**Scripts run on Cartesius for microbiome analysis**

* **DNA data**

/home/mbaron/2018\_02\_smb' -> '/home/cbmucl17/2018\_02\_smb'

* **Demultiplexing**
* Joining Read1 and Read2 (forward and reverse strands)

#!/bin/bash

#SBATCH -t 1:00:00

#SBATCH -n 24

#SBATCH -p short

#Load modules

module load eb

module load Miniconda2

# loading virtualenv

source activate qiime

# setting temporary directory

export TMPDIR=~/qiime\_tmp

# joining Illumina sequences

echo "join paired ends"

time join\_paired\_ends.py -m SeqPrep -b Index.fastq.gz -f Read1.fastq.gz -r Read2.fastq.gz -o SeqPrep\_joined

# deactivating environment

source deactivate

* Split libraries according to barcodes specified in mapping file

#!/bin/bash

#SBATCH -t 1:00:00

#SBATCH -n 24

#SBATCH -p short

#Load modules and qiime

module load eb

module load Miniconda2

source activate qiime

# setting temporary directory

export TMPDIR=~/qiime\_tmp

split\_libraries\_fastq.py \

-m ~/2018\_02\_smb/map\_incomplete.tsv \

-i ~/2018\_02\_smb/SeqPrep\_joined/seqprep\_assembled.fastq.gz \

-b ~/2018\_02\_smb/SeqPrep\_joined/seqprep\_assembled.fastq\_barcodes.fastq \

-o dem1 \

-q 19 \

--rev\_comp\_barcode \

--rev\_comp\_mapping\_barcodes \

#deactivating environment

source deactivate

* **Picking OTUS**

#!/bin/bash

#SBATCH -t 1:00:00

#SBATCH -n 24

#SBATCH -p short

#Load modules and qiime

module load eb

module load Miniconda2

source activate qiime

# setting temporary directory

export TMPDIR=~/qiime\_tmp

# picking OTUs

echo “Picking OTUs with closed reference”

pick\_closed\_reference\_otus.py \

-i /dem1/seqs.fna \

-o /OTUs

#deactivating environment

source deactivate

* Copy SILVA in home directory

cp /Macintosh HD/Users/Tanguybesson/Downloads/SILVA\_132\_SSURef\_12\_12\_17\_opt.arb /2018\_02\_smb/Silva

* Using SILVA instead of default QIIME greengene database

#!/bin/bash

#SBATCH -t 1:00:00

#SBATCH -n 24

#SBATCH -p short

#Load modules and qiime

module load eb

module load Miniconda2

source activate qiime

# setting temporary directory

export TMPDIR=~/qiime\_tmp

# picking OTUs

echo “Picking OTUs with closed reference”

pick\_closed\_reference\_otus.py \

-i ~/dem1/seqs.fna \

-r ~/2018\_02\_smb/SILVA\_128\_QIIME\_release/rep\_set/rep\_set\_16S\_only/97/97\_otus\_16S.fasta \

-o ~/OTUs2

#deactivating environment

source deactivate

* Using open references

#!/bin/bash

#SBATCH -t 1:00:00

#SBATCH -n 24

#SBATCH -p short

#Load modules and qiime

module load eb

module load Miniconda2

source activate qiime

# setting temporary directory

export TMPDIR=~/qiime\_tmp

# picking OTUs

echo "Picking OTUs with open reference"

pick\_open\_reference\_otus.py \

-i ~/dem1/seqs.fna \

-o ~/2018\_02\_smb/OTUs4 \

-r ~/2018\_02\_smb/SILVA\_128\_QIIME\_release/rep\_set/rep\_set\_16S\_only/97/97\_otus\_16S.fasta

-a -O 24 \

#deactivating environment

source deactivate

* **Analyse the Biom table**

#!/bin/bash

#SBATCH -t 1:00:00

#SBATCH -n 24

#SBATCH -p short

#Load modules and qiime

module load eb

module load Miniconda2

source activate qiime

# setting temporary directory

export TMPDIR=~/qiime\_tmp

biom summarize-table -i ~/OTUs2/otu\_table.biom

#deactivating environment

source deactivate

* **Analyse diversity**

#!/bin/bash

#SBATCH -t 1:00:00

#SBATCH -n 24

#SBATCH -p short

#Load modules and qiime

module load eb

module load Miniconda2

source activate qiime

# setting temporary directory

export TMPDIR=~/qiime\_tmp

# diversity analyses

echo "core diversity analyses"

core\_diversity\_analyses.py \

-i ~/2018\_02\_smb/OTUs4/otu\_table\_mc2\_w\_tax\_no\_pynast\_failures.biom \

-m ~/2018\_02\_smb/map\_incomplete.tsv \

-o ~/2018\_02\_smb/Core\_Diversity \

-e 627 --recover\_from\_failure \

-t ~/2018\_02\_smb/OTUs4/rep\_set.tre

#deactivating environment

source deactivate

* **extract file**

scp -r cartesius:~/2018\_02\_smb /Users/Tanguybesson/Documents

scp –r Documents/complete\_map.tsv cartesius:~/2018\_02\_smb

* **Compare alpha diversity for metadata categories**

#!/bin/bash

#SBATCH -t 1:00:00

#SBATCH -n 24

#SBATCH -p short

#Load modules and qiime

module load eb

module load Miniconda2

source activate qiime

# setting temporary directory

export TMPDIR=~/qiime\_tmp

# alpha diversity comparison

echo "compare alpha diversity"

compare\_alpha\_diversity.py \

-i ~/2018\_02\_smb/Core\_Diversity/arare\_max627/alpha\_div\_collated/PD\_whole\_tree.txt \

-m ~/2018\_02\_smb/complete\_map.tsv \

-c SamplePhosphorus \

-o ~/2018\_02\_smb/Alpha/Alpha\_diversity\_PD\_whole\_tree.txt/SamplePhosphorus

#deactivating environment

source deactivate

* **COMPARE Categories**

#!/bin/bash

#SBATCH -t 1:00:00

#SBATCH -n 24

#SBATCH -p short

#Load modules and qiime

module load eb

module load Miniconda2

source activate qiime

# setting temporary directory

export TMPDIR=~/qiime\_tmp

# compare\_categories

echo "analyse statistical significance of sample groupings using distance matrices"

compare\_categories.py \

--method adonis

-I ~/2018\_02\_smb/Core\_Diversity/bdiv\_even627/unweighted\_unifrac\_dm.txt -m ~/2018\_02\_smb/complete\_map.tsv \

-m ~/2018\_02\_smb/complete\_map.tsv \

-c SamplePh

-o ~/2018\_02\_smb/Adonis\_potassium

#deactivating environment

source deactivate

* Make an OTU network to further use the output into cytoscape

#!/bin/bash

#SBATCH -t 1:00:00

#SBATCH -n 24

#SBATCH -p short

#Load modules and qiime

module load eb

module load Miniconda2

source activate qiime

# setting temporary directory

export TMPDIR=~/qiime\_tmp

#make otu network

echo "otu network"

make\_otu\_network.py \

-i ~/2018\_02\_smb/OTUs4/otu\_table\_mc2\_w\_tax\_no\_pynast\_failures.biom \

-m ~/2018\_02\_smb/complete\_map.tsv \

-o ~/2018\_02\_smb/OTU\_network

#deactivating environment

source deactivate

* **Split the OUT table by taxonomy at Level 2 corresponding to phylum classification**

#!/bin/bash

#SBATCH -t 1:00:00

#SBATCH -n 24

#SBATCH -p short

#Load modules and qiime

module load eb

module load Miniconda2

source activate qiime

# setting temporary directory

export TMPDIR=~/qiime\_tmp

#split\_otu\_by\_taxonomy

echo "otu\_table\_by\_taxonomy"

split\_otu\_table\_by\_taxonomy.py \

-i ~/2018\_02\_smb/OTUs4/otu\_table\_mc2\_w\_tax\_no\_pynast\_failures.biom \

-o ~/2018\_02\_smb/OTU\_table\_by\_taxonomy \

-L 2

#deactivating environment

source deactivate

* Make another OTU network at L2

#!/bin/bash

#SBATCH -t 1:00:00

#SBATCH -n 24

#SBATCH -p short

#Load modules and qiime

module load eb

module load Miniconda2

source activate qiime

# setting temporary directory

export TMPDIR=~/qiime\_tmp

#make otu network

echo "otu network"

make\_otu\_network.py \

-i ~/2018\_02\_smb/summarize\_taxa/otu\_table\_mc2\_w\_tax\_no\_pynast\_failures\_L2.biom \

-m ~/2018\_02\_smb/complete\_map.tsv \

-o ~/2018\_02\_smb/OTU\_network\_L2

#deactivating environment

source deactivate

* **Make an OTU heatmap at level 2**

#!/bin/bash

#SBATCH -t 1:00:00

#SBATCH -n 24

#SBATCH -p short

#Load modules and qiime

module load eb

module load Miniconda2

source activate qiime

# setting temporary directory

export TMPDIR=~/qiime\_tmp

#make otu heatmap

echo "otu heatmap"

make\_otu\_heatmap.py \

-I ~/2018\_02\_smb/summarize\_taxa/otu\_table\_mc2\_w\_tax\_no\_pynast\_failures\_L2.biom \

-o ~/heatmaps \

-t ~/2018\_02\_smb/SILVA\_128\_QIIME\_release/trees/97/97\_otus.tre \

-m ~/2018\_02\_smb/complete\_map.tsv \

-c SamplePh

#deactivating environment

source deactivate

* **Group significance**

#!/bin/bash

#SBATCH -t 1:00:00

#SBATCH -n 24

#SBATCH -p short

#Load modules and qiime

module load eb

module load Miniconda2

source activate qiime

# setting temporary directory

export TMPDIR=~/qiime\_tmp

#groupsignificance

echo "pH group significance"

group\_significance.py \

-I ~/2018\_02\_smb/summarize\_taxa/otu\_table\_mc2\_w\_tax\_no\_pynast\_failures\_L2.biom \

-o ~/2018\_02\_smb/group\_significance\_pH \

-m ~/2018\_02\_smb/complete\_map.tsv \

-c SamplePh

#deactivating environment

source deactivate

* **Filter taxa for the the 10 most abundant phylum**

#!/bin/bash

#SBATCH -t 1:00:00

#SBATCH -n 24

#SBATCH -p short

#Load modules and qiime

module load eb

module load Miniconda2

source activate qiime

# setting temporary directory

export TMPDIR=~/qiime\_tmp

#filter\_taxa

echo "filter taxa"

filter\_taxa\_from\_otu\_table.py \

-I ~/2018\_02\_smb/summarize\_taxa/otu\_table\_mc2\_w\_tax\_no\_pynast\_failures\_L2.biom \

-o ~/2018\_02\_smb/filter\_taxa \

-p p\_Proteobacteria, p\_Acidobacteria, p\_Actinobacteria, p\_Verrucomicrobia, p\_Bacteriodetes, p\_Plantomyetes, p\_Gemmatimonadetes, p\_Chloroflexi, p\_Crenarchaeota, p\_Nitrospirae, p\_Firmicutes

#deactivating environment

source deactivate