# MetCleaning v0.99.0

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## Introduction

*MetCleaning* provides a comprehensive pipeline for data cleaning and statistical analysis of large-scale mass spectrometry (MS) based-metabolomics data. It includes missing value (MV) filtering and imputation, zero value filtering, detection of sample outliers, data normalization, data integration, data quality assessment, and common statistical analysis such as univariate and multivariate statistical analysis. This document describes the step-by-step processing metabolomics data using *MetCleaning*.

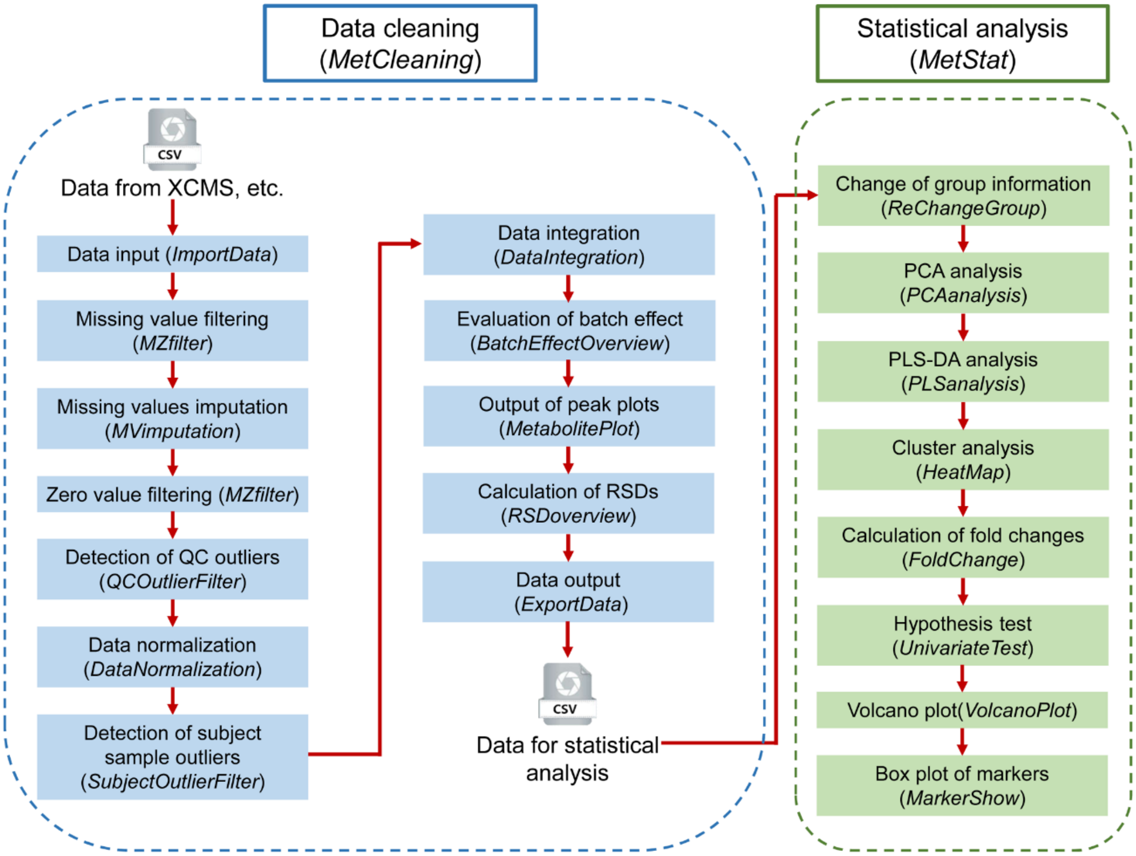

Data cleaning (MetCleaning)
Statistical analysis (MetStat)
csv
Data from XCMS, etc.
Data input (ImportData)
Missing value filtering (MZfilter)
Missing values imputation (MVimputation)
Zero value filtering (MZfilter)
Detection of QC outliers (QCOutlierFilter)
Data normalization (DataNormalization)
Detection of subject sample outliers (SubjectOutlierFilter)
Data integration (DataIntegration)
Evaluation of batch effect (BatchEffectOverview)
Output of peak plots (MetabolitePlot)
Calculation of RSDs (RSDoverview)
Data output (ExportData)
csv
Data for statistical analysis
Change of group information (ReChangeGroup)
PCA analysis (PCAanalysis)
PLS-DA analysis (PLSanalysis)
Cluster analysis (HeatMap)
Calculation of fold changes (FoldChange)
Hypothesis test (UnivariateTest)
Volcano plot(VolcanoPlot)
Box plot of markers (MarkerShow)

***Figure1.*** *The detailed data cleaning pipeline for large-scale mass spectrometry-based untargeted metabolomics using the R package MetCleaning.*

## Installation and help document

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

code 1: Installation of *MetCleaning*

```
##pcaMethods, pathifier and impute should be installed from bioconductor
##installation of pcaMethods
source("http://bioconductor.org/biocLite.R")
    biocLite("pcaMethods")
## installation of pathifier
source("http://bioconductor.org/biocLite.R")
    biocLite("pathifier")
## installation of 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")
```

## Demo data

Demo data in *MetCleaning* is from a study to discover metabolite biomarkers for screening of esophagus cancer (EC). The participants were screened using endoscope and iodine staining for EC (golden standard for diagnosis of EC). The participants were divided into two classes according to their reaction to iodine staining: screening positive and screening negative.

**In *MetCleaning* package, we selected a two-batch dataset as an example.** The dataset contains 1401 metabolic peaks and 606 samples (536 subject samples and 70 QC samples). See the detailed information in Table 1. In *MetCleaning*, metabolomics data is named as "data.csv" and sample information is named as "sample.information.csv".

***Table1.*** *The basic information of demo data in MetCleaning.*

| **Variable** | Screen negative | Screen positive | QC number | Total |
|---|---|---|---|---|
| **Sample number** | 297 | 239 | 70 | 606 |
| **Batch 1** | 141 | 116 | 34 | 291 |
| **Batch 2** | 156 | 123 | 36 | 315 |

## Data cleaning

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

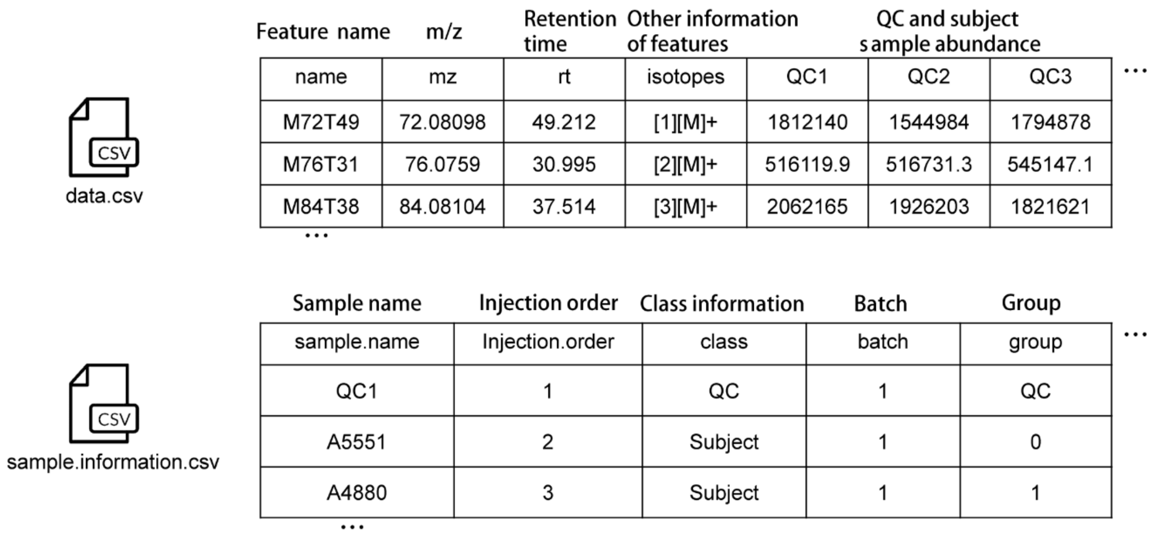
code 2: Demo data of *MetCleaning*

```r
##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 as Figure 2 shows. It contains two files, "data.csv" and "sample.information.csv".

1. **"data.csv"** is the raw metabolomics dataset. Rows are metabolic peaks, and columns are metabolic peak abundance of samples and information of metabolic peaks. The information of metabolic peaks must contain "name" (peak name), "mz" (mass to change ratio) and "rt" (retention time). Other information of metabolic peaks is 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, but "210a" or "210-a" are invalid.

2. **"sample.information.csv"** is sample information for metabolomics dataset. Column 1 is "sample.name" which is the name 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".


data.csv
Feature name
m/z
Retention time
Other information of features
QC and subject sample abundance
sample.information.csv
Sample name
Injection order
Class information
Batch
Group

| name | mz | rt | isotopes | QC1 | QC2 | QC3 | ··· |
|---|---|---|---|---|---|---|---|
| M72T49 | 72.08098 | 49.212 | [1][M]+ | 1812140 | 1544984 | 1794878 | |
| M76T31 | 76.0759 | 30.995 | [2][M]+ | 516119.9 | 516731.3 | 545147.1 | |
| M84T38 | 84.08104 | 37.514 | [3][M]+ | 2062165 | 1926203 | 1821621 | |
| ··· | | | | | | | |

| sample.name | Injection.order | class | batch | group | ··· |
|---|---|---|---|---|---|
| QC1 | 1 | QC | 1 | QC | |
| A5551 | 2 | Subject | 1 | 0 | |
| A4880 | 3 | Subject | 1 | 1 | |
| ··· | | | | | |

***Figure2**. Data organization and data format of MetCleaning.*

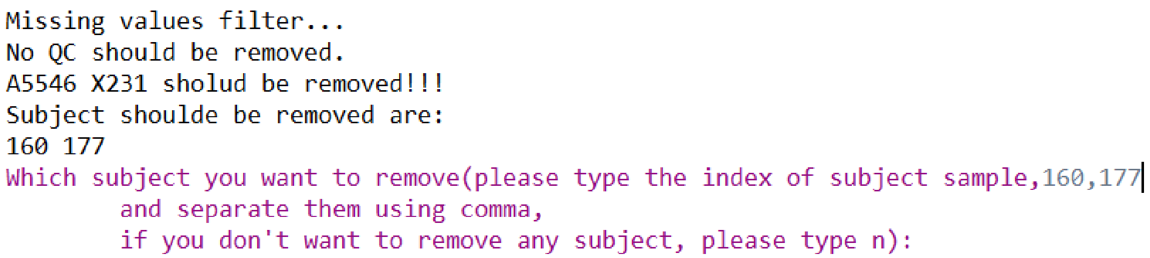
Then you can run *MetCleaning* function to do data cleaning of data. All the arguments of *MetCleaning* can be found in *MetCleaning*. You can use **help(package = "MetCleaning")** to see the help page of *MetCleaning*.

code 3: Running of *MetCleaning*

```
MetCleaning(polarity = "positive")
```

## Running results of *MetCleaning*

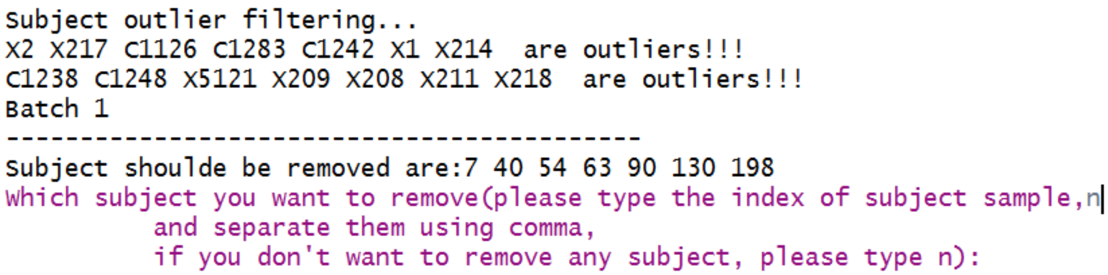
1. Missing or zero values filtering. In the missing or zero value filtering step, if there are samples which beyond the threshold you set, you should decide to remove them or not. We recommend removing all of them as Figure 3 shows.



```
Missing values filter...
No QC should be removed.
A5546 X231 sholud be removed!!!
Subject shoulde be removed are:
160 177
Which subject you want to remove(please type the index of subject sample,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. Detection of sample outliers. In the detection of QC or subject sample outlier step (based on PCA), if there are samples which beyond the threshold you set, you should decide to remove 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.*** *The detection of sample outliers step in MetCleaning.*

3. Output files. Output files of *MetCleaning* 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 information of detection of sample outliers 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 metabolic peak.

(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.

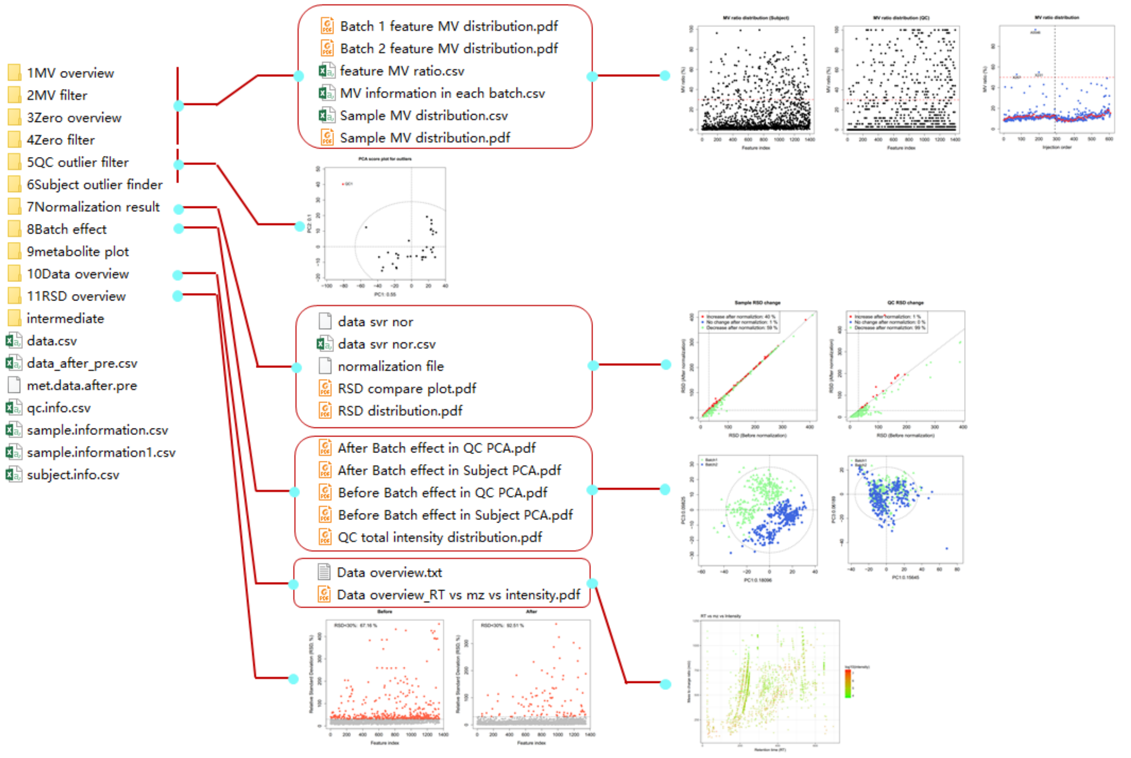


***Figure5.*** *Output files of MetCleaning.*

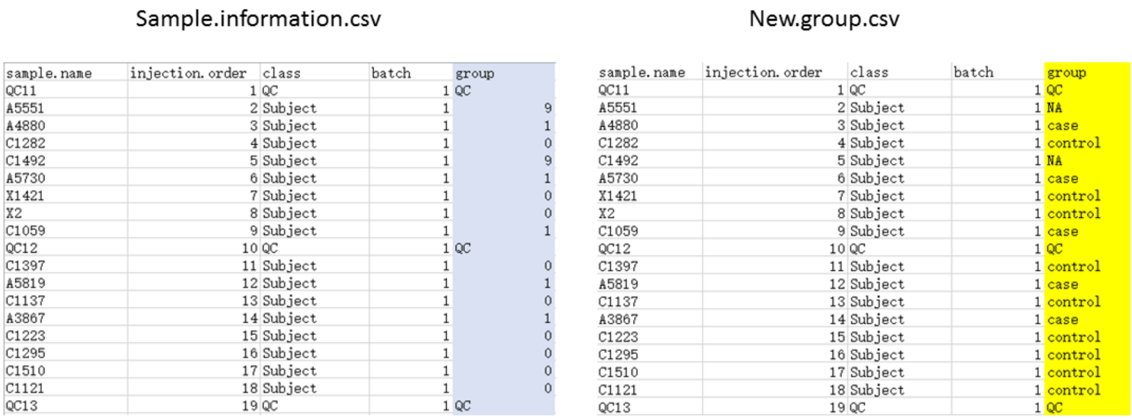
# Statistical analysis

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*

```r
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 |

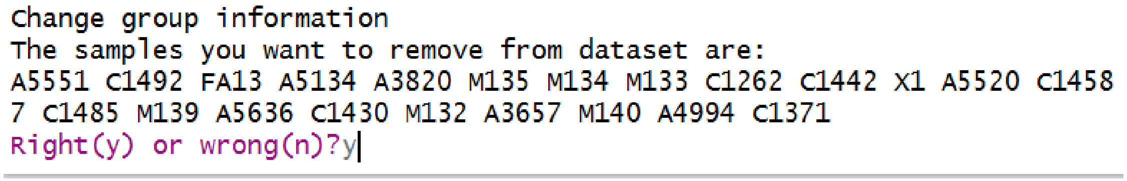
***Figure6****. Group information for statistical analysis.*

code 5: Running of *MetStat*

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

## Running results of *MetStat*

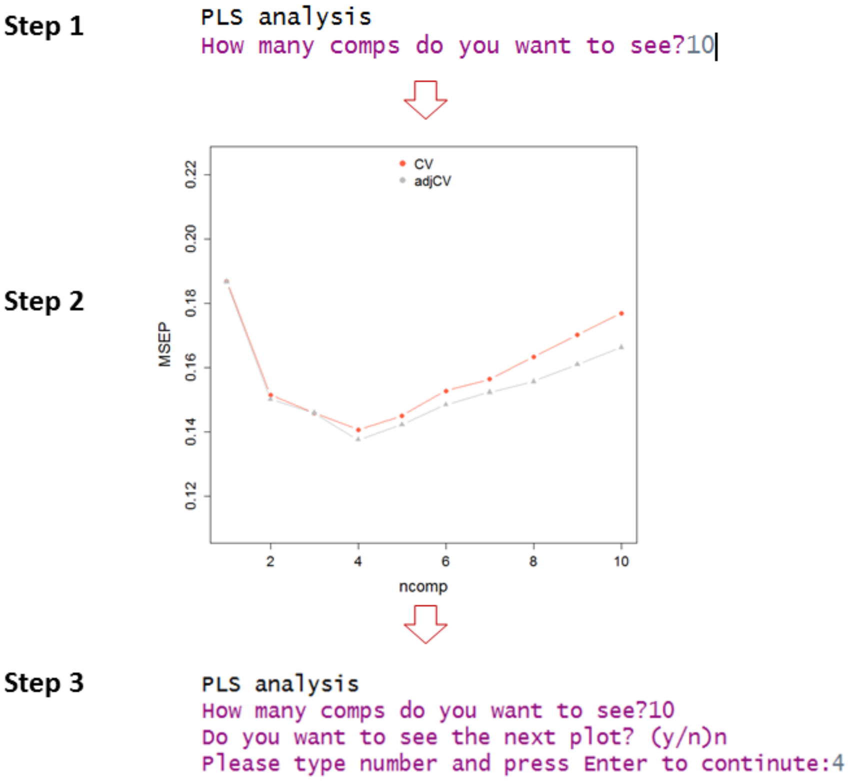
1. Sample removing. Firstly, you need to confirm the samples which you want to remove form dataset as Figure 7 shows.



```
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.*** *The confirmation of the samples you want to remove.*

2. The selection of best number of component 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.*** *The selection of best number of component 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.

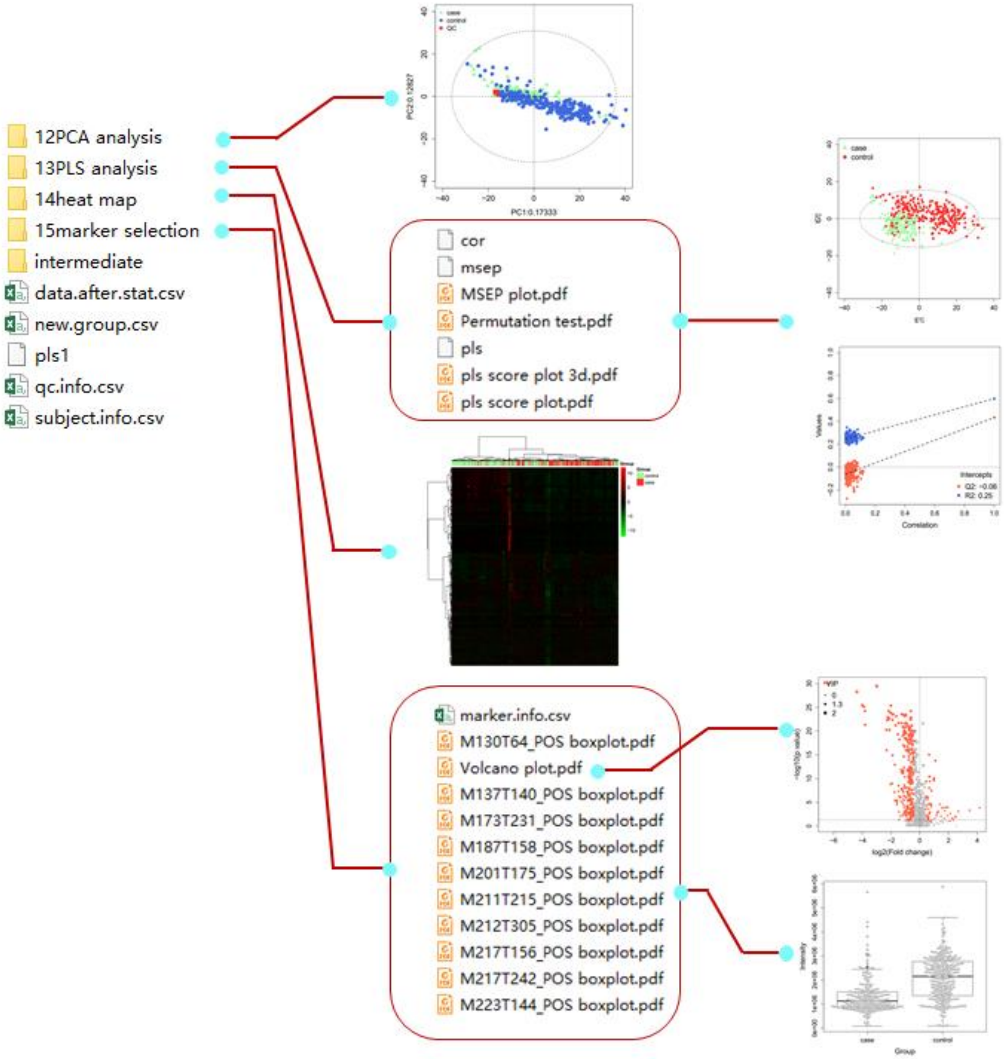


*Figure9. Output files of MetStat.*