```
Mac version

Using ReadLine,Boost,HDF5,GSL
mothur v.1.47.0
Last updated: 1/21/22
by
Patrick D. Schloss

Department of Microbiology & Immunology

University of Michigan
http://www.mothur.org

When using, please cite:
Schloss, P.D., et al., Introducing mothur: Open-source, platform-
independent, community-supported software for describing and comparing
microbial communities. Appl Environ Microbiol, 2009. 75(23):7537-41.

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Type 'help()' for information on the commands that are available

For questions and analysis support, please visit our forum at https://
forum.mothur.org

Type 'quit()' to exit program

[NOTE]: Setting random seed to 19760620.

Interactive Mode



mothur > Unable to open stability.files. Trying MOTHUR_FILES
directory /Users/natalieburkhard/bio-490/independentStudy/Week9/
mothur/stability.files.
/
**********************************************************************
*******/
Unable to open Unable to open stability.files. Trying MOTHUR_FILES
directory /Users/natalieburkhard/bio-490/independentStudy/Week9/
mothur/stability.files.. Trying MOTHUR_FILES directory mothur/
stability.files..
Unable to open mothur/stability.files.. Trying mothur's executable
directory stability.files..
Unable to open stability.files..
[ERROR]: unable to open stability.files. batch file, please correct.
/
**********************************************************************
*******/
```

```
mothur > Unable to open /Users/natalieburkhard/bio-490/
independentStudy/Week9/mothur/stability.files. Trying mothur's
executable directory /Users/natalieburkhard/bio-490/independentStudy/
Week9/mothur/stability.files.
/
**************************************************************************
*******/
Unable to open Unable to open /Users/natalieburkhard/bio-490/
independentStudy/Week9/mothur/stability.files. Trying mothurs
executable directory /Users/natalieburkhard/bio-490/independentStudy/
Week9/mothur/stability.files.. Trying MOTHUR_FILES directory mothur/
stability.files..
Unable to open mothur/stability.files.. Trying mothur's executable
directory stability.files..
Unable to open stability.files..
[ERROR]: unable to open stability.files. batch file, please correct.
/
**************************************************************************
*******/

mothur > Unable to open /Users/natalieburkhard/bio-490/
independentStudy/Week9/mothur/stability.files.
/
**************************************************************************
*******/
Unable to open Unable to open /Users/natalieburkhard/bio-490/
independentStudy/Week9/mothur/stability.files.. Trying MOTHUR_FILES
directory mothur/stability.files..
Unable to open mothur/stability.files.. Trying mothur's executable
directory stability.files..
Unable to open stability.files..
[ERROR]: unable to open stability.files. batch file, please correct.
/
**************************************************************************
*******/

mothur > Unable to open stability.files
/
**************************************************************************
*******/
Unable to open Unable to open stability.files. Trying MOTHUR_FILES
directory mothur/Unable to open stability.files.
Unable to open mothur/Unable to open stability.files. Trying mothur's
executable directory Unable to open stability.files.
Unable to open Unable to open stability.files.
[ERROR]: unable to open Unable to open stability.files batch file,
please correct.
/
**************************************************************************
```

```
*******/

mothur >


It took 0 seconds to run 0 commands from Unable to open
stability.files batch file.



It took 0 seconds to run 0 commands from stability.files. batch file.



It took 0 seconds to run 0 commands from stability.files. batch file.



It took 0 seconds to run 0 commands from stability.files. batch file.

[ERROR]: You are missing (
[ERROR]: Invalid.

mothur > Using 8 processors.
/
**********************************************************************
*******/
Unable to open Using 8 processors.. Trying MOTHUR_FILES directory
mothur/Using 8 processors..
Unable to open mothur/Using 8 processors.. Trying mothur's executable
directory Using 8 processors..
Unable to open Using 8 processors..
[ERROR]: unable to open Using 8 processors. batch file, please
correct.
/
**********************************************************************
*******/

mothur > [ERROR]: did not complete make.contigs.
/
**********************************************************************
*******/
Unable to open [ERROR]: did not complete make.contigs.. Trying
MOTHUR_FILES directory mothur/[ERROR]: did not complete make.contigs..
Unable to open mothur/[ERROR]: did not complete make.contigs.. Trying
mothur's executable directory [ERROR]: did not complete make.contigs..
Unable to open [ERROR]: did not complete make.contigs..
[ERROR]: unable to open [ERROR]: did not complete make.contigs. batch
file, please correct.
/
```

```
**********************************************************************
*******/

mothur >


It took 0 seconds to run 0 commands from [ERROR]: did not complete
make.contigs. batch file.



It took 0 seconds to run 0 commands from Using 8 processors. batch
file.

[ERROR]: You are missing (
[ERROR]: Invalid.

mothur > make.contigs(file=stability.files)

Using 8 processors.

>>>>>   Processing file pair F3D0_S188_L001_R1_001.fastq -
F3D0_S188_L001_R2_001.fastq (files 1 of 1)<<<<<
Making contigs...
Done.

It took 1 secs to assemble 7793 reads.


Group count:
F3D0    7793

Total of all groups is 7793

It took 1 secs to process 7793 sequences.

Output File Names:
stability.trim.contigs.fasta
stability.scrap.contigs.fasta
stability.contigs_report
stability.contigs.count_table


mothur > summary.seqs(fasta=stability.trim.contigs.fasta)

Using 8 processors.

                Start   End     NBases  Ambigs  Polymer NumSeqs
Minimum: 1      249     249     0       3       1
2.5%-tile:      1       252     252     0       4       195
```

```
25%-tile:       1       252     252     0       4       1949
Median:  1      252     252     0       4       3897
75%-tile:       1       253     253     0       5       5845
97.5%-tile:     1       253     253     5       6       7599
Maximum: 1      502     502     241     188     7793
Mean:    1      252     252     0       4
# of Seqs:      7793

It took 0 secs to summarize 7793 sequences.

Output File Names:
stability.trim.contigs.summary


mothur > screen.seqs(fasta=stability.trim.contigs.fasta, maxambig=0,
maxlength=275)

Using 8 processors.

It took 0 secs to screen 7793 sequences, removed 1155.


Output File Names:
stability.trim.contigs.good.fasta
stability.trim.contigs.bad.accnos


It took 0 secs to screen 7793 sequences.

mothur > get.current()

Current RAM usage: 0.131039 Gigabytes. Total Ram: 8 Gigabytes.

Current files saved by mothur:
accnos=stability.trim.contigs.bad.accnos
fasta=stability.trim.contigs.good.fasta
contigsreport=stability.contigs_report
count=stability.contigs.count_table
processors=8
summary=stability.trim.contigs.summary

Current default directories saved by mothur:
        mothur/


Current working directory: /Users/natalieburkhard/bio-490/
independentStudy/Week9/

Output File Names:
current_files.summary
```

```
mothur > unique.seqs(fasta=stability.trim.contigs.good.fasta)
6638    1533

Output File Names:
stability.trim.contigs.good.unique.fasta
stability.trim.contigs.good.count_table


mothur > count.seqs(name=stability.trim.contigs.good.names)
Unable to open stability.trim.contigs.good.names. Trying MOTHUR_FILES
directory mothur/stability.trim.contigs.good.names.
Unable to open mothur/stability.trim.contigs.good.names. Trying
mothur's executable directory stability.trim.contigs.good.names.
Unable to open stability.trim.contigs.good.names.
Unable to open stability.trim.contigs.good.names
You have no current namefile or sharedfile and the name or shared
parameter is required, unless inflating or deflating an existing count
file.
[ERROR]: did not complete count.seqs.

mothur > get_current()
[ERROR]: Invalid command.
[ERROR]: did not complete get_current.

mothur > get.current()

Current RAM usage: 0.131039 Gigabytes. Total Ram: 8 Gigabytes.

Current files saved by mothur:
accnos=stability.trim.contigs.bad.accnos
fasta=stability.trim.contigs.good.unique.fasta
contigsreport=stability.contigs_report
count=stability.trim.contigs.good.count_table
processors=8
summary=stability.trim.contigs.summary

Current default directories saved by mothur:
        mothur/


Current working directory: /Users/natalieburkhard/bio-490/
independentStudy/Week9/

Output File Names:
current_files.summary


mothur > summary.seqs(count=stability.trim.contigs.good.count_table)
```

```
Using stability.trim.contigs.good.unique.fasta as input file for the
fasta parameter.

Using 8 processors.

                Start   End     NBases  Ambigs  Polymer NumSeqs
Minimum: 1      250     250     0       3       1
2.5%-tile:      1       252     252     0       4       166
25%-tile:       1       252     252     0       4       1660
Median:  1      252     252     0       4       3320
75%-tile:       1       253     253     0       5       4979
97.5%-tile:     1       253     253     0       6       6473
Maximum: 1      255     255     0       8       6638
Mean:    1      252     252     0       4
# of unique seqs:       1533
total # of seqs: 6638

It took 0 secs to summarize 6638 sequences.

Output File Names:
stability.trim.contigs.good.unique.summary


mothur > pcr.seqs(fasta=silva.bacteria.fasta, start=11894, end=25319,
keepdots=F)

Using 8 processors.
[NOTE]: no sequences were bad, removing silva.bacteria.bad.accnos

It took 8 secs to screen 14956 sequences.

Output File Names:
silva.bacteria.pcr.fasta



mothur > system(mv silva.bacteria.pcr.fasta silva.v4.fasta)


mothur > summary.seqs(fasta=silva.v4.fasta)

Using 8 processors.

                Start   End     NBases  Ambigs  Polymer NumSeqs
Minimum: 2      13425   270     0       3       1
2.5%-tile:      2       13426   292     0       4       374
25%-tile:       2       13426   293     0       4       3740
Median:  2      13426   293     0       4       7479
75%-tile:       2       13426   293     0       5       11218
97.5%-tile:     2       13426   294     1       6       14583
```

```
Maximum: 4       13426   351     5       9       14956
Mean:    2       13425   292     0       4
# of Seqs:       14956

It took 2 secs to summarize 14956 sequences.

Output File Names:
silva.v4.summary


mothur > align.seqs(fasta=stability.trim.contigs.good.unique.fasta,
reference=silva.v4.fasta)

Using 8 processors.

Reading in the silva.v4.fasta template sequences...        DONE.
It took 4 to read  14956 sequences.

Aligning sequences from stability.trim.contigs.good.unique.fasta ...
It took 0 secs to align 1533 sequences.


It took 1 seconds to align 1533 sequences.

Output File Names:
stability.trim.contigs.good.unique.align
stability.trim.contigs.good.unique.align_report


mothur > summary.seqs(fasta=stability.trim.contigs.good.unique.align,
count=stability.trim.contigs.good.count_table)

Using 8 processors.

                Start   End     NBases  Ambigs  Polymer NumSeqs
Minimum: 1968   11550   250     0       3       1
2.5%-tile:      1969    11551   252     0       4       166
25%-tile:       1969    11551   252     0       4       1660
Median:  1969   11551   252     0       4       3320
75%-tile:       1969    11551   253     0       5       4979
97.5%-tile:     1969    11551   253     0       6       6473
Maximum: 1969   11553   255     0       8       6638
Mean:    1968   11550   252     0       4
# of unique seqs:       1533
total # of seqs: 6638

It took 0 secs to summarize 6638 sequences.

Output File Names:
stability.trim.contigs.good.unique.summary
```

```
mothur > screen.seqs(fasta=stability.trim.contigs.good.unique.align,
count=stability.trim.contigs.good.count_table,
summary=stability.trim.contigs.good.unique.summary, start=1968,
end=11550, maxhomop=8)

Using 8 processors.

It took 0 secs to screen 1533 sequences, removed 1532.

/******************************************/
Running command:
remove.seqs(accnos=stability.trim.contigs.good.unique.bad.accnos.temp,
count=stability.trim.contigs.good.count_table)
Removed 6637 sequences from stability.trim.contigs.good.count_table.

Output File Names:
stability.trim.contigs.good.pick.count_table

/******************************************/

Output File Names:
stability.trim.contigs.good.unique.good.summary
stability.trim.contigs.good.unique.good.align
stability.trim.contigs.good.unique.bad.accnos
stability.trim.contigs.good.good.count_table


It took 0 secs to screen 1533 sequences.

mothur > summary.seqs(fasta=current, count=current)
Using stability.trim.contigs.good.good.count_table as input file for
the count parameter.
Using stability.trim.contigs.good.unique.good.align as input file for
the fasta parameter.

Using 8 processors.

                Start    End     NBases   Ambigs   Polymer  NumSeqs
Minimum: 1968   11553    252     0        5        1
2.5%-tile:      1968     11553   252      0        5        1
25%-tile:       1968     11553   252      0        5        1
Median:  1968   11553    252     0        5        1
75%-tile:       1968     11553   252      0        5        1
97.5%-tile:     1968     11553   252      0        5        1
Maximum: 1968   11553    252     0        5        1
Mean:    1968   11553    252     0        5
# of unique seqs:        1
total # of seqs: 1
```

```
It took 0 secs to summarize 1 sequences.

Output File Names:
stability.trim.contigs.good.unique.good.summary


mothur >
filter.seqs(fasta=stability.trim.contigs.good.unique.good.align,
vertical=T, trump=.)

Using 8 processors.
Creating Filter...
It took 0 secs to create filter for 1 sequences.


Running Filter...
It took 0 secs to filter 1 sequences.



Length of filtered alignment: 252
Number of columns removed: 13174
Length of the original alignment: 13426
Number of sequences used to construct filter: 1

Output File Names:
stability.filter
stability.trim.contigs.good.unique.good.filter.fasta


mothur >
unique.seqs(fasta=stability.trim.contigs.good.unique.good.filter.fasta
, count=stability.trim.contigs.good.good.count_table)
1       1

Output File Names:
stability.trim.contigs.good.unique.good.filter.unique.fasta
stability.trim.contigs.good.unique.good.filter.count_table


mothur >
pre.cluster(fasta=stability.trim.contigs.good.unique.good.filter.uniqu
e.fasta,
count=stability.trim.contigs.good.unique.good.filter.count_table,
diffs=2)

Using 8 processors.
When using running without group information mothur can only use 1
processor, continuing.
```

```
1       1       0
Total number of sequences before precluster was 1.
pre.cluster removed 0 sequences.

/******************************************/
Running command:
get.seqs(fasta=stability.trim.contigs.good.unique.good.filter.unique.f
asta,
accnos=stability.trim.contigs.good.unique.good.filter.unique.precluste
r.fasta.temp)
Selected 1 sequences from
stability.trim.contigs.good.unique.good.filter.unique.fasta.

Output File Names:
stability.trim.contigs.good.unique.good.filter.unique.pick.fasta

/******************************************/
Done.
It took 0 secs to cluster 1 sequences.

Using 8 processors.

Output File Names:
stability.trim.contigs.good.unique.good.filter.unique.precluster.fasta
stability.trim.contigs.good.unique.good.filter.unique.precluster.count
_table
stability.trim.contigs.good.unique.good.filter.unique.precluster.map


mothur >
chimera.vsearch(fasta=stability.trim.contigs.good.unique.good.filter.u
nique.precluster.fasta,
count=stability.trim.contigs.good.unique.good.filter.unique.precluster
.count_table, dereplicate=t)

Using 8 processors.
Unable to open vsearch. Trying MOTHUR_FILES directory mothur/vsearch.
Checking sequences from
stability.trim.contigs.good.unique.good.filter.unique.precluster.fasta
...
When using template=self, mothur can only use 1 processor, continuing.
Error in reading your fastafile, at position -1. Blank name.

mothur >
chimera.vsearch(fasta=stability.trim.contigs.good.unique.good.filter.u
nique.precluster.fasta,
count=stability.trim.contigs.good.unique.good.filter.unique.precluster
.count_table, dereplicate=t)

Using 8 processors.
```

```
Unable to open vsearch. Trying MOTHUR_FILES directory mothur/vsearch.
Using vsearch version v2.16.0.
Checking sequences from
stability.trim.contigs.good.unique.good.filter.unique.precluster.fasta
...
When using template=self, mothur can only use 1 processor, continuing.

It took 0 secs to check your sequences. 0 chimeras were found.

No chimeras found, skipping remove.seqs.

Output File Names:
stability.trim.contigs.good.unique.good.filter.unique.precluster.denov
o.vsearch.chimeras
stability.trim.contigs.good.unique.good.filter.unique.precluster.denov
o.vsearch.accnos


mothur >
remove.seqs(fasta=stability.trim.contigs.good.unique.good.filter.uniqu
e.precluster.fasta,
accnos=stability.trim.contigs.good.unique.good.filter.unique.precluste
r.denovo.vsearch.accnos)
Removed 0 sequences from
stability.trim.contigs.good.unique.good.filter.unique.precluster.fasta
.

Output File Names:
stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.
fasta


mothur >
classify.seqs(fasta=stability.trim.contigs.good.unique.good.filter.uni
que.precluster.pick.fasta,
count=stability.trim.contigs.good.unique.good.filter.unique.precluster
.count_table, reference=trainset9_032012.pds/
trainset9_032012.pds.fasta, taxonomy=trainset9_032012.pds/
trainset9_032012.pds.tax, cutoff=80)

Using 8 processors.
Generating search database...    DONE.
It took 8 seconds generate search database.

Reading in the trainset9_032012.pds/trainset9_032012.pds.tax
taxonomy...      DONE.
Calculating template taxonomy tree...     DONE.
Calculating template probabilities...     DONE.
It took 16 seconds get probabilities.
Classifying sequences from
```

```
stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.
fasta ...

It took 0 secs to classify 1 sequences.


It took 0 secs to create the summary file for 1 sequences.


Output File Names:
stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.
pds.wang.taxonomy
stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.
pds.wang.tax.summary


mothur > current.system(ls)
[ERROR]: Invalid command.
[ERROR]: did not complete current.system.

mothur > system(0ls
[ERROR]: You are missing )
[ERROR]: Invalid.

mothur > system(ls)
F3D0_S188_L001_R1_001.fastq
F3D0_S188_L001_R2_001.fastq
MiSeq_SOP
README.md
commandScreen.output
current_files.summary
independentStudy.Rproj
mothur
mothur.1647394025.logfile
silva.bacteria
silva.bacteria.fasta
silva.v4.8mer
silva.v4.fasta
silva.v4.summary
stability.contigs.count_table
stability.contigs_report
stability.files
stability.filter
stability.scrap.contigs.fasta
stability.trim.contigs.bad.accnos
stability.trim.contigs.fasta
stability.trim.contigs.good.count_table
stability.trim.contigs.good.fasta
stability.trim.contigs.good.good.count_table
stability.trim.contigs.good.unique.align
```

stability.trim.contigs.good.unique.align_report
stability.trim.contigs.good.unique.bad.accnos
stability.trim.contigs.good.unique.fasta
stability.trim.contigs.good.unique.good.align
stability.trim.contigs.good.unique.good.filter.count_table
stability.trim.contigs.good.unique.good.filter.fasta
stability.trim.contigs.good.unique.good.filter.unique.fasta
stability.trim.contigs.good.unique.good.filter.unique.precluster.count_table
stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.accnos
stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.chimeras
stability.trim.contigs.good.unique.good.filter.unique.precluster.fasta
stability.trim.contigs.good.unique.good.filter.unique.precluster.map
stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.fasta
stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pds.wang.tax.summary
stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pds.wang.taxonomy
stability.trim.contigs.good.unique.good.summary
stability.trim.contigs.good.unique.summary
stability.trim.contigs.summary
trainset9_032012.pds