

Using R and Bioconductor for proteomics data analysis

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[Projet PROSPECTOM](#) 19 Nov 2014, Grenoble, France

Version of this document: 5a89930 [2014-11-18 20:14:43 +0000]

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`.

```
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.

```
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, quantitative proteomics, mass spectrometry (MS) and quantitation data processing. Links to other packages and references are also documented.

The vignettes included in the [RforProteomics](#) package also contains useful material.

Exploring available infrastructure

In Bioconductor version 3.0, there are respectively 65 [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 announcements
```

```
##      Data.Set      Publication.Data      Message
## 1 PXD000715 2014-11-18 16:34:39 Updated information
## 2 PXD000837 2014-11-18 16:30:02 Updated information
## 3 PXD001354 2014-11-18 16:29:15 Updated information
## 4 PXD000627 2014-11-18 16:28:07 Updated information
## 5 PXD001125 2014-11-18 16:27:02 Updated information
## 6 PXD001045 2014-11-18 16:26:04 Updated information
## 7 PXD001260 2014-11-18 16:24:55 Updated information
## 8 PXD001414 2014-11-18 16:22:44 Updated information
## 9 PXD000715 2014-11-18 09:35:10 New
## 10 PXD000837 2014-11-18 09:27:36 New
## 11 PXD001260 2014-11-18 09:13:08 Updated information
## 12 PXD001045 2014-11-18 09:12:15 Updated information
## 13 PXD001354 2014-11-18 09:11:16 New
## 14 PXD001125 2014-11-18 09:05:58 New
## 15 PXD001414 2014-11-18 08:51:50 New
```

```
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:

```
pntax(px)
pxurl(px)
pxref(px)
```

Data files can then be downloaded with the `pxget` function as illustrated below.

```
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](#).

Handling raw MS data

The `mzR` package provides an interface to the [proteowizard](#) code base, the legacy RAMP is a non-sequential parser and other C/C++ code to access various raw data files, such as `mzML`, `mzXML`, `netCDF`, and `mzData`. The data is accessed on-disk, i.e it does not get loaded entirely in memory by default. 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.

```
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"
```

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?

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.)

Handling identification data

The `RforProteomics` package distributes a small identification result file (see `?TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzXML`) 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))
```

```
## [1] "/home/lg390/R/x86_64-unknown-linux-gnu-library/3.1/RforProteomics/extdata/TMT_Erwinia.mzid.gz"
```

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

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

```
id
```

```
## An mzID object
##
## Software used:   MS-GF+ (version: Beta (v10072))
##
## Rawfile:        /home/lgatto/dev/00_github/RforProteomics/sandbox/TMT_Erwinia_1uLSike_Top10HCD_isol
##
## 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?

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

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.

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

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

```
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:

```
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.

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 `mzIdentML` 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

PSMresults <- read.delim(system.file("extdata", "human_brain.txt",
                                     package="MSnID"),
                        stringsAsFactors=FALSE)
psms(msnid) <- PSMresults
show(msnid)

## MSnID object
## Working directory: "."
## #Spectrum Files: 1
## #PSMs: 997 at 37 % FDR
## #peptides: 687 at 57 % FDR
## #accessions: 665 at 65 % 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.

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

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

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

```
filtObj.grid <- optimize_filter(filtObj, msnid, fdr.max=0.01,
                              method="Grid", level="peptide",
                              n.iter=500)

show(filtObj.grid)
```

```
## MSnIDFilter object
## (absParentMassErrorPPM < 2.3) & (msmsScore > 7.8)
```

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

```
## MSnID object
## Working directory: "."
## #Spectrum Files: 1
## #PSMs: 346 at 0 % FDR
## #peptides: 160 at 0 % FDR
## #accessions: 132 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 file.

(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: Tue Nov 18 20:15:18 2014
## MSnbase version: 1.14.0
## - - - 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 with the `[` operator. Individual can be accessed with `[[`.

```
length(msexp)
```

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

```
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:evaluate
## 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:specvalue 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>
```

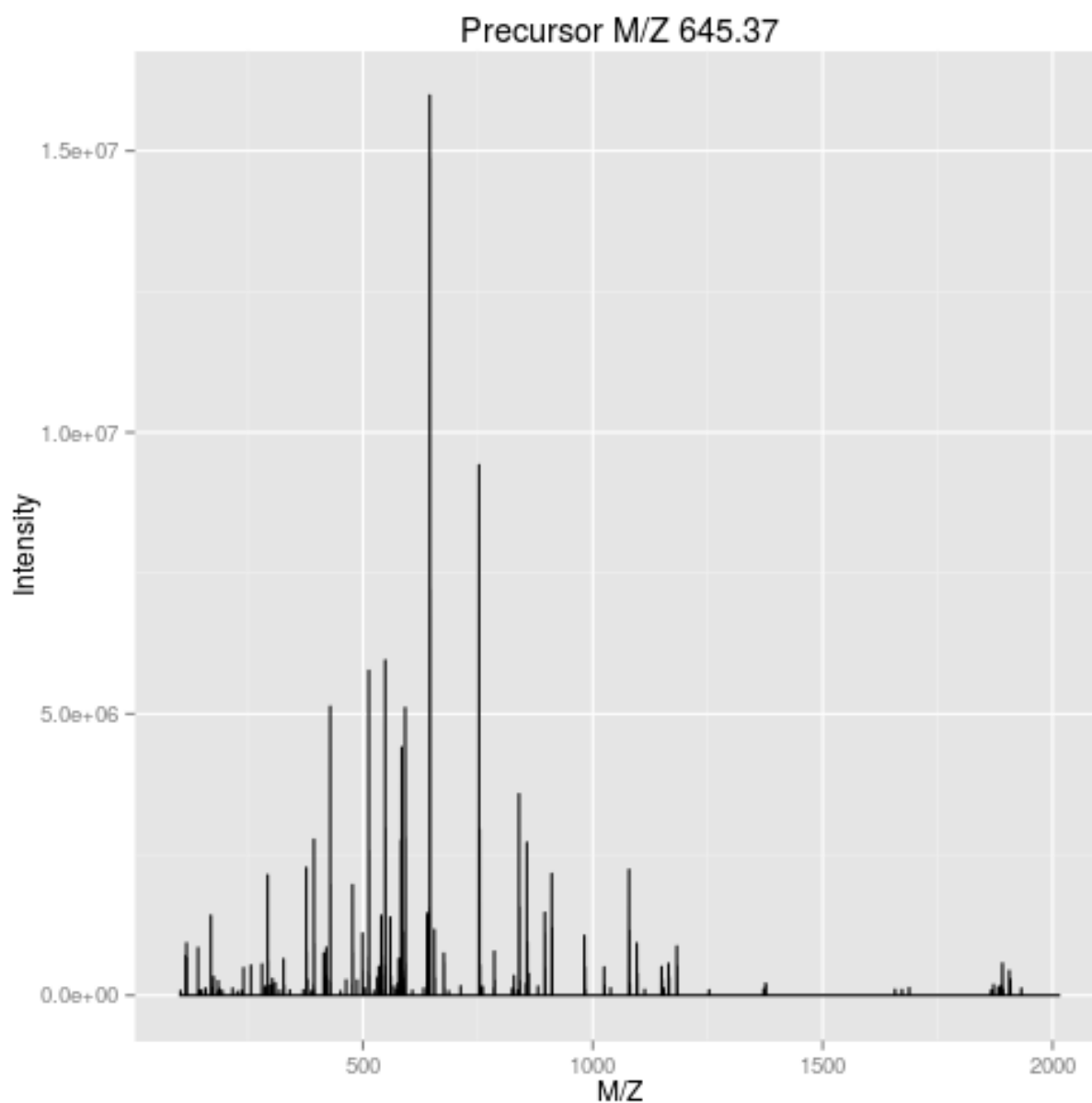
```
## X5.1 putative capsular polysaccharide biosynthesis transferase
## pepseq modified modification databaseFile
## X1.1 VESITARHGEVLQLRPK FALSE NA erwinia_carotovora.fasta
## X2.1 IDGQWVTHQWLKK FALSE NA erwinia_carotovora.fasta
## X3.1 <NA> NA NA <NA>
## X4.1 <NA> NA NA <NA>
## X5.1 LVILLFR FALSE 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
```

Spectra and (parts of) experiments can be extracted 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)
```

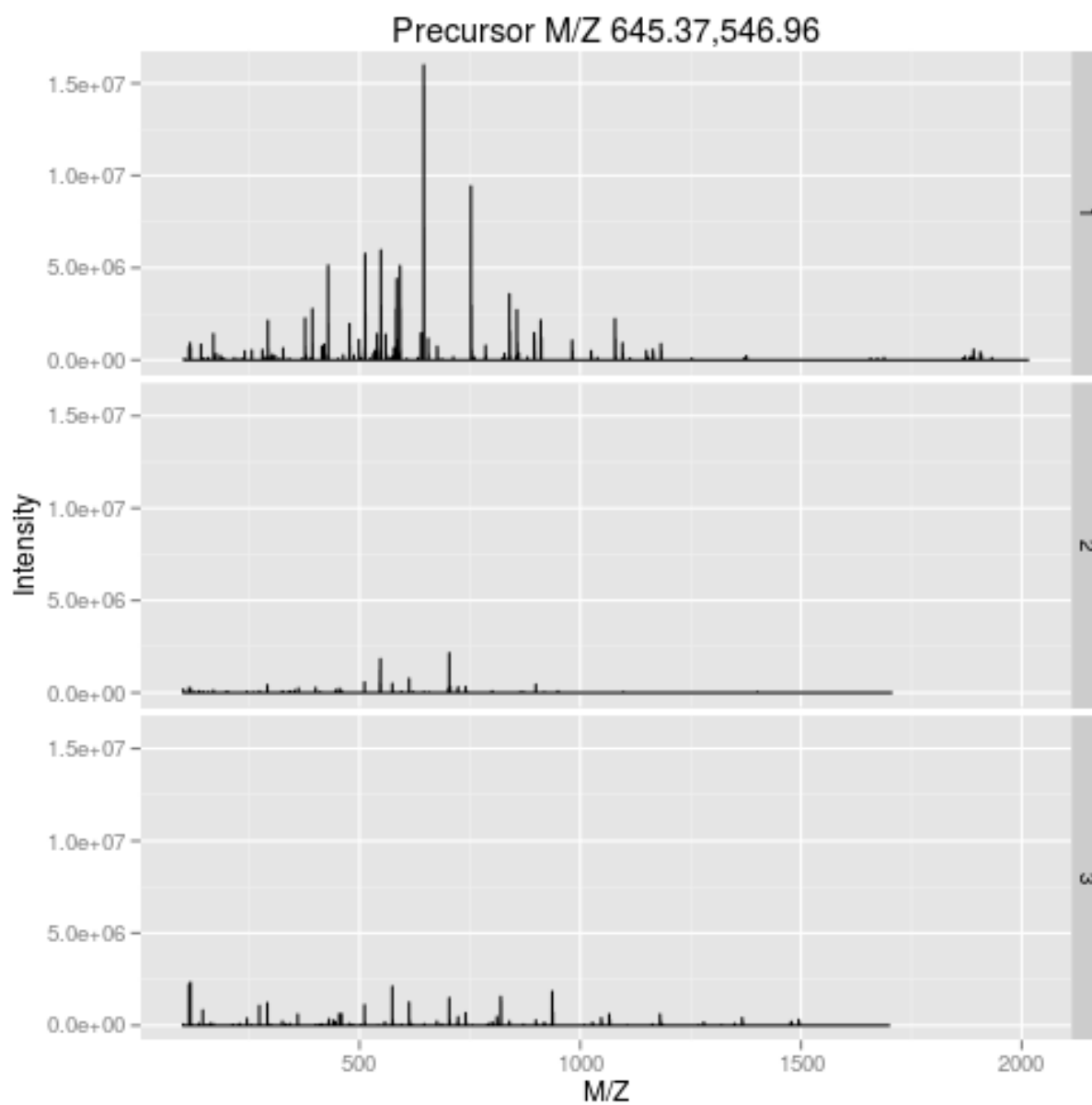


```
msexp[1:3]
```

```
## 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: Tue Nov 18 20:15:18 2014
## Data [numerically] subsetted 3 spectra: Tue Nov 18 20:15:19 2014
## MSnbase version: 1.14.0
## - - - 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

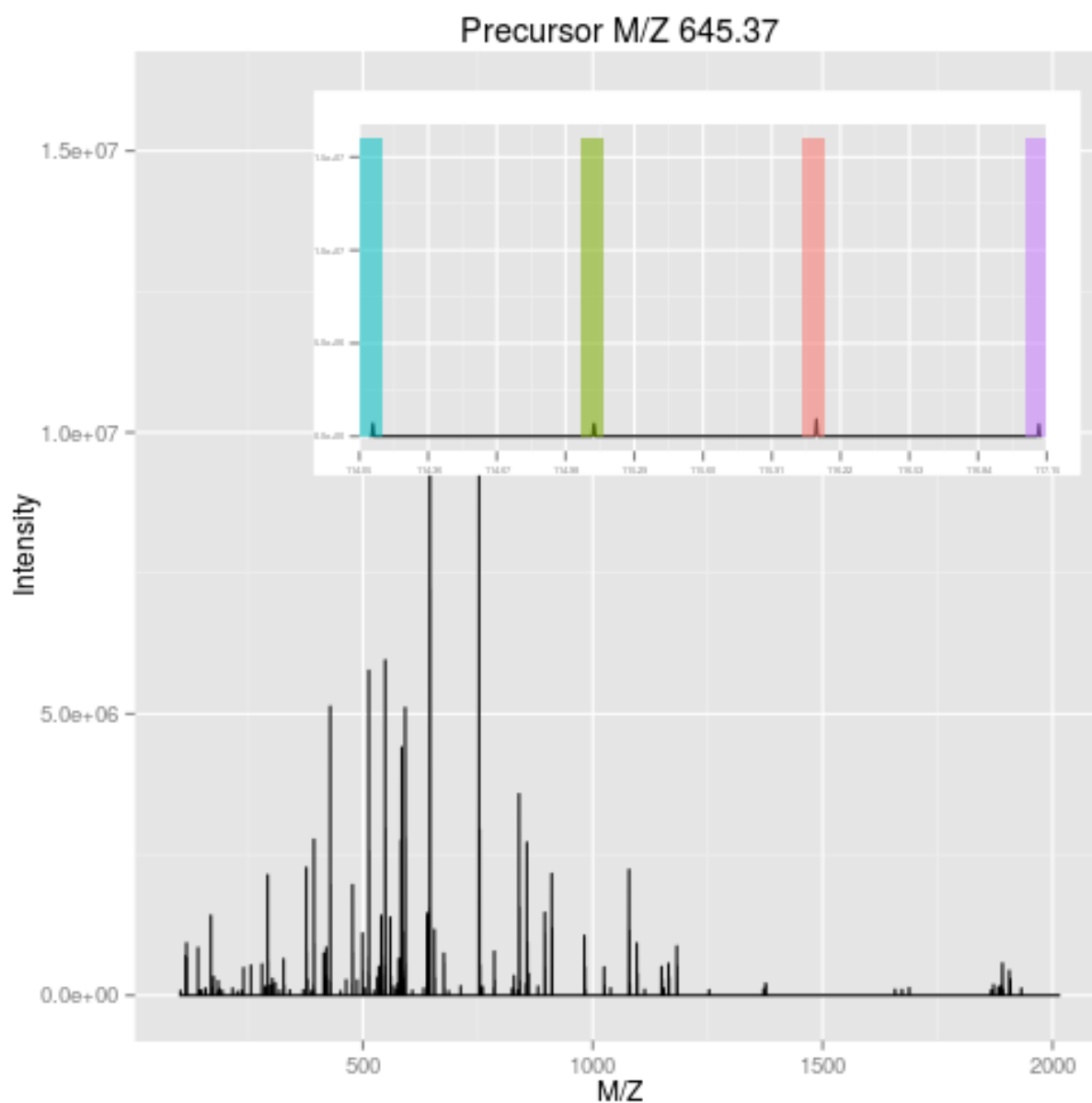
There are a wide range of proteomics quantitation techniques that can broadly be classified as labelled vs. label-free, depending whether the features are labelled prior the MS acquisition and the MS level at which quantitation is inferred, namely MS1 or MS2.

	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).

```
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: Tue Nov 18 20:15:18 2014
## iTRAQ4 quantification by trapezoidation: Tue Nov 18 20:15:20 2014
## MSnbase version: 1.14.0
```

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 quantitative 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: Tue Nov 18 20:15:29 2014
## MSnbase version: 1.14.0
```

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: Tue Nov 18 20:15:31 2014
## Purity corrected: Tue Nov 18 20:15:32 2014
## MSnbase version: 1.1.22

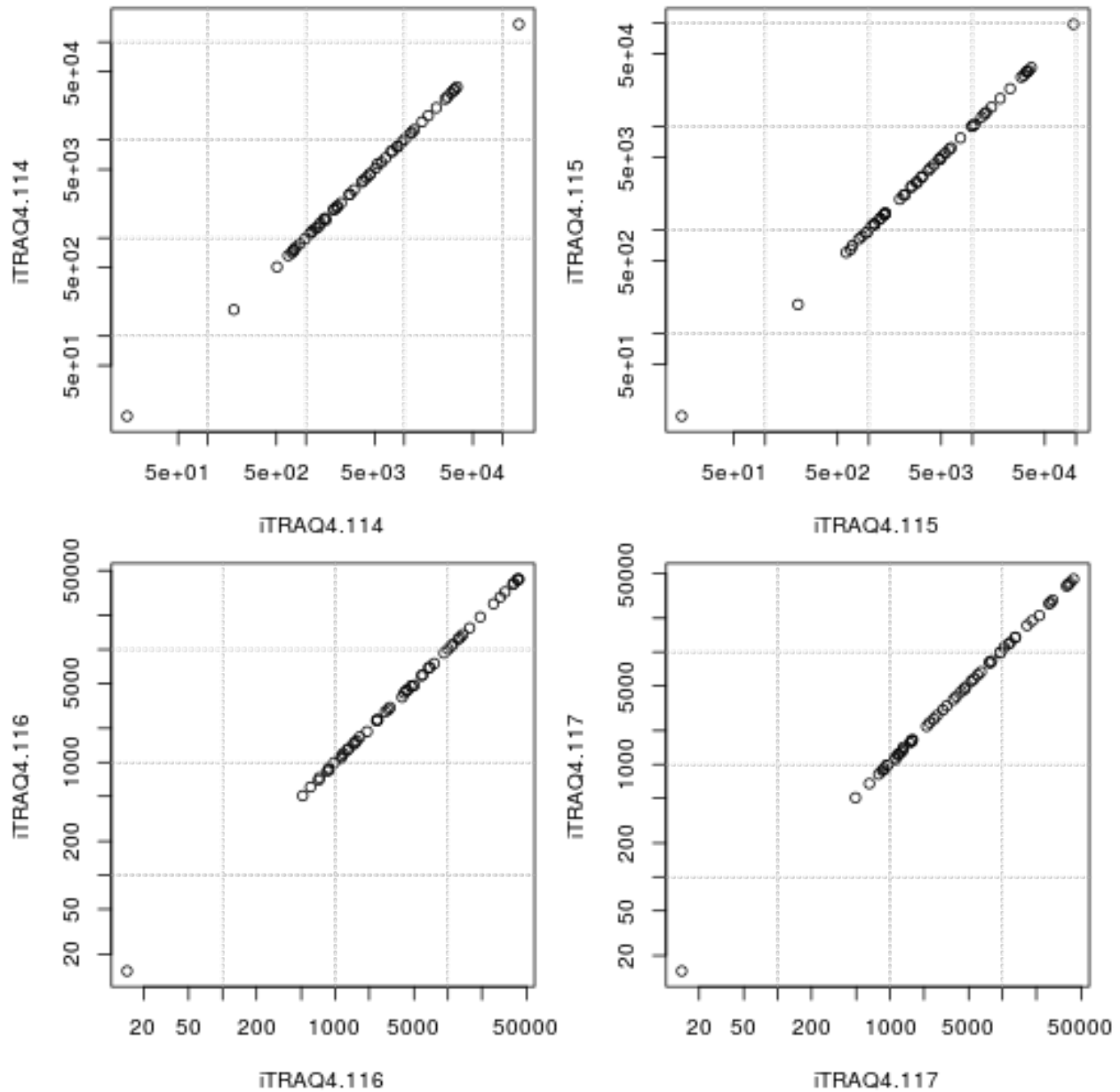
```

```

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: Tue Nov 18 20:15:31 2014  
## Purity corrected: Tue Nov 18 20:15:32 2014  
## Normalised (quantiles): Tue Nov 18 20:15:32 2014  
## Combined 55 features into 3 using sum: Tue Nov 18 20:15:32 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.

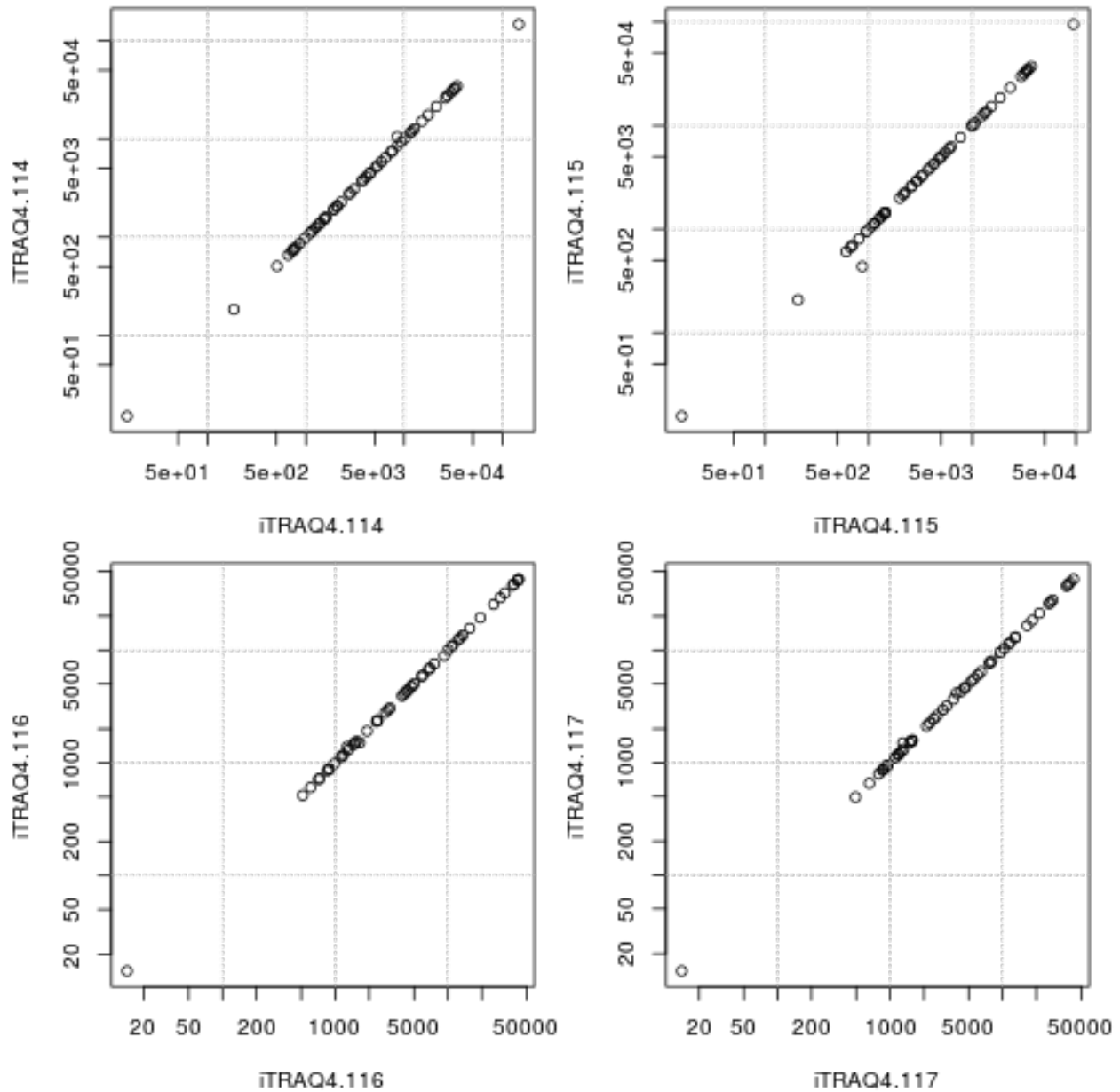
```
set.seed(1)  
qnt0 <- qnt  
exprs(qnt0)[sample(prod(dim(qnt0)), 10)] <- NA  
table(is.na(qnt0))
```

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

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

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

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



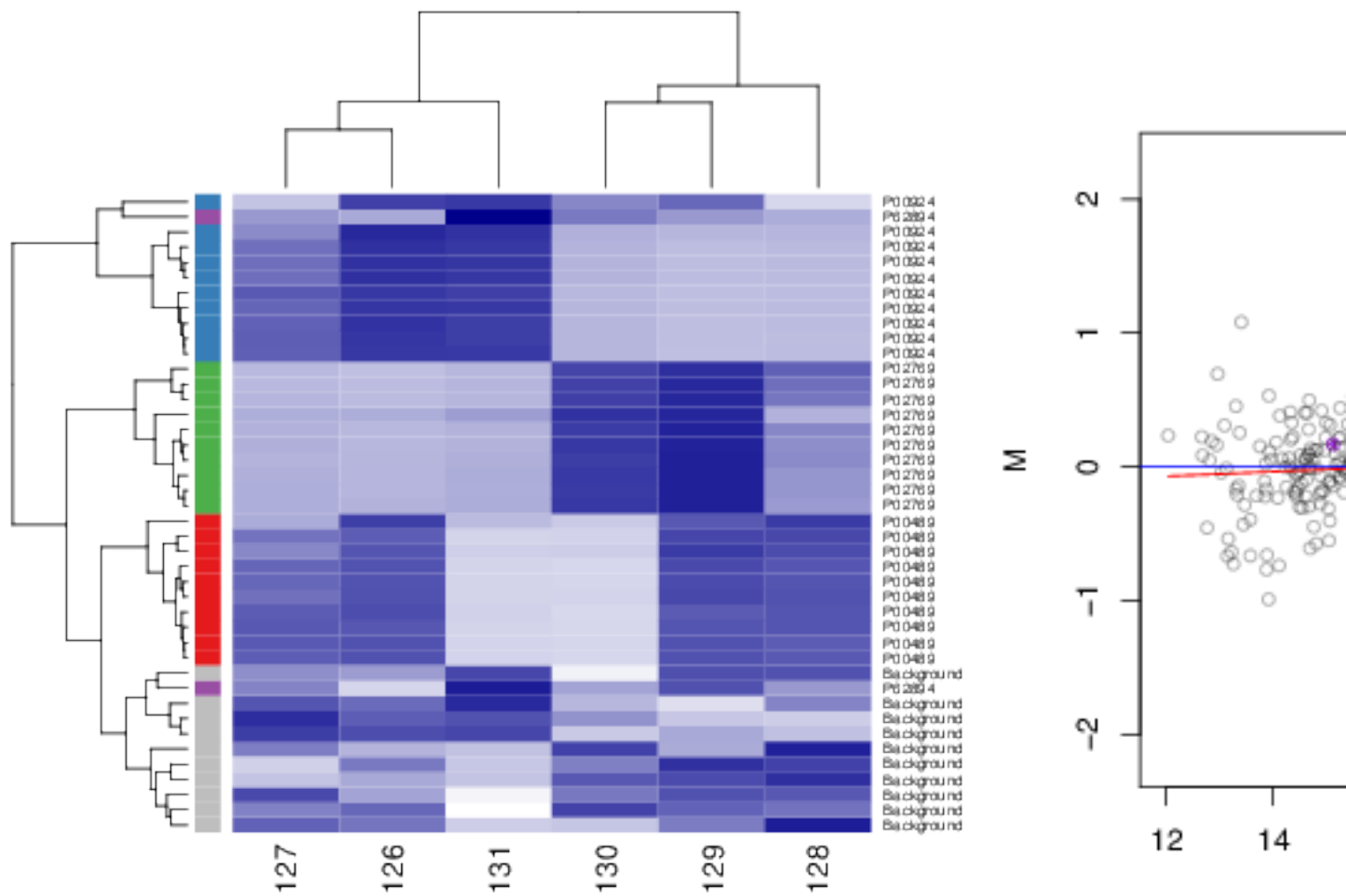
Exercise

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.
- **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.

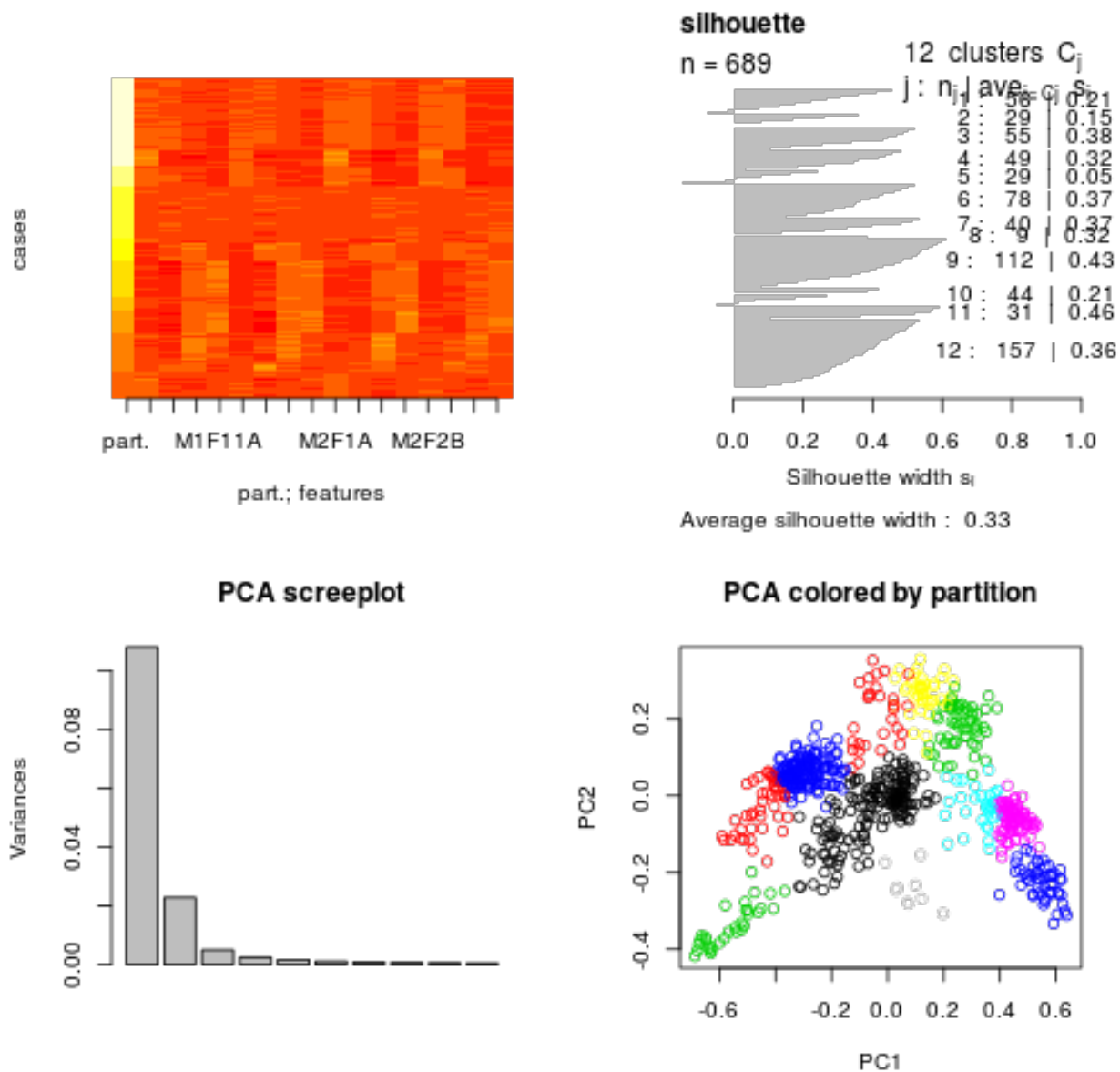
```
library("MLInterfaces")
library("pRoloc")
library("pRolocdata")
data(dunkley2006)
traininds <- which(fData(dunkley2006)$markers != "unknown")
ans <- MLearn(markers ~ ., data = t(dunkley2006), knnI(k = 5), traininds)
ans
```

```
## 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
```

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

```
## 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)
```

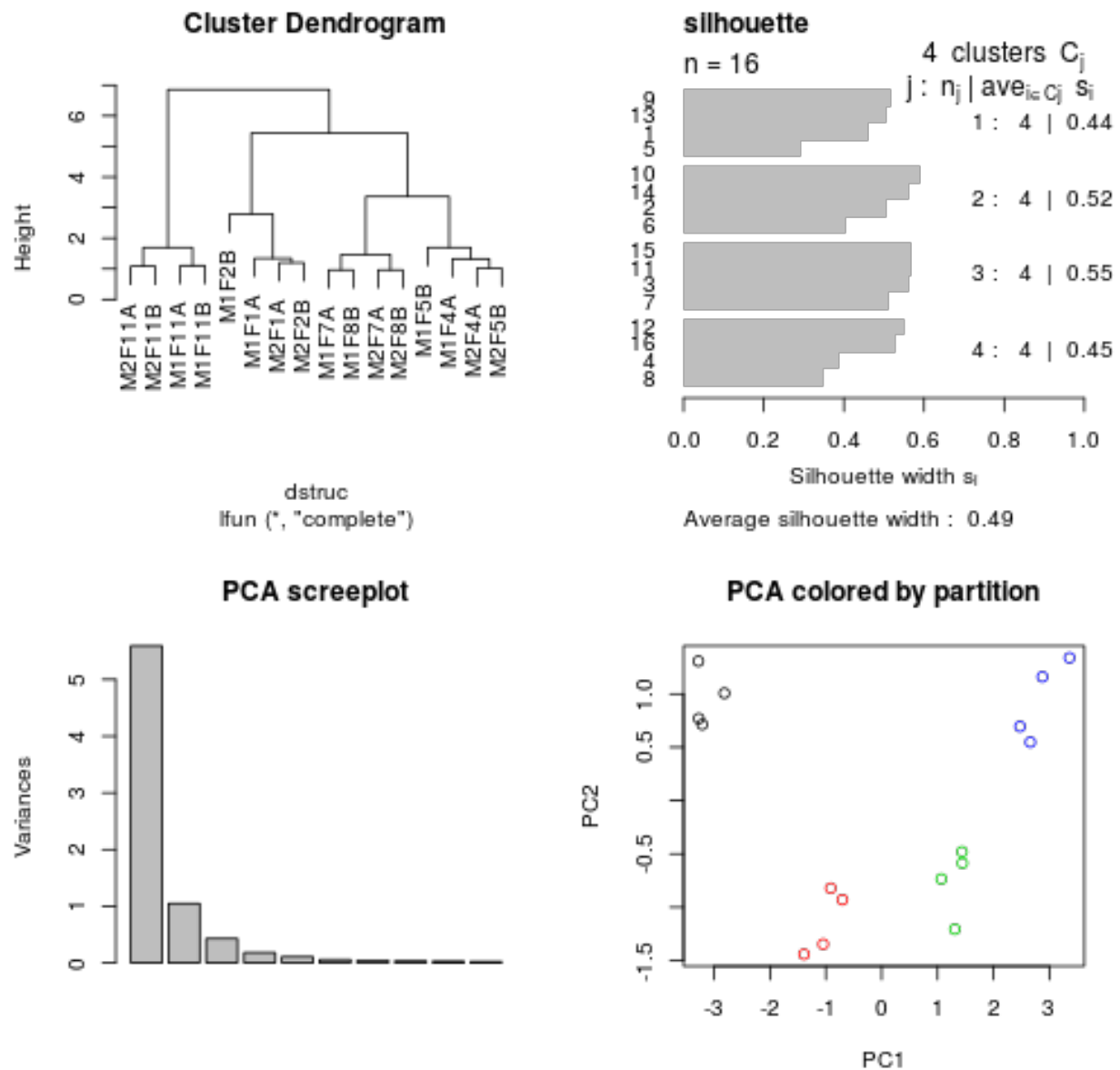
```
plot(kcl, exprs(dunkley2006))
```

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

```
## 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 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 93 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] hpar_1.8.0          rols_1.8.0          MSGFgui_1.0.1
## [4] rTANDEM_1.6.0       data.table_1.9.4    pRolocdata_1.5.2
## [7] pRoloc_1.7.1        MLInterfaces_1.46.0 cluster_1.15.3
## [10] annotate_1.44.0     XML_3.98-1.1        AnnotationDbi_1.28.1
## [13] GenomeInfoDb_1.2.3 IRanges_2.0.0       S4Vectors_0.4.0
## [16] rpx_1.2.0           MSGFplus_1.0.3      MSnID_1.0.0
## [19] mzID_1.4.1          RforProteomics_1.5.2 MSnbase_1.14.0
## [22] BiocParallel_1.0.0 mzR_2.0.0           Rcpp_0.11.3
## [25] Biobase_2.26.0      BiocGenerics_0.12.1 BiocInstaller_1.16.1
## [28] 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] BradleyTerry2_1.0-5  brew_1.0-6
## [9] brglm_0.5-9          car_2.0-21
## [11] caret_6.0-37         Category_2.32.0
## [13] checkmate_1.5.0      chron_2.3-45
## [15] class_7.3-11         codetools_0.2-9
## [17] colorspace_1.2-4     DBI_0.3.1
## [19] digest_0.6.4         doParallel_1.0.8
## [21] e1071_1.6-4          evaluate_0.5.5
## [23] fail_1.2             FNN_1.1
## [25] foreach_1.4.2        formatR_1.0
## [27] gdata_2.13.3         genefilter_1.48.1
## [29] ggplot2_1.0.0        graph_1.44.0
```

## [31] grid_3.1.1	gridSVG_1.4-0
## [33] GSEABase_1.28.0	gtable_0.1.2
## [35] gtools_3.4.1	htmltools_0.2.6
## [37] httpuv_1.3.2	impute_1.40.0
## [39] interactiveDisplay_1.4.0	interactiveDisplayBase_1.4.0
## [41] iterators_1.0.7	kernlab_0.9-19
## [43] labeling_0.3	lattice_0.20-29
## [45] limma_3.22.1	lme4_1.1-7
## [47] lpSolve_5.6.10	MALDIquant_1.11
## [49] MASS_7.3-35	Matrix_1.1-4
## [51] mclust_4.4	mime_0.2
## [53] minqa_1.2.4	munsell_0.4.2
## [55] mvtnorm_1.0-0	nlme_3.1-118
## [57] nloptr_1.0.4	nnet_7.3-8
## [59] pcaMethods_1.56.0	pls_2.4-3
## [61] plyr_1.8.1	preprocessCore_1.28.0
## [63] proto_0.3-10	proxy_0.4-13
## [65] R6_2.0.1	randomForest_4.6-10
## [67] RBGL_1.42.0	R.cache_0.10.0
## [69] RColorBrewer_1.0-5	RCurl_1.95-4.3
## [71] rda_1.0.2-2	reshape2_1.4
## [73] rJava_0.9-6	RJSONIO_1.3-0
## [75] R.methodsS3_1.6.1	R.oo_1.18.0
## [77] rpart_4.1-8	RSQLite_1.0.0
## [79] RUnit_0.4.27	R.utils_1.34.0
## [81] sampling_2.6	scales_0.2.4
## [83] sendmailR_1.2-1	sfsmisc_1.0-26
## [85] shiny_0.10.2.1	shinyFiles_0.4.0
## [87] splines_3.1.1	SSOAP_0.8-0
## [89] stringr_0.6.2	survival_2.37-7
## [91] tools_3.1.1	vsn_3.34.0
## [93] xlsx_0.5.7	xlsxjars_0.6.1
## [95] XMLSchema_0.7-2	xtable_1.7-4
## [97] zlibbioc_1.12.0	