

TXRNAseq_LassoRegressions

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Load libraries

```
library(tidyverse)
library(glmnet)
library(glmnetUtils)
library(caret)
library(Hmisc)

options(max.print=99999)
```

Open files and prep for analysis

```

#log2fc gene data and plant response data
lfc <- read.csv(file="all_DEGs_log2fc.csv",
                header=TRUE, row.names=1, stringsAsFactors=FALSE)
plant <- read.csv(file="RNAseqPlantRespData.csv", header=TRUE, row.names=1, stringsAsFactors=FALSE)

# limit plant data to variables and replicates where RNA was extracted
colnames(plant)

## [1] "Fungus"      "FunCode"     "PhysCode"    "Water"       "Repl"        "rgr"
## [7] "wilt"        "death"       "waterloss"   "totbiom"     "leafcol"

plant_rna <- plant %>%
  dplyr::select(!leafcol) %>%
  dplyr::filter(!is.na(totbiom)) %>%
  dplyr::filter(Fungus != "Alt")

# calculate averages for d calcs
ConD <- plant_rna %>%
  dplyr::filter(Fungus == "Con" & Water == "Drought")
ConD_wilt <- mean(ConD$wilt)
ConD_waterloss <- mean(ConD$waterloss)

ConW <- plant_rna %>%
  dplyr::filter(Fungus == "Con" & Water == "WellWatered")
ConW_wilt <- mean(ConW$wilt)
ConW_waterloss <- mean(ConW$waterloss)

# calculate d values for treatments
plant_rna_d <- plant_rna %>%
  dplyr::filter(Fungus != "Con") %>%
  dplyr::mutate(wilt_d = base::ifelse(Water == "Drought", wilt-ConD_wilt, wilt-ConW_wilt)) %>%
  dplyr::mutate(waterloss_d = base::ifelse(Water == "Drought", waterloss-ConD_waterloss, waterloss-ConW_waterloss))

# check
all(rownames(plant_rna_d) == colnames(lfc))

## [1] TRUE

# transpose
lfc <- t(lfc)
all(rownames(plant_rna_d) == rownames(lfc))

## [1] TRUE

lfc <- as.data.frame(lfc)

# merge
alldata <- merge(plant_rna_d, lfc, by = "row.names", all=TRUE)
alldata <- as.data.frame(alldata)

#export
write.csv(alldata, "TXRNAseq_LassoData.csv")

```

Lasso regressions

Create x variable

```
x <- alldata %>%
  dplyr::select(dplyr::starts_with("PhHal"))
anyNA(x)
```

```
## [1] FALSE
```

```
x <- as.matrix(x)
```

Wilt

```
# assign y
y <- alldata$wilt_d
anyNA(y)
```

```
## [1] FALSE
```

```
set.seed(123)
```

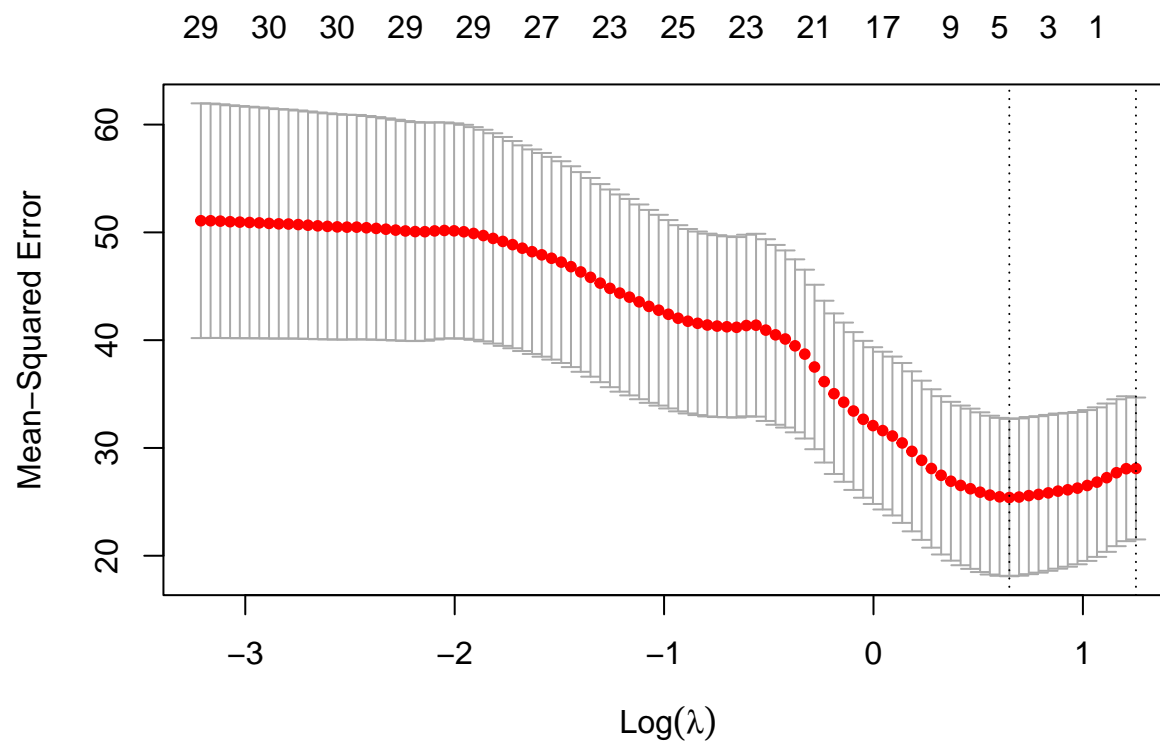
```
# use cross-validation (leave-one-out) to select lambda (minimum)
cvfit_wilt <- glmnet::cv.glmnet(x, y,
                               alpha = 1, nfolds = nrow(x), grouped=FALSE)
cvfit_wilt
```

```
##
## Call:  glmnet::cv.glmnet(x = x, y = y, nfolds = nrow(x), grouped = FALSE,      alpha = 1)
##
## Measure: Mean-Squared Error
##
##      Lambda Index Measure      SE Nonzero
## min  1.913    14   25.40 7.299         4
## 1se  3.502     1   28.09 6.583         0
```

```
r2_wilt <- cvfit_wilt$glmnet.fit$dev.ratio[which(cvfit_wilt$glmnet.fit$lambda == cvfit_wilt$lambda.min)]
r2_wilt
```

```
## [1] 0.3895734
```

```
plot(cvfit_wilt)
```



```
lam_min <- cvfit_wilt$lambda.min
coef(cvfit_wilt, s=lam_min)
```

```
## 2575 x 1 sparse Matrix of class "dgCMatrix"
##               s1
## (Intercept)  -1.536557958
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```

```
#capture.output(print(cvfit_wilt), file = "TXRNAseq_Lasso_wilt.txt")
#capture.output(print(r2_wilt), file = "TXRNAseq_Lasso_wilt.txt", append=TRUE)
#capture.output(coef(cvfit_wilt, s=lam_min), file="TXRNAseq_Lasso_wilt.txt", append=TRUE)
```

Water loss

```
# assign y
y <- alldata$waterloss_d
anyNA(y)
```

```
## [1] FALSE
```

```
set.seed(123)
```

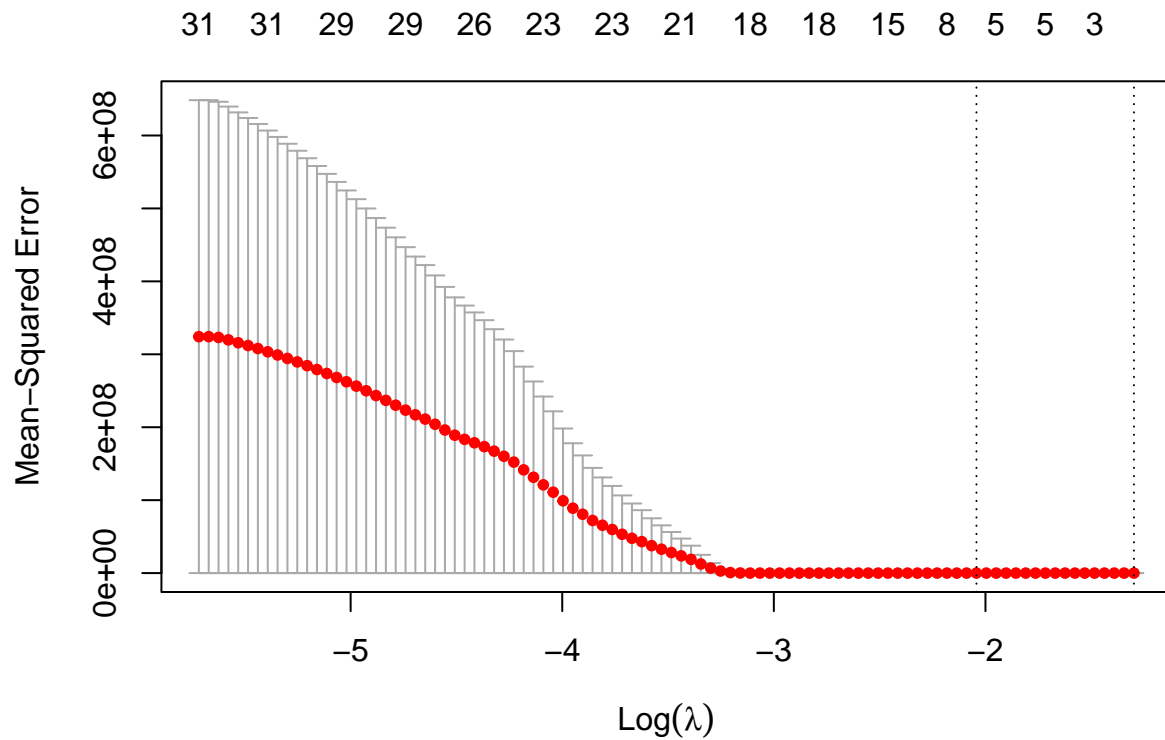
```
# use cross-validation (leave-one-out) to select lambda (minimum)
cvfit_waterloss <- glmnet::cv.glmnet(x, y,
                                     alpha = 1, nfolds = nrow(x), grouped=FALSE)
cvfit_waterloss
```

```
##
## Call:  glmnet::cv.glmnet(x = x, y = y, nfolds = nrow(x), grouped = FALSE,      alpha = 1)
##
## Measure: Mean-Squared Error
##
##      Lambda Index Measure      SE Nonzero
## min 0.1297    17  0.1628 0.03398         7
## 1se 0.2730     1  0.1862 0.03567         0
```

```
r2_waterloss <- cvfit_waterloss$glmnet.fit$dev.ratio[which(cvfit_waterloss$glmnet.fit$lambda == cvfit_w
r2_waterloss
```

```
## [1] 0.5265281
```

```
plot(cvfit_waterloss)
```



```
lam_min <- cvfit_waterloss$lambda.min
coef(cvfit_waterloss, s=lam_min)
```

```
## 2575 x 1 sparse Matrix of class "dgCMatrix"
##                               s1
## (Intercept)    -0.068448448
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```

```
#capture.output(print(cvfit_waterloss), file = "TXRNAseq_Lasso_waterloss.txt")
#capture.output(print(r2_waterloss), file = "TXRNAseq_Lasso_waterloss.txt", append=TRUE)
#capture.output(coef(cvfit_waterloss, s=lam_min), file="TXRNAseq_Lasso_waterloss.txt", append=TRUE)
```

Linear regression for DEGs identified in Lasso

re-open data file if needed

```
alldata <- read.csv("TXRNAseq_LassoData.csv")
```

wilt

```
#PhHAL.7G251400
summary(lm(wilt_d ~ PhHAL.7G251400, data=alldata))
```

```
##
## Call:
## lm(formula = wilt_d ~ PhHAL.7G251400, data = alldata)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -8.4918 -1.0633 -0.0716  1.9053  7.6896
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    -1.3708     0.8032  -1.707  0.09894 .
## PhHAL.7G251400  -3.0438     0.7207  -4.223  0.00023 ***
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 4.148 on 28 degrees of freedom
## Multiple R-squared:  0.3891, Adjusted R-squared:  0.3673
## F-statistic: 17.84 on 1 and 28 DF,  p-value: 0.0002303
```

```
#PhHAL.2G280300
```

```
summary(lm(wilt_d ~ PhHAL.2G280300, data=alldata))
```

```
##
## Call:
## lm(formula = wilt_d ~ PhHAL.2G280300, data = alldata)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -7.674 -1.974 -0.284  2.919  6.112
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -0.2498     0.8414  -0.297   0.769
## PhHAL.2G280300 -2.4406     0.4933  -4.948 3.2e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.877 on 28 degrees of freedom
## Multiple R-squared:  0.4665, Adjusted R-squared:  0.4474
## F-statistic: 24.48 on 1 and 28 DF,  p-value: 3.2e-05
```

```
#PhHAL.2G424800
```

```
summary(lm(wilt_d ~ PhHAL.2G424800, data=alldata))
```

```
##
## Call:
## lm(formula = wilt_d ~ PhHAL.2G424800, data = alldata)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -8.7694 -3.5266  0.4438  2.8907  9.2037
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -2.3163     0.8073  -2.869 0.00774 **
## PhHAL.2G424800 -1.4829     0.4193  -3.537 0.00143 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 4.413 on 28 degrees of freedom
## Multiple R-squared:  0.3088, Adjusted R-squared:  0.2841
## F-statistic: 12.51 on 1 and 28 DF,  p-value: 0.001432
```

```
#PhHAL.2G319800
```

```
summary(lm(wilt_d ~ PhHAL.2G319800, data=alldata))
```

```
##
## Call:
## lm(formula = wilt_d ~ PhHAL.2G319800, data = alldata)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -7.9042 -2.7121 -0.6451  3.2927  6.5473
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.1456     0.9679   0.150 0.881527
## PhHAL.2G319800 -1.9901     0.4600  -4.326 0.000174 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 4.109 on 28 degrees of freedom
## Multiple R-squared:  0.4006, Adjusted R-squared:  0.3792
## F-statistic: 18.71 on 1 and 28 DF,  p-value: 0.0001744
```

water loss

```
#PhHAL.9G036800
summary(lm(waterloss_d ~ PhHAL.9G036800, data=alldata))
```

```
##
## Call:
## lm(formula = waterloss_d ~ PhHAL.9G036800, data = alldata)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.47163 -0.28922  0.01046  0.17136  0.76237
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  -0.03176     0.05952  -0.534   0.598
## PhHAL.9G036800  0.21025     0.04636   4.535 9.88e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3259 on 28 degrees of freedom
## Multiple R-squared:  0.4235, Adjusted R-squared:  0.4029
## F-statistic: 20.57 on 1 and 28 DF,  p-value: 9.88e-05
```

```
#PhHAL.6G027300
summary(lm(waterloss_d ~ PhHAL.6G027300, data=alldata))
```

```
##
## Call:
## lm(formula = waterloss_d ~ PhHAL.6G027300, data = alldata)
##
## Residuals:
```

```
##      Min      1Q   Median      3Q      Max
## -0.52790 -0.26863 -0.03991  0.25304  0.73866
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -0.15007    0.06924   -2.167 0.038866 *
## PhHAL.6G027300  0.11616    0.02970    3.911 0.000534 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3452 on 28 degrees of freedom
## Multiple R-squared:  0.3533, Adjusted R-squared:  0.3302
## F-statistic: 15.29 on 1 and 28 DF,  p-value: 0.0005336
```

```
#PhHAL.7G113600
```

```
summary(lm(waterloss_d ~ PhHAL.7G113600, data=alldata))
```

```
##
## Call:
## lm(formula = waterloss_d ~ PhHAL.7G113600, data = alldata)
##
## Residuals:
##      Min      1Q   Median      3Q      Max
## -0.62014 -0.20444  0.02908  0.21650  0.57189
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -0.03328    0.06093   -0.546 0.589297
## PhHAL.7G113600 -0.23110    0.05398   -4.281 0.000197 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3337 on 28 degrees of freedom
## Multiple R-squared:  0.3957, Adjusted R-squared:  0.3741
## F-statistic: 18.33 on 1 and 28 DF,  p-value: 0.0001967
```

```
#PhHAL.9G394700
```

```
summary(lm(waterloss_d ~ PhHAL.9G394700, data=alldata))
```

```
##
## Call:
## lm(formula = waterloss_d ~ PhHAL.9G394700, data = alldata)
##
## Residuals:
##      Min      1Q   Median      3Q      Max
## -0.43672 -0.25117 -0.03596  0.20987  0.74042
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -0.13516    0.06261   -2.159  0.0396 *
## PhHAL.9G394700  0.21330    0.04608    4.629 7.65e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```



```
##
## Residual standard error: 0.323 on 28 degrees of freedom
## Multiple R-squared:  0.4335, Adjusted R-squared:  0.4133
## F-statistic: 21.43 on 1 and 28 DF,  p-value: 7.646e-05
```

```
#PhHAL.2G014400
```

```
summary(lm(waterloss_d ~ PhHAL.2G014400, data=alldata))
```

```
##
## Call:
## lm(formula = waterloss_d ~ PhHAL.2G014400, data = alldata)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.93490 -0.16902  0.01814  0.25319  0.53366
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  -0.26341    0.09604  -2.743   0.0105 *
## PhHAL.2G014400  0.33755    0.10343   3.264   0.0029 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3653 on 28 degrees of freedom
## Multiple R-squared:  0.2756, Adjusted R-squared:  0.2497
## F-statistic: 10.65 on 1 and 28 DF,  p-value: 0.002897
```

```
#PhHAL.1G151400
```

```
summary(lm(waterloss_d ~ PhHAL.1G151400, data=alldata))
```

```
##
## Call:
## lm(formula = waterloss_d ~ PhHAL.1G151400, data = alldata)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.67035 -0.27527  0.05551  0.26581  0.71055
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  -0.06530    0.06559  -0.996   0.32796
## PhHAL.1G151400  0.11178    0.03158   3.540   0.00142 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3568 on 28 degrees of freedom
## Multiple R-squared:  0.3091, Adjusted R-squared:  0.2845
## F-statistic: 12.53 on 1 and 28 DF,  p-value: 0.001422
```

```
#PhHAL.9G551200
```

```
summary(lm(waterloss_d ~ PhHAL.9G551200, data=alldata))
```

```
##
## Call:
## lm(formula = waterloss_d ~ PhHAL.9G551200, data = alldata)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.49299 -0.33291 -0.01092  0.27231  0.56237
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -0.05169    0.06425   -0.804 0.427904
## PhHAL.9G551200  0.25403    0.06841    3.713 0.000902 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3513 on 28 degrees of freedom
## Multiple R-squared:  0.3299, Adjusted R-squared:  0.306
## F-statistic: 13.79 on 1 and 28 DF,  p-value: 0.0009017
```

Function of genes in lasso

open gene list file and annotation file

```
genelist <- read.csv("TXRNAseq_LassoGenes.csv", header=TRUE)
anno <- read.delim("PhalliiHAL_anno.txt")

gene_anno <- dplyr::left_join(genelist, anno, by="locusName")

write.csv(gene_anno, "TXRNAseq_LassoGeneFunctions.csv")
```

Session Info

```
sessionInfo()

## R version 4.2.2 (2022-10-31 ucrt)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19044)
##
## Matrix products: default
##
## locale:
##  [1] LC_COLLATE=English_United States.utf8
##  [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] Hmisc_4.7-2      Formula_1.2-4      survival_3.4-0      caret_6.0-93
## [5] lattice_0.20-45  glmnetUtils_1.1.8  glmnet_4.1-6        Matrix_1.5-3
## [9] forcats_0.5.2    stringr_1.5.0      dplyr_1.0.10        purrr_1.0.1
## [13] readr_2.1.3      tidyr_1.2.1        tibble_3.1.8        ggplot2_3.4.0
## [17] tidyverse_1.3.2
##
## loaded via a namespace (and not attached):
## [1] googledrive_2.0.0  colorspace_2.1-0    deldir_1.0-6
## [4] ellipsis_0.3.2     class_7.3-20        htmlTable_2.4.1
## [7] base64enc_0.1-3    fs_1.6.0            rstudioapi_0.14
## [10] listenv_0.9.0      prodlim_2019.11.13  fansi_1.0.4
## [13] lubridate_1.9.0    xml2_1.3.3          codetools_0.2-18
## [16] splines_4.2.2      knitr_1.42          jsonlite_1.8.4
## [19] pROC_1.18.0        broom_1.0.3         cluster_2.1.4
## [22] dbplyr_2.3.0       png_0.1-8           compiler_4.2.2
## [25] httr_1.4.4         backports_1.4.1     assertthat_0.2.1
## [28] fastmap_1.1.0      gargle_1.3.0        cli_3.6.0
## [31] htmltools_0.5.4    tools_4.2.2         gtable_0.3.1
## [34] glue_1.6.2         reshape2_1.4.4      Rcpp_1.0.10
## [37] cellranger_1.1.0   vctrs_0.5.2         nlme_3.1-161
## [40] iterators_1.0.14   timeDate_4022.108   gower_1.0.1
## [43] xfun_0.36          globals_0.16.2      rvest_1.0.3
## [46] timechange_0.2.0    lifecycle_1.0.3     googlesheets4_1.0.1
## [49] future_1.31.0      MASS_7.3-58.1       scales_1.2.1
## [52] ipred_0.9-13       hms_1.1.2           parallel_4.2.2
## [55] RColorBrewer_1.1-3 yaml_2.3.6          gridExtra_2.3
## [58] rpart_4.1.19       latticeExtra_0.6-30 stringi_1.7.12
## [61] highr_0.10         foreach_1.5.2       checkmate_2.1.0
## [64] hardhat_1.2.0      lava_1.7.1          shape_1.4.6
## [67] rlang_1.0.6        pkgconfig_2.0.3     evaluate_0.20
## [70] recipes_1.0.4      htmlwidgets_1.6.1   tidyselect_1.2.0
## [73] parallelly_1.34.0  plyr_1.8.8          magrittr_2.0.3
## [76] R6_2.5.1           generics_0.1.3      DBI_1.1.3
## [79] pillar_1.8.1       haven_2.5.1         foreign_0.8-84
## [82] withr_2.5.0        nnet_7.3-18         future.apply_1.10.0
## [85] modelr_0.1.10      crayon_1.5.2        interp_1.1-3
## [88] utf8_1.2.2         tzdb_0.3.0          rmarkdown_2.20
## [91] jpeg_0.1-10        grid_4.2.2          readxl_1.4.1
## [94] data.table_1.14.6  ModelMetrics_1.2.2.2 reprex_2.0.2
## [97] digest_0.6.31      stats4_4.2.2        munsell_0.5.0

```