DNA metabarcoding diet analysis in reindeer is quantitative and integrates feeding over several weeks

Stefaniya Kamenova[a,b,c,*], Pernille Meyer[a], Anne Krag Brysting[a], Leo Rescia[d], Lars P. Folkow[d], Galina Gusarova[a,d,e,f], Eric Coissac[g]

[a] *Centre for Ecological and Evolutionary Synthesis, University of Oslo, Oslo, Norway,*

[b] *Departments of Ecology and Natural Resource Management, Norwegian University of Life Sciences, Ås,*

[c] *National Museum of Natural History, Bulgarian Academy of Sciences, Sofia,*

[d] *Department of Arctic and Marine Biology, The Arctic University of Norway, Tromsø,*

[e] *The Arctic University Museum of Norway, The Arctic University of Norway, Tromsø,*

[f] *Department of Botany, St Petersburg State University, St Petersburg,*

[g] *Laboratoire d'Ecologie Alpine, CNRS, Université Grenoble Alpes, Grenoble,*

Abstract

Filtering of the EUKA02 DNA metabarcoding raw data.

1. Setting up the R environment

1.1. Install missing packages

```r
packages <- c("igraph", "tidyverse", "devtools","R.utils","vegan")
install.packages(setdiff(packages,
                         rownames(installed.packages())),
                 dependencies = TRUE
                )
```

1.2. Loading of the R libraries

- `ROBITools` package is used to read result files produced by OBITools.
- `ROBITaxonomy` package provides function allowing to query OBITools formated taxonomy.

```r
if (!"ROBITools" %in% rownames(installed.packages())) {
  # ROBITools are not available on CRAN and have to be installed
  # from http://git.metabarcoding.org using devtools

  metabarcoding_git <- "https://git.metabarcoding.org/obitools"
```

[*] Corresponding author

Email addresses: `stefaniya.kamenova@gmail.com` (Stefaniya Kamenova), `galina.gusarova@uit.no` (Galina Gusarova), `eric.coissac@metabarcoding.org` (Eric Coissac)

```r
  devtools::install_git(paste(metabarcoding_git,
                              "ROBIUtils.git",
                              sep="/"))

  devtools::install_git(paste(metabarcoding_git,
                              "ROBITaxonomy.git",
                              sep="/"))
  devtools::install_git(paste(metabarcoding_git,
                              "ROBITools.git",
                              sep="/"))
}

library(ROBITools)
library(ROBITaxonomy)
```

- `tidyverse`[1] provides various method for efficient data manipulation and plotting via `ggplot2`[2]

```r
library(tidyverse)
```

```r
library(R.utils)
```

```r
library(vegan)
```

```r
library(magrittr)
```

```r
source("methods.R")
```

```

Attaching package: 'matrixStats'

The following object is masked from 'package:dplyr':

    count


Attaching package: 'vctrs'

The following object is masked from 'package:dplyr':

    data_frame

The following object is masked from 'package:tibble':

    data_frame
```

2. Loading the data

2.1. Load the NCBI taxonomy

```
if (! file.exists("Data/ncbi20210212.adx")) {
  gunzip("Data/ncbi20210212.adx.gz",remove=FALSE)
  gunzip("Data/ncbi20210212.ndx.gz",remove=FALSE)
  gunzip("Data/ncbi20210212.rdx.gz",remove=FALSE)
  gunzip("Data/ncbi20210212.tdx.gz",remove=FALSE)
}
```

```
taxo <- read.taxonomy("Data/ncbi20210212")
```

2.2. Loading the metabarcoding data

```
if (! file.exists("Data/Rawdata/EUKA02_all_paired.ali.assigned.ann.diag.uniq.ann.c1.l10.clean.EMBL.
    gunzip("Data/Rawdata/EUKA02_all_paired.ali.assigned.ann.diag.uniq.ann.c1.l10.clean.EMBL.tag.ann


EUKA02.raw = import.metabarcoding.data("Data/Rawdata/EUKA02_all_paired.ali.assigned.ann.diag.uniq.a
```

2.3. Loading the metadata

```
samples.metadata = read_csv("Data/Faeces/sampling_dates.csv",
                            show_col_types = FALSE)
```

3. Sample description

Normalization of samples names

Extract information relative to PCR replicates and sample names.

```
sample_names_split = strsplit(as.character(sample_names), "_R")

replicate = sapply(sample_names_split, function(x) x[length(x)])
sample_id = sapply(sample_names_split, function(x) x[1])

samples_desc = data.frame(name = samples(EUKA02.raw)$sample, replicate = replicate, sample_id = sam

EUKA02.raw@samples = samples_desc
EUKA02.raw@motus <- EUKA02.raw@motus %>% select(-starts_with("obiclean_status:"))
```

3.1. Categorize MOTUs

DNA Sequence of the synthetic sequence used as EUKA02 positive controls.

```
Standard1 = "taagtctcgcactagttgtgacctaacgaatagagaattctataagacgtgttgtcccat"
```

- Identify which MOTU is corresponding to the positive control sequence and associated it to category `standard1`.

- All the MOTUs exhibiting a similarity with one of the reference SPER01 database greater than 80% is tagged as `EUKA02`
- The remaining sequences are tagged as `Unknown`

```
sequence_type = rep("Unknown", nrow(motus(EUKA02.raw)))
sequence_type[which(motus(EUKA02.raw)$`best_identity:db_EUKA`> 0.80)] = "EUKA02"
sequence_type[which(motus(EUKA02.raw)$sequence == Standard1)] = "standard1"

EUKA02.raw@motus$sequence_type = as.factor(sequence_type)
table(EUKA02.raw@motus$sequence_type)
```

```
 EUKA02 Unknown
 252502  223912
```

```
spermatophyta.taxid <- ecofind(taxo,patterns = "^Spermatophyta$")
lecanoromycetidae.taxid = ecofind(taxo,"^Lecanoromycetidae$")

to_keep = (is.subcladeof(taxo,EUKA02.raw@motus$taxid,spermatophyta.taxid) |
             EUKA02.raw@motus$taxid == spermatophyta.taxid) |
           (is.subcladeof(taxo,EUKA02.raw@motus$taxid,lecanoromycetidae.taxid) |
              EUKA02.raw@motus$taxid == lecanoromycetidae.taxid)

table(to_keep)
```

```
to_keep
 FALSE   TRUE
429806  46077
```

```
EUKA02.plant_lichen <- EUKA02.raw[,which(to_keep)]
```

4. Curation procedure

4.1. Select motus occuring at least at 1% in at least one PCR

```
norare = apply(decostand(reads(EUKA02.plant_lichen),method = "total"),
               MARGIN = 2,
               FUN = max) >= 0.01
table(norare)
```

```
norare
FALSE  TRUE
45862   215
```

```
EUKA02.norare <- EUKA02.plant_lichen[,which(norare)]
```

4.2. Filtering for PCR outliers

Only library 1 and 2 have individually tagged PCR replicates

```
library_3.ids = read.csv("Data/samples_library_3.txt",
                         stringsAsFactors = FALSE,
                         header = FALSE)[,1]
```

```
library3.keep = gsub("_R.?$","_R",rownames(EUKA02.norare)) %in% library_3.ids
EUKA02.lib3 = EUKA02.norare[library3.keep,]
EUKA02.lib12= EUKA02.norare[!library3.keep,]

dim(EUKA02.lib3)
```

```
[1]  63 215
```

```
dim(EUKA02.lib12)
```

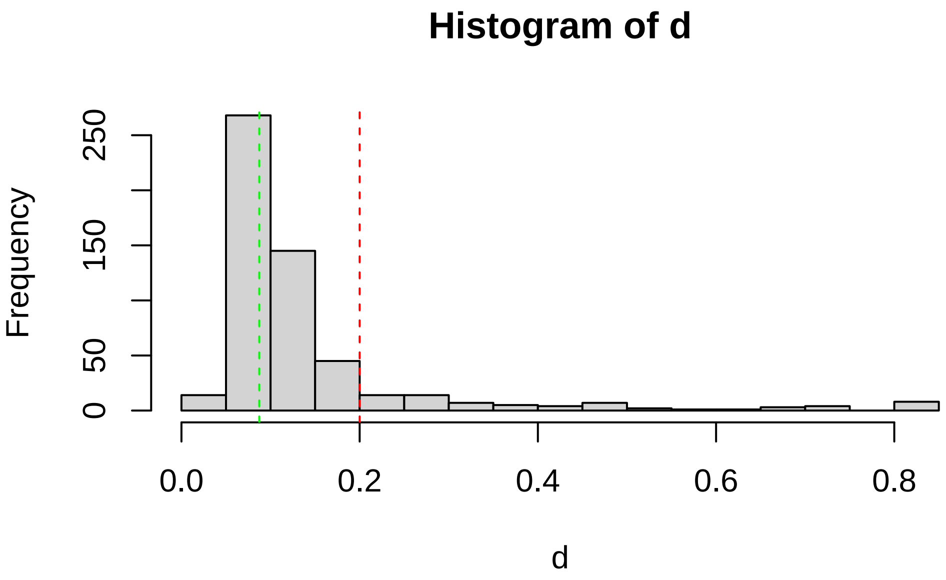
```
[1] 542 215
```

Load the script containing the selection procedure implemented in function `tag_bad_pcr`.

```
source("Select_PCR.R")
```

4.2.1. First selection round

```
keep1 = tag_bad_pcr(samples = samples(EUKA02.lib12)$sample_id,
                   counts = reads(EUKA02.lib12),
                   plot = TRUE,
                   threshold=0.2
                  )
```



Histogram shows the empirical distribution of the PCR replicate distances. The red vertical dashed line indicates the threshold used to discard outlier PCRs. The green vertical dashed line indicates the mode of the observed distribution.

```
table(keep1$keep)
```

```
FALSE  TRUE 
   45   497 
```

FALSEis the count of PCR to discard, TRUE the count of PCR conserved at the end of this selection round.

```
samples(EUKA02.lib12)$name[!keep1$keep]
```

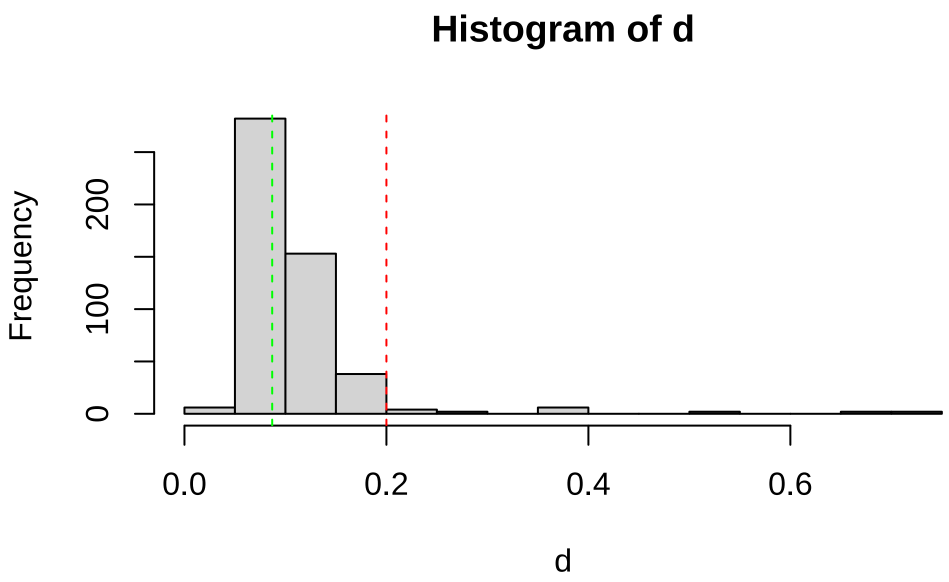
```
 [1] "DNANC11_R3"   "DNANC12_R3"   "DNANC13_R3"   "DNANC15_R3"   "DNANC_10_R2" 
 [6] "DNANC_12_R3"  "DNANC_13_R3"  "DNANC_15_R3"  "DNANC_7_R2"   "DNANC_8_R2"  
[11] "DNANC_9_R2"   "PCRNC_3_R2"   "PCRNC_4_R1"   "PCRNC_4_R2"   "PCRNC_5_R3"  
[16] "PCRNC_6_R3"   "PCRPOS_3_R2"  "X_28_R3"      "X_37_R3"      "X_3_R3"      
[21] "X_9_R3"       "Y_24_R3"      "Y_29_R2"      "Y_2_R2"       "Y_33_R2"     
[26] "Y_36_R2"      "Y_44_R2"      "Y_45_R3"      "Y_46_R2"      "Y_47_R3"     
[31] "Y_48_R2"      "Y_49_R3"      "Y_51_R2"      "Y_52_R1"      "Y_56_R1"     
[36] "Y_8_R1"       "Z_19_R3"      "Z_21_R2"      "Z_30_R1"      "Z_33_R3"     
[41] "Z_45_R3"      "Z_46_R3"      "Z_51_R2"      "Z_53_R2"      "Z_5_R2"      
```

Above is the list of the ids of the discarded PCRs.

```
EUKA02.lib12.k1 = EUKA02.lib12[keep1$keep,]
```

4.2.2. Second selection round

```
keep2 = tag_bad_pcr(samples = samples(EUKA02.lib12.k1)$sample_id,
                   counts = reads(EUKA02.lib12.k1),
                   plot = TRUE,
                   threshold=0.2
                  )
```



```
table(keep2$keep)
```

```
FALSE  TRUE 
   17   480 
```

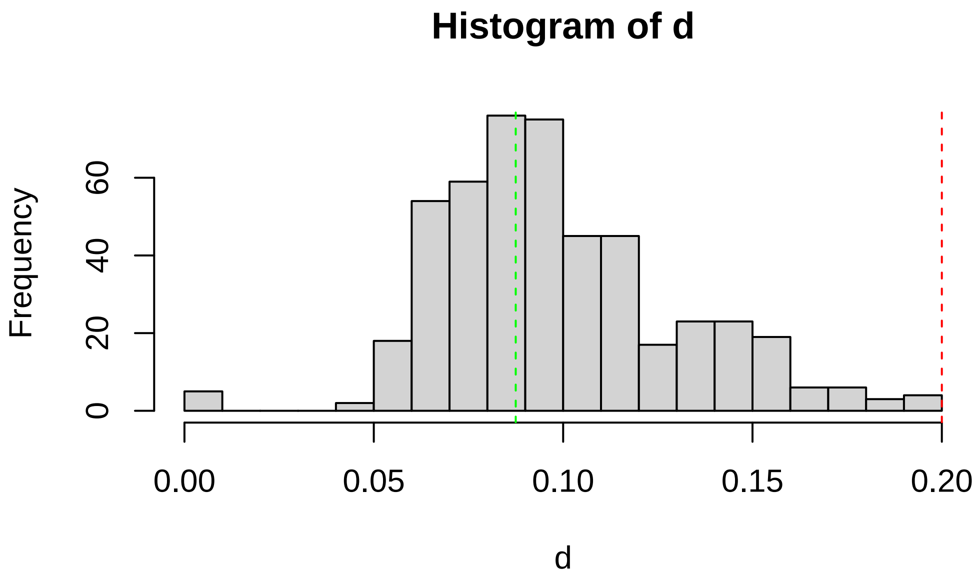
```
samples(EUKA02.lib12.k1)$name[!keep2$keep]
```

```
 [1] "Y_29_R1" "Y_29_R3" "Y_2_R1"  "Y_2_R3"  "Y_44_R1" "Y_44_R3" "Y_46_R3"
 [8] "Y_47_R1" "Y_47_R2" "Y_49_R1" "Y_49_R2" "Y_51_R1" "Y_51_R3" "Z_30_R2"
[15] "Z_30_R3" "Z_53_R1" "Z_53_R3"
```

```
EUKA02.lib12.k2 = EUKA02.lib12.k1[keep2$keep,]
```

4.2.3. Third selection round

```
keep3 = tag_bad_pcr(samples = samples(EUKA02.lib12.k2)$sample_id,
                    counts = reads(EUKA02.lib12.k2),
                    plot = TRUE,
                    threshold=0.2
                   )
```



```
table(keep3$keep)
```

```

FALSE  TRUE 
    1   479 
```

```
keep3[!keep3$keep,]
```

```
        samples distance maximum repeats  keep
Y_46_R1    Y_46        0       0       1 FALSE
```

```
EUKA02.lib12.k3 = EUKA02.lib12.k2[keep3$keep,]
```

4.2.4. Merge remaining PCR replicates

```
freq = decostand(reads(EUKA02.lib12.k3),
                 method = "total")
EUKA02.lib12.k3$count = reads(EUKA02.lib12.k3)
EUKA02.lib12.k3@reads = freq

EUKA02.merged = aggregate(EUKA02.lib12.k3, MARGIN = 1, by = list(sample_id=samples(EUKA02.lib12.k3)
```

4.3. Merge lib 1,2 and 3

4.3.1. Remove controls in library 3

Look for controls left in library 1 and 2

```
rownames(EUKA02.merged)
```

```
  [1] "DNANC_14" "X_10"     "X_11"     "X_12"     "X_14"     "X_15"    
  [7] "X_16"     "X_17"     "X_18"     "X_19"     "X_2"      "X_20"    
 [13] "X_21"     "X_22"     "X_23"     "X_24"     "X_25"     "X_26"    
 [19] "X_27"     "X_28"     "X_29"     "X_3"      "X_30"     "X_31"    
 [25] "X_33"     "X_34"     "X_35"     "X_36"     "X_37"     "X_38"    
 [31] "X_39"     "X_4"      "X_41"     "X_42"     "X_44"     "X_50"    
 [37] "X_51"     "X_53"     "X_54"     "X_56"     "X_57"     "X_59"    
 [43] "X_60"     "X_63"     "X_64"     "X_65"     "X_66"     "X_68"    
 [49] "X_70"     "X_74"     "X_75"     "X_76"     "X_77"     "X_78"    
 [55] "X_79"     "X_80"     "X_9"      "Y_1"      "Y_11"     "Y_13"    
 [61] "Y_14"     "Y_18"     "Y_21"     "Y_23"     "Y_24"     "Y_25"    
 [67] "Y_26"     "Y_28"     "Y_3"      "Y_31"     "Y_32"     "Y_33"    
 [73] "Y_34"     "Y_36"     "Y_38"     "Y_39"     "Y_4"      "Y_40"    
 [79] "Y_41"     "Y_42"     "Y_43"     "Y_45"     "Y_5"      "Y_50"    
 [85] "Y_52"     "Y_53"     "Y_56"     "Y_57"     "Y_58"     "Y_59"    
 [91] "Y_6"      "Y_61"     "Y_69"     "Y_7"      "Y_70"     "Y_71"    
 [97] "Y_72"     "Y_74"     "Y_8"      "Y_9"      "Z_1"      "Z_10"    
[103] "Z_11"     "Z_12"     "Z_13"     "Z_14"     "Z_15"     "Z_16"    
[109] "Z_17"     "Z_18"     "Z_19"     "Z_20"     "Z_21"     "Z_22"    
[115] "Z_23"     "Z_24"     "Z_25"     "Z_27"     "Z_28"     "Z_3"     
[121] "Z_31"     "Z_32"     "Z_33"     "Z_34"     "Z_35"     "Z_36"    
[127] "Z_37"     "Z_38"     "Z_4"      "Z_40"     "Z_42"     "Z_43"    
[133] "Z_44"     "Z_45"     "Z_46"     "Z_48"     "Z_49"     "Z_5"     
[139] "Z_51"     "Z_52"     "Z_54"     "Z_55"     "Z_56"     "Z_59"    
[145] "Z_6"      "Z_60"     "Z_61"     "Z_62"     "Z_63"     "Z_65"    
[151] "Z_66"     "Z_67"     "Z_68"     "Z_69"     "Z_7"      "Z_70"    
[157] "Z_71"     "Z_72"     "Z_73"     "Z_74"     "Z_75"     "Z_76"    
[163] "Z_77"     "Z_78"     "Z_79"     "Z_8"      "Z_80"    
```

4.4. Remove controls in library 3

```r
rownames(EUKA02.lib3)
```

```
 [1] "DNANC_1_R1"  "DNANC_2_R1"  "DNANC_3_R1"  "DNANC_4_R1"  "DNANC_5_R1" 
 [6] "DNANC_6_R1"  "DNANC_6_R2"  "PCRNC_1_R1"  "PCRNC_2_R1"  "PCRPOS_2_R1"
[11] "X_1_R1"      "X_32_R1"     "X_40_R1"     "X_43_R1"     "X_45_R1"    
[16] "X_46_R1"     "X_47_R1"     "X_48_R1"     "X_49_R1"     "X_52_R1"    
[21] "X_55_R1"     "X_58_R1"     "X_5_R1"      "X_62_R1"     "X_67_R1"    
[26] "X_69_R1"     "X_6_R1"      "X_71_R1"     "X_72_R1"     "X_73_R1"    
[31] "X_7_R1"      "X_8_R1"      "Y_10_R1"     "Y_12_R1"     "Y_15_R1"    
[36] "Y_16_R1"     "Y_17_R1"     "Y_19_R1"     "Y_20_R1"     "Y_22_R1"    
[41] "Y_27_R1"     "Y_35_R1"     "Y_37_R1"     "Y_54_R1"     "Y_55_R1"    
[46] "Y_60_R1"     "Y_62_R1"     "Y_63_R1"     "Y_64_R1"     "Y_65_R1"    
[51] "Y_66_R1"     "Y_67_R1"     "Y_68_R1"     "Y_73_R1"     "Z_26_R1"    
[56] "Z_29_R1"     "Z_2_R1"      "Z_41_R1"     "Z_47_R1"     "Z_50_R1"    
[61] "Z_57_R1"     "Z_58_R1"     "Z_9_R1"     
```

```r
EUKA02.lib3.samples = EUKA02.lib3[-(1:10),]
rownames(EUKA02.lib3.samples@reads) = sub("_R.?$","",rownames(EUKA02.lib3.samples))
rownames(EUKA02.lib3.samples)
```

```
 [1] "X_1"  "X_32" "X_40" "X_43" "X_45" "X_46" "X_47" "X_48" "X_49" "X_52"
```

```
[11] "X_55" "X_58" "X_5"  "X_62" "X_67" "X_69" "X_6"  "X_71" "X_72" "X_73"
[21] "X_7"  "X_8"  "Y_10" "Y_12" "Y_15" "Y_16" "Y_17" "Y_19" "Y_20" "Y_22"
[31] "Y_27" "Y_35" "Y_37" "Y_54" "Y_55" "Y_60" "Y_62" "Y_63" "Y_64" "Y_65"
[41] "Y_66" "Y_67" "Y_68" "Y_73" "Z_26" "Z_29" "Z_2"  "Z_41" "Z_47" "Z_50"
[51] "Z_57" "Z_58" "Z_9"
```

4.4.1. Merge library 1, 2 and 3

```
EUKA02.lib123.reads = rbind(EUKA02.merged@reads,
                            decostand(EUKA02.lib3.samples@reads,method = "total"))

common = intersect(names(EUKA02.merged@samples),
                   names(EUKA02.lib3.samples@samples))

EUKA02.lib123.samples = rbind(EUKA02.merged@samples[,common],
                          EUKA02.lib3.samples@samples[,common])

EUKA02.lib123 = metabarcoding.data(reads = decostand(EUKA02.lib123.reads,method = "total"),
                               samples = EUKA02.lib123.samples,
                               motus = EUKA02.merged@motus)


dim(EUKA02.lib123)
```

```
[1] 220 215
```

```
EUKA02.lib123@samples$animal_id = sapply(EUKA02.lib123@samples$sample_id,
                                                function(x) strsplit(as.character(x),"_")[[1]][1])
```

4.4.2. Check for empty MOTUs

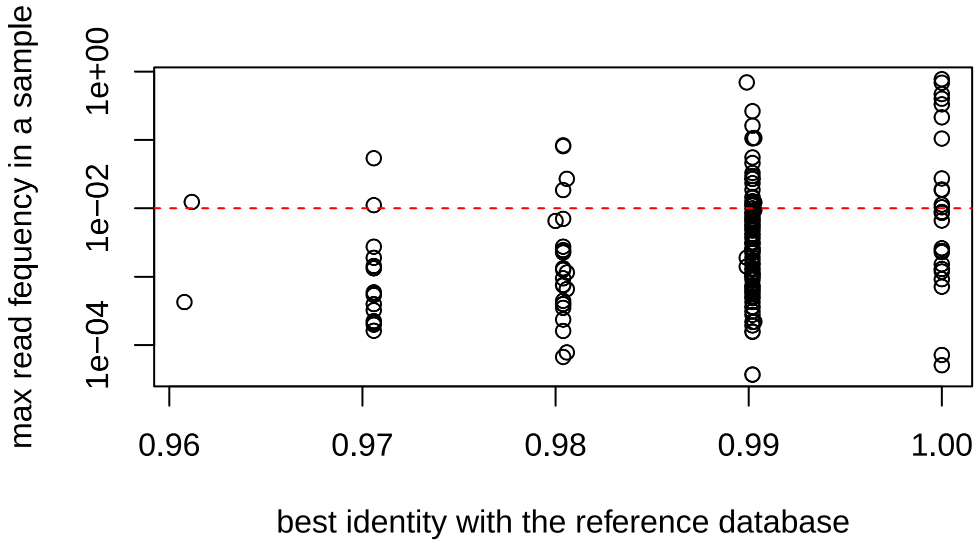
```
zero = colSums(reads(EUKA02.lib123)) == 0
table(zero)
```

```
zero
FALSE  TRUE
  174    41
```

```
EUKA02.nozero = EUKA02.lib123[,!zero]
```

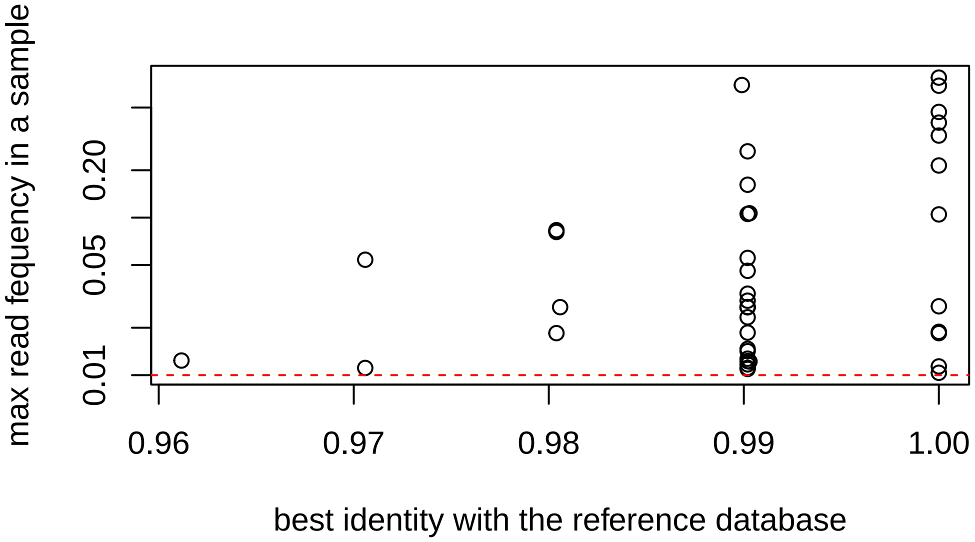
5. Filter out rare species

```
plot(EUKA02.nozero@motus$`best_identity:db_EUKA`,
     apply(reads(EUKA02.nozero),2,max),
     col=as.factor(EUKA02.nozero@motus$sequence_type),
     log="y",
     ylab="max read fequency in a sample",
     xlab="best identity with the reference database")
abline(h=0.01,col="red",lty=2)
abline(v=0.95,col="red",lty=2)
```



```
EUKA02.merged3 = EUKA02.lib123[, apply(reads(EUKA02.lib123),2,max) > 0.01]
```

```
plot(EUKA02.merged3$motus$`best_identity:db_EUKA`,
     apply(reads(EUKA02.merged3),2,max),
     col=as.factor(EUKA02.merged3$motus$sequence_type),
     log="y",
     ylab="max read fequency in a sample",
     xlab="best identity with the reference database")
abline(h=0.01,col="red",lty=2)
abline(v=0.95,col="red",lty=2)
```

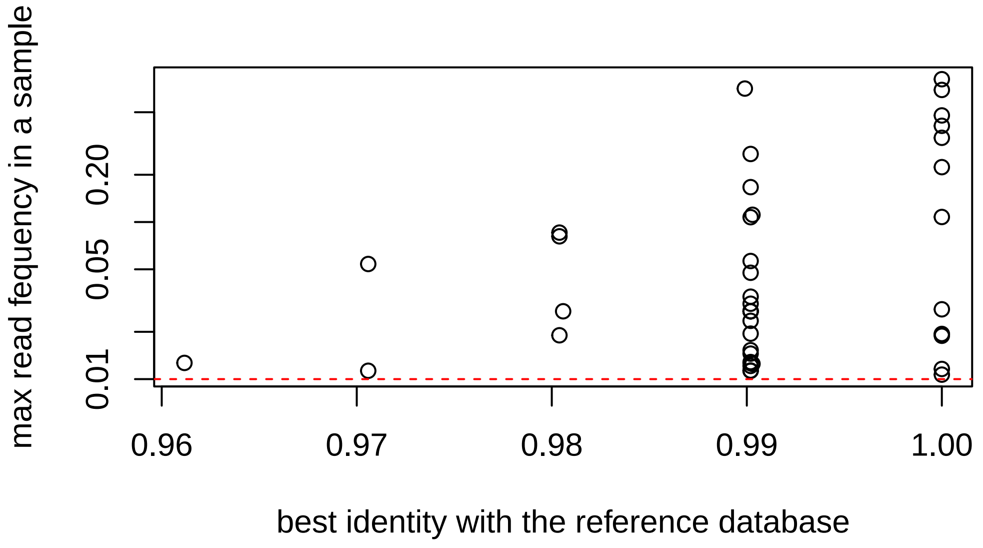


5.1. Keep only MOTUs Strictly identical to one of the reference sequence

5.1.1. First level stringency filter (95% identity)

```
EUKA02.merged4 = EUKA02.merged3[,EUKA02.merged3@motus$`best_identity:db_EUKA` > 0.95]
EUKA02.merged4@reads = decostand(EUKA02.merged4@reads,method = "total")
EUKA02.merged4@motus <- EUKA02.merged4@motus %>% select(-starts_with("obiclean_status:"))
```

```
plot(EUKA02.merged4$motus$`best_identity:db_EUKA`,
     apply(reads(EUKA02.merged4),2,max),
     col=as.factor(EUKA02.merged4$motus$sequence_type),
     log="y",
     ylab="max read fequency in a sample",
     xlab="best identity with the reference database")
abline(h=0.01,col="red",lty=2)
```



5.1.2. High stringency filtering

```
spermatophyta.taxid <- ecofind(taxo,patterns = "^Spermatophyta$")
EUKA02.merged4@motus$is_spermatophyta <- is.subcladeof(taxo,EUKA02.merged4@motus$taxid,spermatophyt
table(EUKA02.merged4@motus$is_spermatophyta)
```

```
FALSE  TRUE
    2    42
```

```
EUKA02.merged4@motus %>% filter(!is_spermatophyta)
```

```
                             id definition best_identity:db_EUKA
EUKAP2_00000018 EUKAP2_00000018                         0.989899
EUKAP1_00050174 EUKAP1_00050174                         1.000000
                best_match:db_EUKA  count family  family_name genus genus_name
EUKAP2_00000018           AJ549807 296011     NA         <NA>    NA       <NA>
EUKAP1_00050174           AF515608    274  39933 Lecanoraceae 39934   Lecanora
                match_count:db_EUKA     rank   scientific_name species
EUKAP2_00000018                  13 subclass Lecanoromycetidae      NA
EUKAP1_00050174                   1    genus          Lecanora      NA

EUKAP2_00000018 ['Usnea florida', 'Pyxine farinosa', 'Cladia aggregata', 'Allocetraria madreporiformis
EUKAP1_00050174
                species_name  taxid
EUKAP2_00000018         <NA> 388435
EUKAP1_00050174         <NA>  39934

EUKAP2_00000018 ataacgaacgagaccttaacctgctaaatagccaggtcagctttggctggccgccggcttcttagagggactatcggctcaagccg
```

```
EUKAP1_00050174 ataacgaacgagaccttaacctgctaaatagccaggccagctccggctggtcgccggcttcttagagggactatcggctcaagccg
                sequence_type is_spermatophyta
EUKAP2_00000018        EUKA02            FALSE
EUKAP1_00050174        EUKA02            FALSE
```

```
to_keep <- EUKA02.merged4@motus$`best_identity:db_EUKA` > 0.95
table(to_keep)
```

```
to_keep
TRUE
  44
```

```
EUKA02.merged4@motus %>% filter(!to_keep)
```

```
 [1] id                     definition            best_identity:db_EUKA
 [4] best_match:db_EUKA     count                 family
 [7] family_name            genus                 genus_name
[10] match_count:db_EUKA    rank                  scientific_name
[13] species                species_list:db_EUKA  species_name
[16] taxid                  sequence              sequence_type
[19] is_spermatophyta
<0 rows> (or 0-length row.names)
```

```
EUKA02.final <- EUKA02.merged4[,which(to_keep)]
EUKA02.final@reads <- decostand(EUKA02.final@reads,method = "total")
```

6. Saving the filtered dataset

6.1. Updating the sample metadata

6.1.1. Adding samples metadata

```
metadata <- read_csv("Data/Faeces/metadata.csv",
                     show_col_types = FALSE)
```

```
EUKA02.final@samples %<>%
  select(sample_id,animal_id) %>%
  left_join(metadata,by = "sample_id") %>%
  mutate(id = sample_id) %>%
  column_to_rownames("id") %>%
  select(sample_id,animal_id,Sample_number,Date,Sample_time,times_from_birch, Fed_biomass)
```

6.1.2. Homogenize time from burch

Adds : - 6 hours to animal X, - 3 hours to animal Y, - 4 hours to animal 2

```
EUKA02.final@samples %<>%
  mutate(times_from_birch = times_from_birch +
           ifelse(animal_id == "X",6,
         ifelse(animal_id == "Y",3,4)))
```

```
EUKA02.final@samples %<>%
  mutate(Animal_id = ifelse(animal_id == "X","9/10",
         ifelse(animal_id == "Y","10/10","12/10")))
```

6.1.3. Adds pellets consumption data

```
pellets <- read_tsv("Data/pellet_weigth.txt", show_col_types = FALSE) %>%
           mutate(Date = str_replace(Date,"2018","18")) %>%
           separate(Date, c("d","m","y"),sep = "/") %>%
           mutate(d = as.integer(d)+1,
                  m = as.integer(m),
                  m = ifelse(d==32,m+1,m),
                  d = ifelse(d==32,1,d),
                  d = sprintf("%02d",d),
                  m = sprintf("%02d",m)) %>%
           unite(col="Date",d,m,y,sep="/") %>%
           pivot_longer(-Date,names_to = "Animal_id",values_to = "pellets")

EUKA02.final@samples %<>%
   left_join(pellets)
```

```
Joining with `by = join_by(Date, Animal_id)`
```

6.2. Add MOTUs Metadata

```
EUKA02.final@motus %<>%
  mutate(category = ifelse(is.subcladeof(taxo,taxid,spermatophyta.taxid),
                            "Plant",
                            "Lichen"))
```

6.3. Only keep samples

```
EUKA02.final <- EUKA02.final[which(str_detect(EUKA02.final@samples$sample_id,"^[XYZ]")),]
```

6.4. Updating count statistics

```
EUKA02.final %<>%
  update_motus_count() %>%
  update_samples_count() %>%
  clean_empty()
```

6.5. Write CSV files

```
write_csv(EUKA02.final@samples,
          file = "Data/Faeces/FE.Eukaryota.samples.samples.csv")
write_csv(EUKA02.final@motus,
          file = "Data/Faeces/FE.Eukaryota.samples.motus.csv")
write_csv(EUKA02.final@reads %>%
            decostand(method = "total") %>%
            as.data.frame()%>%
            rownames_to_column("id"),
          file = "Data/Faeces/FE.Eukaryota.samples.reads.csv")
```

References

[1] H. Wickham, M. Averick, J. Bryan, W. Chang, L. McGowan, R. François, G. Grolemund, A. Hayes, L. Henry, J. Hester, M. Kuhn, T. Pedersen, E. Miller, S. Bache, K. Müller, J. Ooms, D. Robinson, D. Seidel, V. Spinu, K. Takahashi, D. Vaughan, C. Wilke, K. Woo, H. Yutani, Welcome to the tidyverse, Journal of open source software 4 (43) (2019) 1686. doi:10.21105/joss.01686.
URL https://joss.theoj.org/papers/10.21105/joss.01686

[2] H. Wickham, ggplot2: Elegant Graphics for Data Analysis, Springer-Verlag New York, 2016.
URL https://ggplot2.tidyverse.org