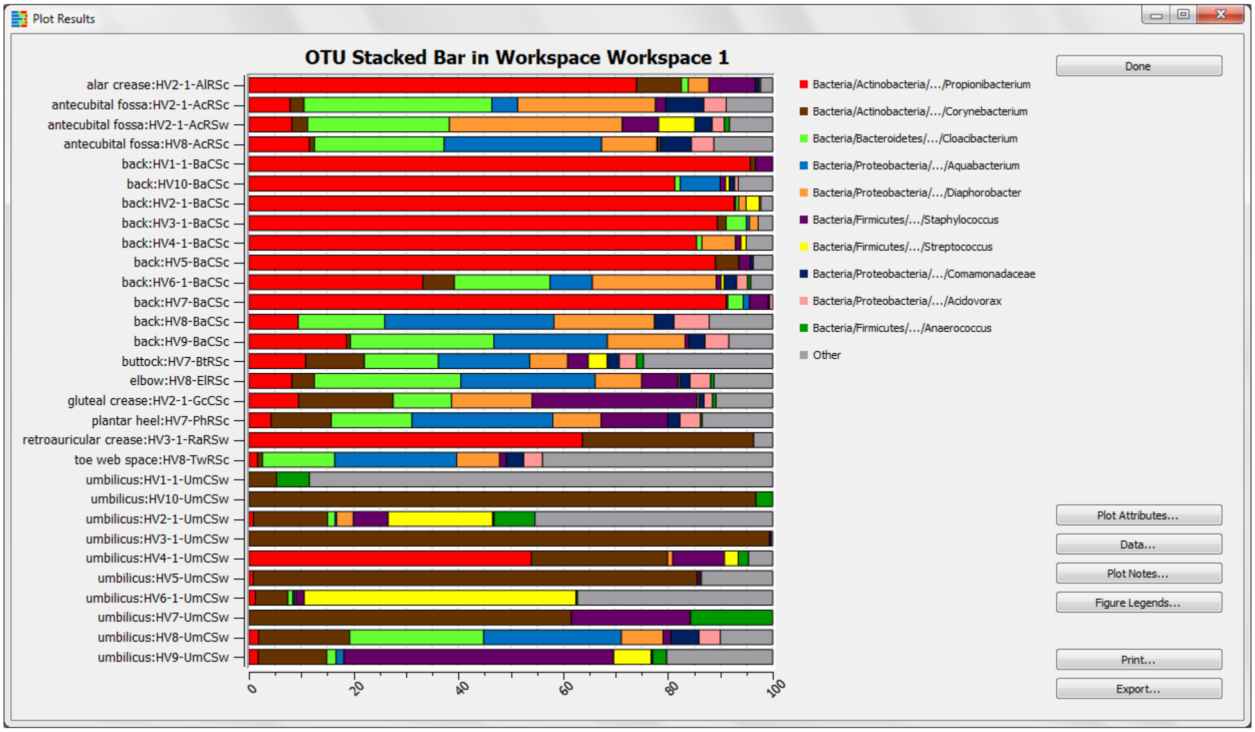
○ % of Total

○ Counts

Libraries:

back:HV8-BaCSc
back:HV9-BaCSc
buttock:HV7-BtRSc
elbow:HV8-ElRSc
gluteal crease:HV2-1-GcCSc
plantar heel:HV7-PhRSc
retroauricular crease:HV3-1-RaRSw
toe web space:HV8-TwRSc
umbilicus:HV1-1-UmCSw
umbilicus:HV10-UmCSw
umbilicus:HV2-1-UmCSw
umbilicus:HV3-1-UmCSw
umbilicus:HV4-1-UmCSw
umbilicus:HV5-UmCSw
umbilicus:HV6-1-UmCSw
umbilicus:HV7-UmCSw
umbilicus:HV8-UmCSw
umbilicus:HV9-UmCSw

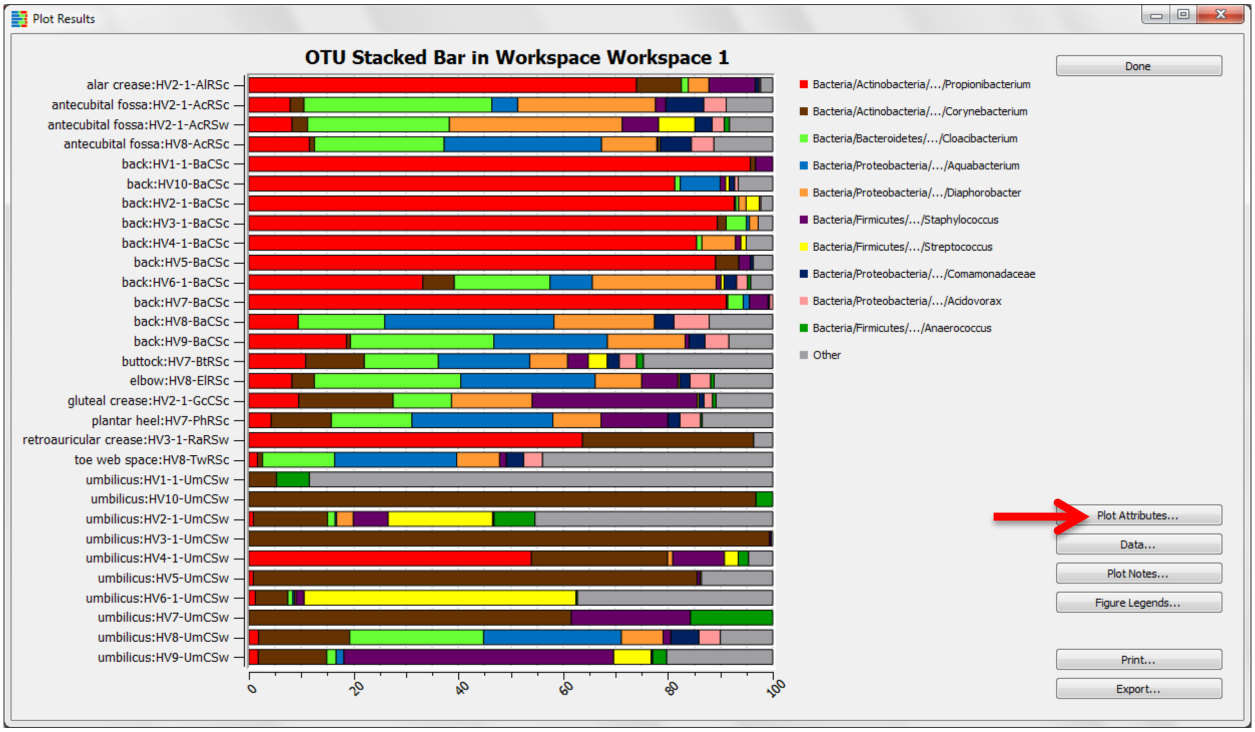
A pop-up window with the OTU stacked bar chart appears



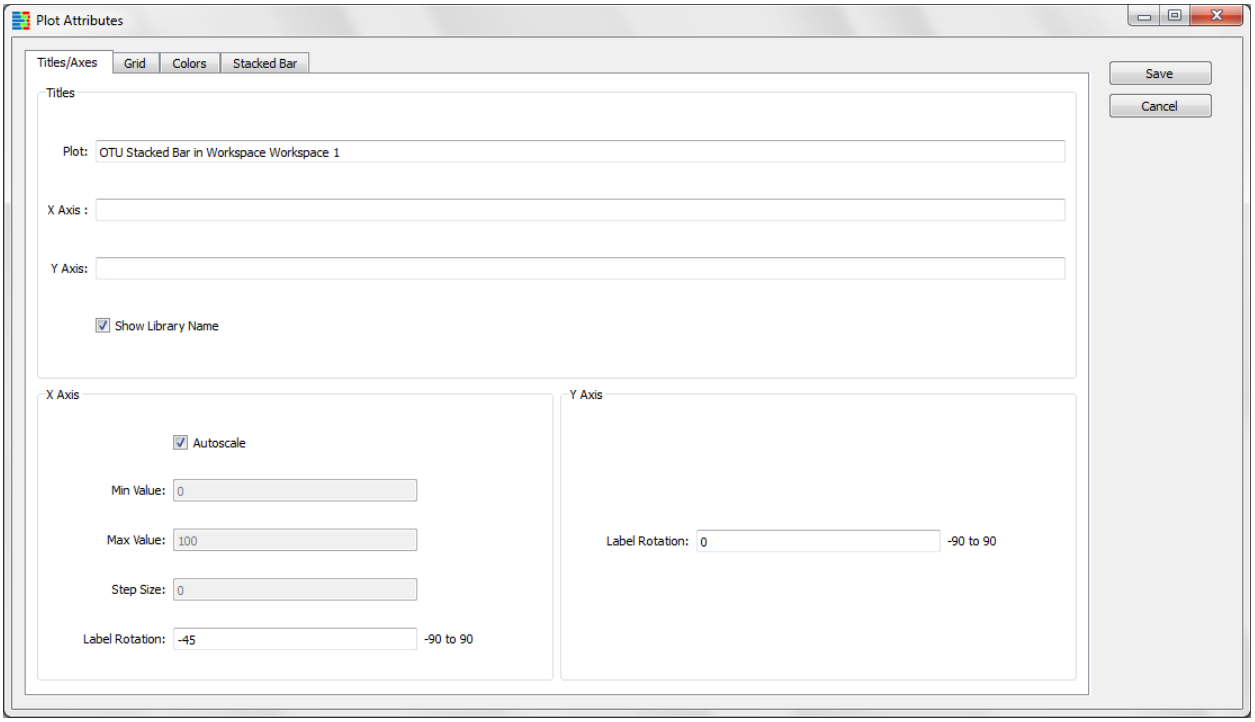
We will now change the default title of the stacked bar chart and add axis labels.

B. Change the Title and Label the Axes

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



A pop-up window will appear



Titles

Plot: OTU Stacked Bar in Workspace Workspace 1

X Axis :

Y Axis:

☑ Show Library Name

X Axis

☑ Autoscale

Min Value: 0

Max Value: 100

Step Size: 0

Label Rotation: -45 -90 to 90

Y Axis

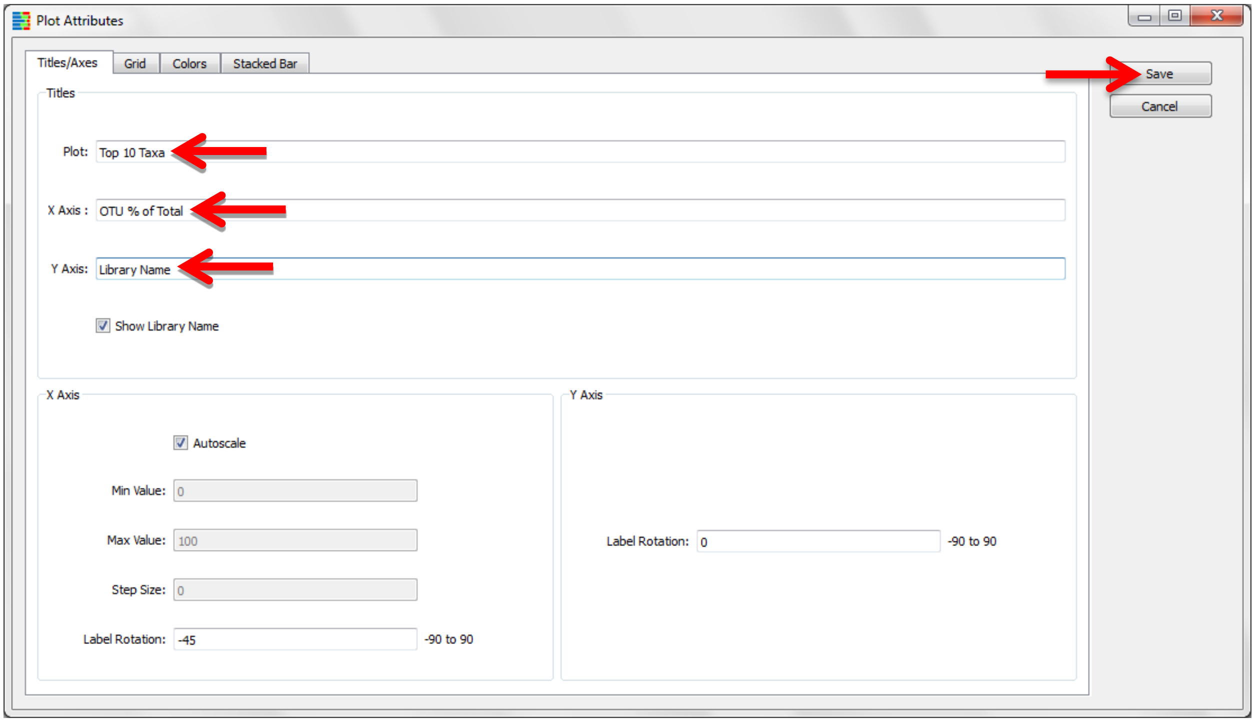
Label Rotation: 0 -90 to 90

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: Top 10 Taxa

X Axis : OTU % of Total

Y Axis: Library Name

☑ Show Library Name

X Axis

☑ Autoscale

Min Value: 0

Max Value: 100

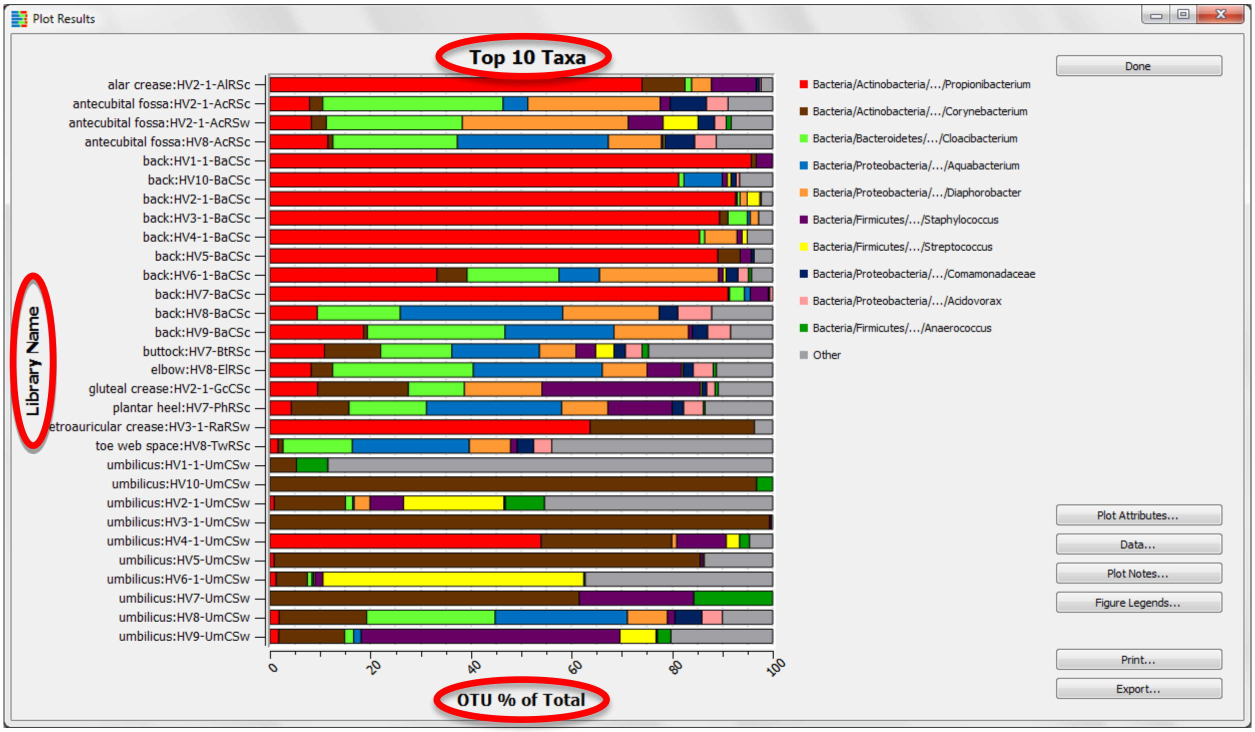
Step Size: 0

Label Rotation: -45 -90 to 90

Y Axis

Label Rotation: 0 -90 to 90

Plot Attributes window will disappear; labels appear on the plot

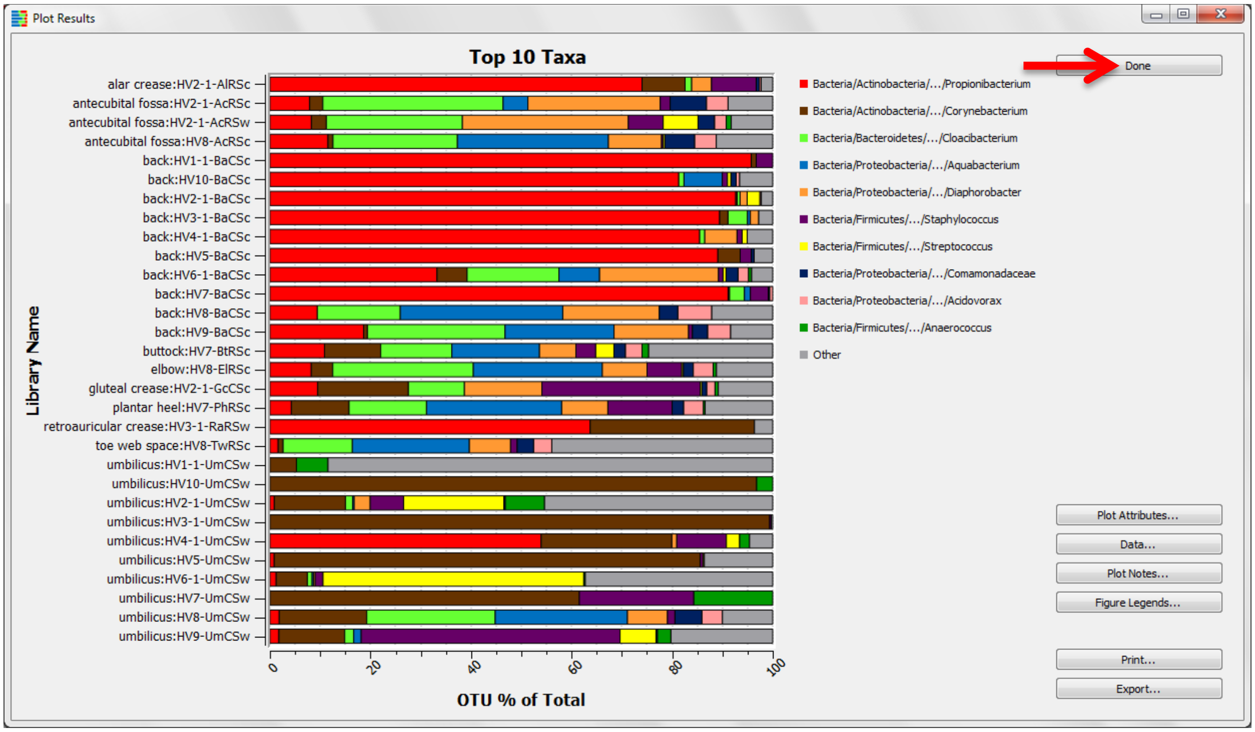


Red and brown appear to be dominant colors in this plot. According to the legend, these colors belong to the "Actinobacteria" phylum. This information may be useful in guiding us toward a hypothesis involving the dominant taxa.

Saving figures in Explicet 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.

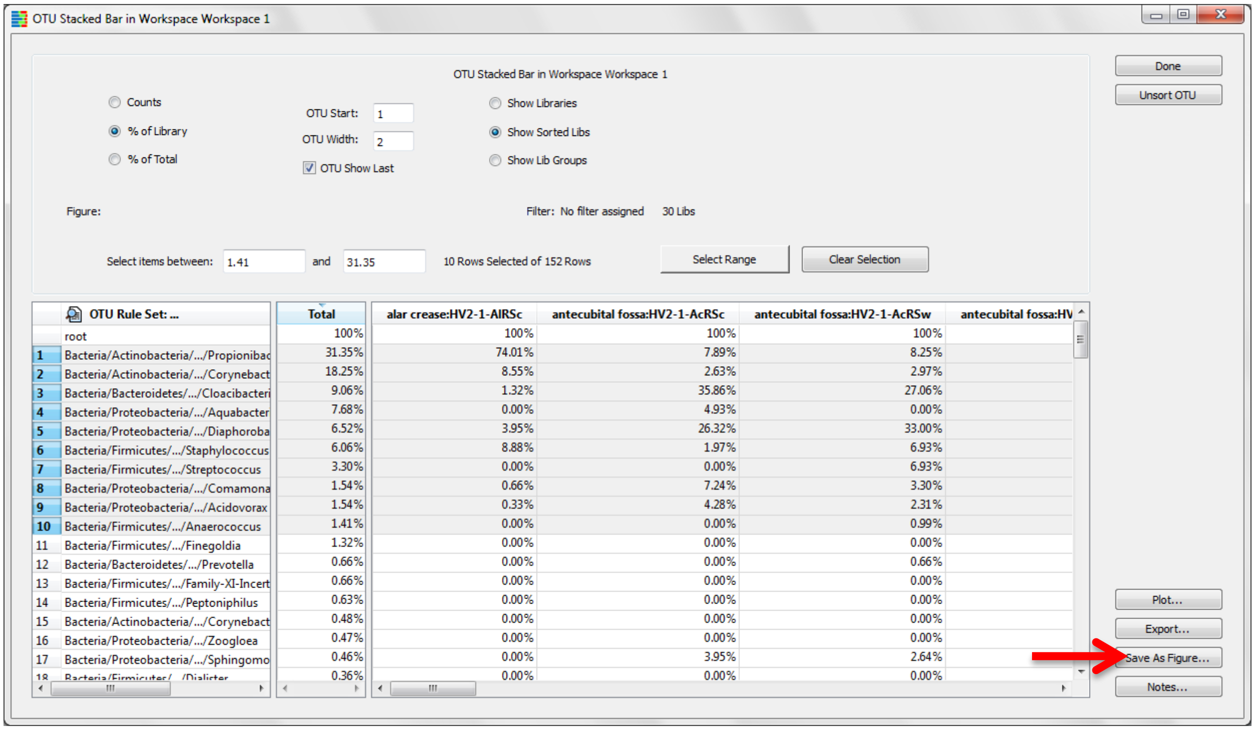
C. Save the OTU Stacked Bar Chart as a Figure

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**



○ Counts
● % of Library
○ % of Total

OTU Start: 1
OTU Width: 2
☑ OTU Show Last

○ Show Libraries
● Show Sorted Libs
○ Show Lib Groups

Figure:

Filter: No filter assigned 30 Libs

Select items between: 1.41 and 31.35 10 Rows Selected of 152 Rows

	OTU Rule Set: ...	Total	alar crease:HV2-1-AlRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fossa:HV
	root	100%	100%	100%	100%	
1	Bacteria/Actinobacteria/.../Propionibac	31.35%	74.01%	7.89%	8.25%	
2	Bacteria/Actinobacteria/.../Corynebact	18.25%	8.55%	2.63%	2.97%	
3	Bacteria/Bacteroidetes/.../Cloacibacteri	9.06%	1.32%	35.86%	27.06%	
4	Bacteria/Proteobacteria/.../Aquabacter	7.68%	0.00%	4.93%	0.00%	
5	Bacteria/Proteobacteria/.../Diaphoroba	6.52%	3.95%	26.32%	33.00%	
6	Bacteria/Firmicutes/.../Staphylococcus	6.06%	8.88%	1.97%	6.93%	
7	Bacteria/Firmicutes/.../Streptococcus	3.30%	0.00%	0.00%	6.93%	
8	Bacteria/Proteobacteria/.../Comamona	1.54%	0.66%	7.24%	3.30%	
9	Bacteria/Proteobacteria/.../Acidovorax	1.54%	0.33%	4.28%	2.31%	
10	Bacteria/Firmicutes/.../Anaerococcus	1.41%	0.00%	0.00%	0.99%	
11	Bacteria/Firmicutes/.../Finegoldia	1.32%	0.00%	0.00%	0.00%	
12	Bacteria/Bacteroidetes/.../Prevotella	0.66%	0.00%	0.00%	0.66%	
13	Bacteria/Firmicutes/.../Family-XI-Incert	0.66%	0.00%	0.00%	0.00%	
14	Bacteria/Firmicutes/.../Peptoniphilus	0.63%	0.00%	0.00%	0.00%	
15	Bacteria/Actinobacteria/.../Corynebact	0.48%	0.00%	0.00%	0.00%	
16	Bacteria/Proteobacteria/.../Zoogloea	0.47%	0.00%	0.00%	0.00%	
17	Bacteria/Proteobacteria/.../Sphingomo	0.46%	0.00%	3.95%	2.64%	
18	Bacteria/Firmicutes/.../Dialister	0.36%	0.00%	0.00%	0.00%	

Enter stacked bar chart name in pop-up window

Click **OK**

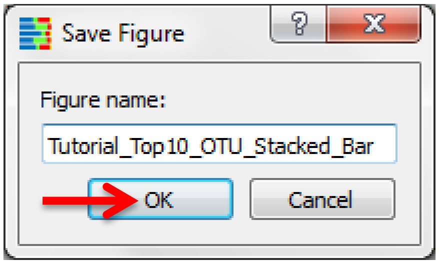
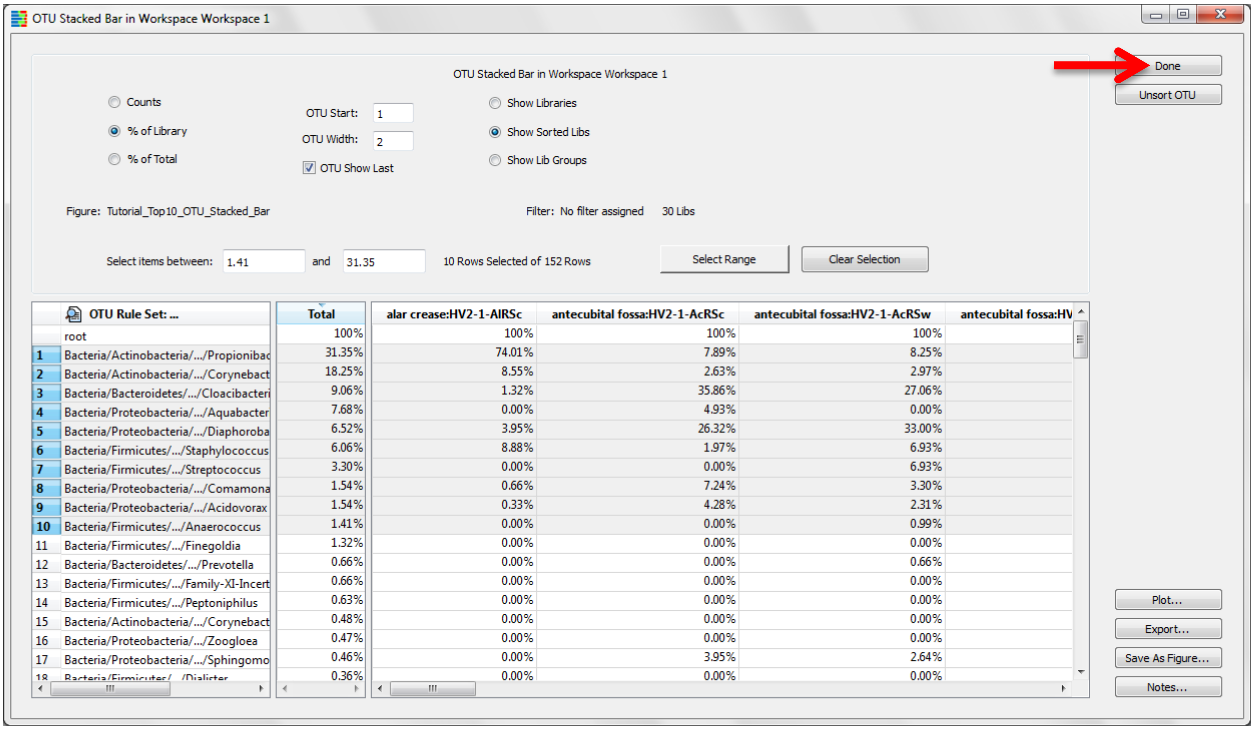


Figure name: Tutorial_Top10_OTU_Stacked_Bar

Click **Done**



OTU Stacked Bar in Workspace Workspace 1

○ Counts
● % of Library
○ % of Total

OTU Start: 1
OTU Width: 2
☑ OTU Show Last

○ Show Libraries
● Show Sorted Libs
○ Show Lib Groups

Figure: Tutorial_Top10_OTU_Stacked_Bar

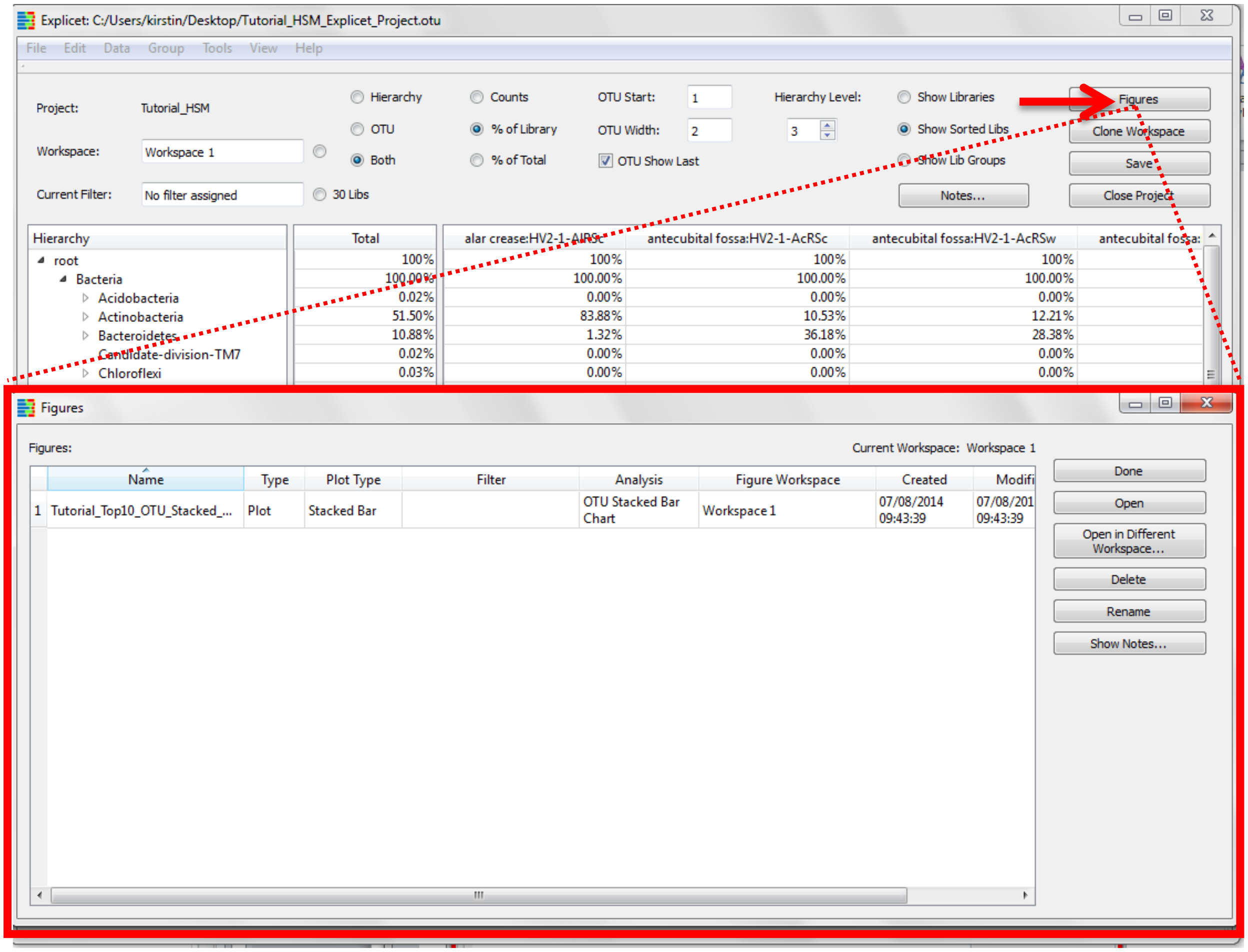
Filter: No filter assigned 30 Libs

Select items between: 1.41 and 31.35

10 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:HV
	root	100%	100%	100%	100%	
1	Bacteria/Actinobacteria/.../Propionibac	31.35%	74.01%	7.89%	8.25%	
2	Bacteria/Actinobacteria/.../Corynebact	18.25%	8.55%	2.63%	2.97%	
3	Bacteria/Bacteroidetes/.../Cloacibacteri	9.06%	1.32%	35.86%	27.06%	
4	Bacteria/Proteobacteria/.../Aquabacter	7.68%	0.00%	4.93%	0.00%	
5	Bacteria/Proteobacteria/.../Diaphoroba	6.52%	3.95%	26.32%	33.00%	
6	Bacteria/Firmicutes/.../Staphylococcus	6.06%	8.88%	1.97%	6.93%	
7	Bacteria/Firmicutes/.../Streptococcus	3.30%	0.00%	0.00%	6.93%	
8	Bacteria/Proteobacteria/.../Comamona	1.54%	0.66%	7.24%	3.30%	
9	Bacteria/Proteobacteria/.../Acidovorax	1.54%	0.33%	4.28%	2.31%	
10	Bacteria/Firmicutes/.../Anaerococcus	1.41%	0.00%	0.00%	0.99%	
11	Bacteria/Firmicutes/.../Finegoldia	1.32%	0.00%	0.00%	0.00%	
12	Bacteria/Bacteroidetes/.../Prevotella	0.66%	0.00%	0.00%	0.66%	
13	Bacteria/Firmicutes/.../Family-XI-Incert	0.66%	0.00%	0.00%	0.00%	
14	Bacteria/Firmicutes/.../Peptoniphilus	0.63%	0.00%	0.00%	0.00%	
15	Bacteria/Actinobacteria/.../Corynebact	0.48%	0.00%	0.00%	0.00%	
16	Bacteria/Proteobacteria/.../Zoogloea	0.47%	0.00%	0.00%	0.00%	
17	Bacteria/Proteobacteria/.../Sphingomo	0.46%	0.00%	3.95%	2.64%	
18	Bacteria/Firmicutes/.../Dialister	0.36%	0.00%	0.00%	0.00%	

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.



Project: Tutorial_HSM

Workspace: Workspace 1

Current Filter: No filter assigned

○ Hierarchy ○ OTU ● Both ○ 30 Libs

○ Counts ● % of Library ○ % of Total

OTU Start: 1

OTU Width: 2

☑ OTU Show Last

Hierarchy Level: 3

○ Show Libraries ● Show Sorted Libs ○ Show Lib Groups

Hierarchy	Total	alar crease:HV2-1-AlRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fossa:
root	100%	100%	100%	100%	
Bacteria	100.00%	100.00%	100.00%	100.00%	
Acidobacteria	0.02%	0.00%	0.00%	0.00%	
Actinobacteria	51.50%	83.88%	10.53%	12.21%	
Bacteroidetes	10.88%	1.32%	36.18%	28.38%	
Candidate-division-TM7	0.02%	0.00%	0.00%	0.00%	
Chloroflexi	0.03%	0.00%	0.00%	0.00%	

Figures

Figures:

Current Workspace: Workspace 1

	Name	Type	Plot Type	Filter	Analysis	Figure Workspace	Created	Modifi
1	Tutorial_Top10_OTU_Stacked_...	Plot	Stacked Bar		OTU Stacked Bar Chart	Workspace 1	07/08/2014 09:43:39	07/08/201 09:43:39

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.

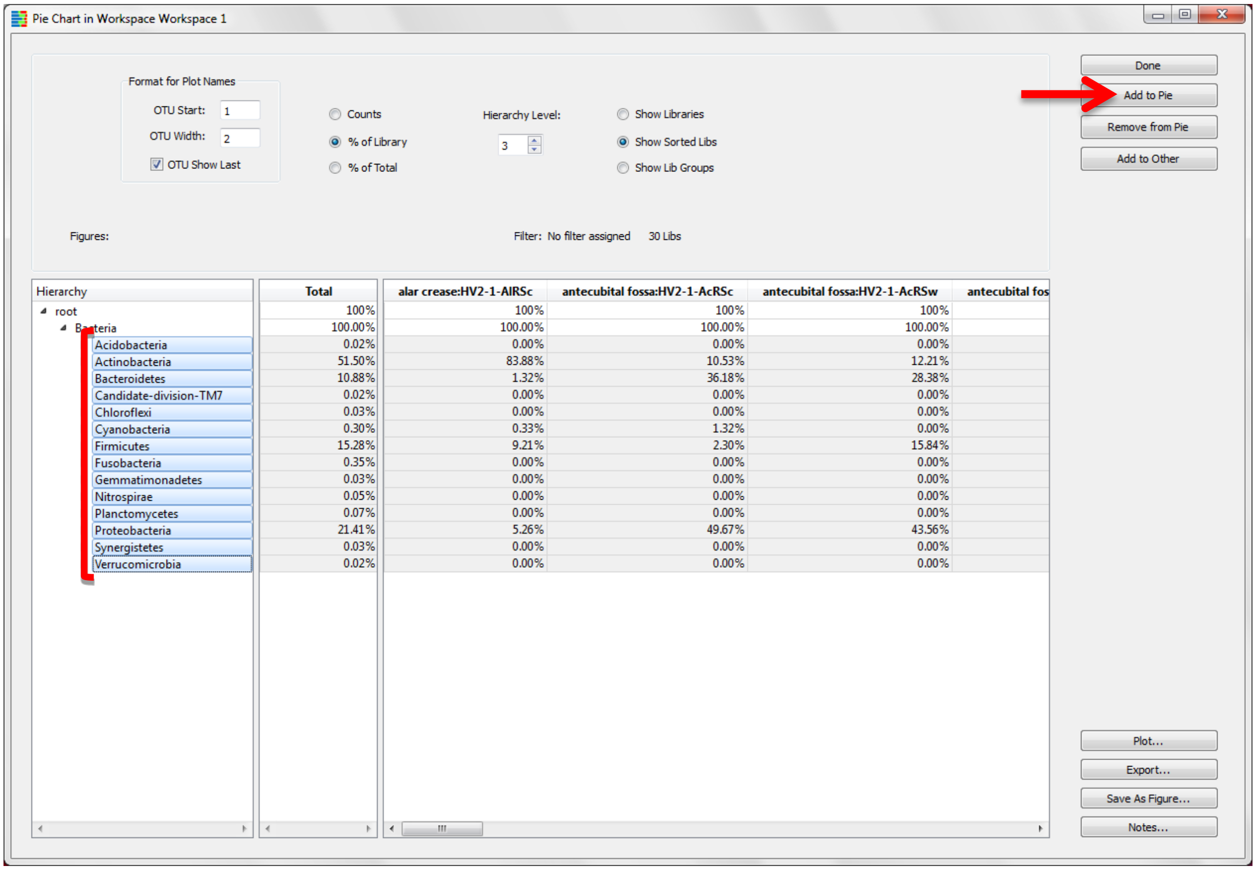
A. Create a Pie Chart of the Project Components

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**



OTU Start: 1
OTU Width: 2
☑ OTU Show Last

○ Counts
● % of Library
○ % of Total

Hierarchy Level: 3

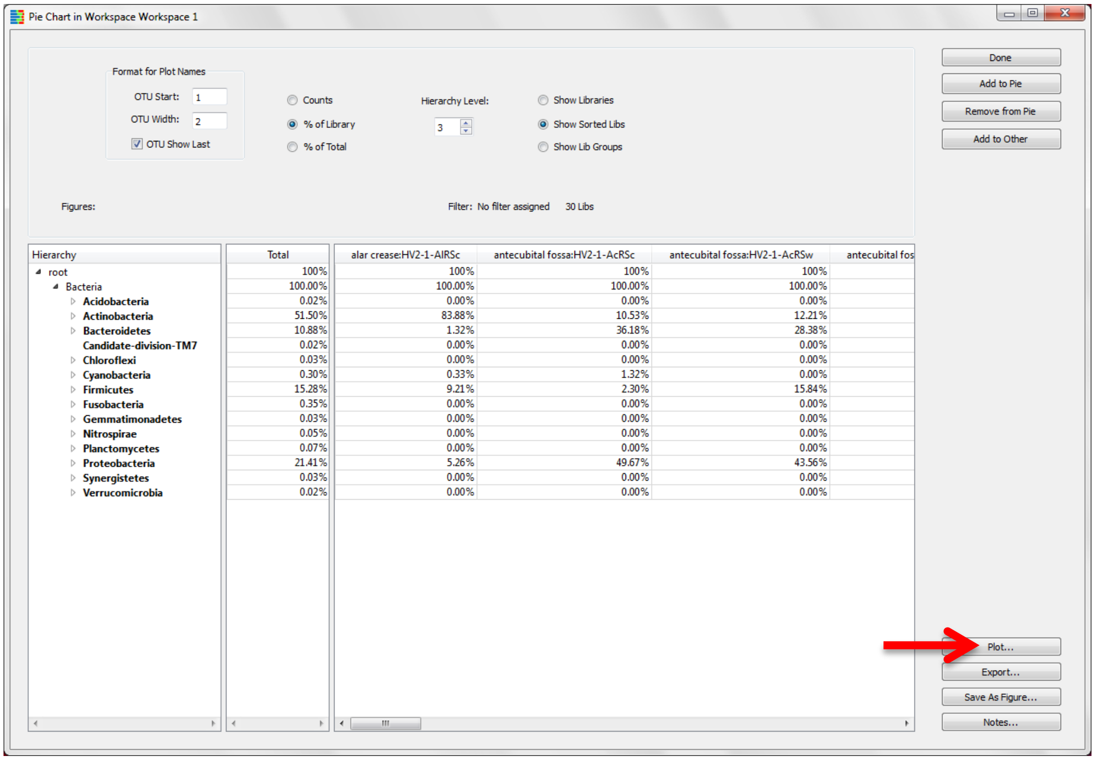
○ Show Libraries
● Show Sorted Libs
○ Show Lib Groups

Filter: No filter assigned 30 Libs

Hierarchy	Total	alar crease:HV2-1-AIRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fos
root	100%	100%	100%	100%	
Bacteria	100.00%	100.00%	100.00%	100.00%	
Acidobacteria	0.02%	0.00%	0.00%	0.00%	
Actinobacteria	51.50%	83.88%	10.53%	12.21%	
Bacteroidetes	10.88%	1.32%	36.18%	28.38%	
Candidate-division-TM7	0.02%	0.00%	0.00%	0.00%	
Chloroflexi	0.03%	0.00%	0.00%	0.00%	
Cyanobacteria	0.30%	0.33%	1.32%	0.00%	
Firmicutes	15.28%	9.21%	2.30%	15.84%	
Fusobacteria	0.35%	0.00%	0.00%	0.00%	
Gemmatimonadetes	0.03%	0.00%	0.00%	0.00%	
Nitrospirae	0.05%	0.00%	0.00%	0.00%	
Planctomycetes	0.07%	0.00%	0.00%	0.00%	
Proteobacteria	21.41%	5.26%	49.67%	43.56%	
Synergistetes	0.03%	0.00%	0.00%	0.00%	
Verrucomicrobia	0.02%	0.00%	0.00%	0.00%	

The selected phyla which 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: 3

○ Show Libraries

● Show Sorted Libs

○ Show Lib Groups

Figures:

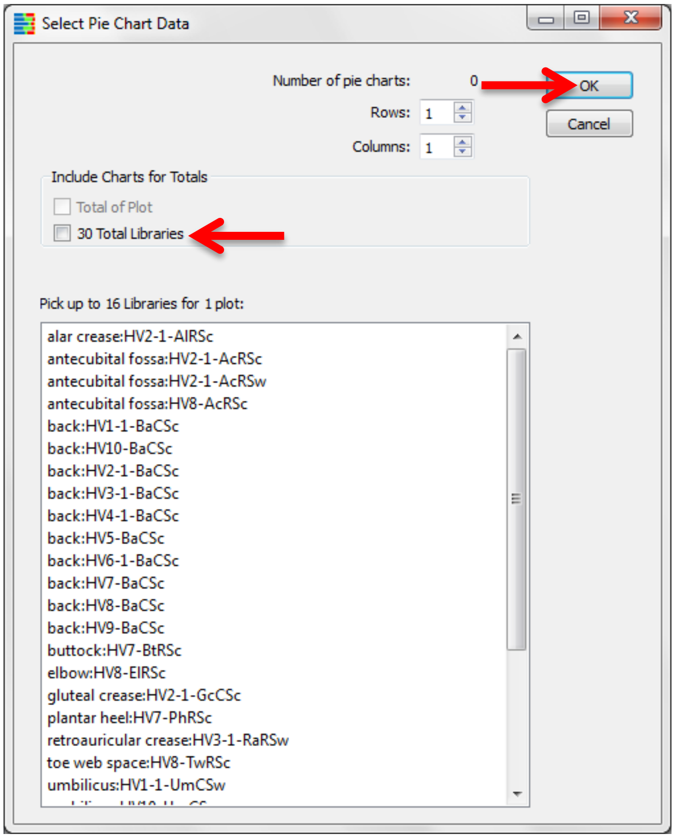
Filter: No filter assigned 30 Libs

Hierarchy	Total	alar crease:HV2-1-AIRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fos
root	100%	100%	100%	100%	
Bacteria	100.00%	100.00%	100.00%	100.00%	
Acidobacteria	0.02%	0.00%	0.00%	0.00%	
Actinobacteria	51.50%	83.88%	10.53%	12.21%	
Bacteroidetes	10.88%	1.32%	36.18%	28.38%	
Candidate-division-TM7	0.02%	0.00%	0.00%	0.00%	
Chloroflexi	0.03%	0.00%	0.00%	0.00%	
Cyanobacteria	0.30%	0.33%	1.32%	0.00%	
Firmicutes	15.28%	9.21%	2.30%	15.84%	
Fusobacteria	0.35%	0.00%	0.00%	0.00%	
Gemmatimonadetes	0.03%	0.00%	0.00%	0.00%	
Nitrospirae	0.05%	0.00%	0.00%	0.00%	
Planctomycetes	0.07%	0.00%	0.00%	0.00%	
Proteobacteria	21.41%	5.26%	49.67%	43.56%	
Synergistetes	0.03%	0.00%	0.00%	0.00%	
Verrucomicrobia	0.02%	0.00%	0.00%	0.00%	

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**



Number of pie charts: 0

Rows: 1

Columns: 1

Include Charts for Totals

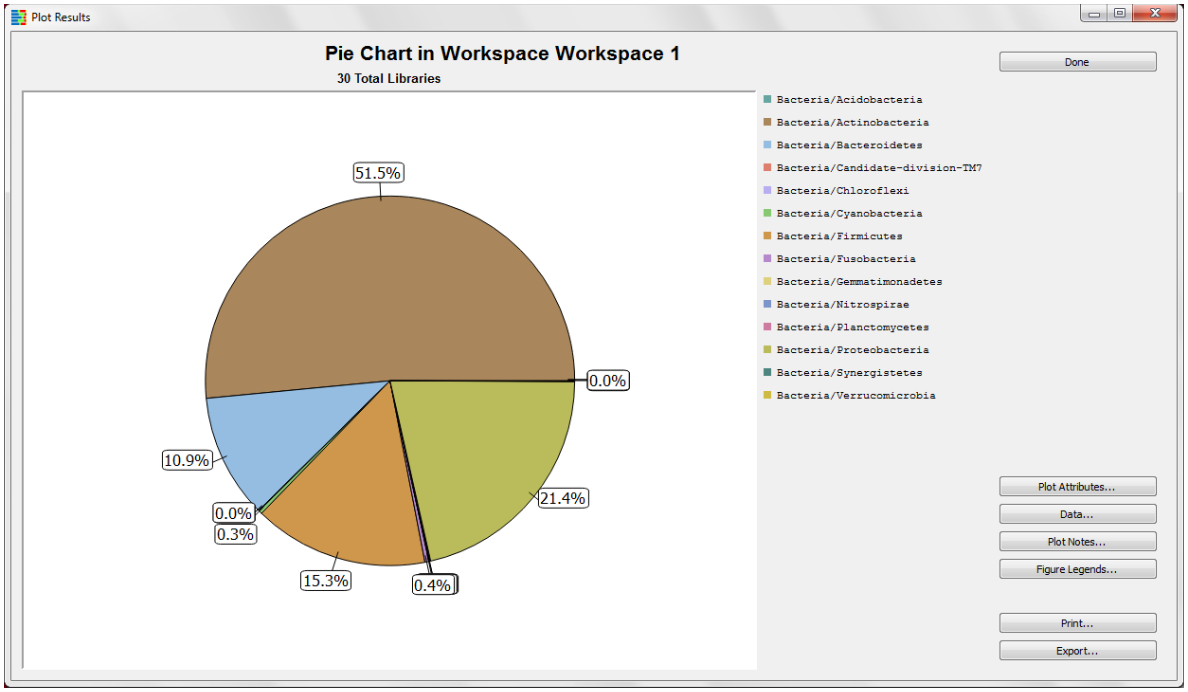
☐ Total of Plot

☐ 30 Total Libraries

Pick up to 16 Libraries for 1 plot:

alar crease:HV2-1-AIRSc
antecubital fossa:HV2-1-AcRSc
antecubital fossa:HV2-1-AcRSw
antecubital fossa:HV8-AcRSc
back:HV1-1-BaCSc
back:HV10-BaCSc
back:HV2-1-BaCSc
back:HV3-1-BaCSc
back:HV4-1-BaCSc
back:HV5-BaCSc
back:HV6-1-BaCSc
back:HV7-BaCSc
back:HV8-BaCSc
back:HV9-BaCSc
buttock:HV7-BtRSc
elbow:HV8-EIRSc
gluteal crease:HV2-1-GcCSc
plantar heel:HV7-PhRSc
retroauricular crease:HV3-1-RaRSw
toe web space:HV8-TwRSc
umbilicus:HV1-1-UmCSw

A pop-up window with the pie chart appears

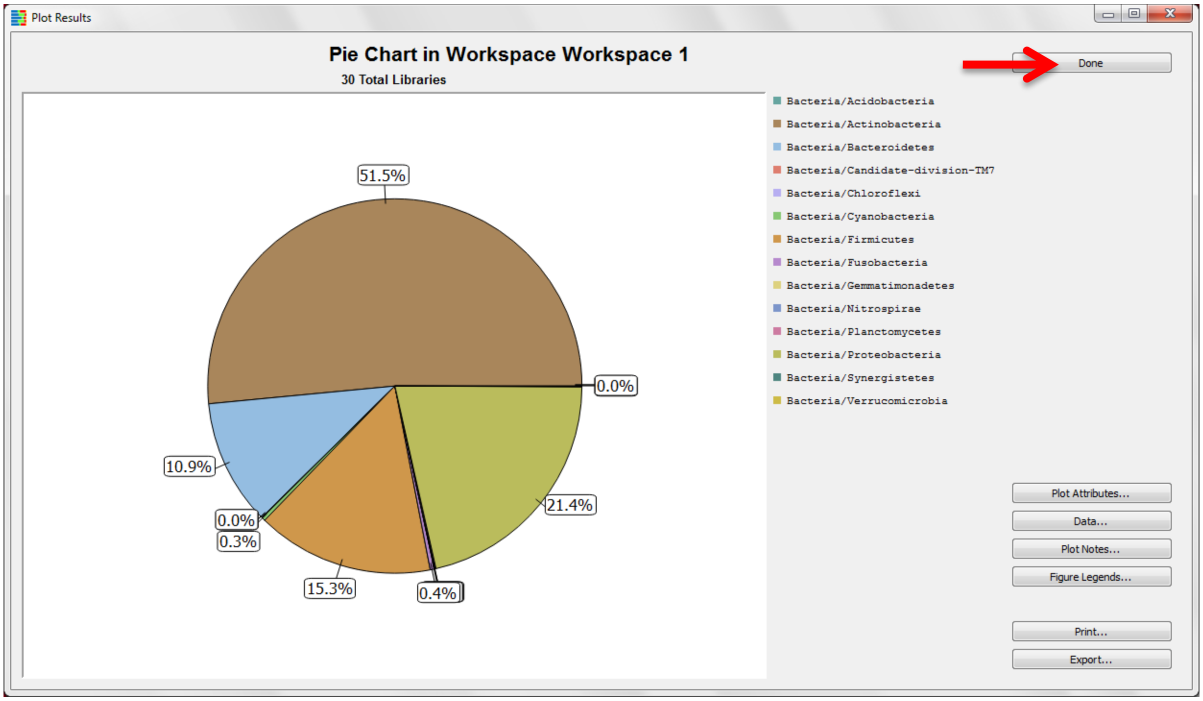


By looking at the pie chart of the phyla, it is clear that the brown wedge, Actinobacteria, is the most prevalent phylum in the data.

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**

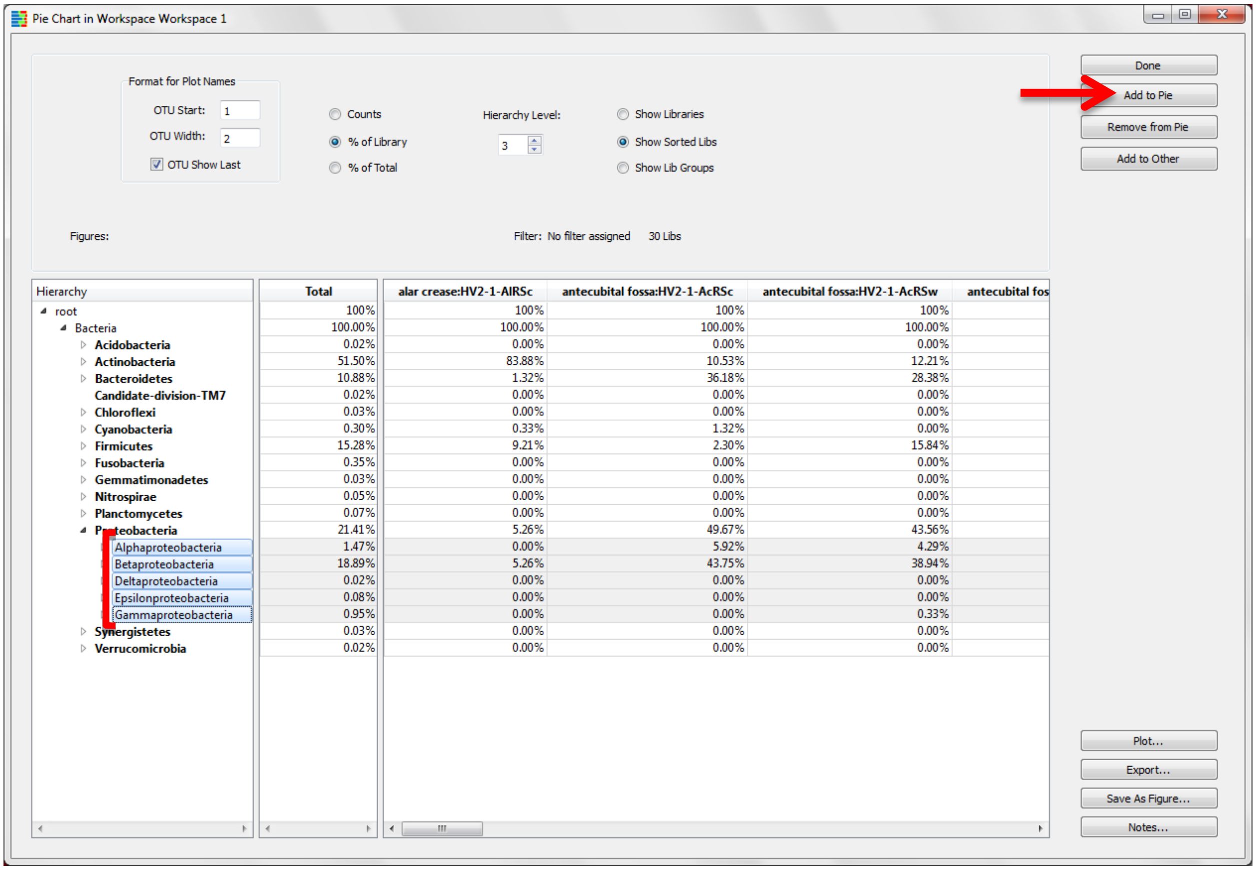


The **Taxonomy Pie Chart** setup window is back on the screen

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: 3

○ Show Libraries

● Show Sorted Libs

○ Show Lib Groups

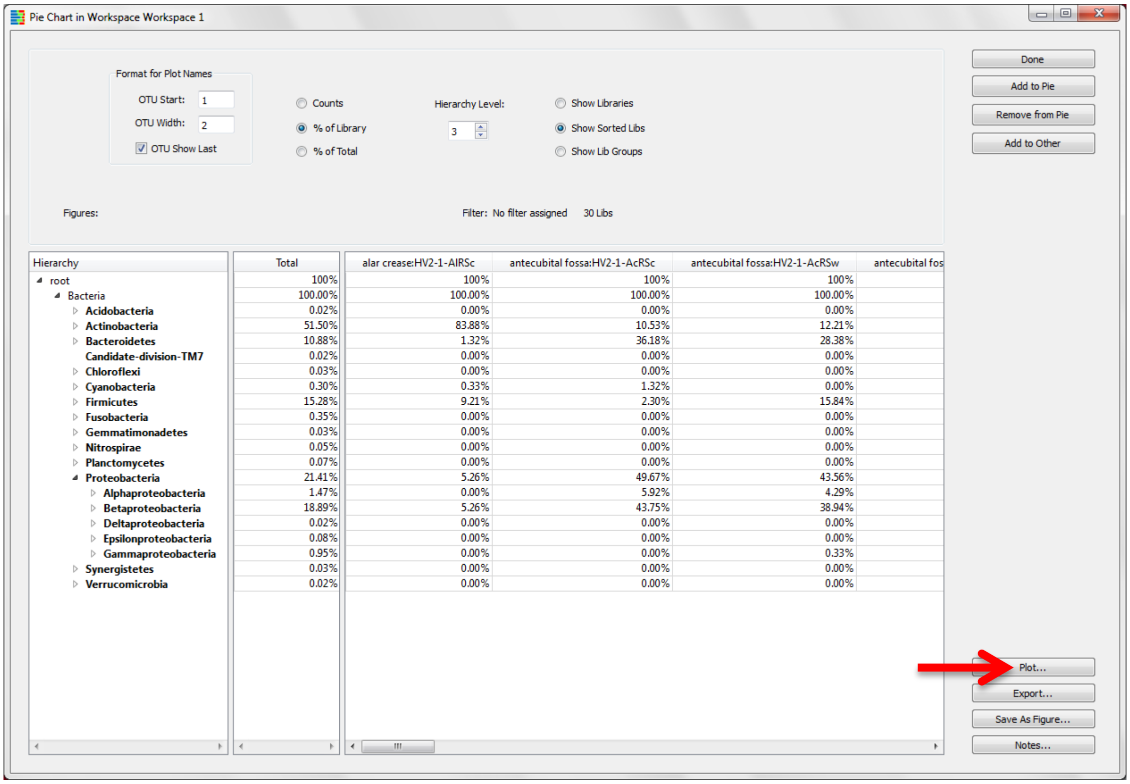
Figures:

Filter: No filter assigned 30 Libs

Hierarchy	Total	alar crease:HV2-1-AIRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fos
root	100%	100%	100%	100%	
Bacteria	100.00%	100.00%	100.00%	100.00%	
Acidobacteria	0.02%	0.00%	0.00%	0.00%	
Actinobacteria	51.50%	83.88%	10.53%	12.21%	
Bacteroidetes	10.88%	1.32%	36.18%	28.38%	
Candidate-division-TM7	0.02%	0.00%	0.00%	0.00%	
Chloroflexi	0.03%	0.00%	0.00%	0.00%	
Cyanobacteria	0.30%	0.33%	1.32%	0.00%	
Firmicutes	15.28%	9.21%	2.30%	15.84%	
Fusobacteria	0.35%	0.00%	0.00%	0.00%	
Gemmatimonadetes	0.03%	0.00%	0.00%	0.00%	
Nitrospirae	0.05%	0.00%	0.00%	0.00%	
Planctomycetes	0.07%	0.00%	0.00%	0.00%	
Proteobacteria	21.41%	5.26%	49.67%	43.56%	
Alphaproteobacteria	1.47%	0.00%	5.92%	4.29%	
Betaproteobacteria	18.89%	5.26%	43.75%	38.94%	
Deltaproteobacteria	0.02%	0.00%	0.00%	0.00%	
Epsilonproteobacteria	0.08%	0.00%	0.00%	0.00%	
Gammaproteobacteria	0.95%	0.00%	0.00%	0.33%	
Synergistetes	0.03%	0.00%	0.00%	0.00%	
Verrucomicrobia	0.02%	0.00%	0.00%	0.00%	

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: 3

○ Show Libraries
● Show Sorted Libs
○ Show Lib Groups

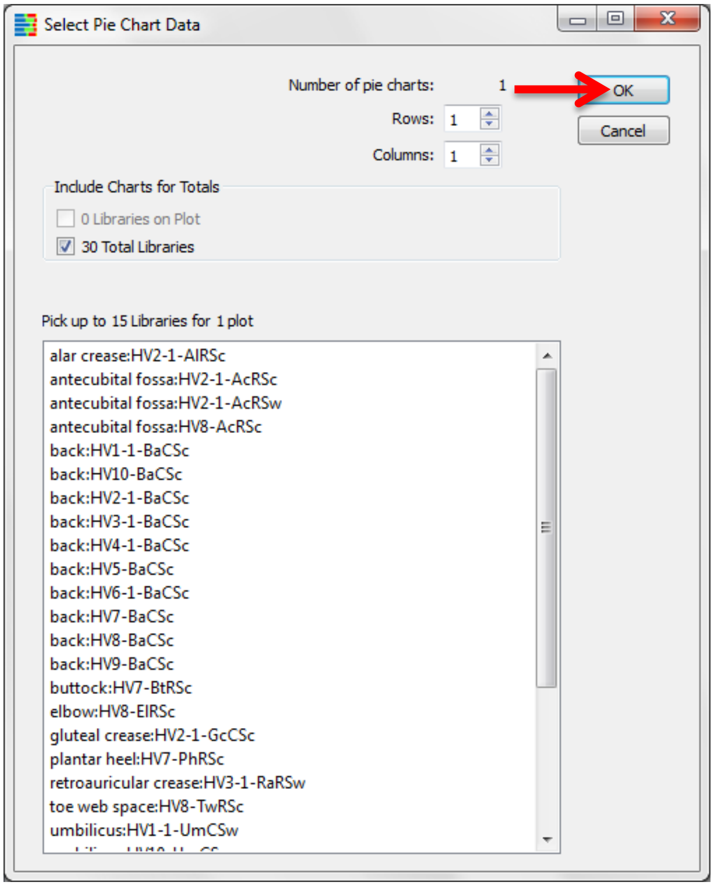
Figures:

Filter: No filter assigned 30 Libs

Hierarchy	Total	alar crease:HV2-1-AIRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fos
root	100%	100%	100%	100%	
Bacteria	100.00%	100.00%	100.00%	100.00%	
Acidobacteria	0.02%	0.00%	0.00%	0.00%	
Actinobacteria	51.50%	83.88%	10.53%	12.21%	
Bacteroidetes	10.88%	1.32%	36.18%	28.38%	
Candidate-division-TM7	0.02%	0.00%	0.00%	0.00%	
Chloroflexi	0.03%	0.00%	0.00%	0.00%	
Cyanobacteria	0.30%	0.33%	1.32%	0.00%	
Firmicutes	15.28%	9.21%	2.30%	15.84%	
Fusobacteria	0.35%	0.00%	0.00%	0.00%	
Gemmatimonadetes	0.03%	0.00%	0.00%	0.00%	
Nitrospirae	0.05%	0.00%	0.00%	0.00%	
Planctomycetes	0.07%	0.00%	0.00%	0.00%	
Proteobacteria	21.41%	5.26%	49.67%	43.56%	
Alphaproteobacteria	1.47%	0.00%	5.92%	4.29%	
Betaproteobacteria	18.89%	5.26%	43.75%	38.94%	
Deltaproteobacteria	0.02%	0.00%	0.00%	0.00%	
Epsilonproteobacteria	0.08%	0.00%	0.00%	0.00%	
Gammaproteobacteria	0.95%	0.00%	0.00%	0.33%	
Synergistetes	0.03%	0.00%	0.00%	0.00%	
Verrucomicrobia	0.02%	0.00%	0.00%	0.00%	

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**



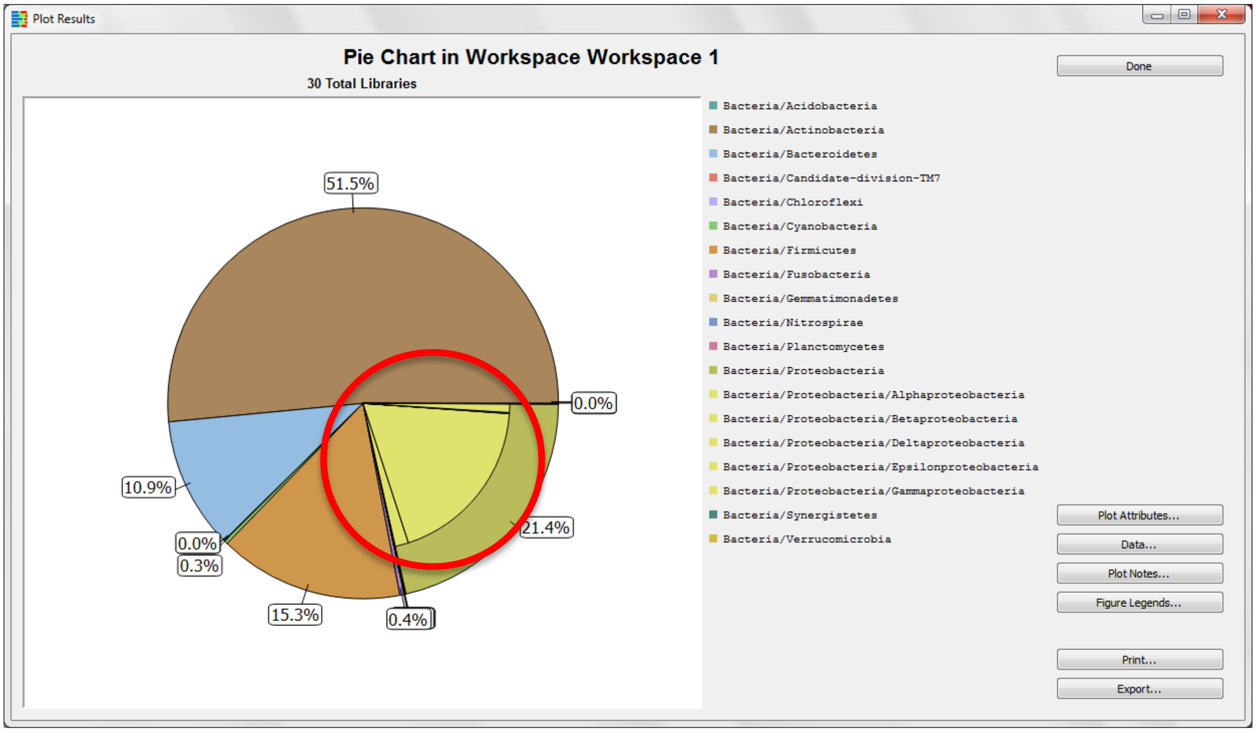
Number of pie charts: 1
Rows: 1
Columns: 1

Include Charts for Totals
☐ 0 Libraries on Plot
☑ 30 Total Libraries

Pick up to 15 Libraries for 1 plot

alar crease:HV2-1-AIRSc
antecubital fossa:HV2-1-AcRSc
antecubital fossa:HV2-1-AcRSw
antecubital fossa:HV8-AcRSc
back:HV1-1-BaCSc
back:HV10-BaCSc
back:HV2-1-BaCSc
back:HV3-1-BaCSc
back:HV4-1-BaCSc
back:HV5-BaCSc
back:HV6-1-BaCSc
back:HV7-BaCSc
back:HV8-BaCSc
back:HV9-BaCSc
buttock:HV7-BtRSc
elbow:HV8-EIRSc
gluteal crease:HV2-1-GcCSc
plantar heel:HV7-PhRSc
retroauricular crease:HV3-1-RaRSw
toe web space:HV8-TwRSc
umbilicus:HV1-1-UmCSw

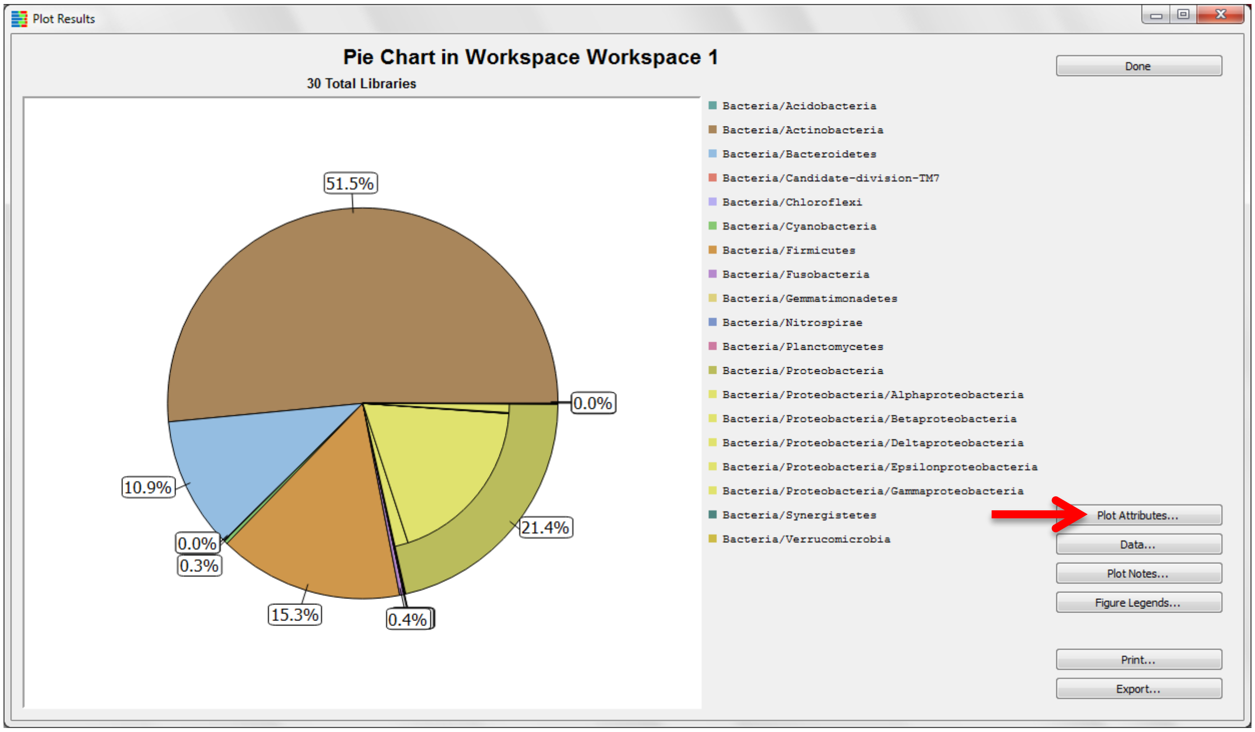
A pop-up window with the pie chart appears. We now see the classes within Proteobacteria represented as sub-wedges.



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

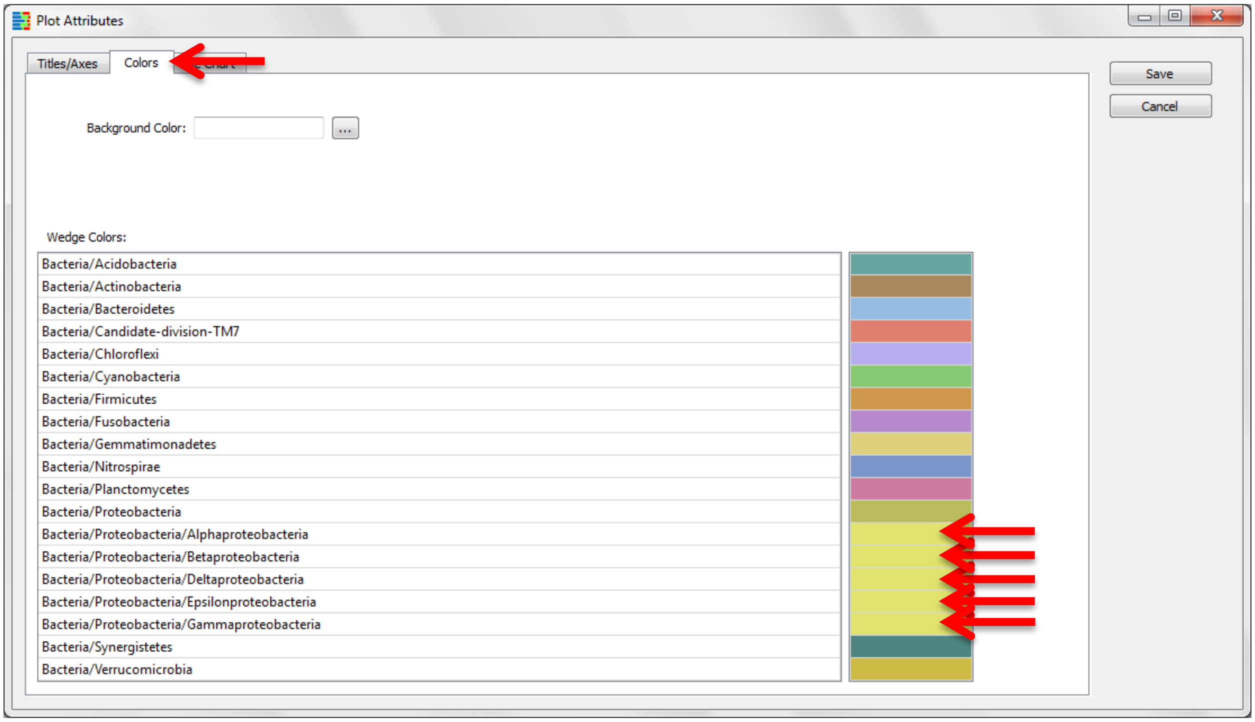
In the pie chart **Plot Results** window, click **Plot Attributes**



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

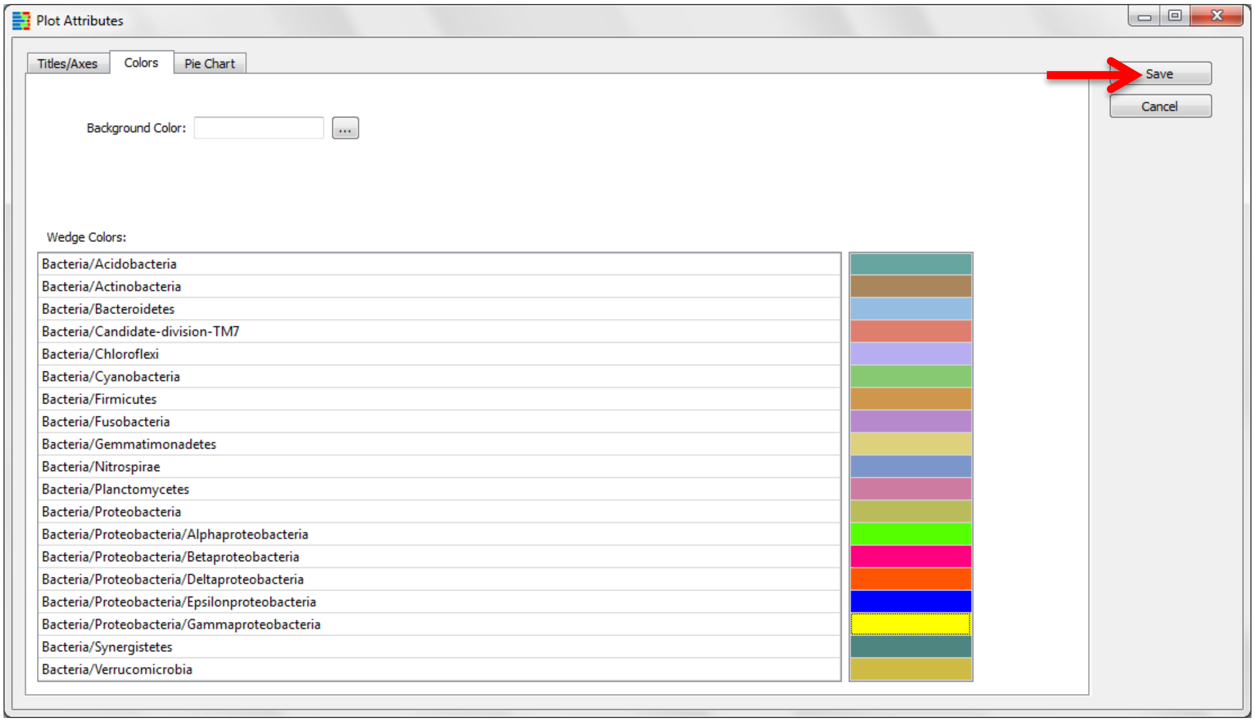


Background Color:

Wedge Colors:

Bacteria/Acidobacteria	🟦
Bacteria/Actinobacteria	🟫
Bacteria/Bacteroidetes	🟦
Bacteria/Candidate-division-TM7	🟥
Bacteria/Chloroflexi	🟪
Bacteria/Cyanobacteria	🟩
Bacteria/Firmicutes	🟧
Bacteria/Fusobacteria	🟪
Bacteria/Gemmatimonadetes	🟨
Bacteria/Nitrospirae	🟦
Bacteria/Planctomycetes	🟪
Bacteria/Proteobacteria	🟨
Bacteria/Proteobacteria/Alphaproteobacteria	🟨
Bacteria/Proteobacteria/Betaproteobacteria	🟨
Bacteria/Proteobacteria/Deltaproteobacteria	🟨
Bacteria/Proteobacteria/Epsilonproteobacteria	🟨
Bacteria/Proteobacteria/Gammaproteobacteria	🟨
Bacteria/Synergistetes	🟦
Bacteria/Verrucomicrobia	🟨

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

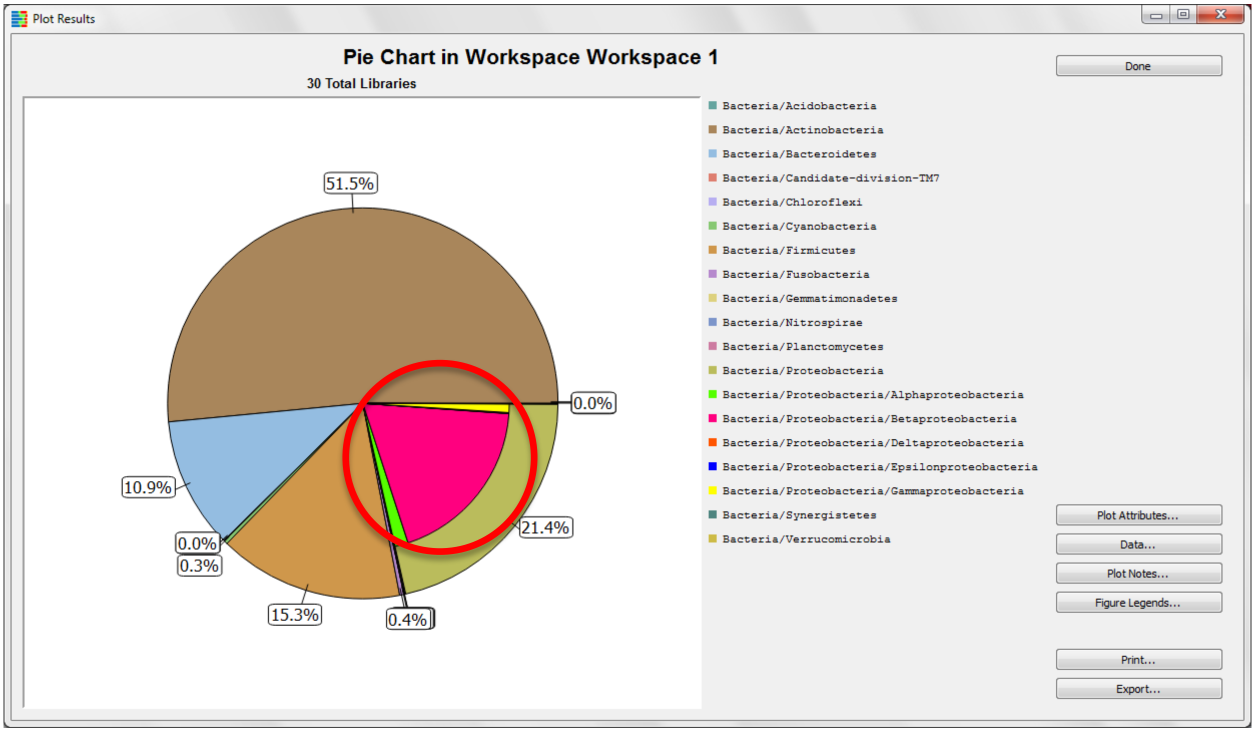


Background Color:

Wedge Colors:

Bacteria/Acidobacteria	🟦
Bacteria/Actinobacteria	🟫
Bacteria/Bacteroidetes	🟦
Bacteria/Candidate-division-TM7	🟥
Bacteria/Chloroflexi	🟪
Bacteria/Cyanobacteria	🟩
Bacteria/Firmicutes	🟧
Bacteria/Fusobacteria	🟪
Bacteria/Gemmatimonadetes	🟨
Bacteria/Nitrospirae	🟦
Bacteria/Planctomycetes	🟪
Bacteria/Proteobacteria	🟨
Bacteria/Proteobacteria/Alphaproteobacteria	🟩
Bacteria/Proteobacteria/Betaproteobacteria	🟥
Bacteria/Proteobacteria/Deltaproteobacteria	🟧
Bacteria/Proteobacteria/Epsilonproteobacteria	🟦
Bacteria/Proteobacteria/Gammaproteobacteria	🟨
Bacteria/Synergistetes	🟦
Bacteria/Verrucomicrobia	🟨

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.

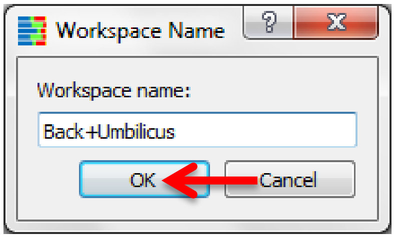
A. Create a New Workspace

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.

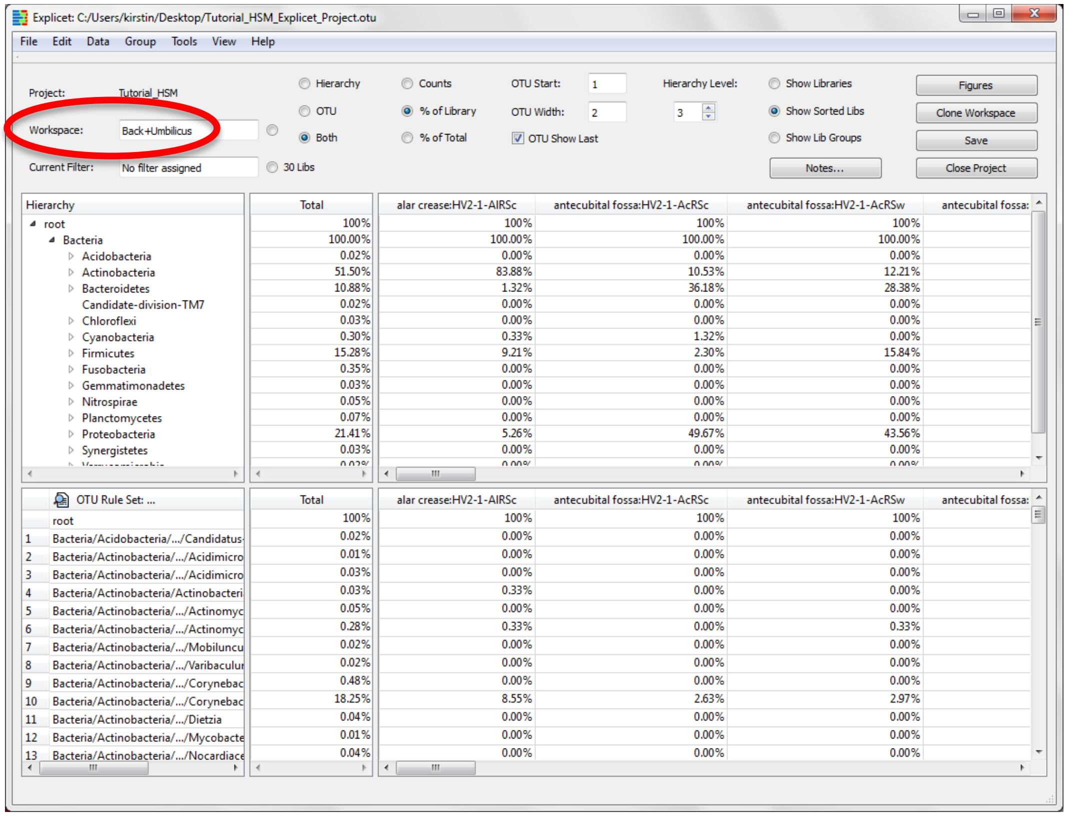
Enter desired workspace name in the pop-up window

Click **OK** to create the new workspace



Workspace name: Back+Umbilicus

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



Project: Tutorial_HSM

Workspace: Back+Umbilicus

Current Filter: No filter assigned

○ 30 Libs

○ Hierarchy
○ OTU
● Both

○ Counts
● % of Library
○ % of Total

OTU Start: 1

OTU Width: 2

☑ OTU Show Last

Hierarchy Level: 3

○ Show Libraries
● Show Sorted Libs
○ Show Lib Groups

Hierarchy	Total	alar crease:HV2-1-AIRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fossa:
root	100%	100%	100%	100%	
Bacteria	100.00%	100.00%	100.00%	100.00%	
Acidobacteria	0.02%	0.00%	0.00%	0.00%	
Actinobacteria	51.50%	83.88%	10.53%	12.21%	
Bacteroidetes	10.88%	1.32%	36.18%	28.38%	
Candidate-division-TM7	0.02%	0.00%	0.00%	0.00%	
Chloroflexi	0.03%	0.00%	0.00%	0.00%	
Cyanobacteria	0.30%	0.33%	1.32%	0.00%	
Firmicutes	15.28%	9.21%	2.30%	15.84%	
Fusobacteria	0.35%	0.00%	0.00%	0.00%	
Gemmatimonadetes	0.03%	0.00%	0.00%	0.00%	
Nitrospirae	0.05%	0.00%	0.00%	0.00%	
Planctomycetes	0.07%	0.00%	0.00%	0.00%	
Proteobacteria	21.41%	5.26%	49.67%	43.56%	
Synergistetes	0.03%	0.00%	0.00%	0.00%	

	OTU Rule Set: ...	Total	alar crease:HV2-1-AIRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fossa:
	root	100%	100%	100%	100%	
1	Bacteria/Acidobacteria/.../Candidatus-	0.02%	0.00%	0.00%	0.00%	
2	Bacteria/Actinobacteria/.../Acidimicro	0.01%	0.00%	0.00%	0.00%	
3	Bacteria/Actinobacteria/.../Acidimicro	0.03%	0.00%	0.00%	0.00%	
4	Bacteria/Actinobacteria/Actinobacteri	0.03%	0.33%	0.00%	0.00%	
5	Bacteria/Actinobacteria/.../Actinomyc	0.05%	0.00%	0.00%	0.00%	
6	Bacteria/Actinobacteria/.../Actinomyc	0.28%	0.33%	0.00%	0.33%	
7	Bacteria/Actinobacteria/.../Mobiluncu	0.02%	0.00%	0.00%	0.00%	
8	Bacteria/Actinobacteria/.../Varibaculur	0.02%	0.00%	0.00%	0.00%	
9	Bacteria/Actinobacteria/.../Corynebac	0.48%	0.00%	0.00%	0.00%	
10	Bacteria/Actinobacteria/.../Corynebac	18.25%	8.55%	2.63%	2.97%	
11	Bacteria/Actinobacteria/.../Dietzia	0.04%	0.00%	0.00%	0.00%	
12	Bacteria/Actinobacteria/.../Mycobacte	0.01%	0.00%	0.00%	0.00%	
13	Bacteria/Actinobacteria/.../Nocardiace	0.04%	0.00%	0.00%	0.00%	

IX. Apply a Filter

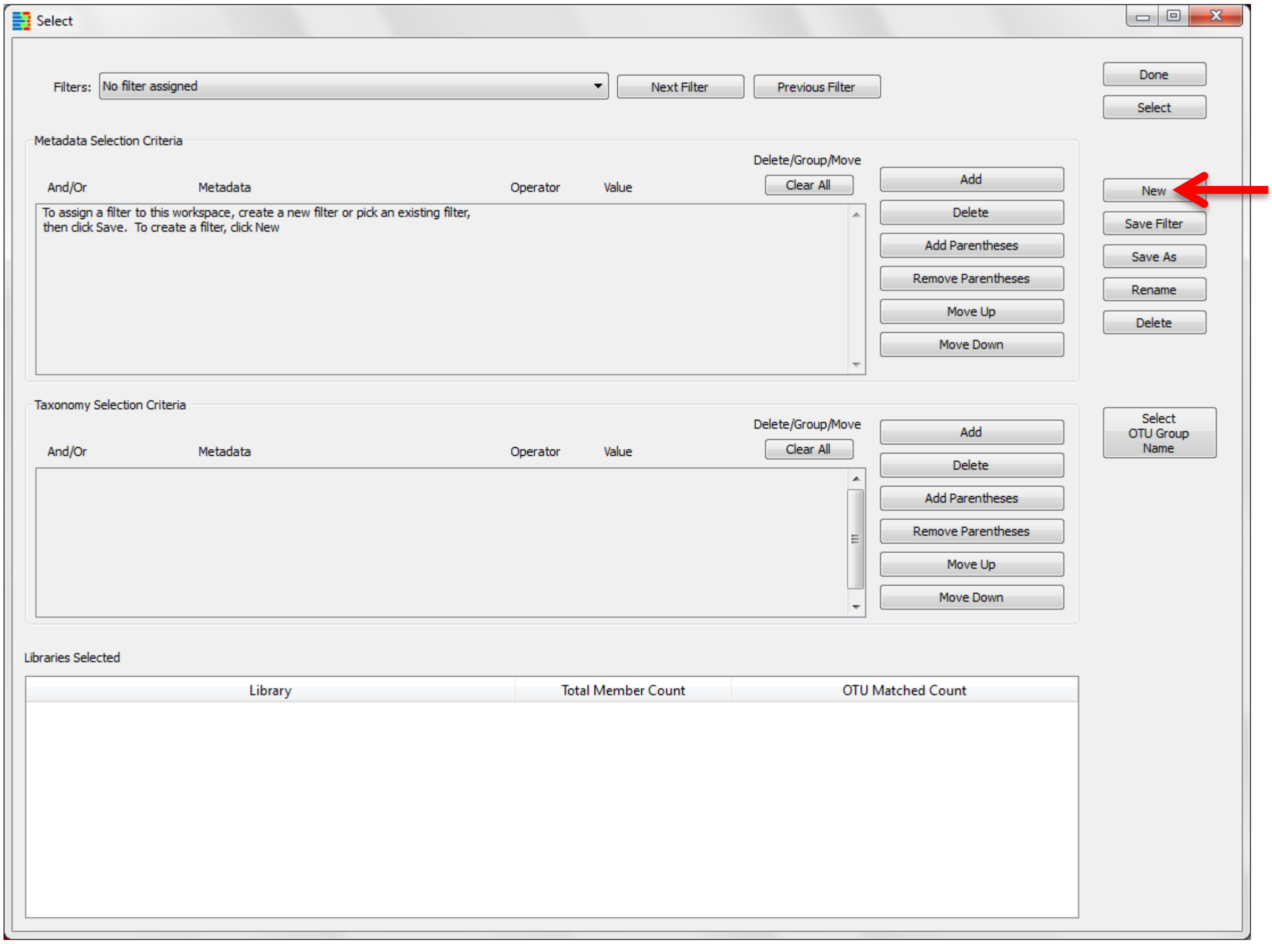
To compare data from only the back and umbilicus, we need to separate these libraries from the other body parts. This is done in Explicet via “filters”.

A. Create a Filter

Data → Select Libraries

New pop-up window appears for creation of filters

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

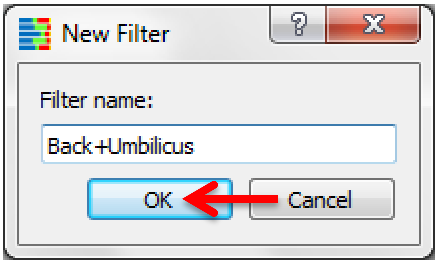


Filters: No filter assigned

To assign a filter to this workspace, create a new filter or pick an existing filter, then click Save. To create a filter, click New

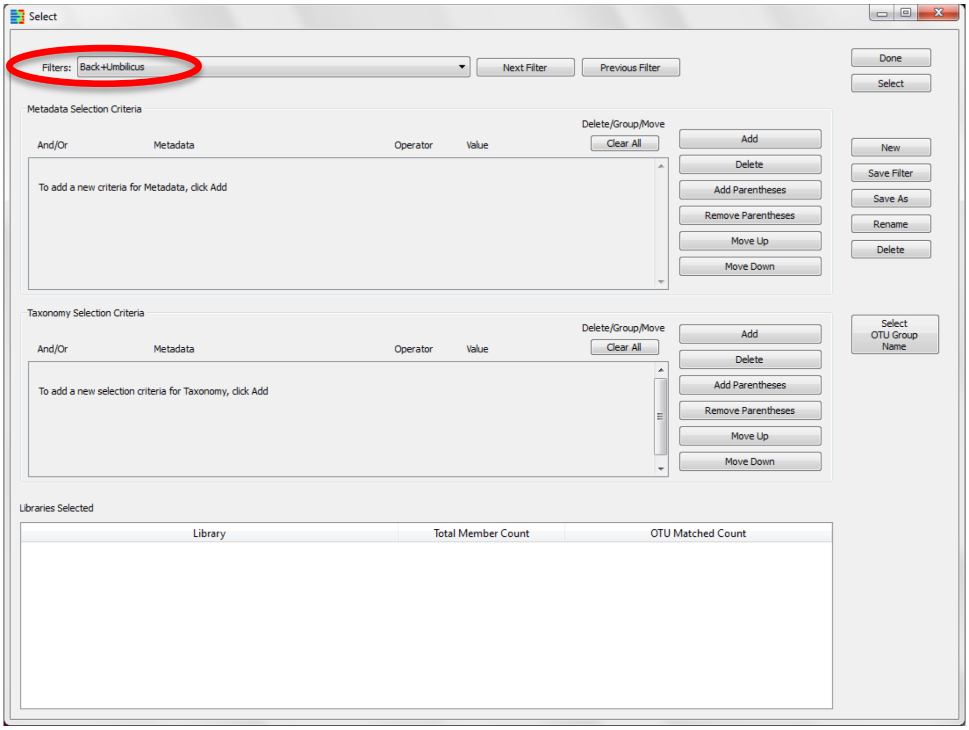
Enter desired filter name in the pop-up window

Click **OK**



Filter name: Back+Umbilicus

The filter name will appear in upper left corner of window



Filters: Back+Umbilicus

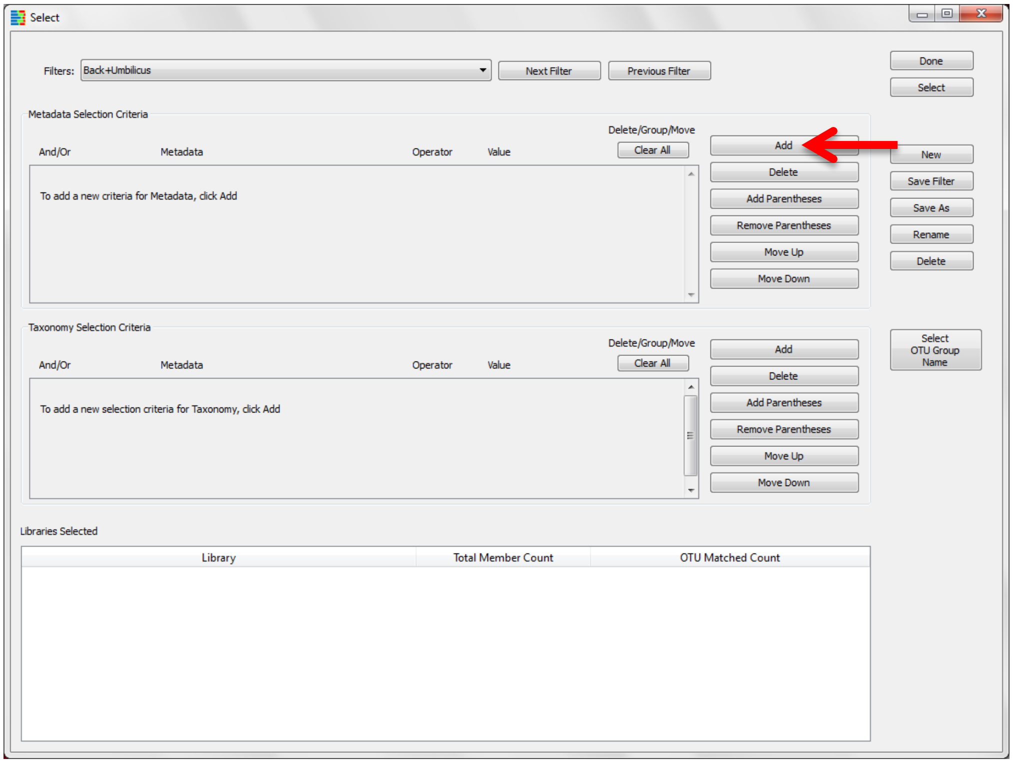
To add a new criteria for Metadata, click Add

To add a new selection criteria for Taxonomy, click Add

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



Filters: Back+Umbilicus

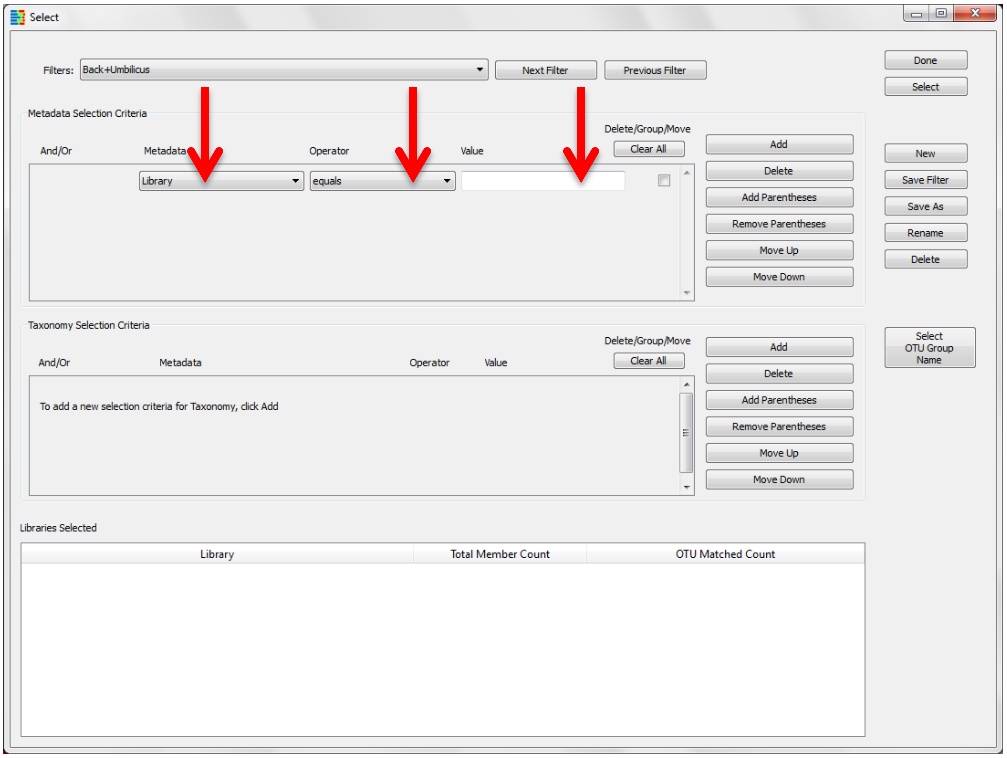
To add a new criteria for Metadata, click Add

To add a new selection criteria for Taxonomy, click Add

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**



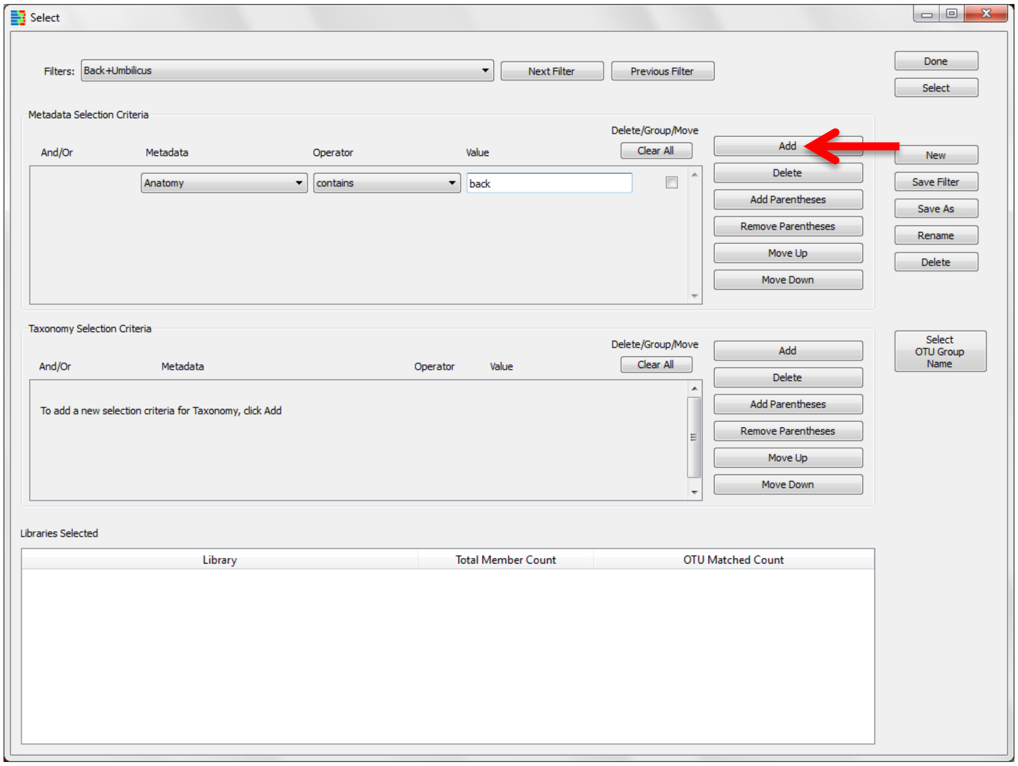
Filters: Back+Umbilicus

Metadata: Library

Operator: equals

To add a new selection criteria for Taxonomy, click Add

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



Filters: Back+Umbilicus

Metadata: Anatomy

Operator: contains

Value: back

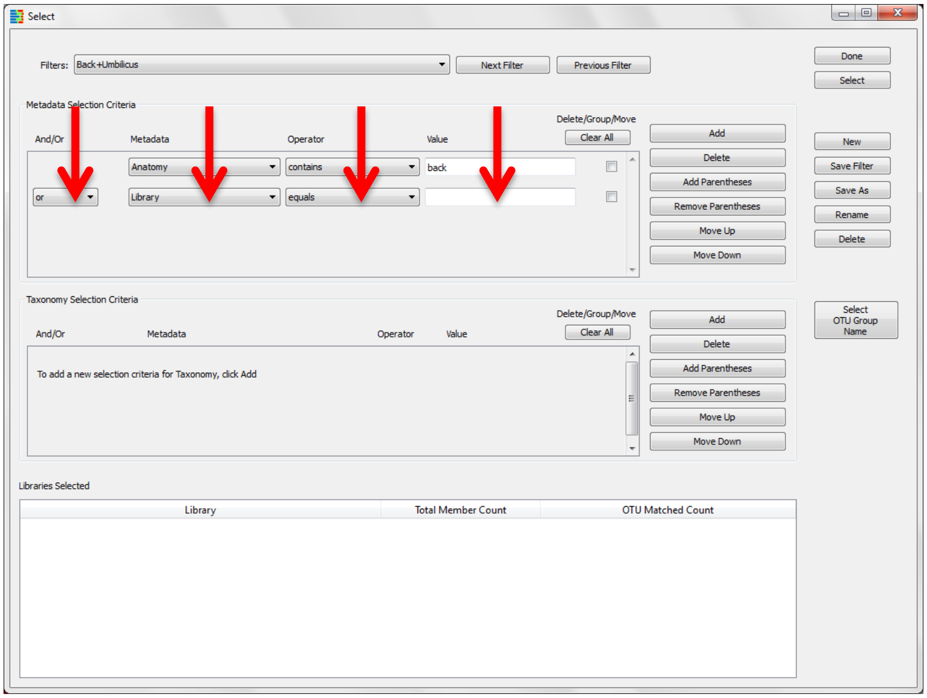
To add a new selection criteria for Taxonomy, click Add

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**



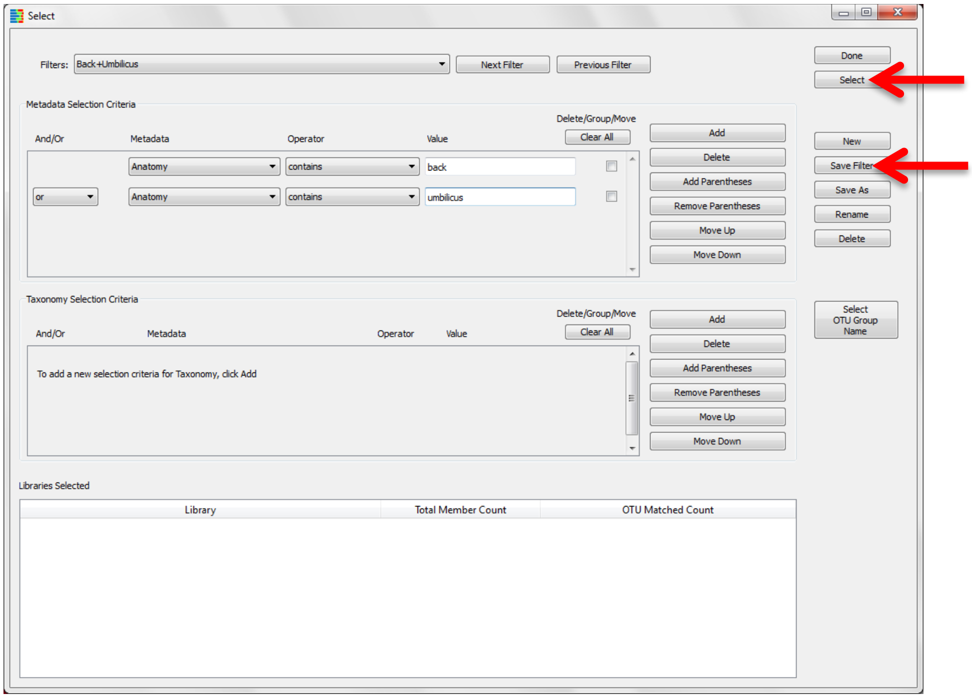
Filters: Back+Umbilicus

And/Or	Metadata	Operator	Value
	Anatomy	contains	back
or	Library	equals	

To add a new selection criteria for Taxonomy, click Add

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

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

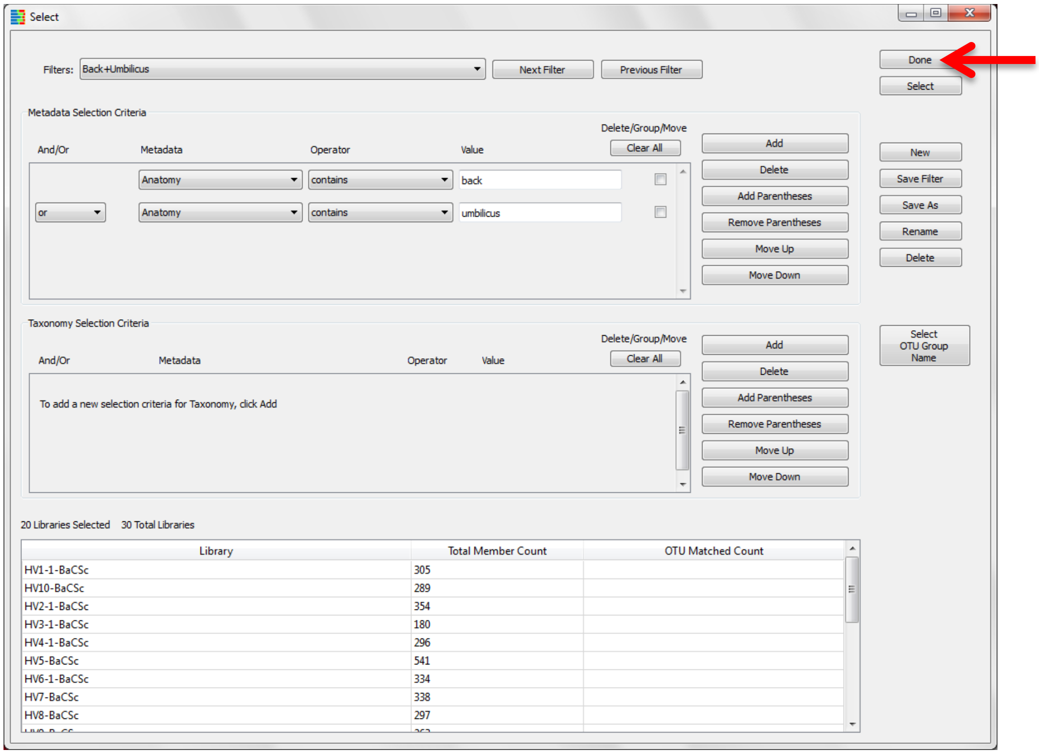


Filters: Back+Umbilicus

And/Or	Metadata	Operator	Value
	Anatomy	contains	back
or	Anatomy	contains	umbilicus

To add a new selection criteria for Taxonomy, click Add

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



Filters: Back+Umbilicus

Metadata Selection Criteria

And/Or	Metadata	Operator	Value	Delete/Group/Move
	Anatomy	contains	back	☐
or	Anatomy	contains	umbilicus	☐

Taxonomy Selection Criteria

And/Or	Metadata	Operator	Value	Delete/Group/Move

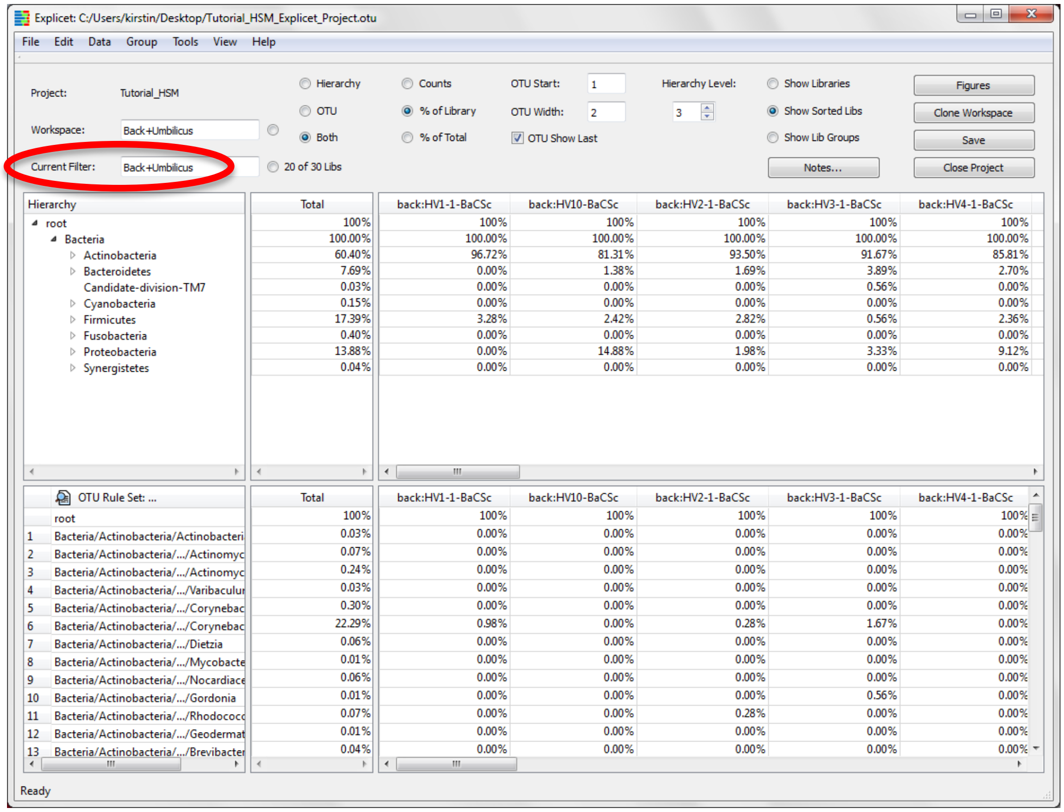
To add a new selection criteria for Taxonomy, click Add

20 Libraries 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	

Pop-up window will disappear

On the current workspace window, 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.



Project: Tutorial_HSM

Workspace: Back+Umbilicus ○

Current Filter: Back+Umbilicus ○ 20 of 30 Libs

○ Hierarchy
○ OTU
● Both

○ Counts
● % of Library
○ % of Total

OTU Start: 1
OTU Width: 2
☑ OTU Show Last

Hierarchy Level: 3

○ Show Libraries
● Show Sorted Libs
○ Show Lib Groups

Hierarchy	Total	back:HV1-1-BaCSc	back:HV10-BaCSc	back:HV2-1-BaCSc	back:HV3-1-BaCSc	back:HV4-1-BaCSc
root	100%	100%	100%	100%	100%	100%
Bacteria	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
Actinobacteria	60.40%	96.72%	81.31%	93.50%	91.67%	85.81%
Bacteroidetes	7.69%	0.00%	1.38%	1.69%	3.89%	2.70%
Candidate-division-TM7	0.03%	0.00%	0.00%	0.00%	0.56%	0.00%
Cyanobacteria	0.15%	0.00%	0.00%	0.00%	0.00%	0.00%
Firmicutes	17.39%	3.28%	2.42%	2.82%	0.56%	2.36%
Fusobacteria	0.40%	0.00%	0.00%	0.00%	0.00%	0.00%
Proteobacteria	13.88%	0.00%	14.88%	1.98%	3.33%	9.12%
Synergistetes	0.04%	0.00%	0.00%	0.00%	0.00%	0.00%

	OTU Rule Set: ...	Total	back:HV1-1-BaCSc	back:HV10-BaCSc	back:HV2-1-BaCSc	back:HV3-1-BaCSc	back:HV4-1-BaCSc
	root	100%	100%	100%	100%	100%	100%
1	Bacteria/Actinobacteria/Actinobacteri	0.03%	0.00%	0.00%	0.00%	0.00%	0.00%
2	Bacteria/Actinobacteria/.../Actinomyc	0.07%	0.00%	0.00%	0.00%	0.00%	0.00%
3	Bacteria/Actinobacteria/.../Actinomyc	0.24%	0.00%	0.00%	0.00%	0.00%	0.00%
4	Bacteria/Actinobacteria/.../Varibaculu	0.03%	0.00%	0.00%	0.00%	0.00%	0.00%
5	Bacteria/Actinobacteria/.../Corynebac	0.30%	0.00%	0.00%	0.00%	0.00%	0.00%
6	Bacteria/Actinobacteria/.../Corynebac	22.29%	0.98%	0.00%	0.28%	1.67%	0.00%
7	Bacteria/Actinobacteria/.../Dietzia	0.06%	0.00%	0.00%	0.00%	0.00%	0.00%
8	Bacteria/Actinobacteria/.../Mycobacte	0.01%	0.00%	0.00%	0.00%	0.00%	0.00%
9	Bacteria/Actinobacteria/.../Nocardiace	0.06%	0.00%	0.00%	0.00%	0.00%	0.00%
10	Bacteria/Actinobacteria/.../Gordonia	0.01%	0.00%	0.00%	0.00%	0.56%	0.00%
11	Bacteria/Actinobacteria/.../Rhodococ	0.07%	0.00%	0.00%	0.28%	0.00%	0.00%
12	Bacteria/Actinobacteria/.../Geodermat	0.01%	0.00%	0.00%	0.00%	0.00%	0.00%
13	Bacteria/Actinobacteria/.../Brevibacter	0.04%	0.00%	0.00%	0.00%	0.00%	0.00%

X. Beta Diversity (Morisita-Horn)

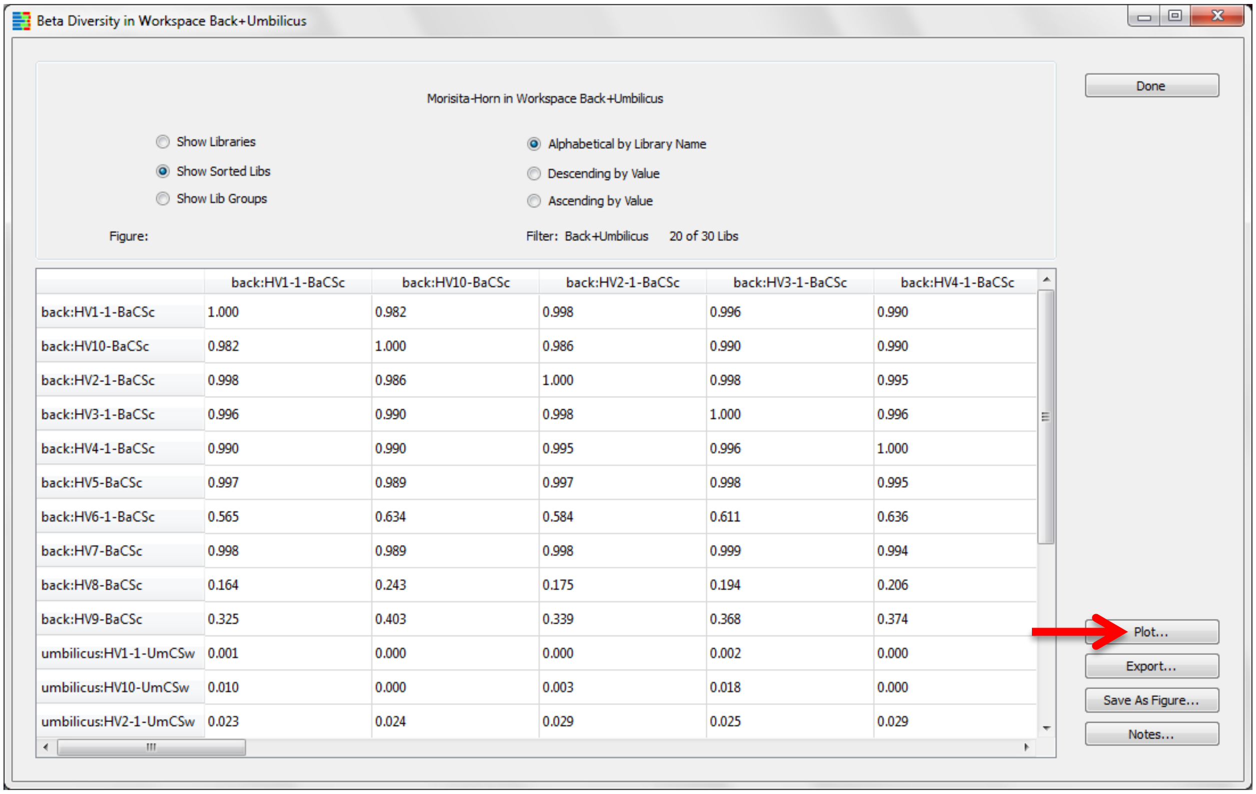
By viewing our libraries in a Morisita-Horn heatmap, we can estimate the similarity of the microbial communities present in the samples at these two anatomical positions. Morisita-Horn is an often used metric that can give insight into how similar or how different sets of samples are from each other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



Morisita-Horn in Workspace Back+Umbilicus

○ Show Libraries
● Show Sorted Libs
○ Show Lib Groups

● Alphabetical by Library Name
○ Descending by Value
○ Ascending by Value

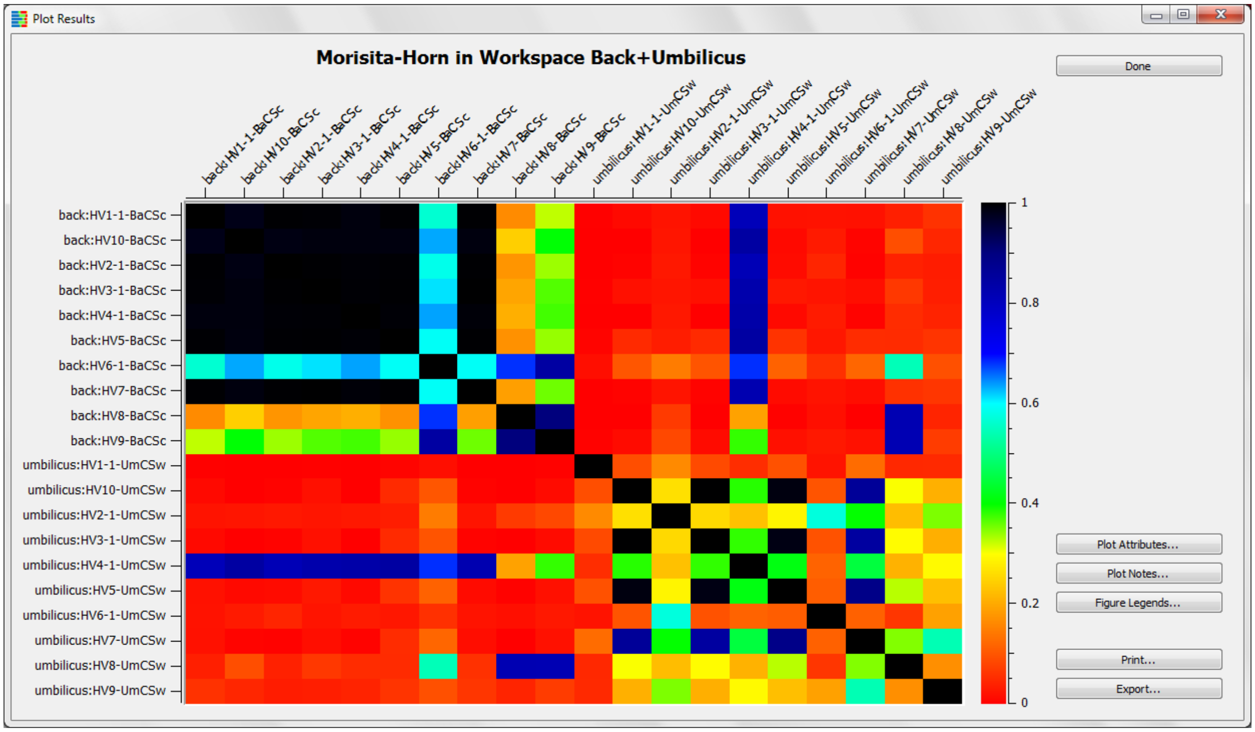
Figure:

Filter: Back+Umbilicus 20 of 30 Libs

	back:HV1-1-BaCSc	back:HV10-BaCSc	back:HV2-1-BaCSc	back:HV3-1-BaCSc	back:HV4-1-BaCSc
back:HV1-1-BaCSc	1.000	0.982	0.998	0.996	0.990
back:HV10-BaCSc	0.982	1.000	0.986	0.990	0.990
back:HV2-1-BaCSc	0.998	0.986	1.000	0.998	0.995
back:HV3-1-BaCSc	0.996	0.990	0.998	1.000	0.996
back:HV4-1-BaCSc	0.990	0.990	0.995	0.996	1.000
back:HV5-BaCSc	0.997	0.989	0.997	0.998	0.995
back:HV6-1-BaCSc	0.565	0.634	0.584	0.611	0.636
back:HV7-BaCSc	0.998	0.989	0.998	0.999	0.994
back:HV8-BaCSc	0.164	0.243	0.175	0.194	0.206
back:HV9-BaCSc	0.325	0.403	0.339	0.368	0.374
umbilicus:HV1-1-UmCSw	0.001	0.000	0.000	0.002	0.000
umbilicus:HV10-UmCSw	0.010	0.000	0.003	0.018	0.000
umbilicus:HV2-1-UmCSw	0.023	0.024	0.029	0.025	0.029

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

Note: In our workspace, we have a filter applied, 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 Explicet 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 Explicet 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.

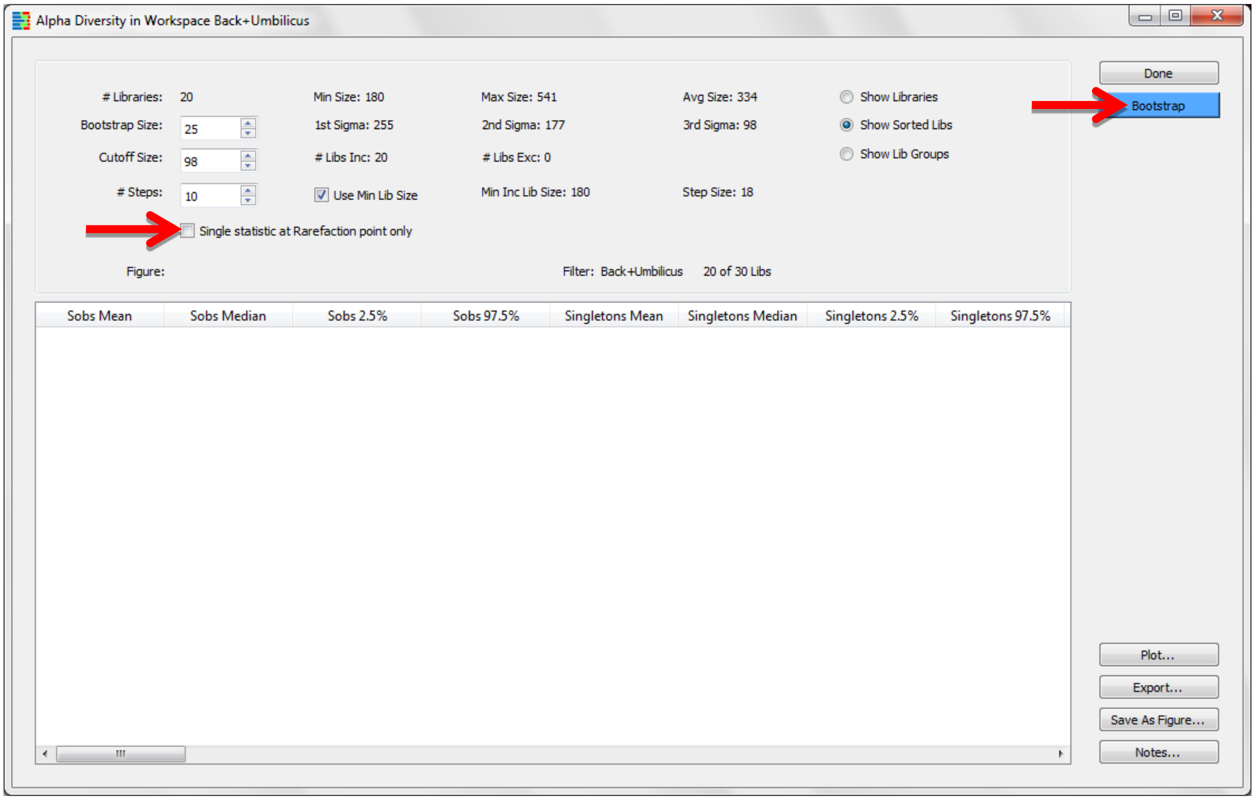
A. Run a Good's Coverage Test

Tools → Analyze → Alpha Diversity

New pop-up window appears

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

Click **Bootstrap**

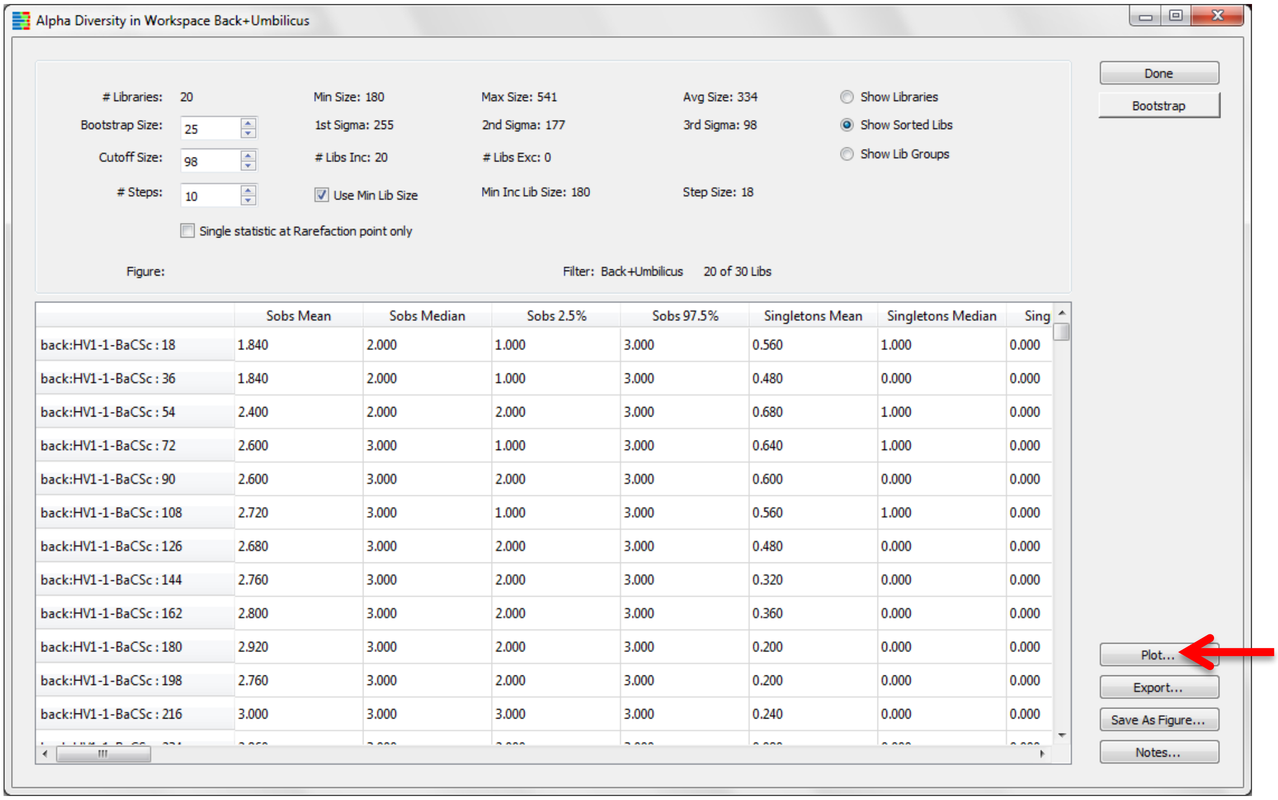


Alpha Diversity in Workspace Back+Umbilicus

Libraries: 20
Min Size: 180
Max Size: 541
Avg Size: 334
Bootstrap Size: 25
1st Sigma: 255
2nd Sigma: 177
3rd Sigma: 98
Cutoff Size: 98
Libs Inc: 20
Libs Exc: 0
Steps: 10
☑ Use Min Lib Size
Min Inc Lib Size: 180
Step Size: 18
○ Show Libraries
● Show Sorted Libs
○ Show Lib Groups
☐ Single statistic at Rarefaction point only
Figure:
Filter: Back+Umbilicus 20 of 30 Libs

Sobs Mean	Sobs Median	Sobs 2.5%	Sobs 97.5%	Singletons Mean	Singletons Median	Singletons 2.5%	Singletons 97.5%

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



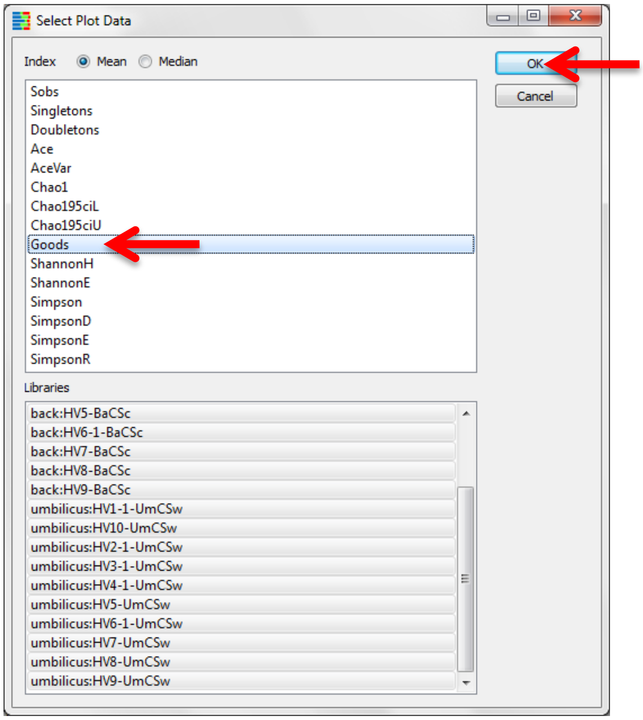
Libraries: 20
Bootstrap Size: 25
Cutoff Size: 98
Steps: 10
Min Size: 180
1st Sigma: 255
Libs Inc: 20
☑ Use Min Lib Size
Max Size: 541
2nd Sigma: 177
Libs Exc: 0
Min Inc Lib Size: 180
Avg Size: 334
3rd Sigma: 98
Step Size: 18
○ Show Libraries
● Show Sorted Libs
○ Show Lib Groups
☐ Single statistic at Rarefaction point only
Figure:
Filter: Back+Umbilicus 20 of 30 Libs

	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
back:HV1-1-BaCSc : 216	3.000	3.000	3.000	3.000	0.240	0.000	0.000

A new pop-up window appears which lists the various alpha diversity tests

Select **Goods**

Click **OK**



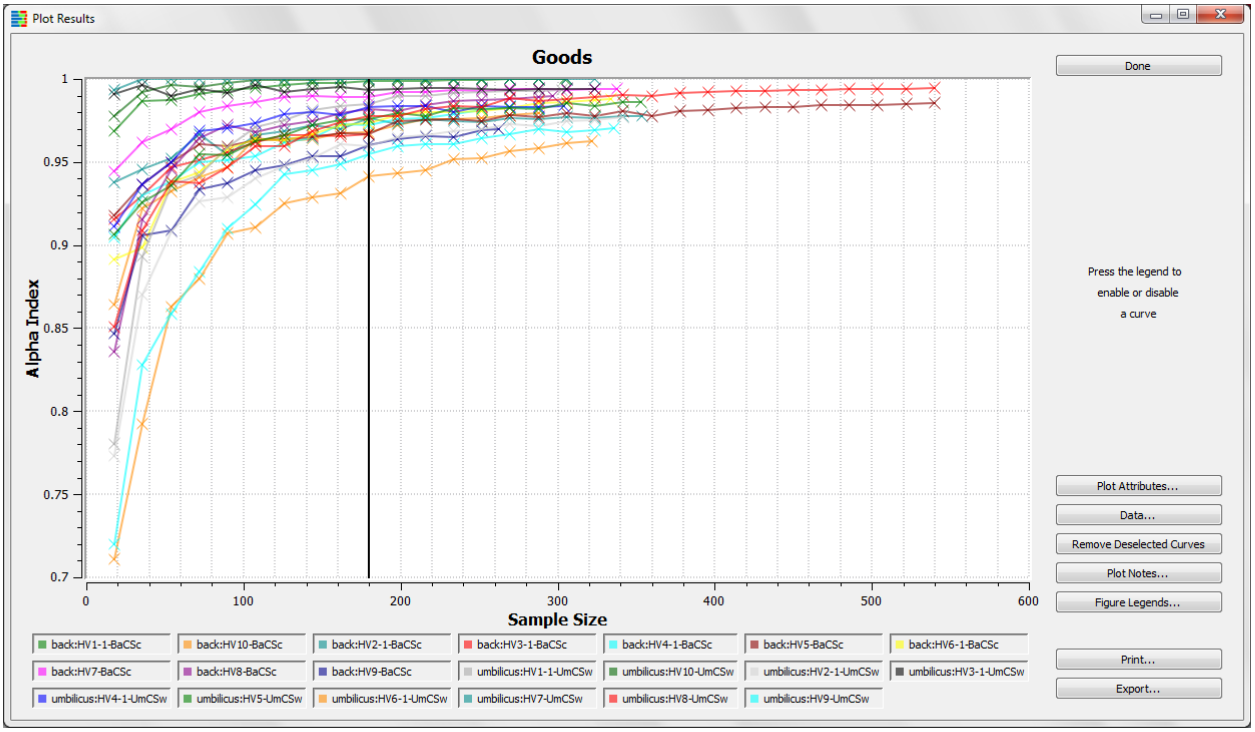
Index ● Mean ○ Median

Sobs
Singletons
Doubletons
Ace
AceVar
Chao1
Chao195ciL
Chao195ciU
Goods
ShannonH
ShannonE
Simpson
SimpsonD
SimpsonE
SimpsonR

Libraries

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

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.

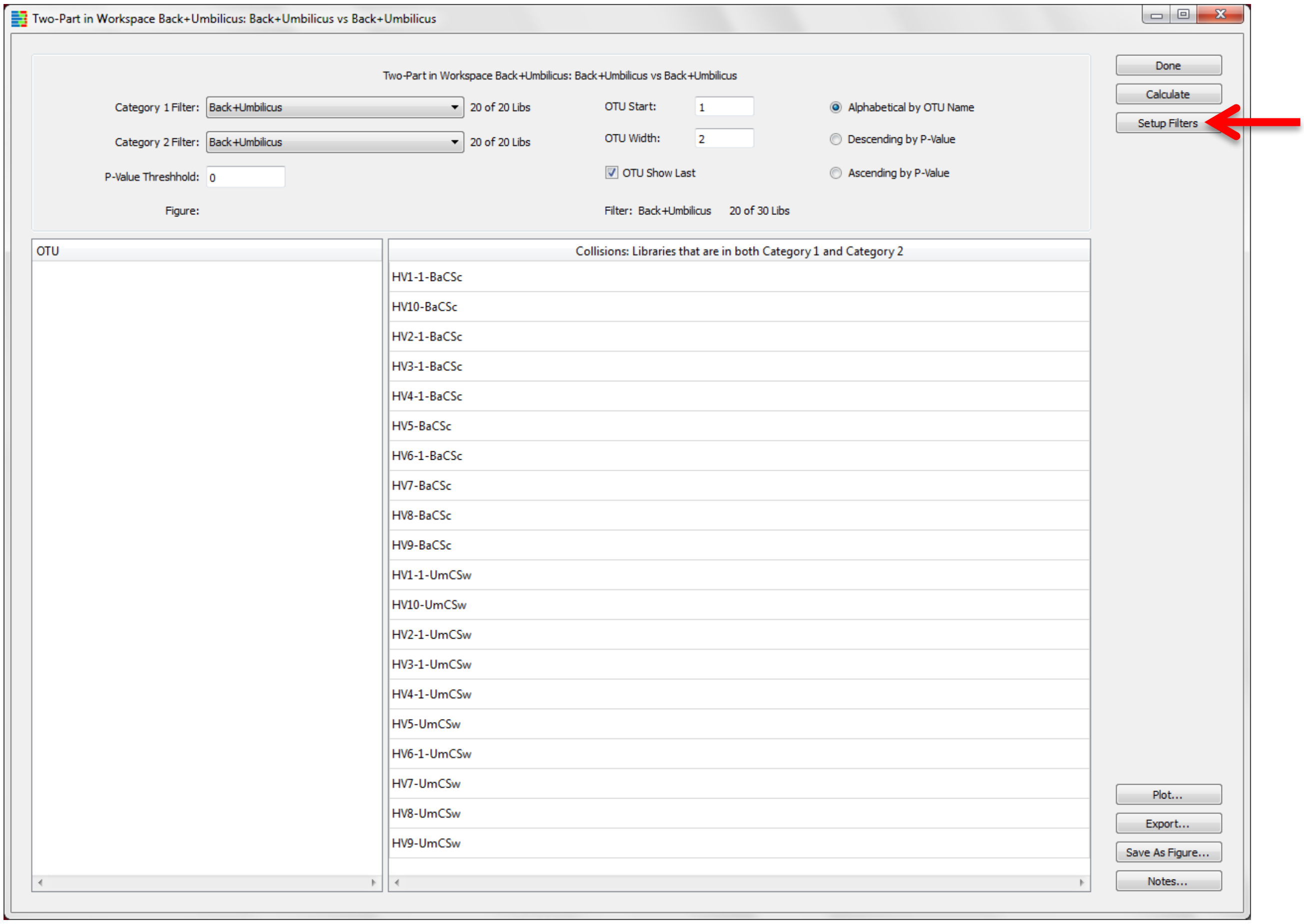
A. Run a Two-Part Test

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

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

Category 1 Filter: Back+Umbilicus 20 of 20 Libs

Category 2 Filter: Back+Umbilicus 20 of 20 Libs

P-Value Threshhold: 0

Figure:

OTU Start: 1

OTU Width: 2

☑ OTU Show Last

Filter: Back+Umbilicus 20 of 30 Libs

● Alphabetical by OTU Name

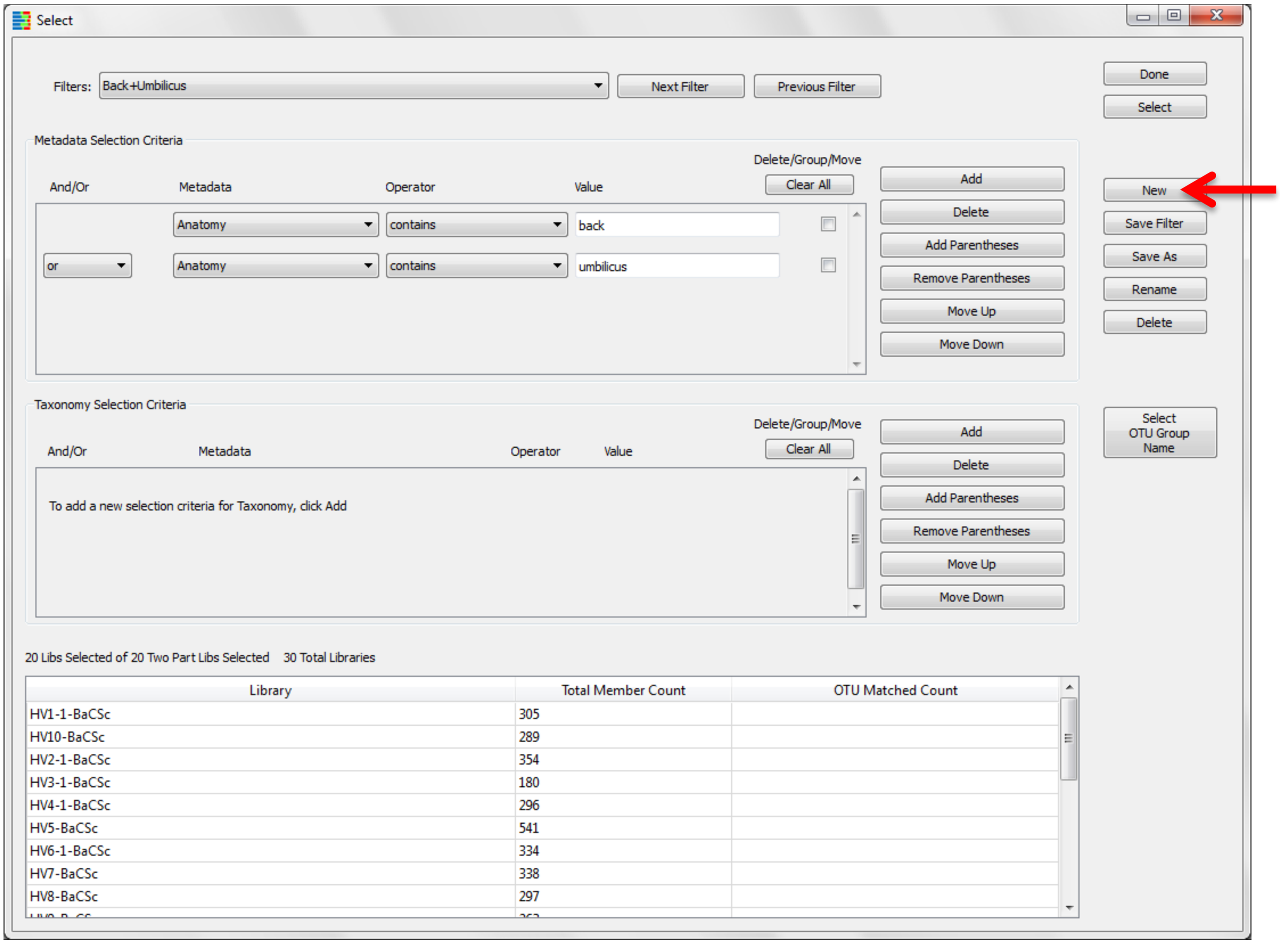
○ Descending by P-Value

○ Ascending by P-Value

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

New pop-up window appears for creation of filters

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



Filters: Back+Umbilicus

And/Or	Metadata	Operator	Value
	Anatomy	contains	back
or	Anatomy	contains	umbilicus

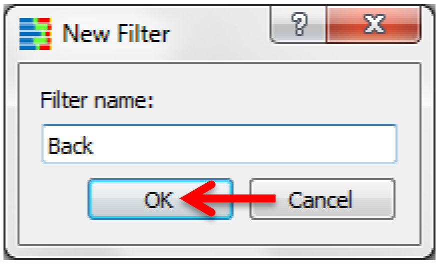
To add a new selection criteria for Taxonomy, click Add

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

Library	Total Member Count	OTU Matched Count
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	

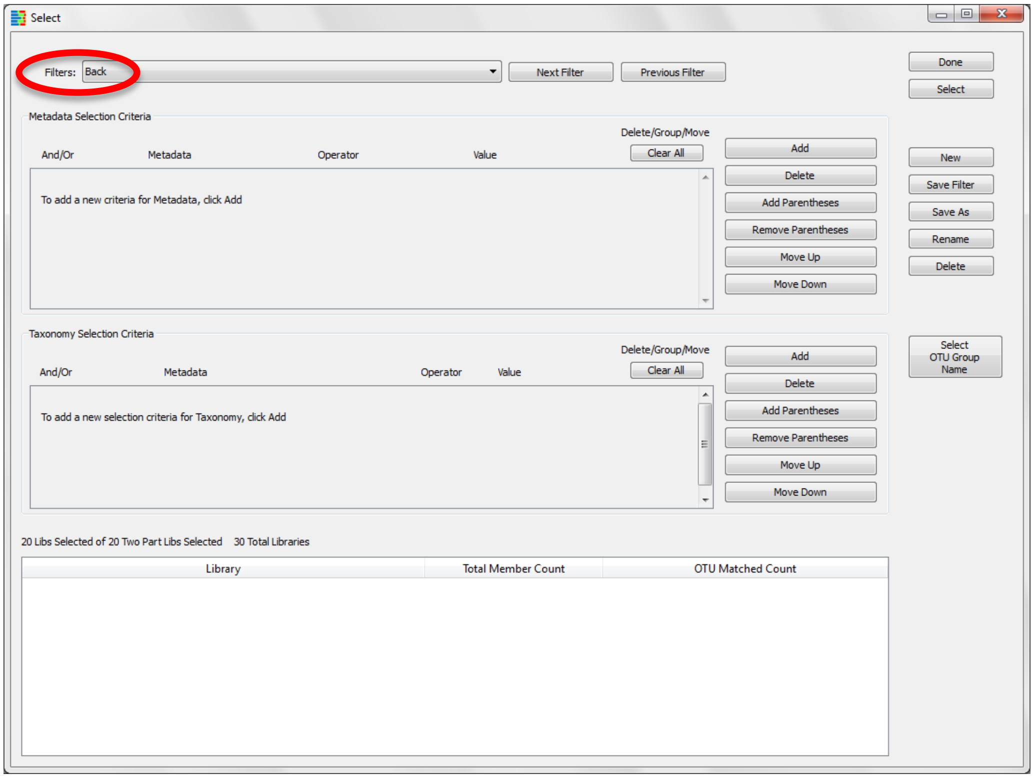
Enter desired filter name in the pop-up window

Click **OK**



Filter name: Back

The filter name will appear in upper left corner of window



To add a new criteria for Metadata, click Add

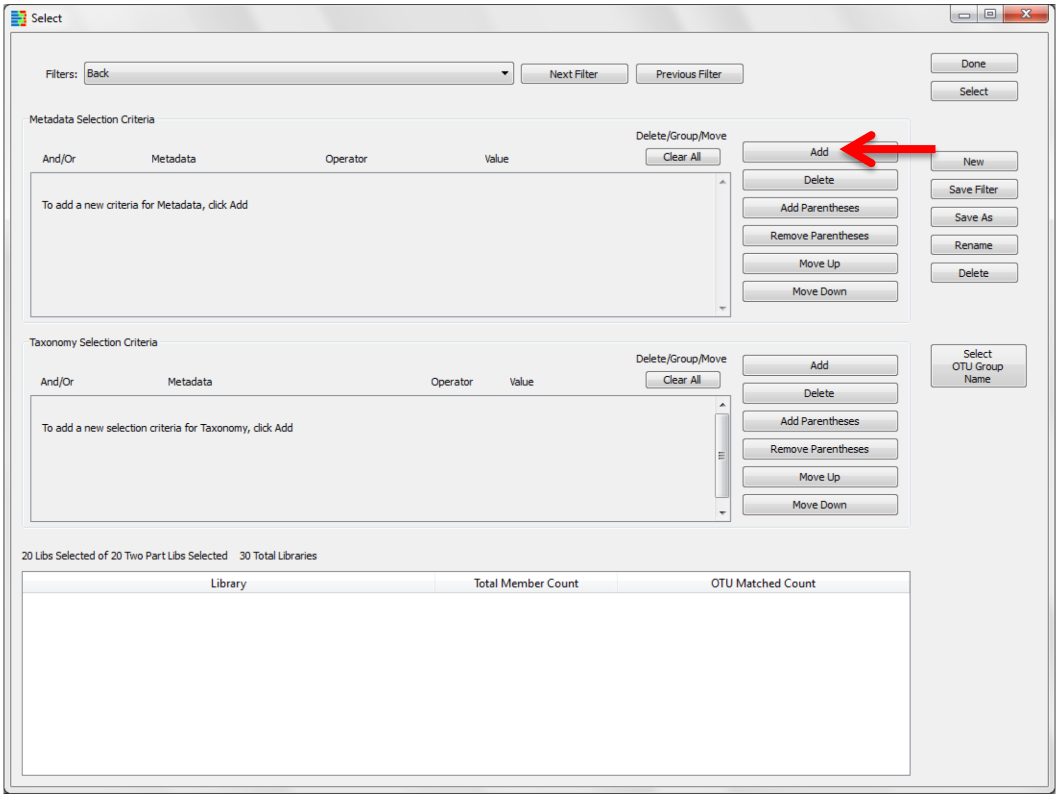
To add a new selection criteria for Taxonomy, click Add

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

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



To add a new criteria for Metadata, click Add

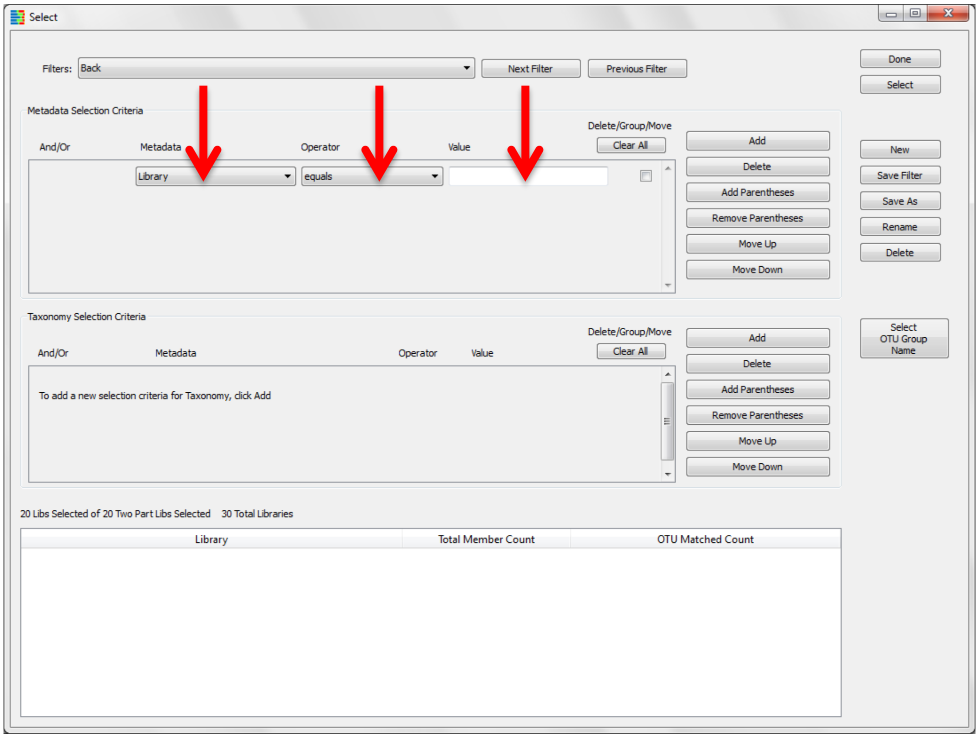
To add a new selection criteria for Taxonomy, click Add

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

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**



Filters: Back

Metadata: Library

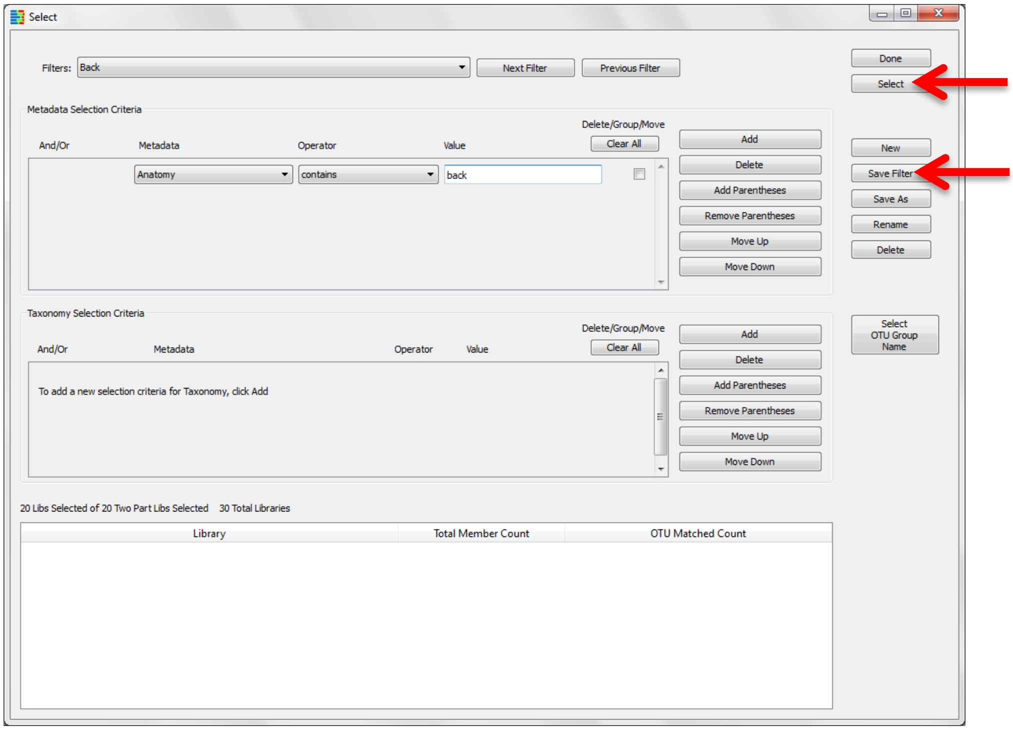
Operator: equals

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

To add a new selection criteria for Taxonomy, click Add

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

Metadata: Anatomy

Operator: contains

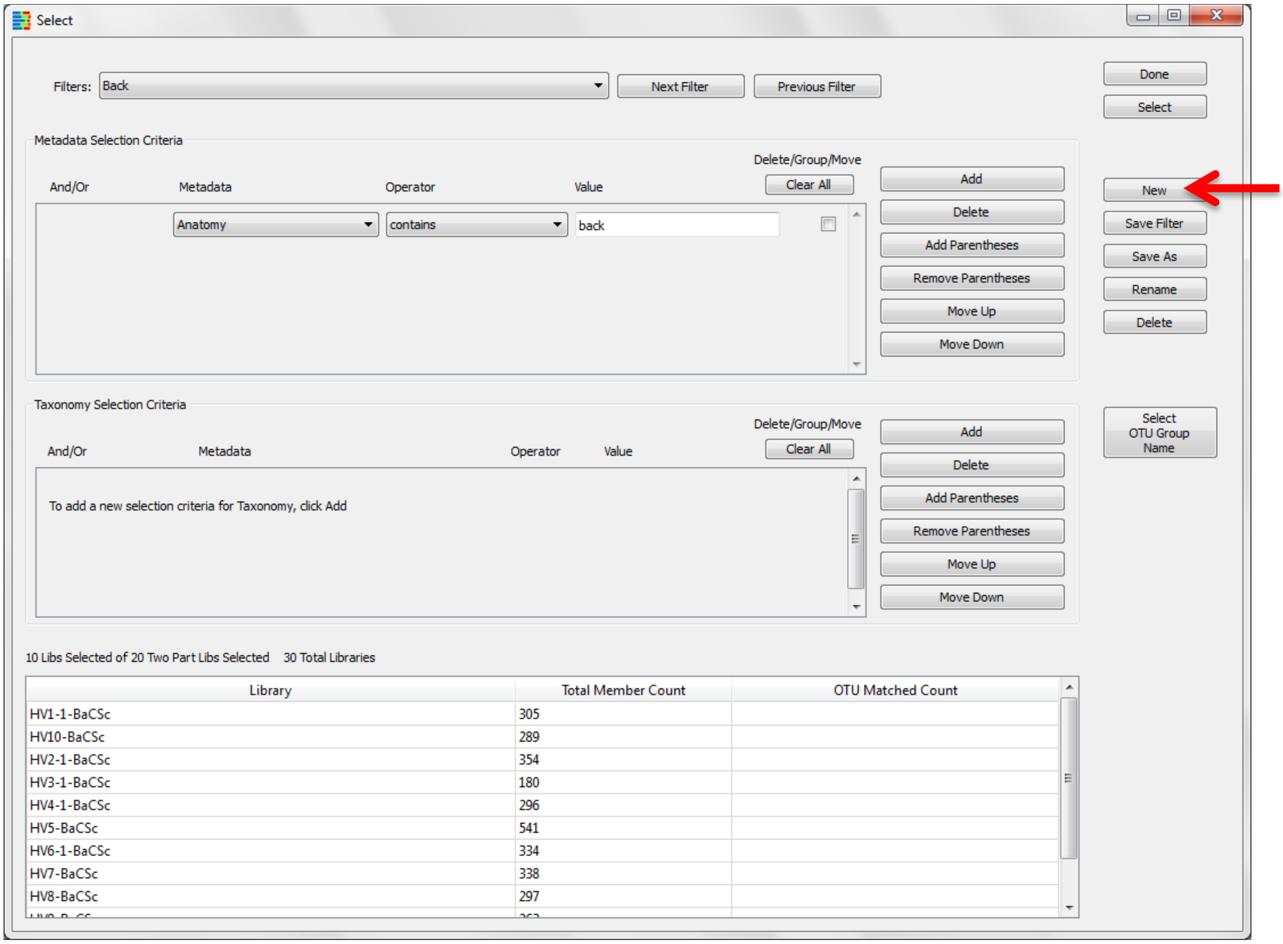
Value: back

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

To add a new selection criteria for Taxonomy, click Add

Now we will create a separate filter for the umbilicus

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



Filters: Back

And/Or	Metadata	Operator	Value
	Anatomy	contains	back

And/Or	Metadata	Operator	Value

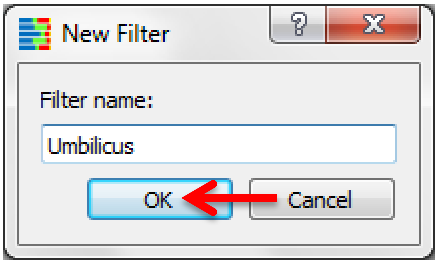
To add a new selection criteria for Taxonomy, click Add

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

Library	Total Member Count	OTU Matched Count
HV1-1-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	

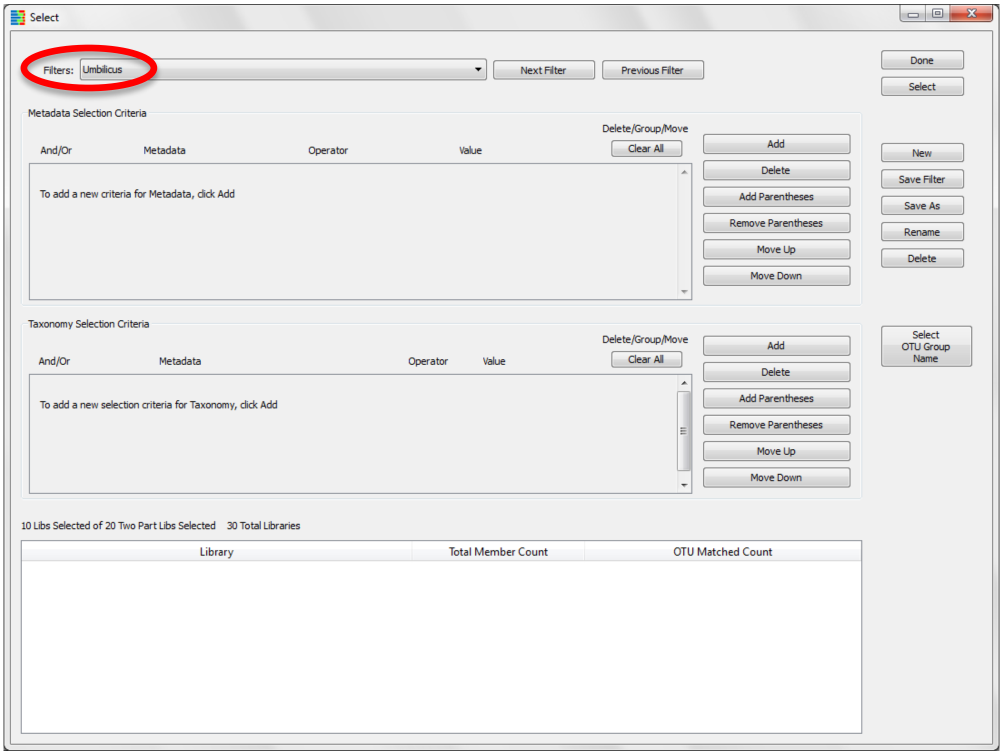
Enter desired filter name in the pop-up window

Click **OK**



Filter name: Umbilicus

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



Filters: Umbilicus

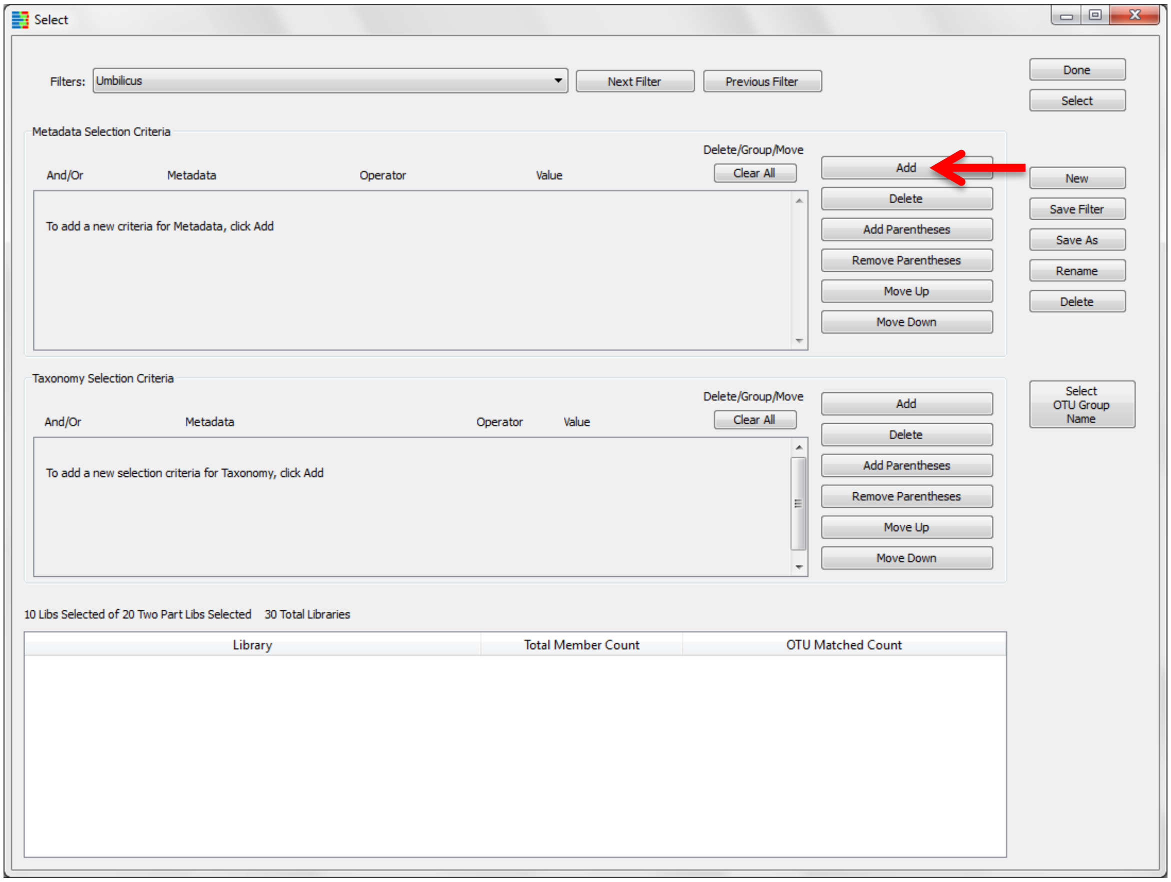
To add a new criteria for Metadata, click Add

To add a new selection criteria for Taxonomy, click Add

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

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



Filters: Umbilicus

To add a new criteria for Metadata, click Add

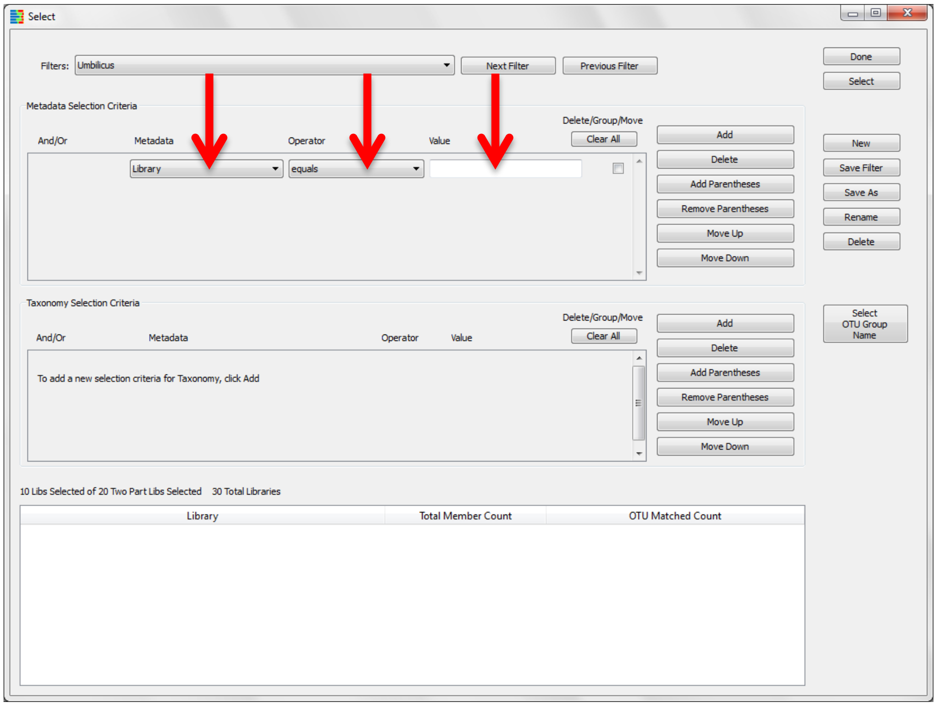
To add a new selection criteria for Taxonomy, click Add

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

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

Metadata: Library

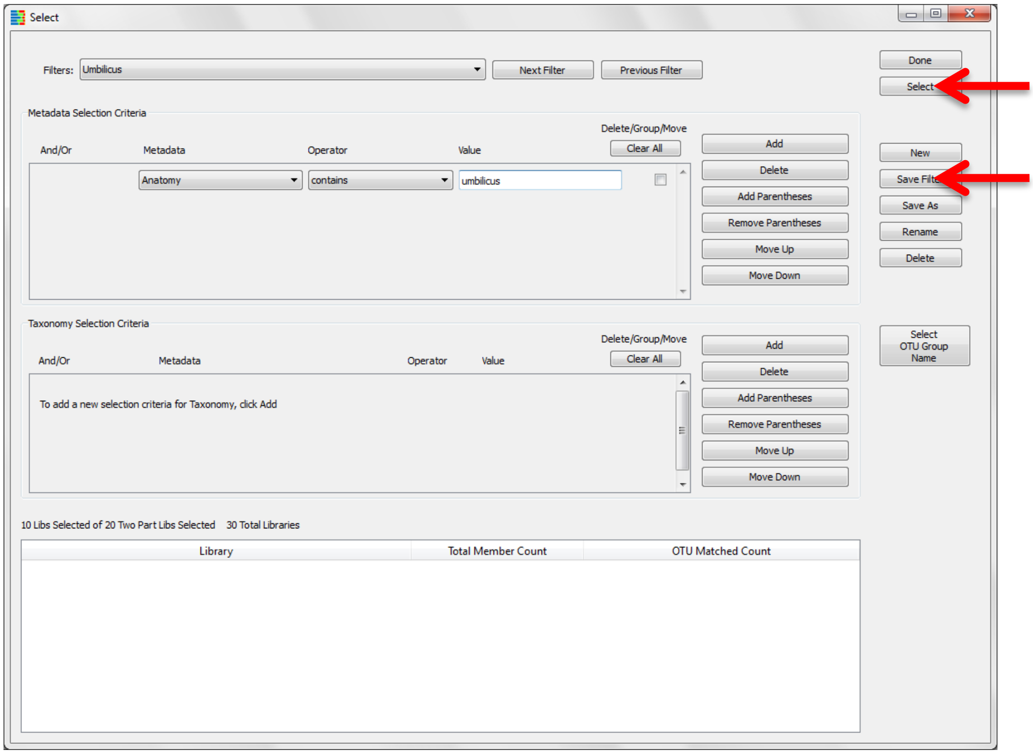
Operator: equals

To add a new selection criteria for Taxonomy, click Add

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

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: Umbilicus

Metadata: Anatomy

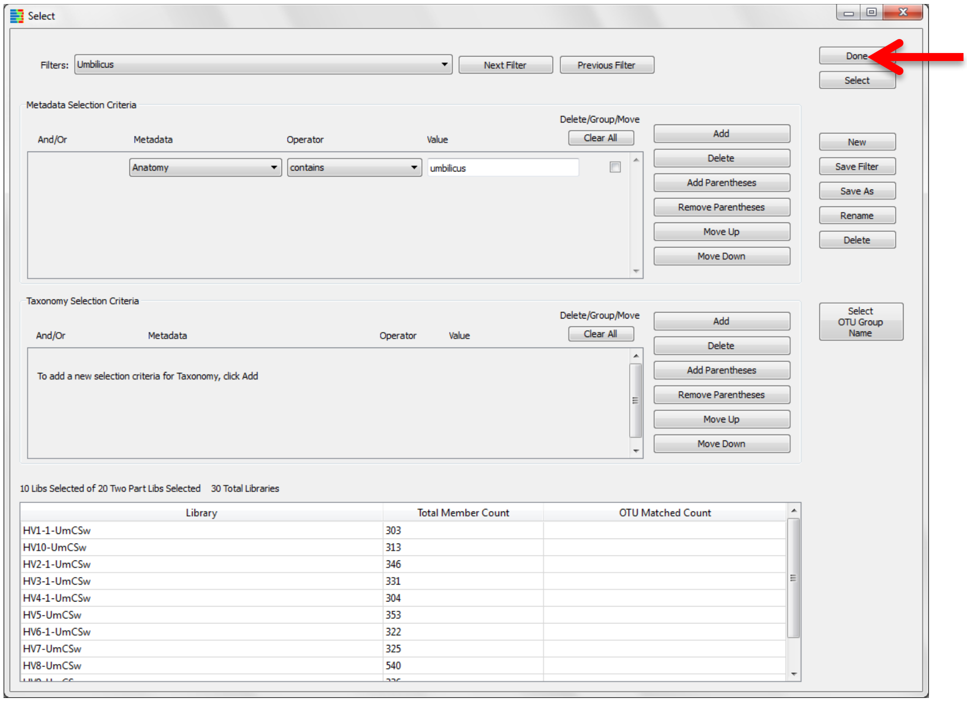
Operator: contains

Value: umbilicus

To add a new selection criteria for Taxonomy, click Add

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

Click **Done** to return to the Two-Part test setup window



Filters: Umbilicus

Metadata Selection Criteria

And/Or	Metadata	Operator	Value	Delete/Group/Move
	Anatomy	contains	umbilicus	☐

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

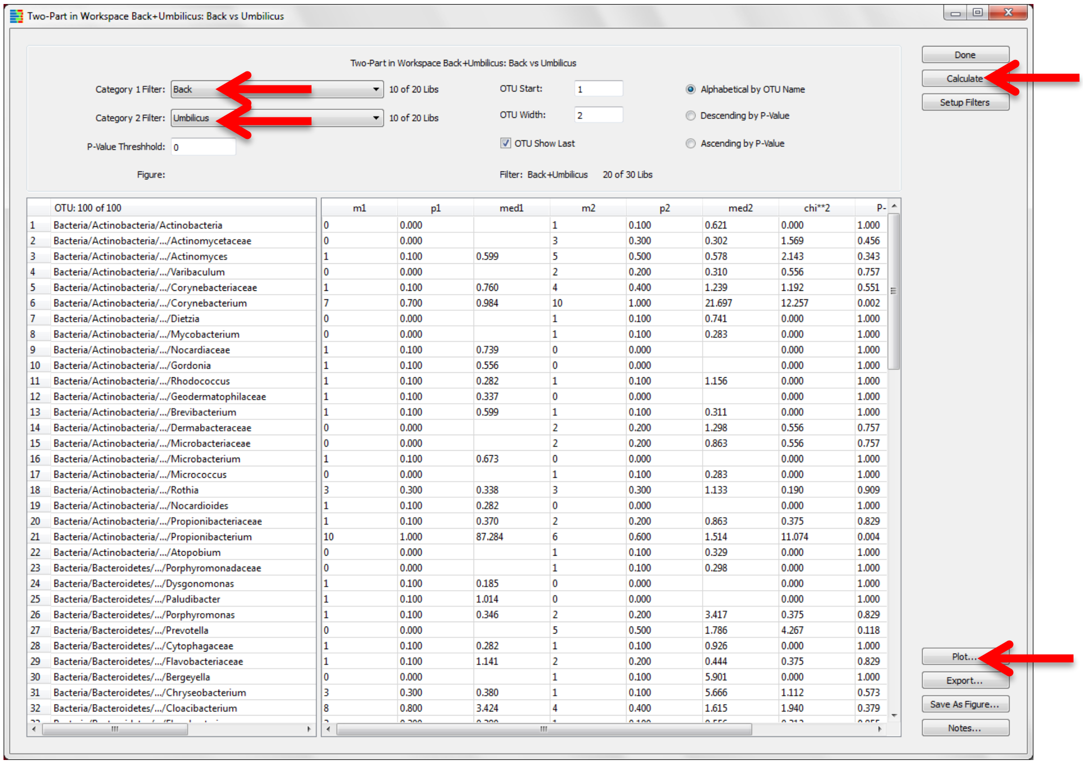
Library	Total Member Count	OTU Matched Count
HV1-1-UmCSw	303	
HV10-UmCSw	313	
HV2-1-UmCSw	346	
HV3-1-UmCSw	331	
HV4-1-UmCSw	304	
HV5-UmCSw	353	
HV6-1-UmCSw	322	
HV7-UmCSw	325	
HV8-UmCSw	540	

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

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

Click **Calculate**

Click **Plot**



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

Category 1 Filter: Back 10 of 20 Libs

Category 2 Filter: Umbilicus 10 of 20 Libs

P-Value Threshhold: 0

Figure:

OTU Start: 1

OTU Width: 2

☑ OTU Show Last

Filter: Back+Umbilicus 20 of 30 Libs

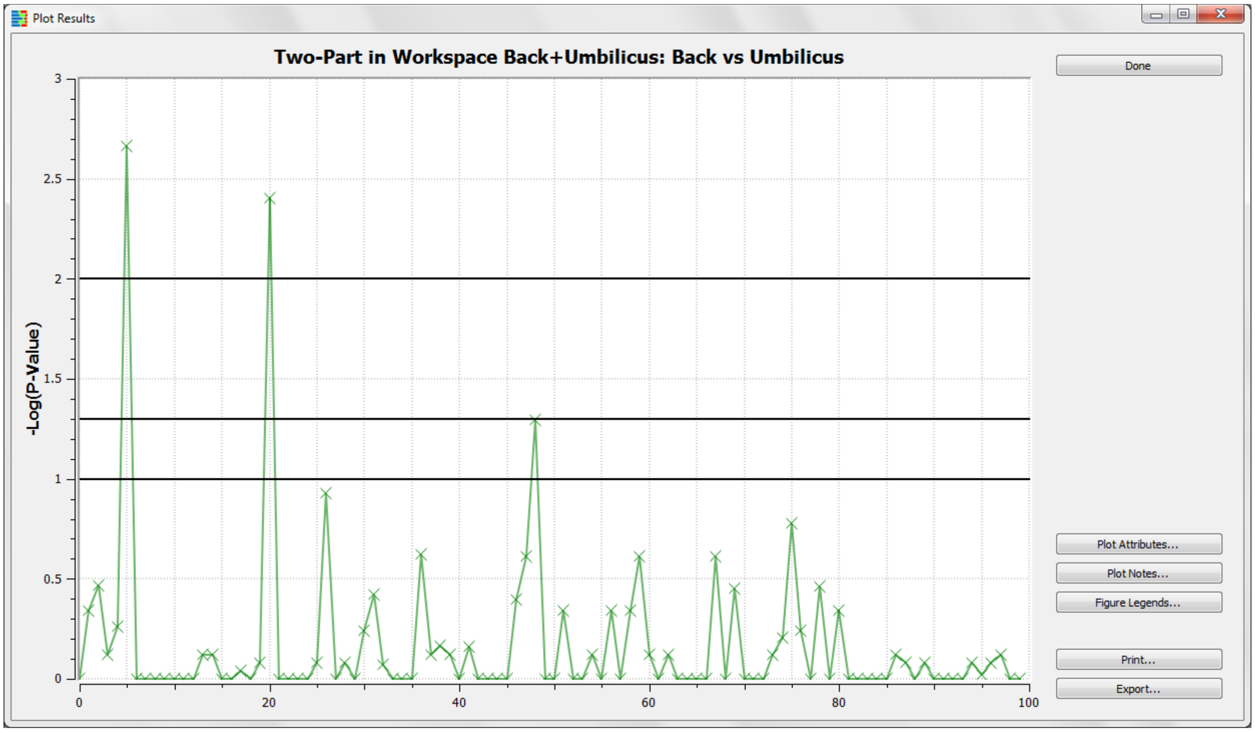
● Alphabetical by OTU Name

○ Descending by P-Value

○ Ascending by P-Value

	OTU: 100 of 100	m1	p1	med1	m2	p2	med2	chi**2	P-
1	Bacteria/Actinobacteria/Actinobacteria	0	0.000		1	0.100	0.621	0.000	1.000
2	Bacteria/Actinobacteria/.../Actinomycetaceae	0	0.000		3	0.300	0.302	1.569	0.456
3	Bacteria/Actinobacteria/.../Actinomyces	1	0.100	0.599	5	0.500	0.578	2.143	0.343
4	Bacteria/Actinobacteria/.../Varibaculum	0	0.000		2	0.200	0.310	0.556	0.757
5	Bacteria/Actinobacteria/.../Corynebacteriaceae	1	0.100	0.760	4	0.400	1.239	1.192	0.551
6	Bacteria/Actinobacteria/.../Corynebacterium	7	0.700	0.984	10	1.000	21.697	12.257	0.002
7	Bacteria/Actinobacteria/.../Dietzia	0	0.000		1	0.100	0.741	0.000	1.000
8	Bacteria/Actinobacteria/.../Mycobacterium	0	0.000		1	0.100	0.283	0.000	1.000
9	Bacteria/Actinobacteria/.../Nocardiaceae	1	0.100	0.739	0	0.000		0.000	1.000
10	Bacteria/Actinobacteria/.../Gordonia	1	0.100	0.556	0	0.000		0.000	1.000
11	Bacteria/Actinobacteria/.../Rhodococcus	1	0.100	0.282	1	0.100	1.156	0.000	1.000
12	Bacteria/Actinobacteria/.../Geodermatophilaceae	1	0.100	0.337	0	0.000		0.000	1.000
13	Bacteria/Actinobacteria/.../Brevibacterium	1	0.100	0.599	1	0.100	0.311	0.000	1.000
14	Bacteria/Actinobacteria/.../Dermabacteraceae	0	0.000		2	0.200	1.298	0.556	0.757
15	Bacteria/Actinobacteria/.../Microbacteriaceae	0	0.000		2	0.200	0.863	0.556	0.757
16	Bacteria/Actinobacteria/.../Microbacterium	1	0.100	0.673	0	0.000		0.000	1.000
17	Bacteria/Actinobacteria/.../Micrococcus	0	0.000		1	0.100	0.283	0.000	1.000
18	Bacteria/Actinobacteria/.../Rothia	3	0.300	0.338	3	0.300	1.133	0.190	0.909
19	Bacteria/Actinobacteria/.../Nocardioides	1	0.100	0.282	0	0.000		0.000	1.000
20	Bacteria/Actinobacteria/.../Propionibacteriaceae	1	0.100	0.370	2	0.200	0.863	0.375	0.829
21	Bacteria/Actinobacteria/.../Propionibacterium	10	1.000	87.284	6	0.600	1.514	11.074	0.004
22	Bacteria/Actinobacteria/.../Atopobium	0	0.000		1	0.100	0.329	0.000	1.000
23	Bacteria/Bacteroidetes/.../Porphyromonadaceae	0	0.000		1	0.100	0.298	0.000	1.000
24	Bacteria/Bacteroidetes/.../Dysgonomonas	1	0.100	0.185	0	0.000		0.000	1.000
25	Bacteria/Bacteroidetes/.../Paludibacter	1	0.100	1.014	0	0.000		0.000	1.000
26	Bacteria/Bacteroidetes/.../Porphyromonas	1	0.100	0.346	2	0.200	3.417	0.375	0.829
27	Bacteria/Bacteroidetes/.../Prevotella	0	0.000		5	0.500	1.786	4.267	0.118
28	Bacteria/Bacteroidetes/.../Cytophagaceae	1	0.100	0.282	1	0.100	0.926	0.000	1.000
29	Bacteria/Bacteroidetes/.../Flavobacteriaceae	1	0.100	1.141	2	0.200	0.444	0.375	0.829
30	Bacteria/Bacteroidetes/.../Bergeyella	0	0.000		1	0.100	5.901	0.000	1.000
31	Bacteria/Bacteroidetes/.../Chryseobacterium	3	0.300	0.380	1	0.100	5.666	1.112	0.573
32	Bacteria/Bacteroidetes/.../Cloacibacterium	8	0.800	3.424	4	0.400	1.615	1.940	0.379

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.

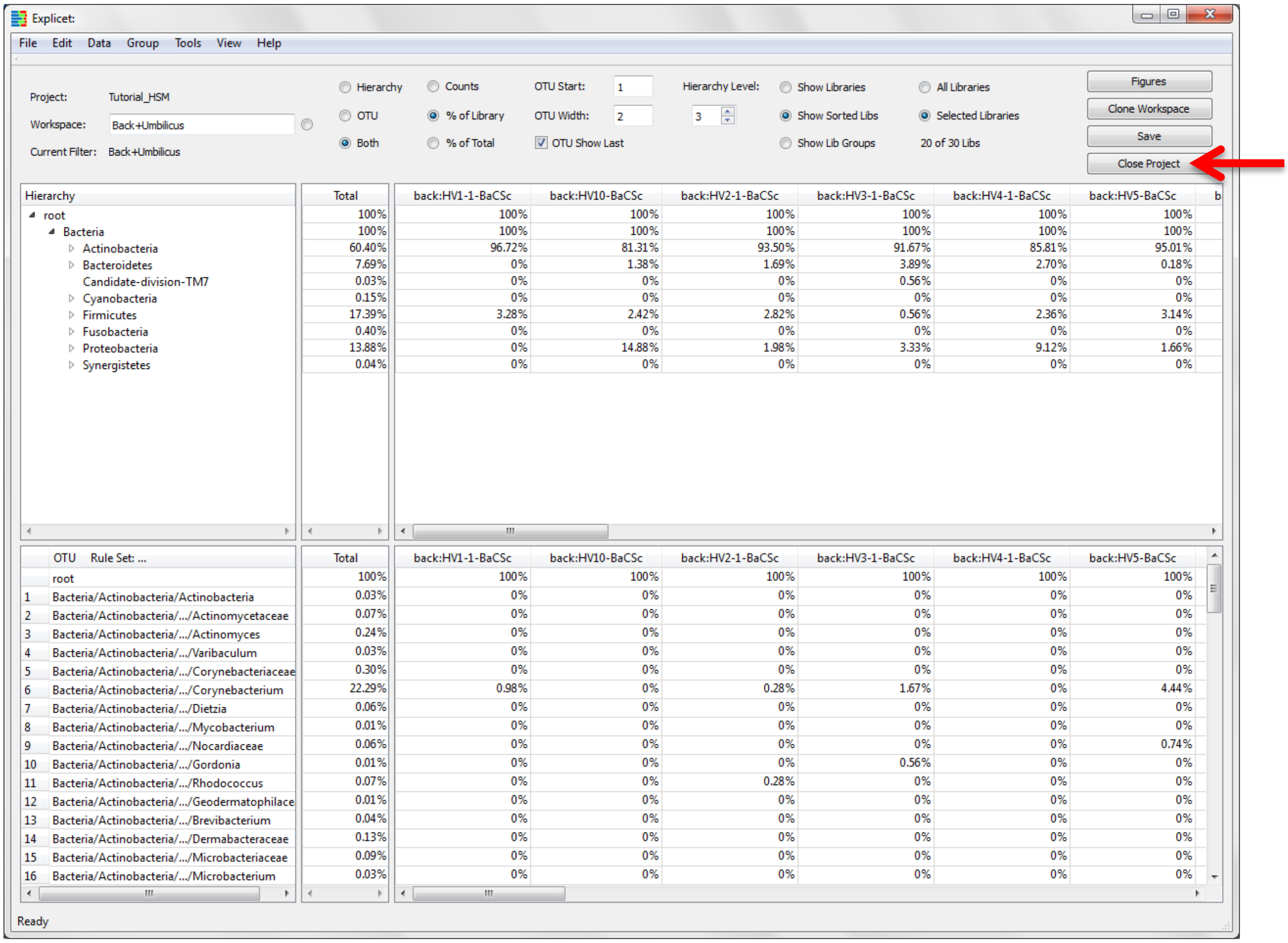
Data can be exported from the **Plot Results** window as tab delimited text using the export button (available in all graphics windows). The data incorporated for each taxon in the Two-Part statistic are summarized for each category. The number of samples with sequences belonging to an OTU within each 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 Explicet. For more complete information on Explicet capabilities, please see the Explicet 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



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

○ Hierarchy
○ OTU
● Both

○ Counts
● % of Library
○ % of Total

OTU Start: 1
OTU Width: 2
☑ OTU Show Last

Hierarchy Level: 3

○ Show Libraries
● Show Sorted Libs
○ Show Lib Groups

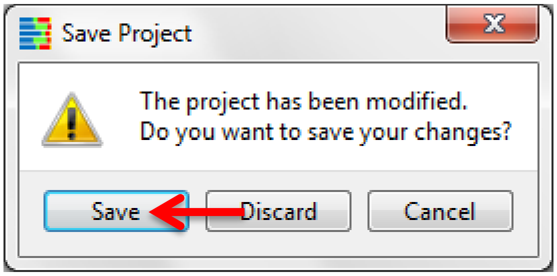
○ All Libraries
● Selected Libraries
20 of 30 Libs

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	100%	100%	100%	100%	100%	100%	100%
Bacteria	100%	100%	100%	100%	100%	100%	100%
Actinobacteria	60.40%	96.72%	81.31%	93.50%	91.67%	85.81%	95.01%
Bacteroidetes	7.69%	0%	1.38%	1.69%	3.89%	2.70%	0.18%
Candidate-division-TM7	0.03%	0%	0%	0%	0.56%	0%	0%
Cyanobacteria	0.15%	0%	0%	0%	0%	0%	0%
Firmicutes	17.39%	3.28%	2.42%	2.82%	0.56%	2.36%	3.14%
Fusobacteria	0.40%	0%	0%	0%	0%	0%	0%
Proteobacteria	13.88%	0%	14.88%	1.98%	3.33%	9.12%	1.66%
Synergistetes	0.04%	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	100%	100%	100%	100%	100%	100%	100%
1	Bacteria/Actinobacteria/Actinobacteria	0.03%	0%	0%	0%	0%	0%	0%
2	Bacteria/Actinobacteria/.../Actinomycetaceae	0.07%	0%	0%	0%	0%	0%	0%
3	Bacteria/Actinobacteria/.../Actinomyces	0.24%	0%	0%	0%	0%	0%	0%
4	Bacteria/Actinobacteria/.../Varibaculum	0.03%	0%	0%	0%	0%	0%	0%
5	Bacteria/Actinobacteria/.../Corynebacteriaceae	0.30%	0%	0%	0%	0%	0%	0%
6	Bacteria/Actinobacteria/.../Corynebacterium	22.29%	0.98%	0%	0.28%	1.67%	0%	4.44%
7	Bacteria/Actinobacteria/.../Dietzia	0.06%	0%	0%	0%	0%	0%	0%
8	Bacteria/Actinobacteria/.../Mycobacterium	0.01%	0%	0%	0%	0%	0%	0%
9	Bacteria/Actinobacteria/.../Nocardiaceae	0.06%	0%	0%	0%	0%	0%	0.74%
10	Bacteria/Actinobacteria/.../Gordonia	0.01%	0%	0%	0%	0.56%	0%	0%
11	Bacteria/Actinobacteria/.../Rhodococcus	0.07%	0%	0%	0.28%	0%	0%	0%
12	Bacteria/Actinobacteria/.../Geodermatophilace	0.01%	0%	0%	0%	0%	0%	0%
13	Bacteria/Actinobacteria/.../Brevibacterium	0.04%	0%	0%	0%	0%	0%	0%
14	Bacteria/Actinobacteria/.../Dermabacteraceae	0.13%	0%	0%	0%	0%	0%	0%
15	Bacteria/Actinobacteria/.../Microbacteriaceae	0.09%	0%	0%	0%	0%	0%	0%
16	Bacteria/Actinobacteria/.../Microbacterium	0.03%	0%	0%	0%	0%	0%	0%

A pop-up window will open

Click **Save**



The project has been modified.
Do you want to save your changes?

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