**ok128**

**#Paired+Single\_end\_sequences**

*#bash ../qiime2/scripts/run\_paired\_single.sh*

#data\_dir=$1

#barcodes\_file=$2

bash ../qiime2/scripts/run\_paired\_single.sh ok128 ok128\_metadata.tsv

#time: 76 min

**#DADA2- quality filtering step**

qiime dada2 denoise-single \

--i-demultiplexed-seqs ok128\_single\_end\_sequences/demux\_single.qza \

--p-trim-left 0 \

--p-trunc-len 233 \

--o-representative-sequences ok128\_rep-seqs-dada2.qza \

--o-table ok128\_table.qza \

--o-denoising-stats ok128\_stats-dada2.qza

qiime feature-table summarize \

--i-table ok128\_table.qza \

--o-visualization ok128\_table.qzv \

--m-sample-metadata-file ok128\_metadata.tsv

#time: 16 min

**#Filtering features after merging**

qiime feature-table filter-features \  
--i-table ok128\_table.qza \  
--p-min-frequency 10 \  
--o-filtered-table ok128\_filtered\_table.qza

qiime feature-table filter-samples \  
--i-table ok128\_filtered\_table.qza \  
--p-min-frequency 10 \  
--o-filtered-table ok128\_filtered\_table.qza

qiime feature-table summarize \  
--i-table ok128\_filtered\_table.qza \  
--o-visualization ok128\_filtered\_table.qzv \  
--m-sample-metadata-file ok128\_metadata.tsv

*##Filtered out samples: 1, 2, 16, 47, 20, 52*

**#Run phylogenetic tree, taxonomy, taxonomy-based cleaning**

*#bash ../qiime2/scripts/run\_tree\_taxonomy\_clean.sh*

*#rep\_seqs=$1*

*#i\_table=$2*

*#metadata=$3*

bash ../qiime2/scripts/run\_tree\_taxonomy\_clean.sh \  
ok128\_rep-seqs-dada2.qza \  
ok128\_filtered\_table.qza \  
ok128\_metadata.tsv

#time: 2 min

**#Feature-table filtering for Metadata**

*#--p-where "Subject='subject-1'"*

*#--p-where "BodySite IN ('left palm', 'right palm')"*

*#--p-where "Subject='subject-1' AND BodySite='gut'"  
#--p-where "BodySite='gut' OR ReportedAntibioticUsage='Yes'"  
#--p-where "Subject='subject-1' AND NOT BodySite='gut'"*

qiime feature-table filter-samples \  
--i-table ok128\_clean\_table.qza \  
--m-metadata-file ok128\_metadata.tsv \  
--p-where "[genotype]=*'wt'*"\  
--o-filtered-table ok128\_clean\_table\_genotype\_wt.qza

qiime feature-table filter-samples \  
--i-table ok128\_clean\_table.qza \  
--m-metadata-file ok128\_metadata.tsv \  
--p-where "[genotype]=*'bko'*"\  
--o-filtered-table ok128\_clean\_table\_genotype\_bko.qza

**#Run diversity, ancom, aldex2**

*#bash ../qiime2/scripts/run\_diversity\_ancom\_aldex2.sh*

*#rooted\_tree=$1*

*#i\_table=$2*

*#p\_sampling\_depth=$3*

*#metadata=$4*

*#dir=$5*

*#column=$6  
#taxonomy=$7*

bash ../qiime2/scripts/run\_diversity\_ancom\_aldex2.sh \

ok128\_tree.qza \  
ok128\_clean\_table.qza \  
27992 \  
ok128\_metadata.tsv \  
ok128\_genotypes \  
genotype \  
ok128\_taxonomy\_clean.qza

bash ../qiime2/scripts/run\_diversity\_ancom.sh \

ok128\_tree.qza \  
ok128\_clean\_table\_genotype\_wt.qza \  
27992 \  
ok128\_metadata.tsv \  
ok128\_genotype\_wt \  
Days\_of\_experiment \  
ok128\_taxonomy\_clean.qza

bash ../qiime2/scripts/run\_diversity\_ancom.sh \

ok128\_tree.qza \  
ok128\_clean\_table\_genotype\_bko.qza \  
27992 \  
ok128\_metadata.tsv \  
ok128\_genotype\_bko \  
Days\_of\_experiment \  
ok128\_taxonomy\_clean.qza

**#LME diversity**

#bash ../qiime2/scripts/run\_LME\_diversity.sh

#metadata=$1

#dir=$2

#group\_column=$3

#state\_column=$4

#subject\_id=$5

#random\_effects=$6

bash ../qiime2/scripts/run\_LME\_diversity.sh \  
ok128\_metadata.tsv \  
ok128\_genotypes \  
genotype \

time \  
Mouse\_num

**#Pairwise longitudinal study**

*#bash ../qiime2/scripts/run\_diversity\_pairwise.sh*

*#dir=$1*

*#metadata=$2*

*#group\_column=$3*

*#state\_column=$4*

*#state\_1=$5*

*#state\_2=$6*

*#subject\_id=$7*

bash ../qiime2/scripts/run\_diversity\_pairwise.sh \

ok128\_genotypes \

ok128\_metadata.tsv \

genotype \

time 1 2 \

Mouse\_num

bash ../qiime2/scripts/run\_diversity\_pairwise.sh \

ok128\_genotypes \

ok128\_metadata.tsv \

genotype \

time 2 3 \

Mouse\_num

bash ../qiime2/scripts/run\_diversity\_pairwise.sh \

ok128\_genotypes \

ok128\_metadata.tsv \

genotype \

time 3 4 \

Mouse\_num

bash ../qiime2/scripts/run\_diversity\_pairwise.sh \

ok128\_genotypes \

ok128\_metadata.tsv \

genotype \

time 4 5 \

Mouse\_num