# Comparative metagenomic analysis using VIROME

### Jaysheel Bhavsar, Shawn Polson, K. Eric Wommack

#### **Abstract**

This tutorial demonstrates the use of VIROME for comparing viral metagenome libraries analyzed with the VIROME pipeline as well as comparative analysis of viral communities using the Quantitative Insights in Microbial Ecology package (QIIME).

Wommack, K. E., J. Bhavsar, S. W. Polson, J. Chen, M. Dumas, S. Srinivasiah, M. Furman, S. Jamindar, and D. J. Nasko. 2012. VIROME: a standard operating procedure for analysis of viral metagenome sequences. Standards in Genomic Sciences 6:427-439 [PMC3558967]

See site for more information.

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protocols.io

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#### **Guidelines**

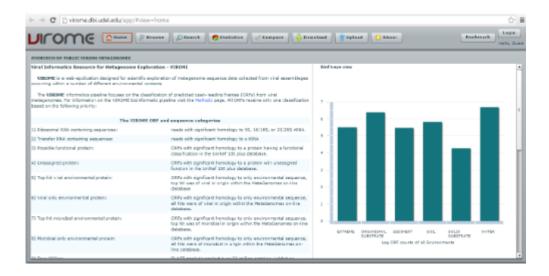
See full video below for more information.

Youtube link: <a href="https://www.youtube.com/watch?v=mL0gRR1uYUM">https://www.youtube.com/watch?v=mL0gRR1uYUM</a>

### **Protocol**

#### Step 1.

Go to VIROME web application.



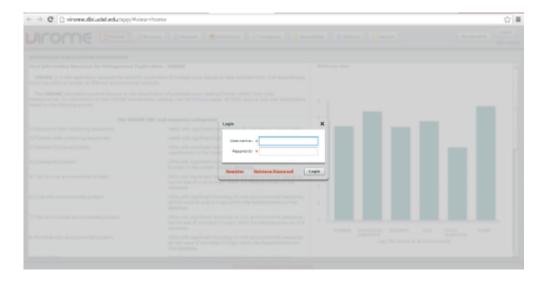
# Step 2.

Click the login button.



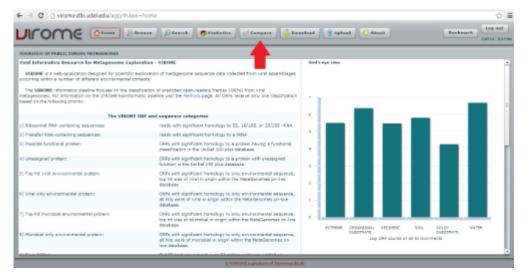
#### Step 3.

Enter username and password to authenticate into the application.



### Step 4.

Click the compare button.



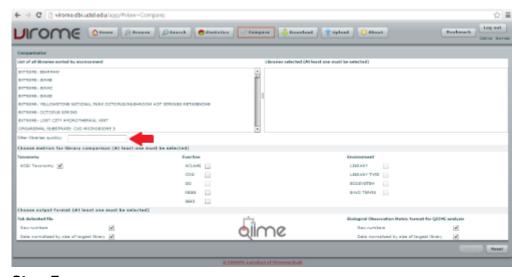
#### Step 5.

The VIROME Comparinator will appear with a list of both private and public libraries.



#### Step 6.

Using the dialogue box below the list you can filter the libraries based on a search term.



#### Step 7.

Double click on library names to choose them for analysis.

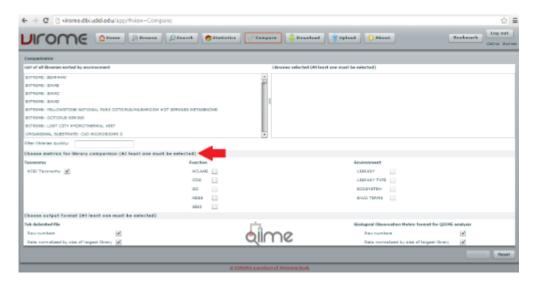
#### **P** NOTES

#### Bonnie Hurwitz 16 Nov 2015

They will appear in the window panel to the right of the list.

### Step 8.

Choose desired metric(s) for library comparison.



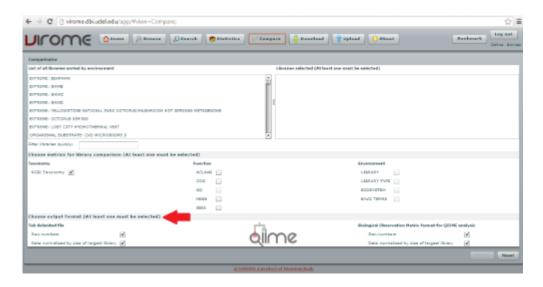
#### NOTES

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Taxonomy, function, and environment options can be selected.

#### Step 9.

Choose the output format(s).



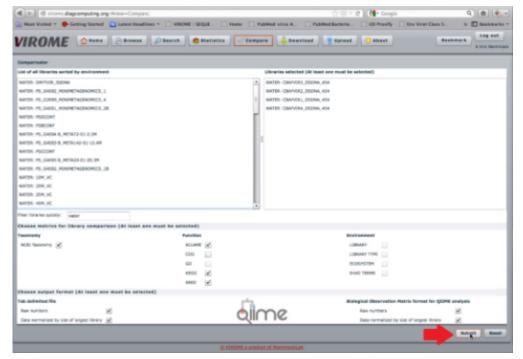
#### NOTES

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Tab delimited file and Biological Obervation Matrix format for QIME analysis options can be selected.

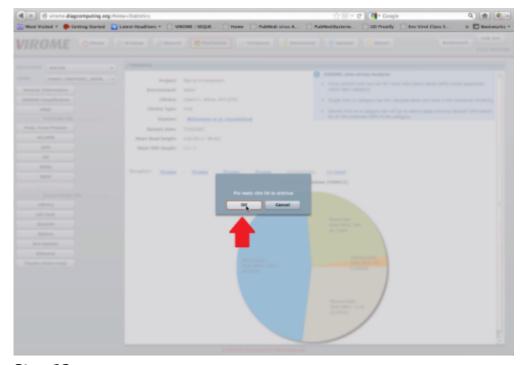
#### Step 10.

Click "Submit".



Step 11.

Click "OK" to download data.



**Step 12.** 

Once prompted to save the file, do not change the file name!

#### **Step 13.**

Navigate to the Finder.

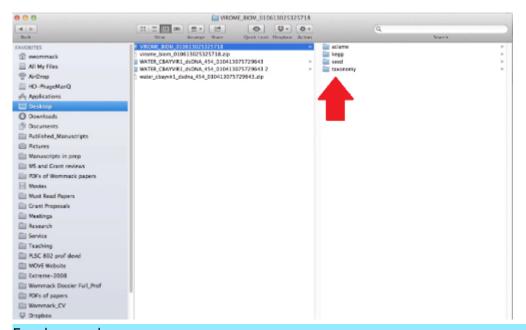


### **Step 14.**

Double click to unzip the file.

### Step 15.

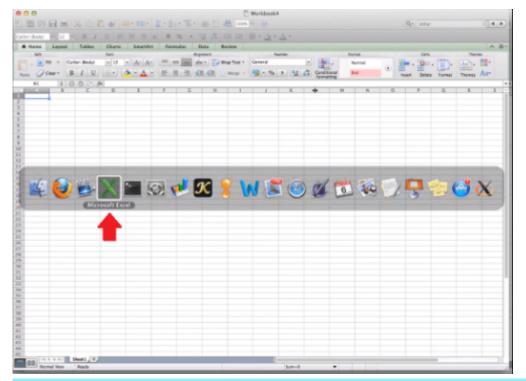
One file for each metric selected should appear.



### Excel example

#### **Step 16.**

Tab delimited files can be opened in Excel spreadsheet.



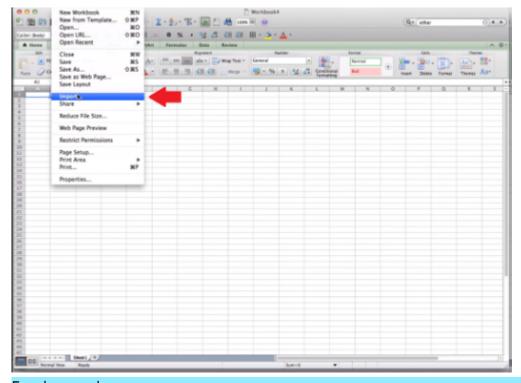
#### **Step 17.**

Navigate to the folder with the file.

# Excel example

### **Step 18.**

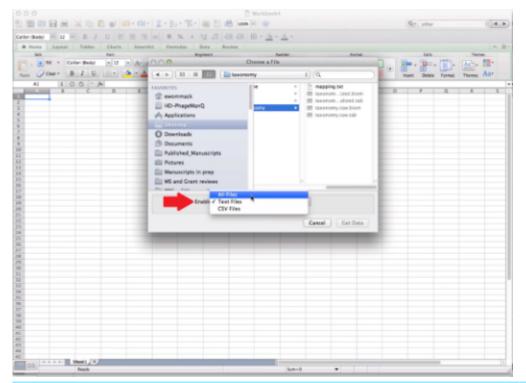
Import as text file.



### Excel example

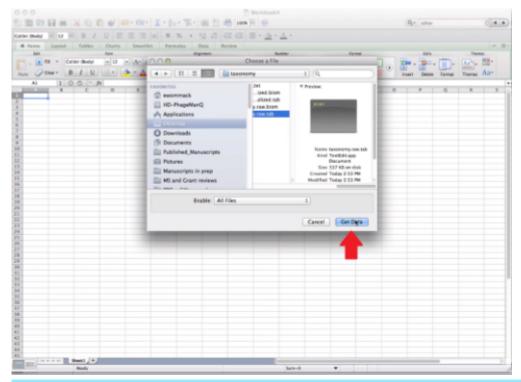
### Step 19.

Enable "All file" types.



# Step 20.

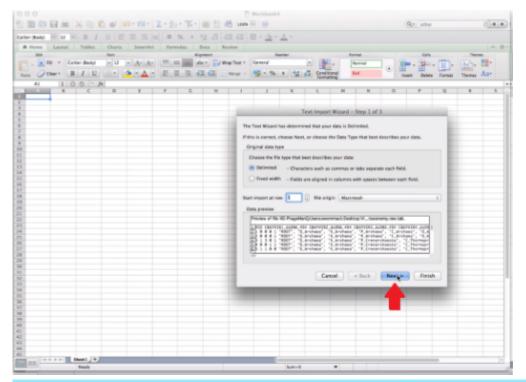
Click "Get Data".



### Excel example

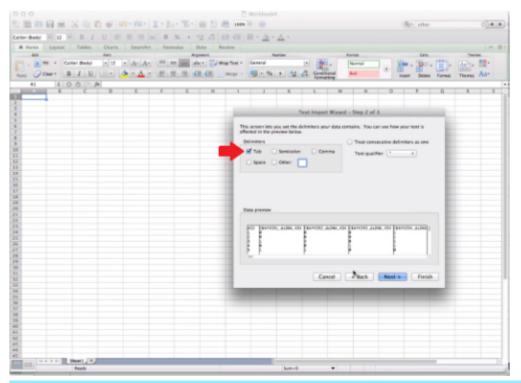
### Step 21.

Click "Next".



#### Step 22.

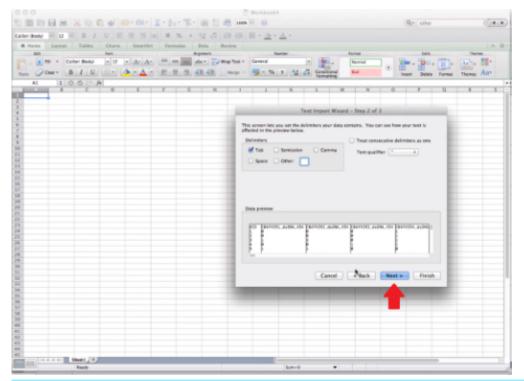
Select "Tab" option.



### Excel example

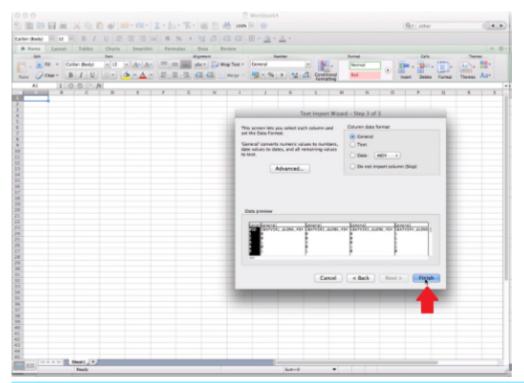
### Step 23.

Click "Next".



# Step 24.

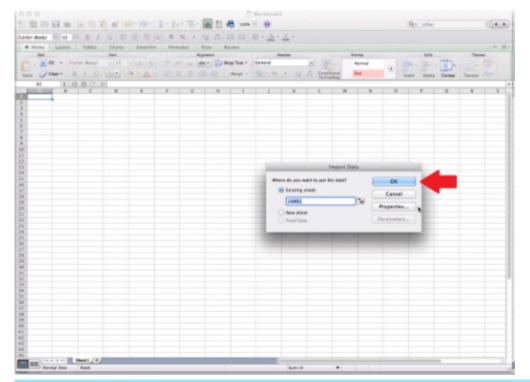
Click "Finish".



# Excel example

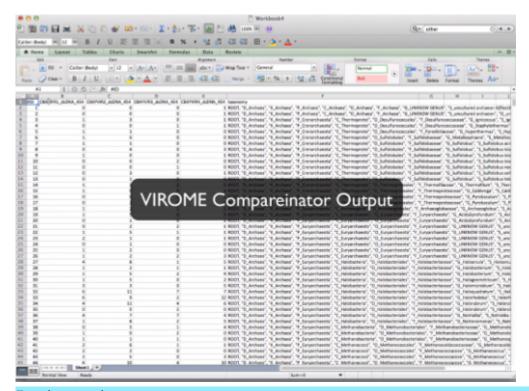
# Step 25.

Click "OK".



#### Step 26.

VIROME Comparinator output will appear.



### Excel example

### **Step 27.**

For more detailed description see video.

@ LINK:

https://www.youtube.com/watch?v=mL0gRR1uYUM&feature=youtu.be&t=2m48s

### QIIME example

# Step 28.

# Open QIIME terminal window.

### NOTES

**Bonnie Hurwitz** 19 Nov 2015 MacQIIME used for this example.

# QIIME example

# Step 29.

Run summarize taxa through plots script.

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```
| Martin | M
```

 $_{\text{cmd}}$  COMMAND

summarize\_taxa\_through\_plots.py

# QIIME example

# Step 30.

Choose input file. (Example command below)

```
| Marcillet | Impre-man-quistumes | Feb | Marcillet | Life D-20122120 | SSIS. 6-Copy-Surger-454-Vacious | Marcillet | Impre-man-quistumes | Marcillet | Life D-20122220 | SSIS. 6-Copy-Surger-454-Vacious | Marcillet | Marcil
```

cmd COMMAND

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-i taxonomy.normalized.biom

# QIIME example

# **Step 31.**

Choose output file. (Example command below)

```
| Marcille | Decision | Section | Section | Decision |
```

cmd COMMAND

-o CB454tax\_summ

# QIIME example

### **Step 32.**

Designate the mapping file.

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cmd COMMAND

-m mapping.txt

# QIIME example

# Step 33.

Run full script. (Example command below)

```
Applications/sequiples 5 of ...

Applications/sequiples 6 of ...
```

cmd COMMAND

### QIIME example

# Step 34.

QIIME will generate stacked bar plots based on the taxonomic data created in VIROME.

### QIIME example

#### Step 35.

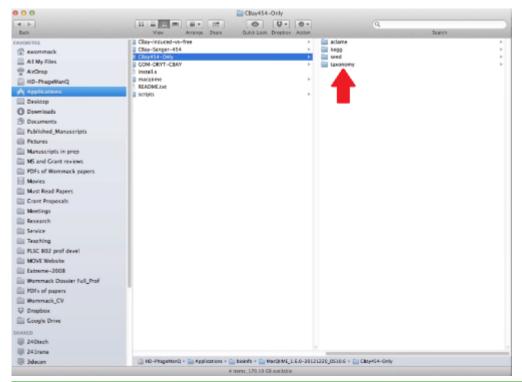
Navigate to the Finder.



### QIIME example

### **Step 36.**

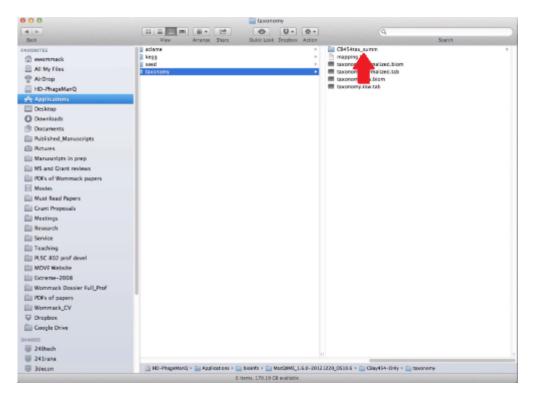
Look in taxonomy folder.



#### QIIME example

#### **Step 37.**

Open taxonomic summary folder.



#### NOTES

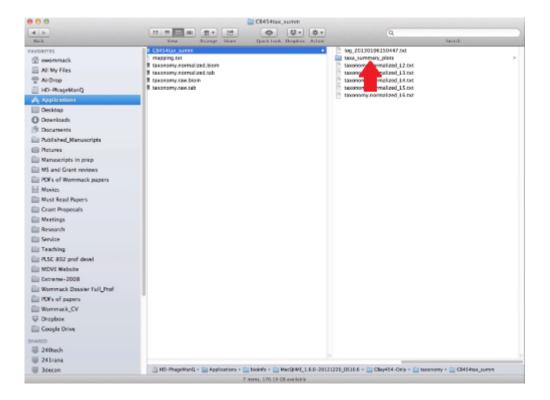
### Bonnie Hurwitz 19 Nov 2015

The name should match the output file designated in QIIME.

#### QIIME example

#### **Step 38.**

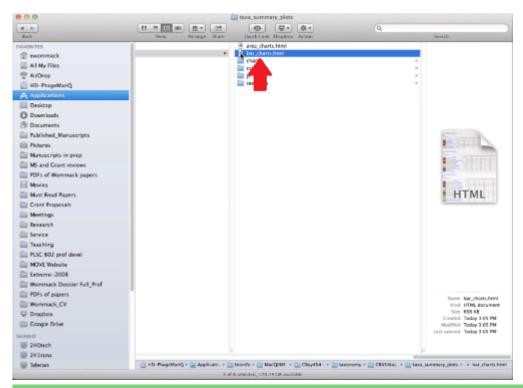
Open taxonomy summary plots folder.



### QIIME example

# Step 39.

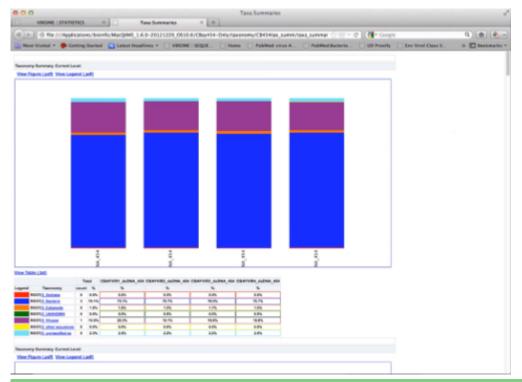
Open the bar charts with a web browser.



### QIIME example

### Step 40.

Output of libraries that were compared can be seen according to different taxonomic levels that occurred in the biom file.



#### QIIME example

#### **Step 41.**

In the VIROME Comparinator, click on the QIIME logo for a direct link to the QIIME homepage with information on the package. (Alternatively use link provided in this step)



**&** LINK:

http://qiime.org/

### **₽** NOTES

#### **Bonnie Hurwitz** 17 Nov 2015

QIIME can produce many outputs including rarefaction curves to compare alpha diversity pipeline.

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Can also run beta diversity pipeline to compare libraries in principal component analysis in the King viewer.

#### QIIME example

### Step 42.

For more detailed example see video.

#### Step 43.

For full video tutorial see guidelines.

### **ELINK:**

https://www.youtube.com/watch?v=mL0qRR1uYUM

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