Statistical Methods for Quantitative MS-based Proteomics: Part I. Preprocessing

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This is part of the online course Proteomics Data Analysis (PDA)

• Playlist PDA Preprocessing

Outline

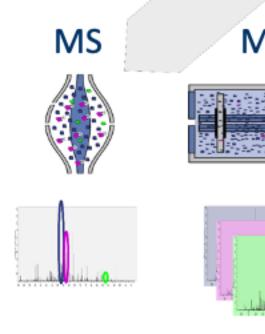
- 1. Introduction
- 2. Preprocessing
 - Log-transformation
 - Filtering
 - Normalization
 - Summarization

Note, that the R-code is included for learners who are aiming to develop R/markdown scripts to automate their quantitative proteomics data analyses. According to the target audience of the course we either work with a graphical user interface (GUI) in a R/shiny App msqrob2gui (e.g. Proteomics Bioinformatics course of the EBI and the Proteomics Data Analysis course at the Gulbenkian institute) or with R/markdowns scripts (e.g. Bioinformatics Summer School at UCLouvain or the Statistical Genomics Course at Ghent University).

1 Intro: Challenges in Label-Free Quantitative Proteomics

1.1 MS-based workflow





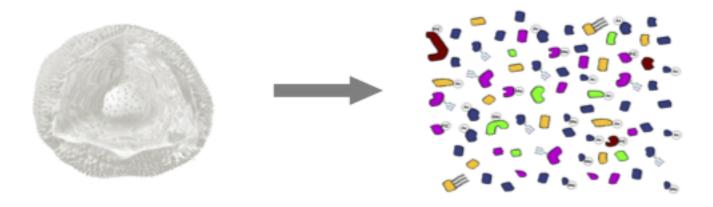
Quantification Ident

- Peptide Characteristics
 - Modifications

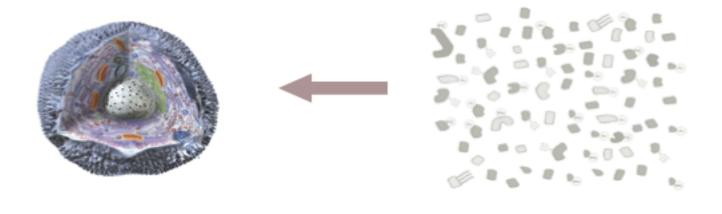
- Ionisation Efficiency: huge variability
- Identification
 - * Misidentification \rightarrow outliers
 - * MS 2 selection on peptide abundance
 - * Context depending missingness
 - * Non-random missingness
- \rightarrow Unbalanced pepide identifications across samples and messy data

1.2 Level of quantification

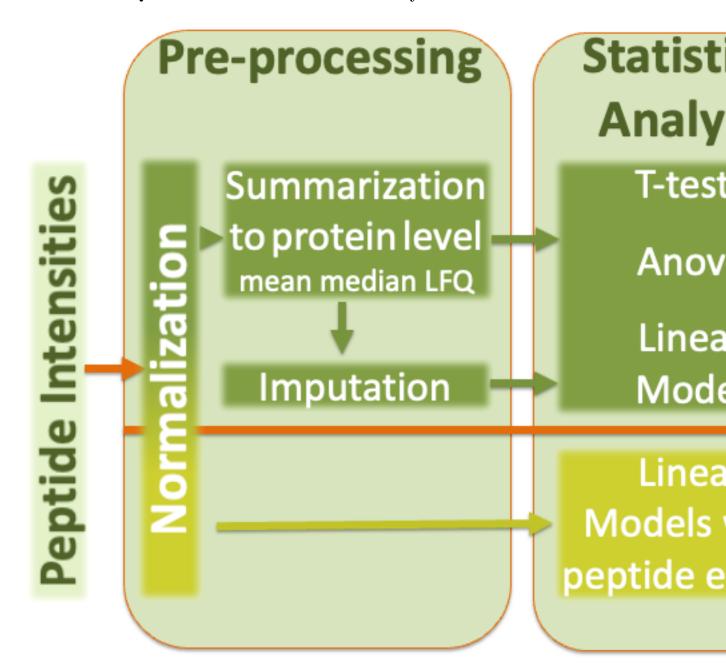
• MS-based proteomics returns peptides: pieces of proteins



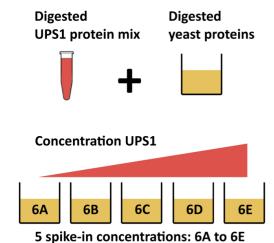
• Quantification commonly required on the protein level

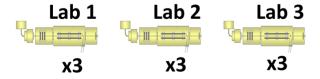


1.3 Label-free Quantitative Proteomics Data Analysis Workflows



1.4 CPTAC Spike-in Study

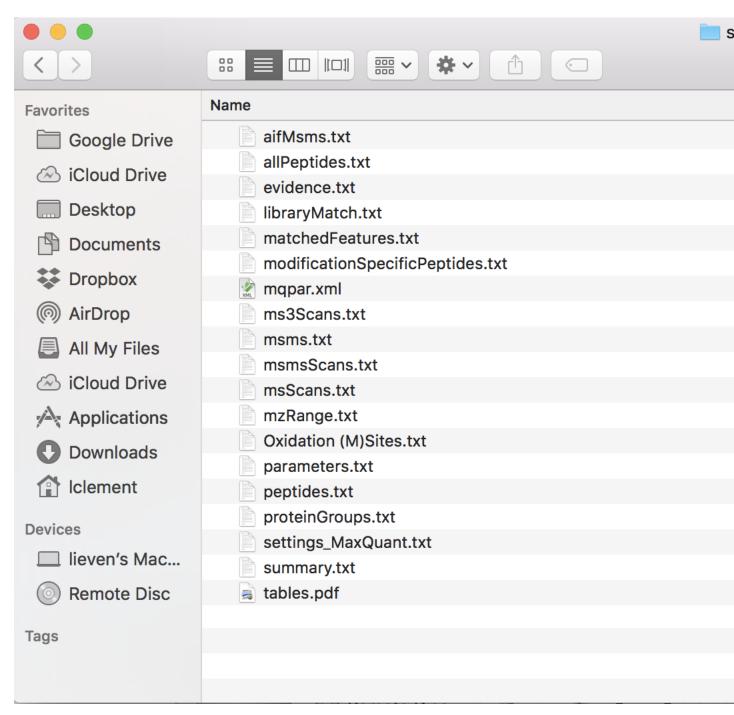




- Same trypsin-digested yeast proteome background in each sample
- Trypsin-digested Sigma UPS1 standard: 48 different human proteins spiked in at 5 different concentrations (treatment A-E)
- Samples repeatedly run on different instruments in different labs
- After MaxQuant search with match between runs option
 - -41% of all proteins are quantified in all samples
 - 6.6% of all peptides are quantified in all samples

 $[\]rightarrow$ vast amount of missingness

1.5 Maxquant output



2 Import the data in R

2.1 Data infrastructure

Click to see background on data infrastructure used in R to store proteomics data

- We use the QFeatures package that provides the infrastructure to
 - store,
 - process,
 - manipulate and
 - analyse quantitative data/features from mass spectrometry experiments.
- It is based on the SummarizedExperiment and MultiAssayExperiment classes.
- Assays in a QFeatures object have a hierarchical relation:
 - proteins are composed of peptides,
 - themselves produced by spectra
 - relations between assays are tracked and recorded throughout data processing

2.2 Import data in R

2.2.1 Load libraries

Click to see code

```
library(tidyverse)
library(limma)
library(QFeatures)
library(msqrob2)
library(plotly)
library(ggplot2)
```

2.2.2 Read data

Click to see background and code

1. We use a peptides.txt file from MS-data quantified with maxquant that contains MS1 intensities summarized at the peptide level.

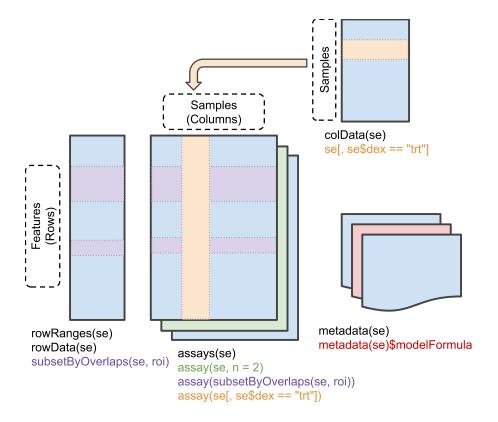
```
peptidesFile <- "https://raw.githubusercontent.com/statOmics/PDA22GTPB/data/quantification/fullCptacDat</pre>
```

2. Maxquant stores the intensity data for the different samples in columns that start with Intensity. We can retreive the column names with the intensity data with the code below:

```
ecols <- grep("Intensity\\.", names(read.delim(peptidesFile)))</pre>
```

3. Read the data and store it in QFeatures object

22/06/2021 SE.svg



file: ///Users/lclement/Dropbox/statOmics/PDA21/figures/SE.svg

1/1

Figure 1: Conceptual representation of a 'SummarizedExperiment' object. Assays contain information on the measured omics features (rows) for different samples (columns). The 'rowData' contains information on the omics features, the 'colData' contains information on the samples, i.e. experimental design etc.

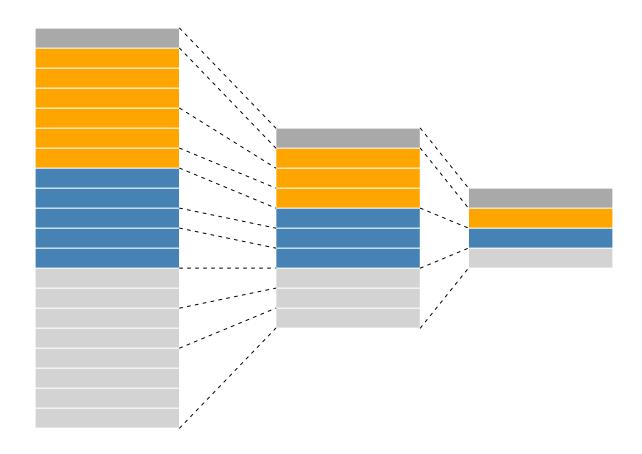


Figure 2: Conceptual representation of a ${\tt QFeatures}$ object and the aggregative relation between different assays.

```
pe <- readQFeatures(
  table = peptidesFile,
  fnames = 1,
  ecol = ecols,
  name = "peptideRaw", sep="\t")</pre>
```

2.2.3 Explore object

Click to see background and code

• The rowData contains information on the features (peptides) in the assay. E.g. Sequence, protein, ...

rowData(pe[["peptideRaw"]])

```
## DataFrame with 11466 rows and 143 columns
##
                             Sequence N.term.cleavage.window C.term.cleavage.window
                          <character>
                                                  <character>
                                                                          <character>
## AAAAGAGGAGDSGDAVTK AAAAGAGGAG...
                                                EHQHDEQKAA...
                                                                        DSGDAVTKIG...
## AAAALAGGK
                            AAAALAGGK
                                                                       AAALAGGKKS...
                                                QQLSKAAKAA...
## AAAALAGGKK
                           AAAALAGGKK
                                                                       AALAGGKKSK...
                                                QQLSKAAKAA...
## AAADALSDLEIK
                        AAADALSDLE...
                                                MPKETPSKAA...
                                                                        ALSDLEIKDS...
## AAADALSDLEIKDSK
                        AAADALSDLE...
                                                MPKETPSKAA...
                                                                        DLEIKDSKSN...
                        YYSIYDLGNN...
                                                VGDAFLRKYY...
                                                                        NNAVGLAKAI...
## YYSIYDLGNNAVGLAK
## YYTFNGPNYNENETIR
                        YYTFNGPNYN...
                                                FKDGSYPKYY...
                                                                        YNENETIRHI...
## YYTITEVATR
                           YYTITEVATR
                                                QEWDINERYY...
                                                                        TITEVATRAK...
                        YYTVFDRDNN...
                                                LGDVFIGRYY...
## YYTVFDRDNNR
                                                                        VFDRDNNRVG...
## YYTVFDRDNNRVGFAEAAR YYTVFDRDNN...
                                                LGDVFIGRYY...
                                                                        VGFAEAARL_...
##
                        Amino.acid.before First.amino.acid Second.amino.acid
                              <character>
                                                <character>
                                                                   <character>
## AAAAGAGGAGDSGDAVTK
                                                                              Α
## AAAALAGGK
                                                                              Α
## AAAALAGGKK
                                         K
## AAADALSDLEIK
                                         K
                                                                              Α
## AAADALSDLEIKDSK
                                         K
                                                                              Α
                                       . . .
## YYSIYDLGNNAVGLAK
                                         K
                                                           Y
                                                                              Y
                                                          Y
## YYTFNGPNYNENETIR
                                         K
                                                                              Y
                                                          Y
## YYTITEVATR
                                         R
                                                                              Y
## YYTVFDRDNNR
                                         R
                                                                              Y
                                                           Υ
## YYTVFDRDNNRVGFAEAAR
                                         R.
##
                        Second.last.amino.acid Last.amino.acid Amino.acid.after
##
                                   <character>
                                                    <character>
                                                                      <character>
## AAAAGAGGAGDSGDAVTK
                                              Т
                                                                                 Ι
                                                               K
## AAAALAGGK
                                              G
                                                               K
                                                                                 K
                                              K
                                                                                 S
## AAAALAGGKK
                                                               K
## AAADALSDLEIK
                                              Ι
                                                                                 D
                                                               K
## AAADALSDLEIKDSK
                                              S
                                                               K
                                                                                 S
## ...
                                            . . .
                                                             . . .
                                                                               . . .
## YYSIYDLGNNAVGLAK
                                                               K
                                                                                 Α
                                              Α
## YYTFNGPNYNENETIR
                                              Ι
                                                               R
                                                                                 Η
                                              Т
## YYTITEVATR
                                                               R.
                                                                                 Α
```

	YYTVFDRDNNR			N	R		V
	YYTVFDRDNNRVGFAEAAR		D 9	A	R	a a .	L
##		A.Count	R.Count	N.Count	D.Count	C.Count	Q.Count
##				<integer></integer>	_		
	AAAAGAGGAGDSGDAVTK	7	0	0	2	0	0
	AAAALAGGK AAAALAGGKK	5 5	0	0	0	0	0
	AAADALSDLEIK	4	0	0	0 2	0	0
	AAADALSDLEIK	4	0	0	3	0	0
##							
	YYSIYDLGNNAVGLAK	2	0	2	1	0	0
	YYTFNGPNYNENETIR	0	1	4	0	0	0
	YYTITEVATR	1	1	0	0	0	0
	YYTVFDRDNNR	0	2	2	2	0	0
	YYTVFDRDNNRVGFAEAAR	3	3	2	2	0	0
##		E.Count	G.Count	H.Count	I.Count	L.Count	K.Count
##		<integer></integer>	<integer></integer>	<integer></integer>	<integer></integer>	<integer></integer>	<integer></integer>
##	AAAAGAGGAGDSGDAVTK	0	5	0	0	0	1
##	AAAALAGGK	0	2	0	0	1	1
##	AAAALAGGKK	0	2	0	0	1	2
##	AAADALSDLEIK	1	0	0	1	2	1
##	AAADALSDLEIKDSK	1	0	0	1	2	2
##							
##	YYSIYDLGNNAVGLAK	0	2	0	1	2	1
##	YYTFNGPNYNENETIR	2	1	0	1	0	0
##	YYTITEVATR	1	0	0	1	0	0
##	YYTVFDRDNNR	0	0	0	0	0	0
##	YYTVFDRDNNRVGFAEAAR	1	1	0	0	0	0
##		M.Count	F.Count	P.Count	S.Count	T.Count	W.Count
##		<integer></integer>	<integer></integer>	<integer></integer>	<integer></integer>	<integer></integer>	<integer></integer>
##	AAAAGAGGAGDSGDAVTK	0	0	0	1	1	0
##	AAAALAGGK	0	0	0	0	0	0
	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	0	0	0	0	0	0
	AAADALSDLEIK	0	0	0	1	0	0
	AAADALSDLEIKDSK	0	0	0	2	0	0
##	YYSIYDLGNNAVGLAK						0
		_		0	1		
	YYTFNGPNYNENETIR YYTITEVATR	0	1 0	1 0	0	2	0
	YYTVFDRDNNR	0	1	0	0	1	0
	YYTVFDRDNNRVGFAEAAR	0	2	0	0	1	0
##	TTT VI DICDINITO GI ILDIIIIO	Y.Count	V.Count	U.Count	_	Missed.cle	•
##				<pre><integer></integer></pre>	_		iteger>
	AAAAGAGGAGDSGDAVTK	0	1	0	18		0
	AAAALAGGK	0	0	0	9		0
##	AAAALAGGKK	0	0	0	10		1
##	AAADALSDLEIK	0	0	0	12		0
##	AAADALSDLEIKDSK	0	0	0	15		1
##							
##	YYSIYDLGNNAVGLAK	3	1	0	16		0
##	YYTFNGPNYNENETIR	3	0	0	16		0
##	YYTITEVATR	2	1	0	10		0
##	YYTVFDRDNNR	2	1	0	11		1
##	YYTVFDRDNNRVGFAEAAR	2	2	0	19		2

```
##
                                        Proteins Leading.razor.protein
##
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                                     <character>
                                                            <character>
                                                          sp|P38915|...
## AAAAGAGGAGDSGDAVTK
                         1445.675 sp|P38915|...
## AAAALAGGK
                          728.418 sp|Q3E792|...
                                                          sp|Q3E792|...
## AAAALAGGKK
                          856.513 sp|Q3E792|...
                                                          sp|Q3E792|...
## AAADALSDLEIK
                         1215.635 sp|P09938|...
                                                          sp|P09938|...
## AAADALSDLEIKDSK
                         1545.789 sp|P09938|...
                                                          sp|P09938|...
## ...
## YYSIYDLGNNAVGLAK
                          1759.88 sp|P07267|...
                                                          sp|P07267|...
## YYTFNGPNYNENETIR
                          1993.88 sp|Q00955|...
                                                          sp|Q00955|...
## YYTITEVATR
                          1215.61 sp|P38891|...
                                                          sp|P38891|...
                          1461.66 P07339ups|...
                                                          P07339ups|...
## YYTVFDRDNNR
                          2263.08 P07339ups|...
  YYTVFDRDNNRVGFAEAAR
                                                          P07339ups|...
##
                        Start.position End.position Unique..Groups.
##
                                           <integer>
                             <integer>
                                                          <character>
## AAAAGAGGAGDSGDAVTK
                                     97
                                                 114
                                                                  yes
## AAAALAGGK
                                     13
                                                  21
                                                                  yes
## AAAALAGGKK
                                     13
                                                  22
                                                                  ves
## AAADALSDLEIK
                                     9
                                                  20
                                                                  yes
## AAADALSDLEIKDSK
                                     9
                                                  23
                                                                  yes
## ...
## YYSIYDLGNNAVGLAK
                                   388
                                                 403
                                                                  ves
## YYTFNGPNYNENETIR
                                   1275
                                                1290
                                                                  yes
## YYTITEVATR
                                   311
                                                 320
                                                                  yes
## YYTVFDRDNNR
                                   225
                                                 235
                                                                  yes
                                                                  yes
## YYTVFDRDNNRVGFAEAAR
                                   225
                                                 243
                        Unique...Proteins.
                                               Charges
                                                               PEP
                                                                       Score
                              <character> <character>
                                                        <numeric> <numeric>
## AAAAGAGGAGDSGDAVTK
                                                      2 1.1843e-05
                                                                      82.942
## AAAALAGGK
                                                      2 7.4562e-06
                                                                     134.810
                                       no
## AAAALAGGKK
                                        no
                                                      2 3.3094e-09
                                                                     143.730
## AAADALSDLEIK
                                                      2 9.1593e-23
                                                                     182.230
                                       yes
## AAADALSDLEIKDSK
                                                      3 1.5319e-04
                                                                      73.927
                                       yes
##
                                       . . .
## YYSIYDLGNNAVGLAK
                                                     2 7.7415e-37
                                                                     174.240
                                       ves
## YYTFNGPNYNENETIR
                                                     2 4.2208e-21
                                       yes
                                                                     147.750
## YYTITEVATR
                                       yes
                                                     2 1.3566e-04
                                                                     109.160
## YYTVFDRDNNR
                                                     2 6.1425e-04
                                                                     110.930
                                       yes
## YYTVFDRDNNRVGFAEAAR
                                                      3 8.9859e-04
                                       yes
                                                                      59.728
##
                        Identification.type.6A_1 Identification.type.6A_2
##
                                      <character>
                                                                <character>
## AAAAGAGGAGDSGDAVTK
                                   By matchin...
                                                                   By MS/MS
## AAAALAGGK
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                                                              By matchin...
## AAAALAGGKK
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## AAADALSDLEIK
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## AAADALSDLEIKDSK
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## YYTFNGPNYNENETIR
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## YYTITEVATR
                                         By MS/MS
                                                              By matchin...
## YYTVFDRDNNR
                                   By matchin...
                                                              By matchin...
## YYTVFDRDNNRVGFAEAAR
                                   By matchin...
                                                              By matchin...
##
                        Identification.type.6A_3 Identification.type.6A_4
##
                                      <character>
                                                                <character>
```

```
## AAAAGAGGAGDSGDAVTK
                                   By matchin...
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## AAAALAGGK
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## AAADALSDLEIK
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## AAADALSDLEIKDSK
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                                   By matchin...
                                                                  By MS/MS
## YYTITEVATR
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                                                             By matchin...
## YYTVFDRDNNR
                                   By matchin...
                                                             By matchin...
## YYTVFDRDNNRVGFAEAAR
                                   By matchin...
                                                             By matchin...
##
                       Identification.type.6A_5 Identification.type.6A_6
                                     <character>
                                                               <character>
## AAAAGAGGAGDSGDAVTK
                                   By matchin...
                                                             By matchin...
## AAAALAGGK
                                   By matchin...
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## AAAALAGGKK
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## AAADALSDLEIK
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## AAADALSDLEIKDSK
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## YYSIYDLGNNAVGLAK
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## YYTITEVATR
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## YYTVFDRDNNR
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## YYTVFDRDNNRVGFAEAAR
                                   By matchin...
                                                             By matchin...
##
                        Identification.type.6A_7 Identification.type.6A_8
                                     <character>
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## AAAAGAGGAGDSGDAVTK
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## AAAALAGGK
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## AAAALAGGKK
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## AAADALSDLEIK
                                        By MS/MS
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## AAADALSDLEIKDSK
                                        By MS/MS
                                                                  By MS/MS
## ...
                                             . . .
## YYSIYDLGNNAVGLAK
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                                                             By matchin...
## YYTFNGPNYNENETIR
                                   By matchin...
                                                             By matchin...
## YYTITEVATR
                                        By MS/MS
                                                             By matchin...
## YYTVFDRDNNR
                                   By matchin...
                                                             By matchin...
## YYTVFDRDNNRVGFAEAAR
                                   By matchin...
                                                             By matchin...
##
                        Identification.type.6A_9 Identification.type.6B_1
##
                                     <character>
                                                               <character>
## AAAAGAGGAGDSGDAVTK
                                        By MS/MS
                                                             By matchin...
## AAAALAGGK
                                        By MS/MS
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## AAAALAGGKK
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## AAADALSDLEIK
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## AAADALSDLEIKDSK
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## YYSIYDLGNNAVGLAK
                                   By matchin...
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## YYTFNGPNYNENETIR
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## YYTITEVATR
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## YYTVFDRDNNR
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## YYTVFDRDNNRVGFAEAAR
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##
                       Identification.type.6B_2 Identification.type.6B_3
                                     <character>
                                                               <character>
## AAAAGAGGAGDSGDAVTK
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## AAAALAGGK
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```
## AAAALAGGKK
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## YYTITEVATR
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## YYTVFDRDNNR
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                                                             By matchin...
## YYTVFDRDNNRVGFAEAAR
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##
                        Identification.type.6B_4 Identification.type.6B_5
                                     <character>
                                                               <character>
## AAAAGAGGAGDSGDAVTK
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## AAAALAGGK
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## AAADALSDLEIK
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## AAADALSDLEIKDSK
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## YYSIYDLGNNAVGLAK
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## YYTFNGPNYNENETIR
                                        By MS/MS
                                                                  By MS/MS
## YYTITEVATR
                                        By MS/MS
                                                                  By MS/MS
## YYTVFDRDNNR
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                                                             By matchin...
## YYTVFDRDNNRVGFAEAAR
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                                                             By matchin...
##
                        Identification.type.6B_6 Identification.type.6B_7
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## AAAALAGGK
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## AAAALAGGKK
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## AAADALSDLEIK
                                        By MS/MS
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## AAADALSDLEIKDSK
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                                             . . .
## YYSIYDLGNNAVGLAK
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## YYTFNGPNYNENETIR
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## YYTITEVATR
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## YYTVFDRDNNR
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## YYTVFDRDNNRVGFAEAAR
                                                             By matchin...
                        Identification.type.6B_8 Identification.type.6B_9
##
                                     <character>
                                                               <character>
## AAAAGAGGAGDSGDAVTK
                                                                  By MS/MS
                                        By MS/MS
## AAAALAGGK
                                        By MS/MS
                                                                  By MS/MS
## AAAALAGGKK
                                        By MS/MS
                                                                  By MS/MS
## AAADALSDLEIK
                                   By matchin...
                                                             By matchin...
## AAADALSDLEIKDSK
                                        By MS/MS
                                                                  By MS/MS
## YYSIYDLGNNAVGLAK
                                   By matchin...
                                                             By matchin...
## YYTFNGPNYNENETIR
                                   By matchin...
                                                             By matchin...
## YYTITEVATR
                                        By MS/MS
                                                             By matchin...
## YYTVFDRDNNR
                                   By matchin...
                                                             By matchin...
## YYTVFDRDNNRVGFAEAAR
                                   By matchin...
                                                             By matchin...
##
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## AAAAGAGGAGDSGDAVTK
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## AAAALAGGK
                                   By matchin...
                                                                  By MS/MS
## AAAALAGGKK
                                   By matchin...
                                                                  By MS/MS
## AAADALSDLEIK
                                        By MS/MS
                                                             By matchin...
```

```
## AAADALSDLEIKDSK
                                   By matchin...
                                                             By matchin...
## ...
## YYSIYDLGNNAVGLAK
                                   By matchin...
                                                             By matchin...
## YYTFNGPNYNENETIR
                                                             By matchin...
                                   By matchin...
## YYTITEVATR
                                   By matchin...
                                                             By matchin...
## YYTVFDRDNNR
                                   By matchin...
                                                             By matchin...
## YYTVFDRDNNRVGFAEAAR
                                   By matchin...
                                                             By matchin...
                       Identification.type.6C_3 Identification.type.6C_4
##
                                     <character>
                                                               <character>
## AAAAGAGGAGDSGDAVTK
                                   By matchin...
                                                             By matchin...
## AAAALAGGK
                                   By matchin...
                                                                  By MS/MS
## AAAALAGGKK
                                   By matchin...
                                                                  By MS/MS
## AAADALSDLEIK
                                        By MS/MS
                                                                  By MS/MS
## AAADALSDLEIKDSK
                                   By matchin...
                                                                  By MS/MS
## YYSIYDLGNNAVGLAK
                                   By matchin...
                                                                  By MS/MS
                                                                  By MS/MS
## YYTFNGPNYNENETIR
                                   By matchin...
## YYTITEVATR
                                        By MS/MS
                                                             By matchin...
## YYTVFDRDNNR
                                   By matchin...
                                                             By matchin...
## YYTVFDRDNNRVGFAEAAR
                                   By matchin...
                                                             By matchin...
##
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                                     <character>
                                                               <character>
## AAAAGAGGAGDSGDAVTK
                                        By MS/MS
                                                             By matchin...
## AAAALAGGK
                                   By matchin...
                                                             By matchin...
## AAAALAGGKK
                                   By matchin...
                                                             By matchin...
## AAADALSDLEIK
                                        By MS/MS
                                                                  By MS/MS
## AAADALSDLEIKDSK
                                        By MS/MS
                                                                  By MS/MS
                                             . . .
                                                                       . . .
## YYSIYDLGNNAVGLAK
                                        By MS/MS
                                                                  By MS/MS
## YYTFNGPNYNENETIR
                                   By matchin...
                                                             By matchin...
## YYTITEVATR
                                   By matchin...
                                                             By matchin...
## YYTVFDRDNNR
                                   By matchin...
                                                             By matchin...
## YYTVFDRDNNRVGFAEAAR
                                   By matchin...
                                                             By matchin...
                       Identification.type.6C_7 Identification.type.6C_8
                                     <character>
                                                               <character>
## AAAAGAGGAGDSGDAVTK
                                        By MS/MS
                                                             By matchin...
## AAAALAGGK
                                        By MS/MS
                                                                  By MS/MS
## AAAALAGGKK
                                        By MS/MS
                                                                  By MS/MS
## AAADALSDLEIK
                                   By matchin...
                                                                  By MS/MS
## AAADALSDLEIKDSK
                                        By MS/MS
                                                                  By MS/MS
                                                                   ...
## YYSIYDLGNNAVGLAK
                                   By matchin...
                                                             By matchin...
## YYTFNGPNYNENETIR
                                   By matchin...
                                                             By matchin...
## YYTITEVATR
                                                                  By MS/MS
                                   By matchin...
## YYTVFDRDNNR
                                   By matchin...
                                                             By matchin...
## YYTVFDRDNNRVGFAEAAR
                                   By matchin...
                                                             By matchin...
##
                        Identification.type.6C_9 Identification.type.6D_1
##
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## AAAAGAGGAGDSGDAVTK
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                                                             By matchin...
## AAAALAGGK
                                        By MS/MS
                                                             By matchin...
## AAAALAGGKK
                                        By MS/MS
                                                             By matchin...
## AAADALSDLEIK
                                        By MS/MS
                                                                  By MS/MS
## AAADALSDLEIKDSK
                                        By MS/MS
                                                                  By MS/MS
## ...
                                             . . .
                                                                       . . .
```

```
By matchin...
## YYSIYDLGNNAVGLAK
                                                             By matchin...
## YYTFNGPNYNENETIR
                                   By matchin...
                                                             By matchin...
## YYTITEVATR
                                        By MS/MS
                                                             By matchin...
## YYTVFDRDNNR
                                   By matchin...
                                                             By matchin...
## YYTVFDRDNNRVGFAEAAR
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                                                             By matchin...
                        Identification.type.6D 2 Identification.type.6D 3
                                     <character>
                                                               <character>
## AAAAGAGGAGDSGDAVTK
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## AAAALAGGK
                                   By matchin...
                                                             By matchin...
## AAAALAGGKK
                                   By matchin...
                                                             By matchin...
## AAADALSDLEIK
                                   By matchin...
                                                             By matchin...
## AAADALSDLEIKDSK
                                        By MS/MS
                                                             By matchin...
## YYSIYDLGNNAVGLAK
                                   By matchin...
                                                             By matchin...
## YYTFNGPNYNENETIR
                                   By matchin...
                                                             By matchin...
## YYTITEVATR
                                        By MS/MS
                                                                  By MS/MS
## YYTVFDRDNNR
                                   By matchin...
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## YYTVFDRDNNRVGFAEAAR
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                                                             By matchin...
                        Identification.type.6D_4 Identification.type.6D_5
##
                                     <character>
                                                               <character>
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                                                             By matchin...
## AAAALAGGK
                                   By matchin...
                                                             By matchin...
## AAAALAGGKK
                                                             By matchin...
                                        By MS/MS
## AAADALSDLEIK
                                        By MS/MS
                                                                  By MS/MS
## AAADALSDLEIKDSK
                                        By MS/MS
                                                                  By MS/MS
## YYSIYDLGNNAVGLAK
                                        By MS/MS
                                                                  By MS/MS
                                        By MS/MS
                                                                  By MS/MS
## YYTFNGPNYNENETIR
## YYTITEVATR
                                   By matchin...
                                                             By matchin...
## YYTVFDRDNNR
                                   By matchin...
                                                             By matchin...
## YYTVFDRDNNRVGFAEAAR
                                   By matchin...
                                                             By matchin...
##
                        Identification.type.6D_6 Identification.type.6D_7
##
                                     <character>
                                                               <character>
## AAAAGAGGAGDSGDAVTK
                                        By MS/MS
                                                             By matchin...
                                                                  By MS/MS
## AAAALAGGK
                                   By matchin...
## AAAALAGGKK
                                   By matchin...
                                                                  By MS/MS
## AAADALSDLEIK
                                        By MS/MS
                                                             By matchin...
## AAADALSDLEIKDSK
                                                                  By MS/MS
                                   By matchin...
## ...
                                                                        . . .
                                              . . .
## YYSIYDLGNNAVGLAK
                                        By MS/MS
                                                             By matchin...
## YYTFNGPNYNENETIR
                                        By MS/MS
                                                             By matchin...
## YYTITEVATR
                                   By matchin...
                                                             By matchin...
## YYTVFDRDNNR
                                   By matchin...
                                                                  By MS/MS
## YYTVFDRDNNRVGFAEAAR
                                                             By matchin...
                                   By matchin...
                        Identification.type.6D_8 Identification.type.6D_9
##
                                     <character>
                                                               <character>
## AAAAGAGGAGDSGDAVTK
                                   By matchin...
                                                             By matchin...
## AAAALAGGK
                                        By MS/MS
                                                                  By MS/MS
## AAAALAGGKK
                                        By MS/MS
                                                                  By MS/MS
## AAADALSDLEIK
                                        By MS/MS
                                                                  By MS/MS
## AAADALSDLEIKDSK
                                        By MS/MS
                                                                  By MS/MS
## YYSIYDLGNNAVGLAK
                                   By matchin...
                                                             By matchin...
## YYTFNGPNYNENETIR
                                   By matchin...
                                                             By matchin...
```

```
## YYTITEVATR
                                        By MS/MS
                                                             By matchin...
## YYTVFDRDNNR
                                        By MS/MS
                                                             By matchin...
## YYTVFDRDNNRVGFAEAAR
                                   By matchin...
                                                             By matchin...
                        Identification.type.6E_1 Identification.type.6E_2
                                     <character>
                                                                <character>
## AAAAGAGGAGDSGDAVTK
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## AAAALAGGK
                                   By matchin...
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## AAAALAGGKK
                                   By matchin...
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## AAADALSDLEIK
                                        By MS/MS
                                                                   By MS/MS
## AAADALSDLEIKDSK
                                        By MS/MS
                                                                   By MS/MS
                                                                       . . .
                                             . . .
## YYSIYDLGNNAVGLAK
                                   By matchin...
                                                             By matchin...
## YYTFNGPNYNENETIR
                                   By matchin...
                                                             By matchin...
## YYTITEVATR
                                   By matchin...
                                                             By matchin...
## YYTVFDRDNNR
                                   By matchin...
                                                             By matchin...
## YYTVFDRDNNRVGFAEAAR
                                   By matchin...
                                                             By matchin...
##
                        Identification.type.6E_3 Identification.type.6E_4
##
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## AAAAGAGGAGDSGDAVTK
                                   By matchin...
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## AAAALAGGK
                                   By matchin...
                                                                   By MS/MS
## AAAALAGGKK
                                   By matchin...
                                                             By matchin...
## AAADALSDLEIK
                                                                   By MS/MS
                                   By matchin...
## AAADALSDLEIKDSK
                                        By MS/MS
                                                             By matchin...
                                                                        . . .
## YYSIYDLGNNAVGLAK
                                   By matchin...
                                                                   By MS/MS
## YYTFNGPNYNENETIR
                                   By matchin...
                                                                   By MS/MS
## YYTITEVATR
                                   By matchin...
                                                             By matchin...
## YYTVFDRDNNR
                                   By matchin...
                                                                   By MS/MS
## YYTVFDRDNNRVGFAEAAR
                                   By matchin...
                                                             By matchin...
##
                        Identification.type.6E_5 Identification.type.6E_6
##
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## AAAAGAGGAGDSGDAVTK
                                   By matchin...
                                                             By matchin...
## AAAALAGGK
                                   By matchin...
                                                             By matchin...
## AAAALAGGKK
                                   By matchin...
                                                             By matchin...
## AAADALSDLEIK
                                        By MS/MS
                                                             By matchin...
## AAADALSDLEIKDSK
                                        By MS/MS
                                                                   By MS/MS
## ...
                                                                       . . .
## YYSIYDLGNNAVGLAK
                                        By MS/MS
                                                                   By MS/MS
## YYTFNGPNYNENETIR
                                        By MS/MS
                                                                   By MS/MS
## YYTITEVATR
                                   By matchin...
                                                             By matchin...
## YYTVFDRDNNR
                                                             By matchin...
                                        By MS/MS
## YYTVFDRDNNRVGFAEAAR
                                   By matchin...
                                                                   By MS/MS
                        Identification.type.6E_7 Identification.type.6E_8
##
                                     <character>
                                                                <character>
## AAAAGAGGAGDSGDAVTK
                                   By matchin...
                                                             By matchin...
## AAAALAGGK
                                        By MS/MS
                                                                   By MS/MS
## AAAALAGGKK
                                        By MS/MS
                                                                   By MS/MS
## AAADALSDLEIK
                                        By MS/MS
                                                                   By MS/MS
## AAADALSDLEIKDSK
                                   By matchin...
                                                                   By MS/MS
## YYSIYDLGNNAVGLAK
                                   By matchin...
                                                             By matchin...
## YYTFNGPNYNENETIR
                                   By matchin...
                                                             By matchin...
## YYTITEVATR
                                   By matchin...
                                                             By matchin...
## YYTVFDRDNNR
                                        By MS/MS
                                                                   By MS/MS
```

## ##	YYTVFDRDNNRVGFAEAAR	By Identification	matchin	Experi	•	atchin. Experi	
##			character>	_	<integer></integer>	_	<integer></integer>
##	AAAAGAGGAGDSGDAVTK	Ву	matchin		NA		1
##	AAAALAGGK		By MS/MS		NA		1
##	AAAALAGGKK		By MS/MS		NA		1
	AAADALSDLEIK		By MS/MS		1		1
##	AAADALSDLEIKDSK		By MS/MS		1		1
##		_					
	YYSIYDLGNNAVGLAK	Ву	matchin		NA		NA
	YYTFNGPNYNENETIR	D	By MS/MS		NA		NA
	YYTITEVATR	Вў	matchin		1 NA		NA NA
	YYTVFDRDNNR YYTVFDRDNNRVGFAEAAR	D++	By MS/MS		NA NA		NA NA
##	IIIVFDRDNNRVGFAEAAR	Experiment.6A_	matchin 3 Evperimen	n+ 6/ /		+ 61 5	IVA
##		<pre>cinteger</pre>	=	teger>	=	teger>	
	AAAAGAGGAGDSGDAVTK	•	A	1	\ <u>11</u>	1	
	AAAALAGGK		2	1		1	
	AAAALAGGKK	N	Ā	1		NA	
##	AAADALSDLEIK		1	1		1	
##	AAADALSDLEIKDSK	N	Α	1		1	
##			•				
##	YYSIYDLGNNAVGLAK	N	A	1		1	
##	YYTFNGPNYNENETIR	N	Α	1		1	
##	YYTITEVATR		1	NA		NA	
	YYTVFDRDNNR	N		NA		NA	
	YYTVFDRDNNRVGFAEAAR	N		NA	_	NA	
##		Experiment.6A_	=		=		
##		<integer< th=""><th></th><th>teger></th><th><1r</th><th>teger></th><th></th></integer<>		teger>	<1r	teger>	
	AAAAGAGGAGDSGDAVTK		1	1 2		1	
	AAAALAGGK AAAALAGGKK		1 1	1		1 1	
	AAADALSDLEIK		1	1		1	
	AAADALSDLEIK		1	1		1	
##							
##	YYSIYDLGNNAVGLAK		1	NA		NA	
##	YYTFNGPNYNENETIR		1	1		NA	
##	YYTITEVATR		1	1		NA	
##	YYTVFDRDNNR	N	A	NA		NA	
##	${\tt YYTVFDRDNNRVGFAEAAR}$	N	Α	NA		NA	
##		Experiment.6A_	_		_		
##		<integer< th=""><th>> <ir< th=""><th>iteger></th><th><ir< th=""><th>teger></th><th></th></ir<></th></ir<></th></integer<>	> <ir< th=""><th>iteger></th><th><ir< th=""><th>teger></th><th></th></ir<></th></ir<>	iteger>	<ir< th=""><th>teger></th><th></th></ir<>	teger>	
	AAAAGAGGAGDSGDAVTK		1	NA		NA	
	AAAALAGGK		1	1		1	
	AAAALAGGKK AAADALSDLEIK		1	NA 1		1	
	AAADALSDLEIK		1	1 NA		1 1	
	ANADALDILEINDON		1			1	
	YYSIYDLGNNAVGLAK	 N	Α	NA		NA	
	YYTFNGPNYNENETIR		1	NA NA		NA	
	YYTITEVATR	N		1		1	
	YYTVFDRDNNR	N		NA		NA	
	YYTVFDRDNNRVGFAEAAR	N		NA		NA	
##		Experiment.6B_	3 Experimer	nt.6B_4	Experimen	nt.6B_5	

##		<integer></integer>	<integer></integer>	<integer></integer>
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##	AAAALAGGK	1	2	1
##	AAAALAGGKK	1	1	NA
##	AAADALSDLEIK	1	1	1
##	AAADALSDLEIKDSK	NA	1	1
##	• • •			
##	YYSIYDLGNNAVGLAK	NA	1	1
##	YYTFNGPNYNENETIR	NA	1	1
##	YYTITEVATR	1	1	1
##	YYTVFDRDNNR	NA	NA	NA
##	YYTVFDRDNNRVGFAEAAR	NA	NA	NA
##		${\tt Experiment.6B_6}$	Experiment.6B_7	Experiment.6B_8
##		<integer></integer>	<integer></integer>	<integer></integer>
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##	AAAALAGGK	NA	2	1
##	AAAALAGGKK	NA	1	1
##	AAADALSDLEIK	1	1	1
##	AAADALSDLEIKDSK	1	1	1
##	• • •	• • •		
##	YYSIYDLGNNAVGLAK	1	NA	NA
##	YYTFNGPNYNENETIR	1	1	NA
##	YYTITEVATR	1	NA	1
##	YYTVFDRDNNR	NA	NA	NA
##	YYTVFDRDNNRVGFAEAAR	NA	NA	NA
##		-	Experiment.6C_1	-
##		<integer></integer>	<integer></integer>	<integer></integer>
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##	AAAALAGGK	2	NA	1
	AAAALAGGKK	1	NA	1
##	AAADALSDLEIK	1	1	1
##	AAADALSDLEIKDSK	1	1	1
##				
##	YYSIYDLGNNAVGLAK	NA	NA	NA
	YYTFNGPNYNENETIR	NA	NA	NA
	YYTITEVATR	NA	1	1
	YYTVFDRDNNR YYTVFDRDNNRVGFAEAAR	NA NA	NA NA	NA NA
##	IIIVFDRDNNRVGFALAAR	NA Evenomiment 6C 3	NA Evenomiment 6C 4	NA Evanciment 6C F
##		<pre>cxperiment.oc_3 <integer></integer></pre>	<pre><integer></integer></pre>	<pre>Experiment.6C_5</pre>
	AAAAGAGGAGDSGDAVTK	NA	\Integer>	1
	AAAALAGGK	2	2	NA
	AAAALAGGKK	NA	1	NA
	AAADALSDLEIK	1	1	1
	AAADALSDLEIKDSK	1	1	1
##				
	YYSIYDLGNNAVGLAK	NA	1	1
	YYTFNGPNYNENETIR	NA	1	1
	YYTITEVATR	1	1	NA
	YYTVFDRDNNR	NA	NA	NA
	YYTVFDRDNNRVGFAEAAR	NA	NA	NA
##				Experiment.6C_8
##		<pre>- <integer></integer></pre>	<pre>- <integer></integer></pre>	<pre>- <integer></integer></pre>
##	AAAAGAGGAGDSGDAVTK	1	1	1

	AAAALAGGK	NA	2	1
	AAAALAGGKK	NA	1	1
	AAADALSDLEIK	1	1	1
	AAADALSDLEIKDSK	1	1	1
##	• • •	• • •	• • •	• • •
##	YYSIYDLGNNAVGLAK	1	NA	NA
##	YYTFNGPNYNENETIR	1	1	1
##	YYTITEVATR	1	NA	1
	YYTVFDRDNNR	1	NA	1
##	YYTVFDRDNNRVGFAEAAR	NA	NA	NA
##		-	Experiment.6D_1	Experiment.6D_2
##		<integer></integer>	<integer></integer>	<integer></integer>
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##	AAAALAGGK	1	NA	1
##	AAAALAGGKK	1	NA	NA
##	AAADALSDLEIK	1	1	1
##	AAADALSDLEIKDSK	1	1	1
##	• • •			
##	YYSIYDLGNNAVGLAK	NA	NA	NA
##	YYTFNGPNYNENETIR	1	NA	NA
##	YYTITEVATR	1	NA	1
##	YYTVFDRDNNR	NA	NA	NA
##	YYTVFDRDNNRVGFAEAAR	NA	NA	NA
##		Experiment.6D_3	Experiment.6D_4	Experiment.6D_5
##		<integer></integer>	<integer></integer>	<integer></integer>
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##	AAAALAGGK	1	1	1
##	AAAALAGGKK	NA	1	NA
##	AAADALSDLEIK	1	1	1
##	AAADALSDLEIKDSK	1	1	1
##				
##	YYSIYDLGNNAVGLAK	NA	1	1
##	YYTFNGPNYNENETIR	NA	1	1
##	YYTITEVATR	1	1	1
##	YYTVFDRDNNR	NA	1	1
##	YYTVFDRDNNRVGFAEAAR	NA	1	NA
##		Experiment.6D_6	Experiment.6D_7	Experiment.6D_8
##		<integer></integer>	<integer></integer>	<integer></integer>
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##	AAAALAGGK	NA	2	1
##	AAAALAGGKK	NA	1	1
##	AAADALSDLEIK	1	1	1
##	AAADALSDLEIKDSK	1	1	1
##	• • •			
##	YYSIYDLGNNAVGLAK	1	1	NA
	YYTFNGPNYNENETIR	1	1	1
	YYTITEVATR	1	NA	1
##	YYTVFDRDNNR	1	1	1
##	YYTVFDRDNNRVGFAEAAR	NA	NA	NA
##			Experiment.6E_1	
##		<pre><integer></integer></pre>	<pre><integer></integer></pre>	<pre><integer></integer></pre>
	AAAAGAGGAGDSGDAVTK	NA	NA	1
	AAAALAGGK	2	NA	1
	AAAALAGGKK	1	NA	NA.
		-	NA	IVA

```
## AAADALSDLEIK
## AAADALSDLEIKDSK
                                   1
                                                   1
                                                                   1
                                  . . .
                                                 . . .
                                                                 . . .
## YYSIYDLGNNAVGLAK
                                 NA
                                                  NA
                                                                  NΑ
## YYTFNGPNYNENETIR
                                  1
                                                  NA
## YYTITEVATR
                                  NA
                                                  NA
                                                                  1
## YYTVFDRDNNR
                                  1
## YYTVFDRDNNRVGFAEAAR
                                  NA
                                                  NA
##
                      Experiment.6E_3 Experiment.6E_4 Experiment.6E_5
##
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                                                           <integer>
## AAAAGAGGAGDSGDAVTK
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                                                  NA
## AAAALAGGK
                                   2
                                                   2
                                                                   1
## AAAALAGGKK
                                  NA
                                                                  NA
## AAADALSDLEIK
                                   1
## AAADALSDLEIKDSK
                                   1
                                                   1
                                                                   1
## ...
                                 . . .
                                                 . . .
## YYSIYDLGNNAVGLAK
                                  1
                                                   1
                                                                   1
## YYTFNGPNYNENETIR
                                  NA
## YYTITEVATR
                                  1
## YYTVFDRDNNR
                                   1
## YYTVFDRDNNRVGFAEAAR
                                  NA
                                                   1
                      Experiment.6E_6 Experiment.6E_7 Experiment.6E_8
##
                                           <integer>
                            <integer>
                                                           <integer>
## AAAAGAGGAGDSGDAVTK
                                   1
                                                  NA
## AAAALAGGK
                                                                   2
                                  NΑ
## AAAALAGGKK
                                  NA
## AAADALSDLEIK
                                   1
                                                   1
## AAADALSDLEIKDSK
                                   1
                                                  NA
## YYSIYDLGNNAVGLAK
                                  1
                                                  NA
                                                                  NA
## YYTFNGPNYNENETIR
                                  1
                                                  1
                                                                  1
## YYTITEVATR
                                  NA
                                                  NA
                                1
## YYTVFDRDNNR
                                                  1
                                                                  1
## YYTVFDRDNNRVGFAEAAR
                                   1
                                                   1
                                                                  1
                      Experiment.6E 9 Intensity
                                                 Reverse Potential.contaminant
##
                            ## AAAAGAGGAGDSGDAVTK
                                  NA
                                       1190800
## AAAALAGGK
                                   1 280990000
## AAAALAGGKK
                                   1 33360000
## AAADALSDLEIK
                                   1 54622000
## AAADALSDLEIKDSK
                                  1 18910000
## ...
## YYSIYDLGNNAVGLAK
                                 NA
                                       2145900
## YYTFNGPNYNENETIR
                                  1
                                       5608800
## YYTITEVATR
                                  NA 13034000
## YYTVFDRDNNR
                                       8702500
                                   1
## YYTVFDRDNNRVGFAEAAR
                                        2391100
##
                             id Protein.group.IDs Mod..peptide.IDs Evidence.IDs
                      <integer>
                                     <character>
                                                      <character> <character>
## AAAAGAGGAGDSGDAVTK
                              0
                                                                0 0;1;2;3;4;...
                                             859
## AAAALAGGK
                             1
                                             230
                                                                1 24;25;26;2...
                             2
                                             230
## AAAALAGGKK
                                                               2 74;75;76;7...
## AAADALSDLEIK
                              3
                                             229
                                                               3 99;100;101...
## AAADALSDLEIKDSK
                             4
                                             229
                                                              4 144;145;14...
```

```
## ...
                               . . .
                                                  . . .
                                                                    . . .
                                                 196
## YYSIYDLGNNAVGLAK
                            11461
                                                                  12240 331367;331...
## YYTFNGPNYNENETIR
                            11462
                                                 1254
                                                                  12241 331384;331...
## YYTITEVATR
                                                 854
                                                                  12242 331411;331...
                            11463
## YYTVFDRDNNR
                            11464
                                                   34
                                                                  12243 331439;331...
## YYTVFDRDNNRVGFAEAAR
                                                                  12244 331455;331...
                            11465
                                                   34
                            MS.MS.IDs Best.MS.MS Oxidation..M..site.IDs MS.MS.Count
##
                          <character>
                                        <integer>
                                                               <character>
                                                                             <integer>
## AAAAGAGGAGDSGDAVTK 0;1;2;3;4;...
                                                                                     10
                                                 0
## AAAALAGGK
                        10;11;12;1...
                                                21
                                                                                     18
## AAAALAGGKK
                        30;31;32;3...
                                                31
                                                                                     21
                                               72
                                                                                     29
## AAADALSDLEIK
                        51;52;53;5...
## AAADALSDLEIKDSK
                        85;86;87;8...
                                               94
                                                                                     32
## YYSIYDLGNNAVGLAK
                        169138;169...
                                           169147
                                                                                     13
## YYTFNGPNYNENETIR
                        169151;169...
                                           169159
                                                                                     14
                                                                                     12
## YYTITEVATR
                        169165;169...
                                           169173
## YYTVFDRDNNR
                        169177;169...
                                           169180
                                                                                      7
## YYTVFDRDNNRVGFAEAAR
                                           169184
                                                                                      1
                                169184
```

• The colData contains information on the samples

colData(pe)

- ## DataFrame with 45 rows and 0 columns
 - No information is stored yet on the design.

```
pe %>% colnames
```

```
## CharacterList of length 1
## [["peptideRaw"]] Intensity.6A_1 Intensity.6A_2 ... Intensity.6E_9
```

- Note, that the sample names include the spike-in condition.
- They also end on a number.
 - -1-3 is from lab 1,
 - 4-6 from lab 2 and
 - 7-9 from lab 3.
- We update the colData with information on the design

```
colData(pe)$lab <- rep(rep(paste0("lab",1:3),each=3),5) %>% as.factor
colData(pe)$condition <- pe[["peptideRaw"]] %>% colnames %>% substr(12,12) %>% as.factor
colData(pe)$spikeConcentration <- rep(c(A = 0.25, B = 0.74, C = 2.22, D = 6.67, E = 20),each = 9)</pre>
```

• We explore the colData again

colData(pe)

```
## DataFrame with 45 rows and 3 columns
##
                       lab condition spikeConcentration
##
                  <factor> <factor>
                                              <numeric>
                                                    0.25
## Intensity.6A_1
                      lab1
## Intensity.6A_2
                      lab1
                                   Α
                                                    0.25
## Intensity.6A_3
                      lab1
                                   Α
                                                    0.25
## Intensity.6A_4
                      lab2
                                                    0.25
                                   Α
## Intensity.6A_5
                      lab2
                                                    0.25
                                   Α
                      . . .
                                 . . .
                                                     . . .
## Intensity.6E_5
                      lab2
                                   Ε
                                                      20
## Intensity.6E_6
                      lab2
                                   Ε
                                                      20
## Intensity.6E_7
                      lab3
                                   Ε
                                                      20
## Intensity.6E_8
                      lab3
                                   Ε
                                                      20
                                   Ε
## Intensity.6E_9
                      lab3
                                                      20
```

3 Preprocessing

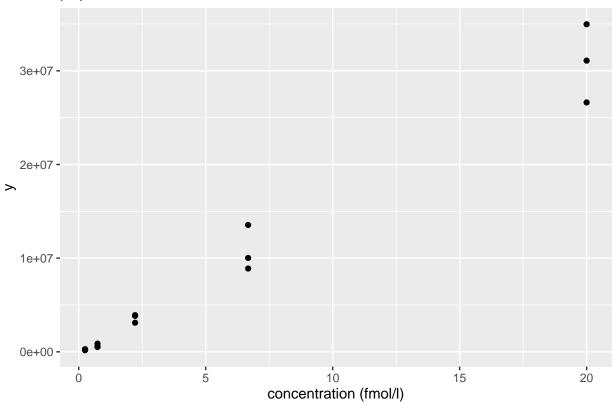
3.1 Log-transformation

3.1.1 Explore the data with plots

Peptide AALEELVK from spiked-in UPS protein P12081. We only show data from lab1.

Click to see code to make plot

peptide AALEELVK in lab1

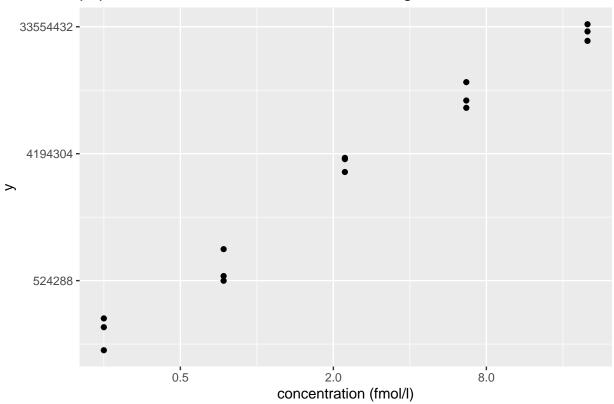


• Variance increases with the mean \rightarrow Multiplicative error structure

Click to see code to make plot

plotLog

peptide AALEELVK in lab1 with axes on log scale



- Data seems to be homoscedastic on log-scale \rightarrow log transformation of the intensity data
- In quantitative proteomics analysis on log₂
- \rightarrow Differences on a \log_2 scale: \log_2 fold changes

$$\begin{split} \log_2 B - \log_2 A &= \log_2 \frac{B}{A} = \log F C_{\text{B - A}} \\ log_2 F C &= 1 \rightarrow F C = 2^1 = 2 \\ log_2 F C &= 2 \rightarrow F C = 2^2 = 4 \end{split}$$

3.1.2 log-transformation of the data

Click to see code to log-transfrom the data

• We calculate how many non zero intensities we have for each peptide and this can be useful for filtering.

Peptides with zero intensities are missing peptides and should be represent with a NA value rather than
 0.

```
pe <- zeroIsNA(pe, "peptideRaw") # convert 0 to NA</pre>
```

• Logtransform data with base 2

```
pe <- logTransform(pe, base = 2, i = "peptideRaw", name = "peptideLog")</pre>
```

3.2 Filtering

- Reverse sequences
- Only identified by modification site (only modified peptides detected)
- Razor peptides: non-unique peptides assigned to the protein group with the most other peptides
- Contaminants
- Peptides few identifications
- Proteins that are only identified with one or a few peptides

Filtering does not induce bias if the criterion is independent from the downstream data analysis! Click to see code to filter the data

1. Handling overlapping protein groups

In our approach a peptide can map to multiple proteins, as long as there is none of these proteins present in a smaller subgroup.

```
pe <- filterFeatures(pe, ~ Proteins %in% smallestUniqueGroups(rowData(pe[["peptideLog"]])$Proteins))</pre>
```

2. Remove reverse sequences (decoys) and contaminants

We now remove the contaminants, peptides that map to decoy sequences, and proteins which were only identified by peptides with modifications.

```
pe <- filterFeatures(pe,~Reverse != "+")
pe <- filterFeatures(pe,~ Potential.contaminant != "+")</pre>
```

3. Drop peptides that were only identified in one sample

We keep peptides that were observed at last twice.

```
pe <- filterFeatures(pe,~ nNonZero >=2)
nrow(pe[["peptideLog"]])
```

[1] 10478

We keep 10478 peptides upon filtering.

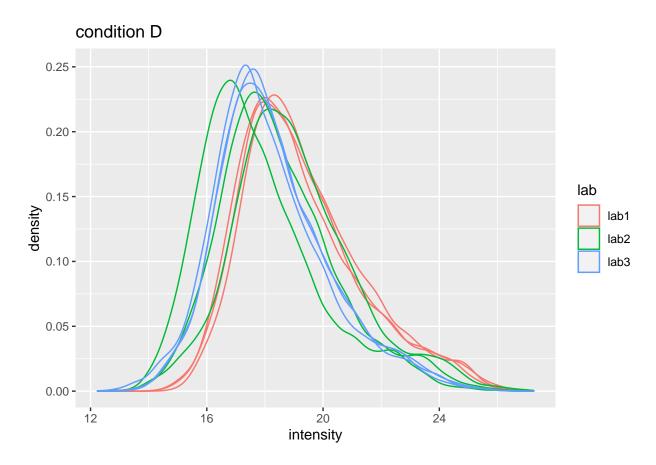
3.3 Normalization

Click to see code to make plot

```
densityConditionD <- pe[["peptideLog"]][,colData(pe)$condition=="D"] %>%
  assay %>%
  as.data.frame() %>%
  gather(sample, intensity) %>%
  mutate(lab = colData(pe)[sample, "lab"]) %>%
  ggplot(aes(x=intensity,group=sample,color=lab)) +
    geom_density() +
    ggtitle("condition D")
densityLab2 <- pe[["peptideLog"]][,colData(pe)$lab=="lab2"] %>%
  assay %>%
  as.data.frame() %>%
  gather(sample, intensity) %>%
  mutate(condition = colData(pe)[sample, "condition"]) %>%
  ggplot(aes(x=intensity,group=sample,color=condition)) +
    geom_density() +
    ggtitle("lab2")
```

 ${\tt densityConditionD}$

Warning: Removed 39179 rows containing non-finite values (stat_density).



Warning: Removed 44480 rows containing non-finite values (stat_density).

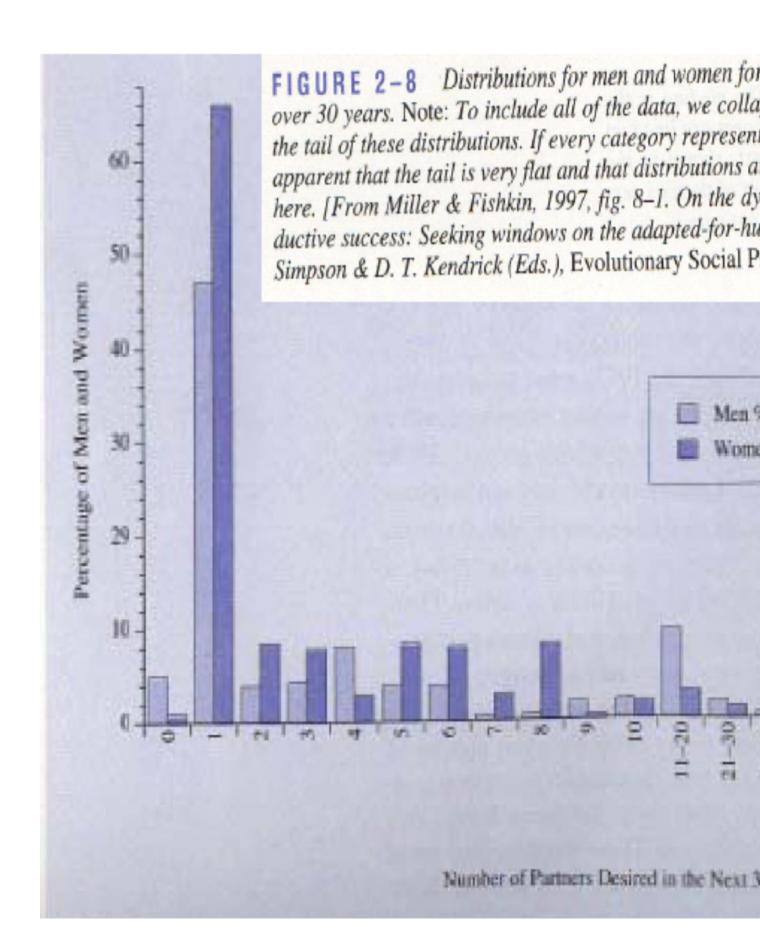


- Even in very clean synthetic dataset (same background, only 48 UPS proteins can be different) the marginal peptide intensity distribution across samples can be quite distinct
 - Considerable effects between and within labs for replicate samples
 - Considerable effects between samples with different spike-in concentration
- \rightarrow Normalization is needed

3.3.1 Mean or median?

- Miller and Fishkin (1997) reported that over a period of 30 years males would like to have on average 64.3 partners and females 2.8.
- Miller and Fishkin (1997) reported that the median number of partners someone would like to have over a period of 30 years males is 1 for both males and females.

Mean is very sensitive to outliers!



3.3.2 Normalization of the data by median centering

$$y_{ip}^{\text{norm}} = y_{ip} - \hat{\mu}_i$$

with $\hat{\mu}_i$ the median intensity over all observed peptides in sample i.

Click to see R-code to normalize the data

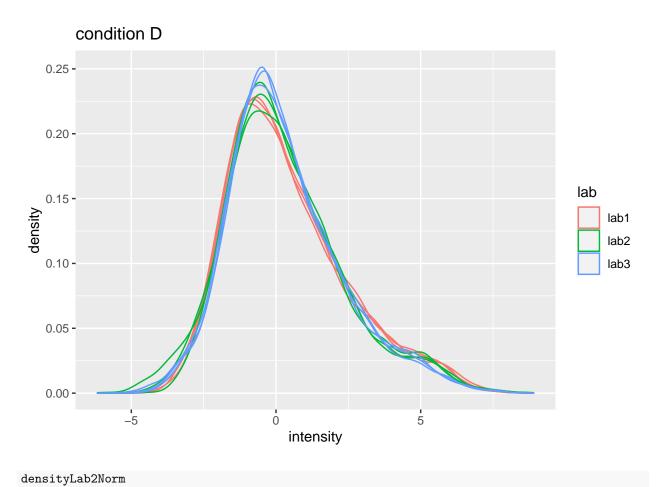
3.3.3 Plots of normalized data

Click to see code to make plot

```
densityConditionDNorm <- pe[["peptideNorm"]][,colData(pe)$condition=="D"] %>%
  assay %>%
  as.data.frame() %>%
  gather(sample, intensity) %>%
  mutate(lab = colData(pe)[sample,"lab"]) %>%
  ggplot(aes(x=intensity,group=sample,color=lab)) +
   geom_density() +
   ggtitle("condition D")
densityLab2Norm <- pe[["peptideNorm"]][,colData(pe)$lab=="lab2"] %>%
  assay %>%
  as.data.frame() %>%
  gather(sample, intensity) %>%
  mutate(condition = colData(pe)[sample, "condition"]) %>%
  ggplot(aes(x=intensity,group=sample,color=condition)) +
   geom_density() +
   ggtitle("lab2")
```

 ${\tt densityConditionDNorm}$

Warning: Removed 39179 rows containing non-finite values (stat_density).



Warning: Removed 44480 rows containing non-finite values (stat_density).



- Upon normalization the marginal distributions of the peptide intensities across samples are much more comparable
- We still see deviations
- This can be due to technical variability
- In micro-array literature, quantile normalisation is used to force the median and all other quantiles to be equal across samples
- In proteomics quantile normalisation often introduces artifacts due to a difference in missing peptides across samples
- More advanced methods should be developed for normalizing proteomics data
- If there are differences in the width of the marginal distributions of the data across samples. They can also be standardized by using a robust estimator for location and scale, i.e.

$$y_{ip}^{\rm norm} = \frac{y_{ip} - \mu_i}{s_i}$$

3.4 Summarization

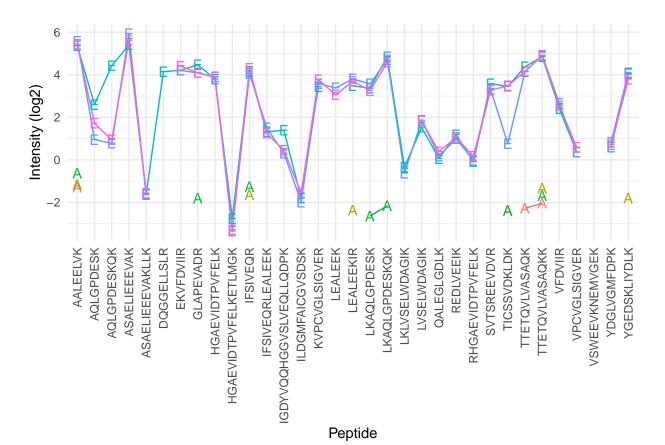
• We illustrate summarization issues using a subset of the cptac study (Lab 2, condition A and E) for a spiked protein (UPS P12081).

Click to see code to make plot

```
summaryPlot <- pe[["peptideNorm"]][
    rowData(pe[["peptideNorm"]])$Proteins == "P12081ups|SYHC_HUMAN_UPS",
    colData(pe)$lab=="lab2"&colData(pe)$condition %in% c("A","E")] %>%
    assay %>%
    as.data.frame %>%
    rownames_to_column(var = "peptide") %>%
    gather(sample, intensity, -peptide) %>%
    mutate(condition = colData(pe)[sample,"condition"]) %>%
    ggplot(aes(x = peptide, y = intensity, color = sample, group = sample, label = condition), show.legengeom_line(show.legend = FALSE) +
    geom_text(show.legend = FALSE) +
    theme_minimal() +
    theme(axis.text.x = element_text(angle = 90, vjust = 0.5, hjust = 1)) +
    xlab("Peptide") +
    ylab("Intensity (log2)")
```

summaryPlot

- ## Warning: Removed 10 row(s) containing missing values (geom_path).
- ## Warning: Removed 90 rows containing missing values (geom_text).



We observe:

• intensities from multiple peptides for each protein in a sample

- Strong peptide effect -Unbalanced peptide identification
- Pseudo-replication: peptide intensities from a particular protein in the same sample are correlated, i.e. they more alike than peptide intensities from a particular protein between samples.
- \rightarrow Summarize all peptide intensities from the same protein in a sample into a single protein expression value Commonly used methods are
 - Mean summarization

$$y_{ip} = \beta_i^{\text{samp}} + \epsilon_{ip}$$

- Median summarization
- Maxquant's maxLFQ summarization (in protein groups file)
- Model based summarization:

$$y_{ip} = \beta_i^{\text{samp}} + \beta_p^{\text{pep}} + \epsilon_{ip}$$

Click to see R-code to normalize the data

We use the standard sumarization in aggregateFeatures, which is robust model based summarization.

```
pe <- aggregateFeatures(pe,
   i = "peptideNorm",
   fcol = "Proteins",
   na.rm = TRUE,
   name = "protein")</pre>
```

Your quantitative and row data contain missing values. Please read the ## relevant section(s) in the aggregateFeatures manual page regarding the ## effects of missing values on data aggregation.

Other summarization methods can be implemented by using the fun argument in the aggregateFeatures function.

- fun = MsCoreUtils::medianPolish() to fits an additive model (two way decomposition) using Tukey's median polish procedure using stats::medpolish()
- fun = MsCoreUtils::robustSummary() to calculate a robust aggregation using MASS::rlm() (default)
- fun = base::colMeans() to use the mean of each column
- fun = matrixStats::colMedians() to use the median of each column
- fun = base::colSums() to use the sum of each column

4 Exercise

- 1. We will evaluate different summarization methods (Maxquant maxLFQ, median and robust model based) in the tutorial session before discussing on their advantages/disadvantages.
- 2. Can you anticipate on potential problems related to the summarization?

5 Software & code

- Our R/Bioconductor package msqrob2 can be used in R markdown scripts or with a GUI/shinyApp in the msqrob2gui package.
- The GUI is intended as a introduction to the key concepts of proteomics data analysis for users who have no experience in R.
- However, learning how to code data analyses in R markdown scripts is key for open en reproducible science and for reporting your proteomics data analyses and interpretation in a reproducible way.
- More information on our tools can be found in our papers (L. J. Goeminne, Gevaert, and Clement 2016), (L. J. E. Goeminne et al. 2020) and (Sticker et al. 2020). Please refer to our work when using our tools.

5.1 Code

- 1. Data infrastructure
- 2. Import proteomics data
- 3. Preprocessing
 - Log-transformation
 - Filtering
 - Normalisation
 - Summarization

5.2 Data analysis with the GUI/shinyApp msqrob2gui

References

- Goeminne, L. J. E., A. Sticker, L. Martens, K. Gevaert, and L. Clement. 2020. "MSqRob Takes the Missing Hurdle: Uniting Intensity- and Count-Based Proteomics." *Anal Chem* 92 (9): 6278–87.
- Goeminne, L. J., K. Gevaert, and L. Clement. 2016. "Peptide-level Robust Ridge Regression Improves Estimation, Sensitivity, and Specificity in Data-dependent Quantitative Label-free Shotgun Proteomics." *Mol Cell Proteomics* 15 (2): 657–68.
- Sticker, A., L. Goeminne, L. Martens, and L. Clement. 2020. "Robust Summarization and Inference in Proteome-wide Label-free Quantification." *Mol Cell Proteomics* 19 (7): 1209–19.