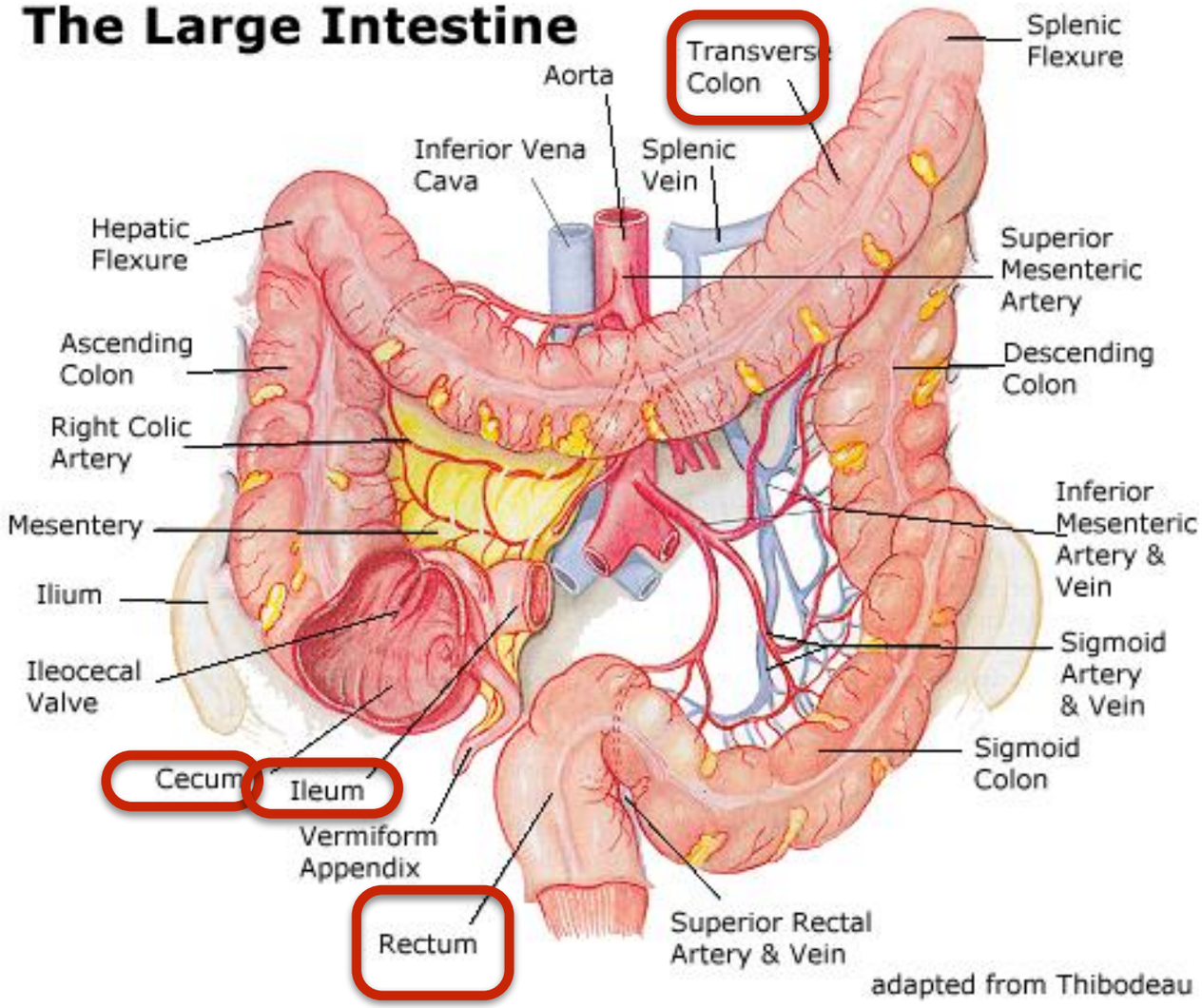
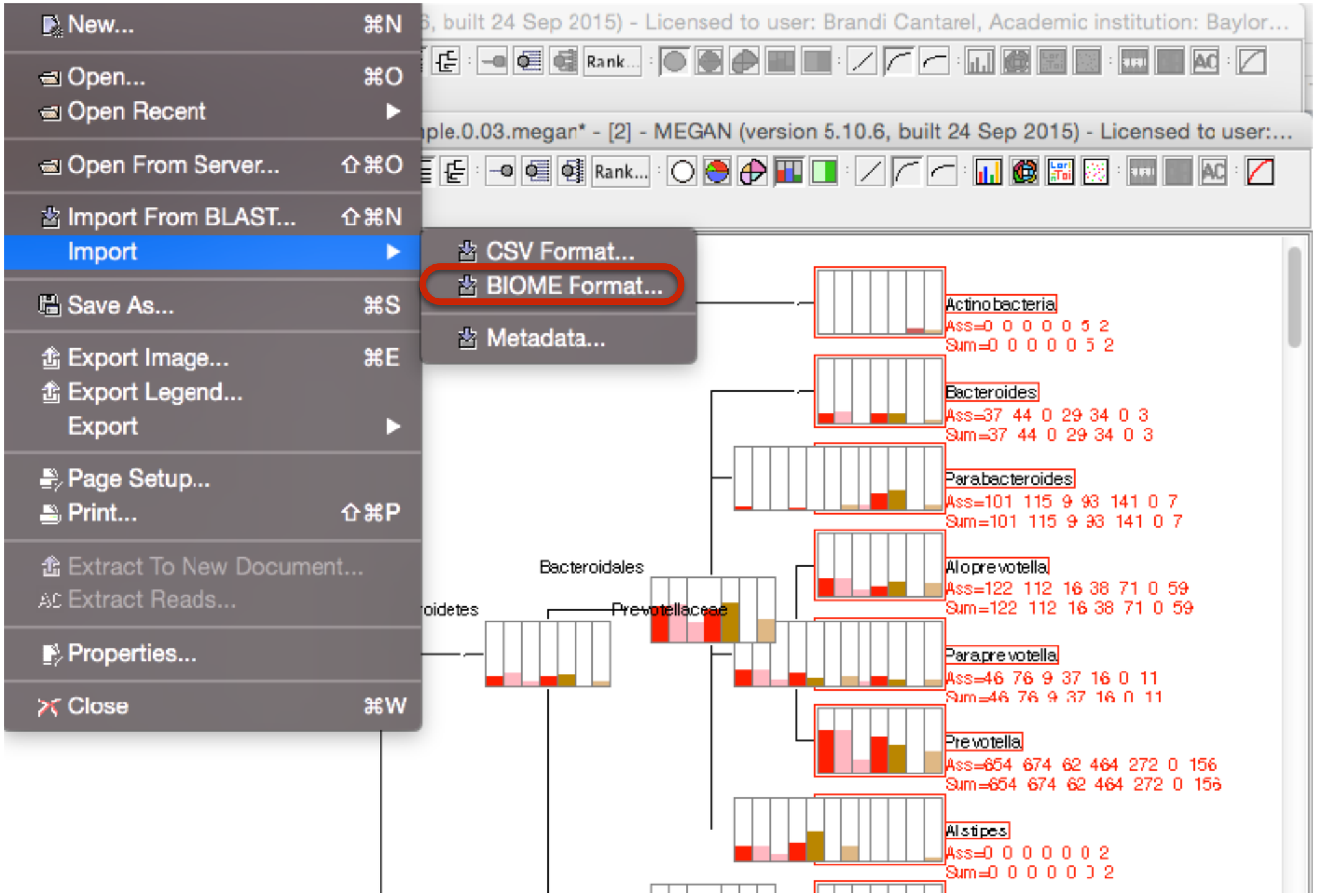
Metagenomic Visualization

Using Visualization Tools Megan and Taxonomer

The Large Intestine



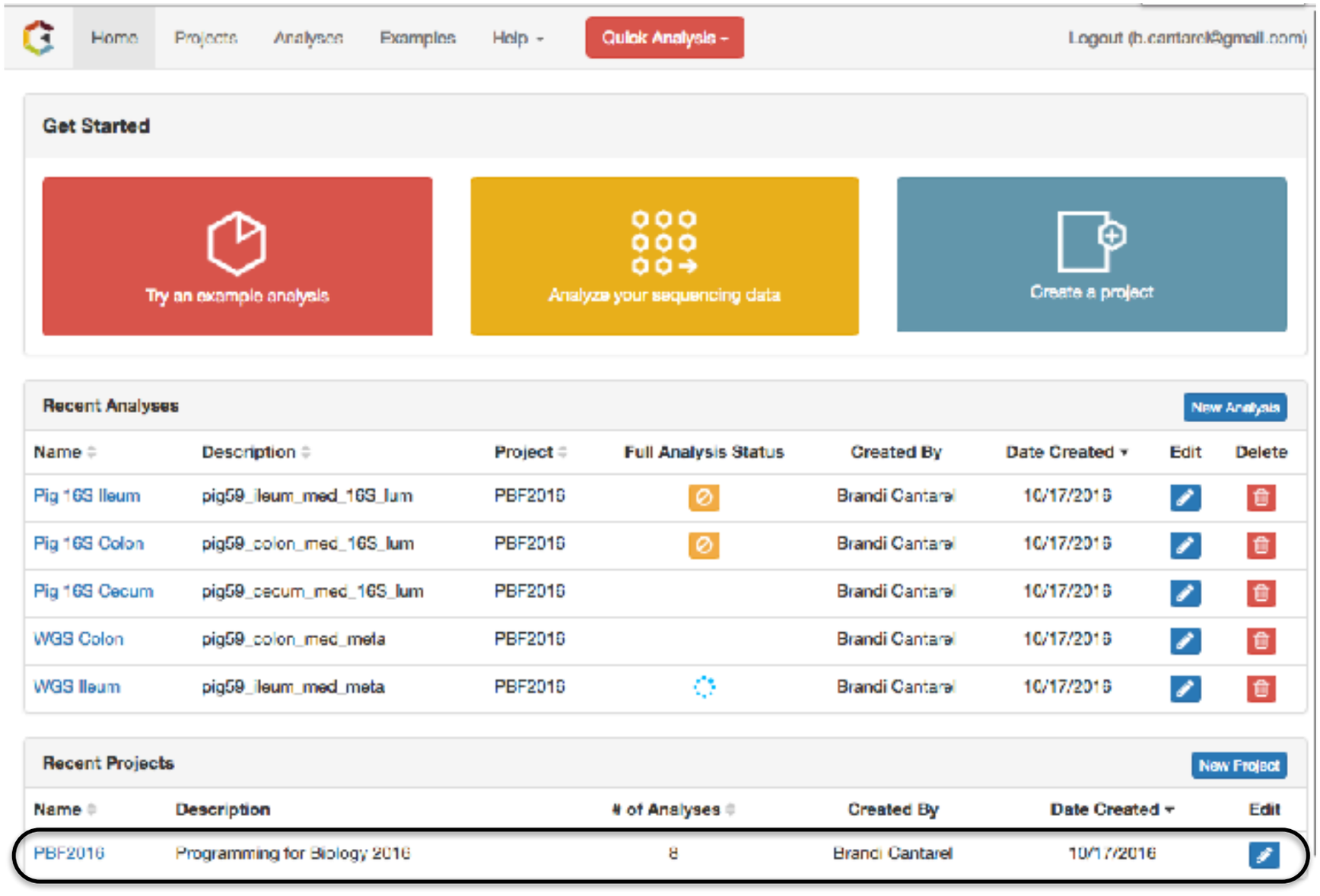
adapted from Thibodeau



http://ab.inf.uni-tuebingen.de/data/software/megan6/download/manual.pdf

Taxonomer

- Go to web site: https://www.taxonomer.com/
- Login as me: b.cantarel@gmail.com
- Password pfb2016
- Select the Project PFB2016
- Select a 16S Sample For Comparison with Mother Results

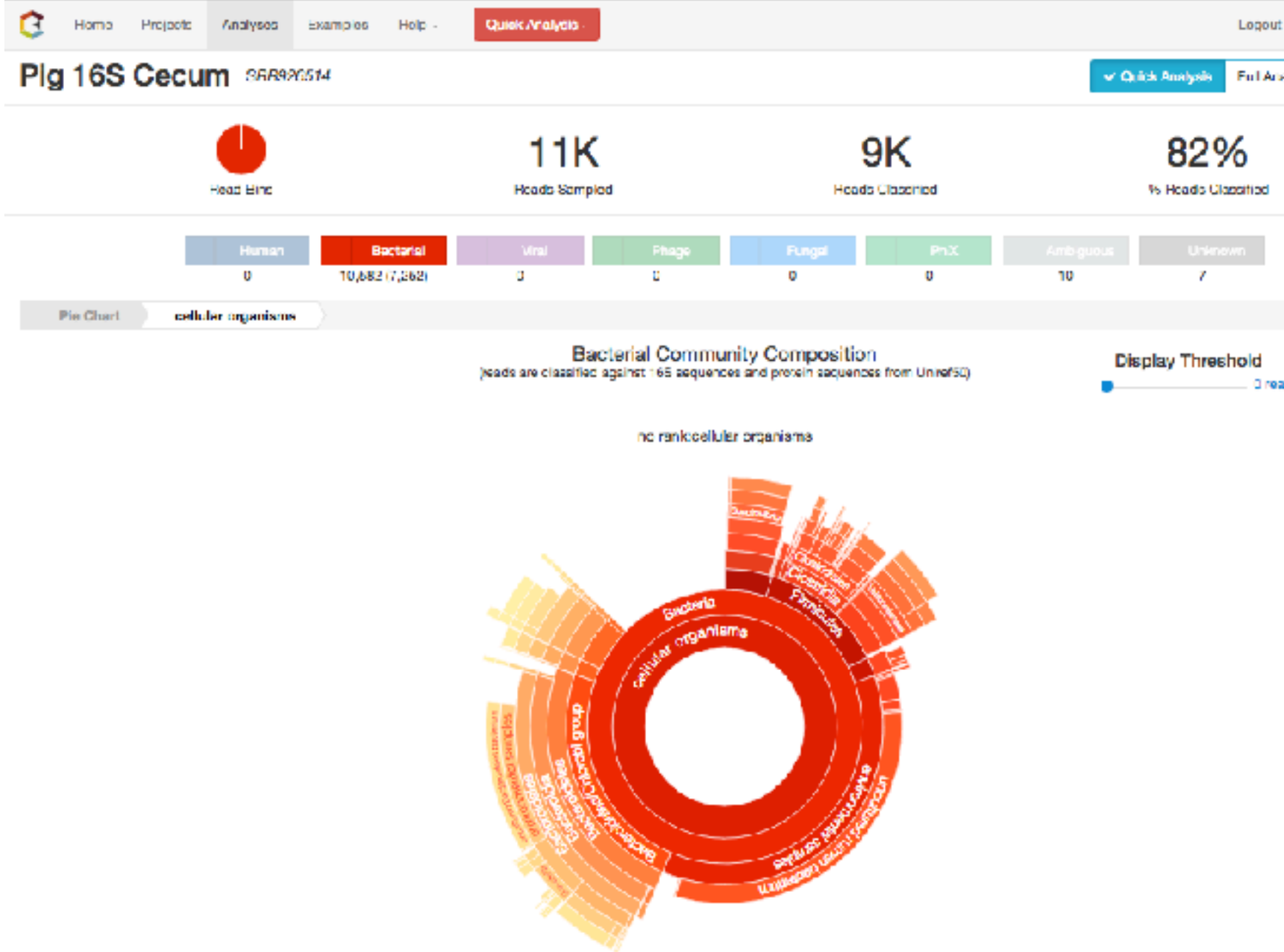


Also on iobio http://taxonomer.iobio.io/

Taxonomer

- You will see something similar to the page on the right

- You can hover over each classification to get the number of reads assigned or click to see the subset of the data

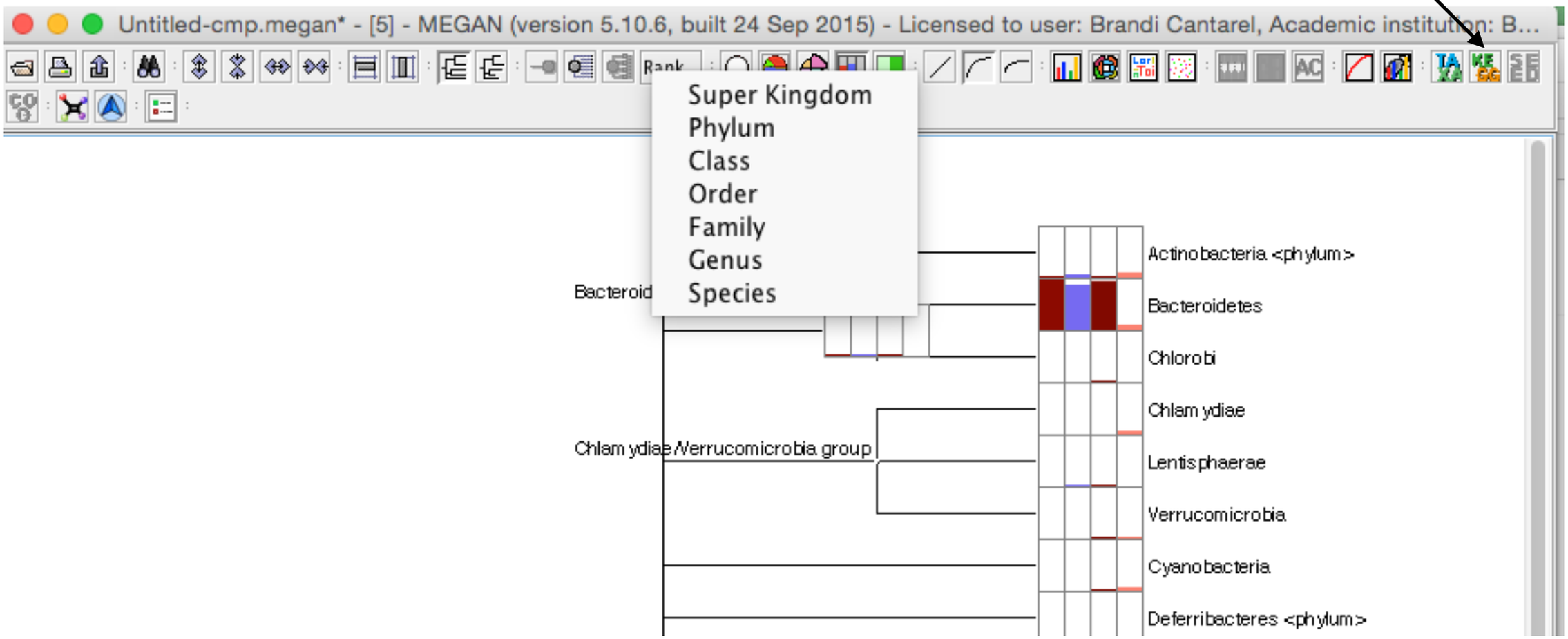


16S Taxonomic Comparison

- What are the dominate phyla, family and genera for each sample?
- Are sites that are closer (spatially) also closer taxonomically (use cluster analysis)?
- What fraction of each samples is “unclassifed" taxonomically?
- Are the taxonomer results similar to the mothur results for your chosen sample?

Some Tool Options In Megan

Gene Annotation

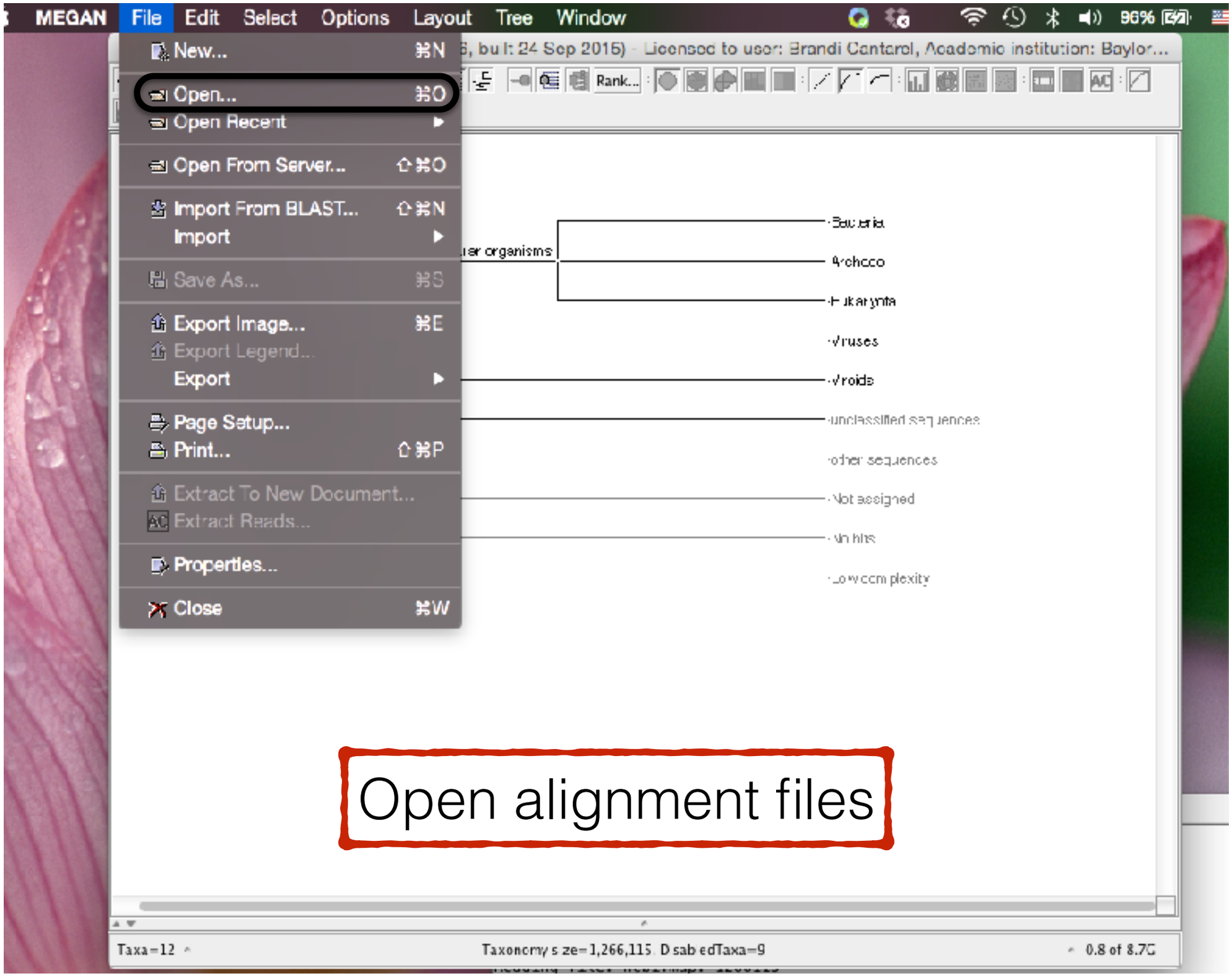


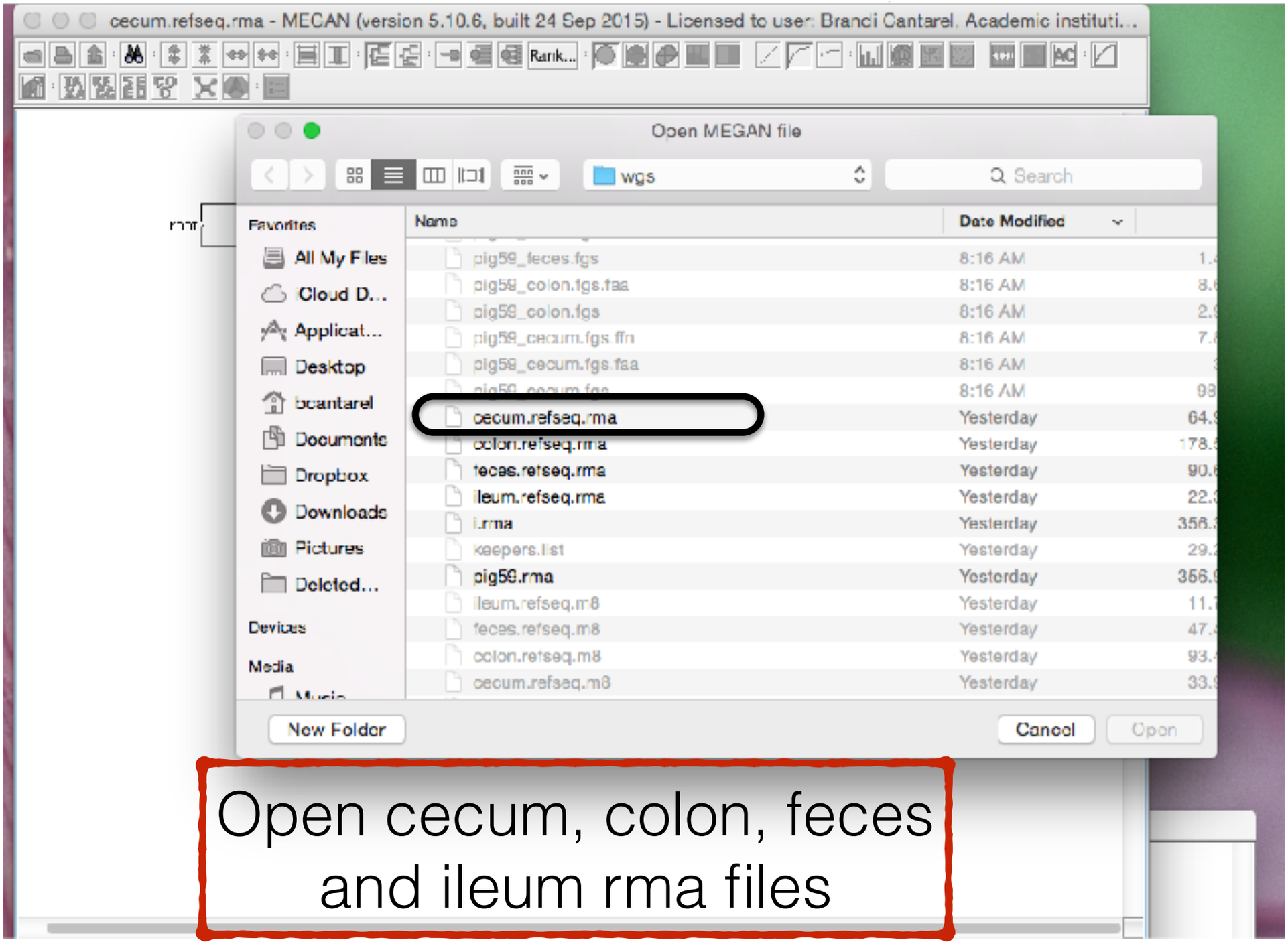
Organism Annotation

Cluster Analysis

Visualization Options

Rarefaction


MEGAN
File
Edit
Select
Options
Layout
Tree
Window
96%
New... ⌘N
Open... ⌘O
Open Recent
Open From Server... ⇧⌘O
Import From BLAST... ⇧⌘N
Import
Save As... ⌘S
Export Image... ⌘E
Export Legend...
Export
Page Setup...
Print... ⇧⌘P
Extract To New Document...
Extract Reads...
Properties...
Close ⌘W
Rank...
Bacteria
Archaea
Eukaryota
Viruses
Viroids
unclassified sequences
other sequences
Not assigned
No hits
Low complexity
Taxa=12
0.8 of 8.7
Open alignment files


cecum.refseq.rma - MEGAN (version 5.10.6, built 24 Sep 2015) - Licensed to user: Branci Cantarel, Academic instituti...
Rank...
Open MEGAN file
wgs
Search
Favorites
All My Files
Cloud D...
Applicat...
Desktop
bcantarel
Documents
Dropbox
Downloads
Pictures
Deleted...
Devices
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Name
Date Modified
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pig59_colon.fgs.faa
8:16 AM
pig59_colon.fgs
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8:16 AM
pig59_cecum.fgs.faa
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Yesterday
colon.refseq.rma
Yesterday
feces.refseq.rma
Yesterday
ileum.refseq.rma
Yesterday
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Yesterday
keepers.list
Yesterday
pig59.rma
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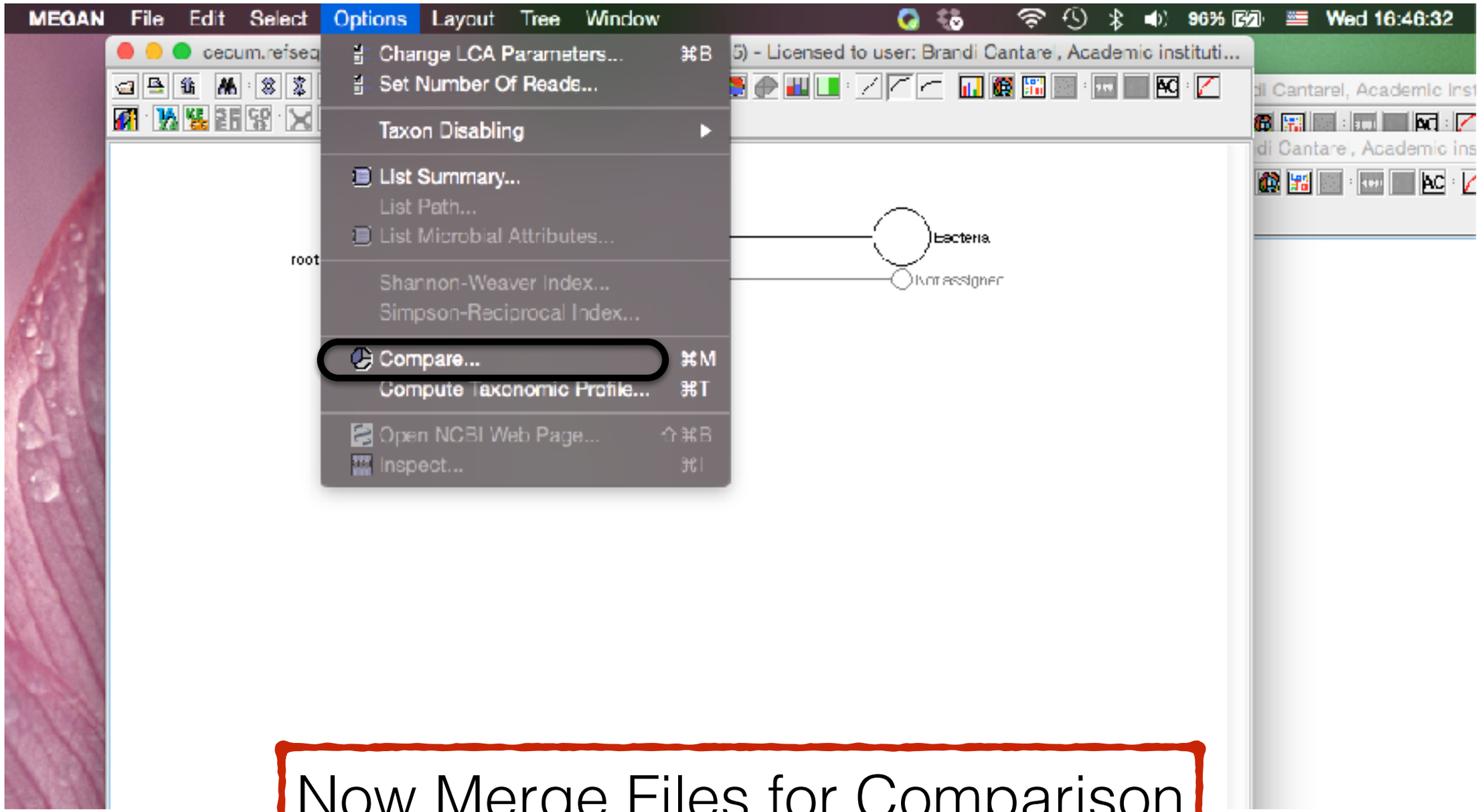
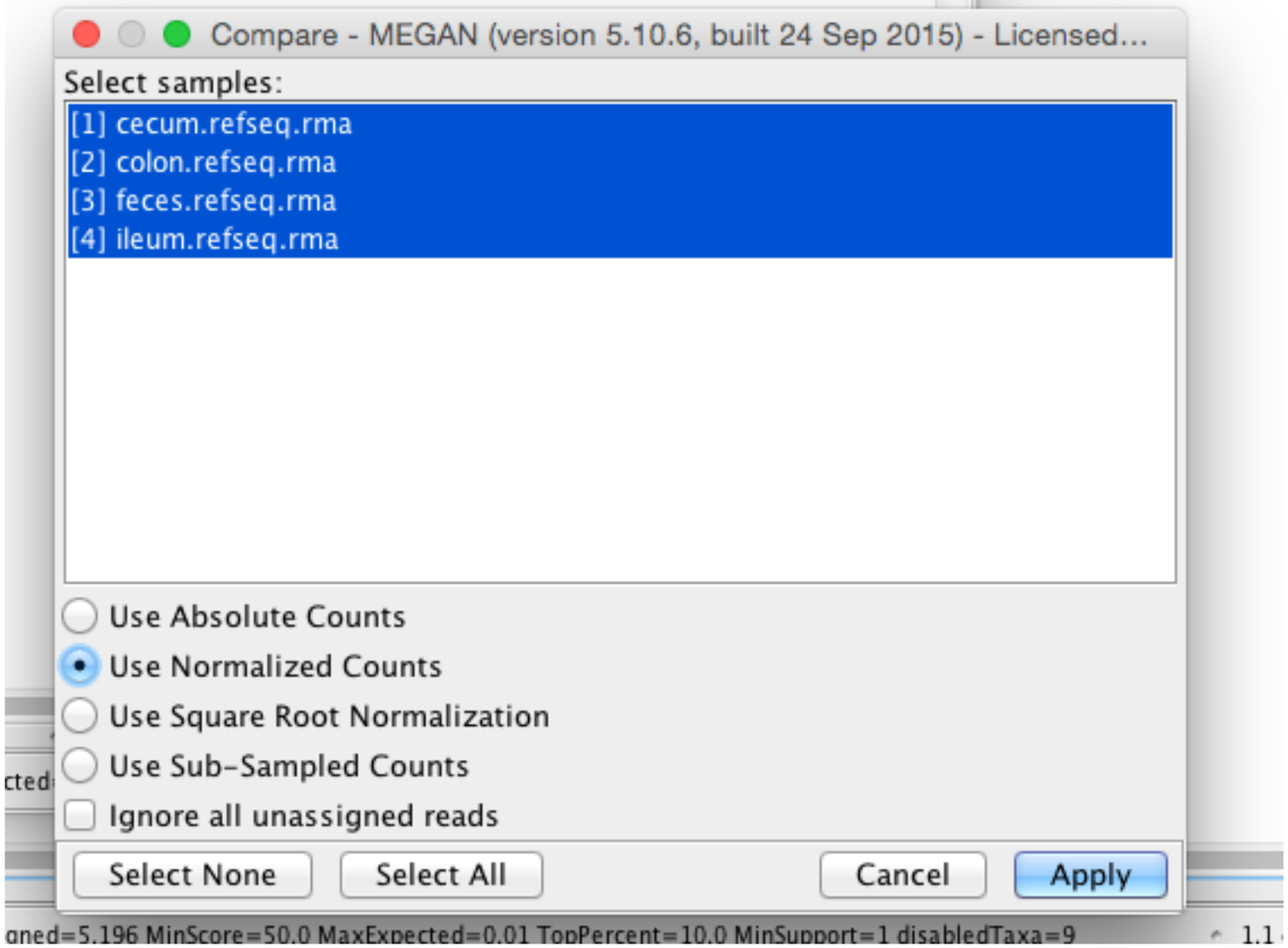
ileum.refseq.m8
Yesterday
feces.refseq.m8
Yesterday
colon.refseq.m8
Yesterday
cecum.refseq.m8
Yesterday
New Folder
Cancel
Open
Open cecum, colon, feces and ileum rma files



Now Merge Files for Comparison



Select all files and select normalized counts

Taxonomer

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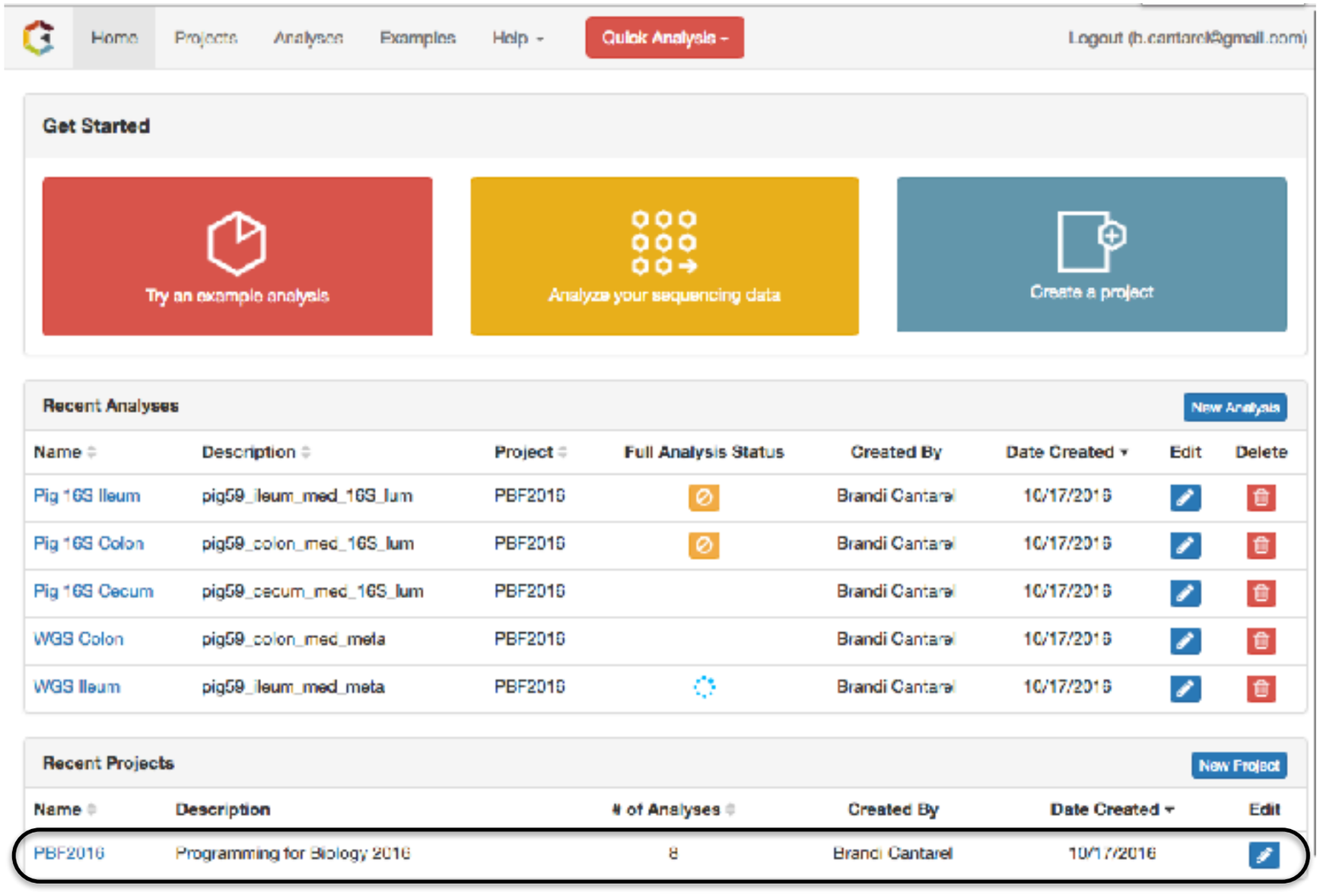

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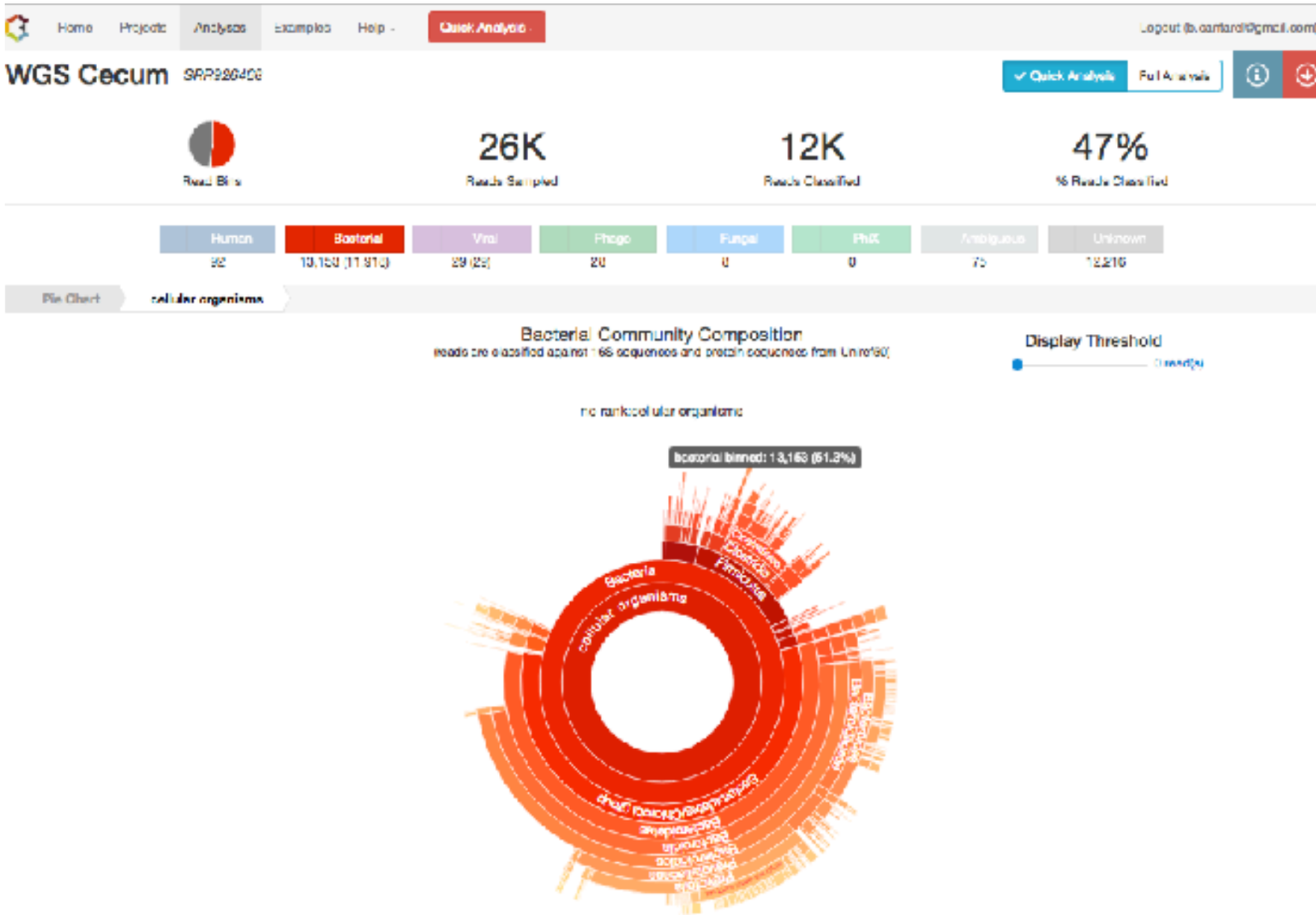
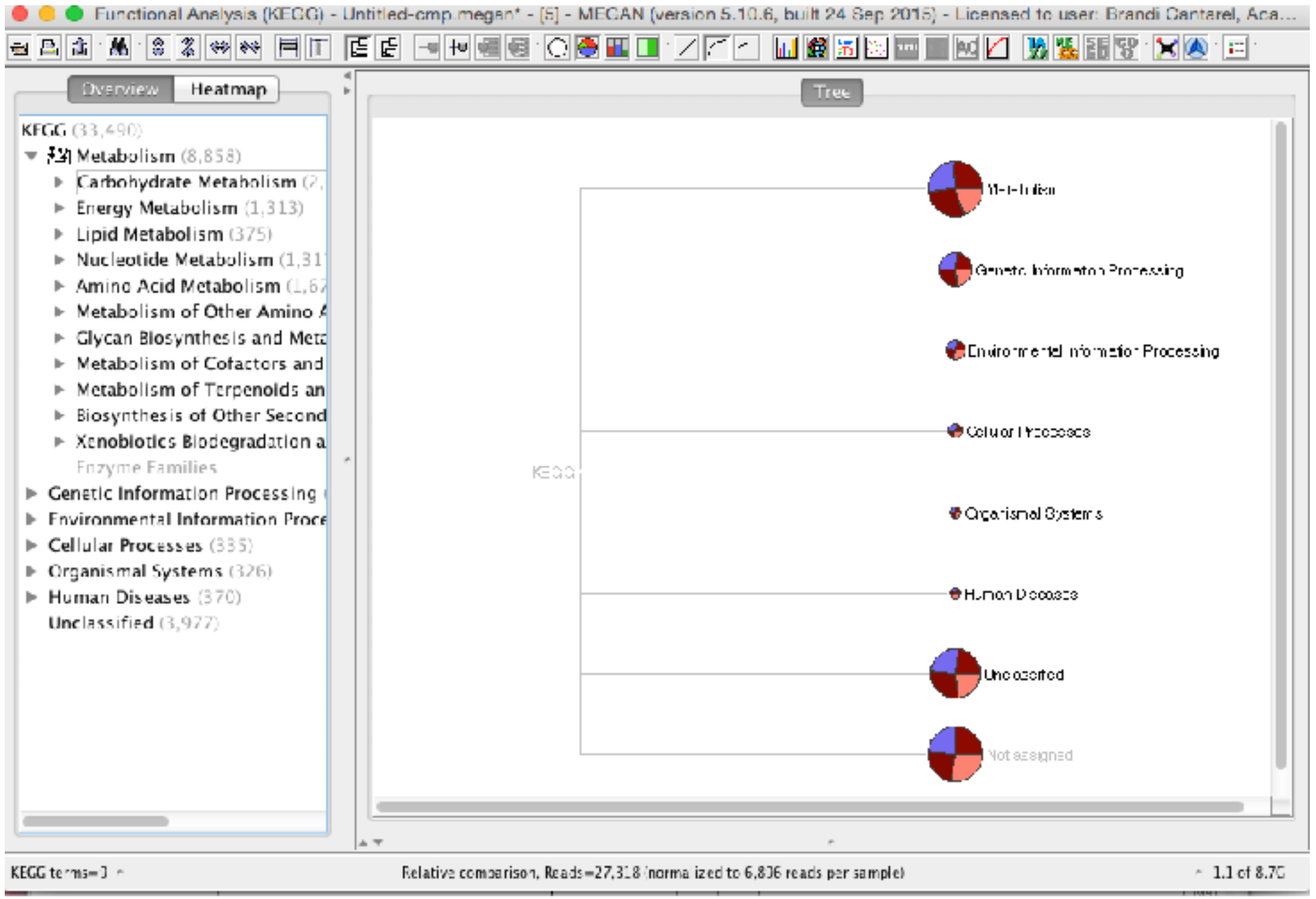


WGS Taxonomic Comparison

- What are the dominate phyla, family and genera for each sample?
- Are these dominate taxa consistent with 16S results?
- Are sites that are closer (spatially) also closer taxonomically (use cluster analysis)?
- What fraction of each samples is “unclassifed" taxonomically?
- Are the taxonomer results similar to the Megan and Mothur results for your chosen sample and bodysite?

Functional Exploration



Functional Exploration

- Pick a pathway from Carbohydrate Metabolism or Glycan Biosynthesis and Metabolism.
- How does the abundances of the genes in your pathway compare between the 4 body sites?
- Now pick a pathway from Genetic Information Processing
- Are the abundances more or less similar between body sites in your chosen sugar metabolism pathway compared to Genetic Information Processing?
- Run KEGG Cluster analysis, how does functional clustering compare to clustering by Taxa?
- What fraction of each samples is “unclassifed" functionally?

Explore the Mothur 16S Analysis:

- Plot the PCoA (R or excel)
    - *.axes* is the PCoA analysis, samples can be plotted by the axis1 vs axis2
    - How does the unifrac plots (final.phylip.tre1.weighted.ave.pcoa.axes and final.phylip.1.unweighted.ave.pcoa.axes) compare to the distance plot (final.an.unique_list.thetayc.0.03.lt.pcoa.axes), are the same samples grouped together?
    - More about Unifrac http://aem.asm.org/content/71/12/8228.full
- Open the SVG files in your web browser
    - How do the different distance methods differ in their heatmap?
- Rarefaction is a method for assess species richness, plot the rarefaction (R or excel), using the file final.an.unique_list.groups.rarefaction
    - The first column represents the number of sequences samples and each column represents the number of OTUs observed when randomly selecting that many reads
    - How does this compare to the Megan results, how many samples are needed to reach OTU saturation?