R implementation

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Loaded functions:

```
#source("/media/Data/Dropbox/humanR/01funcs.R")
rm(list=ls())
#setwd("/media/Data/Dropbox/humanR/PD/")
#setwd("~/Dropbox/humanR/PD/")
###load("PD.Rdata", .GlobalEnv)
#lsos(pat="")
```

Load packages.

```
pkgs <- c('gdata','caret','leaps','glmnet','lattice','latticeExtra',
          'ggplot2', 'dplyr', 'tidyr', 'RColorBrewer','igraph',
          'DescTools')
lapply(pkgs, require, character.only = TRUE)

Warning in library(package, lib.loc = lib.loc, character.only = TRUE, logical.return
= TRUE, : there is no package called 'gdata'
Warning in library(package, lib.loc = lib.loc, character.only = TRUE, logical.return
= TRUE, : there is no package called 'caret'
Warning in library(package, lib.loc = lib.loc, character.only = TRUE, logical.return
= TRUE, : there is no package called 'glmnet'
Warning in library(package, lib.loc = lib.loc, character.only = TRUE, logical.return
= TRUE, : there is no package called 'igraph'
```

1 Data structure

Data is from patients with Lymphoma tumors, either undergone or not a Rituximab CHOP treatment. Some patients show relapse after treatment. Tumors migrate though nodal (lymphnodes) or extranodal tissues. Tumors involve two different subtypes of cells of origin, ABC or GCB. **The first aim is to find correlation genes that respond differently to treatment, nodal transmission, and cell subtypes.**

```
metadata <- read.table("data/phenodata", sep = "\t", header = T)
```

```
head(metadata)
```

```
  SAMPLE_ID PATIENT_ID Timepoint OTHER_ID   res_id INCLUDE_MATCHING
1 CNR1001T1    CNR1001        T1          01-18186              YES
2 CNR1002T1    CNR1002        T1          01-26575              YES
3 CNR1002T2    CNR1002        T2          01-26575              YES
4 CNR1003T1    CNR1003        T1          02-10117              YES
5 CNR1006T1    CNR1006        T1 DLC_0304 03-11110              YES
6 CNR1007T1    CNR1007        T1 DLC_0193 03-26640              YES
  INCLUDED_SUBMISSION_TCAG             GROUP SITE Normalization Score
1                      YES CNS_RELAPSE_RCHOP   SO            37   789
2                      YES CNS_RELAPSE_RCHOP   GA            60  3548
3                      YES CNS_RELAPSE_RCHOP  CNS            62  3941
4                      YES CNS_RELAPSE_RCHOP   SO            79  -355
5                      YES CNS_RELAPSE_RCHOP   LN           843  -245
6                      YES CNS_RELAPSE_RCHOP   SO           143  3469
  ABClikelihood Prediction BCL2_BA BCL6_BA MYC_BA DH COMMENT CODE_OS
1             0        GCB       0       0      1  0               1
2             1        ABC       0       0      0  0               1
3             1        ABC       0       0      0  0               1
4             0        GCB       1       1      1  1               1
5             0        GCB       1       0      0  0               1
6             1        ABC       0       0      0  0               1
  CODE_DSS CODE_PFS CODE_TTP CODE_CNS Overall.survival..y.
1        1        1        1        1                 0.87
2        1        1        1        1                 2.98
3        1        1        1        1                 2.98
4        1        1        1        1                 0.60
5        1        1        1        1                 0.42
6        1        1        1        1                 4.64
  Disease.specific.survival..y. Progression.free.survival..y.
1                          0.87                           0.52
2                          2.98                           0.38
3                          2.98                           0.38
4                          0.60                           0.31
5                          0.42                           0.13
6                          4.64                           0.54
  Time.to.progression..y. Time.to.CNS.relapse..y. SEX AGE STAGE
1                    0.52                    0.52   F  82    4B
2                    0.38                    0.38   F  77    4A
3                    0.38                    0.38   F  77    4A
4                    0.31                    0.31   F  54    4A
5                    0.13                    0.15   M  59   2BE
6                    0.54                    0.45   M  62   1AE
  STAGEGRP E4SITE PS LDH LDHNORML LDHRATIO MASS IPI IPI_GROUP
1      ADV   BoSo  0 997      415     2.40   14   4         3
2      ADV   GaKi  1  -1      210    -1.00    1  -1         2
3      ADV   GaKi  1  -1      210    -1.00    1  -1         2
4      ADV SoOvUt  4 993      210     4.73   11   4         3
5      ADV     Gi  2 861      540     1.59    5   2         2
6      LIM   BoSo  1 424      210     2.02    7   3         2
  CNS.RiskScore CNS.RiskGrp Rehyb
1             4           3    NO
2            -1          -1   YES
3            -1          -1   YES
4             4           3    NO
5             2           2    NO
6             3           2    NO
```

In the first steps of the analysis, the samples will be classified (supervised) into the following categories.

```
metadata <- read.table("data/phenodata", sep = "\t", header = T) %>%
    dplyr::select(SAMPLE_ID, Timepoint, GROUP, SITE, Score, Prediction, ABClikelihood) %>%
    filter(Timepoint != "T2") %>%
    mutate(Groups = case_when(GROUP %in% c("CNS_RELAPSE_RCHOP",
                                             "CNS_RELAPSE_CHOPorEQUIVALENT",
                                             "CNS_DIAGNOSIS") ~ "CNS",
                                GROUP %in% c("TESTICULAR_NO_CNS_RELAPSE", "NO_RELAPSE") ~ "NOREL",
                                GROUP == "SYTEMIC_RELAPSE_NO_CNS" ~ "SYST",
                                TRUE ~ "CTRL")) %>%
    mutate(ABClassify = case_when(ABClikelihood >= .9 ~ "ABC",
                                   ABClikelihood <= .1 ~ "GCB",
                                   TRUE ~ "U")) %>%
    mutate(ABCScore = case_when(Score > 2412 ~ "ABC",
                                 Score <= 1900 ~ "GCB",
#                                 Score == NA ~ "NA",
                                 TRUE ~ "U")) %>%
    mutate(Nodes = case_when(SITE == "LN" ~ "LN",
                              SITE == "TO" ~ "LN",
                              SITE == "SP" ~ "LN",
                              TRUE ~ "EN")) %>%
    mutate(Lymphnodes = case_when(Nodes == "LN" ~ 1, TRUE ~ 0))

# make sure all samples preserve their ID
metadata$Groups <- as.factor(metadata$Groups)
metadata$ABClassify <- as.factor(metadata$ABClassify)
metadata$ABCScore <- as.factor(metadata$ABCScore)
metadata$Nodes <- as.factor(metadata$Nodes)
metadata$Lymphnodes <- as.factor(metadata$Lymphnodes)

summary(metadata)

     SAMPLE_ID   Timepoint                           GROUP
 CNR1001T1:  1   T1:236    NO_RELAPSE                  :96
 CNR1002T1:  1   T2:  0    SYTEMIC_RELAPSE_NO_CNS      :64
 CNR1003T1:  1             CNS_RELAPSE_RCHOP           :39
 CNR1006T1:  1             TESTICULAR_NO_CNS_RELAPSE   :12
 CNR1007T1:  1             CNS_DIAGNOSIS               :11
 CNR1008T1:  1             CNS_RELAPSE_CHOPorEQUIVALENT: 8
 (Other)  :230             (Other)                     : 6
      SITE         Score       Prediction ABClikelihood    Groups
 LN     :127   Min.   :-881    ABC : 92   Min.   :0.00    CNS  : 58
 SO     : 20   1st Qu.: 676    GCB :103   1st Qu.:0.00    CTRL :  6
 TE     : 18   Median :2106    U   : 39   Median :0.02    NOREL:108
 TO     : 16   Mean   :1820    NA's:  2   Mean   :0.47    SYST : 64
 GI     : 11   3rd Qu.:2941               3rd Qu.:1.00
 SP     :  7   Max.   :4323               Max.   :1.00
 (Other): 37   NA's   :2                  NA's   :4
 ABClassify ABCScore  Nodes    Lymphnodes
 ABC:103    ABC: 92   EN: 86   0: 86
 GCB:117    GCB:103   LN:150   1:150
 U  : 16    U  : 41
```

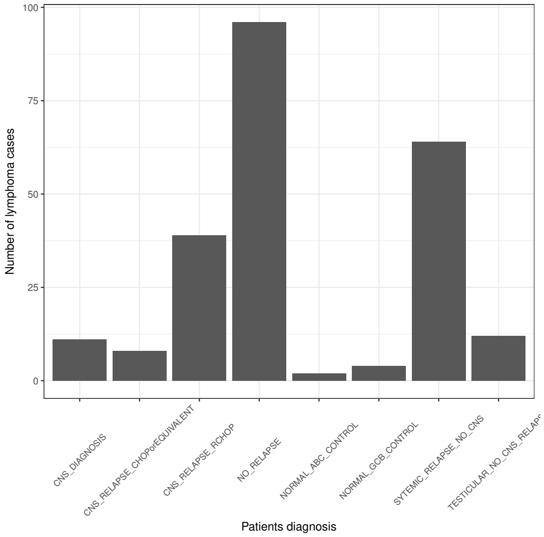
Difference in cases being indexed based on their *cell-of-origin* association subtypes using either of the following features: prediction, ABClassify, ABCScore.

```
x <- metadata %>%
    select(Prediction, ABClassify, ABCScore) %>%
    summary
```

Distribution of samples with different treatments.

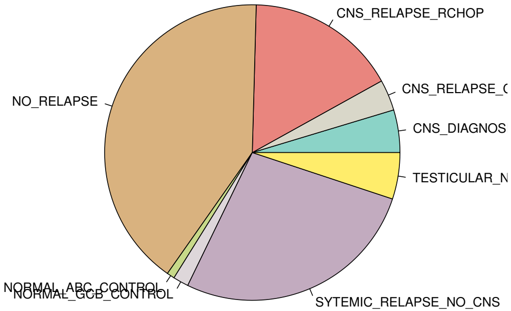
```
metadata %>%
    select(GROUP) %>%
    ggplot(aes(x = GROUP)) +
    geom_histogram(stat = "count") +
    labs(y = "Number of lymphoma cases",
         x = "Patients diagnosis") +
    theme_bw() +
    theme(axis.text.x = element_text(vjust = .5,
                                     angle = 45,
                                     size = 8))

Warning:  Ignoring unknown parameters:  binwidth, bins, pad
```



Or as a pie chart.

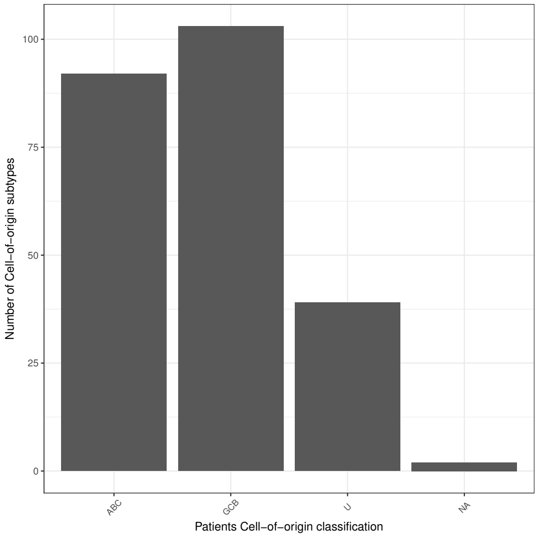
```
palette.pies <- brewer.pal(12, name = "Set3")
palette.pies.adj <- colorRampPalette(palette.pies)(length(unique(metadata$GROUP)))
pie(table(metadata$GROUP), col=palette.pies.adj)
```



Distribution of samples with different cells of origin subtypes.

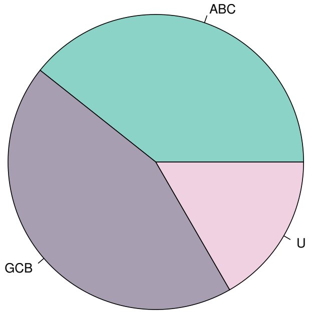
```
metadata %>%
    select(Prediction) %>%
    ggplot(aes(x = Prediction)) +
    geom_histogram(stat = "count") +
    labs(y = "Number of Cell-of-origin subtypes",
         x = "Patients Cell-of-origin classification") +
    theme_bw() +
    theme(axis.text.x = element_text(vjust = .5,
                                     angle = 45,
                                     size = 8))

Warning:  Ignoring unknown parameters:  binwidth, bins, pad
```



Or as pie chart.

```
palette.pies <- brewer.pal(12, name = "Set3")
palette.pies.adj <- colorRampPalette(palette.pies)(length(unique(metadata$Prediction)))
pie(table(metadata$Prediction), col=palette.pies.adj)
```

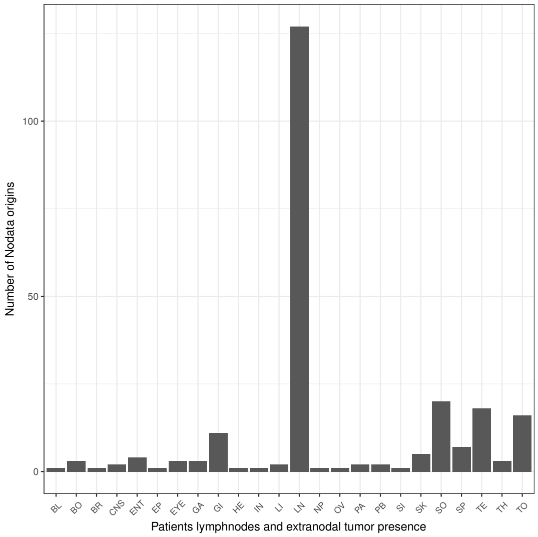


Distribution of samples with different lymphnodes and extranodal cancer metastasis.

```
par(mfrow=c(2,2))
```

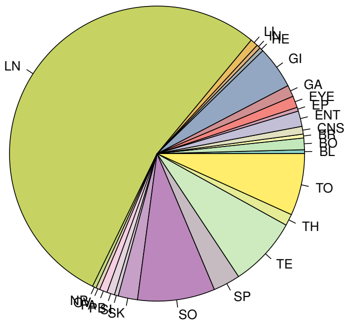
```r
metadata %>%
    select(SITE) %>%
    ggplot(aes(x = SITE)) +
    geom_histogram(stat = "count") +
    labs(y = "Number of Nodata origins",
         x = "Patients lymphnodes and extranodal tumor presence") +
    theme_bw() +
    theme(axis.text.x = element_text(vjust = .5,
                                     angle = 45,
                                     size = 8))
```

```
Warning: Ignoring unknown parameters: binwidth, bins, pad
```



Or as a pie chart.

```r
palette.pies <- brewer.pal(12, name = "Set3")
palette.pies.adj <- colorRampPalette(palette.pies)(length(unique(metadata$SITE)))
pie(table(metadata$SITE), col=palette.pies.adj)
```



2 Differential expression

Genes have been fitted in a model that is based on an Empirical Bayes approach. Ranking of the genes determine if they are statistically significant. Bonferroni correction is used to control the false discovery rate (FDR). Moderated t-statistics, FDR, and fold change (log2) are implemented to reduce selection of false positives.

- **adjpval** is the adjusted P-value to control the FDR using Bonferroni correction. **Genes selected here based on their adjpval are also greater than or equal to the bstat threshold**.

- **avgex** is the average expression the ordinary arithmetic average of the log2-expression values for the probe, across all arrays. **Genes selected here based on their avgex are also greater than or equal to the bstat threshold**.

- **bstat** is the moderated t-statistics using an Empirical Bayes approach generating B-statistics scores.

```
expression <- read.table("data/summary.full.90800.txt", sep = "\t", header = T) %>%
    select(Design, Model, Bthreshold, adjPval, Category, Parameter, Transcripts) %>%
    filter(Category == "total")
summary(expression)

                     Design                             Model
 CNSvsNOREL_ABC          : 54   systemicRelapse             : 54
 CNSvsNOREL_GCB          : 54   systemicRelapseCOOclasses   :162
 CNSvsSYST_ABC           : 54   systemicRelapseCOOprediction:162
 CNSvsSYST_GCB           : 54   systemicRelapseCOOscores    :162
 diffCNSvsNOREL_ABCvsGCB: 54   systemicRelapseNodes        :162
 diffCNSvsSYST_ABCvsGCB : 54
 (Other)                 :378
   Bthreshold        adjPval         Category      Parameter
 Min.   :-2.00   Min.   :0.049   down :  0   adjpval:234
 1st Qu.:-1.00   1st Qu.:0.049   total:702   avgex  :234
 Median : 0.25   Median :0.049   up   :  0   bval   :234
 Mean   : 0.00   Mean   :0.049
 3rd Qu.: 1.00   3rd Qu.:0.049
 Max.   : 1.50   Max.   :0.049

  Transcripts
 Min.   :    0
 1st Qu.:    2
 Median :   46
 Mean   :  580
 3rd Qu.:  463
 Max.   :10578
```

Number of transcripts when comparing B-statistics scores, which represent confidence in selecting each significantly expressed gene.

```
aggregate( Transcripts ~ Bthreshold, data=expression, FUN=range)

  Bthreshold Transcripts.1 Transcripts.2
1       -2.0             0         10578
2       -1.0             0          6448
3        0.0             0          3618
4        0.5             0          2688
5        1.0             0          1976
6        1.5             0          1429
```

Number of transcripts when samples are classed into groups, which are based on clinical data (e.g., cell-of-origin, CNS relapse, and nodal/extranodal tumor transmission).

```
aggregate( Transcripts ~ Model, data=expression, FUN=range)

                         Model Transcripts.1 Transcripts.2
1              systemicRelapse             0          4938
2    systemicRelapseCOOclasses             0         10578
3 systemicRelapseCOOprediction             0         10578
4     systemicRelapseCOOscores             0         10578
5         systemicRelapseNodes             0          6609
```

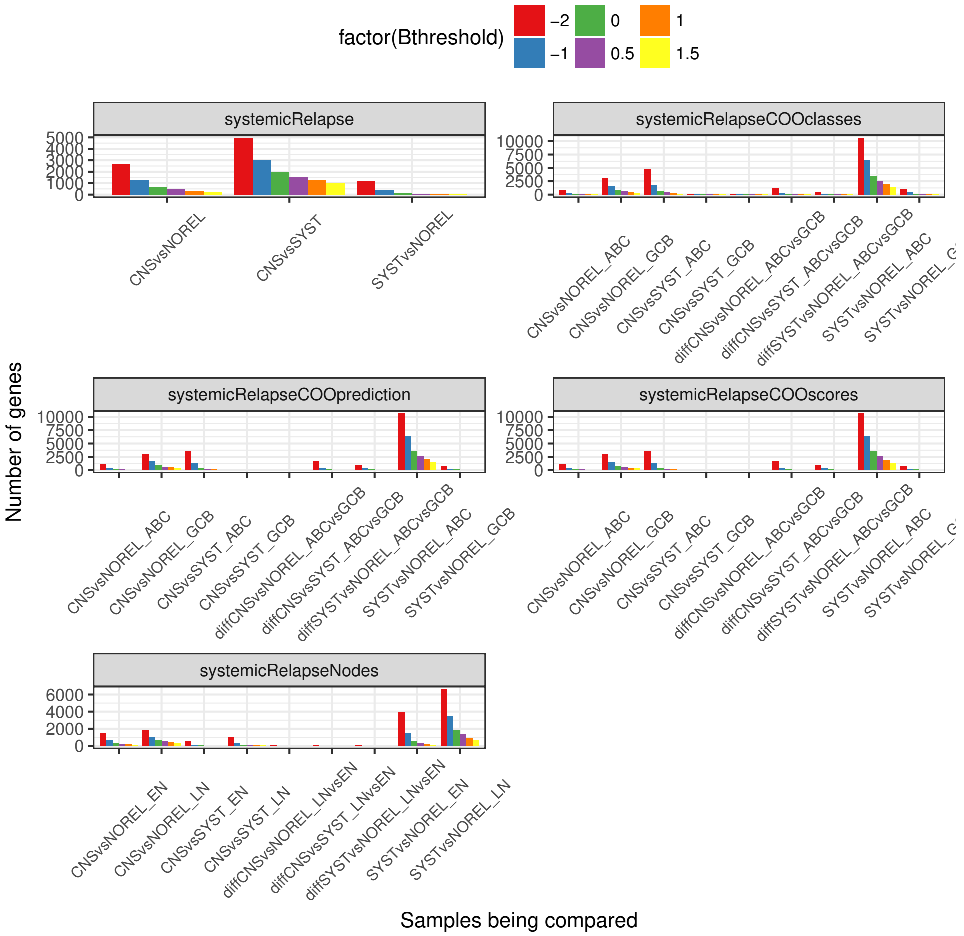
Number of transcripts found when comparing different sample cases indexed based on their clinical data.

```
aggregate( Transcripts ~ Design, data=expression, FUN=range)
```

```
                     Design Transcripts.1 Transcripts.2
1                CNSvsNOREL           116          2678
2            CNSvsNOREL_ABC             2          1082
3             CNSvsNOREL_EN            51          1442
4            CNSvsNOREL_GCB           130          3019
5             CNSvsNOREL_LN           125          1873
6                 CNSvsSYST           441          4938
7             CNSvsSYST_ABC             2          4691
8              CNSvsSYST_EN             3           547
9             CNSvsSYST_GCB             0            98
10             CNSvsSYST_LN             0          1014
11  diffCNSvsNOREL_ABCvsGCB             0            58
12    diffCNSvsNOREL_LNvsEN             0            37
13   diffCNSvsSYST_ABCvsGCB             1          1640
14     diffCNSvsSYST_LNvsEN             0            23
15 diffSYSTvsNOREL_ABCvsGCB             0           868
16   diffSYSTvsNOREL_LNvsEN             0            85
17              SYSTvsNOREL             0          1214
18          SYSTvsNOREL_ABC           704         10578
19           SYSTvsNOREL_EN            35          3907
20          SYSTvsNOREL_GCB             2           994
21           SYSTvsNOREL_LN           295          6609
```

Number of genes that respond to treatment, cell subtypes, and nodal transmission.

```
expression %>%
    ggplot(aes(
        x = Design,
        y = Transcripts,
        fill = factor(Bthreshold))) +
    theme_bw() +
    geom_bar(stat = "identity",
             position = "dodge") +
    facet_wrap( ~ Model,
              ncol = 2,
               scales = "free") +
    scale_fill_brewer(type = "qual", palette = 6) +
    labs(x = "Samples being compared",
         y = "Number of genes") +
    theme(legend.position = "top",
          axis.text.x = element_text(vjust = .5,
                                     angle = 45,
                                     size = 8))
```



3 System Information

The version number of R and packages loaded for generating the vignette were:

```
###save(list=ls(pattern=".*|.*"),file="PD.Rdata")
```

```
sessionInfo()

R version 3.4.3 (2017-11-30)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: elementary OS 0.4.1 Loki

Matrix products: default
BLAS: /usr/lib/libblas/libblas.so.3.6.0
LAPACK: /usr/lib/lapack/liblapack.so.3.6.0

locale:
 [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
 [3] LC_TIME=en_US.UTF-8        LC_COLLATE=en_US.UTF-8
 [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
[7] base

other attached packages:
 [1] Hmisc_4.0-3         Formula_1.2-2       survival_2.41-3
 [4] tabplot_1.3-1       ffbase_0.12.3       ff_2.2-13
 [7] bit_1.1-12          DescTools_0.99.23   knitr_1.17
[10] bindrcpp_0.2        tidyr_0.7.2         dplyr_0.7.4
[13] ggplot2_2.2.1       latticeExtra_0.6-28 RColorBrewer_1.1-2
[16] lattice_0.20-35     leaps_3.0

loaded via a namespace (and not attached):
 [1] tidyselect_0.2.2    purrr_0.2.4         splines_3.4.3
 [4] colorspace_1.3-2    expm_0.999-2        htmltools_0.3.6
 [7] base64enc_0.1-3     rlang_0.1.2         foreign_0.8-69
[10] glue_1.2.0          bindr_0.1           plyr_1.8.4
[13] stringr_1.2.0       munsell_0.4.3       gtable_0.2.0
[16] htmlwidgets_0.9     mvtnorm_1.0-6       evaluate_0.10.1
[19] labeling_0.3        manipulate_1.0.1    htmlTable_1.9
[22] highr_0.6           Rcpp_0.12.13        acepack_1.4.1
[25] scales_0.5.0        backports_1.1.1     checkmate_1.8.5
[28] gridExtra_2.3       fastmatch_1.1-0     digest_0.6.12
[31] stringi_1.1.5       grid_3.4.3          tools_3.4.3
[34] magrittr_1.5        lazyeval_0.2.1      tibble_1.3.4
[37] cluster_2.0.6       pkgconfig_2.0.1     MASS_7.3-47
[40] Matrix_1.2-11       data.table_1.10.4-3 assertthat_0.2.0
[43] R6_2.2.2            boot_1.3-20         rpart_4.1-12
[46] nnet_7.3-12         compiler_3.4.3
```