

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_1pcnt_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 SWATH2stats package can be directly installed from Bioconductor using the commands below (<http://bioconductor.org/packages/devel/bioc/html/SWATH2stats.html>).

```
if (!require("BiocManager"))
  install.packages("BiocManager")
BiocManager::install("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)
```

```
## Registered S3 method overwritten by 'bit64':
##   method          from
##   print.bitstring tools
```

```
library(data.table)
```

```
##
## Attaching package: 'data.table'
##
## The following object is masked from 'package:base':
##
##   %notin%
```

```
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_1pcnt_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_len(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.nodcocy)
```

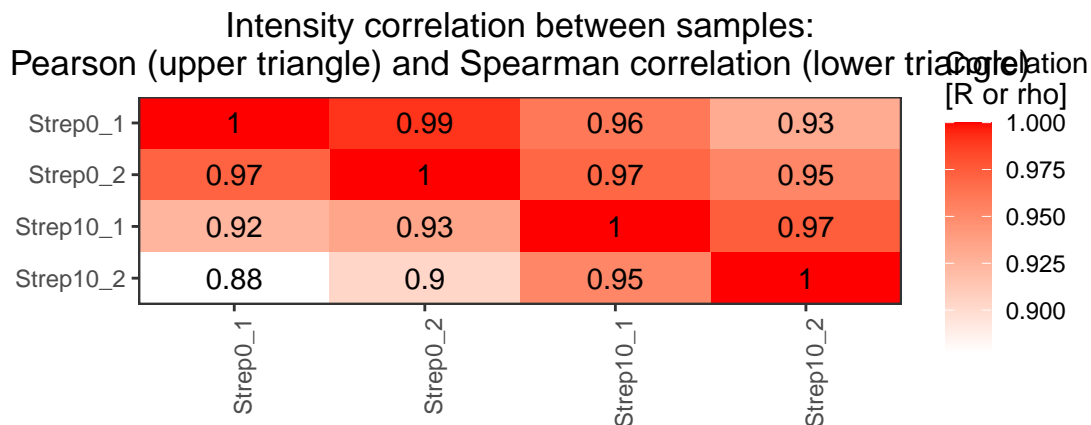
```
##      run_id transition_group_id FullPeptideName ProteinName
## 1 Strep0_1_1           10229           8377       1031
## 2 Strep0_2_2           9716           7970       1003
## 3 Strep10_1_3          8692           7138        943
## 4 Strep10_2_4          8424           6941        910
```

Plot the correlation of the signal intensity.

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

```
## Warning: `aes_string()` was deprecated in ggplot2 3.0.0.
## i Please use tidy evaluation idioms with `aes()`.
## i See also `vignette("ggplot2-in-packages")` for more information.
## i The deprecated feature was likely used in the SWATH2stats package.
## Please report the issue at <https://github.com/peterblattmann/SWATH2stats>.
## This warning is displayed once per session.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.

## Warning: Use of `data.plot$value` is discouraged.
## i Use `value` instead.
```

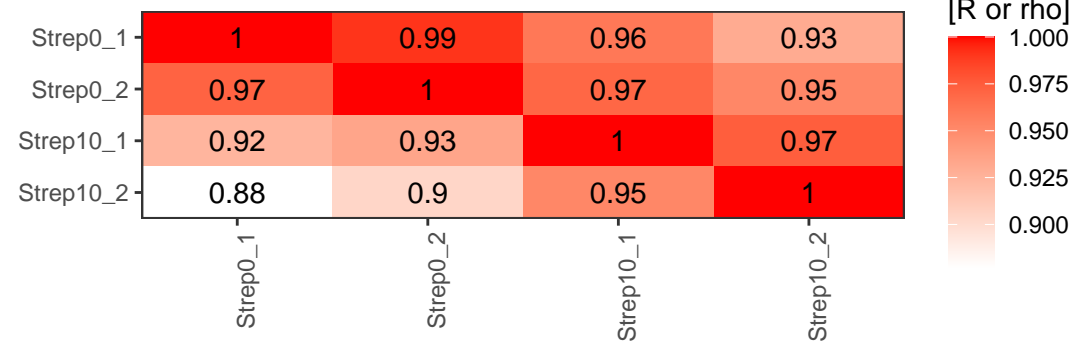


Plot the correlation of the delta_rt, which is the deviation of the retention time from the expected retention time.

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

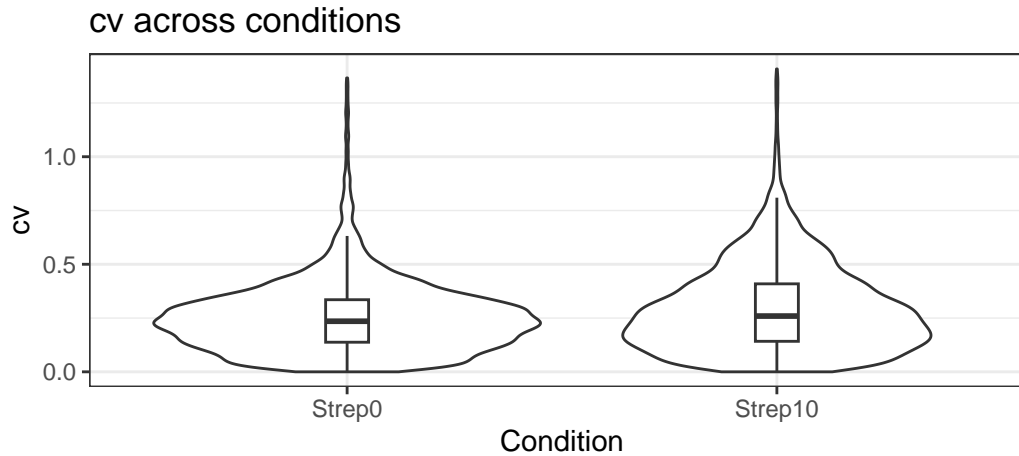
```
## Warning: Use of `data.plot$value` is discouraged.
## i Use `value` instead.
```

Intensity 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)
```

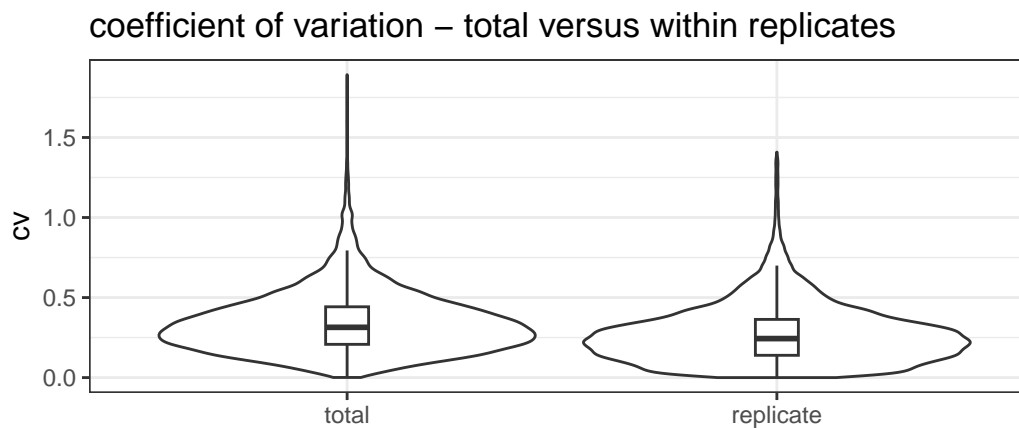


```
variation[[2]]
```

```
## 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)
```

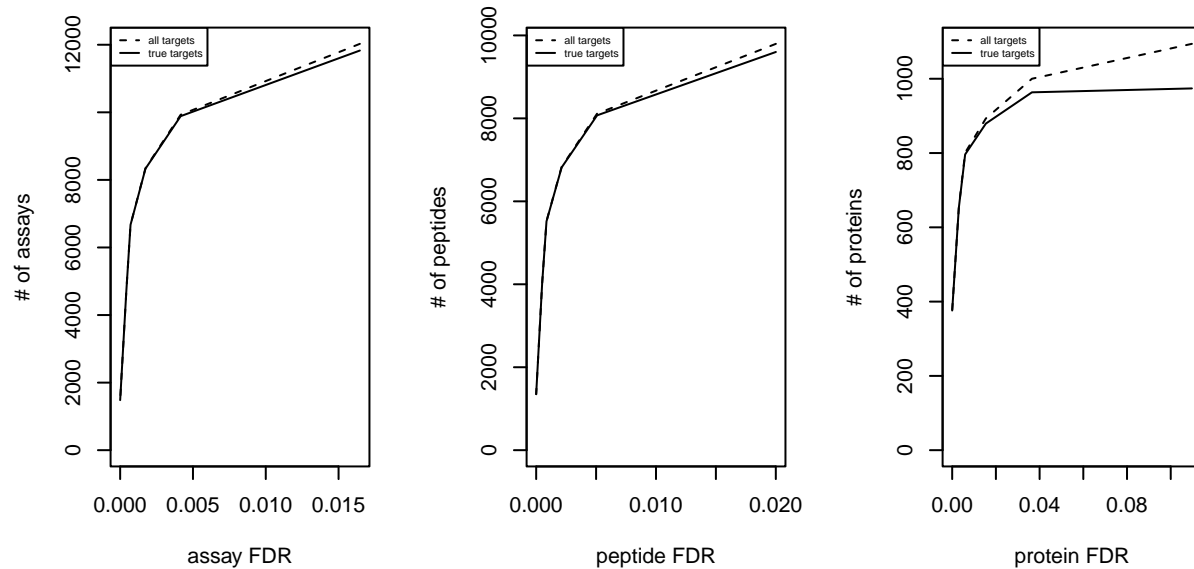
```
## ProteinName Strep0_1_1 Strep0_2_2 Strep10_1_3 Strep10_2_4
## 1 Spyo_Exp3652_DDB_SeqID_1571119 265206 163326 51831 45021
## 2 Spyo_Exp3652_DDB_SeqID_1579753 185725 150672 21483 144314
```

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

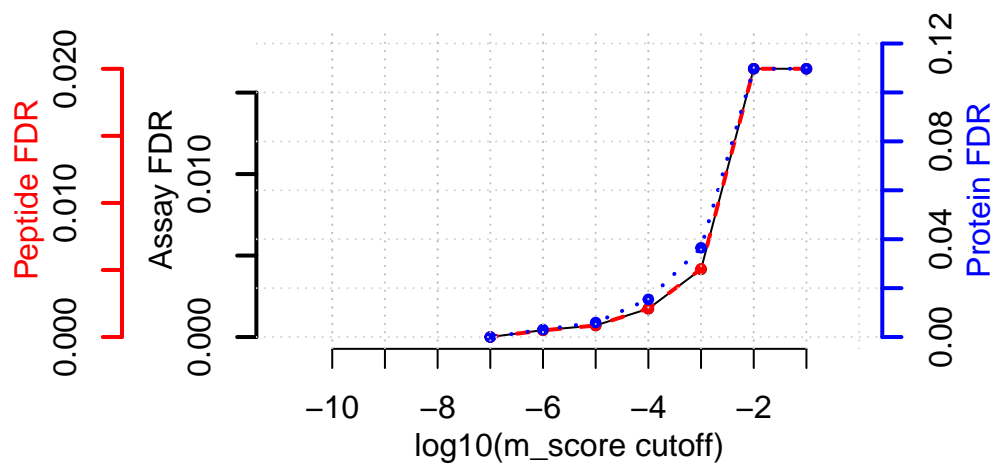
Part 3: FDR estimation

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 with NAs.
## 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)
```

```
## Checking the integrity of the transitions takes a lot of time.
##          To speed up consider changing the option.
```

```
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 4.6.0 RC (2026-04-17 r89917)
## Platform: x86_64-pc-linux-gnu
## Running under: Ubuntu 24.04.4 LTS
##
## Matrix products: default
## BLAS: /home/biocbuild/bbs-3.24-bioc/R/lib/libRblas.so
## LAPACK: /usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.12.0 LAPACK version 3.12.0
##
## locale:
## [1] LC_CTYPE=en_US.UTF-8 LC_NUMERIC=C
## [3] LC_TIME=en_GB 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
##
## time zone: America/New_York
## tzcode source: system (glibc)
##
## attached base packages:
## [1] stats graphics grDevices utils datasets methods base
##
## other attached packages:
## [1] data.table_1.18.2.1 SWATH2stats_1.41.1
##
## loaded via a namespace (and not attached):
## [1] KEGGREST_1.51.1 gtable_0.3.6 xfun_0.57
## [4] ggplot2_4.0.2 httr2_1.2.2 Biobase_2.71.0
## [7] vctrs_0.7.3 tools_4.6.0 generics_0.1.4
## [10] stats4_4.6.0 curl_7.0.0 tibble_3.3.1
## [13] AnnotationDbi_1.73.1 RSQLite_2.4.6 blob_1.3.0
## [16] pkgconfig_2.0.3 dbplyr_2.5.2 RColorBrewer_1.1-3
## [19] S7_0.2.1-1 S4Vectors_0.49.2 lifecycle_1.0.5
## [22] compiler_4.6.0 farver_2.1.2 stringr_1.6.0
## [25] Biostrings_2.79.5 progress_1.2.3 tinytex_0.59
## [28] Seqinfo_1.1.0 htmltools_0.5.9 yaml_2.3.12
## [31] pillar_1.11.1 crayon_1.5.3 cachem_1.1.0
## [34] tidyselect_1.2.1 digest_0.6.39 stringi_1.8.7
## [37] dplyr_1.2.1 reshape2_1.4.5 labeling_0.4.3
## [40] biomaRt_2.67.7 fastmap_1.2.0 grid_4.6.0
## [43] cli_3.6.6 magrittr_2.0.5 dichromat_2.0-0.1
## [46] withr_3.0.2 prettyunits_1.2.0 filelock_1.0.3
## [49] scales_1.4.0 rappdirs_0.3.4 bit64_4.6.0-1
## [52] rmarkdown_2.31 XVector_0.51.0 httr_1.4.8
## [55] bit_4.6.0 otel_0.2.0 png_0.1-9
## [58] hms_1.1.4 memoise_2.0.1 evaluate_1.0.5
## [61] knitr_1.51 IRanges_2.45.0 BiocFileCache_3.1.0
## [64] rlang_1.2.0 Rcpp_1.1.1-1 glue_1.8.1
## [67] DBI_1.3.0 formatR_1.14 BiocGenerics_0.57.1
## [70] R6_2.6.1 plyr_1.8.9

```