Mothur Illumina Tutorial

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Contents

1 Aim of tutorial

This tutorial explain how to process Illumina sequences.

- The first part of the tutorial makes use of R to obtain information on the number and quality of sequences.
- The second part uses mothur to process the sequences and compute the final abundance table.

2 Directory structure

- **/fastq_carbom** : fastq files from the carbom cruise
- **/databases** : Silva alignement and PR2 database files (see Prerequisite above)
- **/mothur/illumina** : Tutorial for Illumina files (carbom cruise)
- **/mothur/454** : Tutorial with 454 files

3 Downloads

Install the following software :

- Terminal program. For Windows MobaXterm is highly recommended : https://mobaxterm.mobatek.net/
- Notepad++ if you are using windows : https://notepad-plus-plus.org/
- Mothur : https://github.com/mothur/mothur/releases/tag/v1.39.5
- R : https://pbil.univ-lyon1.fr/CRAN/
- R studio : https://www.rstudio.com/products/rstudio/download/#download

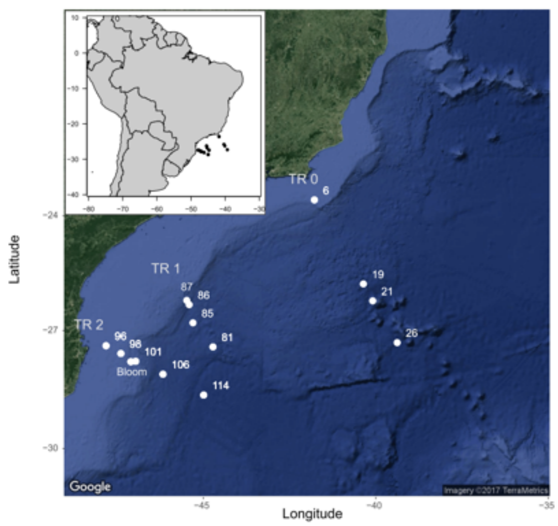
- Download and install the following libraries by running under R studio the following lines

```
install.packages("dplyr")      # To manipulate dataframes
install.packages("stringr")    # To strings

install.packages("ggplot2")    # for high quality graphics

source("https://bioconductor.org/biocLite.R")
biocLite("Biostrings")         # manipulate sequences
biocLite('dada2')              # metabarcode data analysis
```

Download and install in the **/databases directory**

- PR2 database : https://github.com/vaulot/pr2_database/releases/download/4.7.2/pr2_version_4.7.2_mothur.zip .

4 Data used



The samples originate from the CARBOM cruise (2013) off Brazil.

Samples have been sorted by flow cytometry and 3 genes have been PCR amplified :

- 18S rRNA - V4 region
- 16S rNA with plastid
- nifH

The PCR products have been sequenced by 1 run of Illumina 2*250 bp. The data consist of the picoplankton samples from one transect and fastq files have been subsampled with 1000 sequences per sample.

4.1 References

- Gérikas Ribeiro C, Marie D, Lopes dos Santos A, Pereira Brandini F, Vaulot D. (2016). Estimating microbial populations by flow cytometry: Comparison between instruments. Limnol Oceanogr Methods 14:750–758.
- Gérikas Ribeiro C, Lopes dos Santos A, Marie D, Brandini P, Vaulot D. (2018). Relationships between photosynthetic eukaryotes and nitrogen-fixing cyanobacteria off Brazil. ISME J in press.

- Gérikas Ribeiro C, Lopes dos Santos A, Marie D, Helena Pellizari V, Pereira Brandini F, Vaulot D. (2016). Pico and nanoplankton abundance and carbon stocks along the Brazilian Bight. PeerJ 4:e2587.

5 Pre visualization of the fastq files with R

- Load the script file from **/mothur/illumina/R_analyze_fastq.R**

Load the necessary libraries

```
library("dada2")
library("Biostrings") # To manipulate DNA sequences

library("ggplot2")
library("stringr")
library("dplyr")
```

(1) **Set up the directories for the analysis**

```
# change the following line to the path where you unzipped the tutorials
  tutorial_dir <- "C:/Users/vaulot/Google Drive/Scripts/"

# set up working directory
  working_dir <- paste0( tutorial_dir, "metabarcodes_tutorials/mothur/illumina")
  setwd(working_dir)

# ngs directory
  ngs_dir <- paste0( tutorial_dir, "metabarcodes_tutorials/fastq_carbom")

# get a list of all fastq files in the ngs directory and separate R1 and R2
  fns <- sort(list.files(ngs_dir, full.names = TRUE))
  fns <- fns[str_detect( basename(fns),".fastq")]
  fns_R1 <- fns[str_detect( basename(fns),"R1")]
  fns_R2 <- fns[str_detect( basename(fns),"R2")]
```

(2) **Compute number of paired reads in each fastq file**

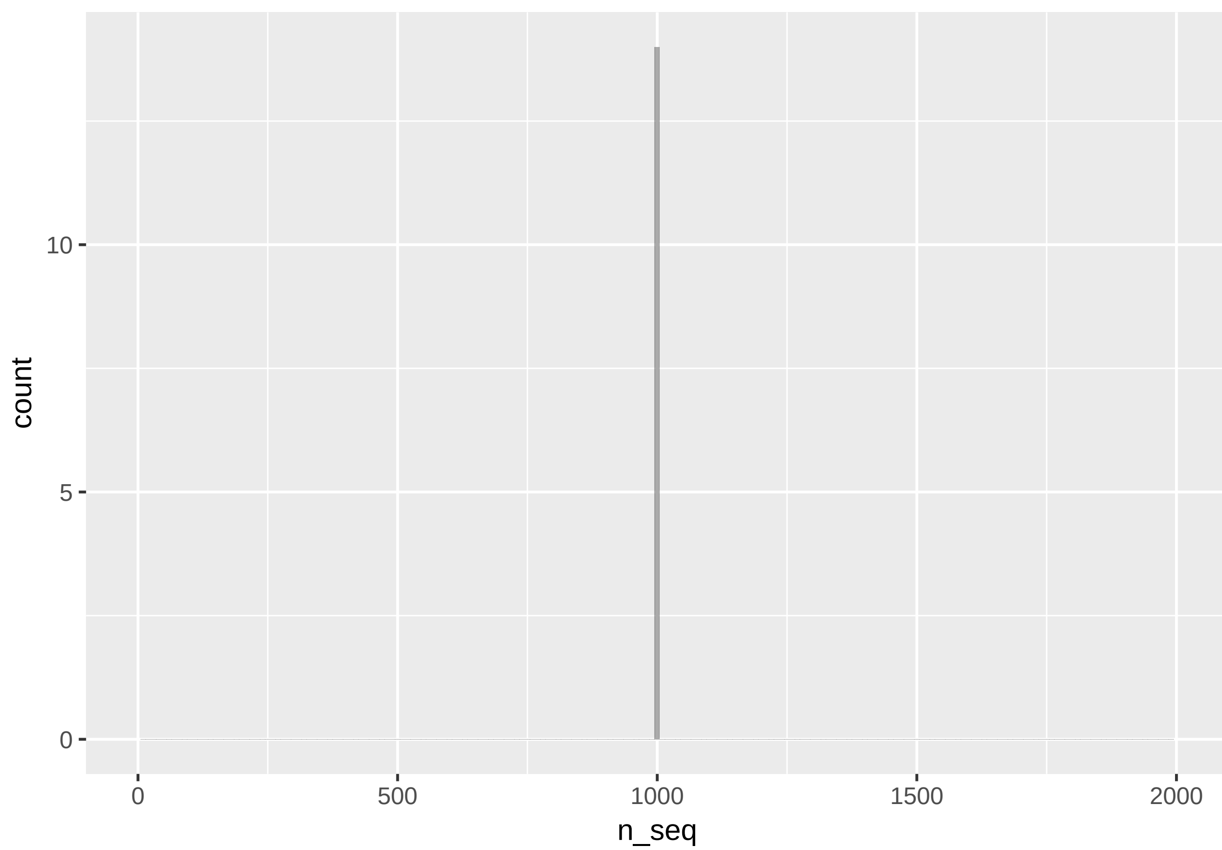
Note that the data have been sub-sampled at 1000 reads per file.

```
# create an empty data frame
  df <- data.frame()

# loop throuh all the R1 files (no need to go through R2 which should be the same)

  for(i in 1:length(fns_R1)) {

    # use the dada2 function fastq.geometry
      geom <- fastq.geometry(fns_R1[i])

    # extract the information on number of sequences and file name
      df_one_row <- data.frame (n_seq=geom[1], file_name=basename(fns[i]) )

    # add one line to data frame
      df <- bind_rows(df, df_one_row)
 }
# display number of sequences and write data to small file
```

```
df
```

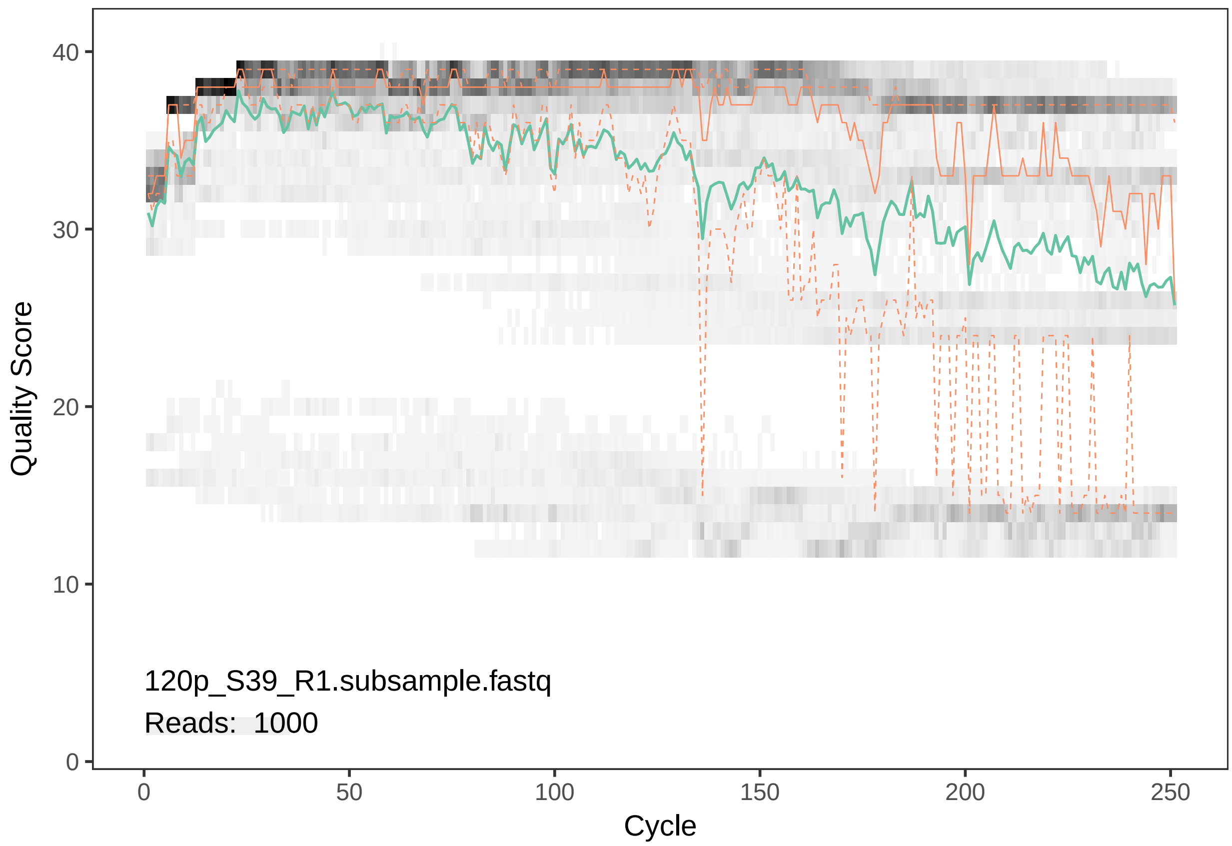
```
##    n_seq                   file_name
## 1   1000 120p_S39_R1.subsample.fastq
## 2   1000 120p_S39_R2.subsample.fastq
## 3   1000 121p_S57_R1.subsample.fastq
## 4   1000 121p_S57_R2.subsample.fastq
## 5   1000  122p_S4_R1.subsample.fastq
## 6   1000  122p_S4_R2.subsample.fastq
## 7   1000 125p_S22_R1.subsample.fastq
## 8   1000 125p_S22_R2.subsample.fastq
## 9   1000 126p_S40_R1.subsample.fastq
## 10  1000 126p_S40_R2.subsample.fastq
## 11  1000  140p_S5_R1.subsample.fastq
## 12  1000  140p_S5_R2.subsample.fastq
## 13  1000 141p_S23_R1.subsample.fastq
## 14  1000 141p_S23_R2.subsample.fastq
```

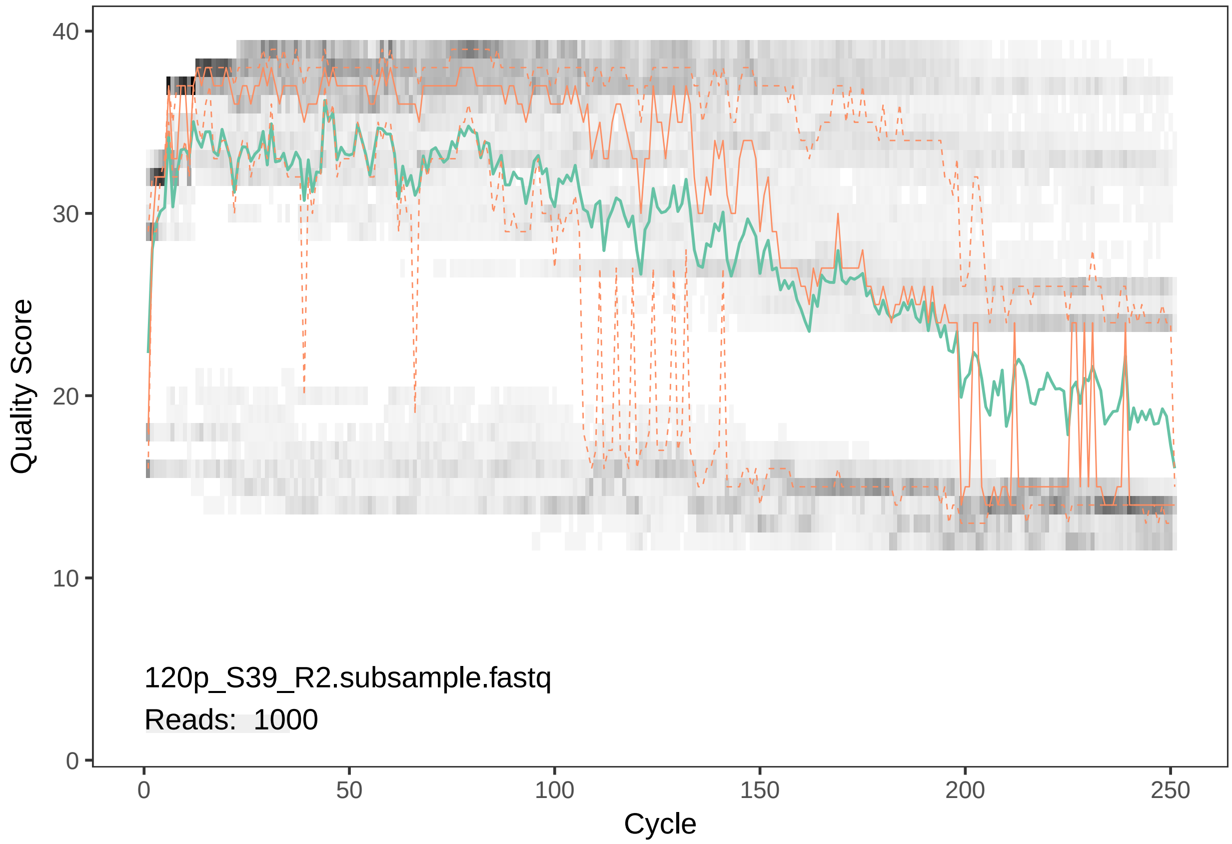
```
  write.table(df, file = paste0(working_dir,"/n_seq.txt"), sep="\t", row.names = FALSE, na="", quote=F

# plot the histogram with number of sequences
  ggplot(df, aes(x=n_seq)) +
        geom_histogram( alpha = 0.5, position="identity", binwidth = 10) +
        xlim(0, 2000)
```



(3) **Plot the quality fo each fastq file**

```
# loop throuh all the R1 files (no need to go through R2 which should be the same)

 for(i in 1:2) {

  # Use dada2 function to plot quality
    p1 <- plotQualityProfile(fns[i])

  # Only plot on screen for first 2 files
    print(p1)

  # save the file as a pdf file
    p1_file <- paste0(ngs_dir,"/qual/",basename(fns[i]),".pdf")
    ggsave( plot=p1, filename= p1_file,
              device = "pdf", width = 15, height = 15, scale=1, units="cm")
  }
```





(4) **Clean up memory**

It is necessary to clean up the memory because the fastq files are quite big and occupy a lot of memory during processing

```
rm(list=ls())
```

6 Analysis with mothur

6.1 Major processing steps

- Build the contigs from the R1 and R2 reads
- Extract the sequences that contain the 2 primers
- Remove sequences in low abundance (singletons in particular)
- Align sequences to a reference alignment
- Remove chimeras
- Assign taxonomy based on PR2
- Compute sequence distance
- Cluster sequences at a given threhold (make OTUs)
- Create a final file with all the information

Note that some of the steps have been removed for simplicity.

6.2 How to run the script

Two files containing all the commands are provided

- mothur_carbom_linux.sh : use on a Linux/Mac
- mothur_carbom_windows.cmd : use on Windows

1. Open the relevant file with an editor such as Notepad++
2. Change lines 2 and 4 to correspond to the location of the fastq files and mothur executable
3. Open either a terminal window (Linux/Mac) or a cmd window (DOS)
4. Copy and paste each line in turn in the terminal window

6.3 Step by step

(1) **First define a few constants to make the script independant of the files**

Under Linux/Mac

```
# 1. Change the path below to the path where you have downloaded the fastQ files
DIR_DATA="/home/metabarcodes_tutorials/fastq_carbom"

# 2. Change the path below to the path where you have downloaded the mothuer fules
MOTHUR="/usr/local/genome2/mothur-1.39.5/mothur"

# Nothing else to change below
FILE_PR2_TAX="../databases/pr2_version_4.72_mothur.tax"
FILE_PR2_FASTA="../databases/pr2_version_4.72_mothur.fasta"
FILE_SILVA="../databases/silva.seed_v123.euk.fasta"
FILE_PR2_END="72"
FILE_OLIGOS="../databases/oligos18s_V4_Zingone.oligos"
PROJECT="carbom"
```

Under Windows syntax is slightly different (for the next steps we use the Linux/Mac syntax).

```
:: 1. Change the path below to the path where you have downloaded the fastQ files
SET DIR_DATA="C:\Users\vaulot\Google Drive\Scripts\metabarcodes_tutorials\fastq_carbom"

:: 2. Change the path below to the path where you have downloaded the mothur program
SET MOTHUR="C:\Program Files (x86)\mothur\mothur.exe"

:: Nothing else to change below

SET FILE_PR2_TAX="..\databases\pr2_version_4.72_mothur.tax"
SET FILE_PR2_FASTA="..\databases\pr2_version_4.72_mothur.fasta"
SET FILE_PR2_END="72"
SET FILE_SILVA="..\databases\silva.seed_v123.euk.fasta"
SET FILE_OLIGOS="..\databases\oligos18s_V4_Zingone.oligos"

SET PROJECT="carbom"
```

(2) **Change directory to where the fastq files are located**

```
cd $DIR_DATA
```

(3) **Make the contigs using the file $PROJECT.txt (= carbom.txt).**

This file has the following structure :

Sample	R1 file	R2 file
120p	120p_S39_R1.subsample.fastq	120p_S39_R2.subsample.fastq
121p	121p_S57_R1.subsample.fastq	121p_S57_R2.subsample.fastq
122p	122p_S4_R1.subsample.fastq	122p_S4_R2.subsample.fastq

```
$MOTHUR "#make.contigs(file=$PROJECT.txt, processors=32)"
```

(4) **Remove sequences that do not satisfy the following conditions:**

- Number of ambiguities = 0
- Minlength=350
- Maxlength=450

```
$MOTHUR "#screen.seqs(fasta=$PROJECT.trim.contigs.fasta,group=$PROJECT.contigs.groups,
                      maxambig=0,minlength=350, maxlength=450, processors=32)"
```

(5) **Extract the sequences based on the presence of forward and reverse primers**

- Mismatches allowed on the forward primer - pdiffs=2,
- Mismatches allowed on the reverse primer - rdiffs=2
- Oligo file : oligos18s_V4_Zingone.oligos

Keyword	Primer forward	Primer reverse	Name of primer
primer	CCAGCASCYGCGGTAATTCC	ACTTTCGTTCTTGATYRATGA	18S_V4_Zingone

```
$MOTHUR "#pcr.seqs(fasta=$PROJECT.trim.contigs.good.fasta,
                   group=$PROJECT.contigs.good.groups,
                   oligos=$FILE_OLIGOS,
                   pdiffs=2, rdiffs=2,
                   processors=32)"
```

(6) **Shorten file names and indicate gene name**

```
cp $PROJECT.trim.contigs.good.pcr.fasta $PROJECT_18S.fasta
cp $PROJECT.contigs.good.pcr.groups $PROJECT_18S.groups
```

(7) **Dereplicate unique sequences**

```
$MOTHUR "#unique.seqs(fasta=$PROJECT_18S.fasta)"
```

(8) **Create a count file**

This file create a table which as the following structure. For each unique sequence, it provides the total number of sequences and the number of sequences in each sample.

```
Representative_Sequence total   120p    121p    122p    125p    126p
M02439_22_000000000-AD0LA_1_1101_14247_1437 277 46  35  0   12  20
M02439_22_000000000-AD0LA_1_1101_12787_1647 2   2   0   0   0   0
M02439_22_000000000-AD0LA_1_1101_17899_1772 2   2   0   0   0   0
M02439_22_000000000-AD0LA_1_1101_13893_1778 1   1   0   0   0   0
```

This step saves disk space and speed up analysis

```
$MOTHUR "#count.seqs(name=$PROJECT_18S.names,
                     group=$PROJECT_18S.groups, processors=32)"
```

(9) **Remove singletons**

One can change the settings with the cutoff parameter.

```
$MOTHUR "#split.abund(count=$PROJECT_18S.count_table,
                      fasta=$PROJECT_18S.unique.fasta,
                      cutoff=1, accnos=true)"
```

(10) **Align sequences to reference alignement**

The file to be used can be downloaded from the mothur web site : https://www.mothur.org/w/images/a/a4/Silva.seed_v128.tgz. It is best to :

- extract only the eukaryotes using mothur command: `get.lineage(taxonomy=$SILVA.tax, taxon=Eukaryota, fasta=$SILVA.align)`
- remove all the gaps that are common to all sequences with mothur command `filter.seqs` (see next line)

```
$MOTHUR "#align.seqs(fasta=$PROJECT_18S.unique.abund.fasta,
                     reference=$FILE_SILVA,
                     flip=T, processors=32)"
```

(11) **Remove all the gaps that are common to all sequences**

```
$MOTHUR "#filter.seqs(fasta=$PROJECT_18S.unique.abund.align, processors=32)"
```

(12) **Precluster the sequences**

The number of differences taken into account can be changed. In general use `diffs=2`. However if one does not want to make OTUS for example to look at fine genetic variation, it is necessary to remove this step.

```
$MOTHUR "#pre.cluster(fasta=$PROJECT_18S.unique.abund.filter.fasta,
                      count=$PROJECT_18S.abund.count_table,
                      diffs=1, processors=32)"
```

(13) **Remove chimeras**

```
$MOTHUR "#chimera.uchime(fasta=$PROJECT_18S.unique.abund.filter.precluster.fasta,
                         count=$PROJECT_18S.unique.abund.filter.precluster.count_table,
                         processors=32)"

$MOTHUR "#remove.seqs(fasta=$PROJECT_18S.unique.abund.filter.precluster.fasta,
                      accnos=$PROJECT_18S.unique.abund.filter.precluster.denovo.uchime.accnos,
                      count=$PROJECT_18S.unique.abund.filter.precluster.count_table)"
```

(14) **Remove sequences in low abundance (here cutoff=2)**

It is critical to remove the sequences in low abundance to speed up processing. In general use `cutoff = 10`.

```
$MOTHUR "#split.abund(count=$PROJECT_18S.unique.abund.filter.precluster.pick.count_table,
                      fasta=$PROJECT_18S.unique.abund.filter.precluster.pick.fasta,
                      cutoff=2, accnos=true)"
```

(15) **Remove sequences that are too short or too long (here minlength=200)**

```
$MOTHUR "#screen.seqs(fasta=$PROJECT_18S.unique.abund.filter.precluster.pick.abund.fasta,
                      count=$PROJECT_18S.unique.abund.filter.precluster.pick.abund.count_table,
                      minlength=200, processors=32)"
```

(16) **Rename files to remember that sequences in low abundance where removed**

```
cp $PROJECT_18S.unique.abund.filter.precluster.pick.abund.good.fasta
   $PROJECT_18S.uniq.preclust.no_chim.more_than_2.fasta
cp $PROJECT_18S.unique.abund.filter.precluster.pick.abund.good.count_table
   $PROJECT_18S.uniq.preclust.no_chim.more_than_2.count_table
```

(17) **Classify the sequences using the PR2 database**

Two files are required

- pr2.fasta
- pr2.taxo

```
$MOTHUR "#classify.seqs(fasta=$PROJECT_18S.uniq.preclust.no_chim.more_than_2.fasta,
            count=$PROJECT_18S.uniq.preclust.no_chim.more_than_2.count_table,
            reference=$FILE_PR2.fasta, taxonomy=$FILE_PR2.tax,
            processors=32,
            probs=T)"
```

(18) **Compute distance matrix**

It is critical to have as few sequences as possible at this step because the computation time is proportionnal to the **square** of the number of sequences.

```
$MOTHUR "#dist.seqs(fasta=$PROJECT_18S.uniq.preclust.no_chim.more_than_2.fasta, processors=32)"
```

(19) **Cluster the sequences to create the OTUs**

Here we use a 0.02 cutoff corresponding to 98% similarity.

```
$MOTHUR "#cluster(column=$PROJECT_18S.uniq.preclust.no_chim.more_than_2.dist,
                  count=$PROJECT_18S.uniq.preclust.no_chim.more_than_2.count_table,
                  cutoff=0.02, processors=32)"
```

(20) **Classify the OTUs based on the classification of the sequences (see above)**

```
$MOTHUR "#classify.otu(taxonomy=$PROJECT_18S.uniq.preclust.no_chim.more_than_2.$FILE_PR2_END.wang.taxonomy,
                       count=$PROJECT_18S.uniq.preclust.no_chim.more_than_2.count_table,
                       list=$PROJECT_18S.uniq.preclust.no_chim.more_than_2.opti_mcc.list,
                       label=0.02, probs=F, basis=sequence)"
```

(21) **Get sequences represnetative of each OTU**

```
$MOTHUR "#get.oturep(fasta=$PROJECT_18S.uniq.preclust.no_chim.more_than_2.fasta,
                     column=$PROJECT_18S.uniq.preclust.no_chim.more_than_2.dist,
                     count=$PROJECT_18S.uniq.preclust.no_chim.more_than_2.count_table,
                     list=$PROJECT_18S.uniq.preclust.no_chim.more_than_2.opti_mcc.list,
                     method=abundance,
                     cutoff=0.02)"
```

(22) **Format the final result in a single synthetic file**

- otu id
- abundance in each sample
- representative sequence
- taxonomy

```
$MOTHUR "#create.database(list=$PROJECT_18S.uniq.preclust.no_chim.more_than_2.opti_mcc.list,
          count=$PROJECT_18S.uniq.preclust.no_chim.more_than_2.opti_mcc.0.02.rep.count_table,
          label=0.02,
          repfasta=$PROJECT_18S.uniq.preclust.no_chim.more_than_2.opti_mcc.0.02.rep.fasta ,
          constaxonomy=$PROJECT_18S.uniq.preclust.no_chim.more_than_2.opti_mcc.0.02.cons.taxonomy)"
```

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	OTUNu	120p	121p	122p	125p	126p	140p	141p	142p	155p	156p	157p	165p	166p	167p	repSeqName	repSeq	OTUConTaxonomy
2	Otu01	72	54	0	14	26	0	0	14	81	64	19	0	38	0	M02439_22_000000(	....................AGCTCCAATAGCG	Eukaryota;Hacrobia;Haptophyta;Prymnesiophyceae;Prymnesiophyceae_X;Braarudosphaeraceae;Braarudosphaeraceae_X;Braarudosp
3	Otu02	0	1	22	8	24	0	58	11	0	12	101	0	0	9	M02439_22_000000(	....................AGCTCCAATAGCG	Eukaryota;Archaeplastida;Chlorophyta;Mamiellophyceae;Mamiellales;Bathycoccaceae;Bathycoccus;Bathycoccus_prasinos;
4	Otu03	0	17	62	39	0	0	0	0	0	0	37	0	0	0	M02439_22_000000(	....................AGCTCCAATAGCG	Eukaryota;Archaeplastida;Chlorophyta;Mamiellophyceae;Mamiellales;Bathycoccaceae;Ostreococcus;Ostreococcus_tauri;
5	Otu04	6	20	0	18	0	0	0	0	0	1	0	9	0	100	M02439_22_000000(	....................AGCTCCAATAGCG	Eukaryota;Stramenopiles;Ochrophyta;Chrysophyceae;Chrysophyceae_X;Chrysophyceae_Clade-G;Chrysophyceae_Clade-G_X;Chryso
6	Otu05	0	0	0	0	0	154	0	0	0	0	0	0	0	0	M02439_22_000000(	....................AGCTCCAATAGCG	Eukaryota;Opisthokonta;Fungi;Basidiomycota;Agaricomycotina;Agaricomycetes;Hyphodontia;Hyphodontia_sp.;
7	Otu06	0	0	0	0	0	0	0	0	0	0	0	0	134	0	M02439_22_000000(	....................AGCTCCAATAGCG	Eukaryota;Alveolata;Dinophyta;Dinophyceae;Dinophyceae_X;Dinophyceae_XX;Gonyaulax;Gonyaulax_polygramma;
8	Otu07	0	29	0	0	0	0	0	74	0	0	0	0	0	0	M02439_22_000000(	....................AGCTCCAATAGCG	Eukaryota;Alveolata;Dinophyta;Dinophyceae;Dinophyceae_X;Dinophyceae_XX;Prorocentrum;Prorocentrum_sp.;
9	Otu08	0	0	0	0	0	0	100	1	0	0	0	0	0	0	M02439_22_000000(	....................AGCTCCAATAGCG	Eukaryota;Archaeplastida;Streptophyta;Klebsormidiophyceae;Klebsormidiophyceae_X;Klebsormidiophyceae_XX;Klebsormidium;Klebs
10	Otu09	0	0	100	0	0	0	0	0	0	0	0	0	0	0	M02439_22_000000(	....................AGCTCCAATAGCG	Eukaryota;Alveolata;Dinophyta;Syndiniales;Dino-Group-III;Dino-Group-III_X;Dino-Group-III_XX;Dino-Group-III_XX_sp.;
11	Otu10	1	0	0	0	0	0	0	88	0	0	0	0	0	0	M02439_22_000000(	....................AGCTCCAATAGCG	Eukaryota;Alveolata;Dinophyta;Dinophyceae;Dinophyceae_X;Dinophyceae_XX;Dinophyceae_XX_unclassified;Dinophyceae_XX_unclas
12	Otu11	0	1	0	5	0	38	0	0	23	6	0	0	0	0	M02439_22_000000(	....................AGCTCCAATAGCG	Eukaryota;Stramenopiles;Ochrophyta;Dictyochophyceae;Dictyochophyceae_X;Pedinellales;Pedinellales_X;Pedinellales_X_sp.;
13	Otu12	0	0	0	0	39	0	0	0	0	0	19	0	0	0	M02439_22_000000(	....................AGCTCCAATAGCG	Eukaryota;Alveolata;Dinophyta;Dinophyceae;Dinophyceae_X;Dinophyceae_XX;Prorocentrum;Prorocentrum_unclassified;
14	Otu13	0	0	0	0	0	0	8	0	0	21	0	20	0	8	M02439_22_000000(	....................AGCTCCAATAGCG	Eukaryota;Stramenopiles;Ochrophyta;Bacillariophyta;Bacillariophyta_X;Raphid-pennate;Raphid-pennate_unclassified;Raphid-pennate_
15	Otu14	0	0	0	0	0	0	0	0	0	51	0	0	0	0	M02439_22_000000(	....................AGCTCCAATAGCG	Eukaryota;Stramenopiles;Stramenopiles_X;Bicoecea;Borokales;Borokaceae;Borokaceae_X;Borokaceae_X_sp.;
16	Otu15	0	0	4	0	0	0	0	2	0	0	0	0	0	41	M02439_22_000000(	....................AGCTCCAATAGCG	Eukaryota;Opisthokonta;Fungi;Ascomycota;Saccharomycotina;Saccharomycetales;Debaryomyces;Debaryomyces_hansenii;
17	Otu16	0	0	0	0	0	0	0	0	0	0	0	44	0	0	M02439_22_000000(	....................AGCTCTAATAGCG	Eukaryota;Hacrobia;Cryptophyta;Cryptophyceae;Cryptophyceae_X;Cryptomonadales;Teleaulax;Teleaulax_sp.;
18	Otu17	0	0	0	0	44	0	0	0	0	0	0	0	0	0	M02439_22_000000(	....................AGCTCCAATAGCG	Eukaryota;Stramenopiles;Ochrophyta;Chrysophyceae;Chrysophyceae_X;Chrysophyceae_X_unclassified;Chrysophyceae_X_unclassifi
19	Otu18	0	0	0	44	0	0	0	0	0	0	0	0	0	0	M02439_22_000000(	....................AGCTCCAATAGCG	Eukaryota;Alveolata;Dinophyta;Dinophyceae;Suessiales;Suessiales_X;Karlodinium;Karlodinium_sp.;
20	Otu19	0	1	0	12	0	0	15	0	0	0	0	10	3	0	M02439_22_000000(	....................AGCTCCAATAGCG	Eukaryota;Stramenopiles;Ochrophyta;Pelagophyceae;Pelagomonadales;Pelagomonadaceae;Pelagomonas;Pelagomonas_calceolata;
21	Otu20	0	0	0	0	0	0	0	0	0	0	0	0	0	40	M02439_22_000000(	....................AGCTCCAATAGCG	Eukaryota;Stramenopiles;Stramenopiles_X;MOCH;MOCH-5;MOCH-5_X;MOCH-5_XX;MOCH-5_XX_sp.;
22	Otu21	0	0	0	0	0	0	0	0	0	0	0	0	0	32	M02439_22_000000(	....................AGCTCCAAGAGCC	Eukaryota;Opisthokonta;Fungi;Ascomycota;Saccharomycotina;Saccharomycetales;Saccharomycetales_unclassified;Saccharomyceta

7 What is next ?

- It is a good practice to confirm the phylogeny of at least the major OTUs by BLAST
- The database format can be easily used by the phyloseq package. A short tutorial can be found here : https://github.com/vaulot/R_tutorials

8 Alternative strategies

- Use the R dada2 package : https://benjjneb.github.io/dada2/tutorial.html
- Use vsearch : https://github.com/torognes/vsearch/wiki/VSEARCH-pipeline