# SPEAQeasy\_example

Josh Stolz

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#### Contents

### 1 Reproducibility

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```
library("pheatmap")
library("tidyr")
library("jaffelab")
library("here")
library("VariantAnnotation")
library("SummarizedExperiment")
library("devtools")
library("BiocStyle")
```

In order to resolve the swaps to our best ability we need four data sets. Here we have load snpGeno\_example which is from our topmed imputed genotype data, a phenotype data sheet (pd\_example), a vcf file of the relevant speaqeasy output (Speaqeasy), and our current genotype sample sheet (brain\_sentrix). This file is wrote in the directory listed below.

```
load(here("sample_selection","snpsGeno_example.RData"), verbose = TRUE)

## Loading objects:
## snpsGeno_example

load(here("sample_selection","pd_example.Rdata"), verbose = TRUE)

## Loading objects:
## pd_example

Speaqeasy<-readVcf(here("pipeline_outputs","merged_variants","mergedVariants.vcf.gz"),genome="hg38")
brain_sentrix<- read.csv(here("brain_sentrix_speaqeasy.csv"))</pre>
```

We can see that the genotype is represented in the form of 0s,1s, and 2s. The rare 2s are a result of multiallelic snps and we will drop those. 0 represent the reference allele with ones representing the alternate. We can see the distribution below.

```
Geno_speaqeasy<-geno(Speaqeasy)$GT
table(Geno_speaqeasy)</pre>
```

```
## Geno_speaqeasy
## ./. 0/1 0/2 1/1 2/2
## 14096 7803 4 6018 9
```

Given this we convert we convert the Genotype data from Speageasy to numeric data. The "./." were values that could not accurately be determined and are replaced with NA.

```
colnames_speaqeasy<- as.data.frame(colnames(Geno_speaqeasy))
colnames(colnames_speaqeasy)<-c("a")
samples<-separate(colnames_speaqeasy,a,into = c("a","b","c"),sep = "_")</pre>
```

## Warning: Expected 3 pieces. Additional pieces discarded in 42 rows [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1 ## 16, 17, 18, 19, 20, ...].

```
samples<-paste0(samples$a,"_",samples$b)
samples<-as.data.frame(samples)
colnames(Geno_speaqeasy)<-samples$samples
Geno_speaqeasy[Geno_speaqeasy == "./."] = NA
Geno_speaqeasy[Geno_speaqeasy == "0/0"] = 0
Geno_speaqeasy[Geno_speaqeasy == "0/1"] = 1
Geno_speaqeasy[Geno_speaqeasy == "1/1"] = 2
class(Geno_speaqeasy) = "numeric"</pre>
```

## Warning in class(Geno\_speaqeasy) = "numeric": NAs introduced by coercion

```
corner(Geno_speaqeasy)
```

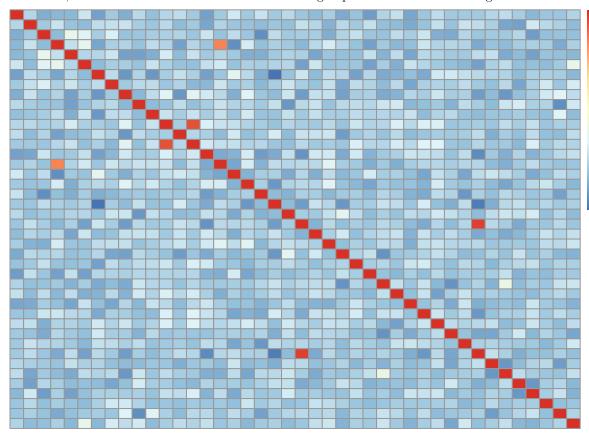
```
R14030 H7K5NBBXX R14184 H7K5NBBXX R13904 H7K5NBBXX R14296 H7JLCBBXX R14247 HF3JYBB
##
## chr1:4712657_G/A
## chr1:7853370_G/A
                                    NA
                                                      NA
                                                                        NA
                                                                                          NA
## chr1:9263851_G/A
                                    NA
                                                      NA
                                                                         1
                                                                                           1
## chr1:13475857_T/C
                                    NA
                                                      NA
                                                                        NA
                                                                                          NA
## chr1:15289643_G/A
                                    NA
                                                      NA
                                                                        NA
                                                                                          NA
## chr1:15341780_G/A
                                    NA
                                                      NA
                                                                        NA
                                                                                          NA
```

We then make a correlation matrix to find the possible mismatches between samples.

```
speaqeasy_Cor = cor(Geno_speaqeasy,use="pairwise.comp")
corner(speaqeasy_Cor)
```

```
##
                    R14030_H7K5NBBXX R14184_H7K5NBBXX R13904_H7K5NBBXX R14296_H7JLCBBXX R14247_HF3JYBBX
## R14030_H7K5NBBXX
                          1.00000000
                                           0.13507812
                                                             0.01657484
                                                                              0.03106472
                                                                                                0.0338491
## R14184_H7K5NBBXX
                          0.13507812
                                           1.00000000
                                                             0.09347004
                                                                              0.18748043
                                                                                                0.0708049
## R13904_H7K5NBBXX
                          0.01657484
                                           0.09347004
                                                             1.00000000
                                                                              0.04958385
                                                                                                0.2879895
## R14296_H7JLCBBXX
                          0.03106472
                                           0.18748043
                                                             0.04958385
                                                                              1.00000000
                                                                                                0.1665380
## R14247_HF3JYBBXX
                          0.03384912
                                           0.07080491
                                                             0.28798955
                                                                              0.16653808
                                                                                                1.0000000
## R15093_HFY2MBBXX
                          0.18938568
                                           0.04720309
                                                             0.25477682
                                                                              0.27251010
                                                                                                0.1409733
```

Here in the heatmap below we can see that several points do not correlate with themselves in a semetrical matrix. This could be mismatches, but it also could be a result of a brain being sequenced twice. We will dig



more into this later on.

We repeat the process for the genotype data from topmed. First creating our numeric data for the genotypes.

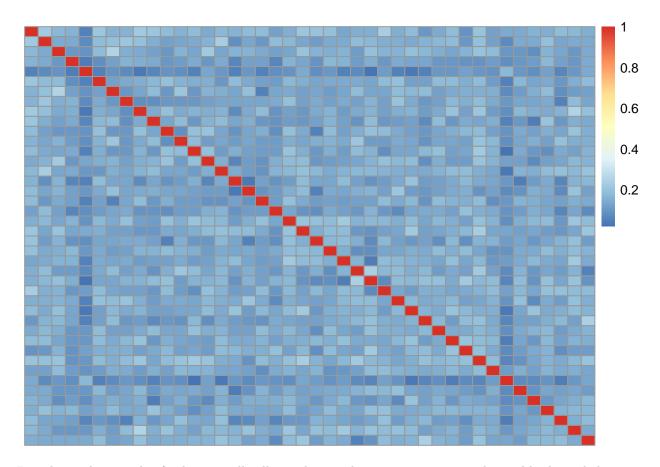
```
## Geno_example
## 0|0 0|1 1|0 1|1
## 9656 6956 7185 7535
```

## 5535506145\_R01C01

##		4463344375_R01C01	4463344375_R01C02	4572348848_R01C02	4572348855_R01C02	5535549043
##	4463344375_R01C01	1.00000000	0.21736687	0.17615291	0.16590842	0.0
##	4463344375_R01C02	0.21736687	1.00000000	0.13803954	0.14670605	0.0
##	4572348848_R01C02	0.17615291	0.13803954	1.00000000	0.10600440	0.0
##	4572348855_R01C02	0.16590842	0.14670605	0.10600440	1.00000000	0.04
##	5535549043_R01C01	0.05367081	0.07697879	0.09356496	0.04874508	1.00
##	5535506145_R01C01	0.21189898	0.18114194	0.17829111	0.08186958	0.0
##		5535506145_R01C01				
##	4463344375_R01C01	0.21189898				
##	4463344375_R01C02	0.18114194				
##	4572348848_R01C02	0.17829111				
##	4572348855_R01C02	0.08186958				
##	5535549043_R01C01	0.05331113				

In this case the data only appears to have samples that match themselves. However there is the potential for a second kind of error where a brain has two samples, however the do not match each other.

1.00000000



In order to dig into this further we will collapse the correlation matrices into a data table shown below

```
corLong = data.frame(cor = signif(as.numeric(correlation_genotype),3))
corLong$rowSample = rep(colnames(snpsGeno_example), times = ncol(snpsGeno_example))
corLong$colSample = rep(colnames(snpsGeno_example), each = ncol(snpsGeno_example))
corLong = corLong[!is.na(corLong$cor),]
head(corLong)
```

```
## cor rowSample colSample
## 1 1.0000 4463344375_R01C01 4463344375_R01C01
## 2 0.2170 4463344375_R01C02 4463344375_R01C01
## 3 0.1760 4572348848_R01C02 4463344375_R01C01
## 4 0.1660 4572348855_R01C02 4463344375_R01C01
## 5 0.0537 5535549043_R01C01 4463344375_R01C01
## 6 0.2120 5535506145_R01C01 4463344375_R01C01

corLong2 = data.frame(cor = signif(as.numeric(speaqeasy_Cor),3))
corLong2$rowSample = rep(colnames(Geno_speaqeasy), times = ncol(Geno_speaqeasy))
corLong2$colSample = rep(colnames(Geno_speaqeasy), each = ncol(Geno_speaqeasy))
corLong2 = corLong2[!is.na(corLong2$cor),]
head(corLong2)
```

```
## cor rowSample colSample
## 1 1.0000 R14030_H7K5NBBXX R14030_H7K5NBBXX
## 2 0.1350 R14184_H7K5NBBXX R14030_H7K5NBBXX
```

```
## 3 0.0166 R13904_H7K5NBBXX R14030_H7K5NBBXX
## 4 0.0311 R14296_H7JLCBBXX R14030_H7K5NBBXX
## 5 0.0338 R14247_HF3JYBBXX R14030_H7K5NBBXX
## 6 0.1890 R15093_HFY2MBBXX R14030_H7K5NBBXX
```

We can check these tables for columns where different brains are strongly correlated and where the same brain fails to match itself. Below is the output of those analysis for the topmed genotypes.

```
##
           cor
                          rowSample
                                               colSample rowBrain colBrain
                                                                              rowBatch
                                                                                          colBatch
                  6017049081_R01C02
                                      5535549043_R01C01
## 180
        0.0825
                                                           Br1652
                                                                     Br1652
                                                                                     1M
                                                                                                1M
        0.0501 201398400130_R04C01
                                      5535549043_R01C01
  196
                                                           Br1652
                                                                     Br1652 2-5-8-v1-3
                                                                                                1M
##
                 5535549043_R01C01
                                      6017049081_R01C02
## 467
        0.0825
                                                           Br1652
                                                                     Br1652
                                                                                     1M
                                                                                                1M
        0.1440 201398400130_R04C01
                                      6017049081_R01C02
## 490
                                                           Br1652
                                                                     Br1652 2-5-8-v1-3
                                                                                                1M
                 5535549043_R01C01 201398400130_R04C01
## 1139 0.0501
                                                           Br1652
                                                                     Br1652
                                                                                     1M 2-5-8-v1-3
                 6017049081 R01C02 201398400130 R04C01
## 1146 0.1440
                                                           Br1652
                                                                     Br1652
                                                                                     1M 2-5-8-v1-3
                 9373406026 R02C01 201398400130 R06C01
## 1212 0.0379
                                                           Br2275
                                                                     Br2275
                                                                                     5M 2-5-8-v1-3
## 1499 0.0379 201398400130_R06C01
                                      9373406026_R02C01
                                                           Br2275
                                                                     Br2275 2-5-8-v1-3
                                                                                                5M
```

```
## [1] cor rowSample colSample rowBrain colBrain rowBatch colBatch
## <0 rows> (or 0-length row.names)
```

And we do this again for the speaqeasy data.

```
## [1] cor rowSample colSample rowBrain colBrain
## <0 rows> (or 0-length row.names)

## [1] cor rowSample colSample rowBrain colBrain
## <0 rows> (or 0-length row.names)
```

We will next compare the correlation between the speaqeasy samples and the topmed samples. In order to do this we need to subset the genotypes for only SNPs that are common between the two. We can see that we have 656 snps common between the 42 samples.

```
## [1] 662 42
## [1] 662 42
```

As we did before we create a correlation matrix this time between the two data sets.

Check to correlation between Speaqeasy and Genotype for mismatches and swaps.

```
##
                         rowSample
                                           colSample colBrain rowBrain
         0.00962 9373408026_R01C01 R14296_H7JLCBBXX
## 165
                                                       Br2473
                                                                 Br2473
         0.11600 5535549043 R01C01 R14129 HCTYLBBXX
## 425
                                                       Br1652
                                                                 Br1652
         0.11800 6017049081_R01C02 R14129_HCTYLBBXX
## 432
                                                       Br1652
                                                                 Br1652
        -0.04270 9373406026_R02C01 R14222_H7JHNBBXX
## 498
                                                       Br2275
                                                                 Br2275
        -0.03790 9373406026 R02C01 R13997 H7JHNBBXX
## 582
                                                       Br2275
                                                                 Br2275
         0.08540 9373408026 R01C01 R14077 HCTYLBBXX
## 669
                                                       Br2473
                                                                 Br2473
         0.04020 9373406026_R01C01 R14290_H7L3FBBXX
## 917
                                                       Br2260
                                                                 Br2260
## 1463
         0.05050 9373406026_R01C01 R14071_HF3JYBBXX
                                                       Br2260
                                                                 Br2260
         0.76500 3998646040_R06C01 R14017_HF5JNBBXX
## 1697
                                                       Br5190
                                                                 Br5190
```

```
##
                      rowSample
                                       colSample colBrain rowBrain
          cor
## 161 0.865 9373406026 R01C01 R14296 H7JLCBBXX
                                                            Br2260
                                                   Br2473
## 665 0.815 9373406026 R01C01 R14077 HCTYLBBXX
                                                   Br2473
                                                            Br2260
## 921 0.866 9373408026_R01C01 R14290_H7L3FBBXX
                                                   Br2260
                                                            Br2473
                                                            Br2473
## 1467 0.915 9373408026 R01C01 R14071 HF3JYBBXX
                                                   Br2260
```

We can see from this from this analysis there are a few swaps present between RNA and DNA samples here. We can categorize them as simple and complex sample swaps. Because the two Br2275 do not match each other and also match nothing else we will be forced to consider this a complex swap and drop the sample. In the case of Br2473 it is a simple swap with Br2260 in both cases. This can be ammended by swapping with in the phenotype data sheet manually. Now we have our accurate data outputs and will need to fix our ranged summarized experiment object for our Speageasy data.

```
load(here('pipeline_outputs', 'count_objects', 'rse_gene_Jlab_experiment_n42.Rdata'))
## drop sample from rse with speageasy data
ids<-pd example$SAMPLE ID[pd example$BrNum =="Br2275"]
rse_gene<-rse_gene[,!rse_gene$SAMPLE_ID ==ids[1]]</pre>
rse_gene<- rse_gene[,!rse_gene$SAMPLE_ID ==ids[2]]</pre>
# resolve swaps and drops in pd_example
pd_example<-pd_example[!pd_example$SAMPLE_ID ==ids[1],]</pre>
pd_example<-pd_example[!pd_example$SAMPLE_ID ==ids[2],]</pre>
ids2<-pd_example$SAMPLE_ID[pd_example$BrNum =="Br2260"]
ids3<-pd_example$SAMPLE_ID[pd_example$BrNum =="Br2473"]
pd_example$SAMPLE_ID[pd_example$Sample_ID == ids2]<- "Br2473"
pd_example$SAMPLE_ID[pd_example$Sample_ID == ids3]<- "Br2260"
# reorder phenotype data by the sample order present in the 'rse_gene' object
pd_example = pd_example[match(rse_gene$SAMPLE_ID, pd_example$SAMPLE_ID),]
# add important colData to 'rse gene'
rse gene$BrainRegion = pd example$BrainRegion
rse_gene$Race = pd_example$Race
rse_gene$PrimaryDx = pd_example$PrimaryDx
rse_gene$Sex = pd_example$Sex
rse_gene$AgeDeath = pd_example$AgeDeath
#add correct BrNum to colData for rse_gene
colData(rse_gene)$BrNum <- pd_example$BrNum</pre>
save(rse_gene, file = "rse_speaqeasy.RData")
```

## 1 Reproducibility

This analysis report was made possible thanks to:

- R (Müller, 2017)
- devtools (Allaire, Xie, McPherson, Luraschi, et al., 2020)
- here (Obenchain, Lawrence, Carey, Gogarten, et al., 2014)
- jaffelab (Boettiger, 2019)
- knitcitations (Collado-Torres, Jaffe, and Burke, 2019)

- pheatmap (Kolde, 2019)
- rmarkdown
- SummarizedExperiment (R Core Team, 2020)
- tidyr (Morgan, Obenchain, Hester, and Pagès, 2020)
- noom.
- VariantAnnotation (Xie, Allaire, and Grolemund, 2018)

#### Bibliography file

- [1] J. Allaire, Y. Xie, J. McPherson, J. Luraschi, et al. *rmarkdown: Dynamic Documents for R.* R package version 2.3. 2020. <URL: https://github.com/rstudio/rmarkdown>.
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- [5] M. Morgan, V. Obenchain, J. Hester, and H. Pagès. SummarizedExperiment: SummarizedExperiment container. R package version 1.19.6. 2020.
- [6] K. Müller. here: A Simpler Way to Find Your Files. https://github.com/krlmlr/here, http://krlmlr.github.io/here. 2017.
- [7] V. Obenchain, M. Lawrence, V. Carey, S. Gogarten, et al. "VariantAnnotation: a Bioconductor package for exploration and annotation of genetic variants". In: *Bioinformatics* 30.14 (2014), pp. 2076-2078. DOI: 10.1093/bioinformatics/btu168.
- [8] R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria, 2020. <URL: https://www.R-project.org/>.
- [9] Y. Xie, J. Allaire, and G. Grolemund. *R Markdown: The Definitive Guide*. ISBN 9781138359338. Boca Raton, Florida: Chapman and Hall/CRC, 2018. <URL: https://bookdown.org/yihui/rmarkdown>.

```
# Time spent creating this report:
diff(c(timestart, Sys.time()))
```

## Time difference of 1.317441 secs

```
# Date this report was generated
message(Sys.time())
```

## 2020-09-25 17:00:20

```
# Reproducibility info
options(width = 120)
devtools::session_info()
```

```
language (EN)
##
   collate en_US.UTF-8
##
    ctype
            en US.UTF-8
            UTC
##
   t.z.
##
   date
            2020-09-25
##
  - Packages ------
##
   package
                        * version date
                                              lib source
##
   AnnotationDbi
                        * 1.51.3
                                    2020-07-25 [1] Bioconductor
##
   askpass
                          1.1
                                    2019-01-13 [2] RSPM (R 4.0.0)
   assertthat
                           0.2.1
                                    2019-03-21 [2] RSPM (R 4.0.0)
##
                          1.1.10
                                    2020-09-15 [2] RSPM (R 4.0.2)
   backports
##
   base64enc
                          0.1 - 3
                                    2015-07-28 [2] RSPM (R 4.0.0)
##
                                   2020-01-02 [1] RSPM (R 4.0.0)
  bibtex
                          0.4.2.2
##
   Biobase
                        * 2.49.1
                                    2020-09-03 [1] Bioconductor
##
   BiocFileCache
                           1.13.1
                                    2020-08-04 [1] Bioconductor
##
                        * 0.35.4
                                    2020-06-04 [1] Bioconductor
   BiocGenerics
##
   BiocManager
                        * 1.30.10
                                   2019-11-16 [2] CRAN (R 4.0.2)
##
  BiocParallel
                          1.23.2
                                    2020-07-06 [1] Bioconductor
##
   BiocStyle
                        * 2.17.0
                                    2020-04-27 [1] Bioconductor
##
   biomaRt
                          2.45.2
                                    2020-07-05 [1] Bioconductor
##
   Biostrings
                        * 2.57.2
                                    2020-06-09 [1] Bioconductor
##
                          4.0.4
                                    2020-08-04 [1] RSPM (R 4.0.2)
   bit
   bit64
                          4.0.5
                                    2020-08-30 [1] RSPM (R 4.0.2)
##
##
                          1.0-6
   bitops
                                   2013-08-17 [1] RSPM (R 4.0.0)
   blob
                          1.2.1
                                    2020-01-20 [1] RSPM (R 4.0.0)
##
   bookdown
                          0.20
                                    2020-06-23 [1] RSPM (R 4.0.2)
                          1.57.6
                                    2020-09-02 [1] Bioconductor
   BSgenome
##
                          1.31.0
                                    2020-04-28 [1] Bioconductor
   bumphunter
##
   callr
                          3.4.4
                                    2020-09-07 [2] RSPM (R 4.0.2)
##
   checkmate
                          2.0.0
                                    2020-02-06 [1] RSPM (R 4.0.0)
##
   cli
                          2.0.2
                                    2020-02-28 [2] RSPM (R 4.0.0)
##
   cluster
                          2.1.0
                                    2019-06-19 [3] CRAN (R 4.0.2)
                                    2020-09-10 [1] Bioconductor
##
                        * 3.17.3
   clusterProfiler
##
    codetools
                          0.2 - 16
                                    2018-12-24 [3] CRAN (R 4.0.2)
##
                          1.4-1
                                    2019-03-18 [1] RSPM (R 4.0.0)
   colorspace
##
    cowplot
                          1.1.0
                                    2020-09-08 [1] RSPM (R 4.0.2)
##
   crayon
                          1.3.4
                                    2017-09-16 [2] RSPM (R 4.0.0)
##
    curl
                          4.3
                                    2019-12-02 [2] RSPM (R 4.0.0)
   data.table
##
                          1.13.0
                                    2020-07-24 [1] RSPM (R 4.0.2)
##
   DBI
                          1.1.0
                                    2019-12-15 [1] RSPM (R 4.0.0)
##
   dbplyr
                          1.4.4
                                    2020-05-27 [1] RSPM (R 4.0.0)
                                    2020-07-14 [1] Bioconductor
##
   DelayedArray
                        * 0.15.7
##
   derfinder
                          1.23.1
                                    2020-05-10 [1] Bioconductor
                                    2020-05-10 [1] Bioconductor
   derfinderHelper
                          1.23.1
                          1.2.0
                                    2018-05-01 [2] RSPM (R 4.0.0)
##
   desc
                                    2020-07-21 [2] RSPM (R 4.0.2)
##
   devtools
                        * 2.3.1
##
                           0.6.25
                                    2020-02-23 [2] RSPM (R 4.0.0)
   digest
##
  DO.db
                           2.9
                                    2020-09-10 [1] Bioconductor
## doRNG
                           1.8.2
                                    2020-01-27 [1] RSPM (R 4.0.0)
##
   DOSE
                           3.15.0
                                    2020-04-27 [1] Bioconductor
##
   downloader
                          0.4
                                    2015-07-09 [1] RSPM (R 4.0.0)
##
   dplyr
                          1.0.2
                                    2020-08-18 [1] RSPM (R 4.0.2)
##
   edgeR
                        * 3.31.4
                                    2020-06-10 [1] Bioconductor
```

```
2020-05-15 [2] RSPM (R 4.0.0)
    ellipsis
                            0.3.1
##
    enrichplot
                            1.9.1
                                     2020-04-29 [1] Bioconductor
##
    europepmc
                            0.4
                                     2020-05-31 [1] RSPM (R 4.0.0)
                                     2019-05-28 [2] RSPM (R 4.0.0)
##
    evaluate
                            0.14
##
    fansi
                            0.4.1
                                     2020-01-08 [2] RSPM (R 4.0.0)
##
    farver
                            2.0.3
                                     2020-01-16 [1] RSPM (R 4.0.0)
    fastmatch
                            1.1-0
                                     2017-01-28 [1] RSPM (R 4.0.0)
                                     2020-04-27 [1] Bioconductor
##
    fgsea
                            1.15.0
##
    foreach
                            1.5.0
                                     2020-03-30 [1] RSPM (R 4.0.0)
                            0.8-80
                                     2020-05-24 [3] CRAN (R 4.0.2)
##
    foreign
    Formula
                            1.2-3
                                     2018-05-03 [1] RSPM (R 4.0.0)
                                     2020-07-31 [2] RSPM (R 4.0.2)
##
    fs
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##
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    ggraph
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    ggrepel
    ggridges
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    Hmisc
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                            0.1-8.1
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##
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2020-06-08 [1] RSPM (R 4.0.2)
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    pkgconfig
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    RColorBrewer
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    RCurl
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##
    stringi
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##
    stringr
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                                     2019-02-10 [2] RSPM (R 4.0.0)
```

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SummarizedExperiment * 1.19.6
                                   2020-07-09 [1] Bioconductor
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##
   survival
                          3.2-3
## testthat
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## tibble
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## tidygraph
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##
  tidyr
                        * 1.1.2
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## tidyselect
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## tinytex
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## triebeard
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## tweenr
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                                   2018-12-14 [1] RSPM (R 4.0.2)
## urltools
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                        * 1.6.3
                                   2020-09-17 [2] RSPM (R 4.0.2)
## usethis
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## vctrs
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## viridisLite
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## withr
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                                   2020-09-09 [2] RSPM (R 4.0.2)
## xfun
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## XML
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## xml2
                                   2020-04-23 [2] RSPM (R 4.0.0)
                          1.3.2
## XVector
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## yaml
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## zlibbioc
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## [1] /usr/local/lib/R/host-site-library
```

- ## [2] /usr/local/lib/R/site-library
- ## [3] /usr/local/lib/R/library