

ECOGEO 'Omics Training: 3.1 Amplicon Analysis - Mothur

Version 2

Ben Tully

Abstract

mothur is a bioinformatics tool for analyzing 16S rRNA gene sequences, which can be used to process data generated by Sanger, PacBio, IonTorrent, 454, and Illumina (MiSeq/HiSeq).

Citation: Ben Tully ECOGEO 'Omics Training: 3.1 Amplicon Analysis - Mothur. **protocols.io**

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Before start

Before starting, please visit the ECOGEO website for more information on this "Introduction to Environmental 'Omics" training series. The site contains a pre-packaged virtual machine that can be downloaded and used to run all of the protocols in this protocols.io collection. In addition to the VM, the website contains video and presentations from our initial "Intro to Env 'Omics" workshop held at the Univ. of Hawai'i at Manoa on 25-26 Jul 2016.

Please email 'ecogeo-join@earthcube.org' to join the ECOGEO listserv for future updates.

Protocol

Step 1.

Move to directory containing mothur and launch mothur

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

Step 2.

To perform rarefaction, open a second terminal window and navigate to /mothurdir. Less output file:

```
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-F3D3 lci-F3D5 hci-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
```

Step 3.

Creates a file with data for rarefaction curve (nano)

cmd **COMMAND**

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

Step 4.

Alpha diversity measures:

cmd **COMMAND**

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

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

If there is no "\$" at the beginning of the command, perform it in mothur.

Step 5.

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 | numOtu | Otu0001 | Otu0002 | Otu0003 | Otu0004 | Otu0005 | Otu0006 | Otu0007 |
|-------|-------|--------|---------|---------|---------|---------|---------|---------|---------|
| 0.03 | F3D0 | 296 | 181 | 116 | 132 | 201 | 164 | 132 | |

NOTES

Elisha Wood-Charlson 09 Aug 2016

0.03.subsample.shared file was missing. Should be fixed in VM 2.0 release

Step 6.

Beta diversity measures:

cmd **COMMAND**

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

Step 7.

Turn distance measure output in to a dendrogram:

cmd **COMMAND**

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

```
mothur > quit()
```

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

```
$ Dendroscope
```

Copy content of less before launching Dendroscope