

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(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.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
```

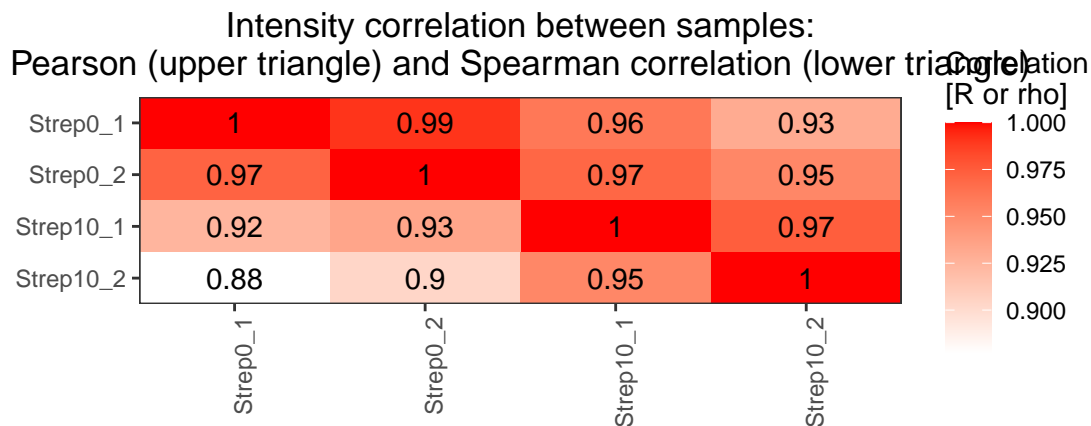
Plot the correlation of the signal intensity.

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

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

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

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



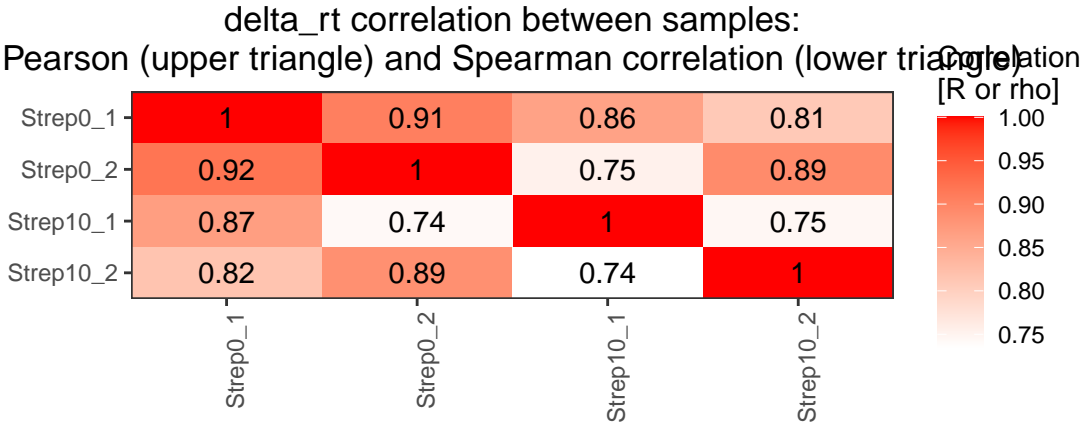
Plot the correlation of the delta_rt, which is the deviation of the retention time from the expected retention time.

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

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

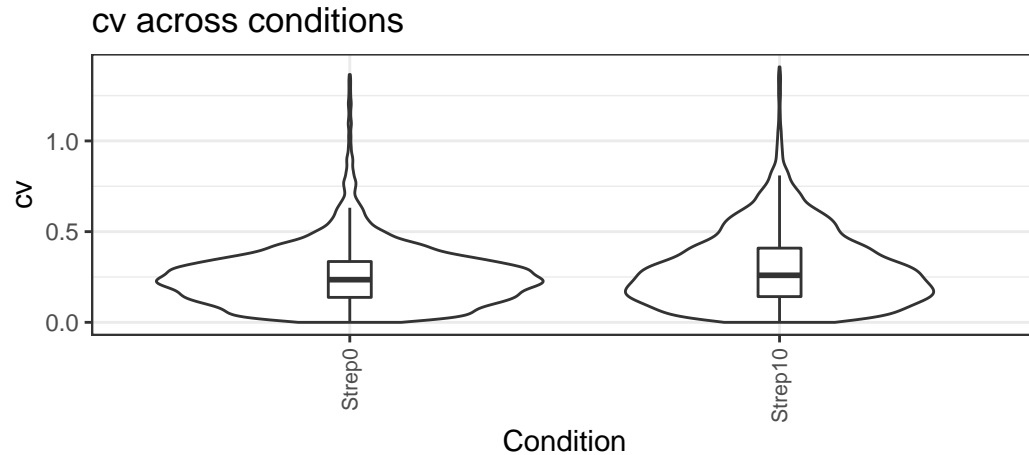
```
## Warning: Use of `data.plot$value` is discouraged. Use `value` instead.
```

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



Plot the variation of the signal across replicates.

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

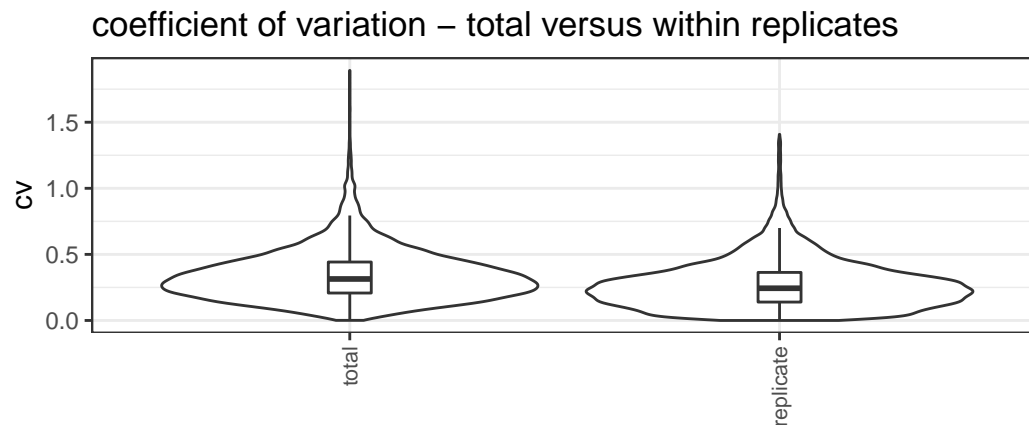


```
variation[[2]]
```

```
## 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.

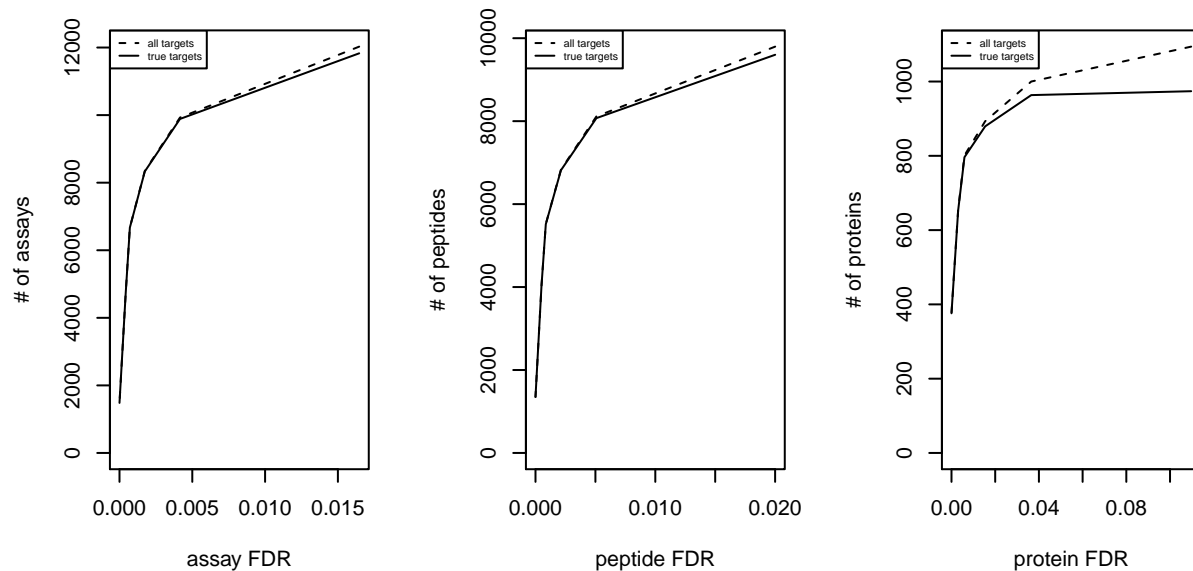
```
peptide_signal <- write_matrix_peptides(data.annotated.nodecoy)
protein_signal <- write_matrix_proteins(data.annotated.nodecoy)
head(protein_signal)
```

##	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

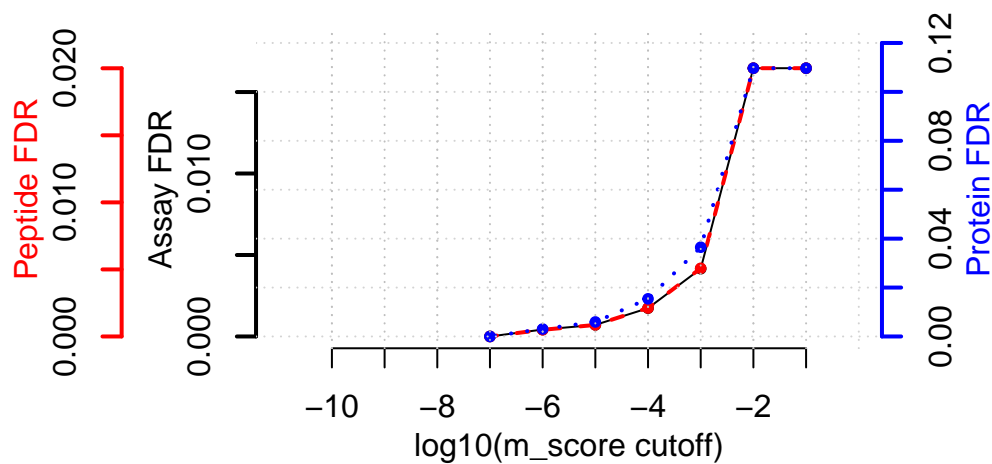
Part 3: FDR estimation

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
## 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 opt.

```
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 4.0.0 (2020-04-24)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
```

```

## Running under: Windows Server 2012 R2 x64 (build 9600)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=C
## [2] LC_CTYPE=English_United States.1252
## [3] LC_MONETARY=English_United States.1252
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_United States.1252
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods   base
##
## other attached packages:
## [1] data.table_1.12.8  SWATH2stats_1.18.0
##
## loaded via a namespace (and not attached):
## [1] progress_1.2.2      tidyselect_1.0.0    xfun_0.13
## [4] purrr_0.3.4         reshape2_1.4.4      colorspace_1.4-1
## [7] vctrs_0.2.4         htmltools_0.4.0     stats4_4.0.0
## [10] BiocFileCache_1.12.0 yaml_2.2.1          blob_1.2.1
## [13] XML_3.99-0.3        rlang_0.4.5         pillar_1.4.3
## [16] glue_1.4.0          DBI_1.1.0           rappdirs_0.3.1
## [19] dbplyr_1.4.3        BiocGenerics_0.34.0 bit64_0.9-7
## [22] lifecycle_0.2.0     plyr_1.8.6          stringr_1.4.0
## [25] munsell_0.5.0       gtable_0.3.0        evaluate_0.14
## [28] memoise_1.1.0       labeling_0.3         Biobase_2.48.0
## [31] knitr_1.28          IRanges_2.22.0      biomaRt_2.44.0
## [34] curl_4.3            parallel_4.0.0      AnnotationDbi_1.50.0
## [37] Rcpp_1.0.4.6        formatR_1.7          openssl_1.4.1
## [40] scales_1.1.0        S4Vectors_0.26.0    farver_2.0.3
## [43] bit_1.1-15.2        ggplot2_3.3.0       hms_0.5.3
## [46] askpass_1.1         digest_0.6.25       stringi_1.4.6
## [49] dplyr_0.8.5         grid_4.0.0          tools_4.0.0
## [52] magrittr_1.5        tibble_3.0.1        RSQLite_2.2.0
## [55] crayon_1.3.4        pkgconfig_2.0.3     ellipsis_0.3.0
## [58] prettyunits_1.1.1   assertthat_0.2.1    rmarkdown_2.1
## [61] httr_1.4.1          R6_2.4.1            compiler_4.0.0

```