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`.

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

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 annoucements
```

```
##     Data.Set    Publication.Data             Message
## 1  PXD000898 2014-11-13 14:42:49                 New
## 2  PXD000922 2014-11-12 11:03:38                 New
## 3  PXD001243 2014-11-12 08:54:07                 New
## 4  PXD001045 2014-11-11 08:20:08                 New
## 5  PXD001090 2014-11-10 13:37:29                 New
## 6  PXD001089 2014-11-10 13:34:47                 New
## 7  PXD001099 2014-11-10 11:47:19 Updated information
## 8  PXD001203 2014-11-10 11:46:31                 New
## 9  PXD001074 2014-11-06 09:52:57                 New
## 10 PXD001165 2014-11-05 15:22:20                 New
## 11 PXD001423 2014-11-04 14:01:46                 New
## 12 PXD001422 2014-11-04 13:57:55                 New
## 13 PXD001421 2014-11-04 13:41:36                 New
## 14 PXD001420 2014-11-04 13:25:20                 New
## 15 PXD001419 2014-11-04 13:21:13                 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.
```

```r
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:

```r
pxtax(px)
pxurl(px)
pxref(px)
```

Data files can then be downloaded with the `pxget` function as illustrated below. Alternatively, the file is available on the workshop's Amazon virtual machine in `/data/Proteomics/data/`.

```r
mzf <- "TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01.mzXML"
if (!file.exists(mzf))
    mzf <- pxget(px, pxfiles(px)[6])
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.

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

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

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

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

```r
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?

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.

```r
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"
```

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

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

```r
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, it 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 `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
```

```
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 and construct an MS experiment file.

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

```
## [1] "/home/lg390/R/x86_64-unknown-linux-gnu-library/3.1/MSnbase/extdata/dummyiTRAQ.mzXML"
```

```
msexp <- readMSData(quantFile, 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: Sun Nov 16 21:59:17 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)'
```

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

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

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

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

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

```
fData(msexp)
```

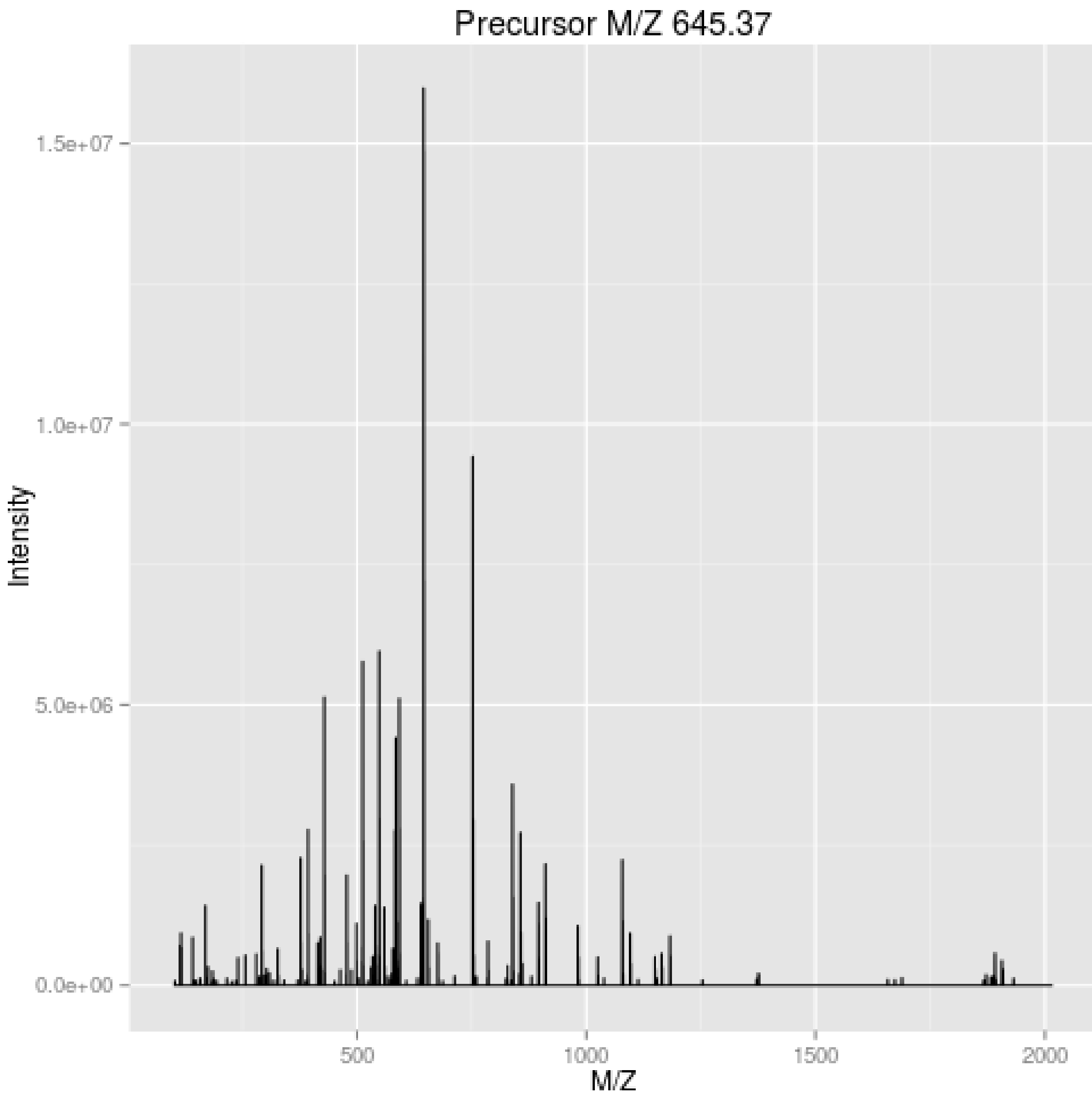
```
##      spectrum scan number(s) passthreshold rank calculatedmasstocharge
## X1.1        1              1          TRUE    1               645.0375
## X2.1        2              2          TRUE    1               546.9633
## X3.1        3             NA            NA   NA                     NA
## 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
```

```
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)
```




```
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

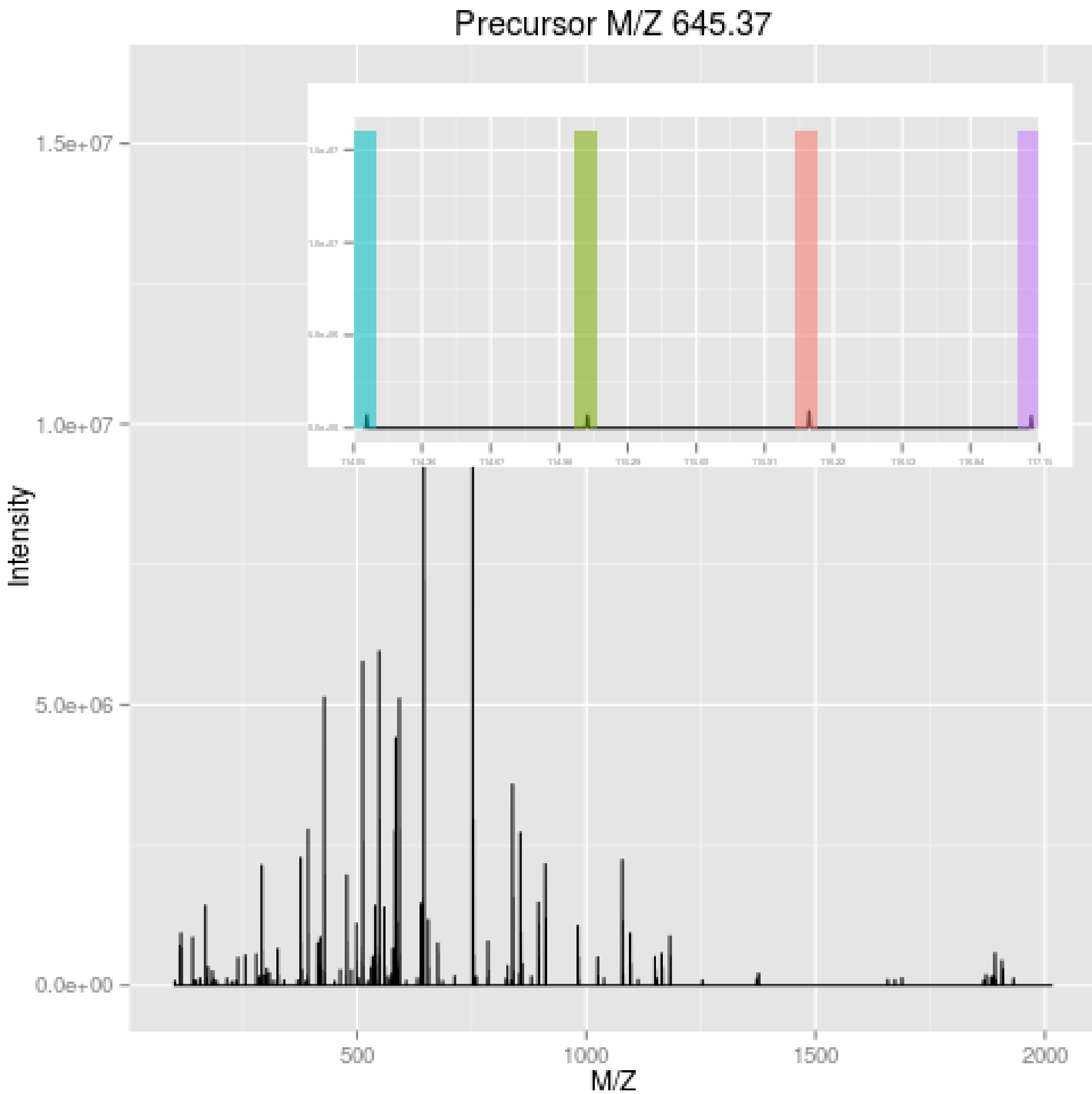
There are a wide range of proteomics quantitation techniques that can broadly be classified as labelled vs. label-free, depending whether the features are labelled prior the MS acquisition and the MS level at which quantitation is inferred, namely MS1 or MS2.

	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: Sun Nov 16 21:59:17 2014
## iTRAQ4 quantification by trapezoidation: Sun Nov 16 21:59:19 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.

Importing third-party 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: Sun Nov 16 21:59:21 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

Processing and normalisation

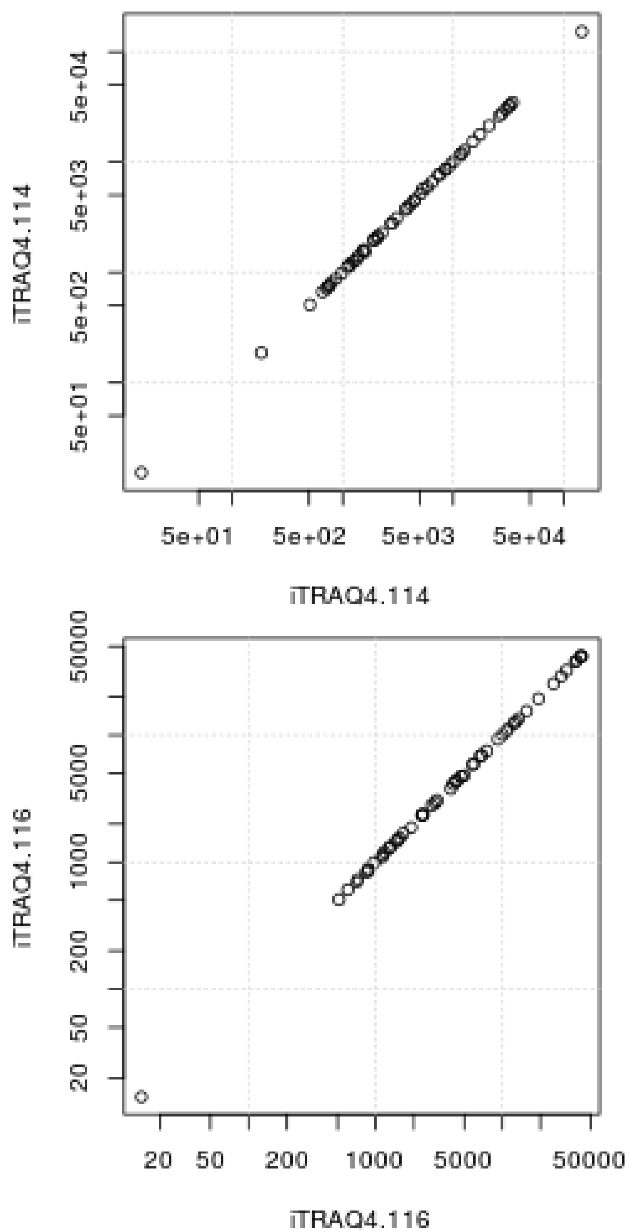
Each different types of quantitative data will require their own pre-processing and normalisation steps. Both `isobar` and `MSnbase` allow to correct for isobaric tag impurities normalise the quantitative data.

```
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: Sun Nov 16 21:59:23 2014
## Purity corrected: Sun Nov 16 21:59:23 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: Sun Nov 16 21:59:23 2014
## Purity corrected: Sun Nov 16 21:59:23 2014
## Normalised (quantiles): Sun Nov 16 21:59:23 2014
## Combined 55 features into 3 using sum: Sun Nov 16 21:59:24 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.

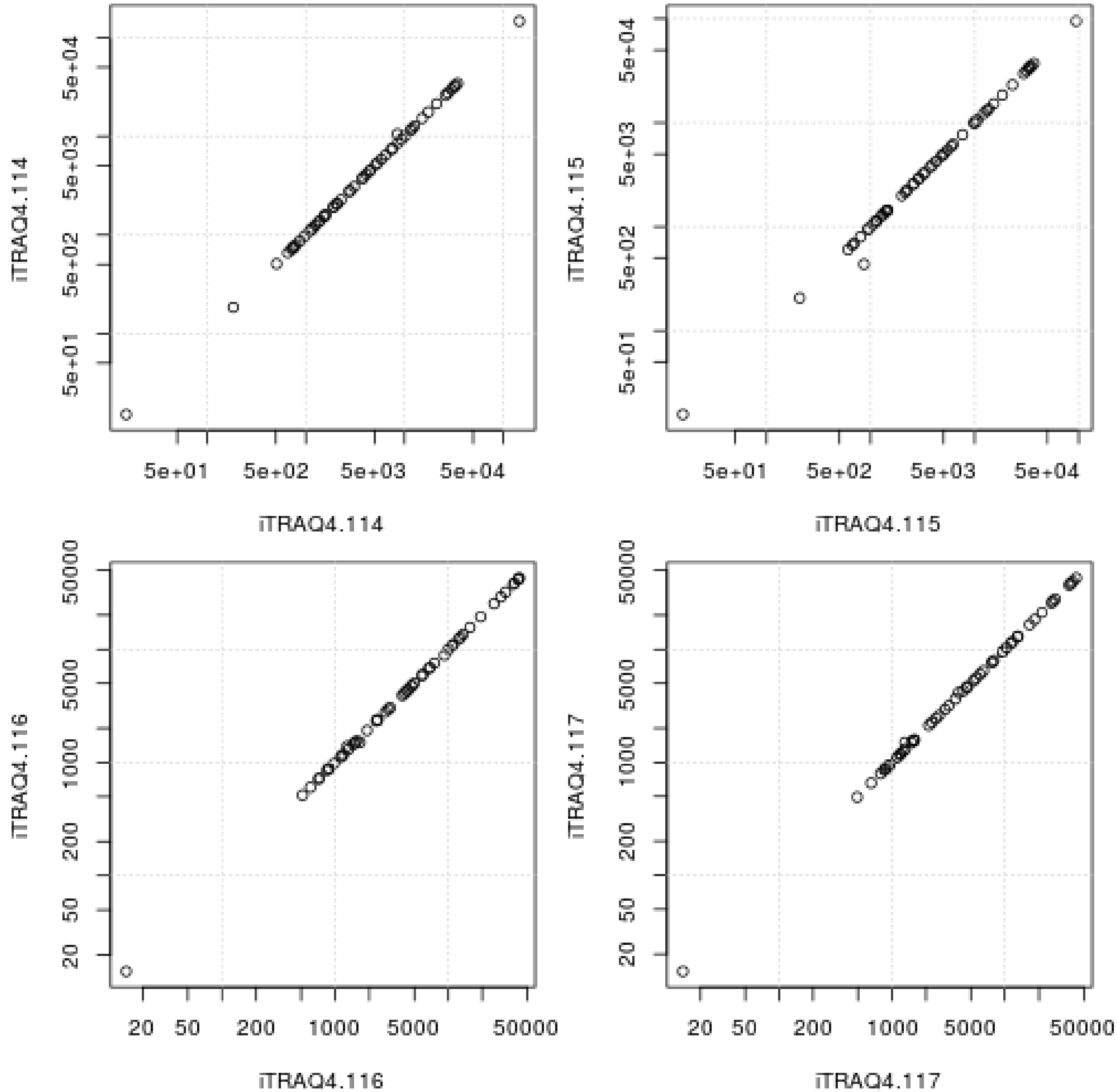
```
set.seed(1)
qnt0 <- qnt
exprs(qnt0)[sample(prod(dim(qnt0)), 10)] <- NA
table(is.na(qnt0))
```

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

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

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

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



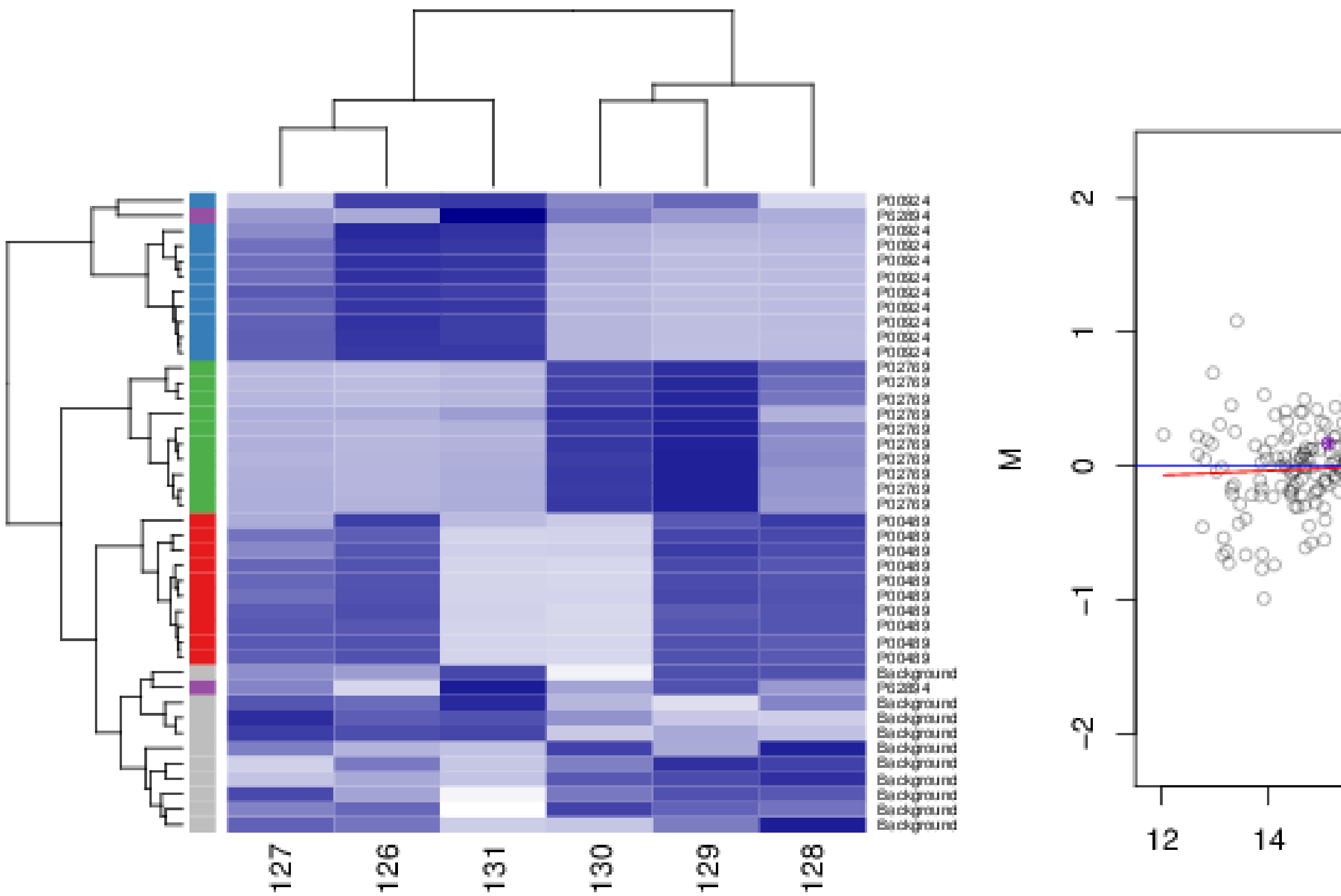
Exercise

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.

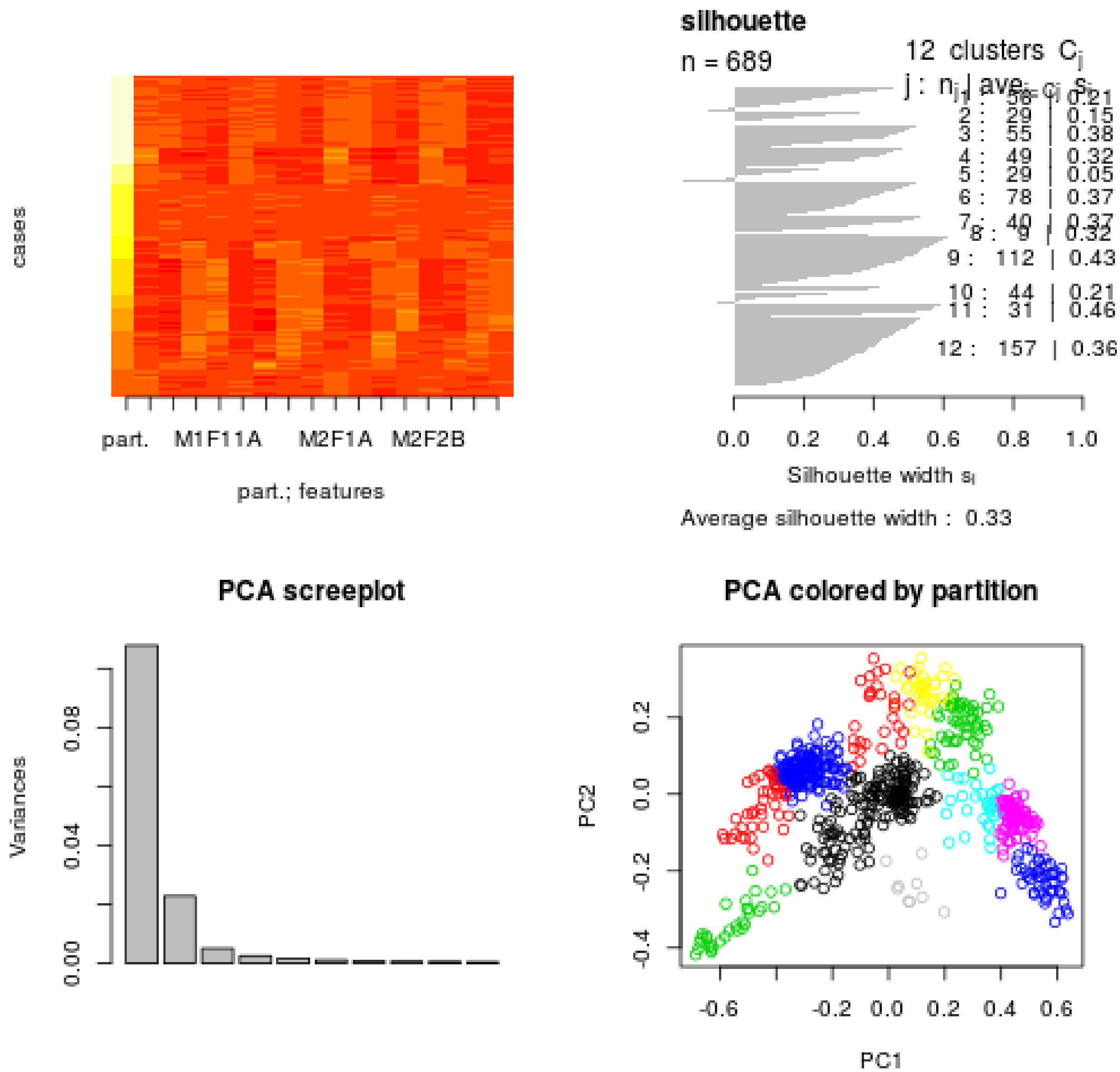
```
library("MLInterfaces")
library("pRoloc")
library("pRolocdata")
data(dunkley2006)
traininds <- which(fData(dunkley2006)$markers != "unknown")
ans <- MLearn(markers ~ ., data = t(dunkley2006), knnI(k = 5), traininds)
ans
```

```
## 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)
```

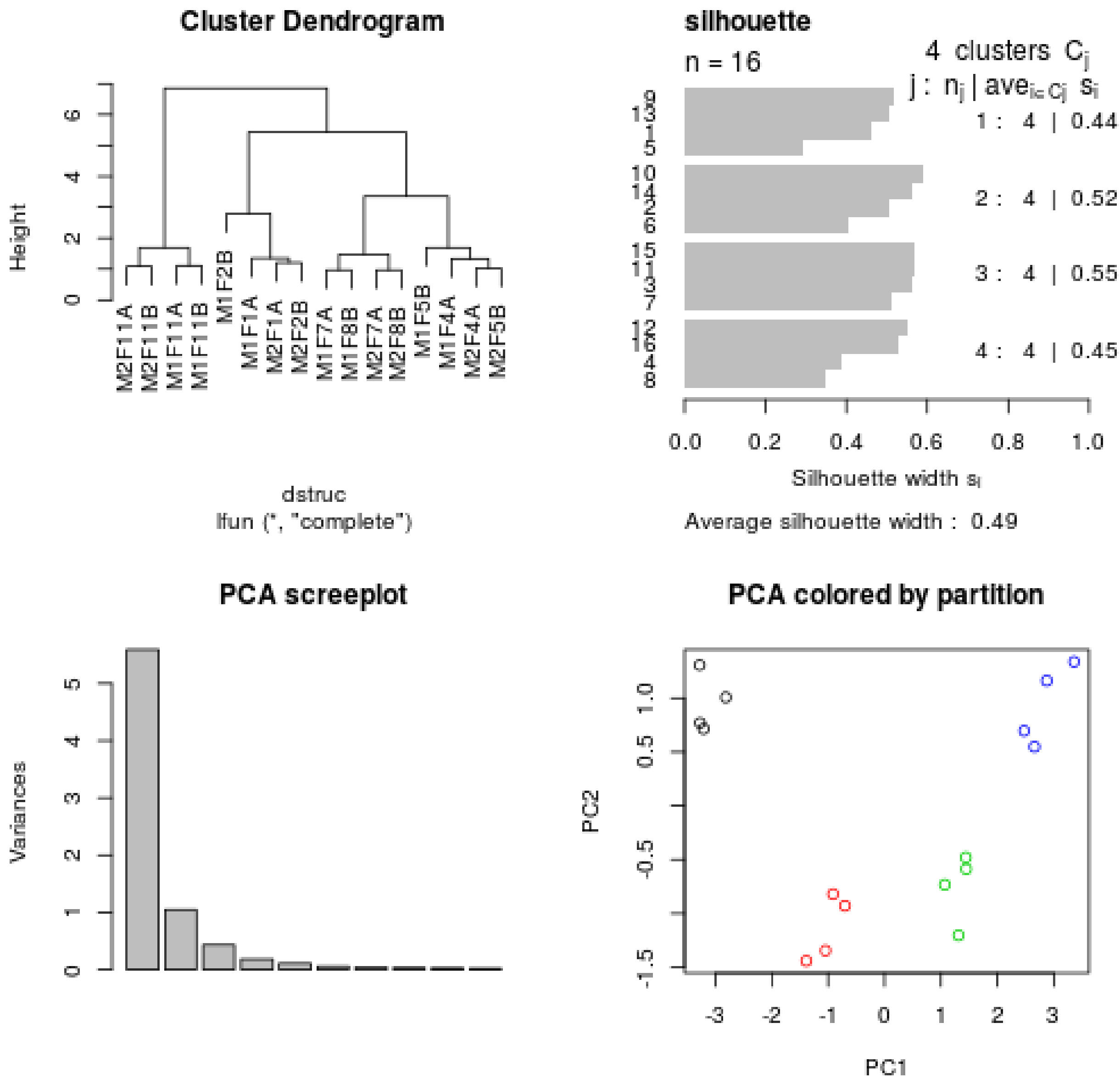
```
plot(kcl, exprs(dunkley2006))
```




```r
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)))
```

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



Annotation

All the Bioconductor annotation infrastructure, such as `biomaRt`, `GO.db`, organism specific annotations, .. are directly relevant to the analysis of proteomics data. 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`. 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] MSGFgui_1.0.1        rTANDEM_1.6.0        data.table_1.9.4
##  [4] pRolocdata_1.5.2     pRoloc_1.7.1         MLInterfaces_1.46.0
##  [7] cluster_1.15.3       annotate_1.44.0      XML_3.98-1.1
## [10] AnnotationDbi_1.28.1 GenomeInfoDb_1.2.3   IRanges_2.0.0
## [13] S4Vectors_0.4.0      rpx_1.2.0            MSGFplus_1.0.3
## [16] MSnID_1.0.0          mzID_1.4.1           RforProteomics_1.5.2
## [19] MSnbase_1.14.0       BiocParallel_1.0.0   mzR_2.0.0
## [22] Rcpp_0.11.3          Biobase_2.26.0       BiocGenerics_0.12.1
## [25] BiocInstaller_1.16.1 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           stringr_0.6.2
## [89] survival_2.37-7         tools_3.1.1
## [91] vsn_3.34.0              xlsx_0.5.7
## [93] xlsxjars_0.6.1          xtable_1.7-4
## [95] zlibbioc_1.12.0
```