**Qiime分析简要流程**

cd new file

ls（表示列表关联）

1 用我们提供的脚本转换为fna文件

split\_libraries\_fastq.py --barcode\_type not-barcoded --sample\_id CK1 -o CK1/ -i CK1.extendedFrags.fastq -m CK1\_map.txt -q 19

2 cat file1.fna file2.fna > seqs.fna

3.\*\*\* move chimeric（去除嵌合体）

chmod 755 /home/qiime/qiime\_software/qiime-1.8.0-release/bin/usearch61

#使usearch61这个程序可以被执行）

identify\_chimeric\_seqs.py -i seqs.fna -m usearch61 -o usearch\_checked\_chimera -r /home/qiime/qiime\_software/gg\_otus-13\_8-release/rep\_set/97\_otus.fasta

#找出chimera，利用usearch算法，与greengene数据比对

filter\_fasta.py -f seqs.fna -s usearch\_checked\_chimera/chimeras.txt -o seqs.nochimera.fna -n

#将找到的chimera从序列文件中筛选掉

4. \*\*\*Picking OTUs \*Building an OTU table\*\*\*

pick\_de\_novo\_otus.py -i seqs.nochimera.fna -o otus

5\*\*\*此步骤为去除biom文件中要删除的seqs

#1)古菌（Archaea），含叶绿素部分及Unclassified，也可以根据自己所需要进行删除，以方便后续操作。\*\*\*

filter\_taxa\_from\_otu\_table.py -i otus/otu\_table.biom -o otus/otu\_table\_non\_ACUU.biom -n k\_\_Archaea,c\_\_Chloroplast,Unclassified,Unassigned

注：ACUU: Archaea, Chloroplast, Unclassified, Unassigned

6\*\*\*去除singletons\*\*\*

#1)

filter\_otus\_from\_otu\_table.py -i otus/otu\_table\_non\_ACUU.biom -o otu\_table\_non\_ACUU\_no\_singletons.biom -n 2

#2)

filter\_otus\_from\_otu\_table.py -i otus/otu\_table\_non\_ACUU.biom -o otu\_table\_non\_ACUU\_min\_count\_fraction\_dob\_sorted.biom --min\_count\_fraction 0.000001

7.\*\*\*最小测序深度:\*\*\*

#1)

biom summarize-table -i otu\_table\_non\_ACUU\_no\_singletons.biom -o otu\_table\_summarize.txt

#2)

biom summarize-table -i otu\_table\_non\_ACUU\_min\_count\_fraction\_dob\_sorted.biom -o otu\_table\_summarize.txt

more otu\_table\_summarize.txt

8\*\*\*sort out\_table by SampleID according to the Mapping file\*\*\*

#1)

sort\_otu\_table.py -i otu\_table\_non\_ACUU\_no\_singletons.biom -o otu\_table\_non\_ACUU\_no\_singletons\_dob\_sorted.biom -l sample\_id\_list.csv

#2)

sort\_otu\_table.py -i otu\_table\_non\_ACUU\_min\_count\_fraction\_dob\_sorted.biom -o otu\_table\_non\_ACUU\_min\_count\_fraction\_dob\_sorted.biom -l sample\_id\_list.csv

这部分是去除了singlton之后的代表序列建立系统发育树

echo "Keep all sequences that are listed as observations in a biom file."

filter\_fasta.py -f otus/pynast\_aligned\_seqs/seqs.nochimera\_rep\_set\_aligned\_pfiltered.fasta -o otus/pynast\_aligned\_seqs/seqs\_rep\_set\_aligned\_pfiltered\_biom\_filtered\_seqs.fasta -b otu\_table\_non\_ACUU\_no\_singletons\_dob\_sorted.biom

make\_phylogeny.py -i otus/pynast\_aligned\_seqs/seqs\_rep\_set\_aligned\_pfiltered\_biom\_filtered\_seqs.fasta -o otus/rep\_mc2\_set.tre

9.\*\*\*Compute Beta Diversity and Generate Beta Diversity Plots\*\*\*

beta\_diversity\_through\_plots.py -i otu\_table\_non\_ACUU\_no\_singletons\_dob\_sorted.biom -m map.txt -o wf\_bdiv\_even4980/ -t otus/rep\_mc2\_set.tre -e 4980

#标准化以后的数据取值应选最小测序深度的整数值（一般只保留百位数或千位数）

把wf\_bdiv\_even4980文件夹中的otu\_table\_non\_ACUU\_no\_singletons\_dob\_sorted\_even4980.biom复制到最初建立的文件夹中

10. \*\*\*Makke OTU Heatmap\*\*\*

make\_otu\_heatmap\_html.py -i otu\_table\_non\_ACUU\_no\_singletons\_dob\_sorted\_even4980.biom -o otus/OTU\_Heatmap/

11. \*\*\*Makke OTU Network\*\*\*

make\_otu\_network.py -m map.txt -i otu\_table\_non\_ACUU\_no\_singletons\_dob\_sorted\_even4980.biom -o otus/OTU\_Network

12. \*\*\*Summarize Communities by Taxonomic Composition\*\*

summarize\_taxa\_through\_plots.py -i otu\_table\_non\_ACUU\_no\_singletons\_dob\_sorted\_even4980.biom -o wf\_taxa\_summary -m map.txt

13.\*\*\*set Alpha Diversity params\*\*\*

multiple\_rarefactions.py -i otu\_table\_non\_ACUU\_no\_singletons\_dob\_sorted.biom -m 10 -x 4980 -s 497 -n 10 -o wf\_arare/rarefaction/

(-m 10 -x 4980 -s 497 -n 10中表示reads数从10开始，4980为第4步中otu\_non\_bac\_firm.biom中最小测序深度，497表示取reads数的步幅，-n 10里的10表示一个步幅中随机取样的次数)

14.\*\*\*Compute Alpha Diversity\*\*\*

*alpha\_diversity.py -i wf\_arare/rarefaction/ -m shannon,chao1,PD\_whole\_tree,observed\_species -o wf\_arare/alpha\_div -t otus/rep\_mc2\_set.tre*

(rarefaction表示稀释曲线，PD\_whole\_tree表示系统发育多样性指数)

15.\*\*\*Collate Rarified OTU Tables\*\*\*#算出多样性指数

collate\_alpha.py -i wf\_arare/alpha\_div/ -o wf\_arare/alpha\_div\_collated/

16.\*\*\*Generate Rarefaction Curves\*\*\*

make\_rarefaction\_plots.py -i wf\_arare/alpha\_div\_collated/ -m map.txt -o wf\_arare/alpha\_rarefaction\_plots/

(\*\*\*Viewing Alpha Diversity Results:open the file wf\_arare/alpha\_rarefaction\_plots/rarefaction\_plots.html \*\*\*)

17.\*\*\*Compute NMDS\*\*\*

nmds.py -i wf\_bdiv\_even4980/unweighted\_unifrac\_dm.txt -o wf\_bdiv\_even4980/nmds\_unweighted

nmds.py -i wf\_bdiv\_even4980/weighted\_unifrac\_dm.txt -o wf\_bdiv\_even4980/nmds\_weighted

18. \*\*\*Generate Bootstrapped Tree\*\*\*

biom convert -i otu\_table\_non\_ACUU\_no\_singletons\_dob\_sorted\_even4980.biom -o otu\_table\_non\_ACUU\_no\_singletons\_dob\_sorted\_even4980.txt -b --table-type 'otu table' --header-key 'taxonomy'

##反转：

biom convert -i otus\_open/otu\_table.txt -o otu\_table.biom --table-type 'otu table' --header-key 'taxonomy'

19.功能预测

pick\_closed\_reference\_otus.py -i seqs.nochimera.fna -r /home/qiime/qiime\_software/gg\_13\_5\_otus/rep\_set/97\_otus.fasta -t /home/qiime/qiime\_software/gg\_13\_5\_otus/taxonomy/97\_otu\_taxonomy.txt -o otus\_closed

filter\_taxa\_from\_otu\_table.py -p list.csv -i otu\_table.biom -o otu\_table\_p.biom

均一化

single\_rarefaction.py -i otu\_table\_s65\_no\_rare.biom -o otu\_table\_s65\_even13950.biom -d 13950

filter\_samples\_from\_otu\_table.py -i A -o B –-sample\_id\_fp C