#Neon Workflow - Closed Referenced 16S OTU Picking - 2015#

```
#!/bin/bash
#SBATCH --job-name=
#SBATCH --output=.out
#SBATCH --error=.err
#SBATCH --time=36:00:00
#SBATCH --partition=amd
#SBATCH --nodes=1
#SBATCH --ntasks=1
#SBATCH --cpus-per-task=1
#SBATCH --mem-per-cpu=5000

module load qiime/1.8

#Unzip Reads
gunzip -c rawseq/16S/Undetermined_S0_L001_I1_001.fastq.gz >
rawseq/16S/barcodes.fastq &&
gunzip -c rawseq/16S/Undetermined_S0_L001_R1_001.fastq.gz >
rawseq/16S/read1.fastq &&
gunzip -c rawseq/16S/Undetermined_S0_L001_R2_001.fastq.gz >
rawseq/16S/read2.fastq

#Join Read 1 and 2
chmod a+x scripts/*

scripts/fastq-join rawseq/16S/read1.fastq rawseq/16S/read2.fastq -o
rawseq/16S/01_joined/out.%.fastq > rawseq/16S/01_joined/out.stats.txt

#Join Barcodes to Paired Reads
scripts/fastq-barcode.pl rawseq/16S/barcodes.fastq
rawseq/16S/01_joined/out.join.fastq >
rawseq/16S/01_joined/out.barcodes.fastq

#Demultiplex Reads (16S joined, ITS single read)
split_libraries_fastq.py -i rawseq/16S/01_joined/out.join.fastq -b
rawseq/16S/01_joined/out.barcodes.fastq -m
mapping/neon_16s_mapping_file.txt -o demultiplex --barcode_type=12 --
max_barcode_errors=0

#Zip 16S Joined Sequences
tar -zcvf 16S_fastq.tar.gz rawseq/16S/01_joined/

#Split FNA file by sample
split_fasta_on_sample_ids.py -i seqs.fna -o 16S_sample_fasta

#Closed Reference OTU Picking in Parallel
pick_closed_reference_otus.py -i demultiplex_16S/seqs.fna -r
/gg_13_8_otus/rep_set/97_otus.fasta -o picked_closed_97 -p
ucrss_params2.txt -a -O 4

#Add Taxonomy to OTU table using Greengenes 13.8
biom add-metadata -i picked_closed_97/16S/otu_table.biom --observation-
metadata-fp /greengenes/gg_13_8_otus/taxonomy/97_otu_taxonomy.txt -o
```

```
picked_closed_97/16S/otu_table_w_tax.biom --sc-separated taxonomy --
observation-header OTUID,taxonomy

#Make Phylogeny using Representative Tree from Greengenes 1.8
make_phylogeny.py -i
picked_otus_97/pynast_aligned_seqs/rep_set_aligned_pfiltered.fasta -o
picked_otus_97/rep_set.tre
```

```
#Neon Workflow - Closed Referenced ITS OTU Picking - 2015#

#!/bin/bash
#SBATCH --job-name=
#SBATCH --output=.out
#SBATCH --error=.err
#SBATCH --time=36:00:00
#SBATCH --partition=amd
#SBATCH --nodes=1
#SBATCH --ntasks=1
#SBATCH --cpus-per-task=1
#SBATCH --mem-per-cpu=5000

module load qiime/1.8

#Unzip Reads
gunzip -c rawseq/ITS/Undetermined_S0_L001_I1_001.fastq.gz >
rawseq/16S/barcodes.fastq &&
gunzip -c rawseq/ITS/Undetermined_S0_L001_R1_001.fastq.gz >
rawseq/16S/read1.fastq &&
gunzip -c rawseq/ITS/Undetermined_S0_L001_R2_001.fastq.gz >
rawseq/16S/read2.fastq

#Demultiplex Reads
split_libraries_fastq.py -i
rawseq/ITS/Undetermined_S0_L001_R1_001.fastq.gz -b
rawseq/ITS/Undetermined_S0_L001_I1_001.fastq.gz -m
mapping/neon_its_mapping_file.txt -o demultiplex_ITS --barcode_type=12 --
max_barcode_errors=0 --rev_comp_mapping_barcodes

#Split FNA file by sample
split_fasta_on_sample_ids.py -i seqs.fna -o ITS_sample_fasta

#Closed Reference OTU Picking in Parallel
pick_closed_reference_otus.py -i demultiplex_ITS/seqs.fna -r
/its_12_11_otus/rep_set/97_otus.fasta -o picked_closed_its_97 -p
ucrss_params_its.txt -a -O 4

#Add Taxonomy to OTU table using UNITE 12.11
biom add-metadata -i picked_closed_97/ITS/otu_table.biom --observation-
metadata-fp /its_12_11_otus/taxonomy/97_otu_taxonomy.txt -o
picked_closed_97/ITS/otu_table_w_tax.biom --sc-separated taxonomy --
observation-header OTUID,taxonomy
```

```
## Parameters file contents ##
ucrss_params.txt: pick_otus:enable_rev_strand_match True;
align_seqs:min_length 75
```