Data Exploration and Preparation

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Contents

1 Load libraries

```
library(survival)
library(RColorBrewer)
library(dplyr)
library(gplots)
library(igraph)
library(ndexr)
library(RCX)
```

2 Patient information

We applied our methods to a large breast cancer patient dataset that we previously studied and preprocessed [27]. That data is compiled out of 10 public microarray datasets measured on Affymetrix Human Genome HG-U133 Plus 2.0 and HG-U133A arrays. The datasets are available from the Gene Expression Omnibus (GEO) [28] data repository (accession numbers GSE25066, GSE20685, GSE19615, GSE17907, GSE16446, GSE17705, GSE2603, GSE11121, GSE7390, GSE6532). The RMA probe-summary algorithm [29] was used to process each of the datasets, and only samples with metadata on metastasis-free survival were selected and combined together on the basis of HG-U133A array probe names. Quantile normalization was applied over all datasets. In the case of several probes mapping to one gene, only the probe with the highest average value was considered.

The patients were assigned to one of two classes: 393 patients with distant metastasis within the first 5 years and 576 patients without metastasis having the last follow-up between 5 and 10 years. Breast cancer molecular subtypes for the patient samples were predicted in [27] utilizing genefu R-package [30].

The constant of labels_GEO_HG.csv

1) People who had metastatic event during the first 0-5 years (correspond to "1"). 393 patients.
2) People who did not have metastatic event during the first five years and who had the last follow up between 5 and 10 years. No metastatic events at all. This class corresponds to "0". 576 patients.

We retrained the Graph-CNN on 872 patients and generated relevances for **97 test patients**.

It is not stated which patients those 97 patients are!!! Use own information

Load the patient information about the 97 patients:

```
patients <- read.csv("data/patient_information.csv", stringsAsFactors = F)
head(patients)
##   Patient.ID label Predicted Concordance subtype mfs.years met.event
## 1  GSM615233     1         1           1    LumA 0.7910000         1
## 2  GSM519226     0         1           0    LumA 9.8000000         0
## 3  GSM411347     1         1           1    LumB 0.6194444         1
## 4  GSM615195     1         1           1   Basal 0.7640000         1
## 5  GSM615097     0         1           0    LumA 5.5520000         0
## 6  GSM615221     1         1           1    LumB 3.3620000         1
```

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The patient data is composed of different subtypes with and without metastatic events:

```
table(patients[, c("subtype", "met.event")])
##         met.event
## subtype   0  1
##   Basal   6 13
##   Her2    1  4
##   LumA   25  3
##   LumB   24 18
##   Normal  2  1
```

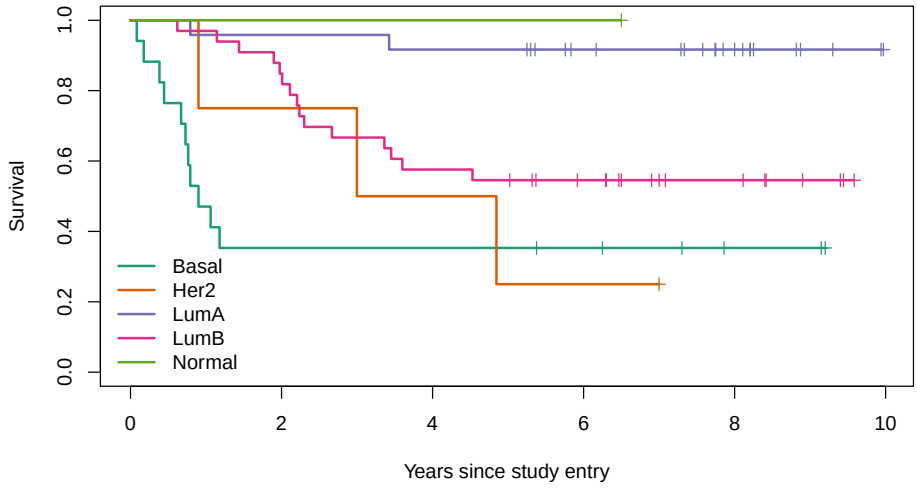
There is a difference between labeled and predicted metastatic event. Therefore the patients are filtered for only correctly predicted metastatic events:

```
## Check colnames
colnames(patients) <- gsub("Patient.ID", "geo_accession", colnames(patients))
colnames(patients) <- gsub("Predicted", "predicted", colnames(patients))
# colnames(patients) = gsub('Concordance',
# 'concordance', colnames(patients))

## Only select patients, that are correctly classified
patients <- patients[patients$Concordance == 1, ]
patients$Concordance <- NULL
patients <- patients[order(patients$subtype),
    ]

## differentiate between the two groups
selMetastatic <- patients$met.event == 1

head(patients)
##    geo_accession label predicted subtype mfs.years met.event
## 4      GSM615195     1         1   Basal 0.7640000         1
## 12     GSM615184     0         0   Basal 7.3020000         0
## 16     GSM519380     0         0   Basal 9.2000000         0
## 31     GSM615651     1         1   Basal 0.7912389         1
## 36     GSM411374     1         1   Basal 0.4444444         1
## 38     GSM411334     1         1   Basal 1.0611111         1
```

Survival by subtype:

```
survival <- survfit(
    Surv(mfs.years, met.event) ~
        subtype, data = patients
)
print(survival)
## Call: survfit(formula = Surv(mfs.years, met.event) ~ subtype, data = patients)
##
##                 n events median 0.95LCL 0.95UCL
## subtype=Basal  17     11   0.90   0.728      NA
## subtype=Her2    4      3   3.92   0.900      NA
## subtype=LumA   24      2     NA      NA      NA
## subtype=LumB   33     15     NA   3.362      NA
## subtype=Normal  1      0     NA      NA      NA
```

```
colors <- brewer.pal(5, "Dark2")
names(colors) <- c("Basal", "Her2", "LumA", "LumB", "Normal")

plot(
    survival, col = colors, lwd = 2, mark.time = TRUE, xlab = "Years since study entry",
    ylab = "Survival"
)
legend(
    "bottomleft", names(colors),
    col = colors, lwd = 2, bty = "n"
)
```



3 Gene expression

http://mypathsem.bioinf.med.uni-goettingen.de/resources/glrp http://mypathsem.bioinf.med.uni-goettingen.de/fileadmin/mypathsem_resources/WP3/GEO_HG_PPI.csv.zip

Gene expression of the whole data set:

```
ge_all <- read.csv("data/GEO_HG_PPI.csv.gz", stringsAsFactors = F)
```

Get the column names for the genes and the patient ids:

```
ge_gene_col <- "probe"
ge_patient_cols <- colnames(ge_all)[colnames(ge_all) !=
    ge_gene_col]
```

Get the mean, standard deviation and boundaries for the 25% and 75% quantile for each gene (probe) based on its expression in all patients.

```
ge_with_statistics <- ge_all %>%
    rowwise() %>%
    do(
        {
            curRow <- unlist(.[ge_patient_cols])
```

```
                result <- data.frame(., stringsAsFactors = F)
                result["mean"] <- mean(curRow)
                result["stdev"] <- sd(curRow)
                quartiles <- quantile(curRow, probs = c(0, 0.25, 0.4, 0.5, 0.6, 0.75, 1))
                result["Q25"] <- quartiles["25%"]
                result["Q50"] <- quartiles["50%"]
                result["Q75"] <- quartiles["75%"]
                result
            }
        )

    print(
        unique(
            gsub("GSM[0-9]+", "GSM*", colnames(ge_with_statistics))
        )
    )
```

```
## [1] "GSM*"  "probe" "mean"  "stdev" "Q25"   "Q50"   "Q75"
```

```
tmp_no_col <- dim(ge_with_statistics)[2]
head(ge_with_statistics[, (tmp_no_col - 5):tmp_no_col])
##    probe     mean     stdev      Q25      Q50      Q75
## 1  RPL41 13.79057 0.2712889 13.67275 13.84122 14.02797
## 2 EEF1A1 13.74600 0.3086460 13.56383 13.79573 13.94892
## 3   TPT1 13.48992 0.2890993 13.30366 13.49129 13.71161
## 4 RPL23A 13.32441 0.3110418 13.09581 13.30366 13.56383
## 5    UBC 13.30906 0.3111604 13.13044 13.28657 13.49129
## 6   RPS2 13.29590 0.2223523 13.15547 13.26993 13.40642
```

This data can be used to calculate the expression level of a gene (LOW, NORMAL or HIGH) using quartile boundaries.

```
ge_expression_level_by_quantiles <- ge_with_statistics %>%
    rowwise() %>%
    do(
        {
            curRow <- unlist(.[ge_patient_cols])
            l <- unlist(.["Q25"])
            h <- unlist(.["Q75"])

            curRow[ge_patient_cols] <- ifelse(
                curRow < l, "LOW", ifelse(curRow > h, "HIGH", "NORMAL")
            )
            curRow <- c(
                unlist(.[ge_gene_col]),
                curRow
            )
            result <- as.data.frame(
                t(curRow),
                stringsAsFactors = F
            )
```

```
                result
            }
        )

    print(
        unique(
            gsub(
                "GSM[0-9]+", "GSM*", colnames(ge_expression_level_by_quantiles)
            )
        )
    )
```

```
    ## [1] "probe" "GSM*"
```

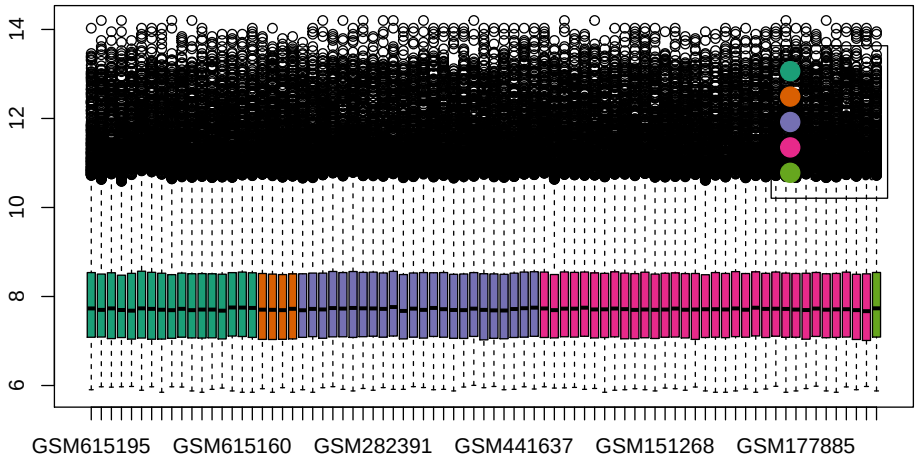
```
    head(ge_expression_level_by_quantiles[, 1:7])
    ##    probe GSM177885 GSM177887 GSM177894 GSM177895 GSM177899 GSM177900
    ## 1  RPL41    NORMAL    NORMAL      HIGH      HIGH      HIGH    NORMAL
    ## 2 EEF1A1       LOW      HIGH    NORMAL    NORMAL       LOW    NORMAL
    ## 3   TPT1    NORMAL    NORMAL    NORMAL       LOW    NORMAL       LOW
    ## 4 RPL23A      HIGH    NORMAL      HIGH      HIGH      HIGH      HIGH
    ## 5    UBC    NORMAL    NORMAL    NORMAL    NORMAL       LOW    NORMAL
    ## 6   RPS2      HIGH    NORMAL    NORMAL    NORMAL       LOW      HIGH
```

Get the gene expression only for the patients and order patients by subtype

```
    ge_patients <- ge_all[, patients$geo_accession[order(patients$subtype)]]
    rownames(ge_patients) <- ge_all$probe
```

Check the gene expression data to be normalized, therefore make a boxplot of the expression data:

```
    boxColors <- sapply(
        colnames(ge_patients),
        function(x) {
            subtype <- patients$subtype[patients$geo_accession ==
                x]
            return(colors[subtype])
        }
    )

    boxplot(ge_patients, col = boxColors)
    par(xpd = TRUE)
    legend(
        "topright", legend = names(colors),
        col = colors, bty = "o", pch = 20, pt.cex = 3, cex = 1,
        horiz = FALSE, inset = c(0.03, 0.1)
    )
```



3.1 Differential gene expression

t-test

```
## perform a t-test for each gene
ttest <- apply(
    ge_patients, 1, function(x) {
        res <- t.test(x[selMetastatic], y = x[!selMetastatic])
        return(res$p.value)
    }
)

## adjust for multiple testing
de <- data.frame(
    prope = names(ttest),
    pvalue = ttest, qvalue = p.adjust(ttest)
)

## re-order by q-value
de <- de[order(de$qvalue),
    ]

## select differentially expressed genes
de_genes <- de$prope[de$qvalue < 0.01]
print(de[de_genes, ])
##       prope       pvalue       qvalue
## MELK   MELK 9.070127e-09 6.247503e-05
## PLAT   PLAT 2.482120e-08 1.709436e-04
## RRM2   RRM2 3.452896e-08 2.377665e-04
## CENPE CENPE 8.659404e-08 5.962000e-04
## TPX2   TPX2 1.076304e-07 7.409279e-04
## PDE4A PDE4A 1.096930e-07 7.550171e-04
## FMOD   FMOD 1.167650e-07 8.035766e-04
## KCND3 KCND3 1.292407e-07 8.893055e-04
## AURKA AURKA 1.322919e-07 9.101685e-04
## HJURP HJURP 1.606460e-07 1.105084e-03
```

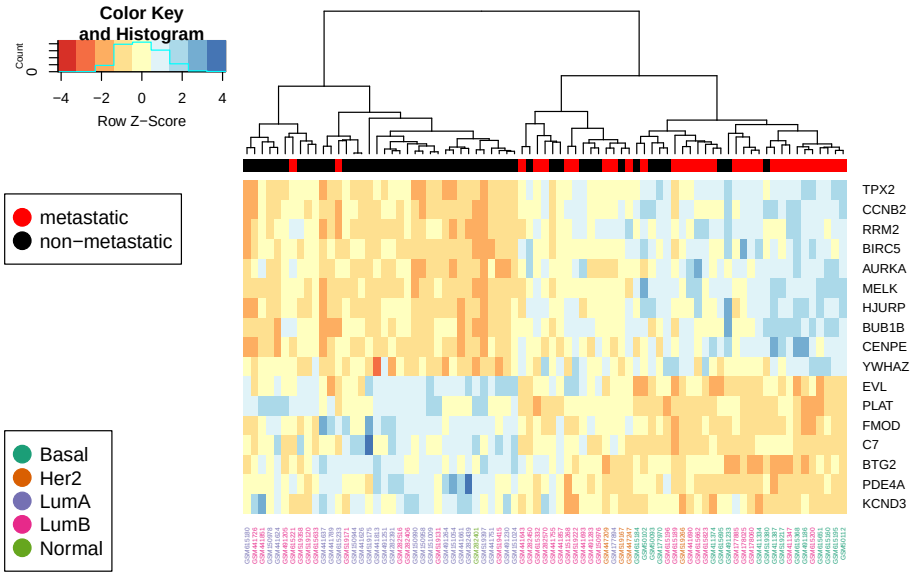
```
## BIRC5 BIRC5 2.889588e-07 1.987459e-03
## CCNB2 CCNB2 4.029074e-07 2.770795e-03
## C7       C7 6.099449e-07 4.193981e-03
## EVL     EVL 7.821289e-07 5.377136e-03
## BTG2   BTG2 1.014067e-06 6.970694e-03
## BUB1B BUB1B 1.034800e-06 7.112181e-03
## YWHAZ YWHAZ 1.176227e-06 8.083035e-03
```

3.2 Heatmap visualization

A heatmap is a graphical representation of data where the individual values contained in a matrix are represented as colors. Now that we have a subset of DE genes, we can use their counts to generate a heatmap. We expect DE genes to be able to separate the samples from different groups into different clusters of the dendrograms.

```
# colors
pal <- brewer.pal(9, "RdYlBu")

# heatmap
heatmap.2(
  as.matrix(ge_patients[de_genes,]) ,
  dist=function(x) {as.dist(1-cor(t(x)))},
  scale="row",
  col=pal,
  colCol = boxColors,
  ColSideColors = ifelse(patients$met.event == 1, "red", "black"),
  trace="none",
  cexRow=1,
  cexCol=0.5,
  dendrogram = "column"
)

## legend
par(xpd=TRUE)

## non-/metastatic
legend("topleft",
       legend = c("metastatic", "non-metastatic") ,
       col = c("red", "black") ,
       bty = "o", pch=20 , pt.cex = 3, cex = 1,
       horiz = FALSE,
       inset=c(-0.1,0.25)
)

## subtypes
legend("bottomleft",
       legend = names(colors) ,
       col = colors ,
       bty = "o", pch=20 , pt.cex = 3, cex = 1,
       horiz = FALSE,
```

```
        inset=c(-0.1,-0.27)
)
```



4 Relevance score

Paper supplement:

```
relevance_score <- read.csv("data/ppi_relevance_score.csv")
print(
    unique(gsub("GSM[0-9]+", "GSM*", colnames(relevance_score)))
)
## [1] "GSM*"  "probe"
```

```
tmp_no_col <- dim(relevance_score)[2]
head(relevance_score[, (tmp_no_col - 6):tmp_no_col])
##      GSM178060    GSM282570    GSM519415    GSM151009    GSM150958    GSM519222  probe
## 1 0.0005343682 0.0005319004 0.0005322866 0.0005033427 0.0005299115 0.0005491435  RPL41
## 2 0.0008184925 0.0008857118 0.0008743374 0.0008016430 0.0008554613 0.0008497383 EEF1A1
## 3 0.0004312475 0.0004374442 0.0004494849 0.0004692455 0.0004679081 0.0004303246   TPT1
## 4 0.0003852157 0.0003426229 0.0003371786 0.0003447103 0.0003699302 0.0003501706 RPL23A
## 5 0.0005625393 0.0005547676 0.0006526647 0.0005729415 0.0006054741 0.0006456108    UBC
## 6 0.0004418110 0.0004219787 0.0004466759 0.0004548862 0.0004237262 0.0004902194   RPS2
```

5 PPI networks

We used the Human Protein Reference Database (HPRD) protein-protein interaction (PPI) network [26] as the molecular network to structure the gene expression data. The database contains protein-protein interaction information based on yeast two-hybrid analysis, in vitro and in vivo methods. The PPI network is an undirected graph with binary interactions between pairs of proteins. The graph is not connected.

[26] Keshava Prasad TS, Goel R, Kandasamy K, Keerthikumar S, Kumar S, Mathivanan S, Telikicherla D, Raju R, Shafreen B, Venugopal A, Balakrishnan L, Marimuthu A, Banerjee S, Somanathan DS, Sebastian A, Rani S, Ray S, Harrys Kishore CJ, Kanth S, Ahmed M, Kashyap MK, Mohmood R, Ramachandra YL, Krishna V, Rahiman BA, Mohan S, Ranganathan P, Ramabadran S, Chaerkady R, Pandey A. Human protein reference database?2009 update. Nucleic Acids Res. 2009; 37:767–72. https://doi.org/10.1093/nar/gkn892.

From website http://hprd.org/ Latest release: Apr 13, 2010

5.1 From publication:

http://mypathsem.bioinf.med.uni-goettingen.de/resources/glrp

http://mypathsem.bioinf.med.uni-goettingen.de/fileadmin/mypathsem_resources/WP3/HPRD_PPI.csv.zip

```
ppi_network_matrix <- read.csv("data/HPRD_PPI.csv.gz", check.names = FALSE)
ppi_network_igraph <- graph_from_adjacency_matrix(
    as.matrix(ppi_network_matrix),
    mode = "undirected"
)

gorder(ppi_network_igraph)
## [1] 6888
gsize(ppi_network_igraph)
## [1] 27859
```

Remove multiple edges and loops from one node to itself from the network.

```
ppi_network_igraph <- simplify(
    ppi_network_igraph, remove.multiple = T, remove.loops = T
)

gorder(ppi_network_igraph)
## [1] 6888
gsize(ppi_network_igraph)
## [1] 27841
```

Prepare the igraph network for converting to RCX, therefore IDs have to be set for nodes and edges. The CX convention is, in contrast to R, that the IDs start at 0.

```
V(ppi_network_igraph)$id <- seq(
    0, gorder(ppi_network_igraph) -
        1
)
E(ppi_network_igraph)$id <- seq(
    0, gsize(ppi_network_igraph) -
        1
)

summary(ppi_network_igraph)
## IGRAPH edae6cb UN-- 6888 27841 --
## + attr: name (v/c), id (v/n), id (e/n)
```

For the conversion from igraph to RCX, the vertex attribute containing the node names has to be specified.

```
ppi_network_rcx <- fromIgraph(ppi_network_igraph, nodeName = "name")
ppi_network_rcx$metaData
## Meta-data:
##    name version idCounter elementCount consistencyGroup
## 1 nodes     1.0      6887         6888                1
## 2 edges     1.0     27840        27841                1
```

Add some information to the network

```
networkAttributes <- createNetworkAttributes(
  name = c("author",
           "name",
           "description",
           "reference",
           "organism",
           "networkType"),
  value = c("Florian J. Auer",
            "Human Protein Reference Database (HPRD) PPI network",
            paste0('Protein-protein interaction (PPI) network from the ',
                   '<a href="http://hprd.org/" target="_blank">',
                   'Human Protein Reference Database (HPRD)</a> ',
                   'used for training and generating subnetworks'),
            paste0('Chereda, H., Bleckmann, A., Menck, K. et al. ',
                   'Explaining decisions of graph convolutional neural networks: ',
                   'patient-specific molecular subnetworks responsible for ',
                   'metastasis prediction in breast cancer. Genome Med 13, 42 (2021). ',
                   '<a href="https://doi.org/10.1186/s13073-021-00845-7" ',
                   'target="_blank">https://doi.org/10.1186/s13073-021-00845-7</a>'),
            "Homo sapiens",
            "Protein-protein interaction")
)
ppi_network_rcx <- updateNetworkAttributes(ppi_network_rcx, networkAttributes)

ppi_network_rcx$metaData
## Meta-data:
##                name version idCounter elementCount consistencyGroup
## 1             nodes     1.0      6887         6888                1
## 2             edges     1.0     27840        27841                1
## 3 networkAttributes     1.0        NA            6                1
```

5.2 Upload to NDEx

To make the network available to for further analyses, we can upload the network to the NDEx platform (https://www.ndexbio.org/). Of course for this an account is required.

```
ndex_con <- ndex_connect(username = "florianjauer", password = "****")
ndexHPRDuuid <- ndex_create_network(ndex_con, ppi_network_rcx)
```

Until now, the network is only visible to the owner. To change that, and make it visible to everyone, we have to update this property:

```
ndex_network_set_systemProperties(ndex_con, ndexHPRDuuid, visibility = TRUE)
```

5.3 Load from NDEx:

This network is also available on the NDEx platform as "Human Protein Reference Database (HPRD) PPI network":

https://www.ndexbio.org/viewer/networks/079f4c66-3b77-11ec-b3be-0ac135e8bacf

The R package `ndexr` can be used to download the network from NDEx:

```
ndex_con <- ndex_connect()
ndexHPRD <- ndex_find_networks(ndex_con, "HPRD AND owner:florianjauer")

print(
    ndexHPRD[c(
        "name", "owner", "externalId", "nodeCount", "edgeCount"
    )]
)
##                                                 name        owner                            externalId no
## 1 Human Protein Reference Database (HPRD) PPI network florianjauer 079f4c66-3b77-11ec-b3be-0ac135e8bacf
```

```
ppi_network_rcx <- ndex_get_network(ndex_con, "079f4c66-3b77-11ec-b3be-0ac135e8bacf")
print(ppi_network_rcx$metaData)
## Meta-data:
##                 name version idCounter elementCount consistencyGroup
## 1              nodes     1.0      6887         6888                1
## 2              edges     1.0     27840        27841                1
## 3 networkAttributes     1.0        NA            7                1
```

```
print(ppi_network_rcx$networkAttributes$name)
## [1] "name"        "description" "version"     "author"      "networkType" "organism"    "reference"
```

NDEx added automatically the version to the network attributes.

6 Session info

```
sessionInfo()
## R version 4.0.3 (2020-10-10)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 20.04.3 LTS
##
## Matrix products: default
## BLAS:   /usr/lib/x86_64-linux-gnu/blas/libblas.so.3.9.0
## LAPACK: /usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.9.0
##
## locale:
```

```
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C               LC_TIME=de_DE.UTF-8        LC_COLLATE=en_US.UT
##  [6] LC_MESSAGES=en_US.UTF-8    LC_PAPER=de_DE.UTF-8       LC_NAME=C                  LC_ADDRESS=C
## [11] LC_MEASUREMENT=de_DE.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
##  [1] XML_3.99-0.8        httr_1.4.2          RJSONIO_1.3-1.6     pacman_0.5.1        devtools_2.4.2      u
##  [8] timeDate_3043.102   pander_0.6.4        xtable_1.8-4        stringr_1.4.0       BiocStyle_2.18.1    k
## [15] igraph_1.2.7        gplots_3.1.1        dplyr_1.0.7         RColorBrewer_1.1-2  survival_3.2-7
##
## loaded via a namespace (and not attached):
##  [1] pkgload_1.2.3       jsonlite_1.7.2      splines_4.0.3       gtools_3.9.2        assertthat_0.2.1    b
##  [8] stats4_4.0.3        remotes_2.4.1       yaml_2.2.1          sessioninfo_1.1.1   pillar_1.6.4        t
## [15] digest_0.6.28       htmltools_0.5.2     Matrix_1.2-18       plyr_1.8.6          pkgconfig_2.0.3     b
## [22] webshot_0.5.2       processx_3.5.2      tibble_3.1.5        generics_0.1.1      ellipsis_0.3.2      b
## [29] cachem_1.0.6        BiocGenerics_0.36.1 cli_3.0.1           mime_0.12           magrittr_2.0.1      c
## [36] memoise_2.0.0       evaluate_0.14       fs_1.5.0            fansi_0.5.0         pkgbuild_1.2.0      g
## [43] tools_4.0.3         formatR_1.11        lifecycle_1.0.1     callr_3.7.0         compiler_4.0.3      j
## [50] tinytex_0.34        rlang_0.4.12        grid_4.0.3          htmlwidgets_1.5.4   crosstalk_1.1.1     b
## [57] testthat_3.1.0      DBI_1.1.1           curl_4.3.2          markdown_1.1        R6_2.5.1            k
## [64] utf8_1.2.2          rprojroot_2.0.2     desc_1.4.0          KernSmooth_2.23-17  stringi_1.7.5       p
## [71] vctrs_0.3.8         tidyselect_1.1.1    xfun_0.27
```