Using R and Bioconductor for proteomics data analysis

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Setup

The follow packages will be used throughout this documents. R version 3.1.1 or higher is required to install all the packages using `BiocInstaller::biocLite`.

```r
library("mzR")
library("mzID")
library("MSnID")
library("MSGFplus")
library("MSnbase")
library("rpx")
library("MLInterfaces")
library("pRoloc")
library("pRolocdata")
library("rTANDEM")
library("MSGFplus")
library("MSGFgui")
library("rols")
library("hpar")
```

The most convenient way to install all the tutorials requirement (and more related content), is to install `RforProteomics` with all its dependencies.

```r
library("BiocInstaller")
biocLite("RforProteomics", dependencies = TRUE)
```

Introduction

This tutorial illustrates R / Bioconductor infrastructure for proteomics. Topics covered focus on support for open community-driven formats for raw data and identification results, packages for peptide-spectrum matching, data processing and analysis:

- Exploring available infrastructure
- Mass spectrometry data
- Getting data from proteomics repositories
- Handling raw MS data
- Handling identification data
- MS/MS database search
- Analysing search results
- High-level data interface
- Quantitative proteomics
- Importing third-party quantitative data
- Data processing and analysis

- Statistical analysis
- Machine learning
- Annotation
- Other relevant packages/pipelines

Links to other packages and references are also documented. In particular, the vignettes included in the `RforProteomics` package also contains relevant material.

Exploring available infrastructure

In Bioconductor version 3.1, there are respectively 66 proteomics, 44 mass spectrometry software packages and 7 mass spectrometry experiment packages. These respective packages can be extracted with the `proteomicsPackages()`, `massSpectrometryPackages()` and `massSpectrometryDataPackages()` and explored interactively.

```
library("RforProteomics")
pp <- proteomicsPackages()
display(pp)
```

Mass spectrometry data

Type	Format	Package
raw	mzML, mzXML, netCDF, mzData	`mzR` (read)
identification	mzIdentML	`mzR` and `mzID` (read)
quantitation	mzQuantML	
peak lists	mgf	`MSnbase` (read/write)
other	mzTab	`MSnbase` (read/write)

Getting data from proteomics repositories

Contemporary MS-based proteomics data is disseminated through the ProteomeXchange infrastructure, which centrally coordinates submission, storage and dissemination through multiple data repositories, such as the PRIDE data base at the EBI for MS/MS experiments, PASSEL at the ISB for SRM data and the MassIVE resource. The `rpx` is an interface to ProteomeXchange and provides a basic and unified access to PX data.

```
library("rpx")
pxannounced()
```

```
## 15 new ProteomeXchange annoucements
```

```
##     Data.Set    Publication.Data             Message
## 1  PXD000402 2014-12-04 15:53:18                 New
## 2  PXD001205 2014-12-04 11:19:40                 New
## 3  PXD000853 2014-12-04 10:40:57                 New
## 4  PXD001259 2014-12-04 10:09:03                 New
## 5  PXD000986 2014-12-04 10:00:18                 New
## 6  PXD000543 2014-12-02 17:44:18                 New
## 7  PXD001075 2014-12-02 17:43:40                 New
## 8  PXD000834 2014-12-02 12:57:00                 New
## 9  PXD001381 2014-12-02 11:21:30                 New
## 10 PXD000801 2014-12-02 08:31:32 Updated information
## 11 PXD000800 2014-12-02 08:31:27 Updated information
## 12 PXD000798 2014-12-02 08:31:17 Updated information
## 13 PXD000471 2014-12-02 08:31:06 Updated information
## 14 PXD000758 2014-12-02 08:31:01 Updated information
## 15 PXD000216 2014-12-02 08:30:30 Updated information
```

Using the unique `PXD000001` identifier, we can retrieve the relevant metadata that will be stored in a `PXDataset` object. The names of the files available in this data can be retrieved with the `pxfiles` accessor function.

```
px <- PXDataset("PXD000001")
px
```

```
## Object of class "PXDataset"
##  Id: PXD000001 with 8 files
##  [1] 'F063721.dat' ... [8] 'erwinia_carotovora.fasta'
##  Use 'pxfiles(.)' to see all files.
```

```
pxfiles(px)
```

```
## [1] "F063721.dat"
## [2] "F063721.dat-mztab.txt"
## [3] "PRIDE_Exp_Complete_Ac_22134.xml.gz"
## [4] "PRIDE_Exp_mzData_Ac_22134.xml.gz"
## [5] "PXD000001_mztab.txt"
## [6] "TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzXML"
## [7] "TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.raw"
## [8] "erwinia_carotovora.fasta"
```

Other metadata for the `px` dataset:

```
pxtax(px)
```

```
## [1] "Erwinia carotovora"
```

```
pxurl(px)
```

```
## [1] "ftp://ftp.pride.ebi.ac.uk/pride/data/archive/2012/03/PXD000001"
```

```
pxref(px)
```

```
## [1] "Gatto L, Christoforou A. Using R and Bioconductor for proteomics data analysis. Biochim Biophys
```

Data files can then be downloaded with the `pxget` function. Below, we retrieve the sixth file, TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzXML. The file is downloaded in the working directory and the name of the file is return by the function and stored in the `mzf` variable for later use.

```
mzf <- pxget(px, pxfiles(px)[6])
```

```
## Downloading 1 file
## TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzXML already present.
```

```
mzf
```

```
## [1] "TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzXML"
```

Exercise

Explore what data files have been deposited by Pandey's recent draft map of the human proteome.

```
library("rpx")
hum <- PXDataset("PXD000561")
hum
```

Solution

```
## Object of class "PXDataset"
##  Id: PXD000561 with 2384 files
##  [1] 'Adult_Adrenalgland_Gel_Elite_49.msf' ... [2384] 'README.txt'
##  Use 'pxfiles(.)' to see all files.
```

```
humf <- pxfiles(hum)
length(humf)
```

```
## [1] 2384
```

```
table(sub("^.+\\.", "", humf))
```

```
##
##  msf  raw  txt  xls  xml
##   85 2212    1    1   85
```

```
rawf <- grep("raw", humf, value = TRUE)
table(sub("_.+$", "", rawf))
```

```
##
## Adult Fetal
##  1715   497
```

Handling raw MS data

The `mzR` package provides an interface to the proteowizard C/C++ code base to access various raw data files, such as `mzML`, `mzXML`, `netCDF`, and `mzData`. The data is accessed on-disk, i.e it is not loaded entirely in memory by default but only when explicitly requested. The three main functions are `openMSfile` to create a file handle to a raw data file, `header` to extract metadata about the spectra contained in the file and `peaks` to extract one or multiple spectra of interest. Other functions such as `instrumentInfo`, or `runInfo` can be used to gather general information about a run.

Below, we access the raw data file downloaded in the previous section and open a file handle that will allow us to extract data and metadata of interest.

```
library("mzR")
ms <- openMSfile(mzf)
ms
```

```
## Mass Spectrometry file handle.
## Filename:  TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzXML
## Number of scans:  7534
```

```
hd <- header(ms)
dim(hd)
```

```
## [1] 7534   21
```

```
names(hd)
```

```
##  [1] "seqNum"                   "acquisitionNum"
##  [3] "msLevel"                  "polarity"
##  [5] "peaksCount"               "totIonCurrent"
##  [7] "retentionTime"            "basePeakMZ"
##  [9] "basePeakIntensity"        "collisionEnergy"
## [11] "ionisationEnergy"         "lowMZ"
## [13] "highMZ"                   "precursorScanNum"
## [15] "precursorMZ"              "precursorCharge"
## [17] "precursorIntensity"       "mergedScan"
## [19] "mergedResultScanNum"      "mergedResultStartScanNum"
## [21] "mergedResultEndScanNum"
```

We can extract metadata and scan data for scan 1000 as follows:

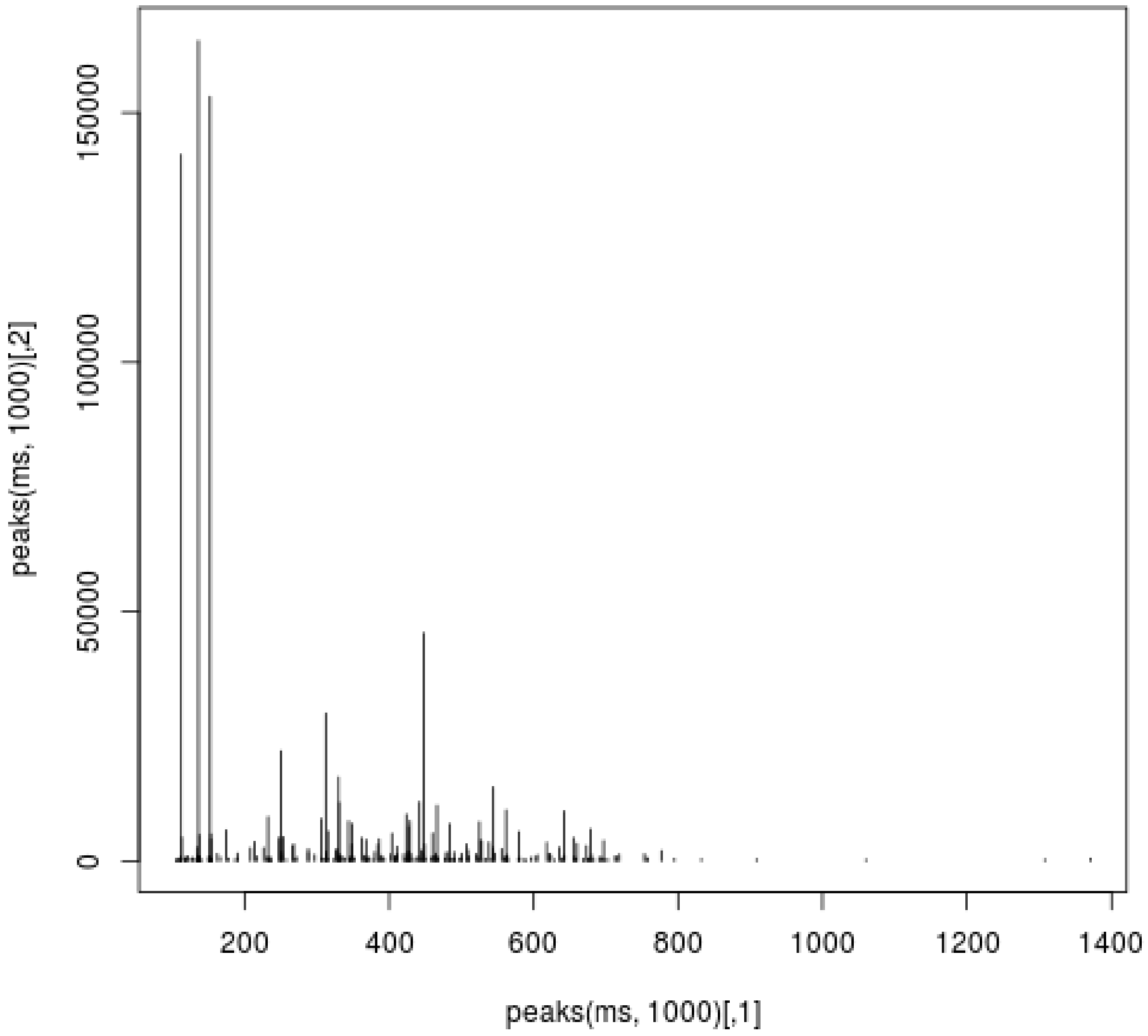
```
hd[1000, ]
```

```
##      seqNum acquisitionNum msLevel polarity peaksCount totIonCurrent
## 1000   1000           1000       2        1        274       1048554
##      retentionTime basePeakMZ basePeakIntensity collisionEnergy
## 1000       1106.92    136.061            164464               0
##      ionisationEnergy    lowMZ   highMZ precursorScanNum precursorMZ
## 1000                0 104.5467 1370.758              992    683.0817
##      precursorCharge precursorIntensity mergedScan mergedResultScanNum
## 1000               2           689443.7          0                   0
##      mergedResultStartScanNum mergedResultEndScanNum
## 1000                        0                      0
```

```r
head(peaks(ms, 1000))
```

```
##           [,1]     [,2]
## [1,] 104.5467 308.9326
## [2,] 104.5684 308.6961
## [3,] 108.8340 346.7183
## [4,] 109.3928 365.1236
## [5,] 110.0345 616.7905
## [6,] 110.0703 429.1975
```

```r
plot(peaks(ms, 1000), type = "h")
```



Exercise

Extract the index of the MS2 spectrum with the highest base peak intensity and plot its spectrum. Is the data centroided or in profile mode?

```
hd2 <- hd[hd$msLevel == 2, ]
i <- which.max(hd2$basePeakIntensity)
hd2[i, ]
```

Solution

```
##      seqNum acquisitionNum msLevel polarity peaksCount totIonCurrent
## 5404   5404           5404       2        1        275    2283283712
##      retentionTime basePeakMZ basePeakIntensity collisionEnergy
## 5404       2751.31   859.5032         354288224               0
##      ionisationEnergy    lowMZ  highMZ precursorScanNum precursorMZ
## 5404                0 100.5031 1995.63             5403    859.1722
##      precursorCharge precursorIntensity mergedScan mergedResultScanNum
## 5404               3          627820480          0                   0
##      mergedResultStartScanNum mergedResultEndScanNum
## 5404                        0                      0
```

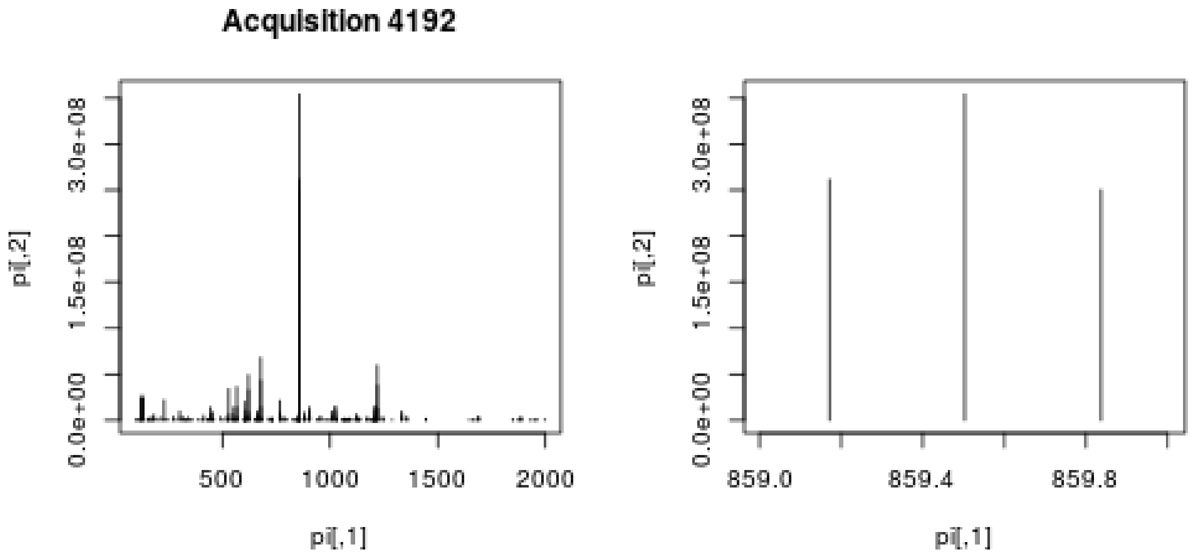
```
head(pi <- peaks(ms, hd2[i, 1]))
```

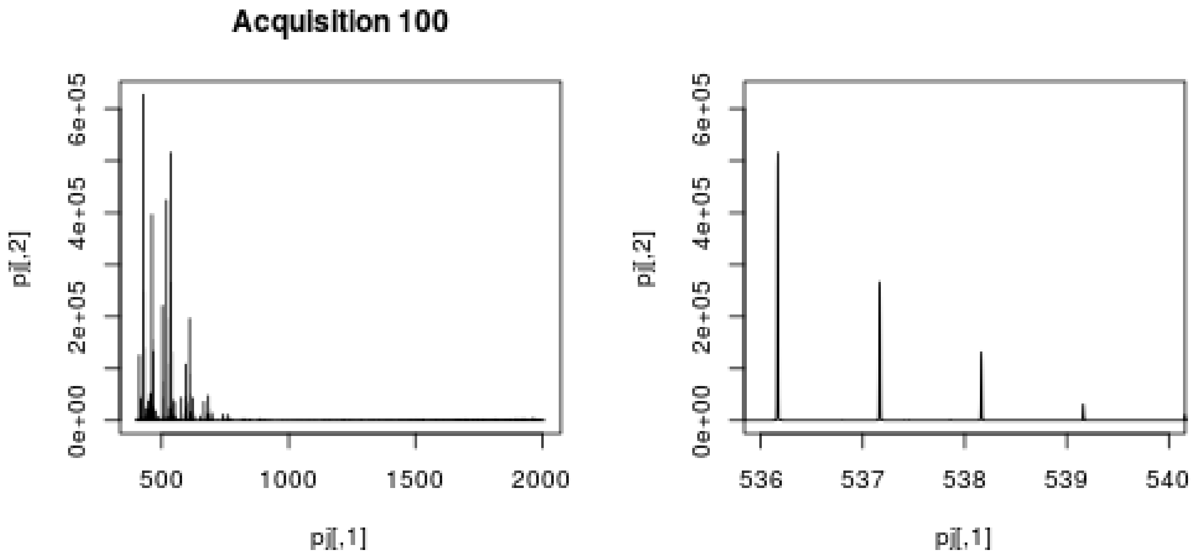
```
##          [,1]      [,2]
## [1,] 100.5031  572248.9
## [2,] 102.3174  463452.2
## [3,] 112.0871 1068157.0
## [4,] 114.9240  526959.1
## [5,] 119.4508  493112.7
## [6,] 120.0810 2219061.0
```

```
mz <- hd2[i, "basePeakMZ"]
mz
```

```
## [1] 859.5032
```

```
par(mfrow = c(2, 2))
plot(pi, type = "h", main = paste("Acquisition", i))
plot(pi, type = "h", xlim = c(mz-0.5, mz+0.5))

pj <- peaks(ms, 100)
plot(pj, type = "l", main = paste("Acquisition", 100))
plot(pj, type = "l", xlim = c(536,540))
```





Exercise

> Read the `MSnbase::MSmap` manual and look at the example to learn how the `mzR` raw data support can be exploited to generate maps of slides of raw MS data. (Note that the `hd` variable containing the raw data header was missing in `MSnbase` version < `1.14.1`.)

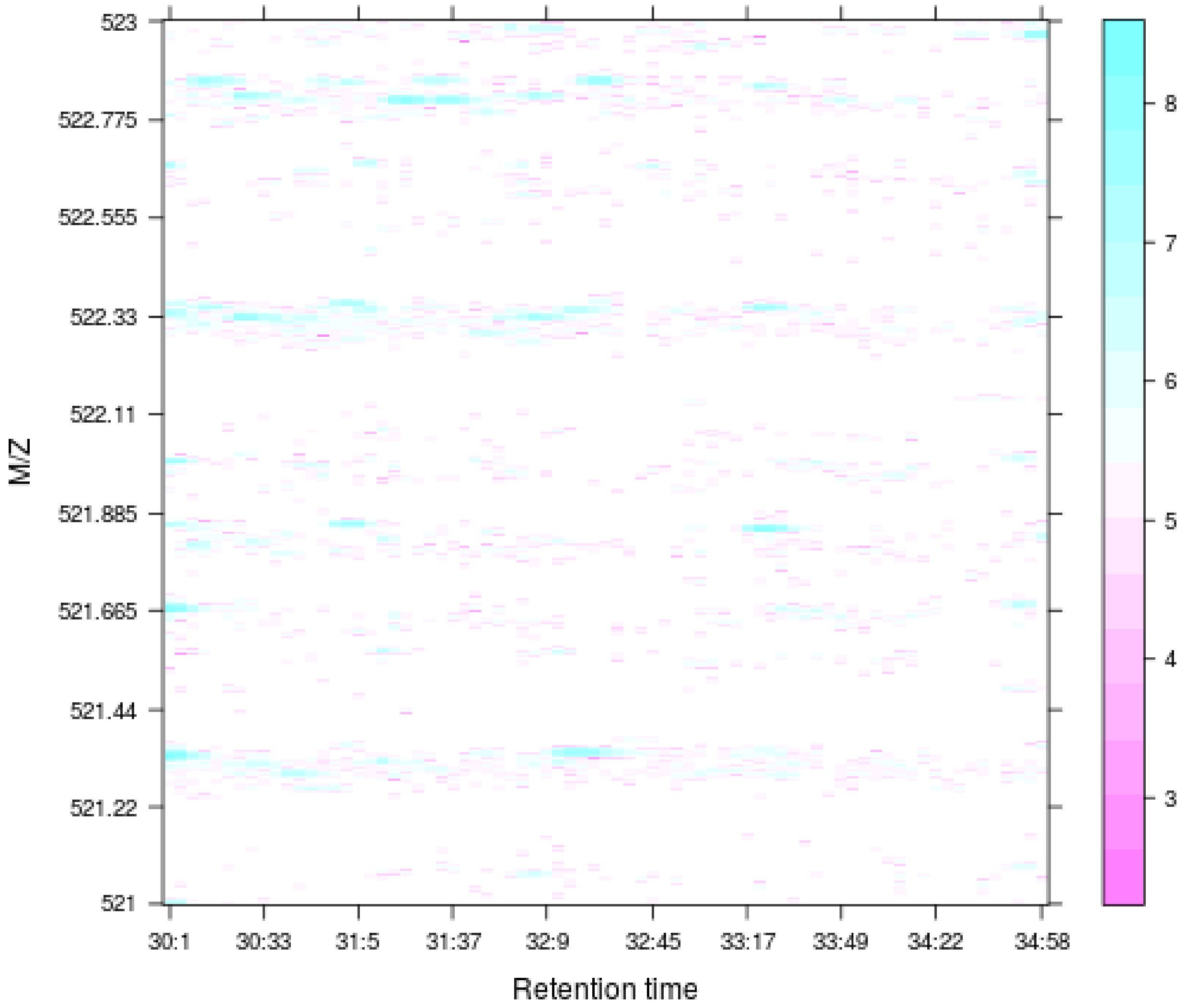
Solution Below we reproduce the example from the `MSmap` function from the `MSnbase` package to plot a specific slice of the raw data using the `mzR` functions we have just described.

```
## a set of spectra of interest: MS1 spectra eluted
## between 30 and 35 minutes retention time
ms1 <- which(hd$msLevel == 1)
```

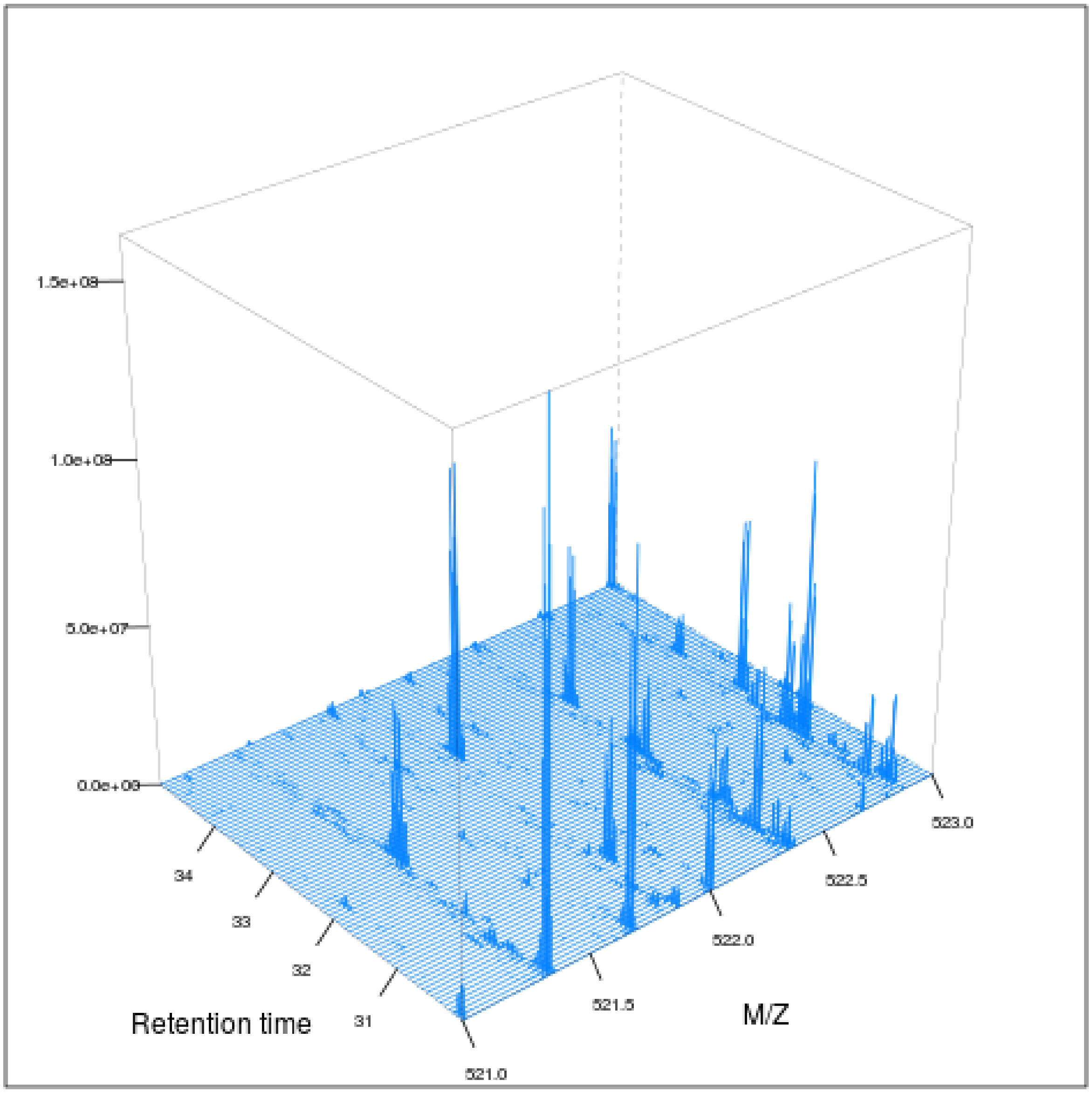
```
rtsel <- hd$retentionTime[ms1] / 60 > 30 &
    hd$retentionTime[ms1] / 60 < 35

## the map
M <- MSmap(ms, ms1[rtsel], 521, 523, .005, hd)
```

```
## 1
```

```
plot(M, aspect = 1, allTicks = FALSE)
```



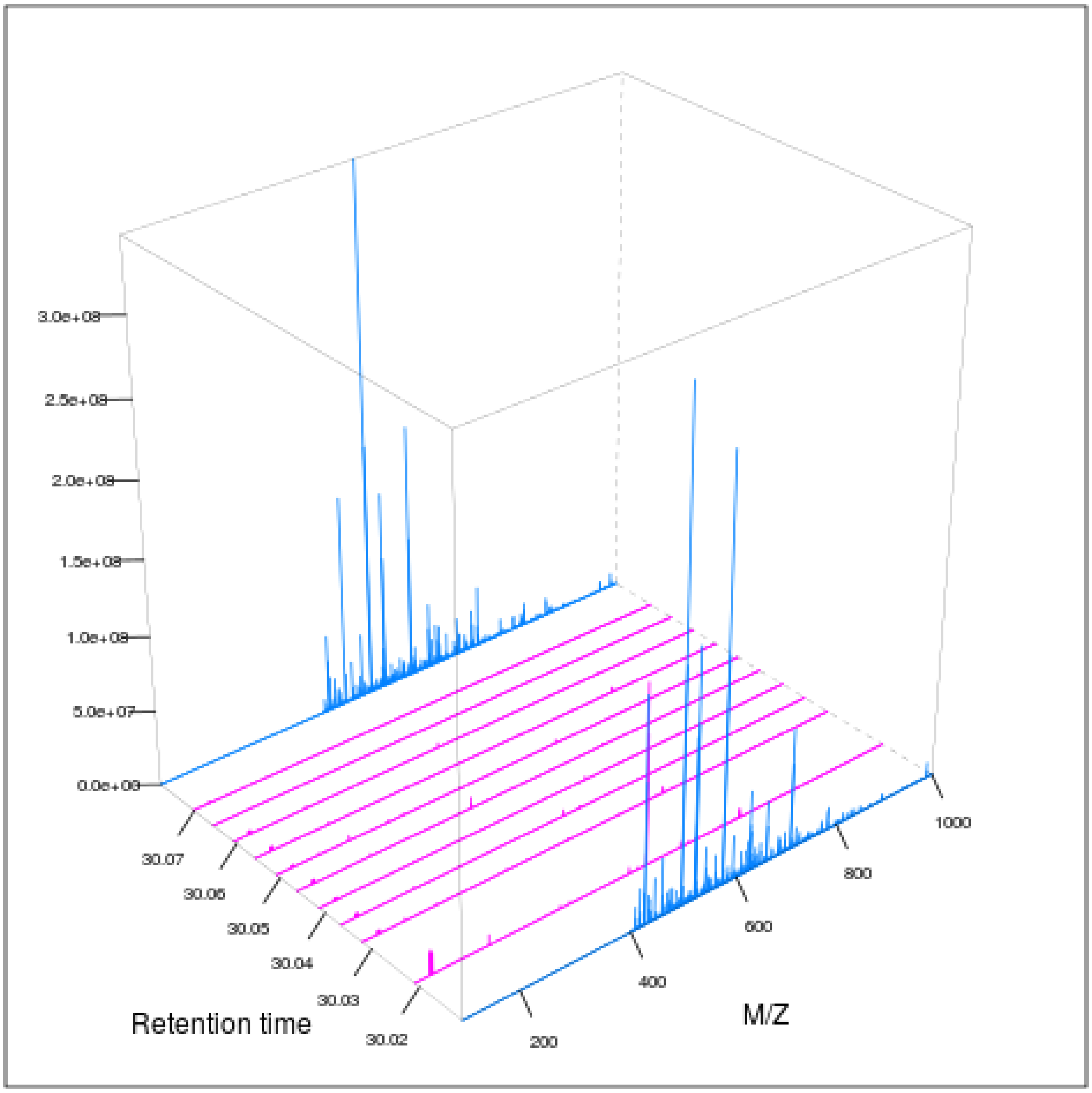
```
plot3D(M)
```



```
## With some MS2 spectra
i <- ms1[which(rtsel)][1]
j <- ms1[which(rtsel)][2]
M2 <- MSmap(ms, i:j, 100, 1000, 1, hd)
```

```
## 1
```

```
plot3D(M2)
```



Handling identification data

The `RforProteomics` package distributes a small identification result file (see `?TMT_Erwinia_1uLSike_Top10HCD_isol2_45ste`
that we load and parse using infrastructure from the `mzID` package.

```
library("mzID")
f <- dir(system.file("extdata", package = "RforProteomics"),
         pattern = "mzid", full.names=TRUE)
basename(f)
```

```
## [1] "TMT_Erwinia.mzid"
```

```
id <- mzID(f)
```

```
## reading TMT_Erwinia.mzid... DONE!
```

```
id
```

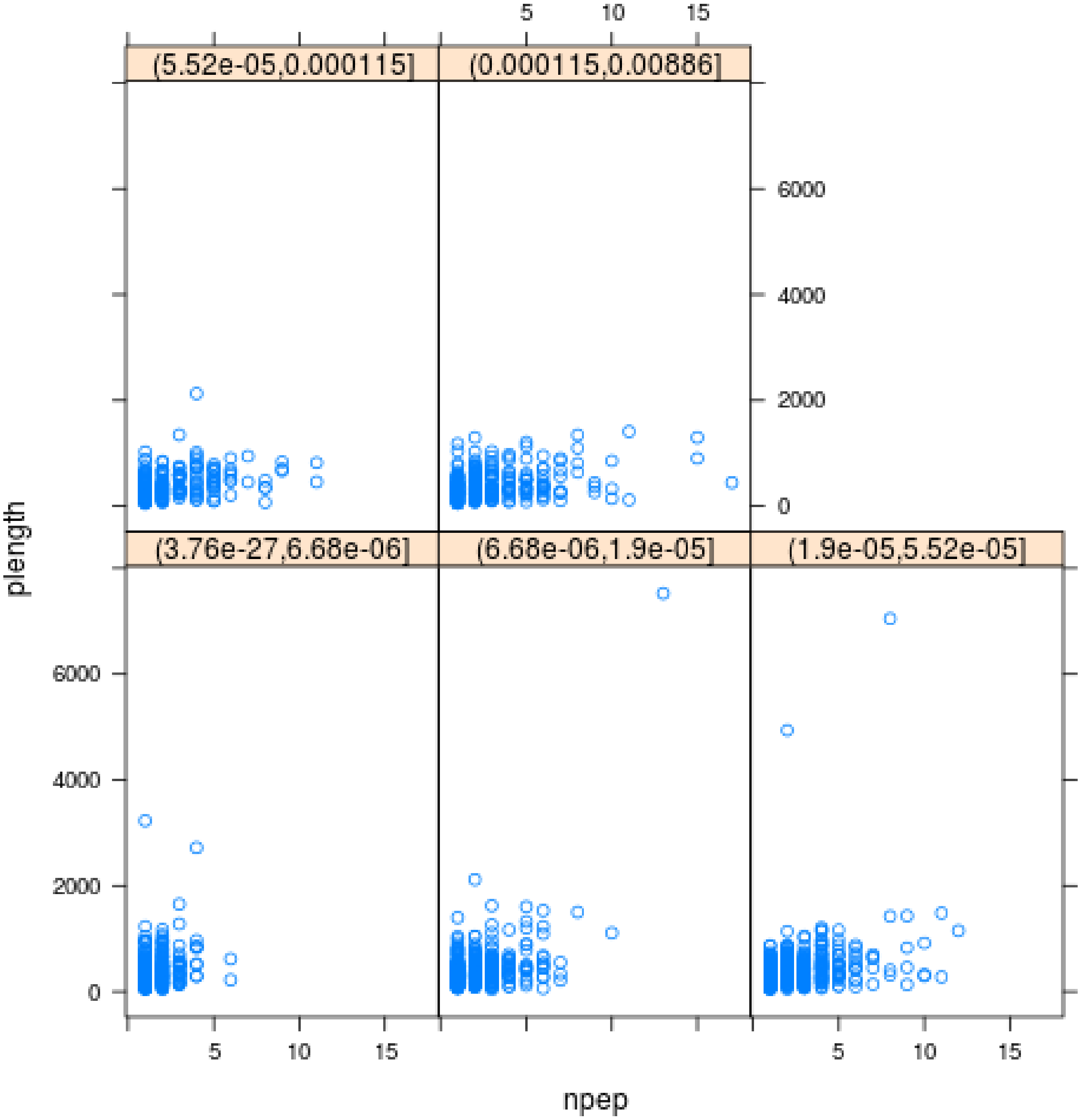
```
## An mzID object
##
## Software used:   MS-GF+ (version: Beta (v10072))
##
## Rawfile:         /home/lgatto/dev/00_github/RforProteomics/sandbox/TMT_Erwinia_1uLSike_Top10HCD_isol2
##
## Database:        /home/lgatto/dev/00_github/RforProteomics/sandbox/erwinia_carotovora.fasta
##
## Number of scans: 5287
## Number of PSM's: 5563
```

Various data can be extracted from the `mzID` object, using one the accessor functions such as `database`, `scans`, `peptides`, … The object can also be converted into a `data.frame` using the `flatten` function.

Exercise

> Is there a relation between the length of a protein and the number of identified peptides, conditioned by the (average) e-value of the identifications?

```
fid <- flatten(id)
x <- by(fid, fid$accession, function(x)
    c(unique(x$length),
      length(unique(x$pepseq)),
      mean(x$'ms-gf:specevalue')))
x <- data.frame(do.call(rbind, x))
colnames(x) <- c("plength", "npep", "eval")
x$bins <- cut(x$eval, summary(x$eval))
library("lattice")
xyplot(plength ~ npep | bins, data = x)
```



Solution

Exercise

The mzR package also support fast parsing of mzIdentML files with the openIDfile function. Compare it, it terms of output and speed with mzID.

```
library("mzR")
library("mzID")
f <- dir(system.file("extdata", package = "RforProteomics"),
         pattern = "mzid", full.names=TRUE)
```

```r
system.time({
    id0 <- mzID(f)
    fid0 <- flatten(id0)
})
```

Solution

```
## reading TMT_Erwinia.mzid.gz... DONE!
```

```
##    user  system elapsed
##  18.810   0.064  18.902
```

```r
head(fid0)
```

```
##   spectrumid scan number(s) acquisitionnum passthreshold rank
## 1  scan=5782           5782           5782          TRUE    1
## 2  scan=6037           6037           6037          TRUE    1
## 3  scan=5235           5235           5235          TRUE    1
## 4  scan=5397           5397           5397          TRUE    1
## 5  scan=6075           6075           6075          TRUE    1
## 6  scan=5761           5761           5761          TRUE    1
##   calculatedmasstocharge experimentalmasstocharge chargestate
## 1              1080.2321                1080.2325           3
## 2              1002.2115                1002.2089           3
## 3              1189.2800                1189.2836           3
## 4               960.5365                 960.5365           3
## 5              1264.3419                1264.3409           3
## 6              1268.6501                1268.6429           2
##   ms-gf:denovoscore ms-gf:evalue ms-gf:rawscore ms-gf:specevalue
## 1               174 5.430080e-21            147     3.764831e-27
## 2               245 9.943751e-20            214     6.902626e-26
## 3               264 2.564787e-19            211     1.778789e-25
## 4               178 2.581753e-18            154     1.792541e-24
## 5               252 2.178423e-17            188     1.510364e-23
## 6               138 2.329453e-17            123     1.618941e-23
##   assumeddissociationmethod isotopeerror isdecoy post pre end start
## 1                       HCD            0   FALSE    S   R  84    50
## 2                       HCD            0   FALSE    R   K 315   288
## 3                       HCD            0   FALSE    A   R 224   192
## 4                       HCD            0   FALSE    -   R 290   264
## 5                       HCD            0   FALSE    F   R 153   119
## 6                       HCD            0   FALSE    Y   K 286   264
##   accession length                                  description
## 1   ECA1932    155                      outer membrane lipoprotein
## 2   ECA1147    434                                  trigger factor
## 3   ECA0013    295                 ribose-binding periplasmic protein
## 4   ECA1731    290                                        flagellin
## 5   ECA1443    298      UTP--glucose-1-phosphate uridylyltransferase
## 6   ECA1444    468 6-phosphogluconate dehydrogenase, decarboxylating
##                               pepseq modified modification
## 1 PVQIQAGEDSNVIGALGGAVLGGFLGNTIGGGSGR    FALSE         <NA>
## 2        TQVLDGLINANDIEVPVALIDGEIDVLR    FALSE         <NA>
## 3   TKGLNVMQNLLTAHPDVQAVFAQNDEMALGALR    FALSE         <NA>
## 4         SQILQQAGTSVLSQANQVPQTVLSLLR    FALSE         <NA>
## 5 PIIGDNPFVVVLPDVVLDESTADQTQENLALLISR    FALSE         <NA>
## 6             WTSQSSLDLGEPLSLITESVFAR    FALSE         <NA>
##                                                   spectrumFile
## 1 TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzXML
## 2 TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzXML
## 3 TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzXML
## 4 TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzXML
## 5 TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzXML
## 6 TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzXML
##               databaseFile
## 1 erwinia_carotovora.fasta
## 2 erwinia_carotovora.fasta
## 3 erwinia_carotovora.fasta
## 4 erwinia_carotovora.fasta
## 5 erwinia_carotovora.fasta
## 6 erwinia_carotovora.fasta
```

```
system.time({
    id1 <- openIDfile(f)
    fid1 <- mzR::psms(id1)
})
```

```
##    user  system elapsed
##   0.320   0.001   0.321
```

```
head(fid1)
```

```
##   spectrumID chargeState rank passThreshold experimentalMassToCharge
## 1  scan=5782           3    1          TRUE                1080.2325
## 2  scan=6037           3    1          TRUE                1002.2089
## 3  scan=5235           3    1          TRUE                1189.2836
## 4  scan=5397           3    1          TRUE                 960.5365
## 5  scan=6075           3    1          TRUE                1264.3409
## 6  scan=5761           2    1          TRUE                1268.6429
##   calculatedMassToCharge                            sequence modNum
## 1              1080.2321 PVQIQAGEDSNVIGALGGAVLGGFLGNTIGGGSGR      0
## 2              1002.2115        TQVLDGLINANDIEVPVALIDGEIDVLR      0
## 3              1189.2800   TKGLNVMQNLLTAHPDVQAVFAQNDEMALGALR      0
## 4               960.5365         SQILQQAGTSVLSQANQVPQTVLSLLR      0
## 5              1264.3419 PIIGDNPFVVVLPDVVLDESTADQTQENLALLISR      0
## 6              1268.6501             WTSQSSLDLGEPLSLITESVFAR      0
##   isDecoy post pre start end DatabaseAccess DatabaseSeq
## 1   FALSE    S   R    50  84        ECA1932
## 2   FALSE    R   K   288 315        ECA1147
## 3   FALSE    A   R   192 224        ECA0013
## 4   FALSE    -   R   264 290        ECA1731
## 5   FALSE    F   R   119 153        ECA1443
## 6   FALSE    Y   K   264 286        ECA1444
##                                         DatabaseDescription
## 1                        ECA1932 outer membrane lipoprotein
## 2                                    ECA1147 trigger factor
## 3                ECA0013 ribose-binding periplasmic protein
## 4                                         ECA1731 flagellin
## 5      ECA1443 UTP--glucose-1-phosphate uridylyltransferase
## 6 ECA1444 6-phosphogluconate dehydrogenase, decarboxylating
```

MS/MS database search

While searches are generally performed using third-party software independently of R or can be started from R using a `system` call, the `rTANDEM` package allows one to execute such searches using the X!Tandem engine. The `shinyTANDEM` provides a interactive interface to explore the search results.

```r
library("rTANDEM")
?rtandem
library("shinyTANDEM")
?shinyTANDEM
```

Similarly, the `MSGFplus` package enables to perform a search using the MSGF+ engine, as illustrated below:

```r
library("MSGFplus")
parameters <- msgfPar(database = 'proteins.fasta',
                      tolerance='20 ppm',
                      instrument='TOF',
                      enzyme='Lys-C')
runMSGF(parameters, c('file1.mzML', 'file2.mzML'))
```

A graphical interface to perform the search the data and explore the results is also available:

```r
library("MSGFgui")
MSGFgui()
```

Exercise

> Search TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzXML against the fasta file from `PXD000001` using, for example, `MSGFplus`/`MSGFgui`.

Solution

1. Get the fasta database:

```r
fas <- pxget(px, pxfiles(px)[8])
```

```
## Downloading 1 file
## erwinia_carotovora.fasta already present.
```

```r
basename(fas)
```

```
## [1] "erwinia_carotovora.fasta"
```

2. One could run MSGF+ from the command-line directly from `R`:

```
msgf <- system.file(package = "MSGFplus", "MSGFPlus", "MSGFPlus.jar")
system(paste0("java -jar ", msgf))
cmd <- paste("java -jar", msgf, "-protocol 2 -inst 1 -s", mzf, "-d", fas)
cmd
```

```
## [1] "java -jar /home/lg390/R/x86_64-unknown-linux-gnu-library/3.1/MSGFplus/MSGFPlus/MSGFPlus.jar -pro
```

```
system(cmd)
```

3. Use MSGFplus:

```
library("MSGFplus")
msgfpar <- msgfPar(database = fas,
                   instrument = 'HighRes',
                   tda = TRUE,
                   enzyme = 'Trypsin',
                   protocol = 'iTRAQ')
idres <- runMSGF(msgfpar, mzf, memory=1000)
```

```
## java -Xmx1000M -jar /home/lg390/R/x86_64-unknown-linux-gnu-library/3.1/MSGFplus/MSGFPlus/MSGFPlus.jar
##
## reading TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzid... DONE!
```

```
idres
```

```
## An mzID object
##
## Software used:   MS-GF+ (version: Beta (v10072))
##
## Rawfile:         /home/lg390/Documents/Teaching/rbioc-proteomics/TMT_Erwinia_1uLSike_Top10HCD_isol2_4
##
## Database:        /home/lg390/Documents/Teaching/rbioc-proteomics/erwinia_carotovora.fasta
##
## Number of scans: 5343
## Number of PSM's: 5656
```

```
## identification file (needed below)
basename(files(idres)$id)
```

```
## [1] "TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzid"
```

(Note that in the `runMSGF` call above, I explicitly reduce the memory allocated to the java virtual machine to 3.5GB. In general, there is no need to specify this argument, unless you experience an error regarding the *maximum heap size*).

4. Through the graphical user interface:

```
library("MSGFgui")
MSGFgui()
```

Analysing search results

The MSnID package can be used for post-search filtering of MS/MS identifications. One starts with the construction of an MSnID object that is populated with identification results that can be imported from a data.frame or from mzIdenML files.

```
library("MSnID")
msnid <- MSnID(".")
```

```
## Note, the anticipated/suggested columns in the
## peptide-to-spectrum matching results are:
## -----------------------------------------------
## accession
## calculatedMassToCharge
## chargeState
## experimentalMassToCharge
## isDecoy
## peptide
## spectrumFile
## spectrumID
```

```
msnid <- read_mzIDs(msnid,
                    "TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzid")
```

```
## Reading from mzIdentMLs ...
```

```
## reading TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzid... DONE!
```

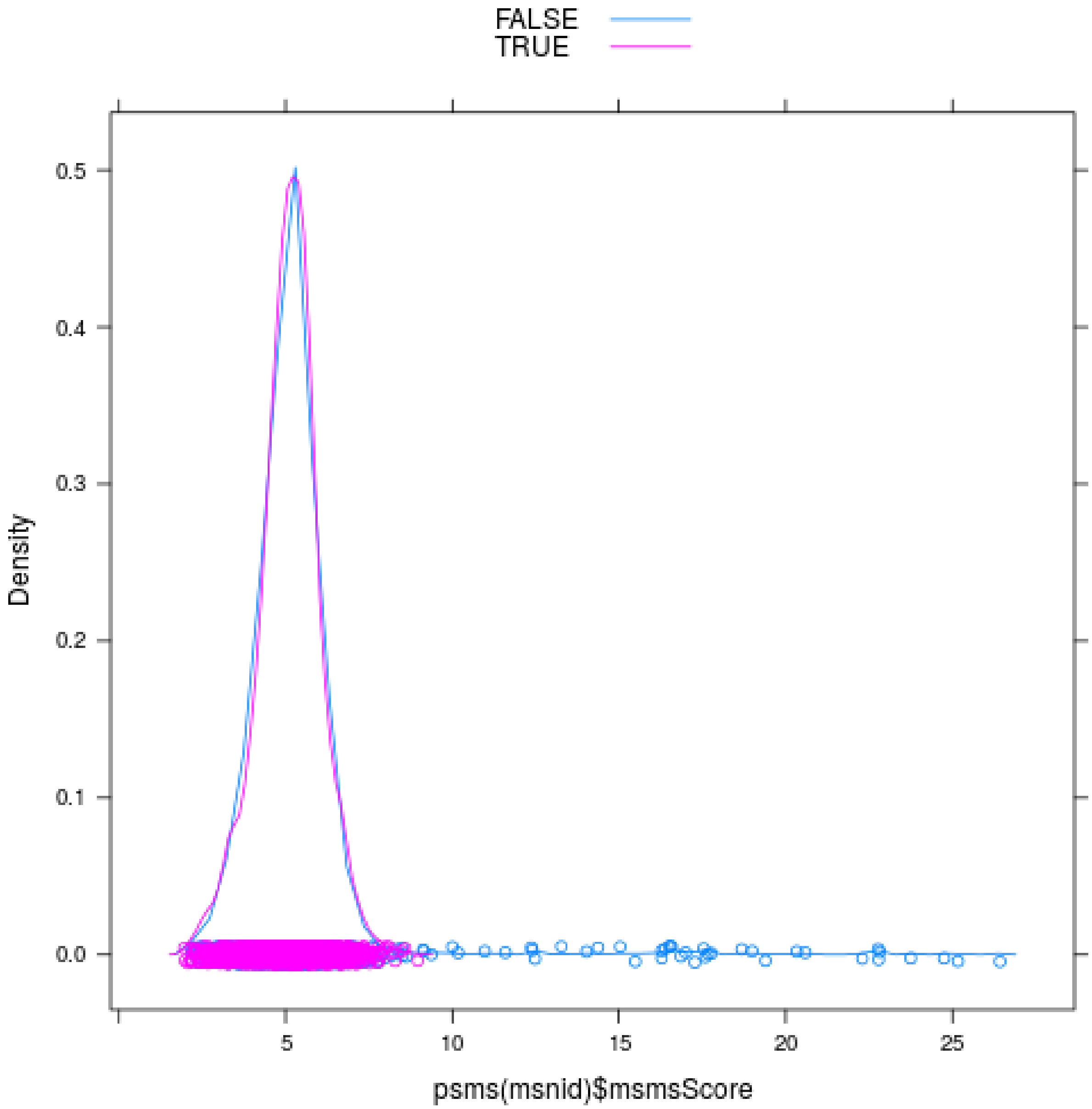
```
show(msnid)
```

```
## MSnID object
## Working directory: "."
## #Spectrum Files:  1
## #PSMs: 5759 at 100 % FDR
## #peptides: 4942 at 99 % FDR
## #accessions: 3148 at 100 % FDR
```

The package then enables to define, optimise and apply filtering based for example on missed cleavages, identification scores, precursor mass errors, etc. and assess PSM, peptide and protein FDR levels. Below, we start by apply a correction of monoisotopic peaks (see ?correct_peak_selection for details) and define two variables to be used for identification filtering.

```
msnid <- correct_peak_selection(msnid)
msnid$msmsScore <- -log10(msnid$`MS-GF:SpecEValue`)
msnid$absParentMassErrorPPM <- abs(mass_measurement_error(msnid))
```

As shown below, this particular spiked-in data set displays few high scoring non-decoy hits



We define a filter object, assigning arbitrary threshold and evaluate it on the `msnid` data

```
filtObj <- MSnIDFilter(msnid)
filtObj$absParentMassErrorPPM <- list(comparison="<", threshold=5.0)
filtObj$msmsScore <- list(comparison=">", threshold=8.0)
filtObj
```

```
## MSnIDFilter object
## (absParentMassErrorPPM < 5) & (msmsScore > 8)
```

```
evaluate_filter(msnid, filtObj)
```

```
##                    fdr  n
## PSM       0.04545455 23
## peptide   0.06250000 17
## accession 0.05882353 18
```

We can also optimise the filtering with a target protein FDR value of 0.01

```
filtObj.grid <- optimize_filter(filtObj, msnid, fdr.max=0.01,
                                method="Grid", level="PSM",
                                n.iter=50000)
filtObj.grid
```

```
## MSnIDFilter object
## (absParentMassErrorPPM < 12) & (msmsScore > 8.1)
```

```
evaluate_filter(msnid, filtObj.grid)
```

```
##           fdr  n
## PSM         0 39
## peptide     0 31
## accession   0 26
```

We can now apply the filter to the data

```
msnid <- apply_filter(msnid, filtObj.grid)
msnid
```

```
## MSnID object
## Working directory: "."
## #Spectrum Files:  1
## #PSMs: 39 at 0 % FDR
## #peptides: 31 at 0 % FDR
## #accessions: 26 at 0 % FDR
```

The resulting data can be exported to a `data.frame` or to a dedicated `MSnSet` data structure for quantitative MS data, described below, and further processed and analyses using appropriate statistical tests.

High-level data interface

The above sections introduced low-level interfaces to raw and identification results. The MSnbase package provides abstractions for raw data through the `MSnExp` class and containers for quantification data via the `MSnSet` class. Both store

1. the actual assay data (spectra or quantitation matrix), accessed with `spectra` (or the `[`, `[[` operators) or `exprs`;
2. sample metadata, accessed as a `data.frame` with `pData`;
3. feature metadata, accessed as a `data.frame` with `fData`.

The figure below give a schematics of an `MSnSet` instance and the relation between the assay data and the respective feature and sample metadata.

Another useful slot is processingData, accessed with processingData(.), that records all the processing that objects have undergone since their creation (see examples below).

The readMSData will parse the raw data, extract the MS2 spectra (by default) and construct an MS experiment object of class MSnExp.

(Note that while readMSData supports MS1 data, this is currently not convenient as all the data is read into memory.)

```
library("MSnbase")
rawFile <- dir(system.file(package = "MSnbase", dir = "extdata"),
               full.name = TRUE, pattern = "mzXML$")
basename(rawFile)
```

```
## [1] "dummyiTRAQ.mzXML"
```

```
msexp <- readMSData(rawFile, verbose = FALSE)
msexp
```

```
## Object of class "MSnExp"
##  Object size in memory: 0.2 Mb
## - - - Spectra data - - -
##  MS level(s): 2
##  Number of MS1 acquisitions: 1
##  Number of MSn scans: 5
##  Number of precursor ions: 5
##  4 unique MZs
##  Precursor MZ's: 437.8 - 716.34
##  MSn M/Z range: 100 2016.66
##  MSn retention times: 25:1 - 25:2 minutes
## - - - Processing information - - -
## Data loaded: Thu Dec  4 19:10:37 2014
##  MSnbase version: 1.14.1
## - - - Meta data  - - -
## phenoData
##   rowNames: 1
##   varLabels: sampleNames
##   varMetadata: labelDescription
## Loaded from:
##   dummyiTRAQ.mzXML
## protocolData: none
## featureData
##   featureNames: X1.1 X2.1 ... X5.1 (5 total)
##   fvarLabels: spectrum
##   fvarMetadata: labelDescription
## experimentData: use 'experimentData(object)'
```

MS2 spectra can be extracted as a list of Spectrum2 objects with the spectra accessor or as a subset of the original MSnExp data with the [operator. Individual spectra can be accessed with [[.

```
length(msexp)
```

```
## [1] 5
```

```
msnexp[1:2]
```

```
## Error in eval(expr, envir, enclos): object 'msnexp' not found
```

```
msexp[[2]]
```

```
## Object of class "Spectrum2"
##  Precursor: 546.9586
##  Retention time: 25:2
##  Charge: 3
##  MSn level: 2
##  Peaks count: 1012
##  Total ion count: 56758067
```

The identification results stemming from the same raw data file can then be used to add PSM matches.

```
fData(msexp)
```

```
##      spectrum
## X1.1        1
## X2.1        2
## X3.1        3
## X4.1        4
## X5.1        5
```

```
## find path to a mzIdentML file
identFile <- dir(system.file(package = "MSnbase", dir = "extdata"),
                 full.name = TRUE, pattern = "dummyiTRAQ.mzid")
basename(identFile)
```

```
## [1] "dummyiTRAQ.mzid"
```

```
msexp <- addIdentificationData(msexp, identFile)
```

```
## reading dummyiTRAQ.mzid... DONE!
```

```
fData(msexp)
```

```
##      spectrum scan number(s) passthreshold rank calculatedmasstocharge
## X1.1        1              1          TRUE    1               645.0375
## X2.1        2              2          TRUE    1               546.9633
## X3.1        3             NA            NA   NA                     NA
## X4.1        4             NA            NA   NA                     NA
## X5.1        5              5          TRUE    1               437.2997
##      experimentalmasstocharge chargestate ms-gf:denovoscore ms-gf:evalue
## X1.1                 645.3741           3                77     79.36958
## X2.1                 546.9586           3                39     13.46615
## X3.1                       NA          NA                NA           NA
## X4.1                       NA          NA                NA           NA
## X5.1                 437.8040           2                 5    366.38422
##      ms-gf:rawscore ms-gf:specevalue assumeddissociationmethod
## X1.1            -39     5.527468e-05                       CID
## X2.1            -30     9.399048e-06                       CID
## X3.1             NA               NA                      <NA>
## X4.1             NA               NA                      <NA>
## X5.1            -42     2.577830e-04                       CID
##      isotopeerror isdecoy post  pre end start       accession length
## X1.1            1   FALSE    A    R 186   170 ECA0984;ECA3829    231
## X2.1            0   FALSE    A    K  62    50         ECA1028    275
## X3.1         <NA>      NA <NA> <NA>  NA    NA            <NA>     NA
## X4.1         <NA>      NA <NA> <NA>  NA    NA            <NA>     NA
## X5.1            1   FALSE    L    K  28    22         ECA0510    166
##                                                                       description
## X1.1 DNA mismatch repair protein;acetolactate synthase isozyme III large subunit
## X2.1          2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
## X3.1                                                                         <NA>
## X4.1                                                                         <NA>
## X5.1                    putative capsular polysacharide biosynthesis transferase
##                 pepseq modified modification             databaseFile
## X1.1 VESITARHGEVLQLRPK    FALSE           NA erwinia_carotovora.fasta
## X2.1     IDGQWVTHQWLKK    FALSE           NA erwinia_carotovora.fasta
## X3.1              <NA>       NA           NA                     <NA>
## X4.1              <NA>       NA           NA                     <NA>
## X5.1           LVILLFR    FALSE           NA erwinia_carotovora.fasta
##      identFile nprot npep.prot npsm.prot npsm.pep
## X1.1         2     2         1         1        1
## X2.1         2     1         1         1        1
## X3.1        NA    NA        NA        NA       NA
## X4.1        NA    NA        NA        NA       NA
## X5.1         2     1         1         1        1
```

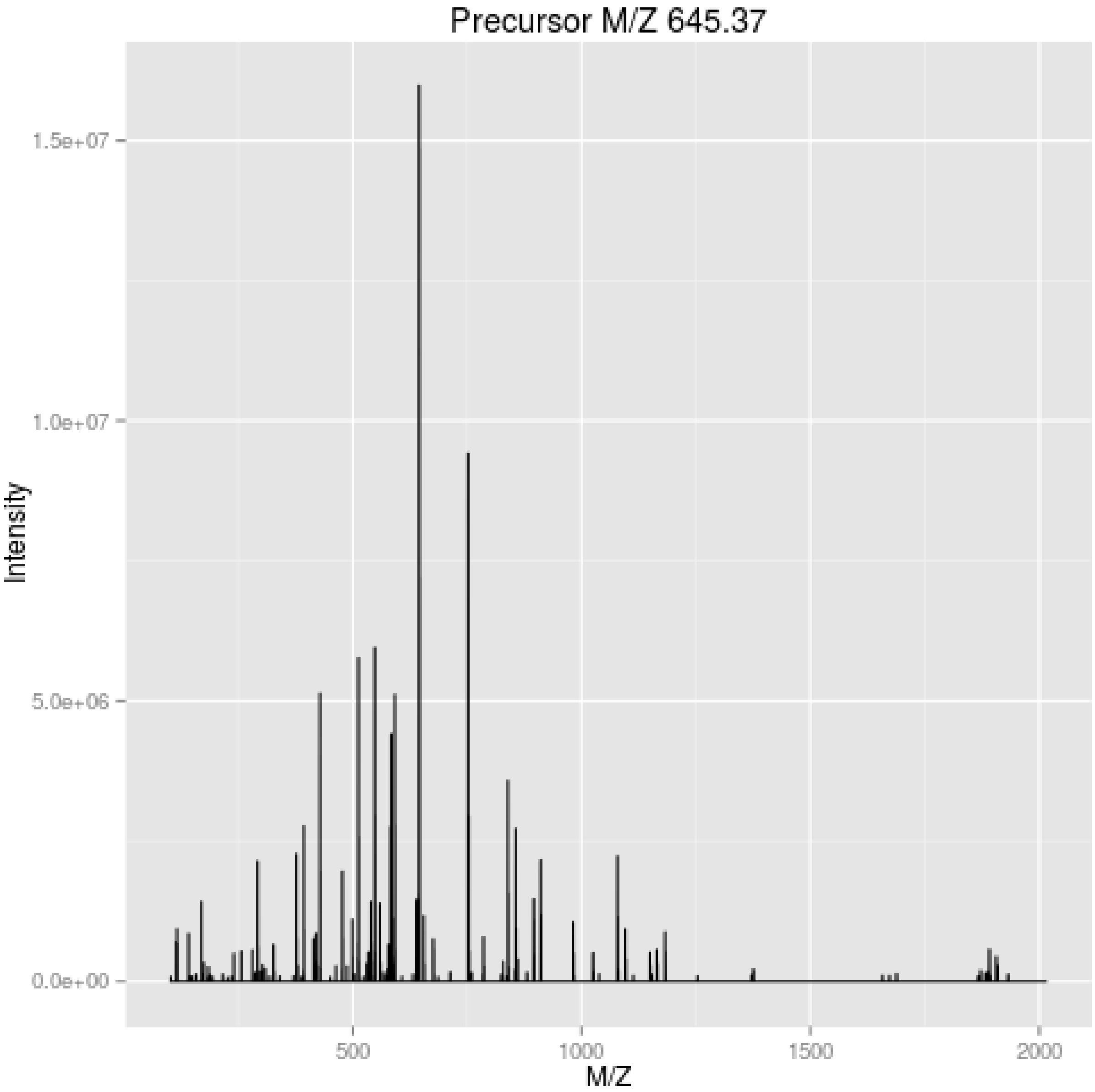
The readMSData and addIdentificationData make use of mzR and mzID packages to access the raw and identification data.

Spectra and (parts of) experiments can be extraced and plotted.

```
msexp[[1]]
```

```
## Object of class "Spectrum2"
##  Precursor: 645.3741
##  Retention time: 25:1
##  Charge: 3
##  MSn level: 2
##  Peaks count: 2921
##  Total ion count: 668170086
```

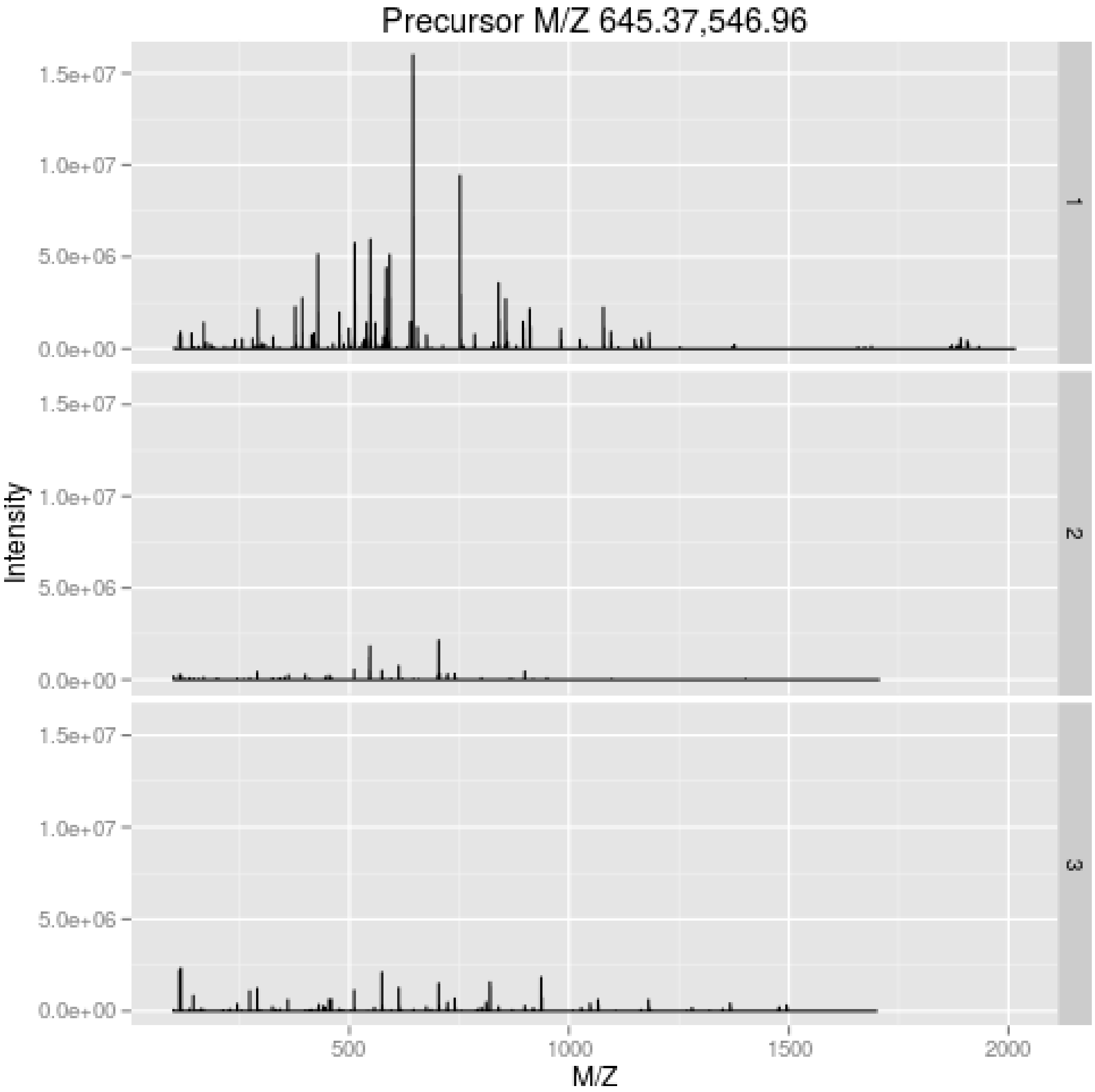
```
plot(msexp[[1]], full=TRUE)
```



```
msexp[1:3]
```

```
## Object of class "MSnExp"
##  Object size in memory: 0.13 Mb
## - - - Spectra data - - -
##  MS level(s): 2
##  Number of MS1 acquisitions: 1
##  Number of MSn scans: 3
##  Number of precursor ions: 3
##  2 unique MZs
##  Precursor MZ's: 546.96 - 645.37
##  MSn M/Z range: 100 2016.66
##  MSn retention times: 25:1 - 25:2 minutes
## - - - Processing information - - -
## Data loaded: Thu Dec  4 19:10:37 2014
## Data [numerically] subsetted 3 spectra: Thu Dec  4 19:10:38 2014
##  MSnbase version: 1.14.1
## - - - Meta data  - - -
## phenoData
##   rowNames: 1
##   varLabels: sampleNames
##   varMetadata: labelDescription
## Loaded from:
##   dummyiTRAQ.mzXML,   dummyiTRAQ.mzid
## protocolData: none
## featureData
##   featureNames: X1.1 X2.1 X3.1
##   fvarLabels: spectrum scan number(s) ... npsm.pep (30 total)
##   fvarMetadata: labelDescription
## experimentData: use 'experimentData(object)'
```

```
plot(msexp[1:3], full=TRUE)
```



Coercion to a `data.frame` is straightforward.

```
as(msexp[[1]], "data.frame")[100:105, ]
```

```
##           mz          i
## 100 141.0990 588594.812
## 101 141.1015 845401.250
## 102 141.1041 791352.125
## 103 141.1066 477623.000
## 104 141.1091 155376.312
## 105 141.1117   4752.541
```

Quantitative proteomics

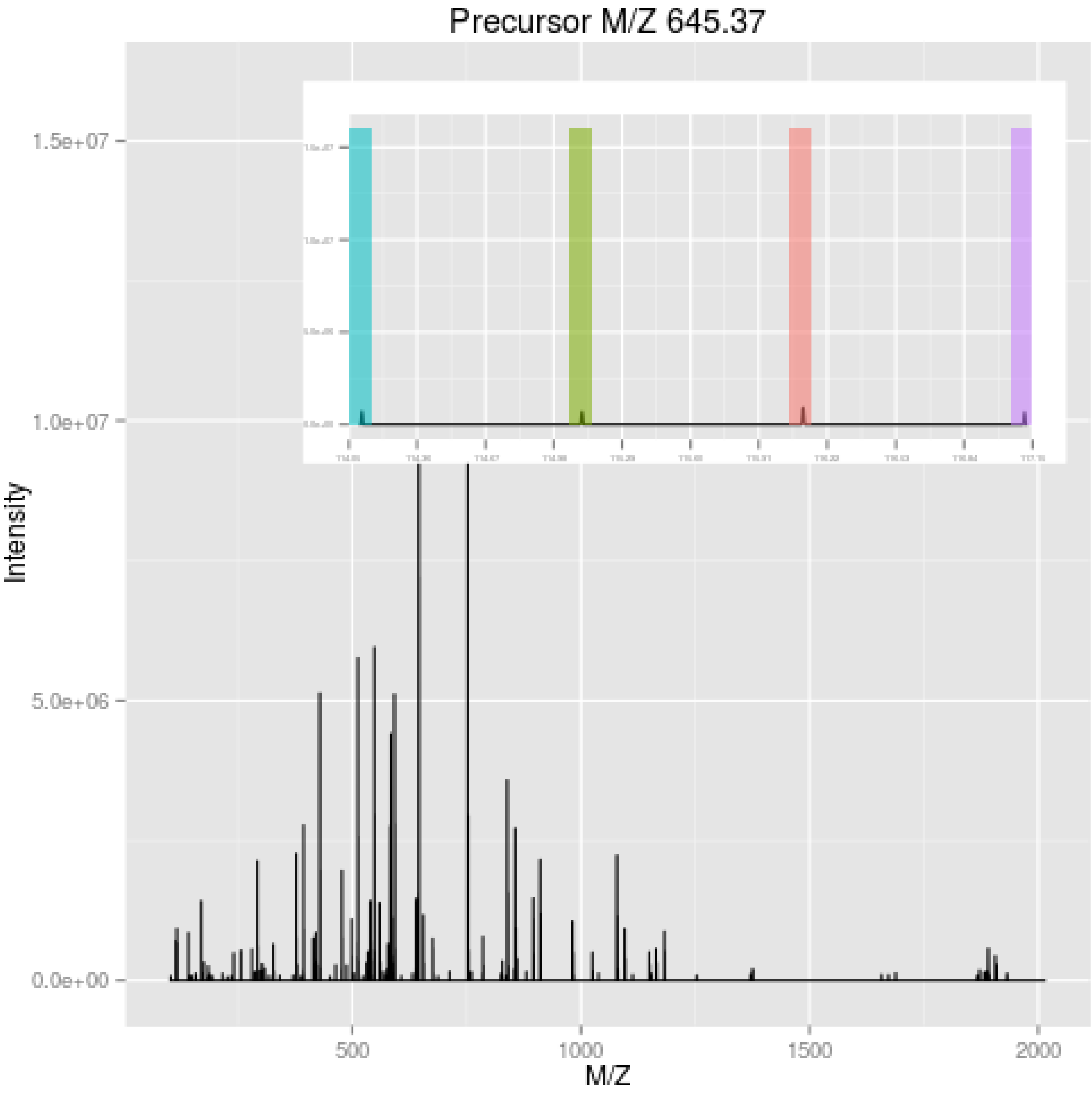
There are a wide range of proteomics quantitation techniques that can broadly be classified as labelled vs. label-free, depending whether the features are labelled prior the MS acquisition and the MS level at which quantitation is inferred, namely MS1 or MS2.

	Label-free	Labelled
MS1	XIC	SILAC, 15N
MS2	Counting	iTRAQ, TMT

In terms of raw data quantitation, most efforts have been devoted to MS2-level quantitation. Label-free XIC quantitation has however been addressed in the frame of metabolomics data processing by the `xcms` infrastructure.

An `MSnExp` is converted to an `MSnSet` by the `quantitation` method. Below, we use the iTRAQ 4-plex isobaric tagging strategy (defined by the `iTRAQ4` parameter; other tags are available).

```r
plot(msexp[[1]], full=TRUE, reporters = iTRAQ4)
```



```
msset <- quantify(msexp, method = "trap", reporters = iTRAQ4, verbose=FALSE)
exprs(msset)
```

```
##       iTRAQ4.114 iTRAQ4.115 iTRAQ4.116 iTRAQ4.117
## X1.1   4483.320   4873.996   6743.441   4601.378
## X2.1   1918.082   1418.040   1117.601   1581.954
## X3.1  15210.979  15296.256  15592.760  16550.502
## X4.1   4133.103   5069.983   4724.845   4694.801
## X5.1  11947.881  13061.875  12809.491  12911.479
```

```
processingData(msset)
```

```
## - - - Processing information - - -
## Data loaded: Thu Dec  4 19:10:37 2014
## iTRAQ4 quantification by trapezoidation: Thu Dec  4 19:10:39 2014
##  MSnbase version: 1.14.1
```

Other MS2 quantitation methods available in `quantify` include the (normalised) spectral index `SI` and (normalised) spectral abundance factor `SAF` or simply a simple count method.

```
exprs(si <- quantify(msexp, method = "SIn"))
```

```
##                   1
## ECA0510 0.003588641
## ECA1028 0.001470129
```

```
exprs(saf <- quantify(msexp, method = "NSAF"))
```

```
##                 1
## ECA0510 0.6235828
## ECA1028 0.3764172
```

Note that spectra that have not been assigned any peptide (`NA`) or that match non-unique peptides (`npsm > 1`) are discarded in the counting process.

See also The `isobar` package supports quantitation from centroided `mgf` peak lists or its own tab-separated files that can be generated from Mascot and Phenyx vendor files.

> Have a look at the `?quantify` documentation file and review the above by walking through the example.

Importing third-party quantitation data

The PSI `mzTab` file format is aimed at providing a simpler (than XML formats) and more accessible file format to the wider community. It is composed of a key-value metadata section and peptide/protein/small molecule tabular sections.

```
mztf <- pxget(px, pxfiles(px)[2])
```

```
## Downloading 1 file
## F063721.dat-mztab.txt already present.
```

```
(mzt <- readMzTabData(mztf, what = "PEP"))
```

```
## Warning in readMzTabData(mztf, what = "PEP"): Support for mzTab version
## 0.9 only. Support will be added soon.
```

```
## Detected a metadata section
## Detected a peptide section
## MSnSet (storageMode: lockedEnvironment)
## assayData: 1528 features, 6 samples
##   element names: exprs
## protocolData: none
## phenoData
##   rowNames: sub[1] sub[2] ... sub[6] (6 total)
##   varLabels: abundance
##   varMetadata: labelDescription
## featureData
##   featureNames: 1 2 ... 1528 (1528 total)
##   fvarLabels: sequence accession ... uri (14 total)
##   fvarMetadata: labelDescription
## experimentData: use 'experimentData(object)'
## Annotation:
## - - - Processing information - - -
## mzTab read: Thu Dec  4 19:10:41 2014
##  MSnbase version: 1.14.1
```

It is also possible to import arbitrary spreadsheets as MSnSet objects into R with the readMSnSet2 function. The main 2 arguments of the function are (1) a text-based spreadsheet and (2) column names of indices that identify the quantitation data.

```
csv <- dir(system.file ("extdata" , package = "pRolocdata"),
           full.names = TRUE, pattern = "pr800866n_si_004-rep1.csv")
getEcols(csv, split = ",")
```

```
##  [1] "\"Protein ID\""               "\"FBgn\""
##  [3] "\"Flybase Symbol\""           "\"No. peptide IDs\""
##  [5] "\"Mascot score\""             "\"No. peptides quantified\""
##  [7] "\"area 114\""                 "\"area 115\""
##  [9] "\"area 116\""                 "\"area 117\""
## [11] "\"PLS-DA classification\""    "\"Peptide sequence\""
## [13] "\"Precursor ion mass\""       "\"Precursor ion charge\""
## [15] "\"pd.2013\""                  "\"pd.markers\""
```

```
ecols <- 7:10
res <- readMSnSet2(csv, ecols)
head(exprs(res))
```

```
##   area.114 area.115 area.116 area.117
## 1 0.379000 0.281000 0.225000 0.114000
## 2 0.420000 0.209667 0.206111 0.163889
## 3 0.187333 0.167333 0.169667 0.476000
## 4 0.247500 0.253000 0.320000 0.179000
## 5 0.216000 0.183000 0.342000 0.259000
## 6 0.072000 0.212333 0.573000 0.142667
```

```
head(fData(res))
```

```
##   Protein.ID        FBgn Flybase.Symbol No..peptide.IDs Mascot.score
## 1    CG10060 FBgn0001104    G-ialpha65A               3       179.86
## 2    CG10067 FBgn0000044         Act57B               5       222.40
## 3    CG10077 FBgn0035720        CG10077               5       219.65
## 4    CG10079 FBgn0003731           Egfr               2        86.39
## 5    CG10106 FBgn0029506        Tsp42Ee               1        52.10
## 6    CG10130 FBgn0010638      Sec61beta               2        79.90
##   No..peptides.quantified PLS.DA.classification Peptide.sequence
## 1                       1                    PM
## 2                       9                    PM
## 3                       3
## 4                       2                    PM
## 5                       1                               GGVFDTIQK
## 6                       3              ER/Golgi
##   Precursor.ion.mass Precursor.ion.charge     pd.2013 pd.markers
## 1                                                  PM    unknown
## 2                                                  PM    unknown
## 3                                             unknown    unknown
## 4                                                  PM    unknown
## 5            626.887                    2 Phenotype 1    unknown
## 6                                            ER/Golgi         ER
```

Data processing and analysis

Raw data processing

For raw data processing look at MSnbases's clean, smooth, pickPeaks, removePeaks and trimMz for MSnExp and spectra processing methods.

The MALDIquant and xcms packages also feautres a wide range of raw data processing methods on their own ad hoc data instance types.

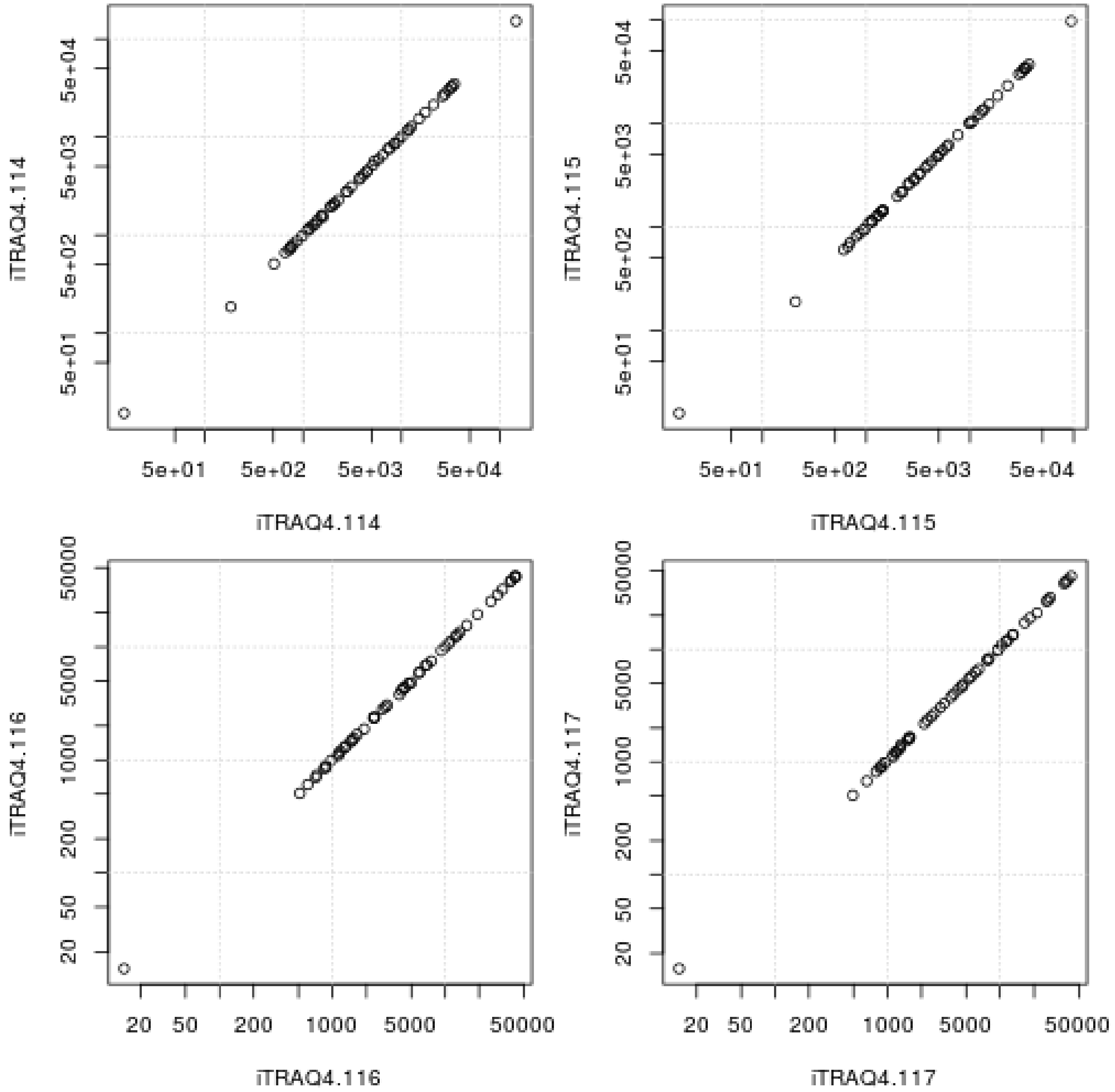
Processing and normalisation

Each different types of quantitative data will require their own pre-processing and normalisation steps. Both isobar and MSnbase allow to correct for isobaric tag impurities normalise the quantitative data.

```
data(itraqdata)
qnt <- quantify(itraqdata, method = "trap",
                reporters = iTRAQ4, verbose = FALSE)
impurities <- matrix(c(0.929,0.059,0.002,0.000,
                       0.020,0.923,0.056,0.001,
                       0.000,0.030,0.924,0.045,
                       0.000,0.001,0.040,0.923),
                     nrow=4, byrow = TRUE)
## or, using makeImpuritiesMatrix()
## impurities <- makeImpuritiesMatrix(4)
qnt.crct <- purityCorrect(qnt, impurities)
processingData(qnt.crct)
```

```
## - - - Processing information - - -
## Data loaded: Wed May 11 18:54:39 2011
## iTRAQ4 quantification by trapezoidation: Thu Dec  4 19:10:43 2014
## Purity corrected: Thu Dec  4 19:10:43 2014
##  MSnbase version: 1.1.22
```

```
plot0 <- function(x, y, main = "") {
    old.par <- par(no.readonly = TRUE)
    on.exit(par(old.par))
    par(mar = c(4, 4, 1, 1))
    par(mfrow = c(2, 2))
    sx <- sampleNames(x)
    sy <- sampleNames(y)
    for (i in seq_len(ncol(x))) {
        plot(exprs(x)[, i], exprs(y)[, i], log = "xy",
             xlab = sx[i], ylab = sy[i])
        grid()
    }
}

plot0(qnt, qnt.crct)
```



Various normalisation methods can be applied the `MSnSet` instances using the `normalise` method: variance stabilisation (`vsn`), quantile (`quantiles`), median or mean centring (`center.media` or `center.mean`), . . .

```
qnt.crct.nrm <- normalise(qnt.crct,"quantiles")
plot0(qnt, qnt.crct.nrm)
```

The `combineFeatures` method combines spectra/peptides quantitation values into protein data. The grouping is defined by the `groupBy` parameter, which is generally taken from the feature metadata (protein accessions, for example).

```
## arbitraty grouping
g <- factor(c(rep(1, 25), rep(2, 15), rep(3, 15)))
prt <- combineFeatures(qnt.crct.nrm, groupBy = g, fun = "sum")
```

```
## Combined 55 features into 3 using sum
```

```
processingData(prt)
```

```
## - - - Processing information - - -
## Data loaded: Wed May 11 18:54:39 2011
## iTRAQ4 quantification by trapezoidation: Thu Dec  4 19:10:43 2014
## Purity corrected: Thu Dec  4 19:10:43 2014
## Normalised (quantiles): Thu Dec  4 19:10:43 2014
## Combined 55 features into 3 using sum: Thu Dec  4 19:10:44 2014
##  MSnbase version: 1.1.22
```

Finally, proteomics data analysis is generally hampered by missing values. Missing data imputation is a sensitive operation whose success will be guided by many factors, such as degree and (non-)random nature of the missingness. Missing value in `MSnSet` instances can be filtered out and imputed using the `filterNA` and `impute` functions.

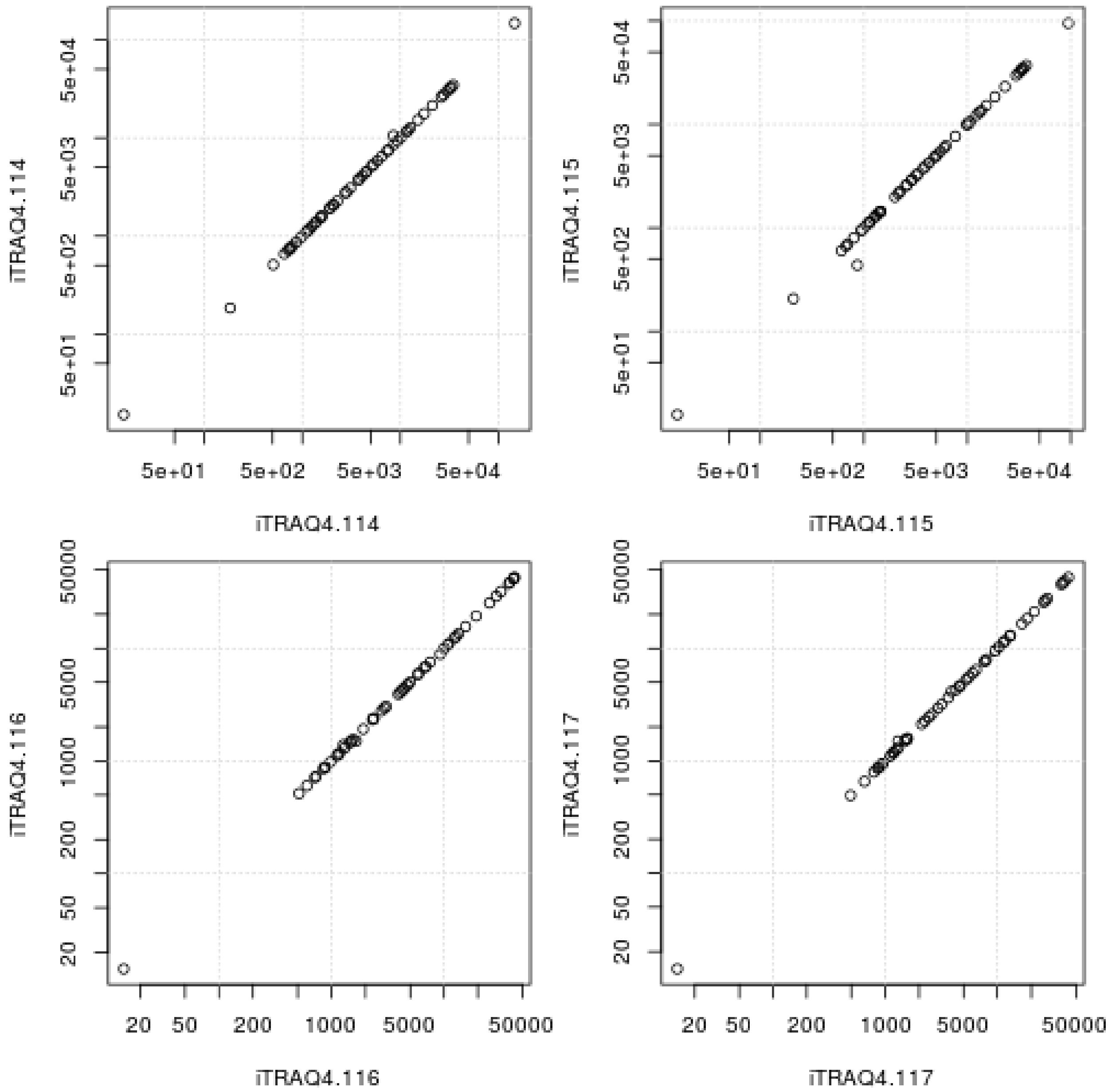
```
set.seed(1)
qnt0 <- qnt
exprs(qnt0)[sample(prod(dim(qnt0)), 10)] <- NA
table(is.na(qnt0))
```

```
##
## FALSE  TRUE
##   209    11
```

```
qnt00 <- filterNA(qnt0)
dim(qnt00)
```

```
## [1] 44  4
```

```
qnt.imp <- impute(qnt0)
plot0(qnt, qnt.imp)
```



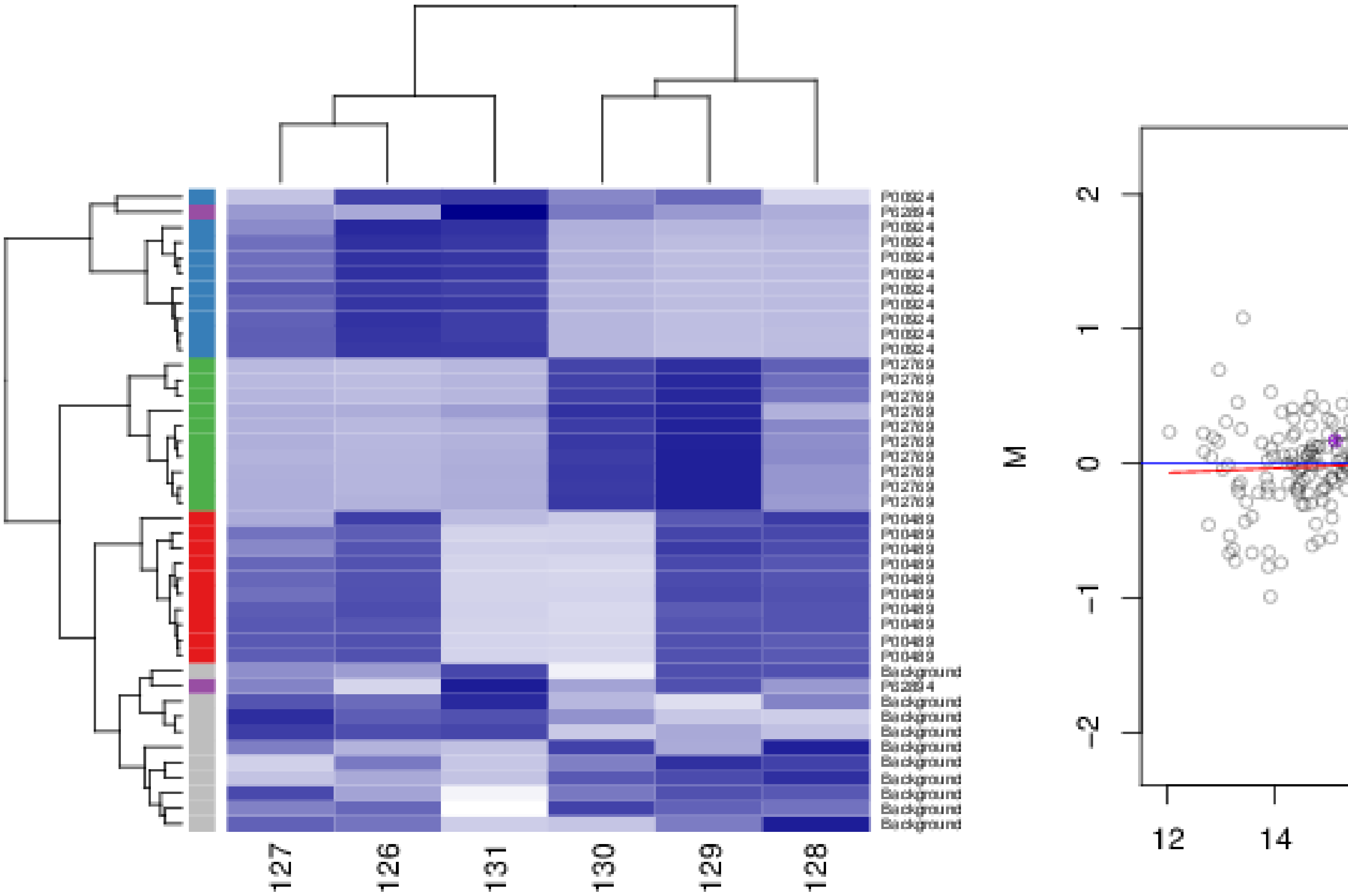
Exercise

The `mzt` instance created from the `mzTab` file has the following is a TMT 6-plex with the following design:

In this TMT 6-plex experiment, four exogenous proteins were spiked into an equimolar *Erwinia carotovora* lysate with varying proportions in each channel of quantitation; yeast enolase (ENO) at 10:5:2.5:1:2.5:10, bovine serum albumin (BSA) at 1:2.5:5:10:5:1, rabbit glycogen phosphorylase (PHO) at 2:2:2:2:1:1 and bovin cytochrome C (CYT) at 1:1:1:1:1:2. Proteins were then digested, differentially labelled with TMT reagents, fractionated by reverse phase nanoflow UPLC (nanoACQUITY, Waters), and analysed on an LTQ Orbitrap Velos mass spectrometer (Thermo Scientific).

Explore the `mzt` data using some of the illustrated functions. The heatmap and MAplot (see

MAplot function), taken from the RforProteomics vignette, have been produced using the same data.



Statistical analysis

R in general and Bioconductor in particular are well suited for the statistical analysis of data. Several packages provide dedicated resources for proteomics data:

- MSstats: A set of tools for statistical relative protein significance analysis in DDA, SRM and DIA experiments.
- msmsTest: Statistical tests for label-free LC-MS/MS data by spectral counts, to discover differentially expressed proteins between two biological conditions. Three tests are available: Poisson GLM regression, quasi-likelihood GLM regression, and the negative binomial of the edgeR package.

```
library(msmsTests)
```

```
## Loading required package: msmsEDA
```

```r
data(msms.dataset)
msms.dataset
```

```
## MSnSet (storageMode: lockedEnvironment)
## assayData: 697 features, 14 samples
##   element names: exprs
## protocolData: none
## phenoData
##   sampleNames: U2.2502.1 U2.2502.2 ... U6.0302.3 (14 total)
##   varLabels: treat batch
##   varMetadata: labelDescription
## featureData: none
## experimentData: use 'experimentData(object)'
##   pubMedIds: http://www.ncbi.nlm.nih.gov/pubmed/22588121
## Annotation:
## - - - Processing information - - -
##  MSnbase version: 1.8.0
```

```r
e <- pp.msms.data(msms.dataset)
e
```

```
## MSnSet (storageMode: lockedEnvironment)
## assayData: 675 features, 14 samples
##   element names: exprs
## protocolData: none
## phenoData
##   sampleNames: U2.2502.1 U2.2502.2 ... U6.0302.3 (14 total)
##   varLabels: treat batch
##   varMetadata: labelDescription
## featureData: none
## experimentData: use 'experimentData(object)'
##   pubMedIds: http://www.ncbi.nlm.nih.gov/pubmed/22588121
## Annotation:
## - - - Processing information - - -
## Subset [697,14][675,14] Thu Dec  4 19:10:44 2014
## Applied pp.msms.data preprocessing: Thu Dec  4 19:10:44 2014
##  MSnbase version: 1.8.0
```

```r
null.f <- "y~batch"
alt.f <- "y~treat+batch"
div <- apply(exprs(e),2,sum)
res <- msms.edgeR(e,alt.f,null.f,div=div,fnm="treat")

head(res)
```

```
##                LogFC         LR     p.value
## YJR104C  0.02689909  0.2691922 0.603874157
## YKL060C -0.12646368  5.5829487 0.018136162
## YDR155C -0.18781161 10.2706901 0.001351602
## YGR192C -0.08495735  2.5941286 0.107260419
## YOL086C -0.11853786  5.7558869 0.016433498
## YLR150W -0.09299164  1.3766331 0.240675481
```

- isobar also provides dedicated infrastructure for the statistical analysis of isobaric data.

Machine learning

The MLInterfaces package provides a unified interface to a wide range of machine learning algorithms. Initially developed for microarray and ExpressionSet instances, the pRoloc package enables application of these algorithms to MSnSet data.

Classification

The example below uses knn with the 5 closest neighbours as an illustration to classify proteins of unknown sub-cellular localisation to one of 9 possible organelles.

```
library("MLInterfaces")
library("pRoloc")
library("pRolocdata")
data(dunkley2006)
traininds <- which(fData(dunkley2006)$markers != "unknown")
ans <- MLearn(markers ~ ., data = t(dunkley2006), knnI(k = 5), traininds)
ans
```

```
## MLInterfaces classification output container
## The call was:
## MLearn(formula = markers ~ ., data = t(dunkley2006), .method = knnI(k = 5),
##     trainInd = traininds)
## Predicted outcome distribution for test set:
##
##      ER lumen   ER membrane         Golgi Mitochondrion       Plastid
##             5           140            67            51            29
##            PM      Ribosome           TGN       vacuole
##            89            31             6            10
## Summary of scores on test set (use testScores() method for details):
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##  0.4000  1.0000  1.0000  0.9332  1.0000  1.0000
```

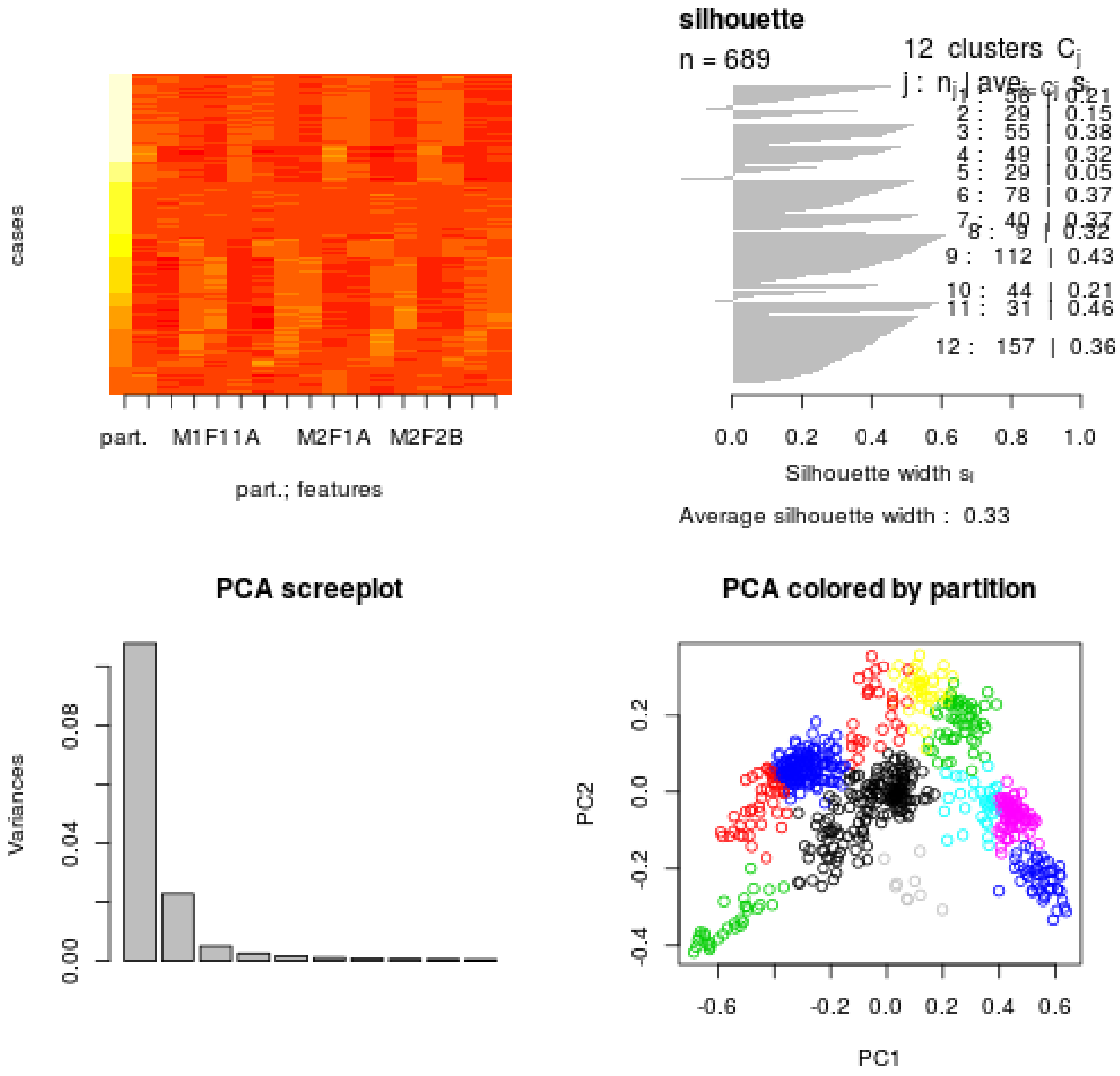
Clustering

```
kcl <- MLearn( ~ ., data = dunkley2006, kmeansI, centers = 12)
kcl
```

kmeans

```
## clusteringOutput: partition table
##
##   1   2   3   4   5   6   7   8   9  10  11  12
##  56  29  55  49  29  78  40   9 112  44  31 157
## The call that created this object was:
## MLearn(formula = ~., data = dunkley2006, .method = kmeansI, centers = 12)
```

```
plot(kcl, exprs(dunkley2006))
```

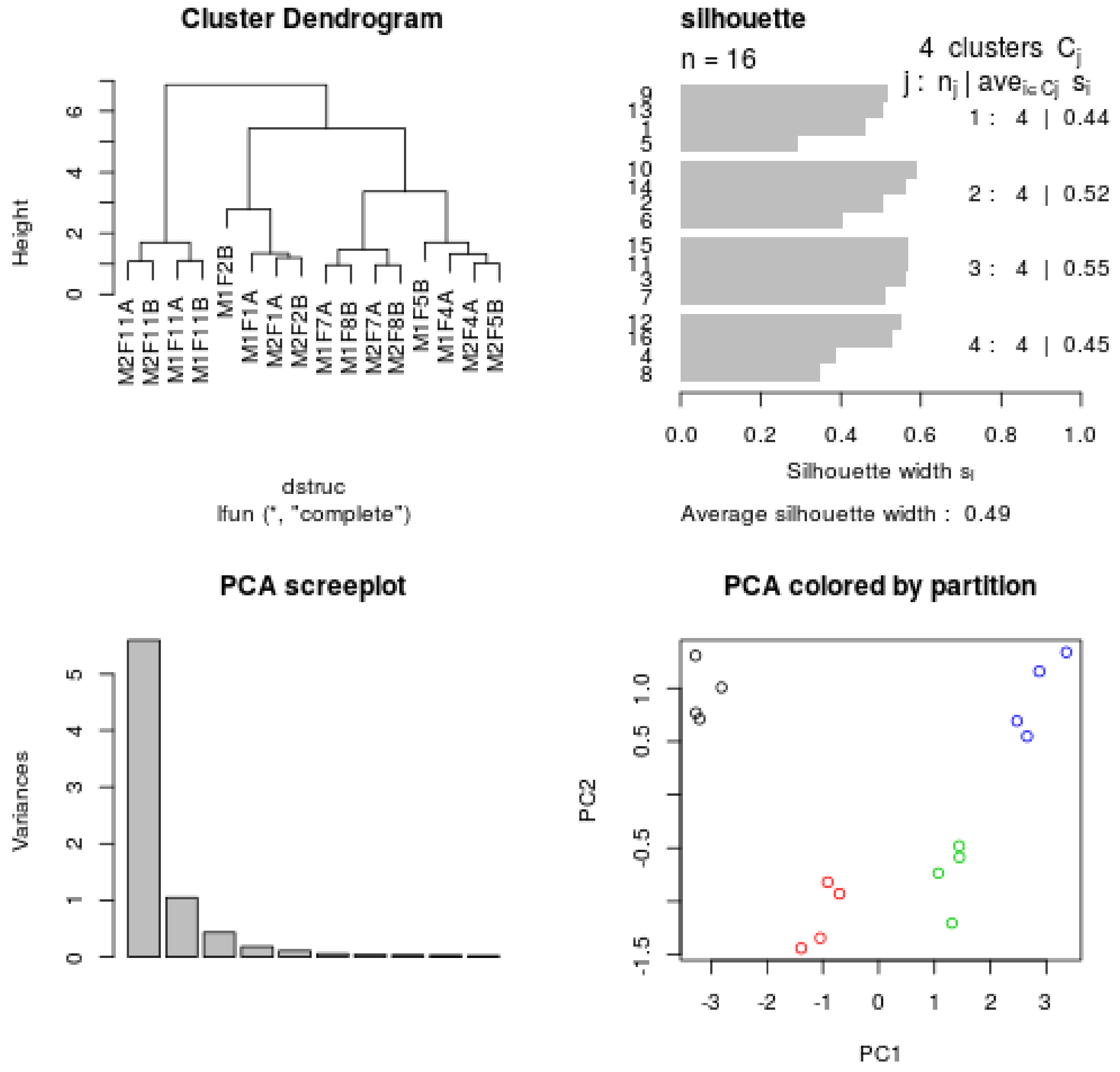


```
hcl <- MLearn( ~ ., data = t(dunkley2006), hclustI(distFun =  dist, cutParm = list(k = 4)))
hcl
```

Hierarchical clustering

```
## clusteringOutput: partition table
##
## 1 2 3 4
## 4 4 4 4
## The call that created this object was:
## MLearn(formula = ~., data = t(dunkley2006), .method = hclustI(distFun = dist,
##     cutParm = list(k = 4)))
```

```
plot(hcl, exprs(t(dunkley2006)))
```



A wide range of classification and clustering algorithms are also available, as described in the ?MLearn documentation page. The pRoloc package also uses MSnSet instances as input and ,while being conceived with the analysis of spatial/organelle proteomics data in mind, is applicable many use cases.

Annotation

All the Bioconductor annotation infrastructure, such as biomaRt, GO.db, organism specific annotations, .. are directly relevant to the analysis of proteomics data. A total of 92 ontologies, including some proteomics-centred annotations such as the PSI Mass Spectrometry Ontology, Molecular Interaction (PSI MI 2.5) or Protein Modifications are available through the rols.

```
library("rols")
olsQuery("ESI", "MS")
```

```
##  MS:1000073  MS:1000162 
##       "ESI" "HiRes ESI" 
```

Data from the Human Protein Atlas is available via the `hpar` package.

Other relevant packages/pipelines

- Analysis of post translational modification with `isobar`.
- Analysis of label-free data from a Synapt G2 (including ion mobility) with `synapter`.
- Analysis of spatial proteomics data with `pRoloc`.
- Analysis of MALDI data with the `MALDIquant` package.
- Access to the Proteomics Standard Initiative Common QUery InterfaCe with the `PSICQUIC` package.

Additional relevant packages are described in the `RforProteomics` vignettes.

Session information

```
## R version 3.1.1 Patched (2014-09-02 r66514)
## Platform: x86_64-unknown-linux-gnu (64-bit)
## 
## attached base packages:
## [1] stats4    parallel  stats     graphics  grDevices utils     datasets 
## [8] methods   base     
## 
## other attached packages:
##  [1] msmsTests_1.4.0      msmsEDA_1.4.0        lattice_0.20-29     
##  [4] hpar_1.8.0           rols_1.8.0           MSGFgui_1.0.1       
##  [7] rTANDEM_1.6.0        data.table_1.9.4     pRolocdata_1.5.2    
## [10] pRoloc_1.7.1         MLInterfaces_1.46.0  cluster_1.15.3      
## [13] annotate_1.44.0      XML_3.98-1.1         AnnotationDbi_1.28.1
## [16] GenomeInfoDb_1.2.3   IRanges_2.0.0        S4Vectors_0.4.0     
## [19] rpx_1.2.0            MSGFplus_1.0.3       MSnID_1.0.1         
## [22] mzID_1.4.1           RforProteomics_1.5.2 MSnbase_1.14.1      
## [25] BiocParallel_1.0.0   mzR_2.0.0            Rcpp_0.11.3         
## [28] Biobase_2.26.0       BiocGenerics_0.12.1  BiocInstaller_1.16.1
## [31] knitr_1.8           
## 
## loaded via a namespace (and not attached):
##   [1] affy_1.44.0                  affyio_1.34.0               
##   [3] base64enc_0.1-2              BatchJobs_1.5               
##   [5] BBmisc_1.8                   biocViews_1.34.1            
##   [7] bitops_1.0-6                 BradleyTerry2_1.0-5         
##   [9] brew_1.0-6                   brglm_0.5-9                 
##  [11] car_2.0-22                   caret_6.0-37                
##  [13] Category_2.32.0              caTools_1.17.1              
##  [15] checkmate_1.5.0              chron_2.3-45                
##  [17] class_7.3-11                 codetools_0.2-9             
##  [19] colorspace_1.2-4             DBI_0.3.1                   
##  [21] digest_0.6.4                 doParallel_1.0.8
##  [23] e1071_1.6-4                  edgeR_3.8.5
##  [25] evaluate_0.5.5               fail_1.2
##  [27] FNN_1.1                      foreach_1.4.2
##  [29] formatR_1.0                  gdata_2.13.3
##  [31] genefilter_1.48.1            ggplot2_1.0.0
##  [33] gplots_2.14.2                graph_1.44.0
##  [35] grid_3.1.1                   gridSVG_1.4-0
##  [37] GSEABase_1.28.0              gtable_0.1.2
##  [39] gtools_3.4.1                 htmltools_0.2.6
##  [41] httpuv_1.3.2                 impute_1.40.0
##  [43] interactiveDisplay_1.4.0     interactiveDisplayBase_1.4.0
##  [45] iterators_1.0.7              kernlab_0.9-19
##  [47] KernSmooth_2.23-13           labeling_0.3
##  [49] limma_3.22.1                 lme4_1.1-7
##  [51] lpSolve_5.6.10               MALDIquant_1.11
##  [53] MASS_7.3-35                  Matrix_1.1-4
##  [55] mclust_4.4                   mime_0.2
##  [57] minqa_1.2.4                  munsell_0.4.2
##  [59] mvtnorm_1.0-1                nlme_3.1-118
##  [61] nloptr_1.0.4                 nnet_7.3-8
##  [63] pcaMethods_1.56.0            pls_2.4-3
##  [65] plyr_1.8.1                   preprocessCore_1.28.0
##  [67] proto_0.3-10                 proxy_0.4-13
##  [69] qvalue_1.40.0                R6_2.0.1
##  [71] randomForest_4.6-10          RBGL_1.42.0
##  [73] R.cache_0.10.0               RColorBrewer_1.0-5
##  [75] RCurl_1.95-4.3               rda_1.0.2-2
##  [77] reshape2_1.4                 rJava_0.9-6
##  [79] RJSONIO_1.3-0                R.methodsS3_1.6.1
##  [81] R.oo_1.18.0                  rpart_4.1-8
##  [83] RSQLite_1.0.0                RUnit_0.4.27
##  [85] R.utils_1.34.0               sampling_2.6
##  [87] scales_0.2.4                 sendmailR_1.2-1
##  [89] sfsmisc_1.0-26               shiny_0.10.2.1
##  [91] shinyFiles_0.4.0             splines_3.1.1
##  [93] SSOAP_0.8-0                  stringr_0.6.2
##  [95] survival_2.37-7              tools_3.1.1
##  [97] vsn_3.34.0                   xlsx_0.5.7
##  [99] xlsxjars_0.6.1               XMLSchema_0.7-2
## [101] xtable_1.7-4                 zlibbioc_1.12.0
```