**ANSC595 Final Project**

**Mothur**

**#preparation**

make.contigs(file=stability.files, processors=20)

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

screen.seqs(fasta=stability.trim.contigs.fasta, group=stability.contigs.groups, summary=stability.trim.contigs.summary, maxambig=0, maxlength=500)

summary.seqs(fasta=stability.trim.contigs.good.fasta)

unique.seqs(fasta=stability.trim.contigs.good.fasta)

count.seqs(name=stability.trim.contigs.good.names, group=stability.contigs.good.groups)

summary.seqs(count=stability.trim.contigs.good.count\_table)

**#alignment V3-4**

#Created redmeat.v34.fasta file according to V3V4 reference alignment in note. align.seqs(fasta=ecoli\_v3.fasta, reference=silva.seed\_v123.align) summary.seqs(fasta=ecoli\_v3.align)

align.seqs(fasta=redmeat.v34.fasta,reference=silva.bacteria.fasta,processors=8)

summary.seqs(fasta=redmeat.v34.align)

pcr.seqs(fasta=silva.bacteria.fasta, start=6388, end=25277, keepdots=F, processors=8)

rename.file(input=silva.bacteria.pcr.fasta, new=silva.v34.fasta)

summary.seqs(fasta=silva.v34.fasta)

align.seqs(fasta=stability.trim.contigs.good.unique.fasta, reference=silva.v34.fasta, flip=t, processors=20)

summary.seqs(fasta=stability.trim.contigs.good.unique.align, count=stability.trim.contigs.good.count\_table, processors=20)

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=11500, maxhomop=8, processors=20)

summary.seqs(fasta=stability.trim.contigs.good.unique.good.align, count=stability.trim.contigs.good.good.count\_table, processors=20)

filter.seqs(fasta=stability.trim.contigs.good.unique.good.align, vertical=T, trump=., processors=20)

unique.seqs(fasta=stability.trim.contigs.good.unique.good.filter.fasta, count=stability.trim.contigs.good.good.count\_table)

pre.cluster(fasta=stability.trim.contigs.good.unique.good.filter.unique.fasta, count=stability.trim.contigs.good.unique.good.filter.count\_table, diffs=2)

chimera.vsearch(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.fasta, count=stability.trim.contigs.good.unique.good.filter.unique.precluster.count\_table, dereplicate=t, processors=20)

remove.seqs(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.fasta, accnos=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.accnos)

summary.seqs(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.fasta, count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.count\_table)

**#preparing for analysis**

classify.seqs(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.fasta, count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.count\_table, reference=trainset16\_022016.rdp.fasta, taxonomy=trainset16\_022016.rdp.tax, cutoff=80)

remove.lineage(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.fasta, count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.count\_table, taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.rdp.wang.taxonomy, taxon=Chloroplast-Mitochondria-unknown-Archaea-Eukaryota)

summary.tax(taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.rdp.wang.taxonomy, count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.count\_table)

**#Did not assess error rate due to missing mock community.**

**#OTUs**

dist.seqs(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.fasta, cutoff=0.03, processors=20)

cluster(column=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.dist, count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.count\_table)

mothur > make.shared(list=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.opti\_mcc.list, count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.count\_table, label=0.03)

classify.otu(list=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.opti\_mcc.list, count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.count\_table, taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.rdp.wang.pick.taxonomy, label=0.03)

**#phylotypes**

phylotype(taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.rdp.wang.pick.taxonomy)

#1 through 6 listed corresponding to genus through kingdom levels.

**# to get the genus-level shared file:**

make.shared(list=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.rdp.wang.pick.tx.list, count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.count\_table, label=1)

classify.otu(list=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.rdp.wang.pick.tx.list, count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.count\_table, taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.rdp.wang.pick.taxonomy, label=1)

1 143

**#Phylogenetic**

dist.seqs(fasta=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.fasta, output=lt, processors=20)

clearcut(phylip=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.phylip.dist)

**#Analysis**

rename.file(taxonomy=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.opti\_mcc.0.03.cons.taxonomy, shared=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.opti\_mcc.shared)

shared=stability.opti\_mcc.shared

taxonomy=stability.taxonomy

mothur > count.groups(shared=stability.opti\_mcc.shared)

SRR9157275\_1 contains 32981.

SRR9157276\_1 contains 16720.

SRR9157277\_1 contains 19569.

SRR9157278\_1 contains 46042.

SRR9157279\_1 contains 128402.

SRR9157280\_1 contains 45714.

SRR9157281\_1 contains 117551.

SRR9157282\_1 contains 45737.

SRR9157284\_1 contains 52642.

SRR9157285\_1 contains 76933.

SRR9157286\_1 contains 45127.

SRR9157287\_1 contains 94318.

SRR9157288\_1 contains 55407.

SRR9157289\_1 contains 162451.

SRR9157290\_1 contains 24513.

SRR9157293\_1 contains 47787.

SRR9157295\_1 contains 60794.

SRR9157297\_1 contains 121728.

SRR9157301\_1 contains 94755.

SRR9157302\_1 contains 75574.

SRR9157305\_1 contains 144365.

SRR9157314\_1 contains 74692.

SRR9157315\_1 contains 47305.

SRR9157316\_1 contains 65970.

SRR9157317\_1 contains 125310.

SRR9157318\_1 contains 130516.

SRR9157319\_1 contains 104690.

SRR9157320\_1 contains 62093.

SRR9157321\_1 contains 107064.

SRR9157322\_1 contains 54573.

SRR9157323\_1 contains 106904.

SRR9157324\_1 contains 41791.

SRR9157325\_1 contains 96695.

SRR9157326\_1 contains 115080.

SRR9157327\_1 contains 127346.

SRR9157328\_1 contains 104196.

SRR9157329\_1 contains 71569.

SRR9157330\_1 contains 78722.

SRR9157332\_1 contains 36555.

SRR9157343\_1 contains 128247.

SRR9157344\_1 contains 24799.

SRR9157346\_1 contains 147904.

SRR9157347\_1 contains 79079.

SRR9157348\_1 contains 90665.

SRR9157349\_1 contains 50208.

Total seqs: 3581083.

Output File Names:

stability.opti\_mcc.count.summary

sub.sample(shared=stability.opti\_mcc.shared, size=16720)

Sampling 16720 from each group.

0.03

**#OTU-based analysis**

**#alpha-diversity**

rarefaction.single(shared=stability.opti\_mcc.shared, calc=sobs, freq=100, processors=20)

summary.single(shared=stability.opti\_mcc.shared, calc=nseqs-coverage-sobs-invsimpson-shannon-ace-chao, subsample=T)

**#beta-diversity**

mothur > dist.shared(shared=stability.opti\_mcc.shared, calc=thetayc-jclass, subsample=t, processors=20)

mothur > pcoa(phylip=stability.opti\_mcc.thetayc.0.03.lt.ave.dist)

Processing...

Rsq 1 axis: 0.56345

Rsq 2 axis: 0.923662

Rsq 3 axis: 0.950131

**#add a third dimension the R-squared value increases to 0.95.**

**#Alternatively, non-metric multidimensional scaling (NMDS)**

nmds(phylip=stability.opti\_mcc.thetayc.0.03.lt.ave.dist, mindim=3, maxdim=3)1

Processing Dimension: 3

 Number of dimensions: 3

Lowest stress : 0.0722233

R-squared for configuration: 0.97336

**#amova**

amova(phylip=stability.opti\_mcc.thetayc.0.03.lt.ave.dist, design=mouse.group.design)

**#homova**

homova(phylip=stability.opti\_mcc.thetayc.0.03.lt.ave.dist, design=mouse.group.design)

**#Signicant OTU**

corr.axes(axes=stability.opti\_mcc.thetayc.0.03.lt.ave.pcoa.axes, shared=stability.opti\_mcc.0.03.subsample.shared, method=spearman, numaxes=3)

**#get community type**

get.communitytype(shared=stability.opti\_mcc.0.03.subsample.shared)

Using 1 processor

0.03

K       NLE             logDet  BIC             AIC             Laplace

1       160321.56       4314.49 176663.56       168907.56       154588.80

2       172936.75       -12366.35       205622.66       190109.75       150972.65

3       188632.75       -29648.37       237662.57       214392.75       **150136.73**

4       209616.13       -46992.58       274989.85       243963.13       154557.07

5       231938.86       -65063.21       313656.49       274872.86       159953.57

6       253974.80       -83351.76       352036.32       305495.80       164954.31

#three community types.

**#population-level analysis**

metastats(shared=stability.opti\_mcc.0.03.subsample.shared, design=mouse.group.design)

**#lefse**

lefse(shared=stability.opti\_mcc.0.03.subsample.shared, design=mouse.group.design)

**Phylogeny-based analysis**

#alpha-diversity

phylo.diversity(tree=stability.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.phylip.tre, count=stability.trim.contigs.good.unique.good.filter.unique.precluster.denovo.vsearch.pick.pick.count\_table, rarefy=T, processors=20)

#beta-diversity

unifrac.unweighted(tree=stability.tre, count=stability.count\_table, distance=lt, processors=2, random=F, subsample=t)  
unifrac.weighted(tree=stability.tre, count=stability.count\_table, distance=lt, processors=2, random=F, subsample=t)