```
Mac version

Using ReadLine,Boost,HDF5,GSL
mothur v.1.47.0
Last updated: 1/21/22
by
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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 > 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:
FD30    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 >
[ERROR]: You are missing (
[ERROR]: Invalid.

mothur > get.current()
```

```
Current RAM usage: 0.13105 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/Lab9/

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 mothur/
stability.trim.contigs.good.names.
Unable to open mothur/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 > count.seqs(name=stability.trim.contigs.good.unique.fasta)

It took 0 secs to create a table for 2300 sequences.

Total number of sequences: 2300

Output File Names:
```

```
stability.trim.contigs.good.unique.count_table


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

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.
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.
Unable to open trainset9_032012.pds/trainset9_032012.pds.fasta. Trying
MOTHUR_FILES directory mothur/trainset9_032012.pds.fasta.
Unable to open mothur/trainset9_032012.pds.fasta. Trying mothur's
executable directory mothur/trainset9_032012.pds.fasta.
Unable to open mothur/trainset9_032012.pds.fasta.
Unable to open trainset9_032012.pds/trainset9_032012.pds.fasta
Unable to open trainset9_032012.pds/trainset9_032012.pds.tax. Trying
MOTHUR_FILES directory mothur/trainset9_032012.pds.tax.
Unable to open mothur/trainset9_032012.pds.tax. Trying mothur's
executable directory mothur/trainset9_032012.pds.tax.
Unable to open mothur/trainset9_032012.pds.tax.
Unable to open trainset9_032012.pds/trainset9_032012.pds.tax
[ERROR]: did not complete classify.seqs.

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
```