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)
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_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.

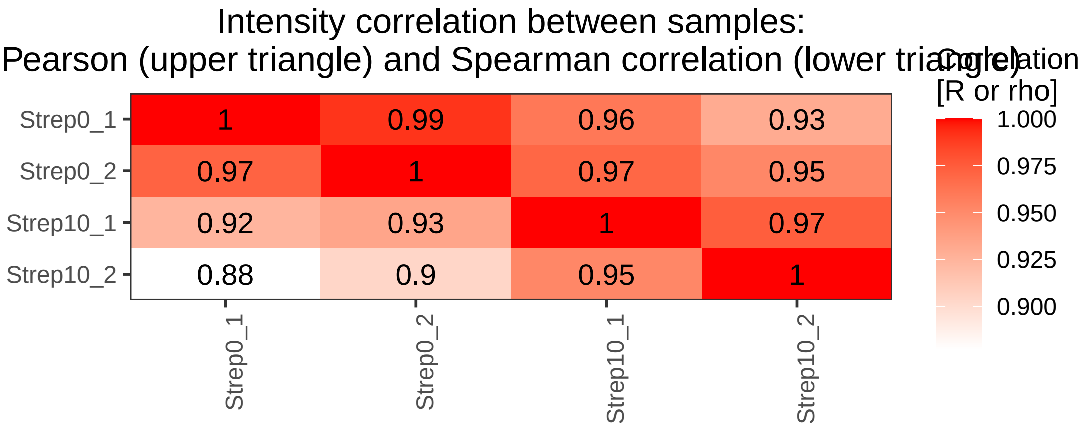
```r
count_analytes(data.annotated.nodecoy)
```

```
##        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.

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

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

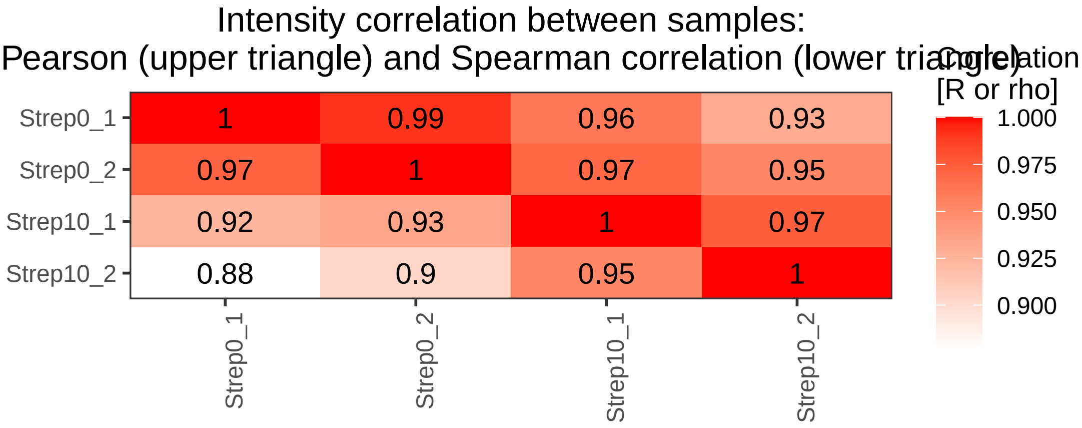


	Strep0_1	Strep0_2	Strep10_1	Strep10_2
Strep0_1	1	0.99	0.96	0.93
Strep0_2	0.97	1	0.97	0.95
Strep10_1	0.92	0.93	1	0.97
Strep10_2	0.88	0.9	0.95	1

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

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

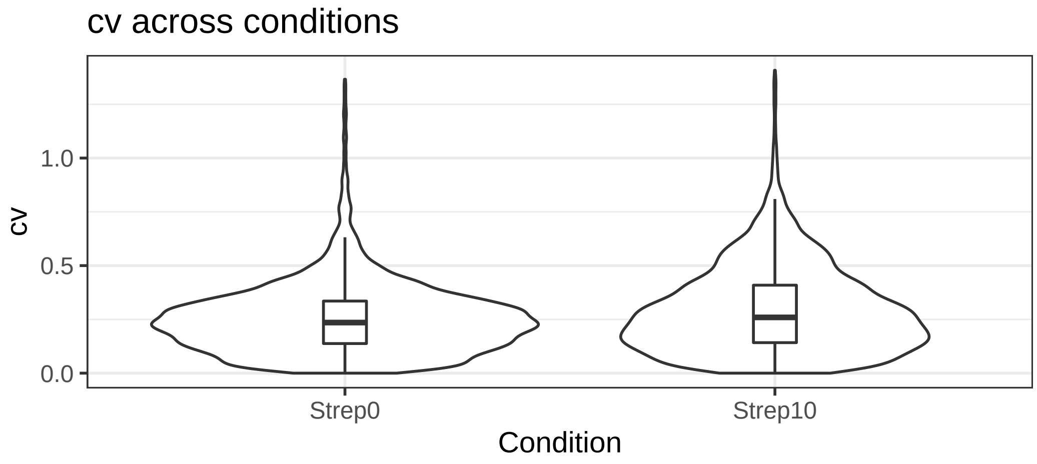
```
## Warning: Use of `data.plot$value` is discouraged. Use `value` instead.
```



	Strep0_1	Strep0_2	Strep10_1	Strep10_2
Strep0_1	1	0.99	0.96	0.93
Strep0_2	0.97	1	0.97	0.95
Strep10_1	0.92	0.93	1	0.97
Strep10_2	0.88	0.9	0.95	1

Plot the variation of the signal across replicates.

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

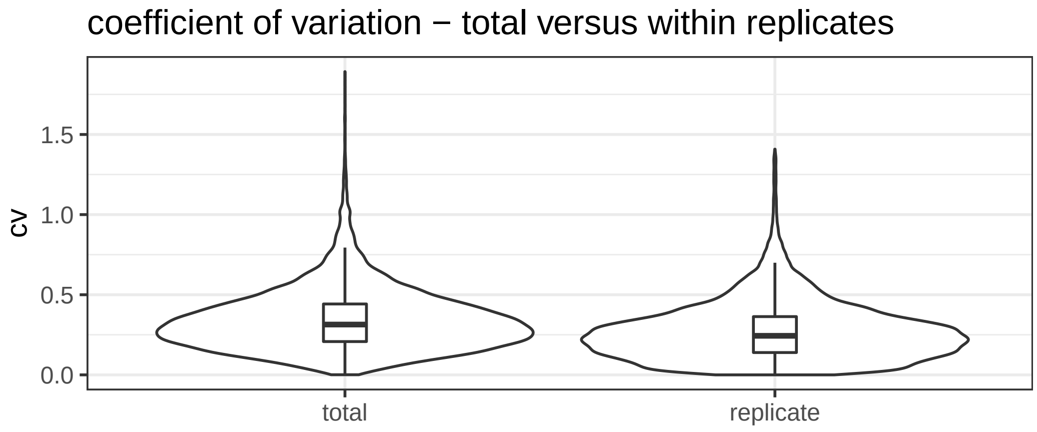


```
variation[[2]]
```

```
##   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.

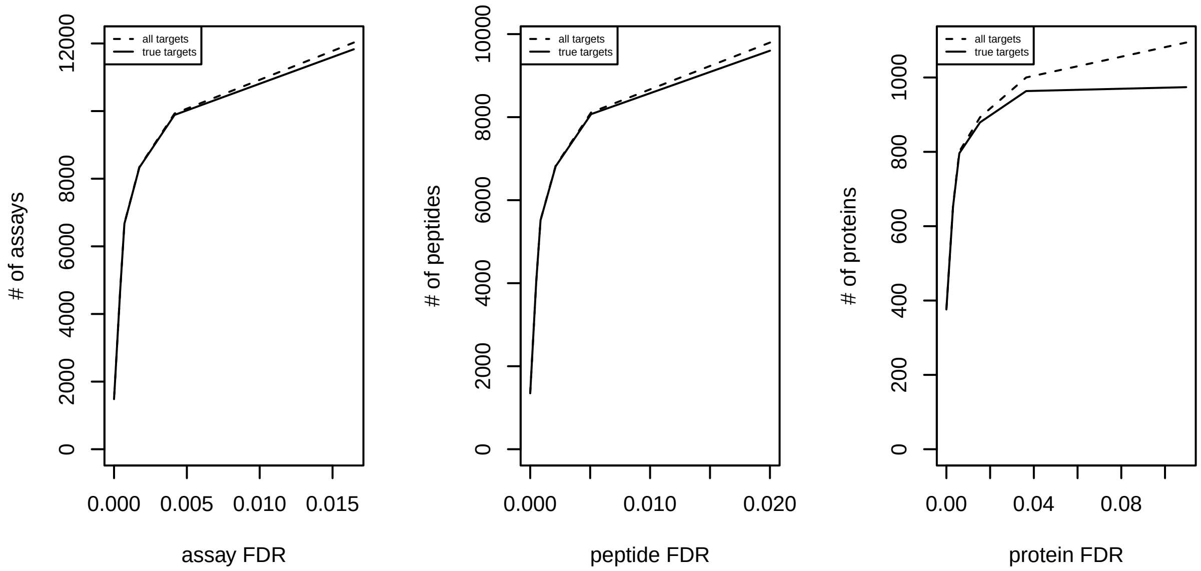
```
peptide_signal <- write_matrix_peptides(data.annotated.nodecoy)
protein_signal <- write_matrix_proteins(data.annotated.nodecoy)
head(protein_signal)
```

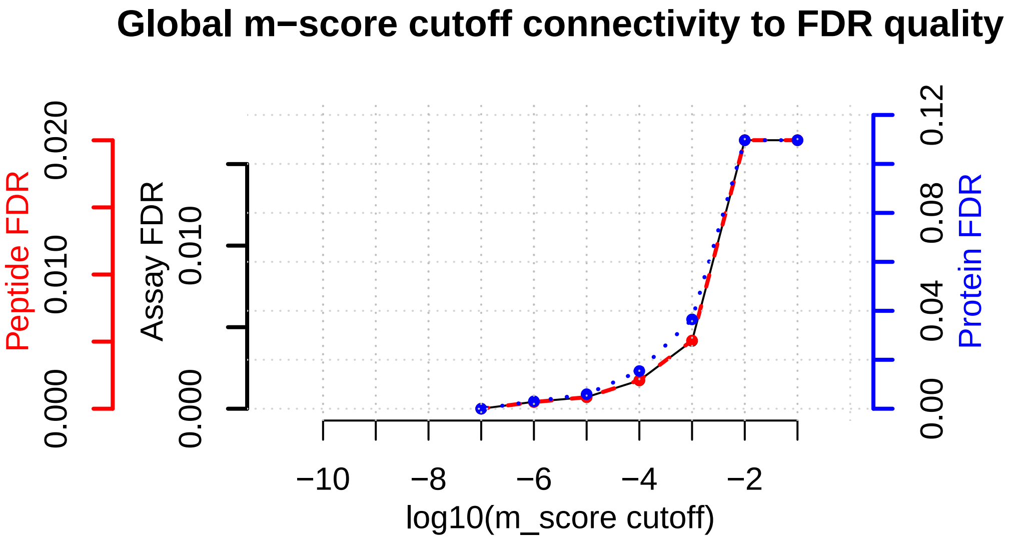
```
##                         ProteinName Strep0_1_1 Strep0_2_2 Strep10_1_3 Strep10_2_4
## 1  Spyo_Exp3652_DDB_SeqID_1571119     265206     163326       51831       45021
## 2  Spyo_Exp3652_DDB_SeqID_1579753     185725     150672       21483      144314
## 3  Spyo_Exp3652_DDB_SeqID_1631459     176686     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.

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





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%.

```r
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.

```r
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.

```r
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 4.2.0 RC (2022-04-19 r82224 ucrt)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows Server x64 (build 20348)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=C
## [2] LC_CTYPE=English_United States.utf8
## [3] LC_MONETARY=English_United States.utf8
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_United States.utf8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] data.table_1.14.2  SWATH2stats_1.26.0
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_1.0.8.3           prettyunits_1.1.1      png_0.1-7
##  [4] Biostrings_2.64.0      assertthat_0.2.1       digest_0.6.29
##  [7] utf8_1.2.2             BiocFileCache_2.4.0    plyr_1.8.7
## [10] R6_2.5.1               GenomeInfoDb_1.32.0    stats4_4.2.0
## [13] RSQLite_2.2.12         evaluate_0.15          highr_0.9
## [16] httr_1.4.2             ggplot2_3.3.5          pillar_1.7.0
## [19] zlibbioc_1.42.0        rlang_1.0.2            progress_1.2.2
## [22] curl_4.3.2             blob_1.2.3             S4Vectors_0.34.0
## [25] rmarkdown_2.14         labeling_0.4.2         stringr_1.4.0
## [28] RCurl_1.98-1.6         bit_4.0.4              biomaRt_2.52.0
## [31] munsell_0.5.0          compiler_4.2.0         xfun_0.30
## [34] pkgconfig_2.0.3        BiocGenerics_0.42.0    htmltools_0.5.2
## [37] tidyselect_1.1.2       KEGGREST_1.36.0        tibble_3.1.6
## [40] GenomeInfoDbData_1.2.8 IRanges_2.30.0         XML_3.99-0.9
## [43] fansi_1.0.3            crayon_1.5.1           dplyr_1.0.8
## [46] dbplyr_2.1.1           bitops_1.0-7           rappdirs_0.3.3
## [49] grid_4.2.0             gtable_0.3.0           lifecycle_1.0.1
## [52] DBI_1.1.2              formatR_1.12           magrittr_2.0.3
## [55] scales_1.2.0           cli_3.3.0              stringi_1.7.6
## [58] cachem_1.0.6           farver_2.1.0           reshape2_1.4.4
## [61] XVector_0.36.0         xml2_1.3.3             ellipsis_0.3.2
## [64] filelock_1.0.2         generics_0.1.2         vctrs_0.4.1
## [67] tools_4.2.0            bit64_4.0.5            Biobase_2.56.0
## [70] glue_1.6.2             purrr_0.3.4            hms_1.1.1
## [73] fastmap_1.1.0          yaml_2.3.5             colorspace_2.0-3
## [76] AnnotationDbi_1.58.0   memoise_2.0.1          knitr_1.38
```