\*\* Based on processing by V. Orphan, D. Case, C. Skennerton (Caltech) \*\*

Start QIIME and Unzip the data

module load qiime

gunzip \*.gz

Joined pair-end reads and perform quality trimming of contigs

From the directory where your data and processing files are, execute:

/export/data1/db/16S\_tag\_processing\_db/QIIME\_join\_trim\_convert\_to\_fasta\_v3.sh

Pick *de novo* OTUs

pick\_otus.py -i all\_seqs\_all\_samples.fasta -s 0.99 -o uclust\_picked\_otus\_99/&

Pick representative sequence for each OTU

pick\_rep\_set.py -i uclust\_picked\_otus\_99/all\_seqs\_all\_samples\_otus.txt -f all\_seqs\_all\_samples.fasta -m most\_abundant&

Assign taxonomy to each OTU

assign\_taxonomy.py -i all\_seqs\_all\_samples.fasta\_rep\_set.fasta -t /export/data1/db/16S\_tag\_processing\_db/SSURef\_NR99\_Silva\_115\_pintailF\_ORPHAN.tax -r /export/data1/db/16S\_tag\_processing\_db/SSURef\_NR99\_Silva\_115\_pintailF\_ORPHAN.fasta --uclust\_similarity 0.9 --uclust\_max\_accepts 10 --uclust\_min\_consensus\_fraction 0.90 -o uclust\_taxa\_0.9\_10\_0.90/&

make\_otu\_table.py -i uclust\_picked\_otus\_99/all\_seqs\_all\_samples\_otus.txt -t uclust\_taxa\_0.9\_10\_0.90/all\_seqs\_all\_samples.fasta\_rep\_set\_tax\_assignments.txt -o uclust\_taxa\_0.9\_10\_0.90/OTU\_table\_Silva\_115\_all\_seqs.biom&

Remove singletons

filter\_otus\_from\_otu\_table.py -i uclust\_taxa\_0.9\_10\_0.90/OTU\_table\_Silva\_115\_all\_seqs.biom -n 2 -o uclust\_taxa\_0.9\_10\_0.90/OTU\_table\_singletonfiltered.biom

Generate abundance table (non-rarified)

summarize\_taxa.py -i uclust\_taxa\_0.9\_10\_0.90/OTU\_table\_singletonfiltered.biom -a -o uclust\_taxa\_0.9\_10\_0.90/singletonfiltered\_taxa\_summary/

To Rarify

biom summarize-table -i uclust\_taxa\_0.9\_10\_0.90/OTU\_table\_singletonfiltered.biom -o table\_summary.txt

pico table\_summary.txt

ctrl-x

single\_rarefaction.py -i uclust\_taxa\_0.9\_10\_0.90/OTU\_table\_singletonfiltered.biom -d 13266 -o uclust\_taxa\_0.9\_10\_0.90/OTU\_AllFiltered\_evendepth.biom

Generate abundance table (rarified)

summarize\_taxa.py -i uclust\_taxa\_0.9\_10\_0.90/OTU\_AllFiltered\_evendepth.biom -a -o uclust\_taxa\_0.9\_10\_0.90/evendepth\_taxa\_summary/