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

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

I. Begin a New Project

An 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

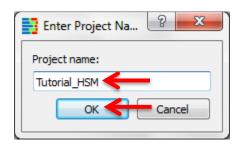
A pop-up window will open with several different options

Click Create Project

Explicet	<u>_ ×</u>
Explicet	
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	
Copyright (C) 2010-2013 Charles E. Robertson and Incubix, Inc. Licensed for use under terms of GNU GPL version	3
Last Project:	
Create Project Open Project Open Last Project Import Cancel	

Enter Project Name, "Tutorial_HSM", when prompted

Click OK



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.

Explicet:									
ile Edit Data	a Group Tools Vie	w Help							
Project: Workspace: Current Filter:	Tutorial_HSM Workspace 1 No filter assigned	0	 Hierarchy OTU Both Libs 	 Counts % of Library % of Total 	OTU Start: OTU Width: I OTU Show	all Last	Hierarchy Level:	Show Libraries Show Sorted Libs Show Lib Groups Notes	Figures Clone Workspace Save Close Project
Hierarchy			Total						
root		Þ e	0	•					,
A OTU Rule S	Set:		Total 0	0					
4		► ₹	4	4					

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

$\textbf{File} \rightarrow \textbf{Import} \rightarrow \textbf{File} \rightarrow \textbf{OTU} \textbf{ Table Counts}$

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

lea	this character as the column delimiter: tab Use this character as the OTL se set the column type to the action you want performed on the column during hange the column type, double click on the column type name.	,	ImportCancel
_	Column Name	Column Type	Rescan
1	Taxonomy	OTU Name	
2	Total	Do not import this column	
3	HV1-1-BaCSc	Library Name and Count	E
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

Explicet: e Edit Data	Group Tools View	Help						
Project:	Tutorial_HSM	Hierarchy	Ounts	OTU Start: 1	Hierarch	y Level: () Show	Libraries	Figures
		Ο ΟΤυ	% of Library	OTU Width: all	3	Show	Sorted Libs	Clone Workspace
Workspace:	Workspace 1	Both	% of Total	OTU Show Last		Show	Lib Groups	Save
Current Filter:	No filter assigned	30 Libs				N	lotes	Close Project
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
Acidol	pacteria	2	0	0	0	0	0	0
Actino	bacteria	5001	295	24	235	303	32	37
Bacter		1056	0	47	4	0	110	86
	date-division-TM7	2	0	0	0	0	0	0
Chloro		3	0	0	0	0	0	0
Cyano		29 1484	0	0 209	0	0 10	4	0 48
 Firmic Fusobi 		34	10	209	0	10	7	48
	acteria iatimonadetes	34	0	0	0	0	0	0
V Gernin Nitros		5	0	0	0	0	0	0
Planct		7	0	0	0	0	0	0
Proteo		2079	0	6	43	0	151	132
Synerg		3	0	3	0	0	0	0
			۰ ۱۱۱	0	٥	0	٥	n
A OTU Ru	le 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
Bacteria/Aci	dobacteria/Acidobacteria/	2	0	0	0	0	0	0
2 Bacteria/Act	inobacteria/Acidimicrobii	1	0	0	0	0	0	0
	inobacteria/Acidimicrobii	3	0	0	0	0	0	0
	inobacteria/Actinobacteri	3	0	0	0	0	0	0
5 Bacteria/Act	inobacteria/Actinobacteri	5	0	0	0	0	0	0
	inobacteria/Actinobacteri	27	0	4	0	0	0	1
			0	0	0	0	0	0
6 Bacteria/Act	inobacteria/Actinobacteri	2					0	0
6 Bacteria/Act 7 Bacteria/Act		2	0	1	0	0	0	
6 Bacteria/Act 7 Bacteria/Act 8 Bacteria/Act	inobacteria/Actinobacteri		0	1	0	0	0	0
6 Bacteria/Act 7 Bacteria/Act 8 Bacteria/Act 9 Bacteria/Act	inobacteria/Actinobacteri inobacteria/Actinobacteri inobacteria/Actinobacteri	2						0
6 Bacteria/Act 7 Bacteria/Act 8 Bacteria/Act 9 Bacteria/Act 10 Bacteria/Act	inobacteria/Actinobacteri inobacteria/Actinobacteri inobacteria/Actinobacteri inobacteria/Actinobacteri	2 47	0	3	0	0	0	-
6 Bacteria/Act 7 Bacteria/Act 8 Bacteria/Act 9 Bacteria/Act 10 Bacteria/Act 11 Bacteria/Act	inobacteria/Actinobacteri inobacteria/Actinobacteri inobacteria/Actinobacteri inobacteria/Actinobacteri inobacteria/Actinobacteri	2 47 1772	0	3	0	0 303	0	9
6 Bacteria/Act 7 Bacteria/Act 8 Bacteria/Act 9 Bacteria/Act 10 Bacteria/Act 11 Bacteria/Act 12 Bacteria/Act	inobacteria/Actinobacteri inobacteria/Actinobacteri inobacteria/Actinobacteri inobacteria/Actinobacteri	2 47 1772 4	0 3 0	3 16 0	0 0 0	0 303 0	0 8 0	9

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

$\textbf{File} \rightarrow \textbf{Import} \rightarrow \textbf{Metadata}$

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

nport Metadata								
			Libraries	not found) Libraries foun	ł		
lect the column which contai	ins the Library name from 5 columns		0 Libraries no	t found in the p	oject for column	Lib		
Column		Sample Data	All Librarie	s found				Import
Lib	HV2-1-AcRSc	<u> </u>						Cancel
Anatomy	antecubital fossa							
Symmetry	Right							
SampleType	Scrape							
Microenvironment	Moist							
			Add missi	ng libraries to th	e project			
tadata which does not mate	ch the Metadata in the project						Ocheck All Clear All	
tadef Namı C	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

efined Met	adata		Assigned Meta	adata				-
Used	Name		Lib	rary Name Anatom	y Aicroenvironmen	SampleType	Symmetry	
30	Anatomy		1 HV1-1-Ba	CSc back	Sebaceous	Scrape	Center	
30	Microenvironment		2 HV1-1-Ur	mCSw umbilicus	Moist	Swab	Center	
30	SampleType		3 HV10-Ba	CSc back	Sebaceous	Scrape	Center	
30	Symmetry		4 HV10-Um	nCSw umbilicus	Moist	Swab	Center	
			5 HV2-1-Ad	RSc antecubital	fossa Moist	Scrape	Right	
		Add ->	6 HV2-1-Ad	RSw antecubital	fossa Moist	Swab	Right	
		Auu ->	7 HV2-1-AI	RSc alar crease	Sebaceous	Scrape	Right	
		<- Remove	8 HV2-1-Ba	CSc back	Sebaceous	Scrape	Center	
			9 HV2-1-Go	CSc gluteal creas	se Moist	Scrape	Center	
			10 HV2-1-Ur	mCSw umbilicus	Moist	Swab	Center	
			11 HV3-1-Ba	CSc back	Sebaceous	Scrape	Center	
			12 HV3-1-Ra	RSw retroauricula	ar c Sebaceous	Swab	Right	
			13 HV3-1-Ur	mCSw umbilicus	Moist	Swab	Center	
			14 HV4-1-Ba	CSc back	Sebaceous	Scrape	Center	
			15 HV4-1-Ur		Moist	Swab	Center	
			16 HV5-BaC	Sc back	Sebaceous	Scrape	Center	
			17 HV5-Um0	CSw umbilicus	Moist	Swab	Center	
			18 HV6-1-Ba	CSc back	Sebaceous	Scrape	Center	
				٠.				4
Metadata	Definition					Сору	Paste	Note: Number of rows/columns to paste to must mate the number of rows/columns copied. One cell may be copied then pasted to multiple cells.
				Enumerated Values: Option	nal			
	Name:					Add Value	Add	
	Type: String that may be	more than 8 characters	•	Values			Replace	
				<new></new>		Delete Value	Delete	
							Clear	1
						Clear Values	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. 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

e Edit Data Group	Tools View H	lelp							
Project: Tutorial F	ISM	Hierarchy	Ounts	OTU Start: 1	Hierarch	y Level: 💿 Show	Libraries	Figures	
		Ο ΟΤυ	% of Library	OTU Width: all	3	Show	Sorted Libs	Clone Workspace	
Workspace: Workspa	ce 1	Both	% of Total	📝 OTU Show Last		Show	Lib Groups	Save	
Current Filter: No filter	assigned	🔘 30 Libs				N	lotes	Close Project	
lierarchy		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-divis	ion-TM7	2	0	0	0	0	0	0	
Chloroflexi		3	0	0	0	0	0	0	Ξ
Cyanobacteria		29	0	0	0	0	4	0	
Firmicutes		1484	10	209	7	10	7	48	_
Fusobacteria		34	0	14	0	0	0	0	_
Gemmatimonad	letes	3	0	0	0	0	0	0	- 11
Nitrospirae		5	0	0	0	0	0	0	- 11
Planctomycetes	_	7	0	0	0	0	0	0	- 11
Proteobacteria		2079	0	6	43	0	151	132	
Synergistetes		3	0	3	0	0	0	0	
	•		< III						F
A 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	=
Bacteria/Acidobacteri	a/Acidobacteria/	2	0	0	0	0	0	0	_
Bacteria/Actinobacter	ia/Acidimicrobii	1	0	0	0	0	0	0	_
Bacteria/Actinobacter	ia/Acidimicrobii	3	0	0	0	0	0	0	
Bacteria/Actinobacter	ia/Actinobacteri	3	0	0	0	0	0	0	
Bacteria/Actinobacter	ia/Actinobacteri	5	0	0	0	0	0	0	
Bacteria/Actinobacter	ia/Actinobacteri	27	0	4	0	0	0	1	
Bacteria/Actinobacter		2	0	0	0	0	0	0	
Bacteria/Actinobacter		2	0	1	0	0	0	0	
Bacteria/Actinobacter	· · · · · · · · · · · · · · · · · · ·	47	0	3	0	0	0	0	-
0 Bacteria/Actinobacter		1772	3	16	0	303	8	9	-
1 Bacteria/Actinobacter		4	0	0	0	0	0	0	-
	· · · · · · · · · · · · · · · · · · ·	1	0	0	0	0	0	0	-
		4	0	0	0	0	0	0	
3 Bacteria/Actinobacter	ia/Actinobacteri		۰ ۱	0	U	U	U		P

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).



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/Acidimicrobia/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.

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ile Edit Data Grou	up Tools View Hel	р						
Project: Tutori	al HSM	🔘 Hierarchy	Counts	OTU Start: 1	Hierarch	y Level: 💿 Show	Libraries	Figures
		⊚ оти	% of Library	OTU Width: 2	3	Show	Sorted Libs	Clone Workspace
Workspace: Work	space 1	 Both 	% of Total	V OTU Show Last		Show	Lib Groups	Save
Current Filter: No fil	ter assigned	30 Libs				N	otes	Close Project
Hierarchy		Total	HV1-1-BaCSc	HV1-1-UmCSw	HV10-BaCSc	HV10-UmCSw	HV2-1-AcRSc	HV2-1-AcRSw
4 root		100%	100%	100%	100%	100%	100%	100%
▲ Bacteria	(2)	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
Acidobacteri		0.02%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Actinobacter		51.50%	96.72%	7.92%	81.31%	96.81%	10.53%	12.21%
Bacteroidete		10.88%	0.00%	15.51%	1.38%	0.00%	36.18%	28.38%
Candidate-d	s ivision-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%
Cyanobacter	ria	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%
Gemmatimo	nadetes	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%
Planctomyce	etes	0.07%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Proteobacter	ria	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%
A 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	ppend last	0.029	 0.00% ↓ Ⅲ 	0.00%	0.00%	0.00%	0.00%	0.00%
OTU Re 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%
	teria// <u>Ca</u> didatus	0.02%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Bacteria/Actinobac		0.01%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	cteria//Acidimicro	0.03%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	cteria/Actinobacteri	0.03%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
		0.05%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	cteria//Actinomyc	0.28%	0.00%	1.32%	0.00%	0.00%	0.00%	0.33%
	cteria//Actinomyc	0.28 %	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	cteria//Mobiluncu							
	cteria//Varibaculur	0.02%	0.00%	0.33%	0.00%	0.00%	0.00%	0.00%
	cteria//Corynebac	0.48%	0.00%	0.99%	0.00%	0.00%	0.00%	0.00%
10 Bacteria/Actinoba	cteria//Corynebac	18.25%	0.98%	5.28%	0.00%	96.81%	2.63%	2.97%
1 Bacteria/Actinoba	cteria//Dietzia	0.04%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
12 Bacteria/Actinobac	cteria//Mycobacte	0.01%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	cteria//Nocardiace	0.04%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
< III	F 4	- F	< III.					

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)

$\textit{View} \rightarrow \textit{Sort Libraries}$

A pop-up window will open

In left panel, select Anatomy

Click Add button between panels

Sort Libraries			
		(Sort Cancel
Select Metadata to Sort by		Sort Libs by	
Select Metadata to Sort by Metadata 1 Anatomy 2 Microenvironment 3 SampleType 4 Symmetry	Add -> <-Remove	Metadata	Sort Order

Name of metadata descriptor will appear in the right panel

Click Sort

Sort Libraries		_	Sort Cancel
Select Metadata to Sort by	So	rt Libs by	
Metadata		Metadata	Sort Order
1 Microenvironment	1	Anatomy	ascending
2 SampleType	-		e
3 Symmetry	Add -> <-Remove		

Pop-up window will disappear

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

ile Edit Data Gr	oup Tools View	Help					
Project: Tuto	rial HSM	O Hierarchy	Counts 01	U Start: 1	Hierarchy Level:	Show Libraries	Figures
	-	© otu	% of Library	U Width: 2	3	Show Sorted Libs	Clone Workspace
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Current Filter: No	filter assigned) 30 Libs				Notes	Close Project
lierarchy		Total	alar crease:HV2-1-AIRSc		sa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fossa:
/ root		100%	100		100%	100%	
▲ Bacteria ▷ Acidobacte		100.00%	100.00		100.00%	100.00%	
 Acidobacte Actinobacte 		51.50%	83.88		10.53%	12.21%	
Actinobact Bacteroidet		10.88%	1.32		36.18%	28.38%	
	division-TM7	0.02%	0.00		0.00%	0.00%	
Chloroflexi		0.03%	0.00		0.00%	0.00%	
Cyanobact		0.30%	0.33	%	1.32%	0.00%	
Firmicutes		15.28%	9.21	%	2.30%	15.84%	
Fusobacter	ia	0.35%	0.00	%	0.00%	0.00%	
Gemmatim	onadetes	0.03%	0.00		0.00%	0.00%	
Nitrospirae		0.05%	0.00		0.00%	0.00%	
Planctomy		0.07%	0.00		0.00%	0.00%	
Proteobact		21.41%	5.26		49.67%	43.56%	
Synergistet		0.03%	0.00		0.00%	0.00%	
< N		<	<	97 T	0.009/	0.00%	Þ
A OTU Rule Se	t:	Total	alar crease:HV2-1-AIRSc	/	sa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fossa
root		100%	100	%	100%	100%	
Bacteria/Acidoba	cteria//Candidatus	0.02%	0.00	%	0.00%	0.00%	
2 Bacteria/Actinob	acteria//Acidimicro	0.01%	0.00	%	0.00%	0.00%	
Bacteria/Actinob	acteria//Acidimicro	0.03%	0.00	%	0.00%	0.00%	
Bacteria/Actinob	acteria/Actinobacteri	0.03%	0.33	%	0.00%	0.00%	
5 Bacteria/Actinob	acteria//Actinomyc	0.05%	0.00	%	0.00%	0.00%	
	acteria//Actinomyc	0.28%	0.33	%	0.00%	0.33%	
	acteria//Mobiluncu	0.02%	0.00	%	0.00%	0.00%	
	acteria//Varibaculur	0.02%	0.00	%	0.00%	0.00%	
	acteria//Corynebac	0.48%	0.00	%	0.00%	0.00%	
	acteria//Corynebac	18.25%	8.55	%	2.63%	2.97%	
	acteria//Dietzia	0.04%	0.00	%	0.00%	0.00%	
		0.01%	0.00	%	0.00%	0.00%	
	accendent internation		0.00	0/	0.00%	0.00%	
12 Bacteria/Actinob	acteria//Nocardiace	0.04%	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

$\textbf{Tools} \rightarrow \textbf{Plot} \rightarrow \textbf{OTU} \textbf{ Stacked Bar}$

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

010	Stacked Bar in Workspace Workspace 1						
			OTU Stacked Bar in	n Workspace Workspace 1			Done
	Counts	OTU Start:	Show L	braries			Unsort OTU
	% of Library		2 Show S	orted Libs			
	% of Total		- Show I	h Groups			
	U Nor Iolai	OTU Show	Last	b di dupa			
	Figure:		Filt	er: No filter assigned 30 Libs			
	Select items between:	and	0 Rows Selected of 1	.52 Rows Select Rang	Clear Selection		
	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//Varibaculun	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%		Plot
15	Bacteria/Actinobacteria//Rhodococce	0.11%	0.66%	0.00%	0.00%		Export
16	Bacteria/Actinobacteria//Geodermate	0.01%	0.00%	0.00%	0.00%		Export
17	Bacteria/Actinobacteria//Blastococcu	0.04%	0.00%	0.00%	0.00%		Save As Figure
18 4	Racteria/Actinohacteria/ /Micrococca	0.01%	0.00%	0.00%	0.00%	₹	Ninter
	4	4 1	< III				Notes

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

	Stacked Bar in Workspace Workspace 1						
			OTU Stacked Bar in	Workspace Workspace 1			Done
	Counts	OTU Start:	Show Li	braries			Unsort OTU
	% of Library	OTU Width:	- O Show S	orted Libs			
	% of Total		- Chow I	b Groups			
	-	OTU Show L	ast				
	Figure:		Filb	er: No filter assigned 30 Libs			
	Select items betwee 1.41	anc 31.35	0 Rows Selected of 1	.52 Rows Select Ran	ge Selection		
	DTU 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%	E	
	Bacteria/Actinobacteria//Propionibac	31.35%	74.01%	7.89%	8.25%		
	Bacteria/Actinobacteria//Corynebact	18.25%	8.55%	2.63%	2.97%		
	Bacteria/Bacteroidetes//Cloacibacteri	9.06%	1.32%	35.86%	27.06%		
	Bacteria/Proteobacteria//Aquabacter	7.68%	0.00%	4.93%	0.00%		
	Bacteria/Proteobacteria//Diaphoroba	6.52%	3.95%	26.32%	33.00%		
	Bacteria/Firmicutes//Staphylococcus	6.06%	8.88%	1.97%	6.93%		
	Bacteria/Firmicutes//Streptococcus	3.30%	0.00%	0.00%	6.93%		
	Bacteria/Proteobacteria//Comamona	1.54%	0.66%	7.24%	3.30%		
	Bacteria/Proteobacteria//Acidovorax	1.54%	0.33%	4.28%	2.31%		
0	Bacteria/Firmicutes//Anaerococcus	1.41%	0.00%	0.00%	0.99%		
1	Bacteria/Firmicutes//Finegoldia	1.32%	0.00%	0.00%	0.00%		
2	Bacteria/Bacteroidetes//Prevotella	0.66%	0.00%	0.00%	0.66%		
3	Bacteria/Firmicutes//Family-XI-Incert	0.66%	0.00%	0.00%	0.00%		
4	Bacteria/Firmicutes//Peptoniphilus	0.63%	0.00%	0.00%	0.00%		Plot
5	Bacteria/Actinobacteria//Corynebact	0.48%	0.00%	0.00%	0.00%		Export
6	Bacteria/Proteobacteria//Zoogloea	0.47%	0.00%	0.00%	0.00%		Export
7	Bacteria/Proteobacteria//Sphingomo	0.46%	0.00%	3.95%	2.64%		Save As Figure.
8	Ractaria/Eirmicutar//Dialictar	0.36%	0.00%	0.00%	0.00%		Notes

The top 10 OTUs are now highlighted

Click Plot

J Stacked Bar in Workspace Workspace 1						
		OTU Stacked Bar i	n Workspace Workspace 1			Done
Counts	OTU Start:	Show L	ibraries			Unsort OTU
% of Library	OTU Width:	 Show S 	orted Libs			
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Figure:		Filt	er: No filter assigned 30 Libs			
Select items between: 1.41	and 31.35	10 Rows Selected of	152 Rows Select Rar	Clear Selection		
🔎 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%	=	
Bacteria/Actinobacteria//Propionibac	31.35%	74.01%	7.89%	8.25%	1	
Bacteria/Actinobacteria//Corynebact	18.25%	8.55%	2.63%	2.97%		
Bacteria/Bacteroidetes//Cloacibacteri	9.06%	1.32%	35.86%	27.06%		
Bacteria/Proteobacteria//Aquabacter	7.68%	0.00%	4.93%	0.00%		
Bacteria/Proteobacteria//Diaphoroba	6.52%	3.95%	26.32%	33.00%		
Bacteria/Firmicutes//Staphylococcus	6.06%	8.88%	1.97%	6.93%		
Bacteria/Firmicutes//Streptococcus	3.30%	0.00%	0.00%	6.93%		
Bacteria/Proteobacteria//Comamona	1.54%	0.66%	7.24%	3.30%		
	1.54%	0.33%	4.28%	2.31%		
Bacteria/Proteobacteria//Acidovorax						
Bacteria/Proteobacteria//Acidovorax Bacteria/Firmicutes//Anaerococcus	1.41%	0.00%	0.00%	0.99%		
	1.41% 1.32%	0.00% 0.00%	0.00% 0.00%	0.99% 0.00%		
Bacteria/Firmicutes//Anaerococcus	1.41% 1.32% 0.66%	0.00% 0.00% 0.00%	0.00% 0.00% 0.00%	0.99% 0.00% 0.66%		
Bacteria/Firmicutes//Anaerococcus Bacteria/Firmicutes//Finegoldia	1.41% 1.32% 0.66% 0.66%	0.00% 0.00% 0.00% 0.00%	0.00% 0.00% 0.00% 0.00%	0.99% 0.00% 0.66% 0.00%		
Bacteria/Firmicutes//Anaerococcus Bacteria/Firmicutes//Finegoldia Bacteria/Bacteroidetes//Prevotella	1.41% 1.32% 0.66%	0.00% 0.00% 0.00%	0.00% 0.00% 0.00%	0.99% 0.00% 0.66%		Plot
Bacteria/Firmicutes//Anaerococcus Bacteria/Firmicutes//Finegoldia Bacteria/Bacteroidetes//Prevotella Bacteria/Firmicutes//Family-XI-Incert	1.41% 1.32% 0.66% 0.66% 0.63% 0.48%	0.00% 0.00% 0.00% 0.00%	0.00% 0.00% 0.00% 0.00% 0.00%	0.99% 0.00% 0.66% 0.00%		
Bacteria/Firmicutes//Anaerococcus Bacteria/Firmicutes//Finegoldia Bacteria/Bacteroidtes//Prevotella Bacteria/Firmicutes//Family-XL-Incert Bacteria/Firmicutes//Peptoniphilus	1.41% 1.32% 0.66% 0.66% 0.63% 0.48% 0.48%	0.00% 0.00% 0.00% 0.00% 0.00% 0.00%	0.00% 0.00% 0.00% 0.00% 0.00% 0.00%	0.99% 0.00% 66% 0.05% 0.00% 0.00% 0.00%		Plot
Bacteria/Firmicutes//Anaerococcus Bacteria/Firmicutes//Finegoldia Bacteria/Bacteroidetes//Prevotella Bacteria/Firmicutes//Family-XI-Incert Bacteria/Firmicutes//Petroiphilus Bacteria/Actinobacteria//Corynebact	1.41% 1.32% 0.66% 0.66% 0.63% 0.48%	0.00% 0.00% 0.00% 0.00% 0.00%	0.00% 0.00% 0.00% 0.00% 0.00%	0.99% 0.00% 0.66% 0.00% 0.00%		

A new window will appear containing stacked bar display options

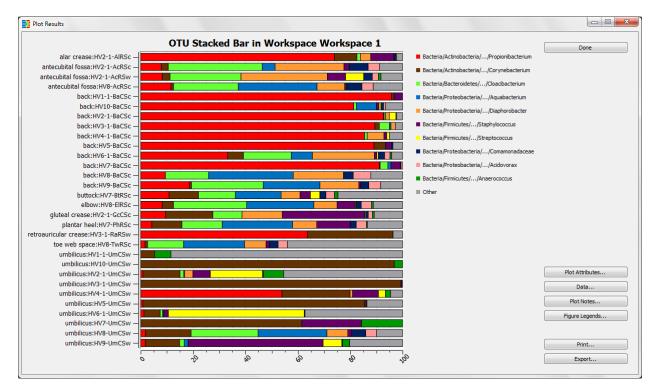
To create a stacked bar chart which displays a big picture of the project components, select % of Total

Click OK

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elect Libraries to Chart		_
Stacked Bar Mode	ОК	-
% of Selected		
% of Total	Cancel	_
Counts		
ibraries:		
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		
umbilicus:HV10-UmCSw		
umbilicus:HV2-1-UmCSw		
umbilicus:HV3-1-UmCSw		
umbilicus:HV4-1-UmCSw	E	
umbilicus:HV5-UmCSw		
umbilicus:HV6-1-UmCSw		
umbilicus:HV7-UmCSw		
umbilicus:HV8-UmCSw		
umbilicus:HV9-UmCSw		

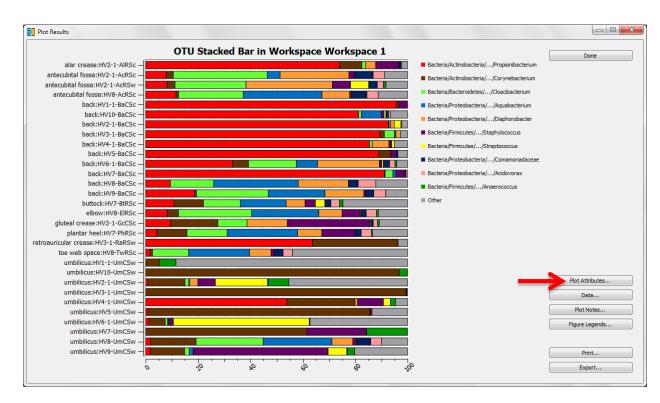
A pop-up window with the OTU stacked bar chart appears



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

tles/Axes	Grid	Colors	tacked Bar					Save
Titles								Cancel
Plot: 01	TU Stacked	Bar in Work	pace Workspace 1	L				
X Axis :								
Y Axis:								
	Show Libra	ry Name						
X Axis					_Y Axis			
		ry Name	e		Y Axis			
X Axis		Autosca	e		⊂Y Axis			
X Axis		Autosca 0	e		Y Axis	-90 to	90	
X Axis	Min Value:	 Autosca 0 100 	e 			-90 to	90	

On the Titles/Axes tab, enter "Top 10 Taxa" into the Plot field

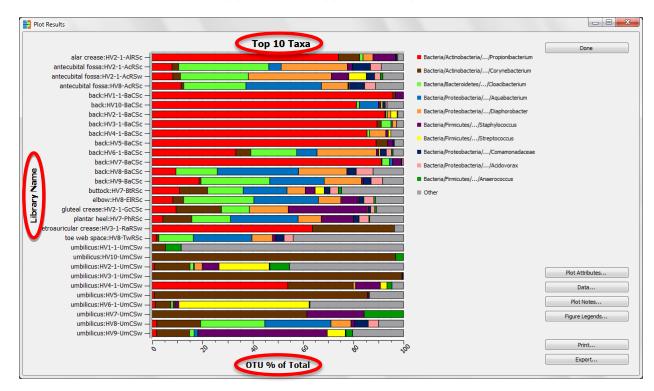
Enter "OTU % of Total" into the X Axis field

Enter "Library Name" into the Y Axis field

Click Save

Titles/Axes Grid Colors Stacked Bar				
Titles			_	Save
Plot: Top 10 Taxa				
X Axis : OTU % of Total				
Y Axis: Library Name				
Show Library Name				
X Axis		Y Axis		
V Autoscale				
Min Value: 0				
Max Value: 100		Label Rotation: 0	-90 to 90	
Step Size: 0				
	-90 to 90			

Plot Attributes window will disappear; labels appear on the plot

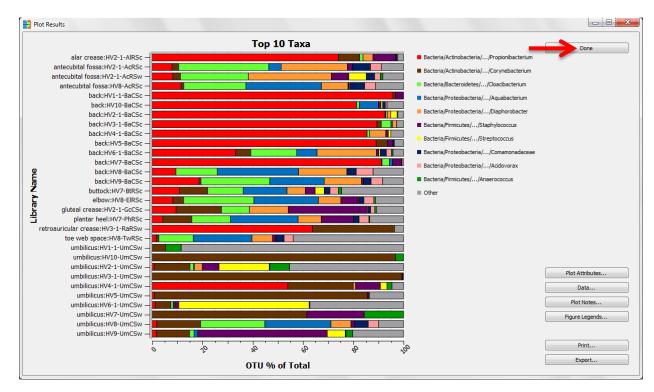


Red and brown appear to be dominant colors in this plot. According to the legend, these colors belong to the "Actinobacteria" phylum. This information may be useful in guiding us toward a hypothesis involving the dominant taxa.

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



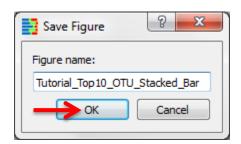
The OTU Stacked Bar setup window is back on the screen

Click Save as Figure

DTU	Stacked Bar in Workspace Workspace 1						
			OTU Stacked Bar i	n Workspace Workspace 1			Done
	Counts	OTU Start:	Show L	ibraries			Unsort OTU
	% of Library	OTU Width:	- O Show S	iorted Libs			
	% of Total		- Chaul	ib Groups			
	0	OTU Show	Last	o o o o o o o			
	Figure:		Filt	er: No filter assigned 30 Libs			
	Select items between: 1.41	and 31.35	10 Rows Selected of	152 Rows Select Ran	ge Clear Selection		
	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%	=	
	Bacteria/Actinobacteria//Propionibac	31.35%	74.01%	7.89%	8.25%		
	Bacteria/Actinobacteria//Corynebact	18.25%	8.55%	2.63%	2.97%		
	Bacteria/Bacteroidetes//Cloacibacteri	9.06%	1.32%	35.86%	27.06%		
	Bacteria/Proteobacteria//Aquabacter	7.68%	0.00%	4.93%	0.00%		
	Bacteria/Proteobacteria//Diaphoroba	6.52%	3.95%	26.32%	33.00%		
	Bacteria/Firmicutes//Staphylococcus	6.06%	8.88%	1.97%	6.93%		
	Bacteria/Firmicutes//Streptococcus	3.30%	0.00%	0.00%	6.93%		
	Bacteria/Proteobacteria//Comamona	1.54%	0.66%	7.24%	3.30%		
	Bacteria/Proteobacteria//Acidovorax	1.54%	0.33%	4.28%	2.31%		
0	Bacteria/Firmicutes//Anaerococcus	1.41%	0.00%	0.00%	0.99%		
1	Bacteria/Firmicutes//Finegoldia	1.32%	0.00%	0.00%	0.00%		
	Bacteria/Bacteroidetes//Prevotella	0.66%	0.00%	0.00%	0.66%		
2		0.66%	0.00%	0.00%	0.00%		
	Bacteria/Firmicutes//Family-XI-Incert			0.000/	0.00%		Plot
3	Bacteria/Firmicutes//Family-XI-Incert Bacteria/Firmicutes//Peptoniphilus	0.63%	0.00%	0.00%			
2 3 4 5		0.48%	0.00%	0.00%	0.00%		Export
3 4	Bacteria/Firmicutes//Peptoniphilus	0.48% 0.47%	0.00% 0.00%	0.00% 0.00%	0.00% 0.00%		Export
3 4 5	Bacteria/Firmicutes//Peptoniphilus Bacteria/Actinobacteria//Corynebact	0.48%	0.00%	0.00%	0.00%		Export Save As Figure

Enter stacked bar chart name in pop-up window

Click OK



Click Done

OTU Stacked Bar in Workspace Workspace 1						
		OTU Stacked Bar i	n Workspace Workspace 1		_	Done
Counts	OTU Start:	Show L	ibraries			Unsort OTU
% of Library	OTU Width:	2 (a) Show 5	Gorted Libs			
% of Total	OTU Show	Last 🔘 Show L	ib Groups			
Figure: Tutorial_Top10_OTU_Stacked_Bar		Filt	er: No filter assigned 30 Libs			
Select items between: 1.41	and 31.35	10 Rows Selected of	152 Rows Select Ran	ge Clear Selection		
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%	=	
Bacteria/Actinobacteria//Propionibac	31.35%	74.01%	7.89%	8.25%		
Bacteria/Actinobacteria//Corynebact	18.25%	8.55%	2.63%	2.97%		
Bacteria/Bacteroidetes//Cloacibacteri	9.06%	1.32%	35.86%	27.06%		
Bacteria/Proteobacteria//Aquabacter	7.68%	0.00%	4.93%	0.00%		
Bacteria/Proteobacteria//Diaphoroba	6.52%	3.95%	26.32%	33.00%		
Bacteria/Firmicutes//Staphylococcus	6.06%	8.88%	1.97%	6.93%		
Bacteria/Firmicutes//Streptococcus	3.30%	0.00%	0.00%	6.93%		
Bacteria/Proteobacteria//Comamona	1.54%	0.66%	7.24%	3.30%		
Bacteria/Proteobacteria//Acidovorax	1.54%	0.33%	4.28%	2.31%		
0 Bacteria/Firmicutes//Anaerococcus	1.41%	0.00%	0.00%	0.99%		
1 Bacteria/Firmicutes//Finegoldia	1.32%	0.00%	0.00%	0.00%		
2 Bacteria/Bacteroidetes//Prevotella	0.66%	0.00%	0.00%	0.66%		
3 Bacteria/Firmicutes//Family-XI-Incert	0.66%	0.00%	0.00%	0.00%		
4 Bacteria/Firmicutes//Peptoniphilus	0.63%	0.00%	0.00%	0.00%		Plot
5 Bacteria/Actinobacteria//Corynebact	0.48%	0.00%	0.00%	0.00%		
6 Bacteria/Proteobacteria//Zoogloea	0.47%	0.00%	0.00%	0.00%		Export
7 Bacteria/Proteobacteria//Sphingomo	0.46%	0.00%	3.95%	2.64%		Save As Figure.
	0.36%	0.00%	0.00%	0.00%		
18 Racteria/Firmicutes/ /Dialister						Notes

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.

Edit Data	s/kirstin/Desktop/ Group Tools		ISM_Explicet_Pro	oject.otu							
roject:	Tutorial_HSM		🔘 Hiera		Counts	OTU Start:	1	lierarchy Level:	Show Lib	_	Figures
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urrent Filter:	No filter assigned		🔘 30 Libs				******	******	Note	es	Close Project
ierarchy			Total	a	lar crease:HV2-1-	AIRSc ante	ubital fossa:HV2-	1-AcRSc	antecubital foss	a:HV2-1-AcRSw	antecubital fossa
root				100%	lar crease:Hv2-1-	100%		100%		10	0%
▲ Bacteria			1(00.00%		100.00%		100.00%		100.0	0%
Acidol	bacteria			0.02%		0.00%		0.00%		0.0	0%
Actino		II.		51.50%		83.88%		10.53%		12.2	
	oidetes			10.88%		1.32%		36.18%		28.3	
	date-division-TM7			0.02%		0.00%		0.00%			0%
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ures:	*								rrent Workspace:	-	Done
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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

$\textbf{Tools} \rightarrow \textbf{Plot} \rightarrow \textbf{Pie Chart}$

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

ie Chart in Wo	orkspace Workspace 1						
	Format for Plot Names						Done
						_	Add to Pie
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 Banteria 	a	100.00%	100.00%	100.00%	100.00%		
	idobacteria	0.02%	0.00%	0.00%	0.00%		
	tinobacteria	51.50%	83.88%	10.53%	12.21%		
	cteroidetes	10.88%	1.32%	36.18%	28.38%		
Car	ndidate-division-TM7	0.02%	0.00%	0.00%	0.00%		
Chl	loroflexi	0.03%	0.00%	0.00%	0.00%		
Cya	anobacteria	0.30%	0.33%	1.32%	0.00%		
Firm	micutes	15.28%	9.21%	2.30%	15.84%		
Fus	sobacteria	0.35%	0.00%	0.00%	0.00%		
Ger	mmatimonadetes	0.03%	0.00%	0.00%	0.00%		
	rospirae	0.05%	0.00%	0.00%	0.00%		
	nctomycetes	0.07%	0.00%	0.00%	0.00%		
	oteobacteria	21.41%	5.26%	49.67%	43.56%		
	nergistetes	0.03%	0.00%	0.00%	0.00%		
Ver	rucomicrobia	0.02%	0.00%	0.00%	0.00%		
							Plot
							Export
							Save As Figure
		•	•			•	Notes

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

Click Plot

e Chart in Workspace Workspace 1						
Format for Plot Names						Done
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/ root	100%	100%	100%	100%		
4 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%		
					_	Plot Export
						Save As Figure
	(F	< III				Notes

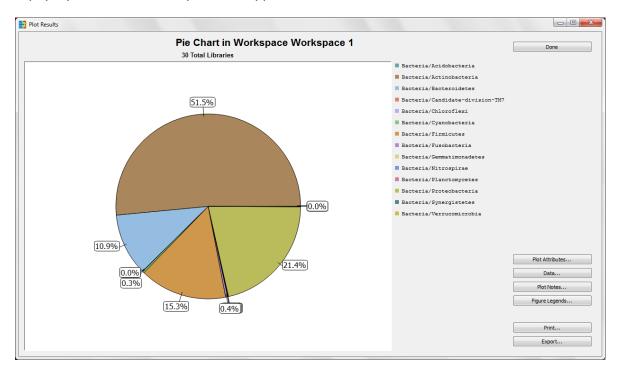
A new window will appear containing pie chart display options

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

Click OK

Select Pie Chart Data				_	
			_		
۱ ۱	lumber of pie ch	arts:	0	_	ОК
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Pick up to 16 Libraries for 1 plot:					
alar crease:HV2-1-AIRSc					
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back:HV8-BaCSc					
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gluteal crease:HV2-1-GcCSc					
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112 1846 11 66				÷	

A pop-up window with the pie chart appears

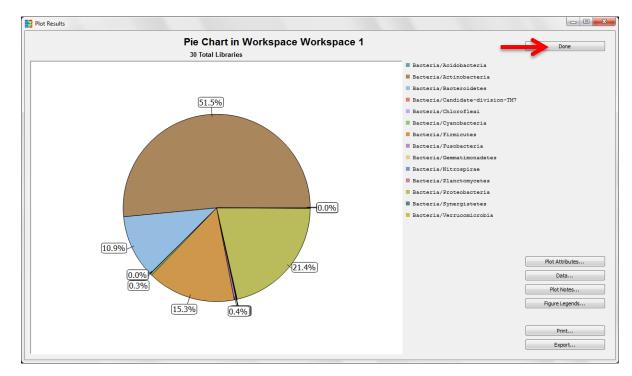


By looking at the pie chart of the phyla, it is clear that the brown wedge, Actinobacteria, is the most prevalent phylum in the data.

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						Done
OTU Start: 1	Counts	Hierarchy Leve	el: O Show Libraries		_	Add to Pie
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erarchy	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%	0.00%		
Synergistetes Verrucomicrobia	0.03 %	0.00%	0.00%	0.00%		
						Plot Export

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

Click Plot

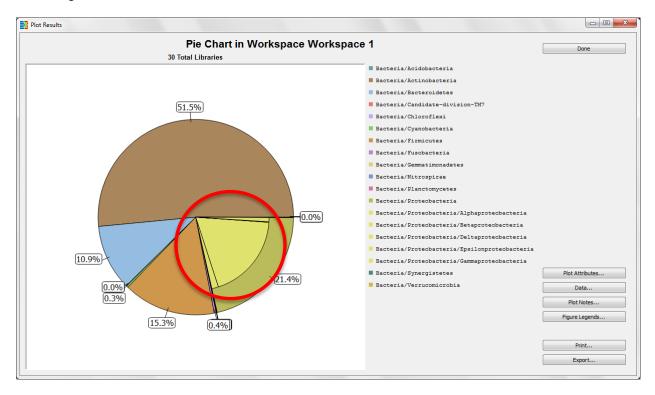
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• Nitrogrine 0.05% 0.00% 0.00% • Planctomycetes 0.07% 0.00% 0.00% • Proteobacteria 21.41% 5.26% 49.57% 43.56% • Alphaproteobacteria 1.47% 0.00% 5.22% 4.29% • Alphaproteobacteria 1.488% 5.26% 43.75% 38.94% • Detaproteobacteria 0.08% 0.00% 0.00% 0.00% • Symergistets 0.03% 0.00% 0.00% 0.00% • Verrucomicrobia 0.02% 0.00% 0.00% 0.00% 0.00%							
Planctomycetes 0.07% 0.00% 0.00% # Proteobacteria 21.41% 5.26% 49.67% 43.56% > Alphapoteobacteria 1.47% 0.00% 5.92% 4.2% > Betapoteobacteria 18.85% 5.26% 43.57% 38.94% > Dettapoteobacteria 0.00% 0.00% 0.00% > Detapoteobacteria 0.02% 0.00% 0.00% > bepilonproteobacteria 0.03% 0.00% 0.00% > Garmapoteobacteria 0.95% 0.00% 0.00% 0.33% > Synergistets 0.02% 0.00% 0.00% 0.00% 0.00% > Verrucomicrobia 0.02% 0.00% 0.00% 0.00% 0.00%							
4 Proteobacteria 22.43% 5.26% 49.67% 43.56% > Alpharoteobacteria 1.47% 0.00% 5.92% 4.35% > Detaproteobacteria 18.89% 5.26% 43.75% 38.94% > Detaproteobacteria 0.02% 0.00% 0.00% 0.00% > Detaproteobacteria 0.08% 0.00% 0.00% 0.00% > Gammaproteobacteria 0.03% 0.00% 0.03% 0.00% > Synergistees 0.03% 0.00% 0.00% 0.00% > Verrucomicrobia 0.02% 0.00% 0.00% 0.00%							
> Alphaproteobacteria 1.47% 0.00% 5.92% 4.37% > Detaproteobacteria 18.89% 5.26% 43.75% 38.94% > Detaproteobacteria 0.02% 0.00% 0.00% 0.00% > Epsilonproteobacteria 0.08% 0.00% 0.00% 0.00% > Symergistetes 0.03% 0.00% 0.00% 0.00% > Verracomicrobia 0.02% 0.00% 0.00% 0.00%							
• Betaproteobacteria 18.8% 5.26% 43.75% 38.34% • Deltaproteobacteria 0.02% 0.00% 0.00% 0.00% • Dipilopproteobacteria 0.02% 0.00% 0.00% 0.00% • Gammaproteobacteria 0.95% 0.00% 0.00% 0.00% • Sammaproteobacteria 0.95% 0.00% 0.00% 0.33% • Synergistets 0.03% 0.00% 0.00% 0.00% • Verrucomicrobia 0.02% 0.00% 0.00% 0.00%							
• Deltaproteobacteria 0.02% 0.00% 0.00% 0.00% • Epsilonproteobacteria 0.08% 0.00% 0.00% 0.00% • Gammaproteobacteria 0.03% 0.00% 0.00% 0.33% • Synergistetes 0.03% 0.00% 0.00% 0.00% • Verrucomicrobia 0.02% 0.00% 0.00% 0.00%							
• Epilonproteobacteria 0.08% 0.00% 0.00% • Gammaroteobacteria 0.95% 0.00% 0.33% • Synergistetes 0.03% 0.00% 0.00% • Verrucomicrobia 0.02% 0.00% 0.00%							
• Gammaproteobacteria 0.95% 0.00% 0.03% 0.33% • Synergistetes 0.03% 0.00% 0.00% 0.00% • Verrucomicrobia 0.02% 0.00% 0.00% 0.00%							
> Synergistetes 0.03% 0.00% 0.00% > Verrucomicrobia 0.02% 0.00% 0.00% 0.00%							
▶ Verrucomicrobia 0.02% 0.00% 0.00% 0.00%							
Plot							
Export						_	Plot
							Export

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

Select Pie Chart Data		
	Number of pie charts: Rows: Columns:	 OK
Include Charts for Totals		
0 Libraries on Plot		
30 Total Libraries		
SU TOTAL LIDRATIES		
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:HV8-BaCSc back:HV9-BaCSc		
back:HV9-BaCSC buttock:HV7-BtRSc		
elbow:HV8-EIRSc		
gluteal crease:HV2-1-GcCSc		
plantar heel:HV7-PhRSc		
1 · · · · · · · · · · · · · · · · · · ·	2Sw	
retroauricular crease:HV3-1-RaF	RSw	
1 · · · · · · · · · · · · · · · · · · ·	RSw	

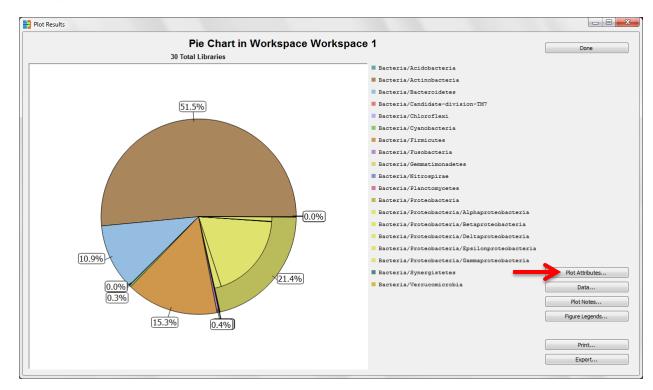
A pop-up window with the pie chart appears. We now see the classes within Proteobacteria represented as sub-wedges.



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

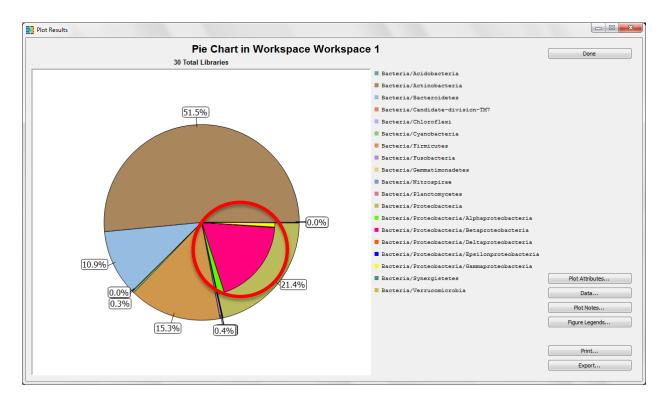
To pick a different wedge color, click on the color, and select a new color from the pop-up display

Plot Attributes		
Titles/Axes Colors		Save
Background Color:		Cancel
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	E	
Bacteria/Proteobacteria/Deltaproteobacteria		
Bacteria/Proteobacteria/Epsilonproteobacteria		
Bacteria/Proteobacteria/Gammaproteobacteria	—	
Bacteria/Synergistetes		
Bacteria/Verrucomicrobia		

When finished, click Save in upper right corner of window

Titles/Axes Colors Pie Chart	2
Background Color:	el
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.

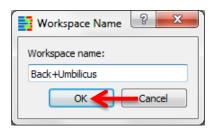
A. Create a New Workspace

$\textbf{File} \rightarrow \textbf{New} \rightarrow \textbf{Workspace from Current Workspace}$

• "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

ile Edit Data Group Tools View ⊢	lelp				
Project: Tutorial_HSM	Mierarchy	Counts OTU S	tart: 1 Hierarchy Level	: 🔘 Show Libraries	Figures
	⊚ οτυ	% of Library OTU W	/idth: 2 3 🖨	Show Sorted Libs	Clone Workspace
Workspace: Back+Umbilicus	Both	🔘 % of Total 🛛 📝 OT	'U Show Last	Show Lib Groups	Save
Current Filter: No filter assigned	③ 30 Libs			Notes	Close Project
Hierarchy	Total	alar crease:HV2-1-AIRSc	antecubital fossa:HV2-1-AcRSc	antecubital fossa:HV2-1-AcRSw	antecubital fossa:
4 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 Cyanobacteria 	0.03%	0.00%	0.00%	0.00%	
 Cyanobacteria 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%	
► V	() 0029/ ()	< III 0.00%	0.0097	0.00.9/	Þ
🔊 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%	
Bacteria/Acidobacteria//Candidatus	0.02%	0.00%	0.00%	0.00%	
Bacteria/Actinobacteria//Acidimicro	0.01%	0.00%	0.00%	0.00%	
Bacteria/Actinobacteria//Acidimicro	0.03%	0.00%	0.00%	0.00%	
Bacteria/Actinobacteria/Actinobacteri	0.03%	0.33%	0.00%	0.00%	
Bacteria/Actinobacteria//Actinomyc	0.05%	0.00%	0.00%	0.00%	
Bacteria/Actinobacteria//Actinomyc	0.28%	0.33%	0.00%	0.33%	
Bacteria/Actinobacteria//Mobiluncu	0.02%	0.00%	0.00%	0.00%	
Bacteria/Actinobacteria//Varibaculur	0.02%	0.00%	0.00%	0.00%	
Bacteria/Actinobacteria//Corynebac	0.48%	0.00%	0.00%	0.00%	
0 Bacteria/Actinobacteria//Corynebac	18.25%	8.55%	2.63%	2.97%	
1 Bacteria/Actinobacteria//Dietzia	0.04%	0.00%	0.00%	0.00%	
2 Bacteria/Actinobacteria//Mycobacte	0.01%	0.00%	0.00%	0.00%	
13 Bacteria/Actinobacteria//Nocardiace	0.04%	0.00%	0.00%	0.00%	
· (III)	() ()	< III			E.

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

$\textbf{Data} \rightarrow \textbf{Select Libraries}$

New pop-up window appears for creation of filters

Click New on far right side of window

Select						
Filters: No filter	r assigned		Next Filter	Previous Filter		Done Select
Metadata Selection	Criteria			Delete/Group/Move		
And/Or	Metadata	Operator	Value	Clear All	Add	New
To assign a filter to then click Save. To	o this workspace, create a new filter or pick an existing filter, o create a filter, click New			*	Delete	Save Filter
					Add Parentheses	Save As
					Remove Parentheses	Rename
					Move Up	Delete
				-	Move Down	
T	0.11-11-					
Taxonomy Selection	Criteria			Delete/Group/Move	Add	Select OTU Group
And/Or	Metadata	Operator	Value	Clear All	Delete	Name
				<u>^</u>	Add Parentheses	
				E	Remove Parentheses	
				=	Move Up	
					Move Down	
				*		
Libraries Selected						
	Library	Tota	al Member Count	OTU	Matched Count	

Enter desired filter name in the pop-up window

Click **OK**

New Filter	? <mark>x</mark>
Filter name:	
Back+Umbilicus	
ок 🧲	Cancel

The filter name will appear in upper left corner of window

Select					
Filters: Back+U	mbilcus	Next Filter	Previous Filter		Done Select
etadata Selection G	Criteria				
And/Or	Metadata	Operator Value	Clear All	Add	New
To add a new crite	eria for Metadata, dick Add			Delete	Save Filter
to add a new cite	ena for metadata, dict Add			Add Parentheses Remove Parentheses	Save As
				Move Up	Rename
				Move Down	Deck
xonomy Selection	Criteria				
And/Or	Metadata	Operator Value	Clear All	Add	Select OTU Group Name
				Delete Add Parentheses	
To add a new sele	ection criteria for Taxonomy, dick Add			Remove Parentheses	
				Move Up	
				Move Down	
aries Selected					
	Library	Total Member Count	OTU Mat	ched Count]

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.

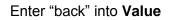
B. Set Up the Filter Parameters

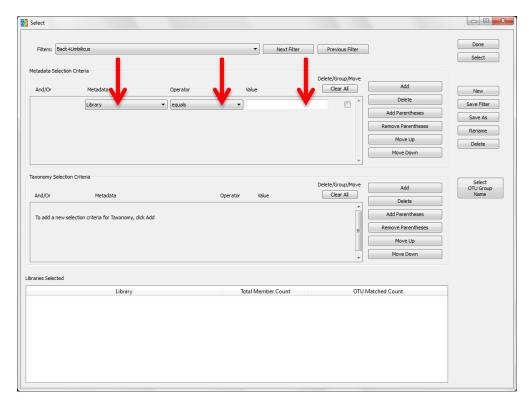
Click Add in the Metadata Criteria pane

Filters: Back+U	mbilicus		Next Filter	Previous Filter		Done Select
etadata Selection (Criteria			Delete/Group/Move		
And/Or	Metadata	Operator	Value	Clear All	Add	New
				^ [Delete	Save Filter
To add a new crite	eria for Metadata, click Add				Add Parentheses	Save As
					Remove Parentheses	Rename
					Move Up	Delete
				-	Move Down	
axonomy Selection	Criteria					
Notionly Delection				Delete/Group/Move	Add	Select OTU Group
And/Or	Metadata	Operator	Value	Clear All	Delete	Name
To add a new sele	ection criteria for Taxonomy, click Add			Î	Add Parentheses	
				=	Remove Parentheses	
					Move Up	
				- (Move Down	
aries Selected						
	Library	Tota	l Member Count	OTU M	atched 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)





Click Add in the Metadata Criteria pane

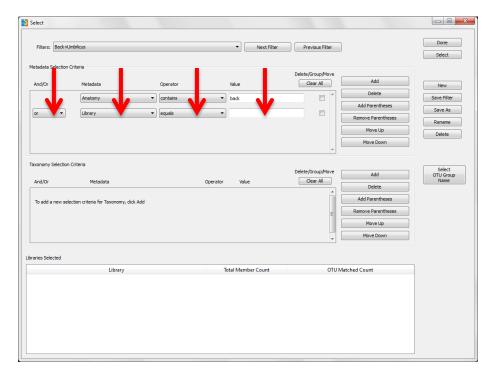
elect							
Filters: Back+Umb	pilicus		•	Next Filter	Previous Filter		Done Select
letadata Selection Cri And/Or	teria Metadata (Anatomy 🗸	Operator Contains •	Value		Delete/Group/Move Clear All	Add Delete Add Parentheses Remove Parentheses Move Up Move Down	New Save Filter Save As Rename Delete
axonomy Selection Cr And/Or To add a new select	iteria Metadata ion criteria for Taxonomy, click Add	Operator		alue	Delete/Group/Move	Add Delete Add Parentheses Remove Parentheses Move Up Move Down	Select OTU Group Name
aries Selected	Library		lotal M	ember Count	OTU	Matched Count	

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

Filters: Back+Umb	dicus		Next F	ilter Previous Filter]	Done
tadata Selection Crit						Select
Ind/Or	Metadata	Operator	Value	Delete/Group/Move Clear All	Add	New
	Anatomy	✓ contains	▼ back		Delete	Save Filter
r 👻	Anatomy	✓ contains	• umbilicus		Add Parentheses Remove Parentheses	Save As
					Move Up	Rename
					Move Down	Delete
onomy Selection Cri nd/Or o add a new selecti	teria Metadata on criteria for Taxonomy, dick Add		Operator Value	Delete/Group/Move	Add Delete Add Parentheses Remove Parentheses Move Up Move Down	Select OTU Group Name
ies Selected						_
	Library		Total Member Count	4 UTO	Matched Count	

Click Done in upper right corner of window

Filters: Back+U	ale the sec		-			Done
Filters: Back+U	nDilicus		 Next Filter 	Previous Filter		Select
						Belect
letadata Selection (Criteria			Delete/Group/Move		
	Metadata			Clear All	Add	
And/Or	Metadata	Operator	Value		Delete	New
	Anatomy 👻	contains 🔹	back		Delete	Save Filter
					Add Parentheses	Save As
or 🔻	Anatomy 🔻	contains 🔹	umbilicus		Remove Parentheses	
					Move Up	Rename
					Move Up	Delete
					Move Down	
To add a new sele	ction criteria for Taxonomy, click Add				Delete Add Parentheses Remove Parentheses Move Up Move Down	
	30 Total Libraries					•
	Library		otal Member Count	OTU Match	ned Count	-
V1-1-BaCSc	Library	305	otal Member Count	OTU Matcl	ned Count	
V1-1-BaCSc V10-BaCSc	Library	305 289	otal Member Count	OTU Matcl	red Count	
V1-1-BaCSc V10-BaCSc V2-1-BaCSc	Library	305 289 354	otal Member Count	OTU Matcl		Ε
V1-1-BaCSc V10-BaCSc V2-1-BaCSc V3-1-BaCSc	Library	305 289 354 180	otal Member Count	OTU Matcl		=
VI-1-BaCSc VI0-BaCSc V2-1-BaCSc V3-1-BaCSc V4-1-BaCSc	Library	305 289 354 180 296	otal Member Count	OTU Matcl		
VI-1-BaCSc VI0-BaCSc V2-1-BaCSc V3-1-BaCSc V4-1-BaCSc V5-BaCSc	Library	305 289 354 180 296 541	otal Member Count	OTU Matel		
VI-1-BaCSc VI0-BaCSc V2-1-BaCSc V3-1-BaCSc V4-1-BaCSc V5-BaCSc V6-1-BaCSc	Library	305 289 354 180 296 541 334	otal Member Count	OTU Matci		
UIV1-1-BaCSc IV10-BaCSc IV2-1-BaCSc IV2-1-BaCSc IV3-1-BaCSc IV4-1-BaCSc IV5-BaCSc IV7-BaCSc IV7-BaCSc	Library	305 289 354 180 296 541	otal Member Count	OTU Matcl		

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.

ile	Edit Da	ata Group Tools	View	Help							
Proje	ect:	Tutorial HSM			Hierarchy	Counts	OTU Start:	1	Hierarchy Level:	Show Libraries	Figures
					otu	% of Library	OTU Width:	2	3	Show Sorted Libs	Clone Workspace
Worl	kspace:	Back+Umbilicus		O	O Both	% of Total	V OTU Show	Last		Show Lib Groups	Save
Curr	ent Filter:	Back+Umbilicus	>	© 2	0 of 30 Libs					Notes	Close Project
liera	archy				Total	back:HV1-1-BaCSc	back:HV1	10-BaCSc	back:HV2-1-BaCSc	back:HV3-1-BaCSc	back:HV4-1-BaCSc
4 10					100%	100	%	100%	100%	100%	100%
	⊿ Bacteria	a			100.00%	100.00		100.00%	100.00%	100.00%	100.00%
		inobacteria			60.40%	96.72		81.31%	93.50%	91.67%	85.81%
		teroidetes			7.69%	0.00		1.38%	1.69%	3.89%	2.70%
	Car	ndidate-division-TM	17		0.03%	0.00	%	0.00%	0.00%	0.56%	0.00%
	⊳ Cya	anobacteria			0.15%	0.00	%	0.00%	0.00%	0.00%	0.00%
	⊳ Firn	nicutes			17.39%	3.28	%	2.42%	2.82%	0.56%	2.36%
	Fus	obacteria			0.40%	0.00	%	0.00%	0.00%	0.00%	0.00%
		teobacteria			13.88%	0.00	%	14.88%	1.98%	3.33%	9.12%
	Pro	leobacteria									
		hergistetes			0.04%	0.00	%	0.00%	0.00%	0.00%	0.00%
<			4	4	0.04%	0.00' < III	%	0.00%	0.00%	0.00%	0.00%
<	⊳ Syn		4	<			back:HV1		0.00%	0.00% back:HV3-1-BaCSc	0.00% back:HV4-1-BaCSc
<	⊳ Syn	nergistetes	4	4	4	<	back:HV1				back:HV4-1-BaCSc
	▷ Syn	nergistetes			Fotal	< m back:HV1-1-BaCSc	back:HV3	10-BaCSc	back:HV2-1-BaCSc	back:HV3-1-BaCSc	back:HV4-1-BaCSc
L	▷ Syn P OTU root Bacteria/A	Rule Set: Actinobacteria/Actir	nobacteri		Total 100%	← m back:HV1-1-BaCSc 100	back:HV3 %	10-BaCSc 100%	back:HV2-1-BaCSc 100%	back:HV3-1-BaCSc 100%	back:HV4-1-BaCSc 100%
L 2	 Syn Syn OTU root Bacteria/ Bacteria/ 	Rule Set: Actinobacteria/Actir Actinobacteria//Ac	nobacteri ctinomyc		Total 100% 0.03%	< m back:HV1-1-BaCSc 100 0.00	back:HVI % %	10-BaCSc 100% 0.00%	back:HV2-1-BaCSc 100% 0.00%	back:HV3-1-BaCSc 100% 0.00%	back:HV4-1-BaCSc 100% 0.00%
L 2 3	▷ Syn P OTU root Bacteria/A Bacteria/A Bacteria/A	Rule Set: Actinobacteria/Actir Actinobacteria//Ac	nobacteri ctinomyc		Total 100% 0.03% 0.07%	# #	back:HVI % % %	10-BaCSc 100% 0.00% 0.00%	back:HV2-1-BaCSc 100% 0.00%	back:HV3-1-BaCSc 100% 0.00% 0.00%	back:HV4-1-BaCSc 100% 0.00% 0.00%
L 2 3	Syn Syn root Bacteria/ Bacteria/ Bacteria/ Bacteria/ Bacteria/	Rule Set: Actinobacteria/Actin Actinobacteria//XA Actinobacteria//XA	nobacteri ctinomyc ctinomyc aribaculu		Total 100% 0.03% 0.24%	* * * * * * * * * * * * * * * * * * *	back:HVI % % %	10-BaCSc 100% 0.00% 0.00%	back:HV2-1-BaCSc 100% 0.00% 0.00%	back:HV3-1-BaCSc 100% 0.00% 0.00%	back:HV4-1-BaCSc 100% 0.00% 0.00%
L 2 3 4 5	 Syn OTU root Bacteria/A Bacteria/A Bacteria/A Bacteria/A 	Rule Set: Actinobacteria/Actin Actinobacteria//Ac Actinobacteria//Ac Actinobacteria//Ac	nobacteri ctinomyc ctinomyc aribaculur orynebac		* Total 100% 0.03% 0.07% 0.24% 0.03%	✓ ////////////////////////////////////	back:HV1 % % % %	10-BaCSc 100% 0.00% 0.00% 0.00%	back:HV2-1-BaCSc 100% 0.00% 0.00% 0.00%	back:HV3-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00%	back:HV4-1-BaCSc 100% 0.00% 0.00% 0.00%
L 2 3 4 5	 Syn OTU root Bacteria// Bacteria// Bacteria// Bacteria// Bacteria// 	Rule Set: Actinobacteria//Ac Actinobacteria//Ac Actinobacteria//C Actinobacteria//C Actinobacteria//C	nobacteri ctinomyc ctinomyc aribaculur orynebac orynebac		* Total 0.03% 0.03% 0.02% 0.03% 0.03%	e m back:HVI-1-BaCSc 100 0.000 0.000 0.000 0.000 0.000 0.000	back:HV3 % % % % %	10-BaCSc 100% 0.00% 0.00% 0.00% 0.00%	back:HV2-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00%	back:HV3-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00%	back:HV4-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00%
L 2 3 4 5 5 7	 Syn Syn Toot Bacteria// Bacteria// Bacteria// Bacteria// Bacteria// Bacteria// Bacteria// Bacteria// 	Rule Set: Actinobacteria/Actir Actinobacteria//Ac Actinobacteria//Xa Actinobacteria//Ca Actinobacteria//Ca	nobacteri ctinomyc ctinomyc aribaculur orynebac orynebac ietzia		* Total 0.03% 0.07% 0.24% 0.30% 0.30% 22.29%	r m back:HV1-1-BaCSc 100' 0.000 0.00' 0.000 0.00' 0.000 0.00' 0.000 0.00' 0.000 0.00'	back:HV3 % % % % % %	10-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00%	back:HV2-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00%	back:HV3-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00% 1.67%	back:HV4-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00%
L 2 3 4 5 5 7 8	 Syn Syn Foot Roteria// Bacteria// Bacteria// Bacteria// Bacteria// Bacteria// Bacteria// Bacteria// 	Rule Set: Actinobacteria/Actin Actinobacteria//Ac Actinobacteria//Ac Actinobacteria//CA Actinobacteria//CA Actinobacteria//CA	nobacteri ctinomyc ctinomyc aribaculur orynebac orynebac ietzia lycobacte		Total 0.03% 0.03% 0.24% 0.03% 0.30% 22.29% 0.06% 0.01%	e m back:HVI-1-BaCSc 100 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000	back:HVJ % % % % % % % % % % %	10-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00%	back:HV2-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00% 0.02% 0.02% 0.00%	back:HV3-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 1.67% 0.00%	back:HV4-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00%
1 2 3 4 5 5 7 8 9	 Syn OTU root Bacteria// 	Rule Set: Actinobacteria//A Actinobacteria//A Actinobacteria//Q Actinobacteria//Q Actinobacteria//Q Actinobacteria//D Actinobacteria//D Actinobacteria//D	nobacteri ctinomyc aribaculur orynebac orynebac ietzia lycobacte ocardiace		Total 100% 0.03% 0.24% 0.03% 0.30% 22.29% 0.06% 0.01%	* ************************************	back:HVJ % % % % % % % % % % %	10-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00%	back:HV2-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00% 0.028% 0.00% 0.00%	back:HV3-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00%	back:HV4-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00%
1 2 3 4 5 5 7 8 9 10	 Syn OTU root Bacteria// 	Rule Set: Actinobacteria/Actir Actinobacteria//Ac Actinobacteria//Xa Actinobacteria//Xa Actinobacteria//Xa Actinobacteria//Xa Actinobacteria//Xa	nobacteri ctinomyc aribacului orynebac orynebac ietzia lycobacte ocardiace ordonia		* Total 0.03% 0.03% 0.03% 0.03% 0.03% 0.06% 0.01% 0.06% 0.01%	✓ m back:HVI-1-BaCSc 1000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000	back:HVJ % % % % % % % % % % % %	10-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00%	back:HV2-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00%	back:HV3-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00%	back:HV4-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00%
1 2 3 4 5 5 7 8 9 10 11	Syn OTU root Bacteria// Bacteria// Bacteria// Bacteria// Bacteria// Bacteria// Bacteria// Bacteria// Bacteria// Bacteria// Bacteria// Bacteria// Bacteria// Bacteria//	Rule Set: Actinobacteria/Actin Actinobacteria//Ac Actinobacteria//Ac Actinobacteria//Qc Actinobacteria//Qc Actinobacteria//Qc Actinobacteria//Qc Actinobacteria//Qc	nobacteri ctinomyc aribacului orynebac orynebac ietzia lycobacte ocardiace ordonia hodococc		* Total 100% 0.03% 0.24% 0.03% 0.24% 0.30% 22.29% 0.06% 0.01% 0.06% 0.01%	e m back:HVI-1-BaCSc 100 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000	back:HVJ % % % % % % % % % % % %	10-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00%	back:HV2-1-BaCSc 100% 0.00%	back:HV3-1-BaCSc 100% 0.00% 0.00% 0.00% 1.67% 0.00% 0.00% 0.00% 0.00%	back:HV4-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00%
1 2 3 4 5 5 7 8 9 10	 Syn Syn Toot Bacteria// 	Rule Set: Actinobacteria/Actir Actinobacteria//Ac Actinobacteria//Xa Actinobacteria//Xa Actinobacteria//Xa Actinobacteria//Xa Actinobacteria//Xa	nobacteri ctinomyc aribaculur orynebac orynebac orynebac ietzia lycobacte ocardiace ordonia hodococc eodermat		* Total 0.03% 0.03% 0.03% 0.03% 0.03% 0.06% 0.01% 0.06% 0.01%	✓ m back:HVI-1-BaCSc 1000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000	back:HVJ % % % % % % % % % % % % % %	10-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00%	back:HV2-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00%	back:HV3-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00% 0.00%	back:HV4-1-BaCSc 100% 0.00% 0.00% 0.00% 0.00% 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

$\textbf{Tools} \rightarrow \textbf{Analyze} \rightarrow \textbf{Beta Diversity} \rightarrow \textbf{Morisita-Horn}$

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

Click Plot

							Done
		Morisita-Horn in We	orkspace Back+Umbilicus				
Show	w Libraries	(Alphabetical by Library Name	e			
 Show Sorted Libs 		(
Show	v Lib Groups	(Ascending by Value				
Figure:		F	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	Ξ	
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		Plot
umbilicus:HV1-1-UmCSw	0.001	0.000	0.000	0.002	0.000		Export
umbilicus:HV10-UmCSw	0.010	0.000	0.003	0.018	0.000		Save As Figure
umbilicus:HV2-1-UmCSw	0.023	0.024	0.029	0.025	0.029	_	Notes

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 \rightarrow Analyze \rightarrow Alpha Diversity

New pop-up window appears

To create curves, deselect Single statistic at Rarefaction point only

Click Bootstrap

oha Diversity in Wor	kspace Back+Umbi	licus					
# Libraries:	20	Min Size: 180	Max Size: 541	Avg Size: 334	Show Libraries	s	Done
Bootstrap Size:	25	1st Sigma: 255	2nd Sigma: 177	3rd Sigma: 98	Show Sorted I		Bootstrap
Cutoff Size:	98	# Libs Inc: 20	# Libs Exc: 0		Show Lib Group	ıps	
# Steps:	10	🔽 Use Min Lib Size	Min Inc Lib Size: 180	Step Size: 18			
	Single statistic a	t Rarefaction point only					
Figure:			Filter: Back+Umbil	icus 20 of 30 Libs			
Sobs Mean	Sobs Median	Sobs 2.5%	Sobs 97.5% Singletons Mean	Singletons Median	Singletons 2.5%	Singletons 97.5%]
							Plot
							Export
							Save As Figure

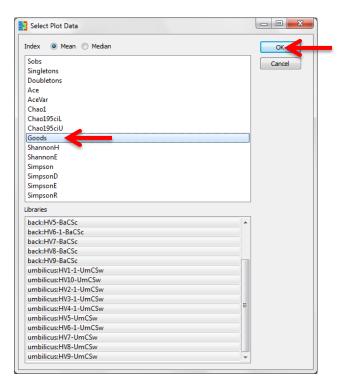
When Bootstrap is finished running, click Plot

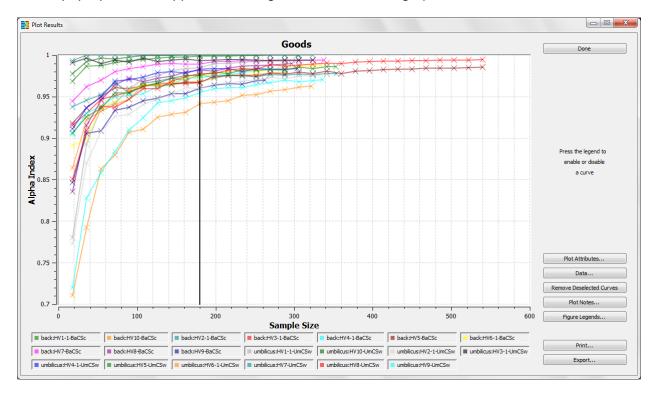
# Libraries: 20	Min Siz	e: 180	Max Size: 541	Avg Size:	334 🔘 S	how Libraries		Done Bootstrap
Bootstrap Size: 25	1st Sig	ma: 255	2nd Sigma: 177	3rd Sigma:	98 O S	how Sorted Libs		boostap
Cutoff Size: 98		Inc: 20	# Libs Exc: 0		© s	how Lib Groups		
# Steps: 10		e Min Lib Size	Min Inc Lib Size: 180	Step Size:	18			
	gle statistic at Rarefactio	n point only						
Figure:			Filter: B	ack+Umbilicus 20 of 3	10 Libs			
	Sobs Mean	Sobs Median	Sobs 2.5%	Sobs 97.5%	Singletons Mean	Singletons Median	Sing 🔺	
ack:HV1-1-BaCSc : 18	1.840	2.000	1.000	3.000	0.560	1.000	0.000	
ack:HV1-1-BaCSc : 36	1.840	2.000	1.000	3.000	0.480	0.000	0.000	
ack:HV1-1-BaCSc : 54	2.400	2.000	2.000	3.000	0.680	1.000	0.000	
ack:HV1-1-BaCSc : 72	2.600	3.000	1.000	3.000	0.640	1.000	0.000	
ack:HV1-1-BaCSc:90	2.600	3.000	2.000	3.000	0.600	0.000	0.000	
ack:HV1-1-BaCSc:108	2.720	3.000	1.000	3.000	0.560	1.000	0.000	
ack:HV1-1-BaCSc:126	2.680	3.000	2.000	3.000	0.480	0.000	0.000	
ack:HV1-1-BaCSc:144	2.760	3.000	2.000	3.000	0.320	0.000	0.000	
ack:HV1-1-BaCSc:162	2.800	3.000	2.000	3.000	0.360	0.000	0.000	
ack:HV1-1-BaCSc:180	2.920	3.000	2.000	3.000	0.200	0.000	0.000	Plot
ack:HV1-1-BaCSc:198	2.760	3.000	2.000	3.000	0.200	0.000	0.000	Export
ack:HV1-1-BaCSc : 216	3.000	3.000	3.000	3.000	0.240	0.000	0.000	Save As Figure

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

Select Goods

Click OK





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

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

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

XII. Two-Part Test

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

A. Run a Two-Part Test

$\textbf{Tools} \rightarrow \textbf{Analyze} \rightarrow \textbf{Two-Part}$

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+Umb	ilicus vs Back+Umbilicus			
	Two-Part in Workspace Back+Umbilicu	s: Back+Umbilicus vs Back+Umbilicus		Done
Category 1 Filter: Back+Umbilicus	▼ 20 of 20 Libs	OTU Start: 1	Alphabetical by OTU Name	Calculate
Category 2 Filter: Back+Umbilicus	▼ 20 of 20 Libs	OTU Width: 2	O Descending by P-Value	Setup Filters
P-Value Threshhold: 0		OTU Show Last	Ascending by P-Value	
Figure:		Filter: Back+Umbilicus 20 of 30	Libs	
UTC	HV1-1-BaCSc	Collisions: Libraries that are in both C	ategory 1 and Category 2	
	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			Plot
	HV8-UmCSw			Export
	HV9-UmCSw			Save As Figure
				Notes

New pop-up window appears for creation of filters

Click New on far right side of window

Select						
Filters: Back+Umbi	ilicus		Next Filter	Previous Filter		Done
Metadata Selection Crite	eria					
Fictuate Sciccoon ena				Delete/Group/Move		
And/Or	Metadata	Operator	Value	Clear All	Add	New
			1		Delete	
	Anatomy 👻	contains 🔹	back		Add Parentheses	Save Filter
or 🔻	Anatomy -	contains 👻	umbilicus			Save As
			,		Remove Parentheses	Rename
					Move Up	
					Move Down	Delete
				- L	Hove bown	
And/Or To add a new selectio	Metadata on criteria for Taxonomy, dick Add	Operator	Value		Add Delete Add Parentheses Remove Parentheses Move Up Move Down	OTU Group Name
D Libs Selected of 20 Two	o Part Libs Selected 30 Total Libraries					
) Libs Selected of 20 Tw	o Part Libs Selected 30 Total Libraries Library		Fotal Member Count	OTU Ma	tched Count	^
			Total Member Count	OTU Ma	tched Count	-
HV1-1-BaCSc		Т	Total Member Count	OTU Ma	tched Count	E
HV1-1-BaCSc HV10-BaCSc HV2-1-BaCSc		T 305	Total Member Count	OTU Ma	tched Count	
HV1-1-BaCSc HV10-BaCSc HV2-1-BaCSc HV3-1-BaCSc		T 305 289 354 180	Total Member Count	OTU Ma	tched Count	
HV1-1-BaCSc HV10-BaCSc HV2-1-BaCSc HV3-1-BaCSc HV3-1-BaCSc HV4-1-BaCSc		T 305 289 354 180 296	fotal Member Count	OTU Ma	tched Count	
HV1-1-BaCSc HV10-BaCSc HV2-1-BaCSc HV3-1-BaCSc HV3-1-BaCSc HV4-1-BaCSc HV5-BaCSc		T 305 289 354 180 296 541	lotal Member Count	OTU Ma	tched Count	
HV1-1-BaCSc HV10-BaCSc HV2-1-BaCSc HV3-1-BaCSc HV4-1-BaCSc HV5-BaCSc HV6-1-BaCSc		T 305 289 354 180 296 541 334	Fotal Member Count	OTU Ma	tched Count	
0 Libs Selected of 20 Twr HV1-1-BaCSc HV10-BaCSc HV2-1-BaCSc HV2-1-BaCSc HV3-1-BaCSc HV4-1-BaCSc HV6-1-BaCSc HV6-T-BaCSc HV7-BaCSc HV7-BaCSc		T 305 289 354 180 296 541	fotal Member Count	OTU Ma	tched Count	

Enter desired filter name in the pop-up window

Click OK

New Filter	8 ×
Filter name:	
Back	
ок	Cancel

The filter name will appear in upper left corner of window

Select						
Filters: Back		•	Next Filter	Previous Filter		Done Select
Metadata Selection Criteria				Delete/Group/Move		
And/Or Metadata	Operator	Value		Clear All	Add	New
					Delete	Save Filter
To add a new criteria for Metadata, click Add					Add Parentheses	Save As
					Remove Parentheses	Rename
					Move Up	Delete
					Move Down	
Taxonomy Selection Criteria				Delete/Group/Move	Add	Select
And/Or Metadata		Operator Va	alue	Clear All	Delete	OTU Group Name
					Add Parentheses	
To add a new selection criteria for Taxonomy, click Add					Remove Parentheses	
				E	Move Up	
					Move Down	
				-	Move Down	
20 Libs Selected of 20 Two Part Libs Selected 30 Total Librari	25					
Library		Total Me	mber Count	OTU N	latched Count	

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".

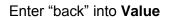
B. Set Up Filter Parameters

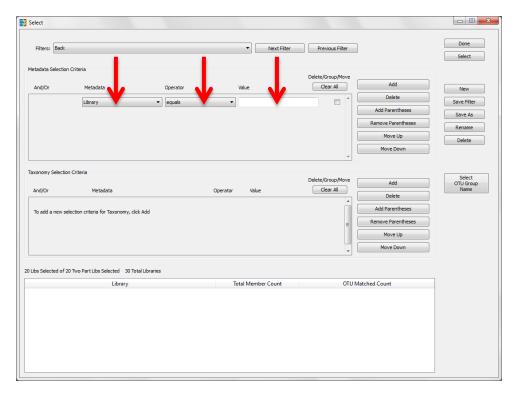
Click Add in the Metadata Criteria pane

Select						
Filters: Back			▼ Ne	xt Filter Previous Filter		Done
etadata Selection	Criteria			Delete/Group/Move		
And/Or	Metadata	Operator	Value	Clear All	Add	New
				*	Delete	Save Filter
To add a new cri	teria for Metadata, click Add				Add Parentheses	Save As
					Remove Parentheses	Rename
					Move Up	Delete
					Move Down	buck
				Ŧ		
xonomy Selection	n Criteria					Select
And/Or	Metadata		Operator Value	Delete/Group/Move	Add	OTU Group Name
Anu/Or	Metadata		Operator value		Delete	, include the second se
To add a new sel	lection criteria for Taxonomy, di	ck Add			Add Parentheses	
				E	Remove Parentheses	
					Move Up	
				*	Move Down	
ibs Selected of 20	0 Two Part Libs Selected 30 To	tal Libraries				
	Library		Total Member Co	unt 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)





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			 Next 	Filter Previous Filter		Done Select
tadata Selection	n Criteria			Delete/Group/Move		
And/Or	Metadata	Operator	Value	Clear All	Add	New
	Anatomy	▼ contains	▼ back	· ·	Delete	Save Filter
					Add Parentheses	Save As
					Remove Parentheses	Rename
					Move Up	Delete
				-	Move Down	
konomy Selectio	- Cileria					
conomy selectio	n Criteria			Delete/Group/Move	Add	Select OTU Group
And/Or	Metadata		Operator Value	Clear All	Delete	Name
To add a pow o	ection criteria for Taxonomy, d	id: Add		<u>^</u>	Add Parentheses	
to add a new se	account citteria for faxonomy, o			E	Remove Parentheses	ĵ
				-	Move Up	
				-	Move Down	
bs Selected of 2	10 Two Part Libs Selected 30 To	otal Libraries				
	Library		Total Member Cour	t OTU I	Matched Count	

Now we will create a separate filter for the umbilicus

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

Select						
Filters: Back			▼ Next Filter	Previous Filter		Done Select
etadata Selection	n Criteria					
				Delete/Group/Move		
And/Or	Metadata	Operator	Value	Clear All	Add	New
	Anatomy	✓ contains	▼ back		Delete	Save Filter
	Anatomy	Contains	Dack		Add Parentheses	
						Save As
					Remove Parentheses	Rename
					Move Up	Delete
				1	Move Down	Decea
				*		
axonomy Selectio	in Criteria			Delete/Group/Move		Select
And/Or	Metadata		Operator Value	Clear All	Add	OTU Group Name
Anu/Or	Meldudid		Operator Value		Delete	
				Ê Î	Add Parentheses	
To add a new se	election criteria for Taxonomy, click A	vdd				
				E	Remove Parentheses	
					Move Up	
					Move Down	
				+	Hove Down	
	20 Two Part Libs Selected 30 Total L	Libraries				
Libs Selected of 2	20 Two Part Libs Selected 50 Total L					
Libs Selected of 2	Library		Total Member Count	OTU Ma	tched Count	
			Total Member Count 305	OTU Ma	tched Count	
W1-1-BaCSc				OTU Ma	tched Count	
V1-1-BaCSc V10-BaCSc			305	OTU Ma	tched Count	
IV1-1-BaCSc IV10-BaCSc IV2-1-BaCSc			305 289	OTU Ma	tched Count	
V1-1-BaCSc V10-BaCSc V2-1-BaCSc V3-1-BaCSc			305 289 354	OTU Ma		
IVI-1-BaCSc IVI0-BaCSc IV2-1-BaCSc IV3-1-BaCSc IV4-1-BaCSc IV5-BaCSc			305 289 354 180	OTU Ma		
IVI-1-BaCSc IVI0-BaCSc IV2-1-BaCSc IV3-1-BaCSc IV4-1-BaCSc IV5-BaCSc			305 289 354 180 296	OTU Ma		
ILIDS Selected of 2 IVI-1-BaCSc IVI0-BaCSc IVI0-BaCSc IVI2-1-BaCSc IVI3-1-BaCSc IVI4-1-BaCSc IVI5-BaCSc IVI6-1-BaCSc IVI7-BaCSc			305 289 354 180 296 541	OTU Ma		

Enter desired filter name in the pop-up window

Click OK

New Filter	8 ×
Filter name:	
Umbilicus	
ок 🧲	Cancel

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

Select						
Filters: Umbi			Next Filter	Previous Filter		Done Select
Metadata Selectio	on Criteria			Delete/Group/Move		
And/Or	Metadata	Operator	Value	Clear All	Add	New
					Delete	Save Filter
To add a new o	criteria for Metadata, click Add				Add Parentheses	
					Remove Parentheses	Save As
						Rename
					Move Up	Delete
					Move Down	
Faxonomy Selecti	ion Criteria			Delete/Group/Move		Select
And/Or	Metadata		Operator Value	Clear All	Add	OTU Group Name
					Delete	
To add a new s	selection criteria for Taxonomy, click	Add			Add Parentheses	
				=	Remove Parentheses	
					Move Up	
					Move Down	
				•	,	
Libs Selected of	20 Two Part Libs Selected 30 Tota	l Libraries				_
	Library		Total Member Count	OTU M	atched Count	

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

elect							
Filters: Umb	vilicus		•	Next Filter	Previous Filter]	Done Select
etadata Selecti	ion Criteria				Delete/Group/Move		
And/Or	Metadata	Operator	Value		Clear All	Add	New
					*	Delete	Save Filter
To add a new	criteria for Metadata, click Add					Add Parentheses	Save As
						Remove Parentheses	Rename
						Move Up	Delete
					÷	Move Down	
xonomy Select	tion Criteria						
					Delete/Group/Move	Add	Select OTU Group
And/Or	Metadata		Operator	/alue	Clear All	Delete	Name
To add a new	selection criteria for Taxonomy, click Add				- A	Add Parentheses	
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						Move Up	
					-	Move Down	
ibs Selected of	f 20 Two Part Libs Selected 30 Total Libra	ries					
	Library		Total M	ember Count	OTU N	Natched 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**)



Select						
Filters: Umbilicus			Next Filter	Previous Filter		Done Select
letadata Selection Crit And/Or	eria Metadata	Operator	Value	Delete/Group/Move	Add	New
	Library	▼ equals	-		Delete Add Parentheses Remove Parentheses	Save Filter Save As
					Move Up Move Down	Rename Delete
ixonomy Selection Cri And/Or To add a new selecti	teria Metadata an criteria for Taxonomy, click Add		Operator Value	Delete/Group/Move	Add Delete Add Parentheses Remove Parentheses Move Up	Select OTU Group Name
ibs Selected of 20 Tw	o Part Libs Selected 30 Total Libra Library	rries	Total Member Count	• • •	Move Down	

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: Umbili	cus		Next Filter	Previous Filter		Select
adata Selection	n Criteria					
And/Or	Metadata	Operator	Value	Clear All	Add	
					Delete	New
	Anatomy	▼ contains	umbilicus		Add Parentheses	Save Filte
					Remove Parentheses	Save As
					Move Up	Rename
					Move Down	Delete
				Ŧ		
onomy Selectio	n Criteria					
				Delete/Group/Move	Add	Select OTU Group
nd/Or	Metadata		Operator Value	Clear All	Delete	Name
a add a paw or	election criteria for Taxonomy, cl	liek Add		Â	Add Parentheses	
o aud a new se	section chiena for faxonomy, c	iot Add			Remove Parentheses	
				=	Move Up	
					Move Down	
				•		
s Selected of 2	0 Two Part Libs Selected 30 To	otal Libraries				
	Library		Total Member Count	OTU M	latched Count]

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

						Done
Filters: Umbilio	CUS		 Next Filte 	er Previous Filter		
						Select
etadata Selection	n Criteria					
				Delete/Group/Move		
And/Or	Metadata	Operator	Value	Clear All	Add	New
	Anatomy	▼ contains	umbilicus		Delete	Save Filter
	And Girly	containa	unblicus		Add Parentheses	
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xonomy Selection	- 000-00					
ixonomy selection	ri Criteria			Delete/Group/Move	Add	Select
And/Or	Metadata		Operator Value	Clear Al	Add	OTU Group Name
ranayon	The constant				Delete	
To add a new se	ection criteria for Taxonomy, click	Add		n E	Add Parentheses	1
	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,				Remove Parentheses	1
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					Move Up	J
					Move Down]
in Coloriad of D	0 Two Part Libs Selected 30 Total	1 Bearing				
Job Selected of 2		cordinea				
	Library		Total Member Count	OTU Match	ned Count	<u>^</u>
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			242			
/10-UmCSw	,		313			
/10-UmCSw /2-1-UmCSw			346			E
/10-UmCSw /2-1-UmCSw /3-1-UmCSw	,		346 331			E
/10-UmCSw /2-1-UmCSw /3-1-UmCSw /4-1-UmCSw	,		346 331 304			E
/10-UmCSw /2-1-UmCSw /3-1-UmCSw /4-1-UmCSw /5-UmCSw			346 331 304 353			E
V1-1-UmCSw V10-UmCSw V2-1-UmCSw V3-1-UmCSw V4-1-UmCSw V5-UmCSw V6-1-UmCSw V7-UmCSw			346 331 304			E

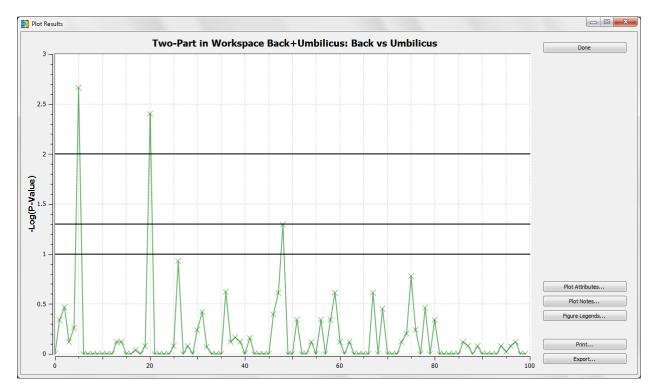
Select "Back" for the Category 1 Filter

Select "Umbilicus" for the Category 2 Filter

Click Calculate

Click Plot

		Two-Pa	rt in Workspace Bad	k+Umbilicus: Back vs	Umbilicus					Done
	Category 1 Filter: Back	-	10 of 20 Libs	OTU Start	: 1		Alphabetical by C	OTU Name		Calculate
	Category 2 Filter: Umbilicus		10 of 20 Libs	OTU Widt	h: 2		O Descending by P	Value		Setup Filters
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	P-Value Threshhold: 0			V OTU S	how Last		Ascending by P-V	/alue		
				5 11 0		60011				
	Figure:			Filter: Ba	ck+Umbilicus 20) of 30 Libs				
_	OTU: 100 of 100	m1	p1	med1	m2	p2	med2	chi**2	p. ^	
	Bacteria/Actinobacteria/Actinobacteria	0	0.000		1	0.100	0.621	0.000	1.000	
	Bacteria/Actinobacteria//Actinomycetaceae	0	0.000		3	0.300	0.302	1.569	0.456	
	Bacteria/Actinobacteria//Actinomyces	1	0.100	0.599	5	0.500	0.578	2.143	0.343	
	Bacteria/Actinobacteria//Varibaculum	0	0.000		2	0.200	0.310	0.556	0.757	
	Bacteria/Actinobacteria//Corynebacteriaceae	1	0.100	0.760	4	0.400	1.239	1.192	0.551 =	
	Bacteria/Actinobacteria//Corynebacterium	7	0.700	0.984	10	1.000	21.697	12.257	0.002	
	Bacteria/Actinobacteria//Dietzia	0	0.000		1	0.100	0.741	0.000	1.000	
	Bacteria/Actinobacteria//Mycobacterium	0	0.000		1	0.100	0.283	0.000	1.000	
	Bacteria/Actinobacteria//Nocardiaceae	1	0.100	0.739	0	0.000		0.000	1.000	
0	Bacteria/Actinobacteria//Gordonia	1	0.100	0.556	0	0.000		0.000	1.000	
1	Bacteria/Actinobacteria//Rhodococcus	1	0.100	0.282	1	0.100	1.156	0.000	1.000	
2	Bacteria/Actinobacteria//Geodermatophilaceae	1	0.100	0.337	0	0.000		0.000	1.000	
3	Bacteria/Actinobacteria//Brevibacterium	1	0.100	0.599	1	0.100	0.311	0.000	1.000	
4	Bacteria/Actinobacteria//Dermabacteraceae	0	0.000		2	0.200	1.298	0.556	0.757	
5	Bacteria/Actinobacteria//Microbacteriaceae	0	0.000		2	0.200	0.863	0.556	0.757	
5	Bacteria/Actinobacteria//Microbacterium	1	0.100	0.673	0	0.000		0.000	1.000	
7	Bacteria/Actinobacteria//Micrococcus	0	0.000		1	0.100	0.283	0.000	1.000	
в	Bacteria/Actinobacteria//Rothia	3	0.300	0.338	3	0.300	1.133	0.190	0.909	
9	Bacteria/Actinobacteria//Nocardioides	1	0.100	0.282	0	0.000		0.000	1.000	
0	Bacteria/Actinobacteria//Propionibacteriaceae	1	0.100	0.370	2	0.200	0.863	0.375	0.829	
1	Bacteria/Actinobacteria//Propionibacterium	10	1.000	87.284	6	0.600	1.514	11.074	0.004	
2	Bacteria/Actinobacteria//Atopobium	0	0.000		1	0.100	0.329	0.000	1.000	
3	Bacteria/Bacteroidetes//Porphyromonadaceae	0	0.000		1	0.100	0.298	0.000	1.000	
4	Bacteria/Bacteroidetes//Dysgonomonas	1	0.100	0.185	0	0.000		0.000	1.000	
5	Bacteria/Bacteroidetes//Paludibacter	1	0.100	1.014	0	0.000		0.000	1.000	
5	Bacteria/Bacteroidetes//Porphyromonas	1	0.100	0.346	2	0.200	3.417	0.375	0.829	
1	Bacteria/Bacteroidetes//Prevotella	0	0.000		5	0.500	1.786	4.267	0.118	
8	Bacteria/Bacteroidetes//Cytophagaceae	1	0.100	0.282	1	0.100	0.926	0.000	1.000	
9	Bacteria/Bacteroidetes//Flavobacteriaceae	1	0.100	1.141	2	0.200	0.444	0.375	0.829	Plot
0	Bacteria/Bacteroidetes//Bergeyella	0	0.000		1	0.100	5.901	0.000	1.000	Export
1	Bacteria/Bacteroidetes//Chryseobacterium	3	0.300	0.380	1	0.100	5.666	1.112	0.573	
2	Bacteria/Bacteroidetes//Cloacibacterium	8	0.800	3.424	4	0.400	1.615	1.940	0.379	Save As Figure
2	n a tan a tan a an	12	0.000	0.000		0.100	0.555	0.010	0.000	Notes



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.

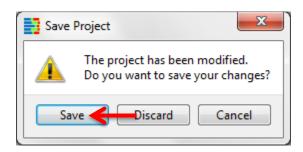
XIII. Close the Project

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

e Edit Data Gr	roup Tools View Help	_							
	· · ·	Mierarch	y Counts	OTU Start: 1	Hierarchy Level: 🔘 S	how Libraries 🔘	All Libraries	Figures	
Project: Tutoria	ial_HSM		, -					Clone Workspace	5
Workspace: Back-	+Umbilicus	© OTU	% of Library	OTU Width: 2	3 🔹 💿 S	how Sorted Libs 💿	Selected Libraries		-
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4 root		100%	100%	100%	100%	100%	100%	100%	
▲ Bacteria		100%	100%	100%	100%	100%	100%	100%	
Actinobact		60.40%	96.72%	81.31%	93.50%	91.67%	85.81%	95.01%	
Bacteroide		7.69%	0%	1.38%	1.69%	3.89%	2.70%	0.18%	
	e-division-TM7	0.03%	0%	0%	0%	0.56%	0%	0%	
Cyanobact		0.15%	0%	0%	0%	0%	0%	0%	_
Firmicutes		17.39% 0.40%	3.28%	2.42%	2.82%	0.56%	2.36%	3.14%	_
 Fusobacter Proteobact 		0.40%	0%	14.88%	1.98%	3.33%	9.12%	1.66%	_
 Proteopact Synergistet 		0.04%	0%	14.00 %	1.98 %	5.55 % 0%	9.12%	1.00 %	_
٠		4	۲ (III) ۱۱۱						4
< 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	•
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root 1 Bacteria/Actinob	 bacteria/Actinobacteria	Total 100% 0.03%	back:HV1-1-BaCSc 100% 0%	100% 0%	100% 0%	100% 0%	100% 0%	100% 0%	
root Bacteria/Actinob Bacteria/Actinob	 bacteria/Actinobacteria bacteria//Actinomycetaceae	Total 100% 0.03% 0.07%	back:HV1-1-BaCSc 100% 0% 0%	100% 0% 0%	100% 0% 0%	100% 0% 0%	100% 0% 0%	100% 0% 0%	
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A pop-up window will open

Click Save



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