

SWATH2stats example script

Example R code showing the usage of the SWATH2stats package. The data processed is the publicly available dataset of *S.pyogenes* (Röst et al. 2014) (<http://www.peptideatlas.org/PASS/PASS00289>). The results file 'rawOpenSwathResults_1pcent_only.tsv' can be found on PeptideAtlas (<ftp://PASS00289@ftp.peptideatlas.org/./Spyogenes/results/>). This is a R Markdown file, showing the result of processing this data. The lines shaded in grey represent the R code executed during this analysis.

The stable release package SWATH2stats can be directly installed from Bioconductor using the commands below. This file here was generated using the current development release SWATH2stats v.1.1.14 that can be downloaded from <http://bioconductor.org/packages/devel/bioc/html/SWATH2stats.html>.

```
## try http:// if https:// URLs are not supported
source('https://bioconductor.org/biocLite.R')
biocLite('SWATH2stats')
```

Part 1: Loading and annotation

Load the SWATH-MS example data from the package, this is a reduced file in order to limit the file size of the package.

```
library(SWATH2stats)
library(data.table)
data('Spyogenes', package = 'SWATH2stats')
```

Alternatively the original file downloaded from the Peptide Atlas can be loaded from the working directory.

```
data <- data.frame(fread('rawOpenSwathResults_1pcent_only.tsv', sep='\t', header=TRUE))
```

Extract the study design information from the file names. Alternatively, the study design table can be provided as an external table.

```
Study_design <- data.frame(FileName = unique(data$align_origfilename))
Study_design$FileName <- gsub(".*strep_align/(.*)_all_peakgroups.*", "\\1",
  Study_design$FileName)
Study_design$Condition <- gsub("(Strep.*)_Repl.*", "\\1", Study_design$FileName)
Study_design$BioReplicate <- gsub(".*Repl([[[:digit:]]).*", "\\1", Study_design$FileName)
Study_design$Run <- seq(1:nrow(Study_design))
head(Study_design)
```

```
##
##      Filename Condition BioReplicate Run
## 1 Strep0_Repl1_R02/split_hroest_K120808 Strep0      1  1
## 2 Strep0_Repl2_R02/split_hroest_K120808 Strep0      2  2
## 3 Strep10_Repl1_R02/split_hroest_K120808 Strep10     1  3
## 4 Strep10_Repl2_R02/split_hroest_K120808 Strep10     2  4
```

The SWATH-MS data is annotated using the study design table.

```
data.annotated <- sample_annotation(data, Study_design, column.file = "align_origfilename")
```

Remove the decoy peptides for a subsequent inspection of the data.

```
data.annotated.nodecoy <- subset(data.annotated, decoy==FALSE)
```

Part 2: Analyze correlation, variation and signal

Count the different analytes for the different injections.

```
count_analytes(data.annotated.nodecoy)
```

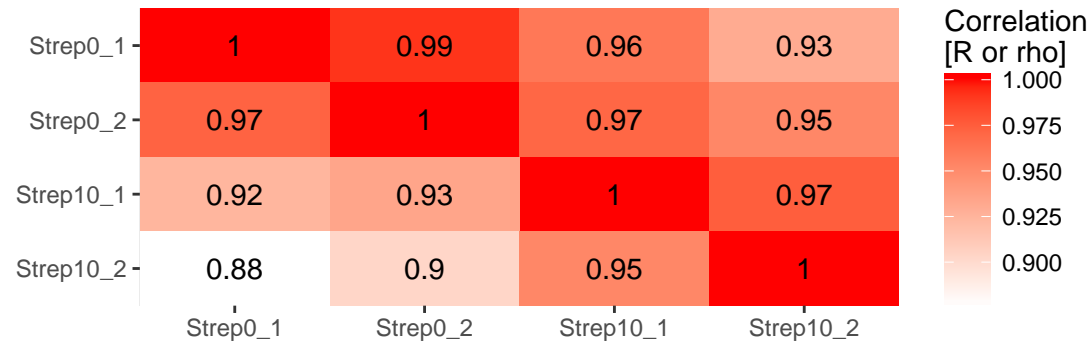
```
##      run_id transition_group_id FullPeptideName ProteinName
## 1 Strep0_1_1           10229           8377      1031
## 2 Strep0_2_2           9716           7970      1003
## 3 Strep10_1_3          8692           7138      943
## 4 Strep10_2_4          8424           6941      910
```

Plot the correlation of the signal intensity.

```
correlation <- plot_correlation_between_samples(data.annotated.nodecoy, column.values = 'Intensity')
```

```
## Warning: Ignoring unknown aesthetics: fill
```

Intensity correlation between samples:
Pearson (upper triangle) and Spearman correlation (lower triangle)



Plot the correlation of the delta_rt, which is the deviation of the retention time from the expected retention time.

```
correlation <- plot_correlation_between_samples(data.annotated.nodecoy, column.values = 'delta_rt')
```

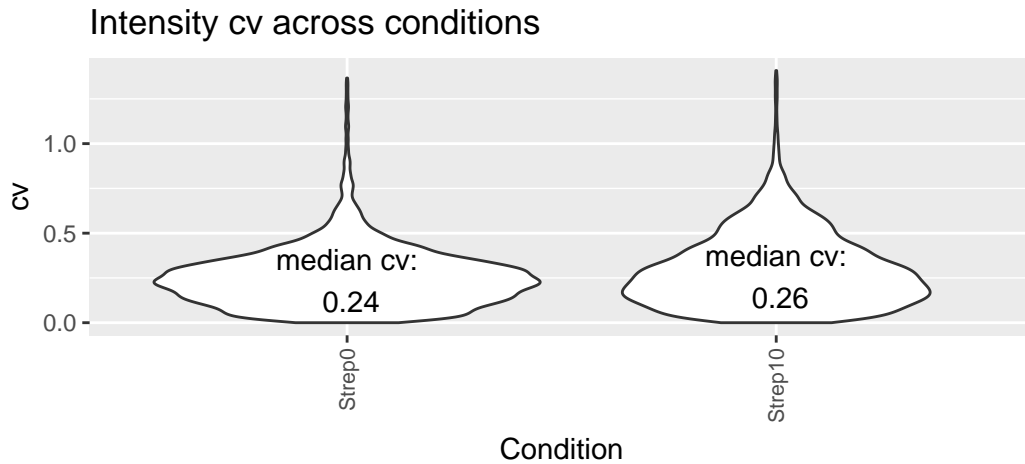
```
## Warning: Ignoring unknown aesthetics: fill
```

delta_rt correlation between samples:
Pearson (upper triangle) and Spearman correlation (lower triangle)



Plot the variation of the signal across replicates.

```
variation <- plot_variation(data.annotated.nodecoy)
```

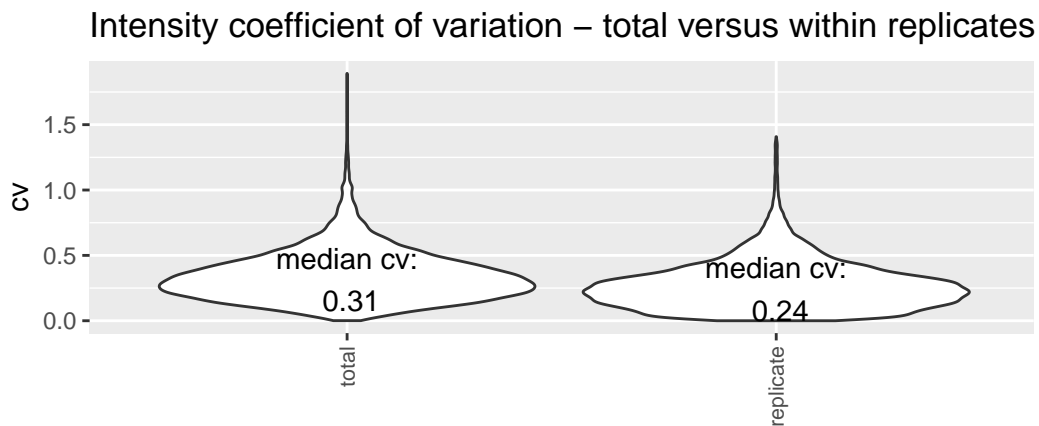


```
variation[[2]]
```

```
## Condition mode_cv mean_cv median_cv  
## 1 Strep0 0.2280372 0.2545450 0.2351859  
## 2 Strep10 0.1706934 0.2947144 0.2592725
```

Plot the total variation versus variation within replicates.

```
variation_total <- plot_variation_vs_total(data.annotated.nodecoy)
```



```
variation_total[[2]]
```

```
## scope mode_cv mean_cv median_cv  
## 1 replicate 0.2209867 0.2728681 0.2438041  
## 2 total 0.2655678 0.3439050 0.3139993
```

Calculate the summed signal per peptide and protein across samples.

```
peptide_signal <- write_matrix_peptides(data.annotated.nodecoy)  
protein_signal <- write_matrix_proteins(data.annotated.nodecoy)  
head(protein_signal)
```

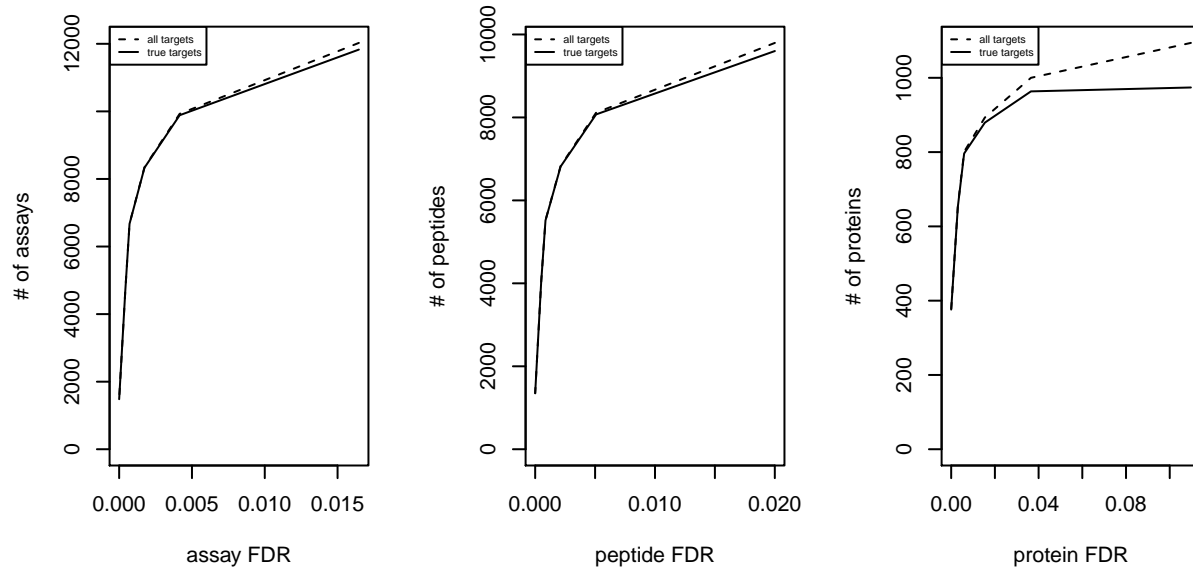
```
## ProteinName Strep0_1_1 Strep0_2_2 Strep10_1_3  
## 1 Spyo_Exp3652_DDB_SeqID_1571119 265206 163326 51831  
## 2 Spyo_Exp3652_DDB_SeqID_1579753 185725 150672 21483
```

## 3	Spyo_Exp3652_DDB_SeqID_1631459	176686	132415	42165
## 4	Spyo_Exp3652_DDB_SeqID_1640263	3310	6617	98550
## 5	Spyo_Exp3652_DDB_SeqID_1709452	852502	747772	503581
## 6	Spyo_Exp3652_DDB_SeqID_17244480	17506	29578	7607
##	Strep10_2_4			
## 1	45021			
## 2	144314			
## 3	32735			
## 4	45169			
## 5	504761			
## 6	2482			

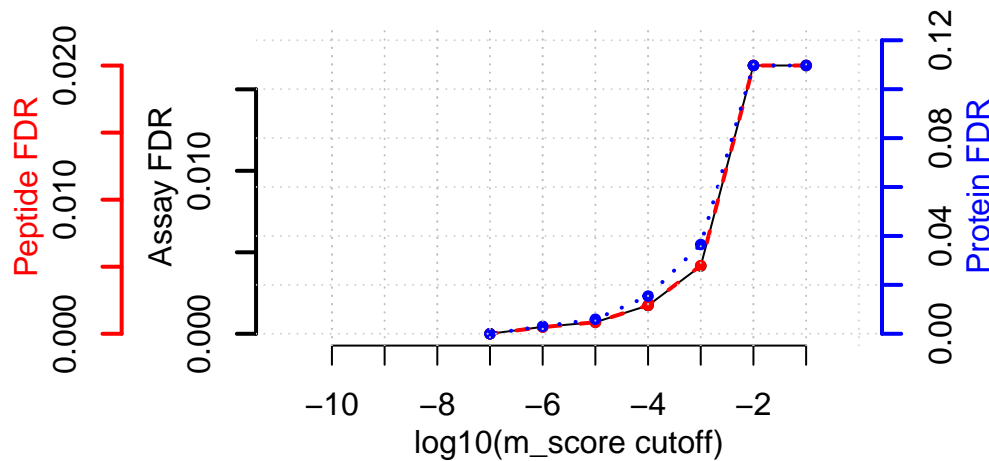
Part 3: FDR estimation

Estimate the overall FDR across runs using a target decoy strategy.

```
par(mfrow = c(1, 3))
fdr_target_decoy <- assess_fdr_overall(data.annotated, n.range = 10, FFT = 0.25, output = 'Rconsole')
```



Global m-score cutoff connectivity to FDR quality



According to this FDR estimation one would need to filter the data with a lower mscore threshold to reach an overall protein FDR of 5%.

```
mscore4protfdr(data, FFT = 0.25, fdr_target = 0.05)
```

```
## Target protein FDR:0.05
## Required overall m-score cutoff:0.0017783
## achieving protein FDR =0.0488
## [1] 0.001778279
```

Part 4: Filtering

Filter data for values that pass the 0.001 mscore criteria in at least two replicates of one condition.

```
data.filtered <- filter_mscore_condition(data.annotated, 0.001, n.replica = 2)
```

```
## Fraction of peptides selected: 0.67
```

```
## Dimension difference: 7226, 0
```

Select only the 10 peptides showing strongest signal per protein.

```
data.filtered2 <- filter_on_max_peptides(data.filtered, n_peptides = 10)
```

```
## Before filtering:
```

```
##   Number of proteins: 884
```

```
##   Number of peptides: 6594
```

```
##
```

```
## Percentage of peptides removed: 29.6%
```

```
##
```

```
## After filtering:
```

```
##   Number of proteins: 884
```

```
##   Number of peptides: 4642
```

Filter for proteins that are supported by at least two peptides.

```
data.filtered3 <- filter_on_min_peptides(data.filtered2, n_peptides = 2)
```

```
## Before filtering:
##   Number of proteins: 884
##   Number of peptides: 4642
##
## Percentage of peptides removed: 3.6%
##
## After filtering:
##   Number of proteins: 717
##   Number of peptides: 4475
```

Part 5: Conversion

Convert the data into a transition-level format (one row per transition measured).

```
data.transition <- disaggregate(data.filtered3)
```

```
## The library contains 6 transitions per precursor.
##
## The data table was transformed into a table containing one row per transition.
```

Convert the data into the format required by MSstats.

```
MSstats.input <- convert4MSstats(data.transition)
```

```
## One or several columns required by MSstats were not in the data. The columns were created and filled
## Missing columns: ProductCharge, IsotopeLabelType
## IsotopeLabelType was filled with light.
## Warning in convert4MSstats(data.transition): Intensity values that were 0,
## were replaced by NA
```

```
head(MSstats.input)
```

```
##           ProteinName      PeptideSequence PrecursorCharge
## 1 Spyo_Exp3652_DDB_SeqID_1571119 AEAAIYQFLEAIGENPNR      3
## 2 Spyo_Exp3652_DDB_SeqID_1571119 AEAAIYQFLEAIGENPNR      3
## 3 Spyo_Exp3652_DDB_SeqID_1571119 AEAAIYQFLEAIGENPNR      3
## 4 Spyo_Exp3652_DDB_SeqID_1571119 AEAAIYQFLEAIGENPNR      3
## 5 Spyo_Exp3652_DDB_SeqID_1571119      AHIAYLPSDGR        2
## 6 Spyo_Exp3652_DDB_SeqID_1571119      AHIAYLPSDGR        2
##           FragmentIon ProductCharge IsotopeLabelType Intensity
## 1 105801_AEAAIYQFLEAIGENPNR/3_y6      NA      light      4752
## 2 105801_AEAAIYQFLEAIGENPNR/3_y6      NA      light      6144
## 3 105801_AEAAIYQFLEAIGENPNR/3_y6      NA      light      3722
## 4 105801_AEAAIYQFLEAIGENPNR/3_y6      NA      light      6624
## 5      118149_AHIAYLPSDGR/2_y8      NA      light      4036
## 6      118149_AHIAYLPSDGR/2_y8      NA      light      1642
##   BioReplicate Condition Run
## 1             2      Strep0  2
## 2             1      Strep10 3
## 3             2      Strep10 4
## 4             1      Strep0  1
```

```
## 5          1      Strep0    1
## 6          1      Strep10   3
```

Convert the data into the format required by mapDIA.

```
mapDIA.input <- convert4mapDIA(data.transition)
head(mapDIA.input)
```

```
##          ProteinName      PeptideSequence
## 1 Spyo_Exp3652_DDB_SeqID_1571119 AEAAIYQFLEAIGENPNR
## 2 Spyo_Exp3652_DDB_SeqID_1571119      AHIAYLPSDGR
## 3 Spyo_Exp3652_DDB_SeqID_1571119      EEFTAVFK
## 4 Spyo_Exp3652_DDB_SeqID_1571119 EKAEAAIYQFLEAIGENPNR
## 5 Spyo_Exp3652_DDB_SeqID_1571119      EQHEDVVIVK
## 6 Spyo_Exp3652_DDB_SeqID_1571119      LTSQIADALVEALNPK
##          FragmentIon Strep0_1 Strep0_2 Strep10_1 Strep10_2
## 1 105801_AEAAIYQFLEAIGENPNR/3_y6    6624    4752    6144    3722
## 2   118149_AHIAYLPSDGR/2_y8        4036    2405    1642     720
## 3    35179_EEFTAVFK/2_y5          2307    1541    1561     NaN
## 4 28903_EKAEAAIYQFLEAIGENPNR/3_y6    3410    2185     NaN    1984
## 5    73581_EQHEDVVIVK/2_b6         2423    1343     NaN     NaN
## 6  115497_LTSQIADALVEALNPK/2_y11    6553    6349     NaN     NaN
```

Convert the data into the format required by aLFQ.

```
aLFQ.input <- convert4aLFQ(data.transition)
head(aLFQ.input)
```

```
##          run_id          protein_id      peptide_id
## 1 Strep0_2_2 Spyo_Exp3652_DDB_SeqID_1571119 AEAAIYQFLEAIGENPNR
## 2 Strep10_1_3 Spyo_Exp3652_DDB_SeqID_1571119 AEAAIYQFLEAIGENPNR
## 3 Strep10_2_4 Spyo_Exp3652_DDB_SeqID_1571119 AEAAIYQFLEAIGENPNR
## 4 Strep0_1_1 Spyo_Exp3652_DDB_SeqID_1571119 AEAAIYQFLEAIGENPNR
## 5 Strep0_1_1 Spyo_Exp3652_DDB_SeqID_1571119      AHIAYLPSDGR
## 6 Strep10_1_3 Spyo_Exp3652_DDB_SeqID_1571119      AHIAYLPSDGR
##          transition_id      peptide_sequence
## 1 AEAAIYQFLEAIGENPNR 105801_AEAAIYQFLEAIGENPNR/3_y6 AEAAIYQFLEAIGENPNR
## 2 AEAAIYQFLEAIGENPNR 105801_AEAAIYQFLEAIGENPNR/3_y6 AEAAIYQFLEAIGENPNR
## 3 AEAAIYQFLEAIGENPNR 105801_AEAAIYQFLEAIGENPNR/3_y6 AEAAIYQFLEAIGENPNR
## 4 AEAAIYQFLEAIGENPNR 105801_AEAAIYQFLEAIGENPNR/3_y6 AEAAIYQFLEAIGENPNR
## 5      AHIAYLPSDGR 118149_AHIAYLPSDGR/2_y8      AHIAYLPSDGR
## 6      AHIAYLPSDGR 118149_AHIAYLPSDGR/2_y8      AHIAYLPSDGR
## precursor_charge transition_intensity concentration
## 1          3          4752          ?
## 2          3          6144          ?
## 3          3          3722          ?
## 4          3          6624          ?
## 5          2          4036          ?
## 6          2          1642          ?
```

Session info on the R version and packages used.

```
sessionInfo()
```

```
## R version 3.5.0 (2018-04-23)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 16.04.4 LTS
##
```



```

## Matrix products: default
## BLAS: /home/biocbuild/bbs-3.7-bioc/R/lib/libRblas.so
## LAPACK: /home/biocbuild/bbs-3.7-bioc/R/lib/libRlapack.so
##
## locale:
## [1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
## [3] LC_TIME=en_US.UTF-8       LC_COLLATE=C
## [5] LC_MONETARY=en_US.UTF-8   LC_MESSAGES=en_US.UTF-8
## [7] LC_PAPER=en_US.UTF-8     LC_NAME=C
## [9] LC_ADDRESS=C              LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods   base
##
## other attached packages:
## [1] data.table_1.11.0  SWATH2stats_1.10.1
##
## loaded via a namespace (and not attached):
## [1] Rcpp_0.12.16      knitr_1.20        magrittr_1.5      munsell_0.4.3
## [5] colorspace_1.3-2  rlang_0.2.0       stringr_1.3.0     plyr_1.8.4
## [9] tools_3.5.0       grid_3.5.0        gtable_0.2.0      htmltools_0.3.6
## [13] yaml_2.1.19       lazyeval_0.2.1    rprojroot_1.3-2   digest_0.6.15
## [17] tibble_1.4.2      formatR_1.5        reshape2_1.4.3    ggplot2_2.2.1
## [21] evaluate_0.10.1   rmarkdown_1.9     labeling_0.3       stringi_1.2.2
## [25] pillar_1.2.2      compiler_3.5.0    scales_0.5.0      backports_1.1.2

```