

MetaOMineR

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Context

`momr`, is the base package of a larger suite of R packages named `MetaOMineR`, which stands for *Mining MetaOmics data in R*. It encompasses many useful functions and modules needed for the analyses of shotgun Quantitative Metagenomics (QM) data. It can be also used for 16S or other types of omics data. Developed since the beginning of the field, `momr` has evolved and is structured around different modules such as preprocessing, analysis, visualization, map-reduce parallel computing, etc. The package comes with a small

subset of a real metagenomics data-set of human gut microbiome from the MetaHIT project (Le Chatelier et al, *Nature*, 2013).

MetaOMineR works with data that can be structured as standalone packages or not. They should contain the needed information to describe a given gene catalog, such as for instance the gene length, annotations, clustering information, etc. In this tutorial we demonstrate some of the functionalities of `momr` with simple examples.

Data processing

In this section we will see how to load the test dataset that comes with the package and how to pre-process it for analysis in a second step. Let us start by loading the `momr` library.

```
library(momr)
library(knitr) # for printing tables
```

To see what data objects are contained in the package we type:

```
data(package="momr")
```

We will see four objects

- `hs_3.3_matahit_genesize` - `hs_3.3_matahit_sample_dat_freq` - `hs_3.3_matahit_sample_dat_raw`
- `mgs_hs_3.3_matahit_sup500`

Loading the data

The files are named following these criteria (*hs* = *homo sapiens*; *3.3_matahit* = the gene catalog from matahit with 3.3M genes). Let us load the raw count data-set after mapping and counting against the (*Qin et al, Nature, 2010*) gene catalog.

```
# Loading the raw count dataset
data("hs_3.3_matahit_sample_dat_raw")
str(hs_3.3_matahit_sample_dat_raw[,10:14])

##  int [1:5000, 1:5] 0 0 0 0 0 0 0 0 0 ...
##  - attr(*, "dimnames")=List of 2
##    ..$ : chr [1:5000] "4955" "4956" "4957" "4958" ...
##    ..$ : chr [1:5] "MH0109" "MH0110" "MH0186" "MH0108" ...
```

As we can see `hs_3.3_matahit_sample_dat_raw` is a data frame containing 5000 features (rows) and 292 samples (columns). This dataset is a subset of the whole 3.3M feature data-frame and as we can see is very sparse.

```
kable(tail(hs_3.3_matahit_sample_dat_raw[,10:14]))
```

	MH0109	MH0110	MH0186	MH0108	MH0236
9904	0	0	0	0	0
9905	0	0	0	0	0
9906	0	0	0	0	0
9907	0	0	0	0	0
9908	38	58	2	12	3
9909	0	0	0	0	0

```

zeroperc <- round(sum(hs_3.3_matahit_sample_dat_raw==0)/
                  length(hs_3.3_matahit_sample_dat_raw)*100)
paste("There are ", zeroperc, "% zeros in this data frame.", sep="")

```

[1] "There are 81% zeros in this data frame."

Normalization

This processing step is necessary to be able to compare abundance among genes and samples. For this reason different normalization procedures are implemented in the package. Note that even from an identical number of reads, the total number of counts can vary when filtering the reads for quality or according to the reference exhaustivity. For the experiment to work well we need to select a gene catalog reference that is representative enough for the different microbial ecosystems sampled in the study. There is not yet a gold standard for normalizing data in quantitative metagenomics and the RPKM method has proven to be good enough in different QM projects. We aim to enrich the package with other normalization approaches shortly in the future.

1. RPKM (**Reads Per Kilobase per Million reads mapped**) is one of the first methods used in QM and was inspired by the RNA-Seq field (Mortazavi et al., Nature Methods, 2008). This approach was initially introduced to facilitate comparisons between genes within a sample and combines between- and within-sample normalization, as it re-scales gene counts to correct for differences in both library sizes and gene length. Let assume that two genes from a given species have different lengths. The longer gene has a higher probability of having more reads mapped to it compared to the shorter one especially when the abundance is low. For this reason we compute a scaling factor which is dependent on the gene length in the normalization process. A second scaling factor applied is the sequencing depth.
2. TC (**Total count**) is a simpler method also used in the 16S datasets. The high variability of sequencing depth among the different samples is inherent of the NGS technology. For this reason it is important to scale the abundance of reads for each sample by the sequencing depth. Technically we can scale each sample by the total number of counts.

```

# Normalization should be performed with the whole dataset (3.3M)
# Loading the gene length information
data(hs_3.3_matahit_genesize)
str(hs_3.3_matahit_genesize)

##  Named int [1:5000] 1083 1746 813 504 162 1356 150 1263 1794 273 ...
##  - attr(*, "names")= chr [1:5000] "4955" "4956" "4957" "4958" ...

norm.data <- normFreqRPKM(dat=hs_3.3_matahit_sample_dat_raw,
                           cat=hs_3.3_matahit_genesize)

## [1] "The dataset is a matrix"

kable(tail(norm.data[,10:14]))

```

	MH0109	MH0110	MH0186	MH0108	MH0236
9904	0.0000000	0.0000000	0.0000000	0.00e+00	0.0000000
9905	0.0000000	0.0000000	0.0000000	0.00e+00	0.0000000

	MH0109	MH0110	MH0186	MH0108	MH0236
9906	0.0000000	0.0000000	0.0000000	0.00e+00	0.0000000
9907	0.0000000	0.0000000	0.0000000	0.00e+00	0.0000000
9908	0.0004564	0.0004709	0.0002019	9.99e-05	0.0001233
9909	0.0000000	0.0000000	0.0000000	0.00e+00	0.0000000

Hereafter we will use a subset of the complete dataset normalized using the 3.3M genes. Note that the scaling factor is lower in the extracted dataset compared to the full dataset due to the lower number of reads sampled for this subset of genes.

```
# Loading the frequency dataset
data("hs_3.3_matahit_sample_dat_freq")
kable(tail(hs_3.3_matahit_sample_dat_freq[, 10:14]))
```

	MH0109	MH0110	MH0186	MH0108	MH0236
9904	0e+00	0e+00	0e+00	0e+00	0e+00
9905	0e+00	0e+00	0e+00	0e+00	0e+00
9906	0e+00	0e+00	0e+00	0e+00	0e+00
9907	0e+00	0e+00	0e+00	0e+00	0e+00
9908	6e-07	1e-06	2e-07	1e-07	2e-07
9909	0e+00	0e+00	0e+00	0e+00	0e+00

Downsizing

Another method to reduce the variability that is generated by the sequencing depths is the **downsizing** also known as **rarefaction**. It consists of drawing randomly the same number of reads for each sample and mapping those to the catalog. For this we need to determine a common level of reads to be drawn (sequencing depth).

```
# Determining the minimal common number of reads
min_nb_reads <- summary(colSums(hs_3.3_matahit_sample_dat_raw))
(min_nb_reads["Min."]); (min_nb_reads["Max."])

## Min.
## 661

## Max.
## 274400

min_nb_reads <- min_nb_reads["Min."]
```

We can notice that the sequencing depth varies greatly in this dataset and this is probably because this is an incomplete dataset. We can perform this for the whole dataset only one time. Next the dataset needs to be normalized as shown above.

```
# Downsizing the whole matrix
data.downsized <- downsizeMatrix(data=hs_3.3_matahit_sample_dat_raw[, 1:5],
                                   level=min_nb_reads, repetitions=1, silent=FALSE)
```

```

## [1] "1 Sample MH0277 with 661 reads and 54 genes"
## [1] "      step 1 with 54 genes"
## [1] "2 Sample MH0087 with 7346 reads and 160 genes"
## [1] "      step 1 with 79 genes"
## [1] "3 Sample MH0444 with 37988 reads and 384 genes"
## [1] "      step 1 with 106 genes"
## [1] "4 Sample MH0156 with 20868 reads and 333 genes"
## [1] "      step 1 with 115 genes"
## [1] "5 Sample MH0333 with 5671 reads and 182 genes"
## [1] "      step 1 with 106 genes"

```

```
kable(tail(data.downsized))
```

	MH0277	MH0087	MH0444	MH0156	MH0333
9904	0	0	0	0	0
9905	0	0	0	0	0
9906	0	0	2	0	0
9907	0	0	0	0	0
9908	0	2	0	1	1
9909	0	0	0	0	0

```
colSums(data.downsized, na.rm=TRUE)
```

```

## MH0277 MH0087 MH0444 MH0156 MH0333
##   661     661     661     661     661

```

Important note: Let assume that most of the samples are sequenced nicely above a sequencing depth we set, but a few samples have a low number of reads for various reasons. Should we still downsize very low (in order to include them) and lose most of the data? The answer is *No!* We recommend setting up downsizing level sufficiently high to maintain a high counting depth. Samples with a total number of reads below the level won't be downsized (NA will be generated instead) and may be discarded or replaced as a proxy by original raw counts before generating the frequency matrix using `normFreqRPKM` function.

Gene richness

A simple number can describe the complexity of an ecosystem that we call here richness. That is the number of genes that are found to be present (`gene_abundance > 0`) in a given sample. Indeed, different studies have shown that the richness is associated with different aspects of the ecosystem (*Le Chatelier et al, Nature, 2013*) and correlates strongly with the number of present microbial species (*Nielsen, Almeida et al, Nat Biotech, 2014*).

```

# Downsizing the genecount
richness <- colSums(hs_3.3_methahit_sample_dat_raw>0, na.rm=TRUE)
summary(richness)

```

```

##      Min. 1st Qu. Median      Mean 3rd Qu.      Max.
## 54.0    720.8  956.0  941.8 1123.0  4909.0

```

Downsizing

Gene richness is very sensitive to the sequencing depth. For this reason we use the downsizing approach to estimate it and perform this multiple times. Finally we compute a mean estimation of the multiple drawings.

```
# Downsizing the matrix multiple times for the computation of gene richness
data.genenb <- downsizeGC(data=hs_3.3_metahit_sample_dat_raw,
                            level=min_nb_reads, repetitions=30, silent=TRUE)
head(apply(data.genenb, 2, mean))
```

```
##      MH0277      MH0087      MH0444      MH0156      MH0333      MH0233
## 54.000000 77.233333 111.700000 109.900000 105.56667 150.80000
```

```
head(apply(data.genenb, 2, sd))
```

```
##      MH0277      MH0087      MH0444      MH0156      MH0333      MH0233
## 0.000000 4.384010 4.793314 6.423824 4.868501 6.466892
```

Notice that the standard deviation is quite small for 30 random drawings.

```
richness.dwnz <- colMeans(data.genenb, na.rm=TRUE)
par(mfrow=c(1,2))
plot(density(richness), main="gene richness", lwd=2, col="darkred")
plot(density(richness.dwnz), main="downsized gene richness", lwd=2, col="darkred")
```

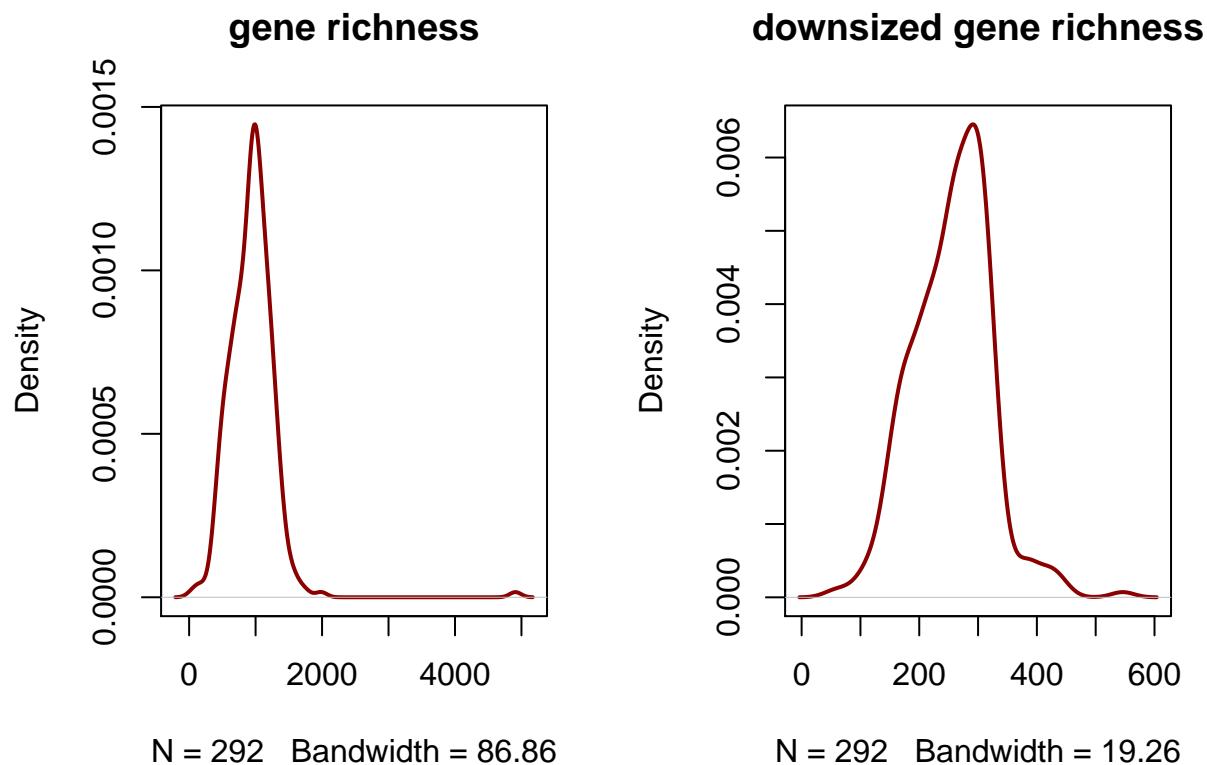


Figure 1: Raw and downsized richness distribution.

In this example (Figure 1) we can see the effect of downsizing on gene richness. For instance one sample had a much higher richness than the rest due to the high variability as mentioned above. After downsizing this sample still remained higher but more comparable with the rest.

```
par(mfrow=c(1,1))
col <- as.character(cut(colSums(hs_3.3_matahit_sample_dat_raw),
                         c(0,2^seq(0, 9, by=1))*1000,
                         labels=paste("gray",seq(100,10,-10),sep="")))
plot(richness, richness.dwnz, main="downsizing effect on richness",
      pch=20,col=col)
```

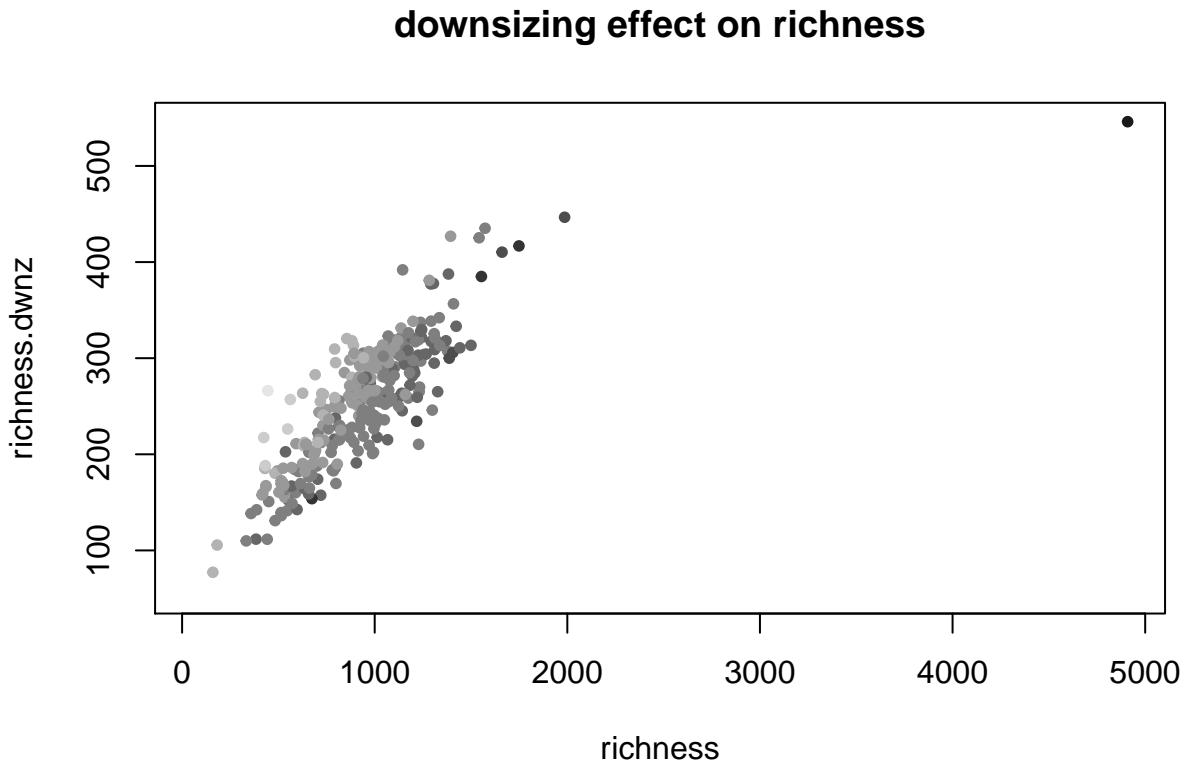


Figure 2: Downsizing effect on gene richness.

This plot (Figure 2) where samples are colored according to read count abundance (the darker the higher) visualize the bias in gene richness estimation due to heterogenous counting depth.

Upsizing

As mentioned above for samples with very low sequencing depth (under the downsizing level) the downsizing process will produce NAs and they will not be exploitable. Based on our observations gene richness downsized at different levels will correlate very strongly among the different levels. This observation led us to propose the *upsizing* approach for gene richness estimation, which allows to estimate a higher level distribution and impute the missing data. In the following example we will use different downsizing levels and show how we can use the up-sizing process to solve this issue.

```
downsize.gc.res <- downsizeGC.all(data = hs_3.3_matahit_sample_dat_raw,
                                    levels = c(600, 5000, 10000, 15000, 20000),
```

```

repetitions = 10, silent = TRUE)
kable(downsized.gc.res[[2]])

```

	down_6e-04M	down_0.005M	down_0.01M	down_0.015M	down_0.02M
	80	146	NA	NA	NA
	77	141	NA	NA	NA
	73	146	NA	NA	NA
	79	147	NA	NA	NA
	74	144	NA	NA	NA
	76	143	NA	NA	NA
	73	144	NA	NA	NA
	80	138	NA	NA	NA
	83	143	NA	NA	NA
	77	146	NA	NA	NA

This function returns a list of samples each containing a matrix of dimension $n=repetitions \times l=levels$ as illustrated above for the second sample. Now let's transform it as a matrix where each column contain the mean-ed downsized values for each repetition.

```

downsize.gc.mat <- downsizedRichnessL2T(richness.list = downsize.gc.res)
kable(head(downsize.gc.mat))

```

	down_6e-04M	down_0.005M	down_0.01M	down_0.015M	down_0.02M
MH0277	49.8	NA	NA	NA	NA
MH0087	77.2	143.8	NA	NA	NA
MH0444	107.0	215.0	260.4	294.9	324.2
MH0156	107.3	227.7	283.1	309.9	329.9
MH0333	100.6	177.4	NA	NA	NA
MH0233	146.6	311.8	376.5	413.4	443.2

Next, we will use the upsizing approach to estimate the missing values as illustrated in Figure 3.

```

upsized <- computeUpsizedGC(richness.table = downsize.gc.mat,
                               keep.real = TRUE)
kable(head(upsized))

```

	down_6e-04M_una	down_0.005M_una	down_0.01M_una	down_0.015M_una	down_0.02M_una
MH0277	50	78	85	87	89
MH0087	77	144	168	180	189
MH0444	107	215	260	295	324
MH0156	107	228	283	310	330
MH0333	101	177	209	226	238
MH0233	147	312	376	413	443

```

reg <- lm(upsized[,2] ~ upsized[,1])
plot(upsized[,2] ~ upsized[,1], main="Regression of the first two levels",

```

```

xlab=("600 reads"), ylab=("5000 reads"), pch=21)
abline(reg,col="red")
points(upsized[is.na(downsized.gc.mat[,2]),2] ~ upsized[is.na(downsized.gc.mat[,2]),1],
      pch=20, col="red")

```

Regression of the first two levels

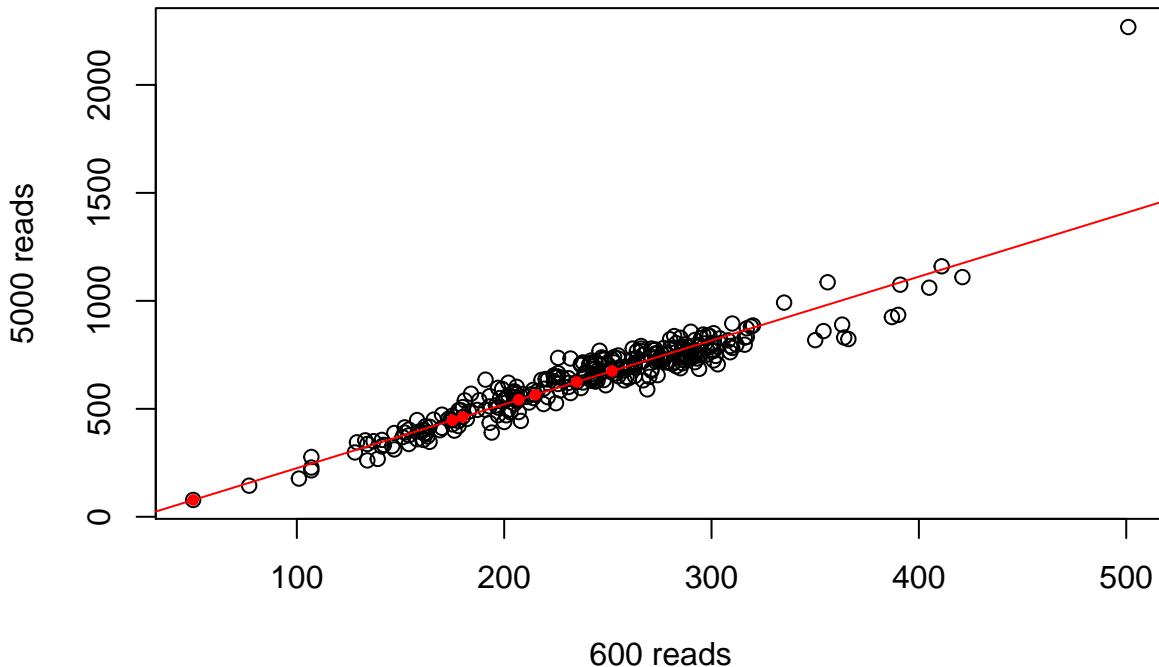


Figure 3: Regression of the first two levels downsizing levels. In red are depicted the points not downsized in the second level.

To compare properly gene richness between samples, we recommend to fix the downsizing/upsizing threshold level in a way that the read counts of most of the samples are above the threshold but also without losing much information with a stringent level.

Sample clustering

Heatmap

Now that the dataset is processed we will relate samples together in order to explore any particular pattern. For this the function `hierClust` will compute the inter-sample distance and use a hierarchical clustering approach cluster samples in a tree. The default distance is computed as `1-cor` where `cor` is the inter-sample spearman correlation. The hierarchical clustering method is the `ward.D`. This function returns a list containing the correlation matrix, the distance object and the hierarchical clustering object. It also displays a heatmap of the correlation matrix with the ward computed dendrogram (Figure 4). These results can be also used as standalone data to fine-tune the analyses.

```
hc.data <- hierClust(data=hs_3.3_metahit_sample_dat_freq[,1:10], side="col", hclust.method = "ward.D")
```

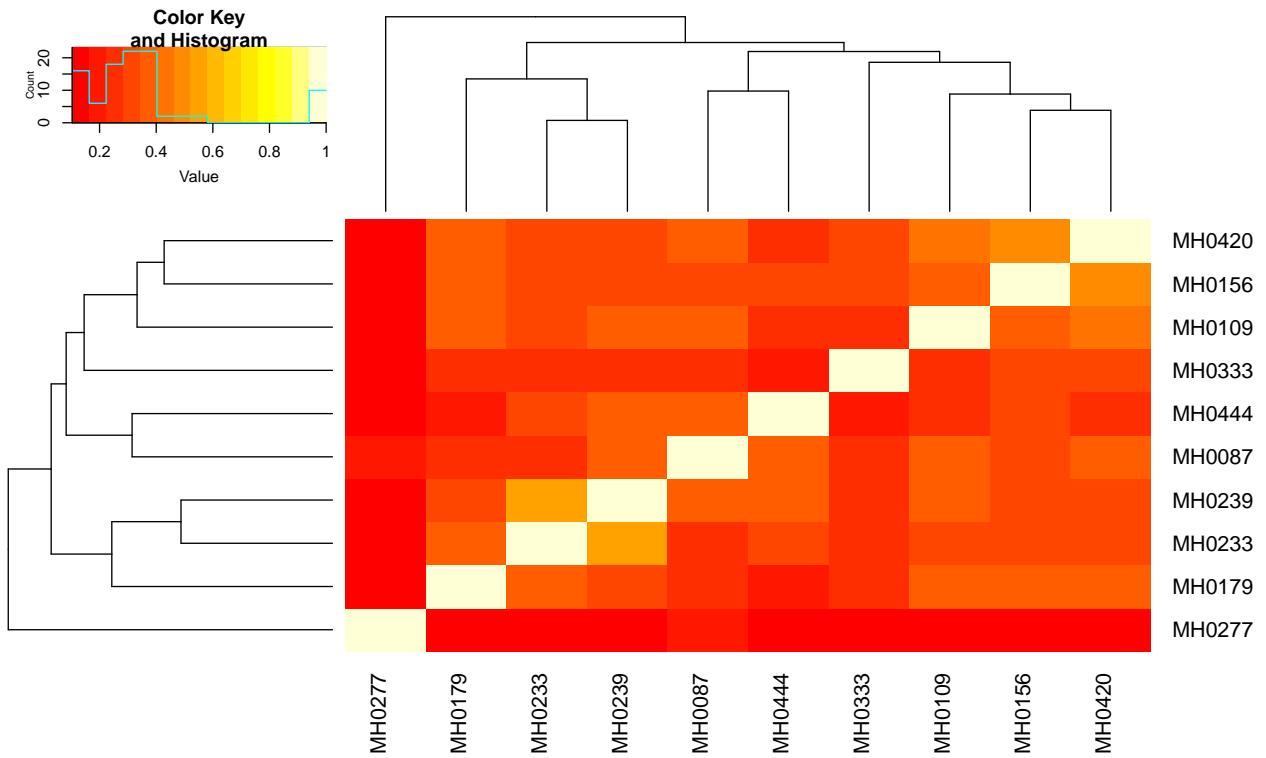


Figure 4: Sample heatmap of the correlation matrix clustered with the ward approach.

```

str(hc.data)

## List of 3
## $ mat.rho   : num [1:10, 1:10] 1 0.182 0.151 0.132 0.104 ...
##   ..- attr(*, "dimnames")=List of 2
##     ...$ : chr [1:10] "MH0277" "MH0087" "MH0444" "MH0156" ...
##     ...$ : chr [1:10] "MH0277" "MH0087" "MH0444" "MH0156" ...
## $ mat.dist  :Class 'dist' atomic [1:45] 0.818 0.849 0.868 0.896 0.874 ...
##   ..- attr(*, "Labels")= chr [1:10] "MH0277" "MH0087" "MH0444" "MH0156" ...
##   ..- attr(*, "Size")= int 10
##   ..- attr(*, "call")= language as.dist.default(m = 1 - mat.rho)
##   ..- attr(*, "Diag")= logi FALSE
##   ..- attr(*, "Upper")= logi FALSE
## $ mat.hclust:List of 7
##   ..$ merge      : int [1:9, 1:2] -6 -4 -10 -2 -7 -5 4 5 -1 -8 ...
##   ..$ height     : num [1:9] 0.471 0.523 0.608 0.623 0.686 ...
##   ..$ order      : int [1:10] 1 7 6 8 2 3 5 10 4 9
##   ..$ labels     : chr [1:10] "MH0277" "MH0087" "MH0444" "MH0156" ...
##   ..$ method     : chr "ward.D"
##   ..$ call       : language hclust(d = mat.dist, method = hclust.method)
##   ..$ dist.method: NULL
##   ..- attr(*, "class")= chr "hclust"

clust.order <- hc.data$mat.hclust$order
# order samples followin the hierarchical clustering
ordered.samples <- colnames(hs_3.3_methhit_sample_dat_freq[,1:10])[clust.order]

```

```
# how close are the two first samples (spearman, rho)
hc.data$mat.rho[ordered.samples[1], ordered.samples[2]]
```

```
## [1] 0.1148695
```

Checking for consistency

When looking for possible contamination or mislabeling in order to make sure that samples in the dataset should not be related ,it is useful to use the `filt.hierClust` function. This routine will extract a subset of the inter-sample correlation matrix and focus on the samples that are closely related (above a given threshold) as illustrated in Figure 5. It also returns a table indicating for each samples the best correlated ones and displays a heatmap of the correlation matrix restricted to the samples correlated above the filter threshold (`plot=TRUE` as default).

```
# Selecting the most closely related observations
close.samples <- filt.hierClust(hc.data$mat.rho, hclust.method = "ward.D",
                                 plot = TRUE, filt = 0.45, size = 4)
```

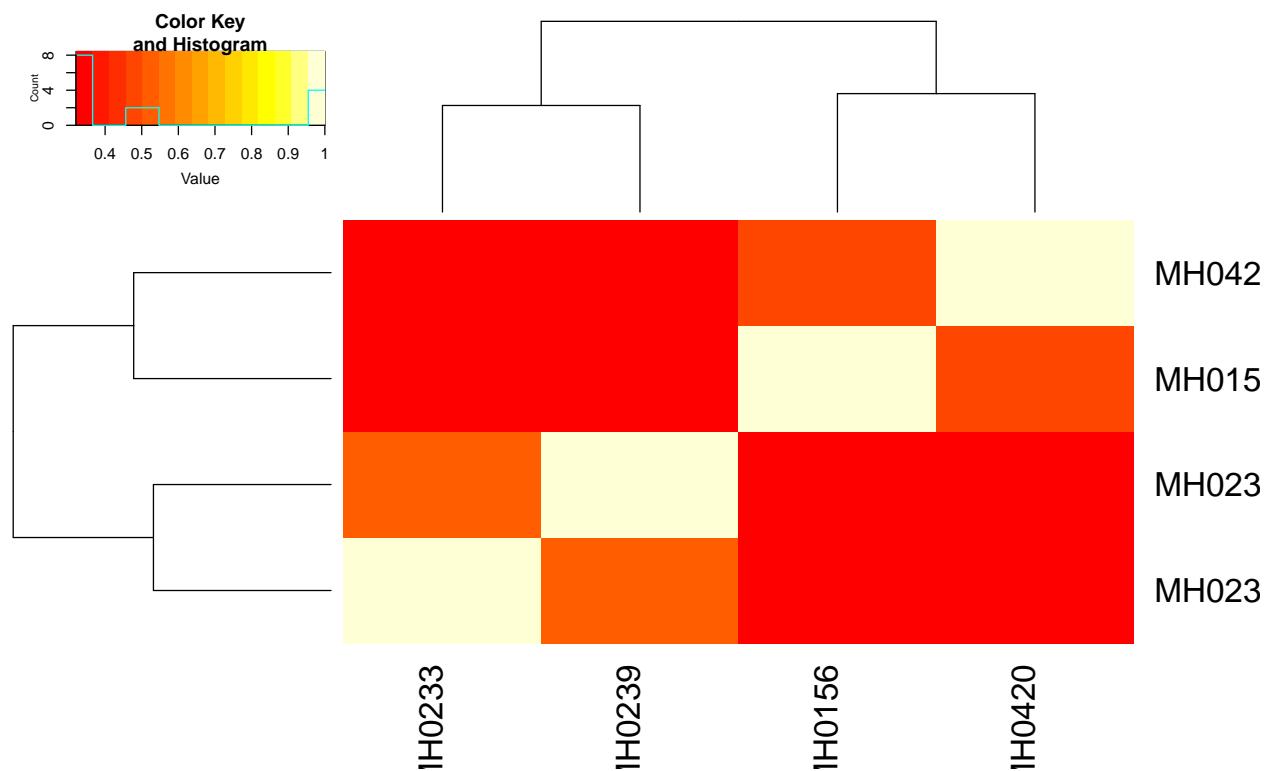


Figure 5: Heatmap of the most correlated observations.

```
kable(head(close.samples)[,1:6])
```

	Hit_1	Hit_rho_1	Hit_2	Hit_rho_2	Hit_3	Hit_rho_3
MH0277	MH0087	0.182	MH0239	0.159	MH0109	0.156
MH0087	MH0444	0.377	MH0109	0.372	MH0420	0.352

	Hit_1	Hit_rho_1	Hit_2	Hit_rho_2	Hit_3	Hit_rho_3
MH0444	MH0239	0.392	MH0087	0.377	MH0156	0.333
MH0156	MH0420	0.477	MH0109	0.387	MH0179	0.350
MH0333	MH0420	0.332	MH0156	0.302	MH0233	0.259
MH0233	MH0239	0.529	MH0179	0.400	MH0420	0.322

Clustering genes - selecting the most correlated samples

Genes as other features of interest can be clustered using different techniques. In QM it makes sense biologically to cluster genes since they are indeed genetically linked together in the same molecular structure - *the genome*. Based on this observation the metagenomic species (MGS) were proposed and published in 2014 (*Nielsen, Almeida et al, Nat Biotech, 2014*). We have build multiple tools that will allow exploring these objects and here is a preview.

The mgs catalog

The MGS catalog can be built using different approaches. We supply in this package a subset of the MGS catalog that was computed in a large dataset in the MetaHIT 3.3M gene catalog. Briefly this is a list of gene (feature) identifiers.

```
# load the curated mgs data for the hs_3.3_matahit catalog
data("mgs_hs_3.3_matahit_sup500")
# the size of each MGS
unlist(lapply(mgs_hs_3.3_matahit_sup500,length))
```

```
## 10763_0_2    10766_2    10770_    10775_    10780_    1_11_2    11747_
##    1708      2249      2778      1436      599       783      4728
##   11752_    11757_    1_20     12719_1  12720_1  12723_    1_3
##   2274      811       2240      1113      2056      2198      827
##  13608_    1_4      1533_1    241_2     273_      319_    4373_16
##   1233      642       694      2931      2241      943      3001
```

Projecting genes onto the MGS catalog

In the following example we will cluster a number of genes in the MGS catalog. We call this: *projecting genes onto the MGS*. The notion of genebag (a bag of genes or features) is recurrent in the architecture of `momr`.

```
# Projecting a list of genes onto the mgs catalogue
genebag <- rownames(hs_3.3_matahit_sample_dat_freq)
mgs <- projectOntoMGS(genebag=genebag, list.mgs=mgs_hs_3.3_matahit_sup500)
length(genebag)
```

```
## [1] 5000
```

```
unlist(lapply(mgs,length))
```

```
##      1533_1        241_2        273_        319_        4373_16
##      131          495          54           80          142
## not_projected
##      4098
```

You can notice that these 5000 genes fall in 5 different MGS and that 4098 genes are not clustered. Indeed only approximately half of the catalog is clustered, due to different stringent criteria for QM purposes. Now that we know which gene is which MGS we can extract their profiles to explore them further.

```
# Extracting the profiles of a list of genes from the whole dataset
mgs.dat <- extractProfiles(mgs, hs_3.3_metahit_sample_dat_freq, silent=FALSE)
```

```
## [1] "Multiple profile extraction"
```

This is a list of data frames where we have a data frame for each MGS.

Visualizing MGS (the barcodes)

The barcode visualization is a very good tool for pattern discovery and recognition (Figure 6). It is a kind of heatmap where white A white color indicates absence and from light blue to dark red an increasing abundance. Each color step is a 4-fold in abundance.

```
# plot the barcodes
par(mfrow=c(length(mgs.dat),1), mar=c(1,0,0,0))
for(i in 1:length(mgs.dat)){
  plotBarcode(mgs.dat[[i]], main=names(mgs.dat)[i])
}
```

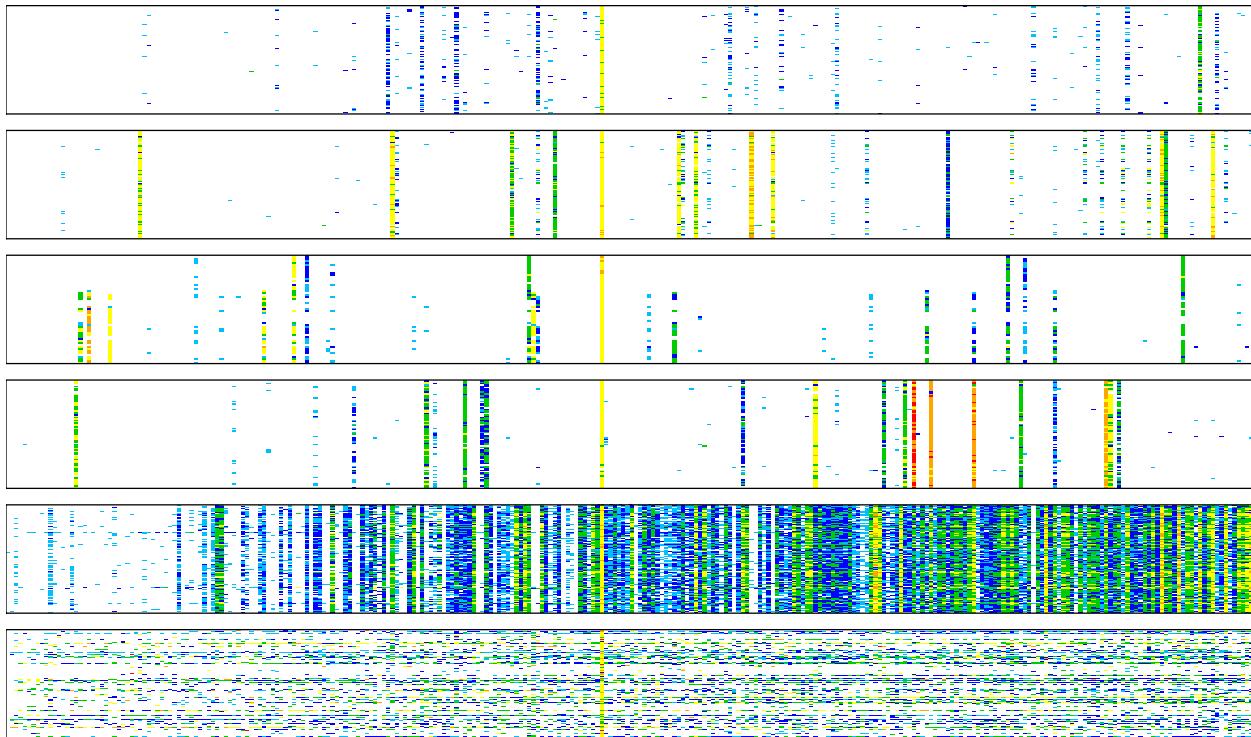


Figure 6: Barcodes of the MGS abundance profiles. Samples are in the columns and genes clustered together in the MGS in the rows.

Reducing dimensions

The MGS can be transformed in simple tracer vectors using `computeFilteredVectors`. This allows to reduce dimensions and apply different statistical learning tools such as clustering (Figure 7). Different metrics can be used to compute this: the mean, the median or the sum are implemented at the moment. The function returns a table of the calculated MGS signal in each sample.

```
# Computing the filtered vectors
mgs.mean.vect <- computeFilteredVectors(profile=mgs.dat, type="mean")
hierClust(t(mgs.mean.vect))
```

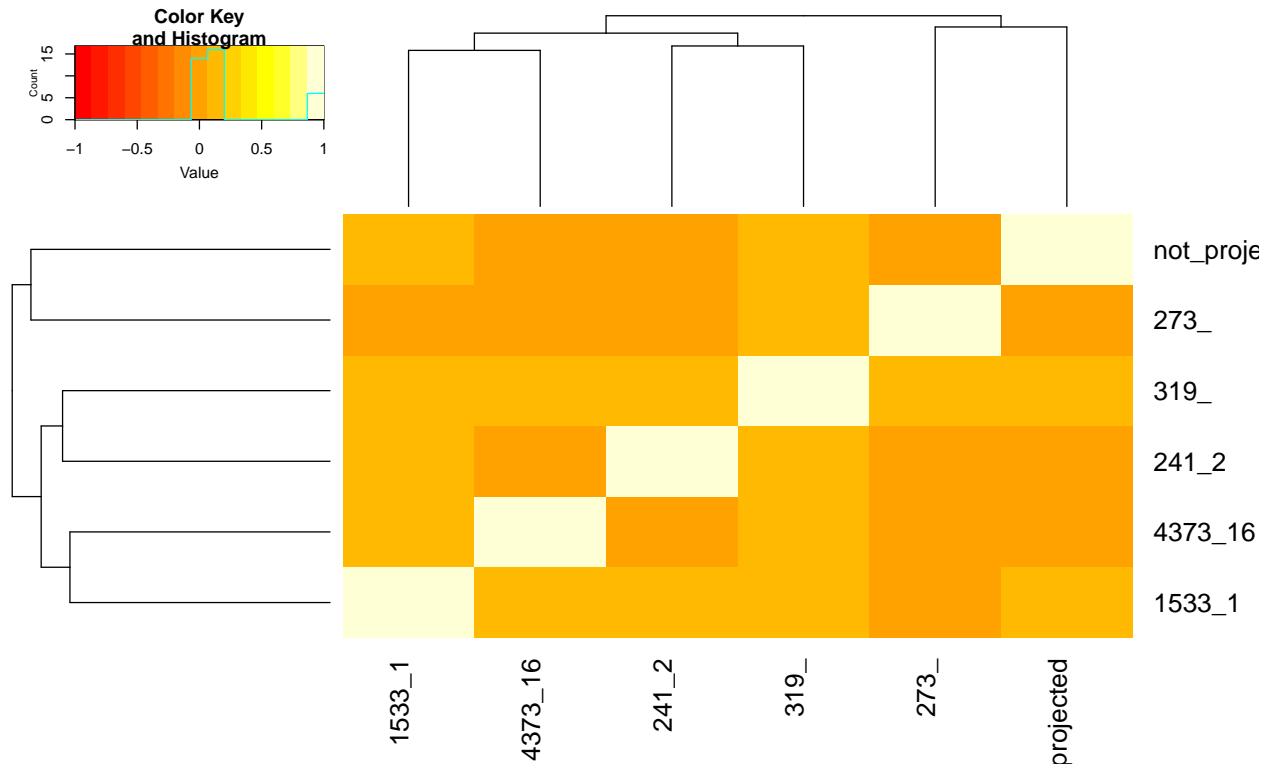


Figure 7: Similarity heatmap and clustering of the MGS.

```
## $mat.rho
##          1533_1      241_2      273_      319_      4373_16
## 1533_1    1.00000000  0.1336279064  0.03183426  0.10000386  0.16266416
## 241_2     0.13362791  1.0000000000  0.05561149  0.13894849  0.05708167
## 273_      0.03183426  0.0556114912  1.00000000  0.07835247  0.01794230
## 319_      0.10000386  0.1389484853  0.07835247  1.00000000  0.15109205
## 4373_16   0.16266416  0.0570816696  0.01794230  0.15109205  1.00000000
## not_projected 0.07064679 -0.0005112535  0.03702869  0.08821160  0.03419235
##          not_projected
## 1533_1     0.0706467852
## 241_2     -0.0005112535
## 273_      0.0370286889
## 319_      0.0882116035
## 4373_16   0.0341923498
## not_projected 1.0000000000
```

```

##  

## $mat.dist  

##          1533_1      241_2      273_      319_    4373_16  

## 241_2      0.8663721  

## 273_      0.9681657 0.9443885  

## 319_      0.8999961 0.8610515 0.9216475  

## 4373_16    0.8373358 0.9429183 0.9820577 0.8489079  

## not_projected 0.9293532 1.0005113 0.9629713 0.9117884 0.9658077  

##  

## $mat.hclust  

##  

## Call:  

## hclust(d = mat.dist, method = hclust.method)  

##  

## Cluster method : ward.D  

## Number of objects: 6

```

Identifying differentially abundant features

Another interesting function is `testRelations`, which allows to identify features (genes, MGS, etc) that are differentially abundant between two groups of samples or correlate with some quantitative variable.

```

# for the first 500 genes
class <- c(rep(1,150),rep(2,142))
res.test <- testRelations(data=hs_3.3_matahit_sample_dat_freq[1:500,],
                           trait=class,type="wilcoxon")
print(paste("There are",sum(res.test$p<0.05, na.rm=TRUE),"significant genes and",
           sum(res.test$q<0.05, na.rm=TRUE), "after adjustment for multiple testing"))

```

```
## [1] "There are 135 significant genes and 93 after adjustment for multiple testing"
```

```

# keep the significant genes
res.test <- res.test[res.test$q < 0.05 & !is.na(res.test$q),]
# sort them by status and q-value
res.test <- res.test[order(res.test$status,res.test$q),]
kable(head(res.test))

```

	rho	rho2	p	q	status
5300	NA	NA	0.0010278	0.0073287	1
5172	NA	NA	0.0010929	0.0076815	1
5272	NA	NA	0.0011985	0.0081897	1
5190	NA	NA	0.0021005	0.0130817	1
5385	NA	NA	0.0033330	0.0202449	1
5191	NA	NA	0.0052412	0.0306984	1

```
table(res.test$status)
```

```

##  

## 1 2

```

```

## 9 84

# test weather the MGS are also differentially abundant with the class
res.test.mgs <- testRelations(data=mgs.mean.vect, trait=class, type="wilcoxon")
kable(res.test.mgs[res.test.mgs$q<0.05,])

```

	rho	rho2	p	q	status
319_	NA	NA	0.0007663	0.0022988	2
4373_16	NA	NA	0.0000000	0.0000000	2

In the example above we tested whether the first 500 genes of this test dataset are differentially abundant between the groups 1 and 2. Indeed there are 9 genes enriched in 1 and 84 in the second group after multiple testing adjustment. We performed this test also on the vectors of the MGS and two of them are also differentially abundant.

Conclusion

momr is a very useful package for quantitative metagenomics and only the main functionalities are described here. The package also allows to perform // computing using the map-reduce principles. We are constantly optimizing algorithms and adding new tools so that it really becomes easy to explore QM datasets. The authors would like to acknowledge the very exciting and fruitful environment that MetaHIT community created.

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