Mothur analysis pipeline

Quality control:

Primers are trimmed

N’s are removed

Chimeras are removed

<30% QC removed

Mothur analysis:

\*\*In Geneious, combine all samples into one large fasta file and export.

\*\*To do analyses at individual sample level later on down the line you need to make a group file. To do this export individual sample files out of geneious and make a group file in mother. This provides a list of each sequence to its corresponding sample ID. (make.group(fasta=sample1.fasta-sample2.fasta….., groups=A-B-C…)­­­­

1. Identify unique sequences
   1. unique.seqs(fasta=\_\_\_\_)
2. Remove singletons
   1. split.abund(fasta=\_\_\_.unique.fasta, names=­\_\_\_\_.names, cutoff=1)
3. Conduct an alignment
   1. align.seqs(candidate=grouped.fasta, template=referencetemplate.fasta)\*
4. Generate distance matrix from the aligned grouped file
   1. dist.seqs
5. Cluster analysis
   1. cluster
6. Get OTU representative for sequences
   1. Get.oturep
7. count.seqs

\*this will give you an output file that can be brought into xcel to see what sequences occur in each sample.

1. make.shared(list=\_\_\_.an.list, group=\_\_groups)

need this function for venn diagrams, rarefaction curves and any further analysis in mother.