Mothur Amplicon Analysis

James Thornton

Abstract

Citation: James Thornton Mothur Amplicon Analysis. **protocols.io**
dx.doi.org/10.17504/protocols.io.feibjce
Published: 25 Jul 2016

Protocol

Step 1.

Move to directory containing Mothur:

cmd COMMAND
```
$ cd /home/c-debi/ecogeo/mothurdir
$ mothur
```

Step 2.

Open a second terminal window.

Step 3.

To perform rarefaction:

cmd COMMAND
```
mothur > rarefaction.single(shared=stability.an.shared, calc=sobs, freq=100)
```

EXPECTED RESULTS

```
numsampled 0.03-F3D0  lci-F3D0   hci-F3D0   0.03-F3D1  lci-F3D1   hci-F3D1   0.03-F3D141     lci-F3D141
     hci-F3D141 0.03-F3D142     lci-F3D142 hci-F3D142 0.03-F3D143     lci-F3D143 hci-F3D143 0.03-F3D144
     lci-F3D144 hci-F3D144 0.03-F3D145     lci-F3D145 hci-F3D145 0.03-F3D146     lci-F3D146 hci-F3D146
     0.03-F3D147     lci-F3D147 hci-F3D147 0.03-F3D148     lci-F3D148 hci-F3D148 0.03-F3D149     lci-F3D149
     hci-F3D149 0.03-F3D150     lci-F3D150 hci-F3D150 0.03-F3D2  lci-F3D2   hci-F3D2   0.03-F3D3  lci-F3D3
     hci-F3D3   0.03-F3Dlci-F3D5hci-F3D5   0.03-F3D6  lci-F3D6   hci-F3D6   0.03-F3D7  lci-F3D7   hci-F3D7
     0.03-F3D8  lci-F3D8   hci-F3D8   0.03-F3D9  lci-F3D9   hci-F3D9
1    1.0000     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000
     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000
     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000
     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000
     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000
     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000     1.0000
     1.0000     1.0000     1.0000
```

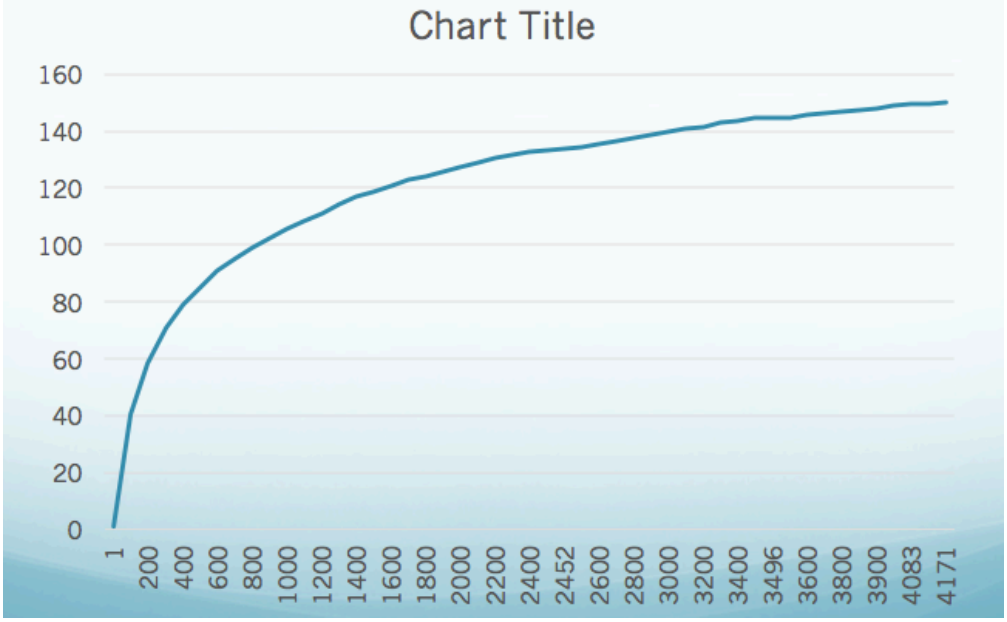
Step 4.

We can make a rarefaction curve in Powerpoint

cmd COMMAND

```
$ cut -f1,2 stability.an.groups.rarefaction > sample1.rarefaction
```

EXPECTED RESULTS



Step 5.

Alpha diversity measures:

Sample	No. seqs used	Observed Species	Inv. Simpson	Confidence interval - L	Confidence interval - H
F3D0	2441	132.734	25.716	24.093	27.574
F3D1	2441	127.36	34.539	32.539	37.032

cmd COMMAND

```
$ mothur > summary.single(shared=stability.an.shared, calc=nseqs-coverage-sobs-
invsimpson, subsample=2441)

$ less stability.an.groups.ave-std.summary
```

Step 6.

What about bar graphs with relative abundance and taxonomy?

cmd COMMAND

```
$ less less stability.an.cons.taxonomy
$ less stability.an.0.03.subsample.shared
```

EXPECTED RESULTS

```
OTU  Size Taxonomy
Otu0001   12328

Bacteria(100);"Bacteroidetes"(100);"Bacteroidia"(100);"Bacteroidales"(100);"Porphyromonadaceae"(1
00);"Porphyromonadaceae"_unclassified(100);"Porphyromonadaceae"_unclassified(100);"Porphyromonada
ceae"_unclassified_unclassified(100);
```

```
label Group numOtus     Otu0001     Otu0002     Otu0003     Otu0004     Otu0005     Otu0006     Otu0007
      0.03  F3D0  296   181   116   132   201   164   132
```

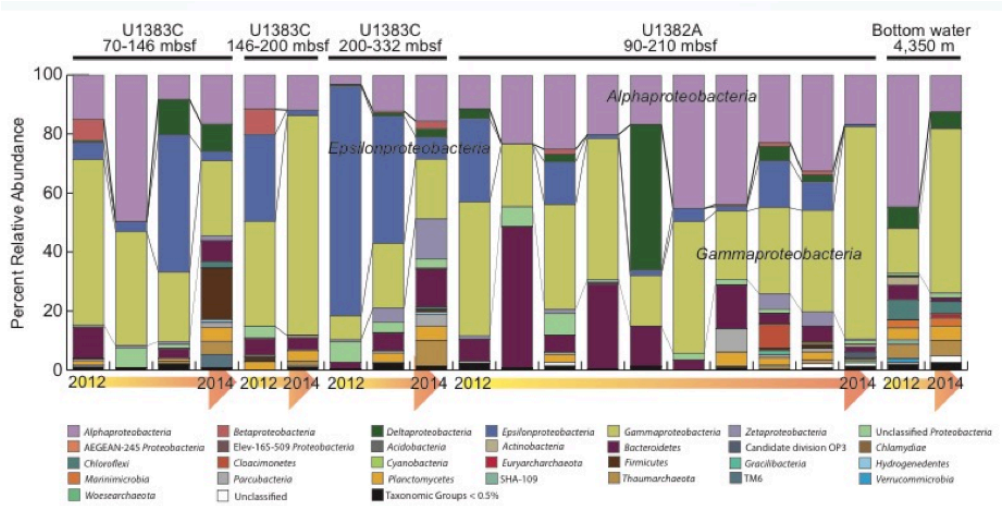
ANNOTATIONS

Elisha Wood-Charlson 03 Aug 2016

0.03.subsample.shared file missing

Step 7.

Unrelated data:



Step 8.

Analysis

- Beta diversity - measured differences between communities
- How related are communities to each other?
- Theta YC (community structure) – considers shared OTUs and relative abundance of OTUs
- Jaccard (community membership) – compared which OTUs are present in samples

Step 9.

Beta diversity measures:

cmd COMMAND

```
$ mothur > dist.shared(shared=stability.an.shared, calc=thetayc-jclass, subsample=2241)
```

Step 10.

Turn distance measure output in to a dendrogram:

cmd COMMAND

```
$ mothur > tree.shared(phylip=stability.an.thetayc.0.03.lt.ave.dist)
$ mothur > quit()

$ Dendroscope
$ less stability.an.thetayc.0.03.lt.ave.tre
```

Step 11.

Statistical significance

- Involves classifying your samples in to categories → good experimental design would have classify these samples before seeing results
- E.g. surface vs deep samples or winter vs. summer
- Generate points to plot Principal Component Analysis or Non-metric Dimensional Scaling plots
- Perform AMOVA statistical tests