**MiSeq 16S Code**

//1 use trimmomatic to do the quality control

//this command is to filter out short sequence

java -jar trimmomatic-0.38.jar PE -threads 4 -phred33 seq\_R1\*.fastq.gz ${path}/seq\_R2\*.fastq.gz ${path}/seq\_R1\_paired.fastq.gz ${path}/seq\_R1\_unpaired.fastq.gz ${path}/seq\_R2\_paired.fastq.gz ${path}/seq\_R2\_unpaired.fastq.gz MINLEN:100

//this command is to cut off low quality part from the seq

java -jar trimmomatic-0.38.jar SE -threads 4 -phred33 seq\_R2\_paired.fastq.gz seq\_R2\_trimmed.fastq.gz CROP:150

//this command is to remove adapter and cut off low quality bases from seqs

java -jar seq\_R1.fastq.gz seq\_R2.fastq.gz seq\_R1.paired.fastq.gz seq\_R1.unpaired.fastq.gz seq\_R2.paired.fastq.gz seq\_R2.unpaired.fastq.gz ILLUMINACLIP: ../adapters/NexteraPE-PE.fa:2:30:10 SLIDINGWINDOW:4:20 MINLEN:60

tips: how to set the parameters of trimmomatic depending on the sequence quality which can be accessed by QC

//2. use flash to mesh paired reads

 ./flash -m 30 -M 150 -x 0.1 -t 1 -o ${name\_output} -d ${path\_output} seq\_R1.paired.fastq.gz seq\_R2.paired.fastq.g

//3. chimera remove

usearch -uchime\_ref paired.extendedFrags.fastq -db ${path}/rRNA16S.gold.foruchime.fasta -uchimeout paired\_chimeraout.txt -nonchimeras paired.fasta -strand plus

//4. rename the seqs generated after usearch

for F in \*.fasta

do

echo $F

MAP=${F%.fasta}

sed "-es!^>\(.\*\)!>\1;barcodelabel=${MAP##\*/}!" "-es! !-!g" < $F > ${MAP}\_barcode.fasta

done

//5. put all sequence into one file(make preparations for otu analysis)

cat \*barcode.fasta>Cat.fasta

//6. deduplicate

usearch -fastx\_uniques Cat.fasta -fastaout uniques.fasta -sizeout

//7. sort the sequence by abundance

usearch -sortbysize uniques.fasta -fastaout sorted.fasta -minsize 4

//8. generate otu fasta file

usearch -cluster\_otus sorted.fasta -otus otus.fasta

//9. make otu\_table

python fasta\_number.py otus.fasta OTU\_ > otuRef.fasta

usearch -usearch\_global Cat.fasta -db otuRef.fasta -strand plus -id 0.97 -uc map.uc

python uc2otutab.py map.uc > otu\_table.txt

//10. make rdp\_table

java -Xmx1g -jar classifier.jar classify -o rdpout.tsv otus.fasta -f filterbyconf -c 0.8