Introduction to MS-based proteomics and Bioconductor infrastructure

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Outline

Proteomics and MS data

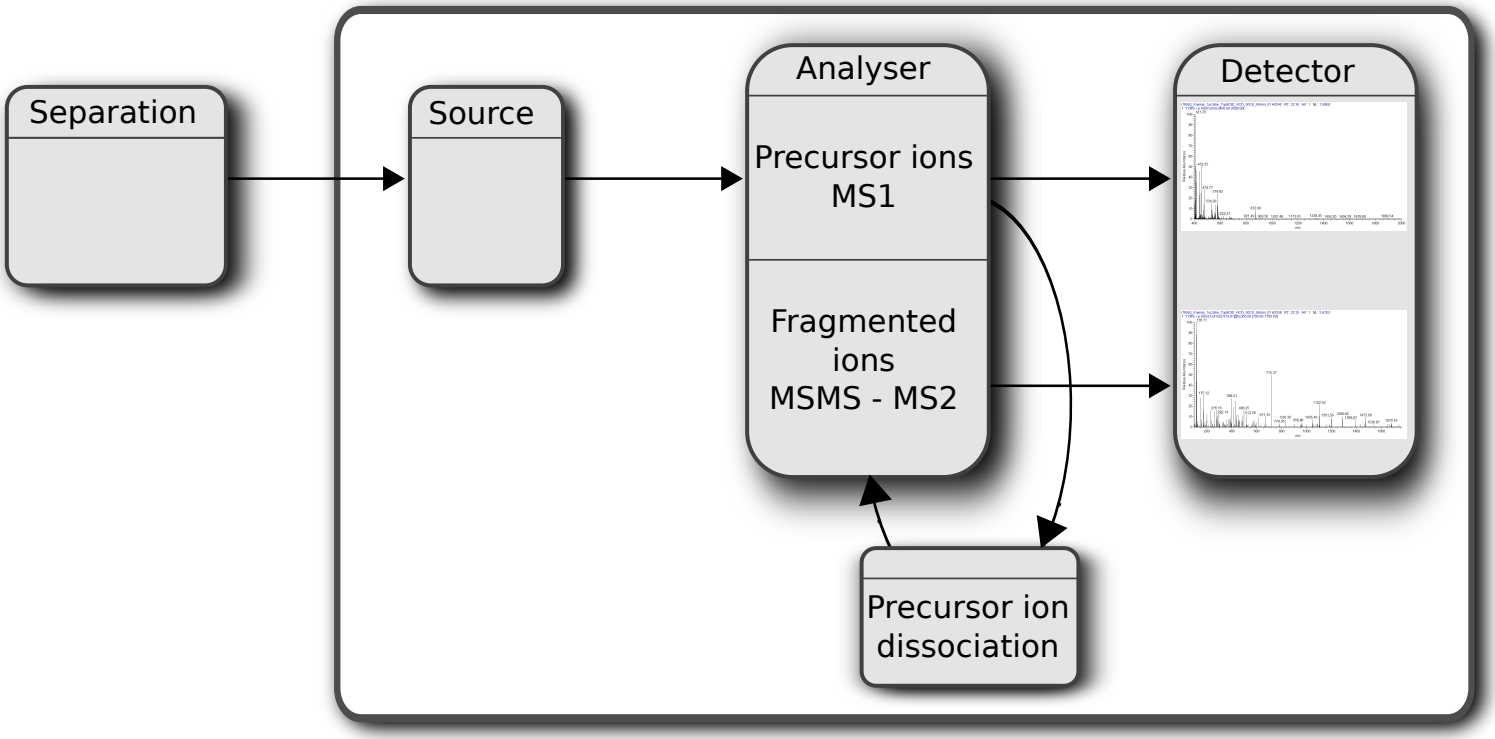
Bioconductor infrastructure

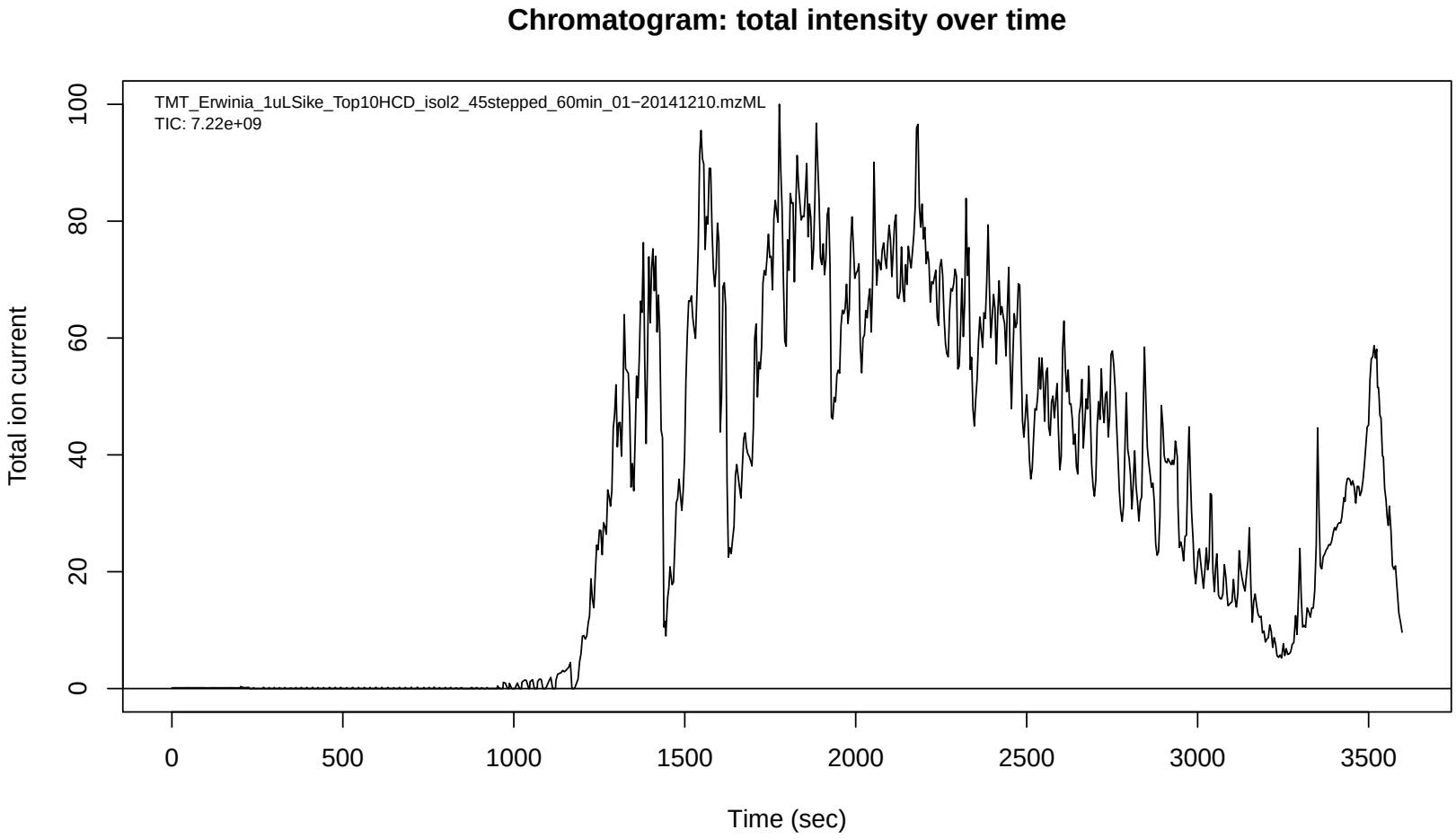
Examples

Ranges infrastructure

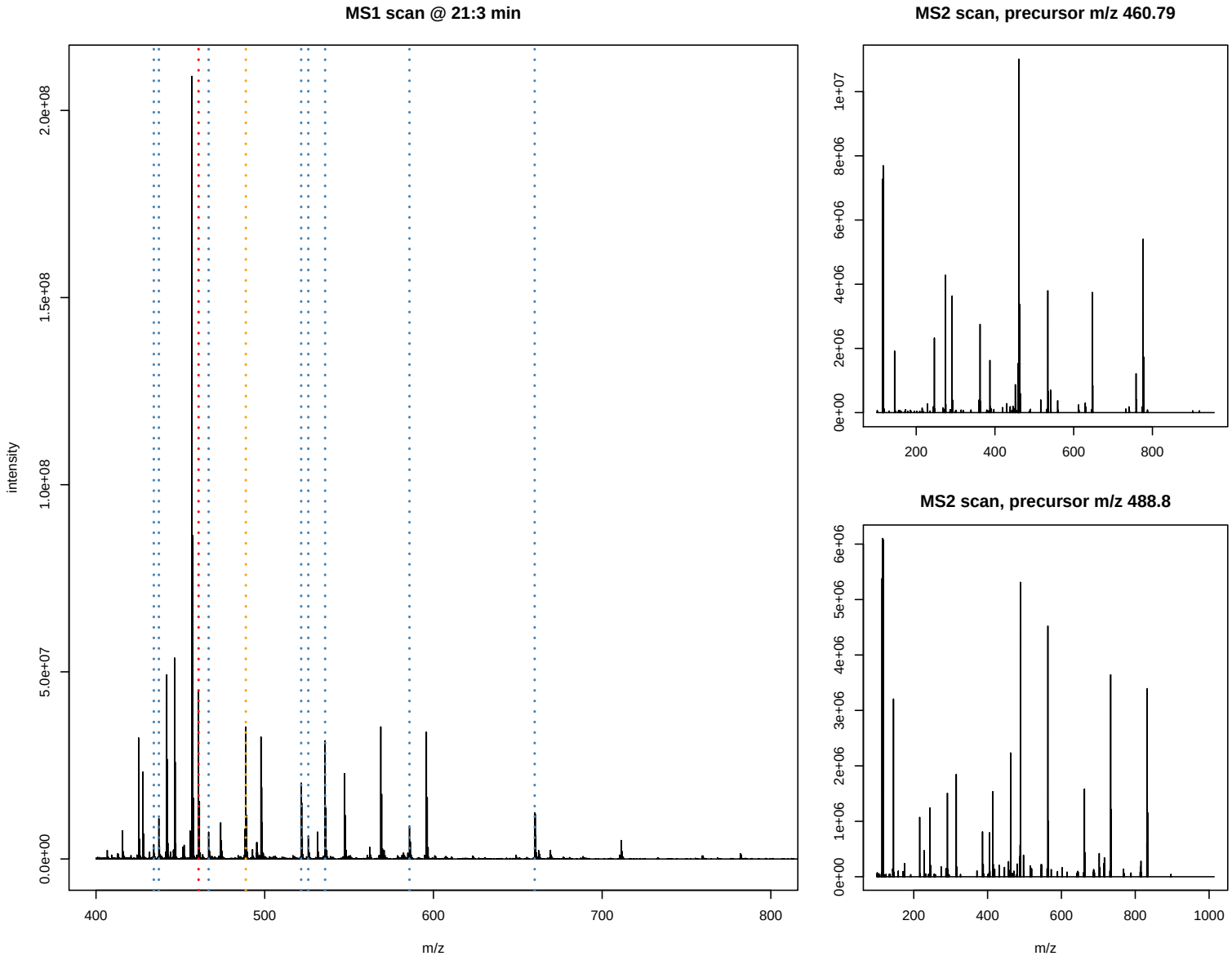
Application: spatial proteomics

Mass-spectrometry – LC-**MS**/MS

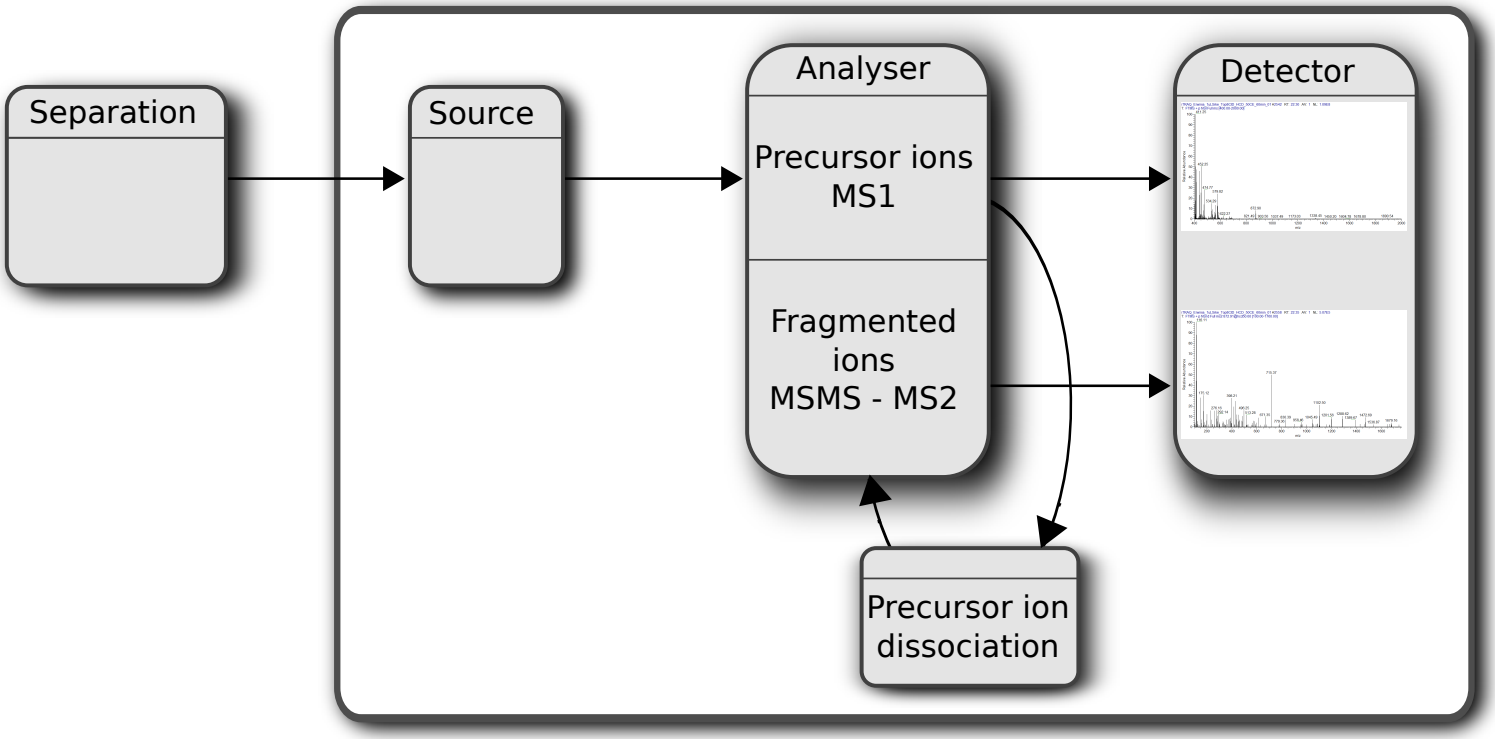



Chromatogram: total intensity over time
TMT_Erwinia_1uLSike_Top10HCD_isol2_45stepped_60min_01−20141210.mzML
TIC: 7.22e+09
Total ion current
0
20
40
60
80
100
0
500
1000
1500
2000
2500
3000
3500
Time (sec)

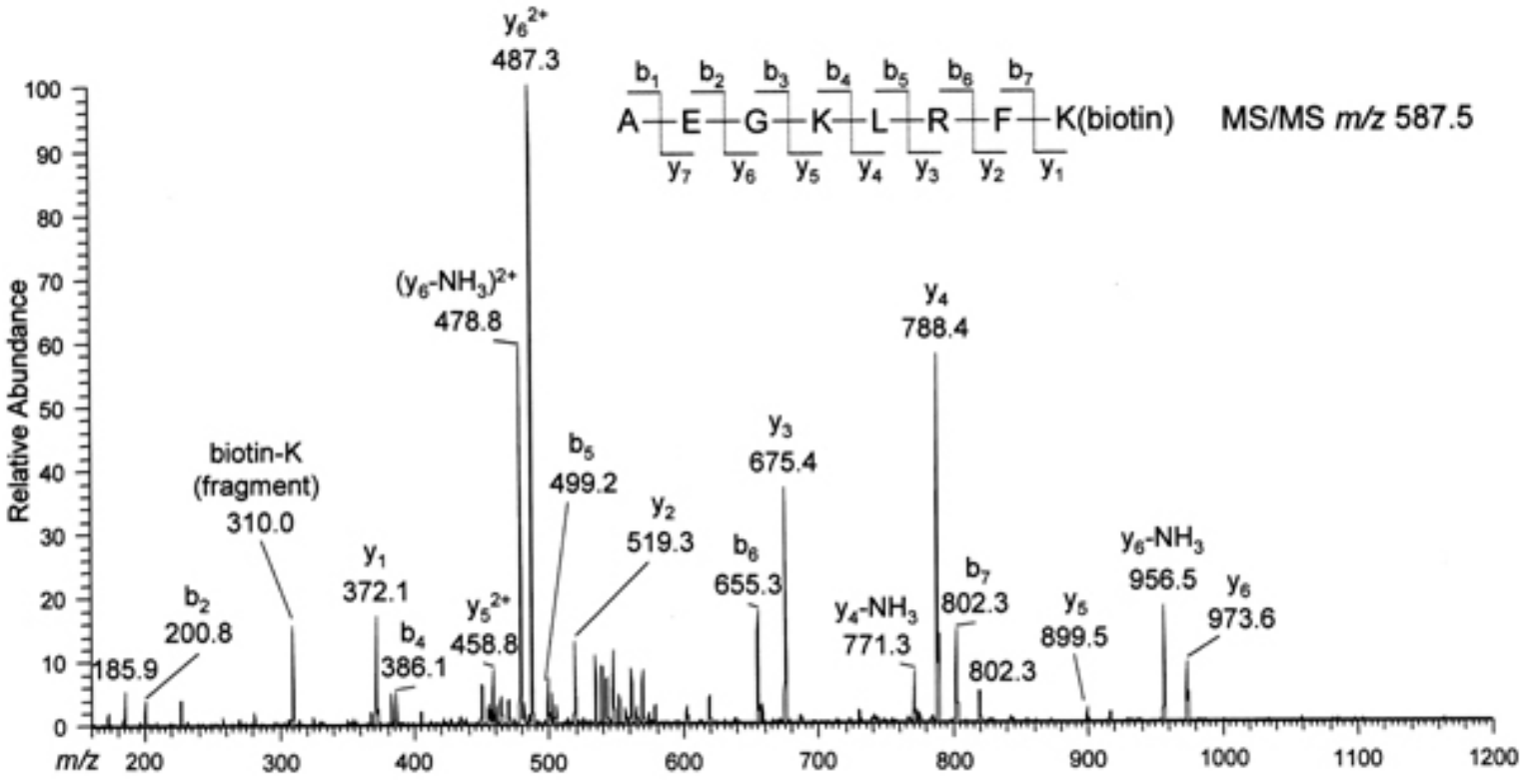
MS1 (and MS2) spectra



Mass-spectrometry – LC-**MS**/MS



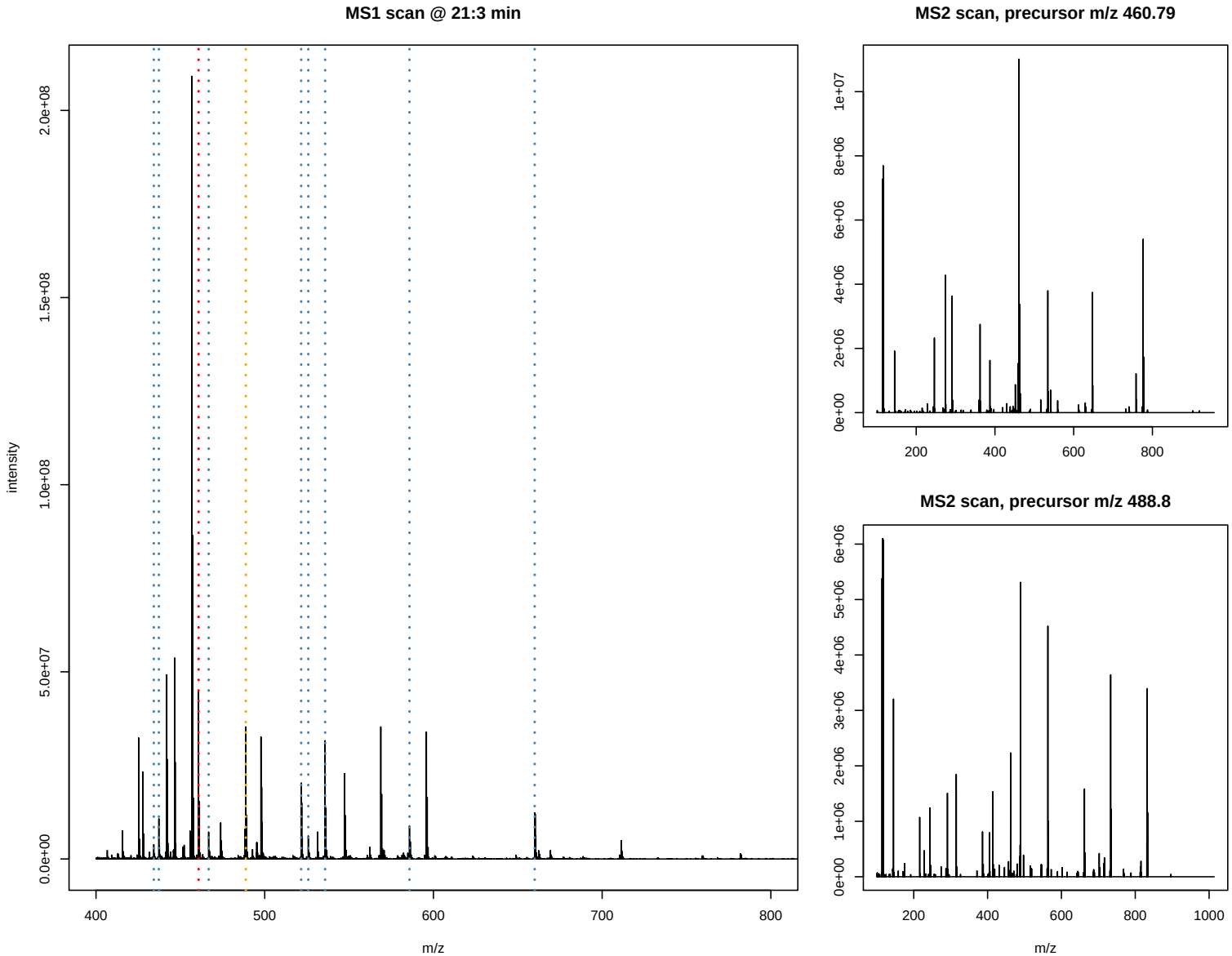
Fragmentation



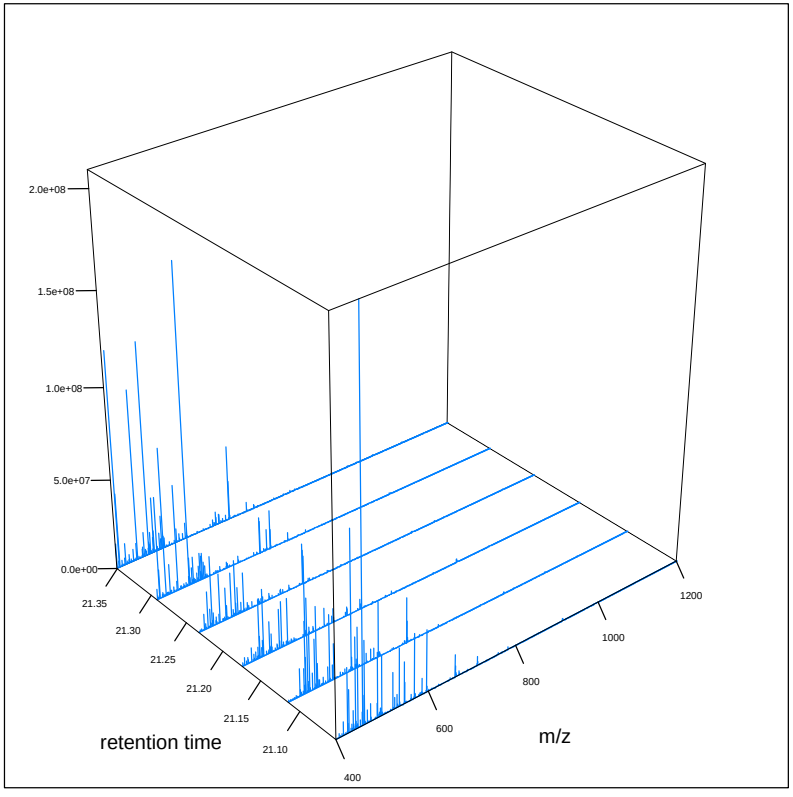
Credit abrg.org

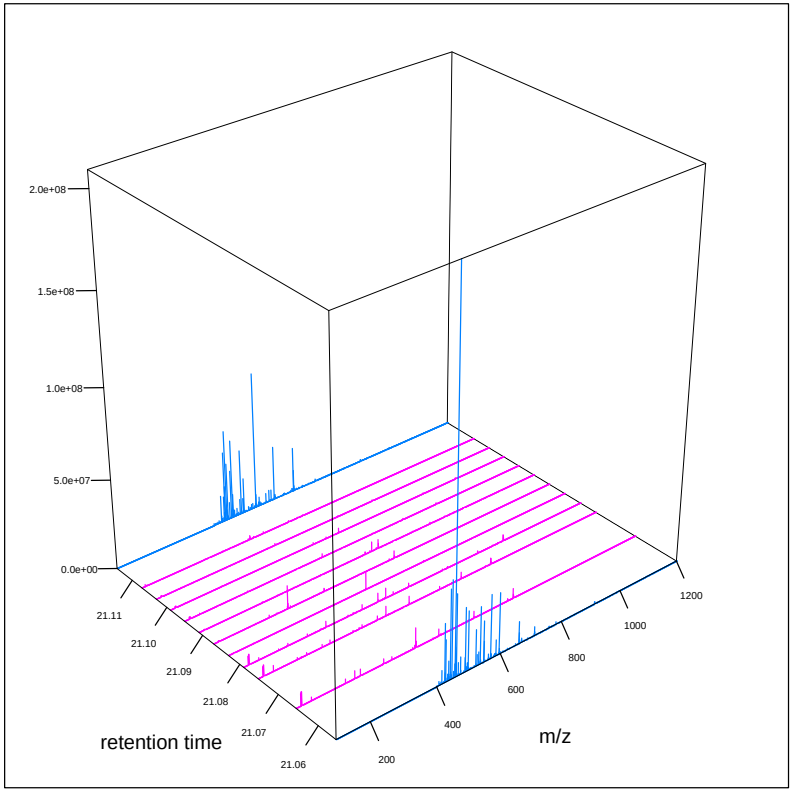
```
cid <- calculateFragments("AEGKLRFK",
                          type=c("b", "y"), z=2)

## Modifications used:  C=160.030649

ht(cid, n = 3)

##           mz ion type pos z seq
## 1   36.52583  b1    b   1 2   A
## 2 101.04713  b2    b   2 2  AE
## 3 129.55786  b3    b   3 2 AEG
## ...
##           mz ion type pos z      seq
## 31 357.7185 y6*   y*   6 2   GKLRFK
## 32 422.2398 y7*   y*   7 2  EGKLRFK
## 33 457.7583 y8*   y*   8 2 AEGKLRFK
```

MS1 *and MS2* spectra



MS1 and MS2 spectra





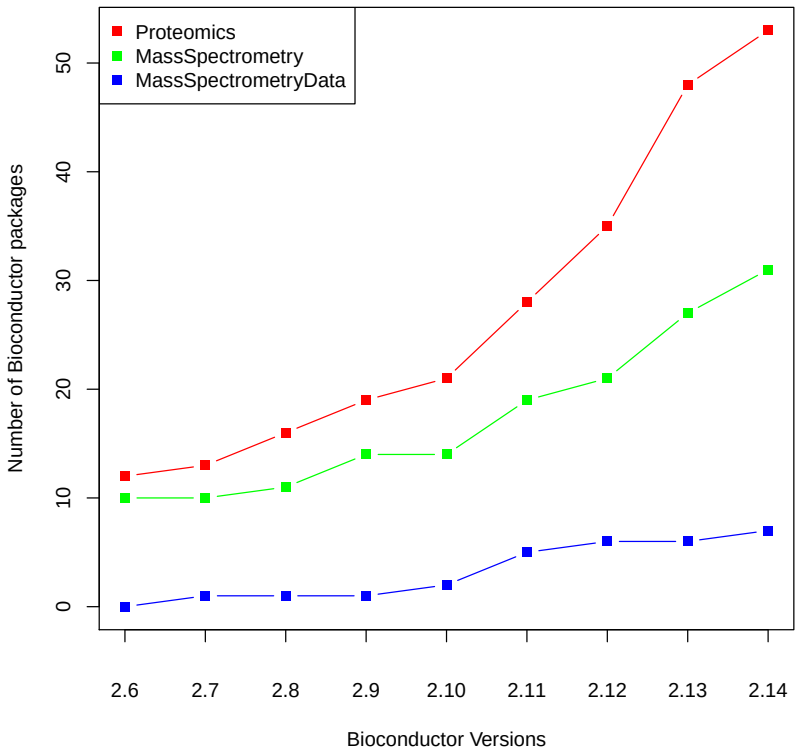
Proteomics data

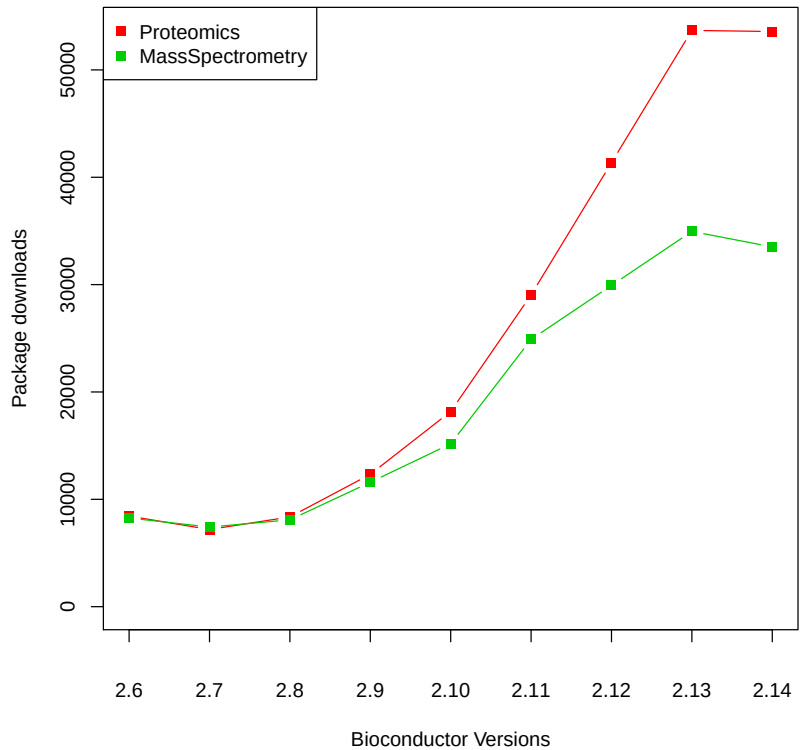
- raw data:
  MS1 and MS2 over retention time
  - identification:
    MS2
  - quantitation:
    MS1 or MS2
- protein database
  (to match MS2 spectra against)

	Status	package
Raw (`mz*ML`)	✓	`mzR`
mzTab	✓	`MSnbase`
mgf	✓	`MSnbase`
mzIdentML	✓	`mzID`, `mzR`
mzQuantML		(?`mzR`)

Bioconductor infrastructure

`biocViews`: Proteomics, MassSpectrometry

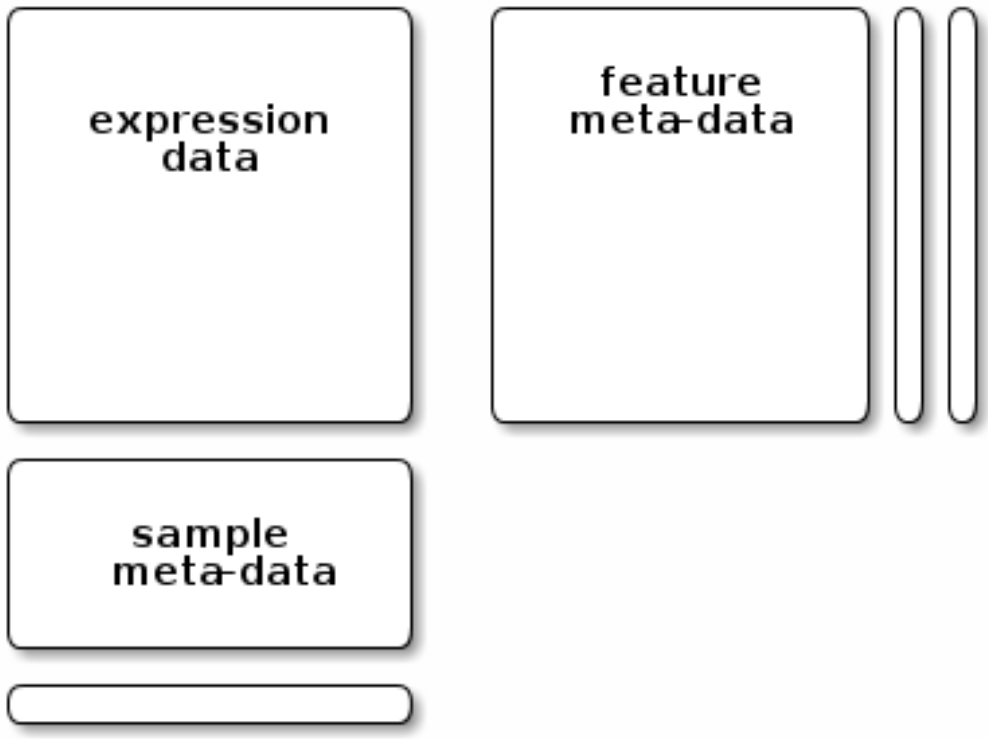




Learning from Bioconductor

genomics	proteomics
eSet (past?)	*MSnSet (present)
Ranges (present)	*Pbase et al. (future)
	PPI (present)
	*localisation (present)

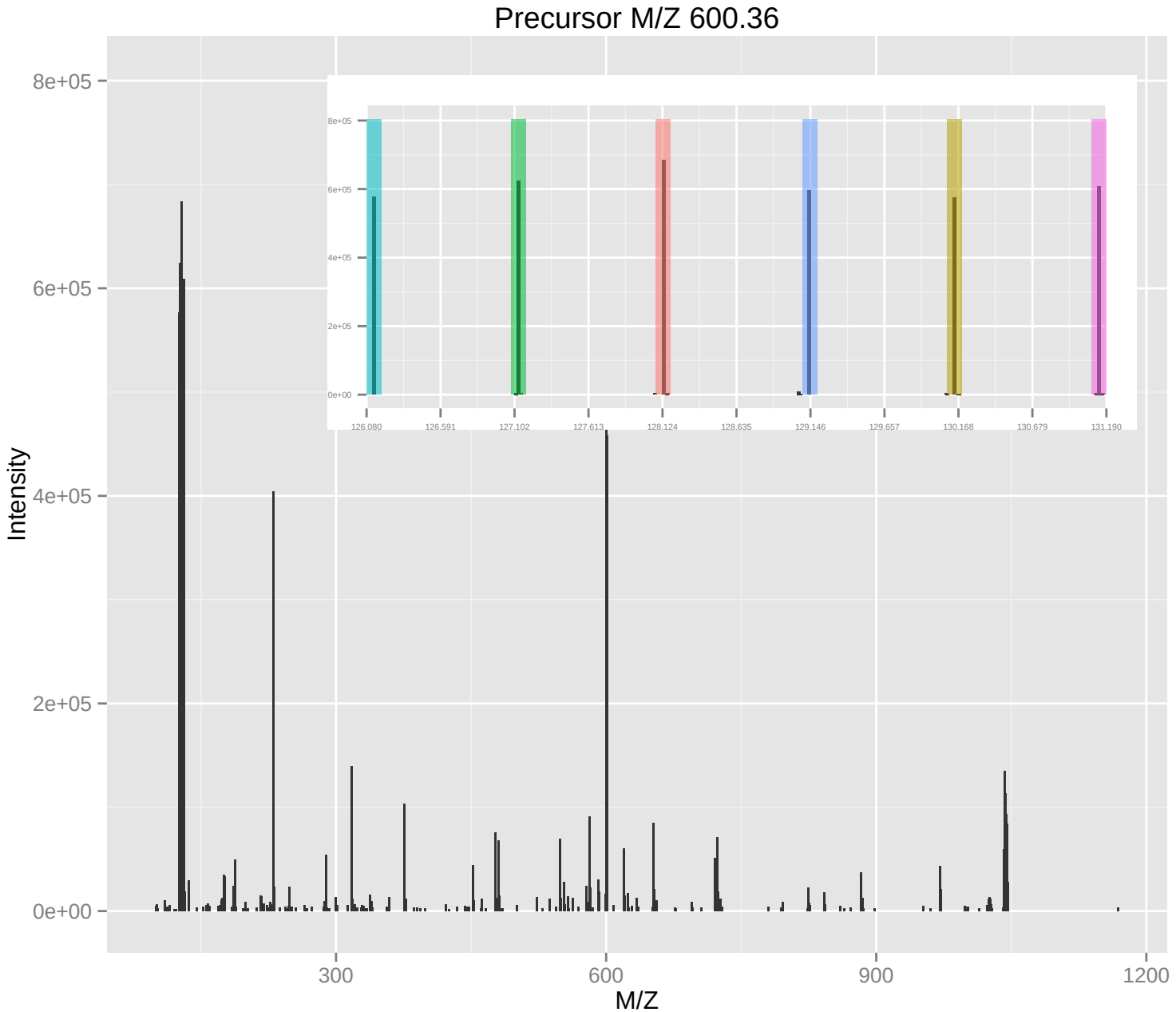
MSnSet



Example

```
library("MSnbase")
rx <- readMSData("rawdata.mzML")
rx <- addIdentificationData(rx, "identification.mzid")
rx <- rx[!is.na(fData(rx)$pepseq)]
plot(rx[[10]], reporters = TMT6, full=TRUE)
```

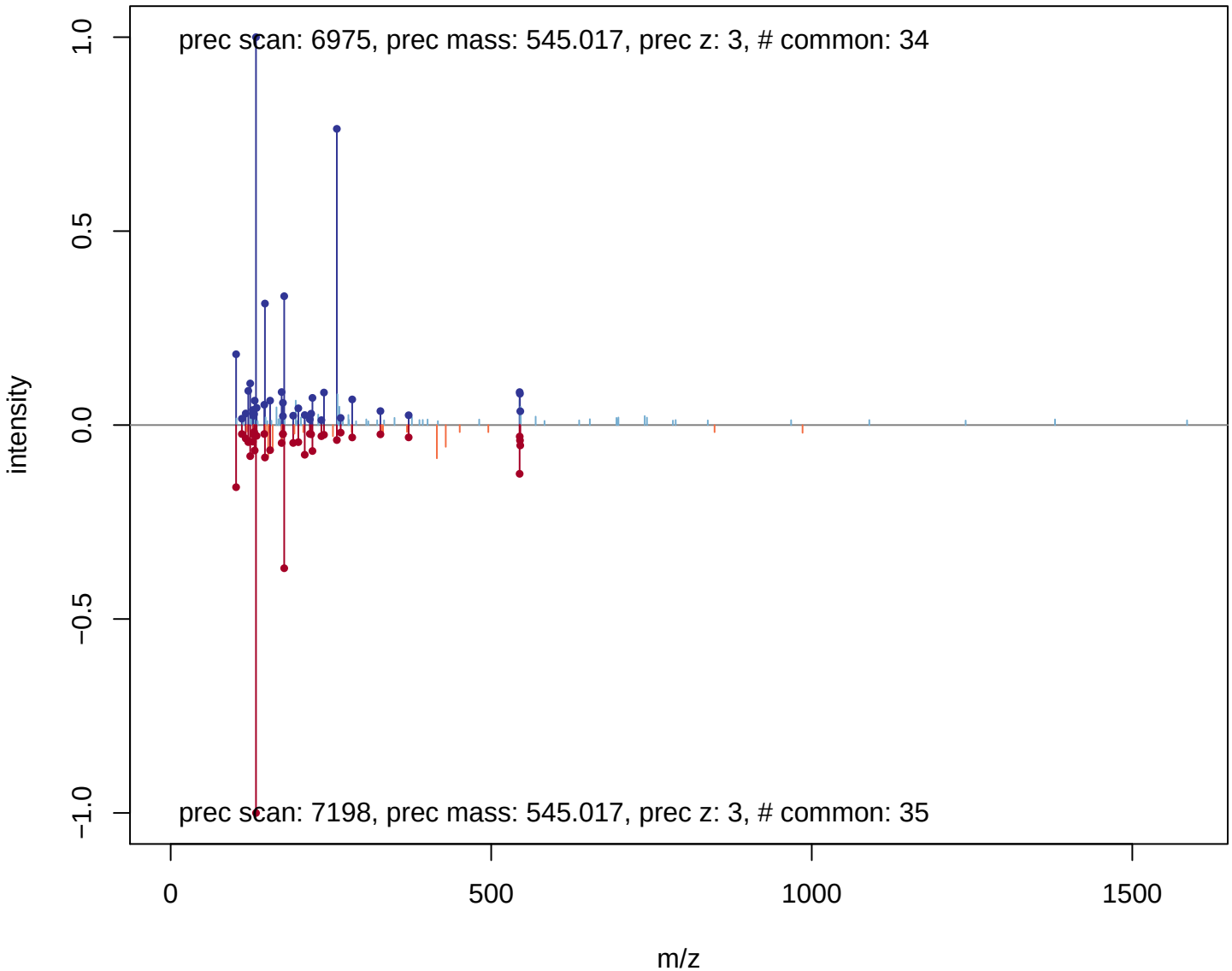
Example


Precursor M/Z 600.36
Intensity
8e+05
6e+05
4e+05
2e+05
0e+00
300
600
900
1200
M/Z
126.080
126.591
127.102
127.613
128.124
128.635
129.146
129.657
130.168
130.679
131.190

Example

```
library("MSnbase")
rx <- readMSData(f, centroided = TRUE)
rx <- addIdentificationData(rx, g)
rx <- rx[!is.na(fData(rx)$pepseq)]
plot(rx[[10]], reporters = TMT6, full=TRUE)
plot(rx[[4730]], rx[[4929]])
```

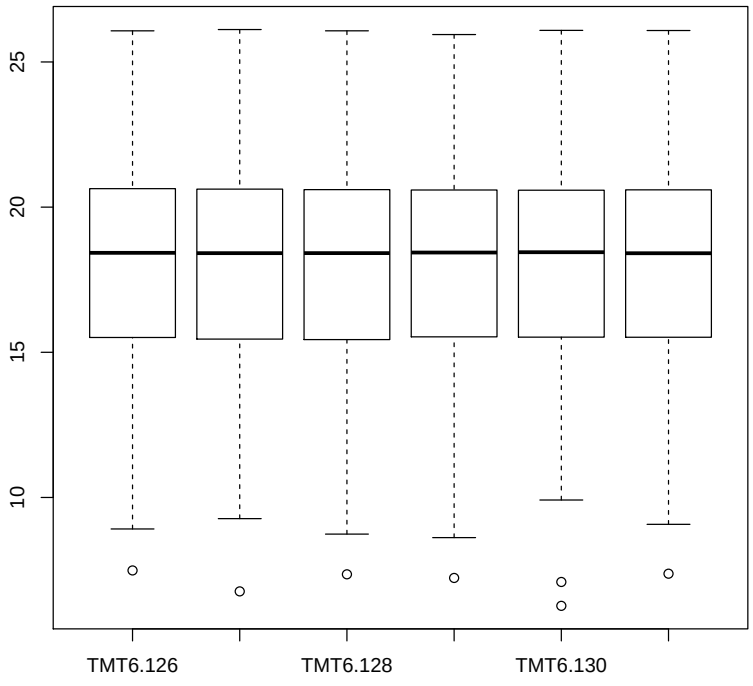
Example

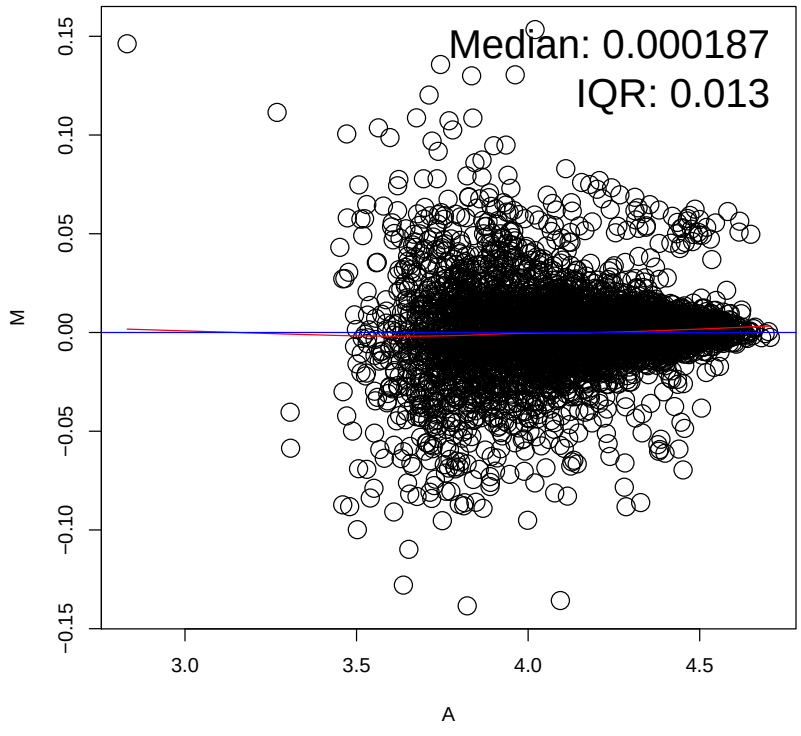

prec scan: 6975, prec mass: 545.017, prec z: 3, # common: 34
prec scan: 7198, prec mass: 545.017, prec z: 3, # common: 35
intensity
1.0
0.5
0.0
−0.5
−1.0
0
500
1000
1500
m/z

Example

```
library("MSnbase")
rx <- readMSData(f, centroided = TRUE)
rx <- addIdentificationData(rx, g)
rx <- rx[!is.na(fData(rx)$pepseq)]
plot(rx[[10]], reporters = TMT6, full=TRUE)
plot(rx[[4730]], rx[[4929]])

qt <- quantify(rx, reporters = TMT6)
## qt <- readMSnSet("quantdata.csv", ecols = 5:11)
nqt <- normalise(qt, method = "vsn")
boxplot(exprs(nqt))
MAplot(nqt[, 1:2])
```

Example


25
20
15
10
TMT6.126
TMT6.128
TMT6.130


Median: 0.000187
IQR: 0.013
M
0.15
0.10
0.05
0.00
−0.05
−0.10
−0.15
3.0
3.5
4.0
4.5
A

More

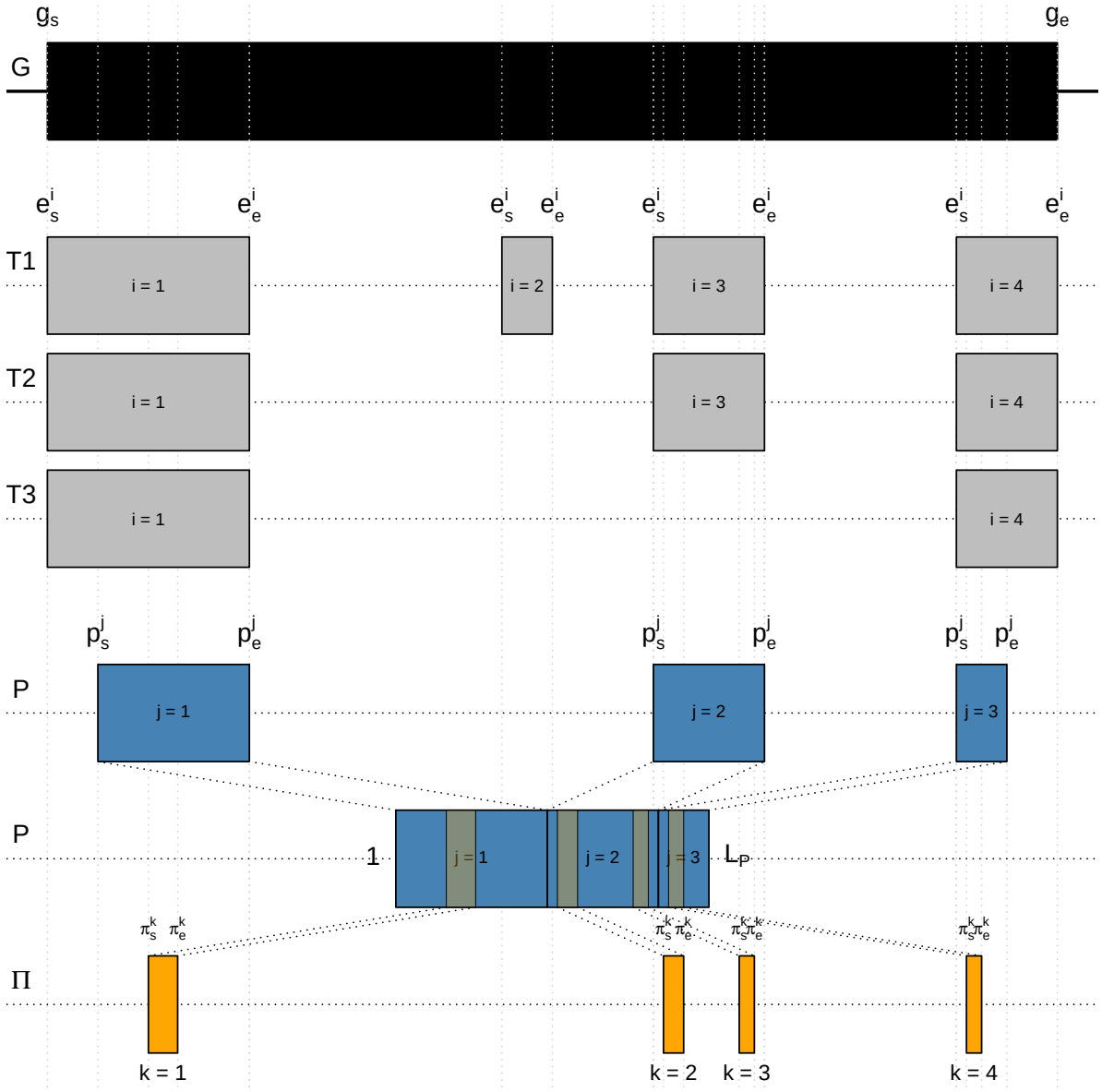
- RforProteomics package

```
library("RforProteomics")
RforProteomics()
RProtVis()
citation(package = "RforProteomics")
```

- Proteomics workflow on the Bioc site
- Lab on Friday

- **protein database**
- raw data
  - quantitation
  - identification

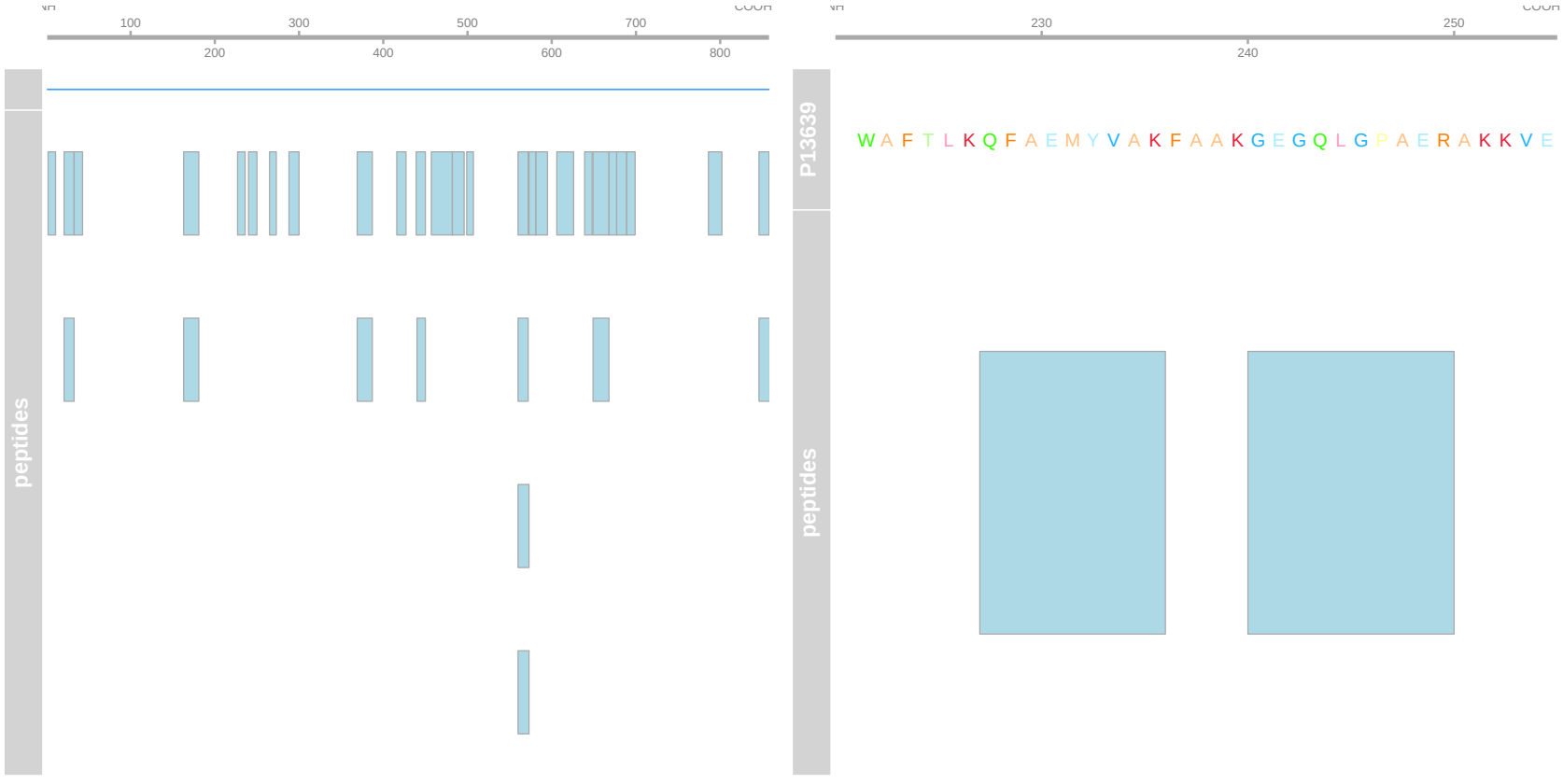
Ranges infrastructure



Pbase package

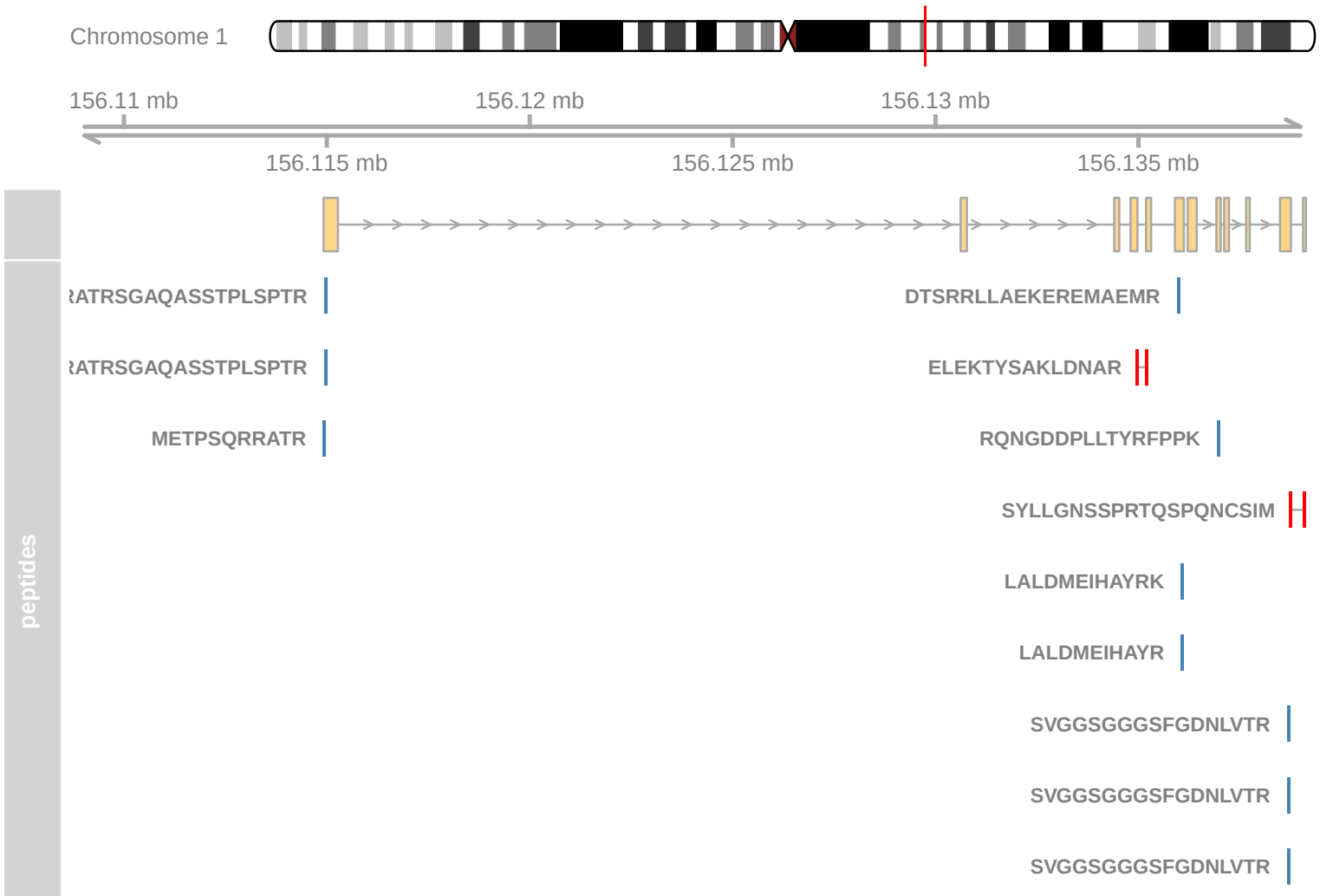
```
library("Pbase")
p <- Proteins("uniprot.fasta")
p <- addIdentificationData(p, "identification.mzid")
aa(p) ## peptides sequences as a AAStringSet
pranges(p) ## peptide ranges as IRangesList
i <- which(acols(p)[, "EntryName"] == "EF2_HUMAN")
plot(p[i])
plot(p[i], from = 155, to = 185)
```

Along protein coordinates



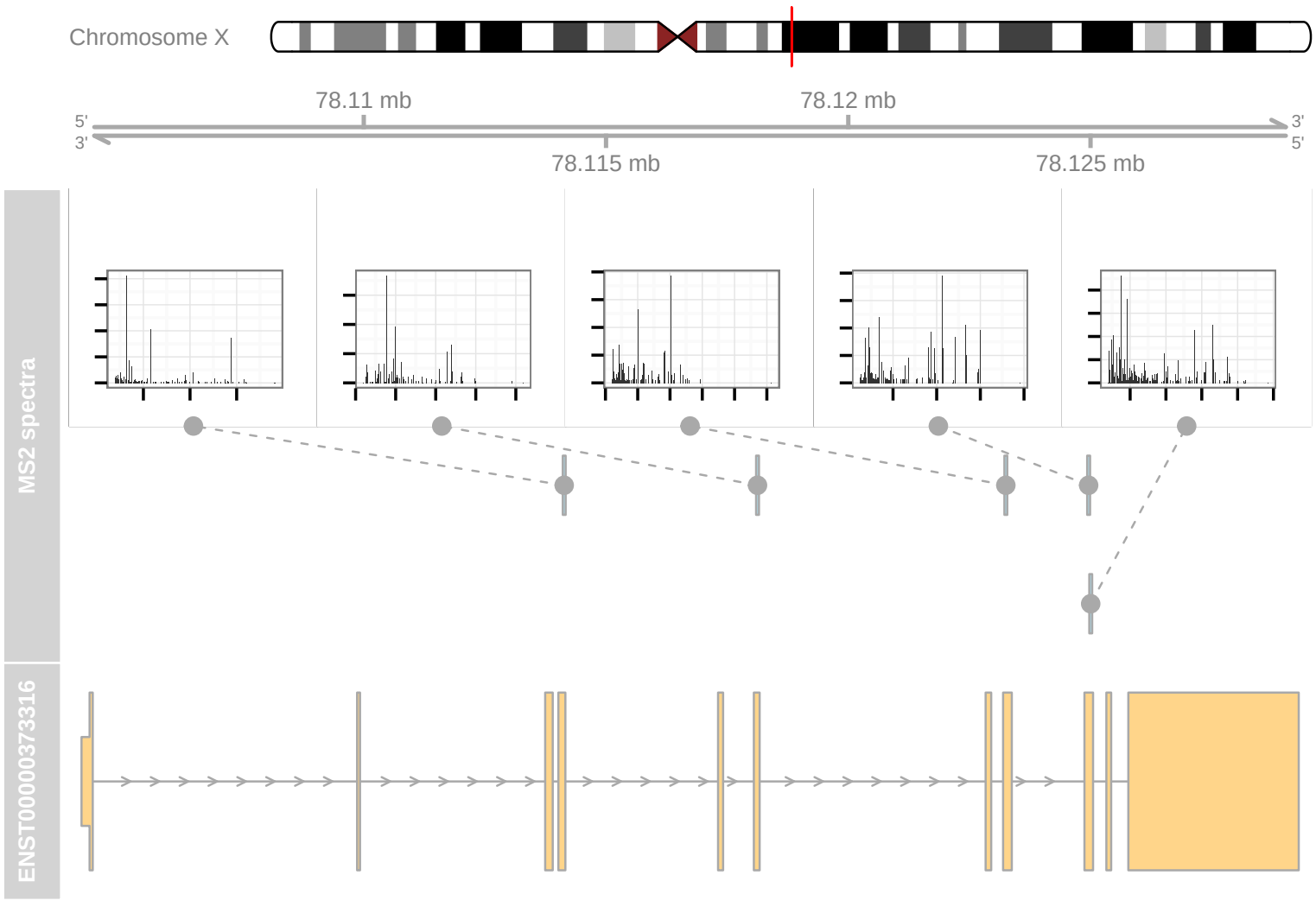
Along genome coordinates

... using transcript models as `GRangesList` and `Gviz` for plotting.



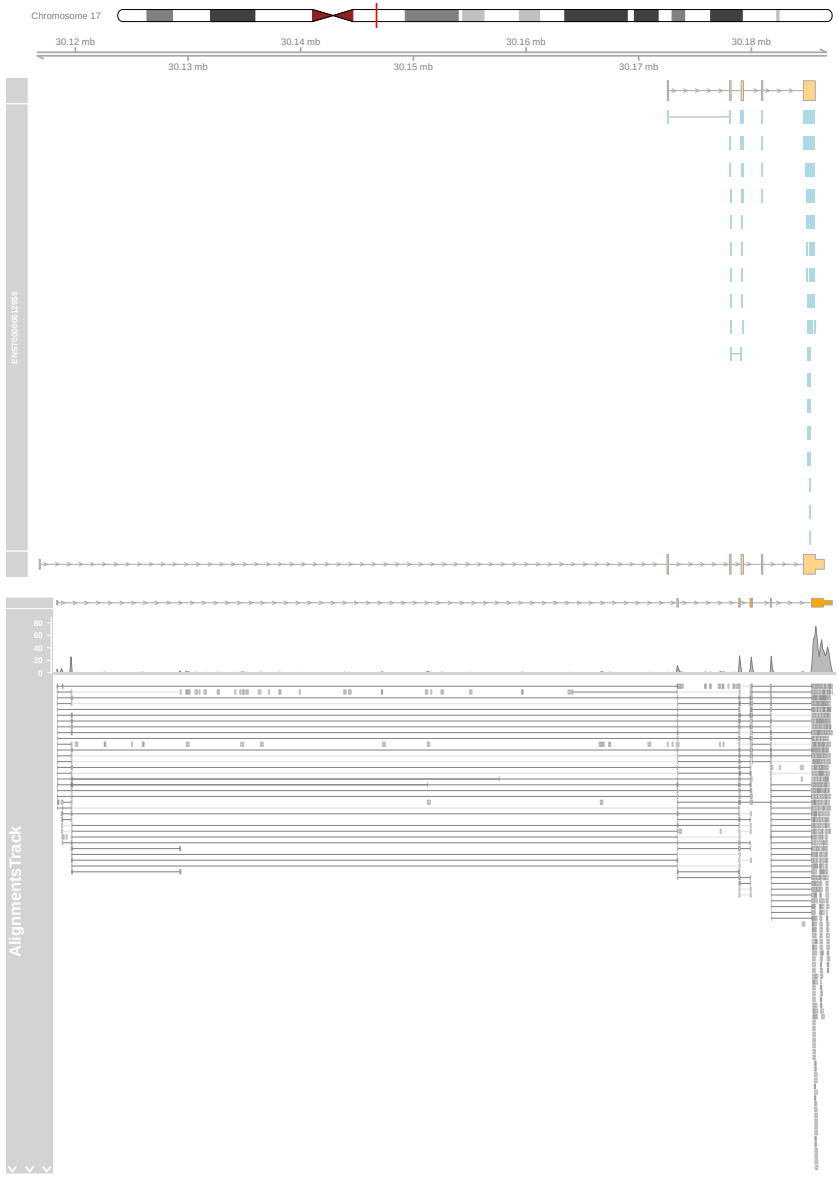
From the `Pbase` mapping vignette.

Along genome coordinates (with raw data)



From the `Pbase` mapping vignette.

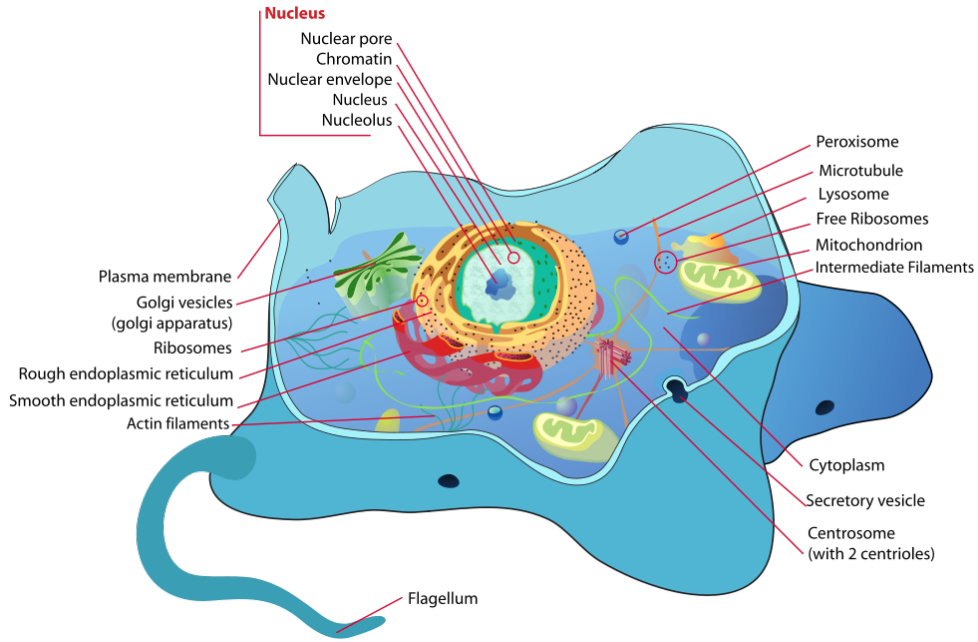
With RNA-Seq reads



From https://github.com/ComputationalProteomicsUnit/Intro-Integ-Omics-Prot

Spatial proteomics

- The cellular sub-division allows cells to establish a range of distinct microenvironments, each favouring different biochemical reactions and interactions and, therefore, allowing each compartment to fulfil a particular functional role.
- Localisation and sequestration of proteins within subcellular niches is a fundamental mechanism for the post-translational regulation of protein function.



Spatial proteomics is the systematic study of protein localisations.

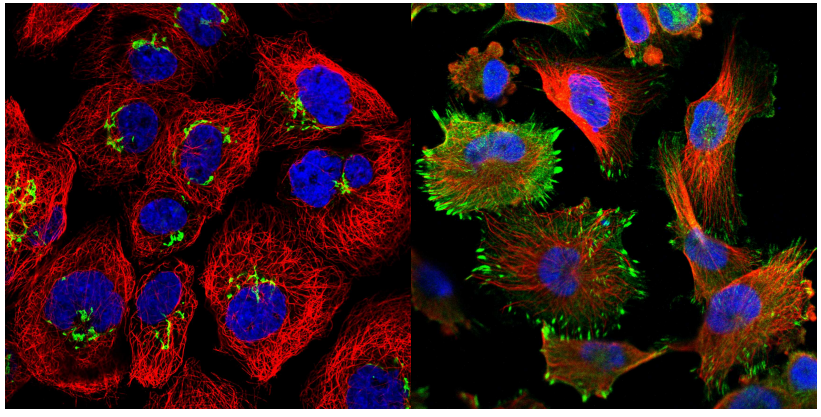


Figure : **Immunofluorescence**: ZFPL1, Golgi (left) and FHL2, mainly localized to actin filaments and focal adhesion sites. Also detected in the nucleus (right). (from the Human Protein Atlas)

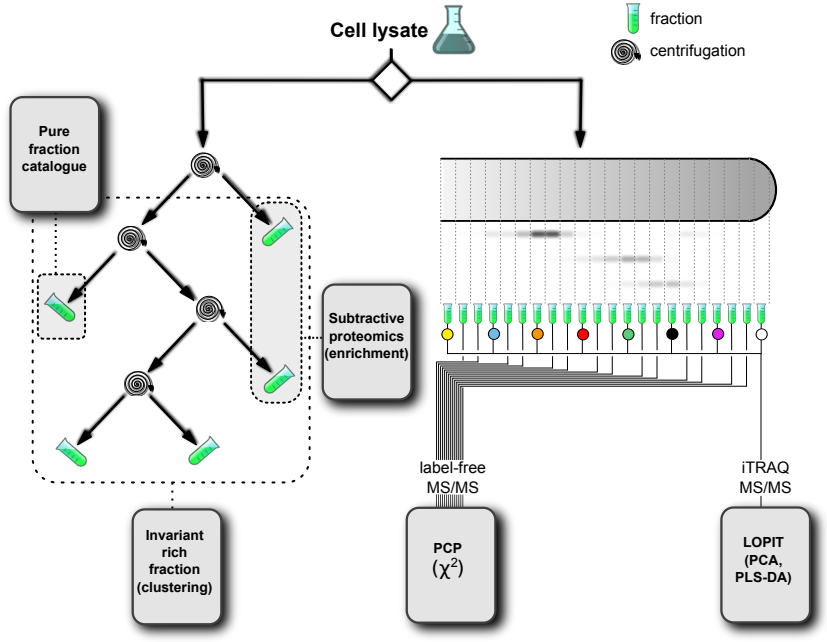
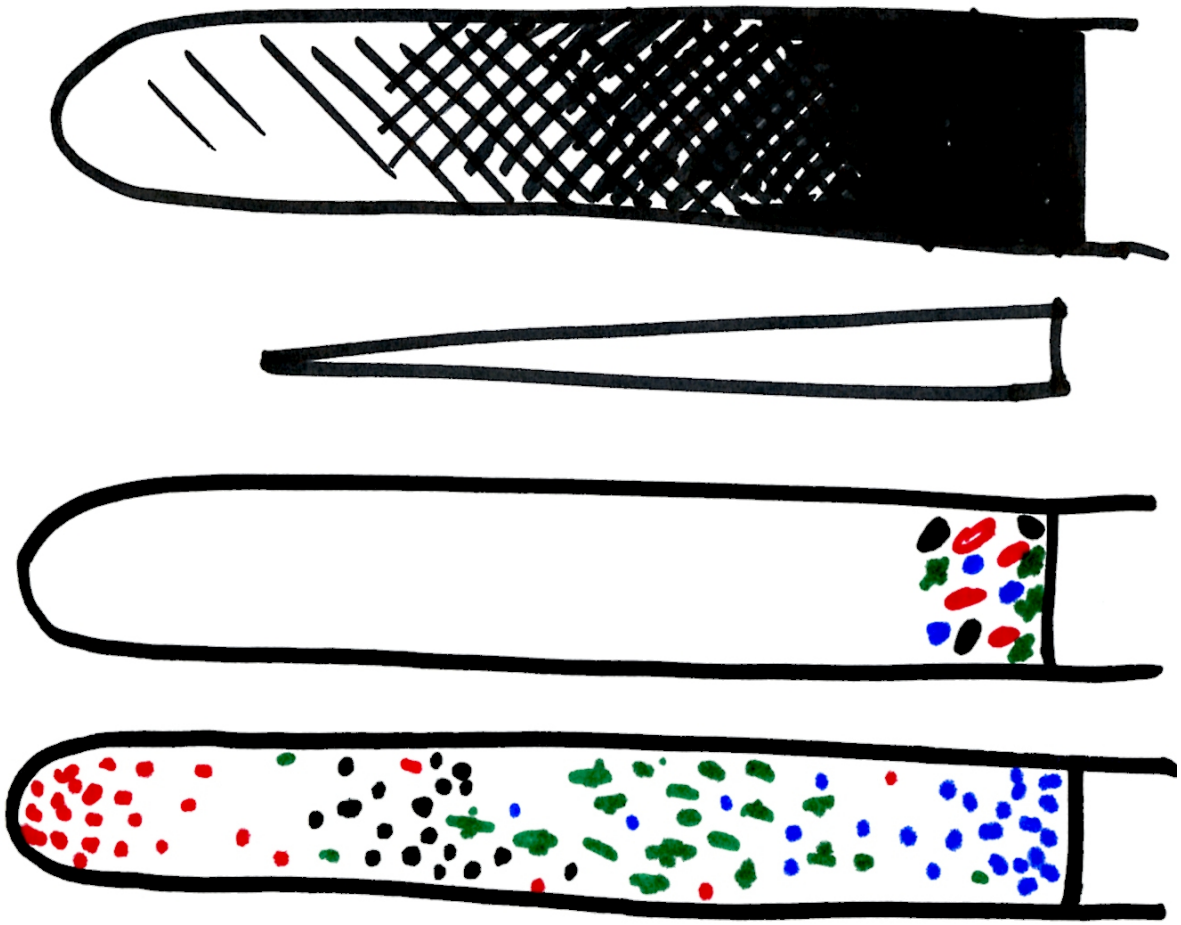


Figure : Mass spectrometry-based approaches based on density gradient subcellular fractionation.

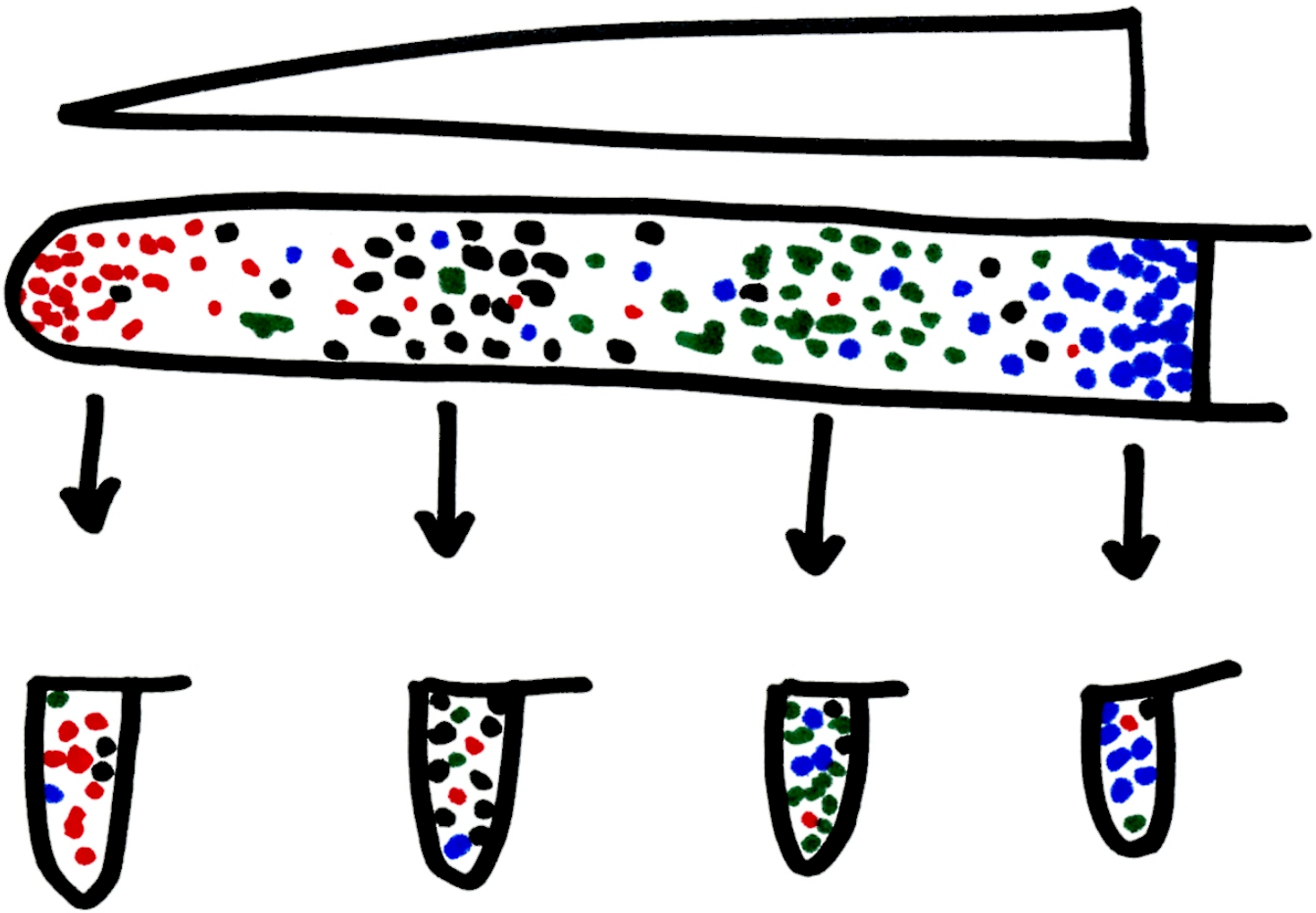
Cell membrane lysis

Mechanical or buffer-induced lysis of the plasma membrane with minimal disruption to intracellular organelles followed by subcellular fractionation.

Density gradient separation



Quantitation by LC-MSMS



Data

	Fraction_1	Fraction_2	...	Fraction_m	markers
p_1	$q_{1,1}$	$q_{1,2}$	...	$q_{1,\,m}$	unknown
p_2	$q_{2,1}$	$q_{2,2}$	...	$q_{2,\,m}$	loc_1
p_3	$q_{3,1}$	$q_{3,2}$	...	$q_{3,\,m}$	unknown
p_4	$q_{4,1}$	$q_{4,2}$	...	$q_{4,\,m}$	loc_k
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$
p_n	$q_{n,1}$	$q_{n,2}$	...	$q_{n,\,m}$	unknown

Data analysis

`MSnbase` for data manipulation, `pRoloc` for clustering, classification and plotting, and `pRolocGUI` for interactive exploration.

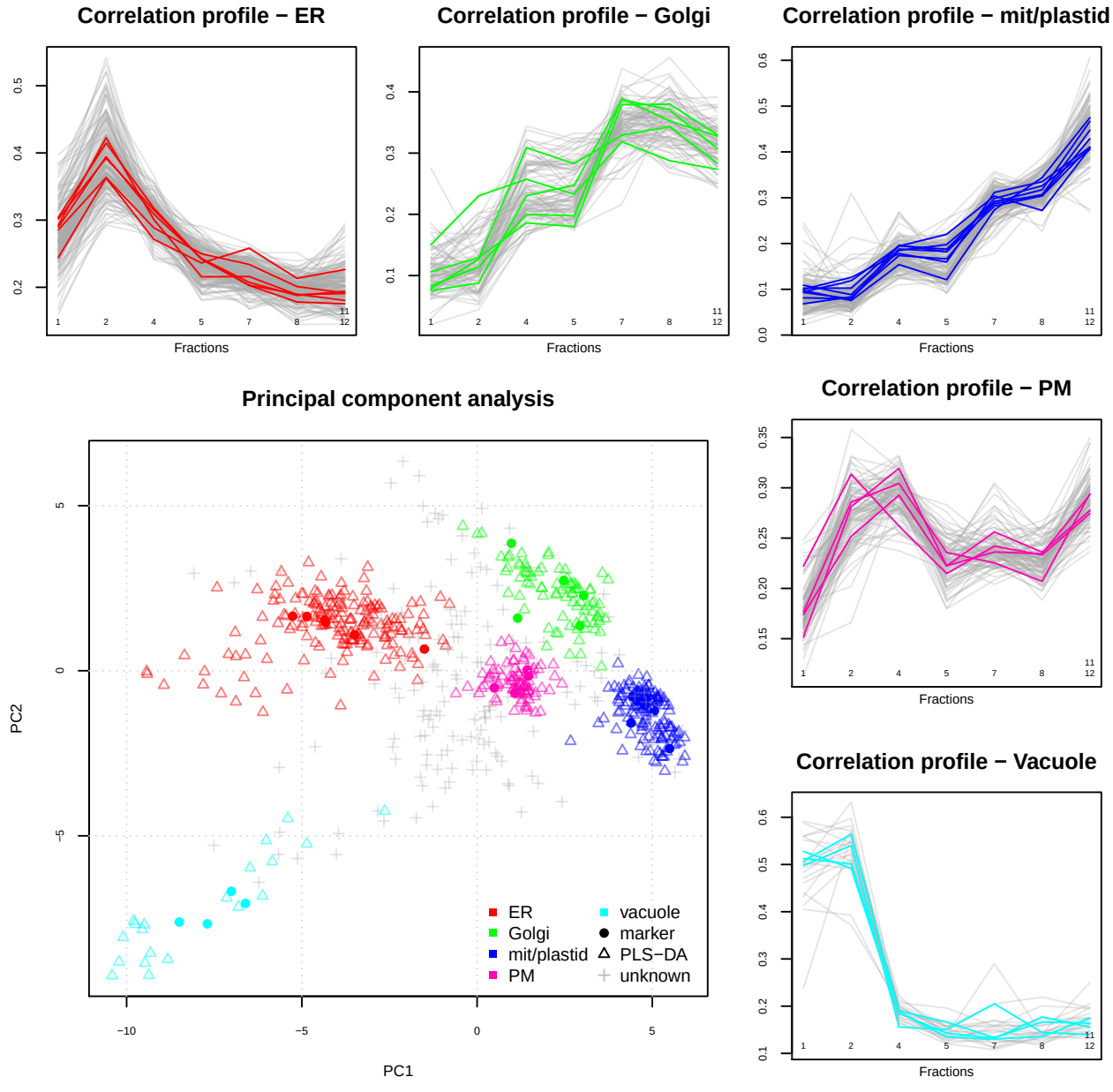


Figure : From Gatto et al. (2010), data from Dunkley et al. (2006).

2009 vs 2013

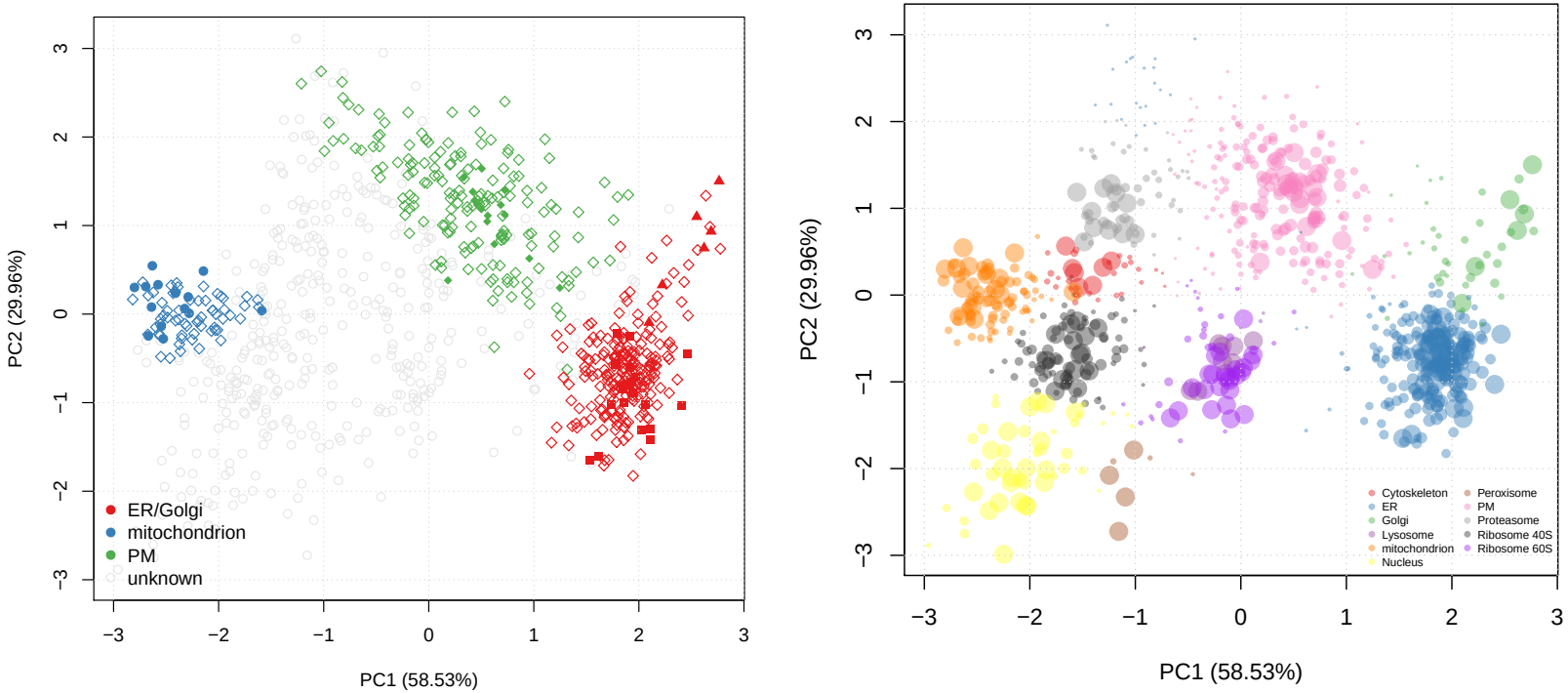
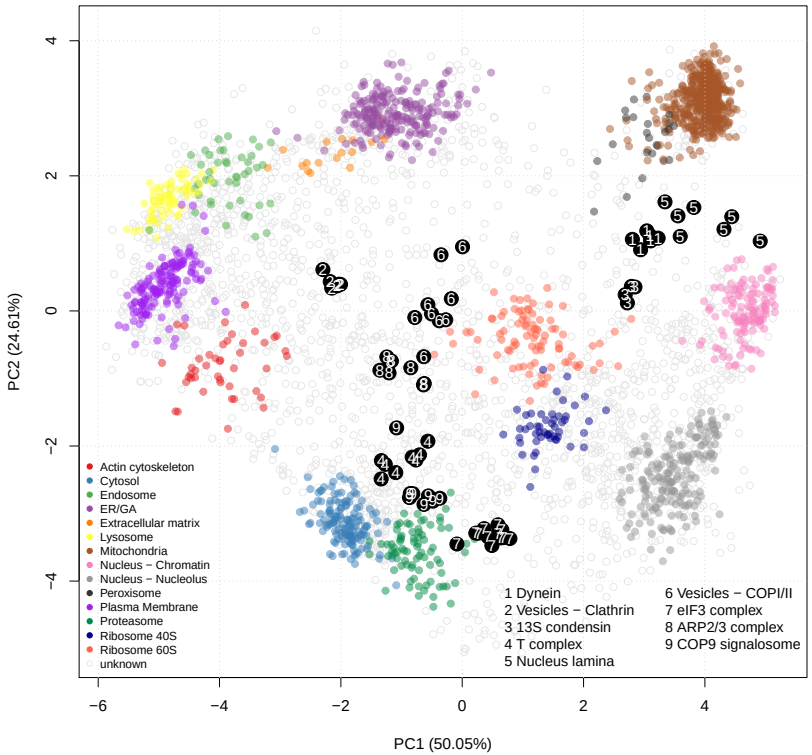
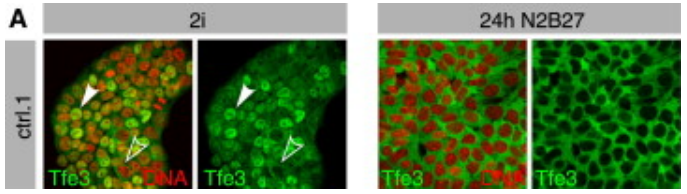


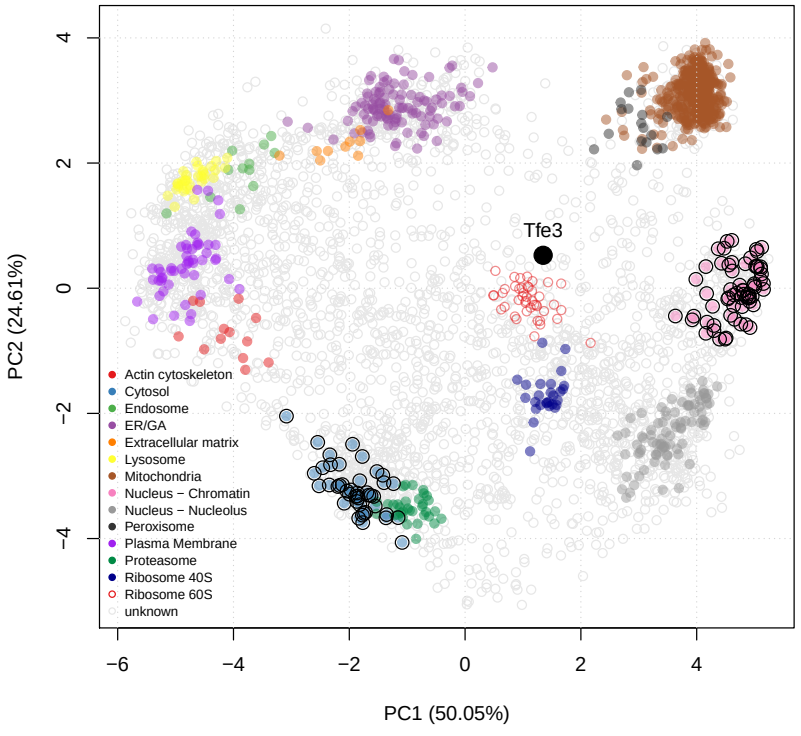
Figure : Semi-supervised approach Breckels et al. (2013). Data from Tan et al (2009).





From Betschinger *et al.* (2013)

Mouse ESC (E14TG2a) in serum LIF



Acknowledgement

- Lisa Breckels
- Sebastien Gibb
- Kathryn Lilley (CCP)


```
## R version 3.2.0 Patched (2015-04-22 r68234)
## Platform: x86_64-unknown-linux-gnu (64-bit)
## Running under: Ubuntu 14.04.2 LTS
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## loaded via a namespace (and not attached):
##  [1] Biobase_2.29.1              vsn_3.37.1
##  [3] splines_3.2.0               foreach_1.4.2
##  [5] Formula_1.2-1               affy_1.47.1
##  [7] Pbase_0.9.0                 highr_0.5
##  [9] stats4_3.2.0                latticeExtra_0.6-26
## [11] BSgenome_1.37.1             Rsamtools_1.21.8
## [13] impute_1.43.0               RSQLite_1.0.0
## [15] lattice_0.20-31             biovizBase_1.17.1
## [17] limma_3.25.9                chron_2.3-45
## [19] digest_0.6.8                GenomicRanges_1.21.15
## [21] RColorBrewer_1.1-2          XVector_0.9.1
## [23] colorspace_1.2-6            preprocessCore_1.31.0
## [25] plyr_1.8.2                  MALDIquant_1.12
## [27] XML_3.98-1.2                biomaRt_2.25.1
## [29] zlibbioc_1.15.0             scales_0.2.4
## [31] affyio_1.37.0               cleaver_1.7.0
## [33] BiocParallel_1.3.25         IRanges_2.3.11
## [35] ggplot2_1.0.1               SummarizedExperiment_0.1.5
## [37] GenomicFeatures_1.21.13     nnet_7.3-9
## [39] Gviz_1.13.2                 BiocGenerics_0.15.2
## [41] proto_0.3-10                survival_2.38-1
## [43] magrittr_1.5                evaluate_0.7
## [45] doParallel_1.0.8            MASS_7.3-40
## [47] foreign_0.8-63              mzR_2.3.1
```