MetCleaning v1.0.0

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Introduction

MetCleaning provides an integrated and automatic pipeline for data cleaning and statistical analysis of large scale mass spectrometry (MS) based-metabolomic data. It includes missing value (MV) filtering and imputation, zero value filtering, data normalization, data integration, data quality assessment, univariate statistical analysis, multivariate statistical analysis such as PCA and PLS-DA, potential marker selection and show. This document describes how to use the integrated functions, *MetClean* and *MetStat* in *MetCleaning* utilizing demo data.

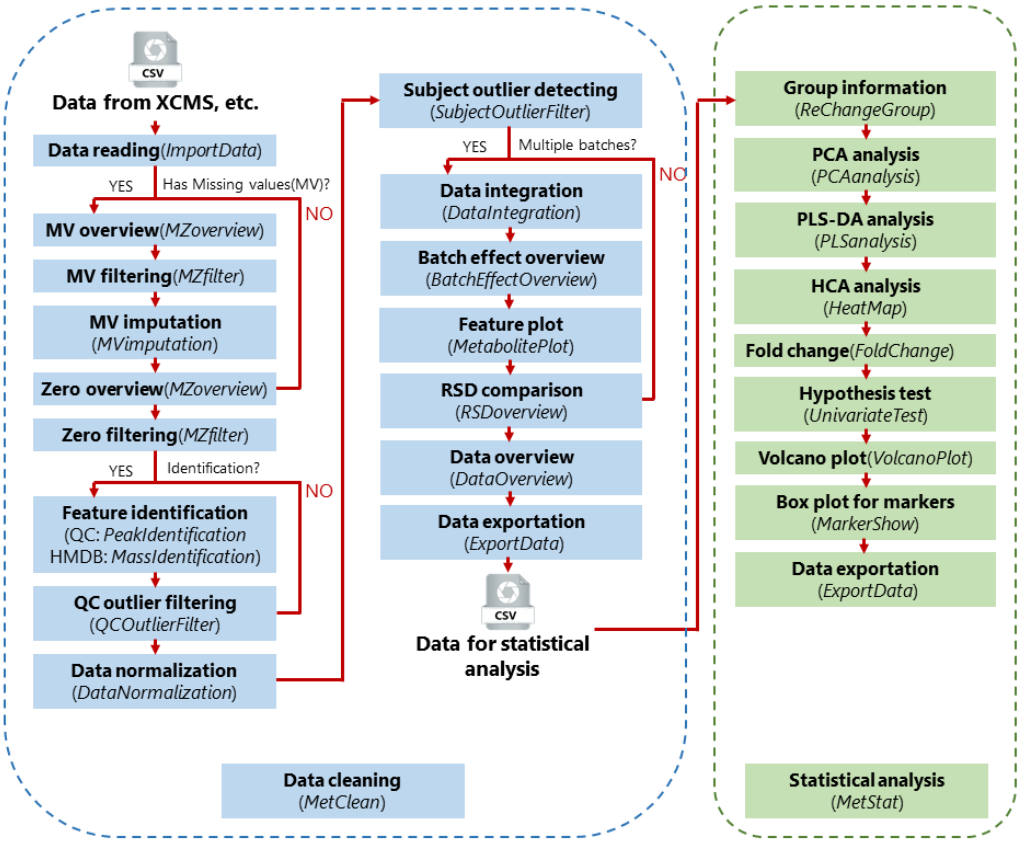


Figure1 Workflow of MetCleaning

Installation and help document

MetCleaning is published in github (link). So you can install it via to github.

code 1: Installation of *MetCleaning*

```
##pcaMethods and impute should be installed from bioconductor
##pcaMethods
source("http://bioconductor.org/biocLite.R")
    biocLite("pcaMethods")
##impute
source("http://bioconductor.org/biocLite.R")
    biocLite("impute")
 if(!require(devtools)) {
  install.packages("devtools")
 }
 library(devtools)
 install_github("jaspershen/MetCleaning")
 library(MetCleaning)
 help(package = "MetCleaning")
```

Data cleaning

Data cleaning is integrated as a function named as *MetClean* in *MetCleaning*. We use the demo data as the example. Copy the code below and paste in you R console.

code 2: Demo data of *MetClean*

```
##demo data
data(data, package = "MetCleaning")
data(sample.information, package = "MetCleaning")

##demo work directory
dir.create("Demo for MetCleaning")
setwd("Demo for MetCleaning")

##write files
write.csv(data, "data.csv", row.names = FALSE)
write.csv(sample.information,"sample.information.csv",row.names=FALSE)
```

The demo data have been added in your work directory and organized in you work directory as Figure 2 shows. It contains two files, "data.csv" and "sample.information.csv".

1. "data.csv" is the metabolomic dataset you want to process. Rows are features and columns are feature abundance of samples and information of features. The information of features must contain "name" (feature name), "mz" (mass to change ratio) and "rt" (retention time). Other information of features are optional, for example "isotopes" and "adducts". The name of sample can contain ".", but cannot contain "-" and space. And the start of sample name cannot be number. For example, "A210.a" and "A210a" are valid, and "210a" or "210-a" are invalid.

2. "sample.information.csv" is sample information for metabolomic dataset. Column 1 is "sample.name" which is the names of subject and QC samples. Please confirm that the sample names in "sample.information.csv" and "data.csv" are completely same. Column 2 is "injection.order" which is the injection order of QC and subject samples. Column 3 is "class", which is used to distinguish "QC" and "Subject" samples. Column 4 is "batch" to provide acquisition batch information for samples. Column 5 is "group", which is used to label the group of subject sample, for example, "control" and "case". The "group" of QC samples is labeled as "QC".

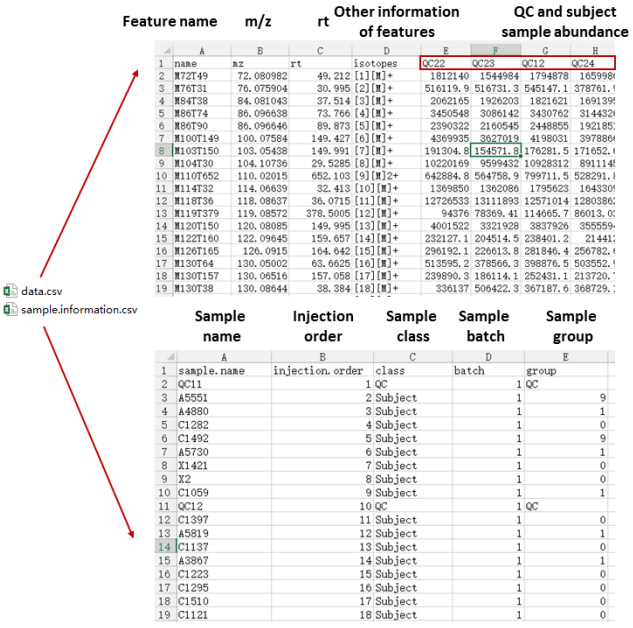


Figure2 Data organization of MetCleaning

Then you can run *MetClean* function to do data cleaning of data. All the arguments of *MetClean* can be found in the other functions in *MetCleaning*. You can use *help(package = "MetCleaning")* to see the help page of *MetCleaning*.

code 3: Running of *MetClean*

```
MetClean(polarity = "positive")
```

Running results of *MetClean*

1. Missing or zero values filtering. In the missing or zero value filtering step, if there are samples which beyond the threshold you set, you should decide to filter them or not. We recommend to remove all of them as Figure 3 shows.

```
Missing values filter...
No QC should be removed.
X257 A5546 X231 sholud be removed!!!
Subject shoulde be removed are:
65 160 177
Which subject you want to remove(please type the index of subject sample,65,160,177
        and separate them using comma,
        if you don't want to remove any subject, please type n):
```

Figure3 Missing or zero value filtering

2. Sample filtering. In the QC or subject sample filtering step (based on PCA), if there are samples which beyond the threshold you set, you should decide to filter them or not. We don't recommend to remove them as Figure 4 shows, because they should be considered combined other information.

```
Subject outlier filtering...
X2 X217 C1126 C1283 C1242 X1 X214  are outliers!!!
C1238 C1248 X5121 X209 X208 X211 X218  are outliers!!!
Batch 1
-----------------------------------------
Subject shoulde be removed are:7 40 54 63 90 130 198
Which subject you want to remove(please type the index of subject sample,n
          and separate them using comma,
          if you don't want to remove any subject, please type n):
```

Figure4 Sample filtering

3. Output files. Output files of MetClean are listed as Figure 5 shows.

(1) "1MV overview", "2MV filter", "3Zero overview" and "4Zero filter" are missing and zero values filtering information.

(2) "5QC outlier filter" and "6Subject outlier filter" are sample filtering based on PCA information.

(3) "7Normalization result" is the data normalization information for each batch.

(4) "8Batch effect" is the batch effect both in before and after data cleaning.

(5) "9metabolite plot" is the scatter plot for each feature.

(6) "10Data overview" is the overview of data.

(7) "11RSD overview" is the RSD distribution for each batch both before and after data cleaning.

(8) "data_after_pre.csv", "qc.info.csv" and "subject.info" are the data and sample information after data cleaning.

(9) "intermediate" is the intermediate data during processing.

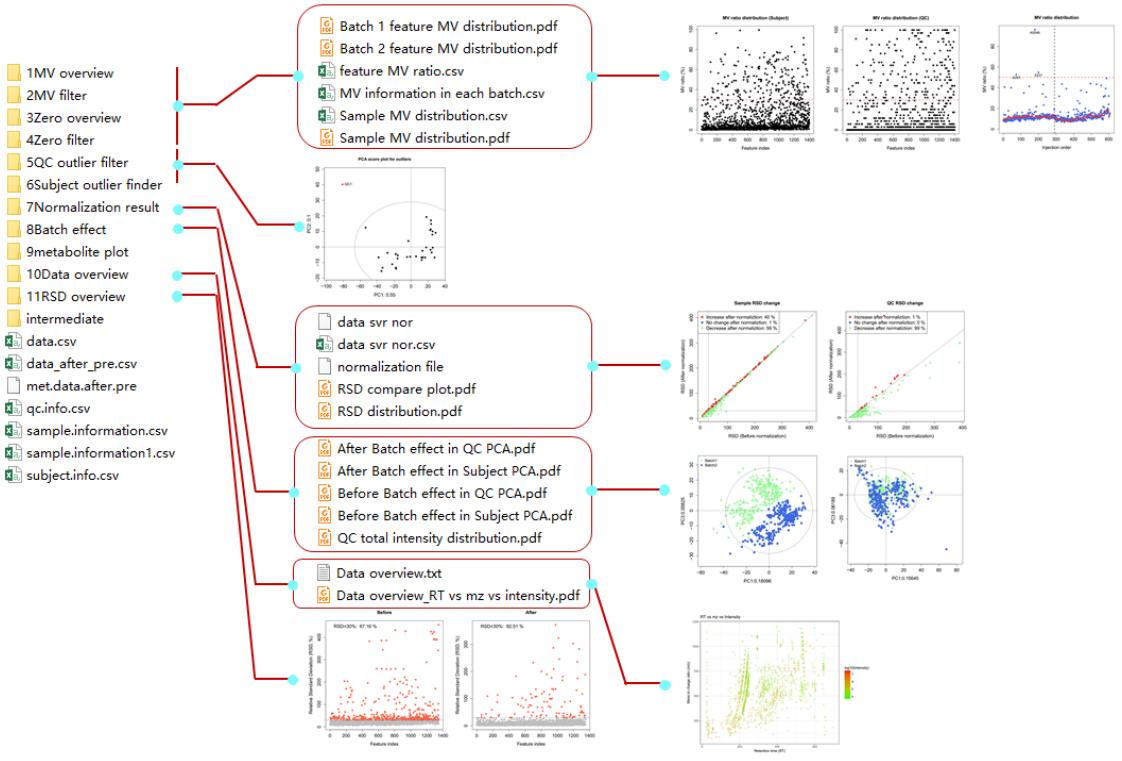


Figure5 Output files of MetClean

Statistical analysis

Data statistical analysis is integrated as a function named as *MetStat* in *MetCleaning*. We use the demo data as the example. Please note that now *MetStat* can only process two class data. Copy the code below and paste in you R console.

code 4: Demo data of *MetStat*

```
data("met.data.after.pre", package = "MetCleaning")
data(new.group, package = "MetCleaning")

##create a folder for MetStat demo
dir.create("Demo for MetStat")
setwd("Demo for MetStat")

## export the demo data as csv
write.csv(new.group, "new.group.csv", row.names = FALSE)
```

The demo data have been added in your work directory. "new.group.csv" is a sample.information which has been changed the group information you want to use for statistical analysis. For the sample which you don't want to use them for statistical analysis, you can set their group information as NA like Figure 6 shows.

Sample.information.csv

sample.name	injection.order	class	batch	group
QC11	1	QC	1	QC
A5551	2	Subject	1	9
A4880	3	Subject	1	1
C1282	4	Subject	1	0
C1492	5	Subject	1	9
A5730	6	Subject	1	1
X1421	7	Subject	1	0
X2	8	Subject	1	0
C1059	9	Subject	1	1
QC12	10	QC	1	QC
C1397	11	Subject	1	0
A5819	12	Subject	1	1
C1137	13	Subject	1	0
A3867	14	Subject	1	1
C1223	15	Subject	1	0
C1295	16	Subject	1	0
C1510	17	Subject	1	0
C1121	18	Subject	1	0
QC13	19	QC	1	QC

New.group.csv

sample.name	injection.order	class	batch	group
QC11	1	QC	1	QC
A5551	2	Subject	1	NA
A4880	3	Subject	1	case
C1282	4	Subject	1	control
C1492	5	Subject	1	NA
A5730	6	Subject	1	case
X1421	7	Subject	1	control
X2	8	Subject	1	control
C1059	9	Subject	1	case
QC12	10	QC	1	QC
C1397	11	Subject	1	control
A5819	12	Subject	1	case
C1137	13	Subject	1	control
A3867	14	Subject	1	case
C1223	15	Subject	1	control
C1295	16	Subject	1	control
C1510	17	Subject	1	control
C1121	18	Subject	1	control
QC13	19	QC	1	QC

Figure6 new group information

code 5: Running of *MetStat*

```
MetStat(MetFlowData = met.data.after.pre, new.group = TRUE)
```

Running results of *MetStat*

1. Sample removing. Firstly, you need to confirm the samples which you want to remove form dataset as Figure 7 shows.

```
Change group information
The samples you want to remove from dataset are:
A5551 C1492 FA13 A5134 A3820 M135 M134 M133 C1262 C1442 X1 A5520 C1458
7 C1485 M139 A5636 C1430 M132 A3657 M140 A4994 C1371
Right(y) or wrong(n)?y
```

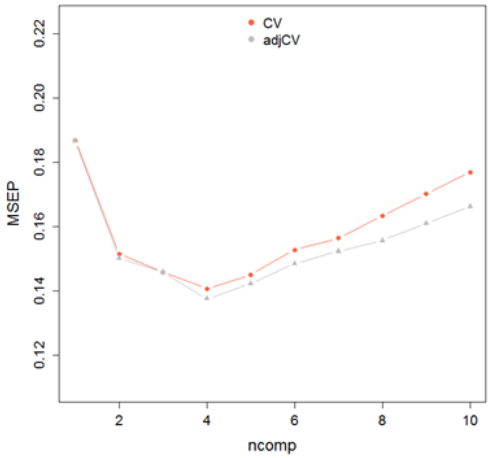
Figure7 sample removing confirmation

2. Number of component selection in PLS-DA analysis. In PLS-DA analysis, you should manually select the best choice of the number of component. When the console show "How many comps do you want to see?", you can type 10 and hit "Enter" key. Then a MSE plot is showing, and the best number of component is the one has the smallest CV values. So type the number (in this example is 4) and hit "Enter" key.

Step 1

```
PLS analysis
How many comps do you want to see?10
```

Step 2



Step 3

```
PLS analysis
How many comps do you want to see?10
Do you want to see the next plot? (y/n)n
Please type number and press Enter to continute:4
```

Figure8 Number of component selection in PLS-DA analysis

3. Output files. Output files of MetStat are listed as Figure 9 shows.

(1) "12PCA analysis" is the PCA score plot.

(2) "13PLS analysis" contains the PLS-DA results.

(3) "14heatmap" is the heatmap.

(4) "15marker selection" contains the information of markers, volcano plot and boxplots of markers.

(5) "data_after_stat.csv", "qc.info.csv" and "subject.info" are the data and sample information after statistical analysis.

(6) "intermediate" is the intermediate data during processing.

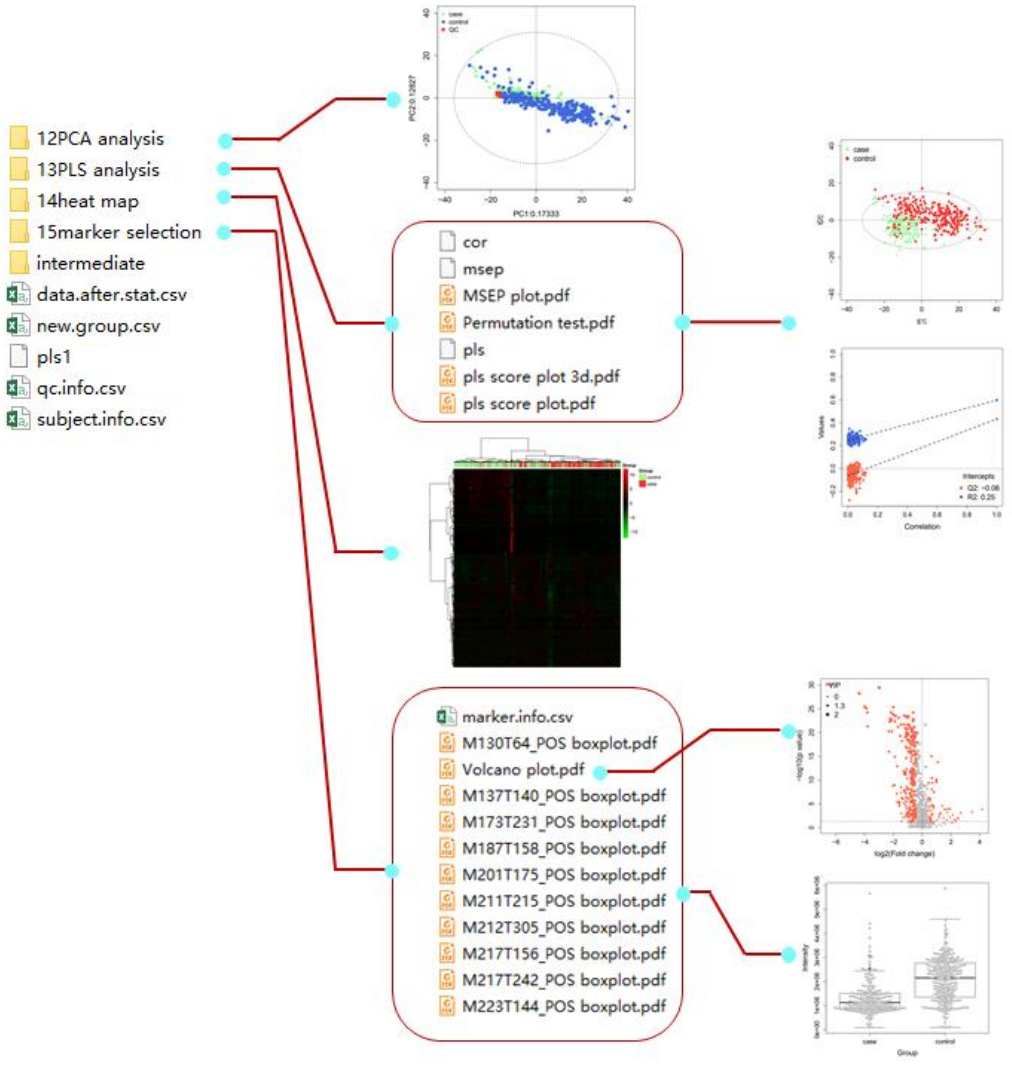


Figure9 Output files of MetStat