mothur > make.contigs(file=DIEL.files, processors=8)

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

mothur > screen.seqs(fasta=DIEL.trim.contigs.fasta, group=DIEL.contigs.groups, summary=DIEL.trim.contigs.summary, maxambig=0, maxlength=254, minlength=251, maxhomop=8)

mothur > unique.seqs(fasta=DIEL.trim.contigs.good.fasta)

mothur > summary.seqs(fasta=DIEL.trim.contigs.good.unique.fasta, name=DIEL.trim.contigs.good.names)

mothur > count.seqs(name=DIEL.trim.contigs.good.names)

mothur > count.groups(group=DIEL.contigs.good.groups)

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

mothur > remove.seqs(fasta=DIEL.trim.contigs.good.unique.align, name=DIEL.trim.contigs.good.names, group=DIEL.contigs.good.groups, accnos=DIEL.trim.contigs.good.unique.flip.accnos)

mothur > count.seqs(name=DIEL.trim.contigs.good.pick.names, group=DIEL.contigs.good.pick.groups)

mothur > summary.seqs(fasta=DIEL.trim.contigs.good.unique.pick.align, count=DIEL.trim.contigs.good.pick.count\_table)

mothur > screen.seqs(fasta=DIEL.trim.contigs.good.unique.pick.align, count=DIEL.trim.contigs.good.pick.count\_table, summary=DIEL.trim.contigs.good.unique.pick.summary, start=1968, end=11550)

mothur > summary.seqs(fasta=DIEL.trim.contigs.good.unique.pick.good.align, count=DIEL.trim.contigs.good.pick.good.count\_table)

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

mothur > unique.seqs(fasta=DIEL.trim.contigs.good.unique.pick.good.filter.fasta, count=DIEL.trim.contigs.good.pick.good.count\_table)

mothur > pre.cluster(fasta=DIEL.trim.contigs.good.unique.pick.good.filter.unique.fasta, count=DIEL.trim.contigs.good.unique.pick.good.filter.count\_table, diffs=2)

chimera.uchime(fasta=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.fasta, count=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.count\_table, dereplicate=t, processors=8)

remove.seqs(fasta=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.fasta, accnos=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.uchime.accnos)

summary.seqs(fasta=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.fasta, count=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.uchime.pick.count\_table)

classify.seqs(fasta=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.fasta, count=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.uchime.pick.count\_table, reference=trainset9\_032012.pds.fasta, taxonomy=trainset9\_032012.pds.tax, cutoff=80)

mothur > remove.lineage(fasta=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.fasta, count=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.uchime.pick.count\_table, taxonomy=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.pds.wang.taxonomy, taxon=Chloroplast-Mitochondria-unknown-Eukaryota)

mothur > set.current(fasta=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.pick.fasta, count=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.uchime.pick.pick.count\_table, taxonomy=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.pds.wang.pick.taxonomy, processors=16)

cluster.split(fasta=current, count=current, taxonomy=current, splitmethod=classify, taxlevel=4, cutoff=0.15, processors=16)

mothur > split.abund(fasta=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.pick.fasta, list=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.pick.an.unique\_list.list, count=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.uchime.pick.pick.count\_table, cutoff=2, label=0.03)

mothur > make.shared(list=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.pick.an.unique\_list.0.03.abund.list, count=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.uchime.pick.pick.0.03.abund.count\_table, label=0.03)

mothur > classify.otu(list=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.pick.an.unique\_list.0.03.abund.list, count=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.uchime.pick.pick.0.03.abund.count\_table, taxonomy=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.pds.wang.pick.taxonomy, label=0.03)

count.groups(shared=diel.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.pick.an.unique\_list.0.03.abund.shared)

sub.sample(shared=diel.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.pick.an.unique\_list.0.03.abund.shared, size=39711) ONE SAMPLE WAS EXCLUDED (SF\_B3\_N1) DUE TO ONLY 267 SEQUENCES AVAILABLE

TO CLASSIFY OTUs FOR TAXONOMIC BREAKDOWN ANALYSIS:

phylotype(taxonomy=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.pds.wang.pick.taxonomy)

make.shared(list=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.pds.wang.pick.tx.list, count=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.uchime.pick.pick.count\_table, label=5)

sub.sample(shared=diel.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.pds.wang.pick.tx.shared, size=40355) THIS SHARED FILE IS WHAT TO ANALYZE

classify.otu(list=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.pds.wang.pick.tx.list, count=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.uchime.pick.pick.count\_table, taxonomy=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.pds.wang.pick.taxonomy, label=5)

get.oturep(method=abundance, list=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.pds.wang.pick.tx.list, count=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.uchime.pick.pick.count\_table, fasta=DIEL.trim.contigs.good.unique.pick.good.filter.unique.precluster.pick.pick.fasta, label=5)