Enrichment analysis for genes in GRN motifs

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2022-08-12

Contents

```
library(here)
## here() starts at /home/faalm/Dropbox/Working_from_home/polyploid_GRNs
library(magrene)
library(tidyverse)
## -- Attaching packages --------------------------------------- tidyverse 1.3.1 --
## v ggplot2 3.3.6     v purrr   0.3.4
## v tibble  3.1.7     v dplyr   1.0.9
## v tidyr   1.2.0     v stringr 1.4.0
## v readr   2.1.2     v forcats 0.5.1
## -- Conflicts ------------------------------------------ tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
library(BioNERO)
##
##
## Attaching package: 'BioNERO'
## The following object is masked from 'package:tidyr':
##
##     replace_na
library(ComplexHeatmap)
## Loading required package: grid
## ========================================
## ComplexHeatmap version 2.12.0
## Bioconductor page: http://bioconductor.org/packages/ComplexHeatmap/
## Github page: https://github.com/jokergoo/ComplexHeatmap
## Documentation: http://jokergoo.github.io/ComplexHeatmap-reference
##
## If you use it in published research, please cite:
## Gu, Z. Complex heatmaps reveal patterns and correlations in multidimensional
##   genomic data. Bioinformatics 2016.
##
## The new InteractiveComplexHeatmap package can directly export static
## complex heatmaps into an interactive Shiny app with zero effort. Have a try!
##
## This message can be suppressed by:
##   suppressPackageStartupMessages(library(ComplexHeatmap))
## ========================================
set.seed(123)
dup_palette <- c(SSD = "#1984c5", WGD = "#ffb400")
```

1 Overview

Here, we will describe the code to perform enrichment analyses to go deeper into the functions of genes in motifs. We will target motifs types differently:

- V and bifan motifs: enrichment for TF families using all TFs in the GRN as background;
- Lambda, delta, and bifan motifs: functional enrichment (GO and InterPro terms) using all targets in the GRN as background.

2 Enrichment analysis

Let's start by loading the required data.

```
# Load annotation
load(here("data", "functional_annotation.rda"))

# Load TFs
load(here("data", "tfs.rda"))

# Load GRN
load(here("products", "result_files", "grns.rda"))

## Create a list of TFs and targets for each GRN to use as
## background
bg_tfs <- lapply(grns, function(x) {
    return(unique(x$Node1))
})
bg_targets <- lapply(grns, function(x) {
    return(unique(x$Node2))
})
rm(grns)

# Load motifs
species <- names(functional_annotation)
files <- here("products", "result_files", "motifs", paste0("motifs_",
    tolower(species), ".rda"))
for (s in files) {
    load(s)
}

# Combine motifs for each species in a single object
motifs <- list(Athaliana = motifs_athaliana, Gmax = motifs_gmax,
    Ptrichocarpa = motifs_ptrichocarpa, Slycopersicum = motifs_slycopersicum,
    Vvinifera = motifs_vvinifera, Osativa = motifs_osativa, Zmays = motifs_zmays)

rm(motifs_athaliana)
rm(motifs_gmax)
rm(motifs_ptrichocarpa)
rm(motifs_slycopersicum)
rm(motifs_vvinifera)
rm(motifs_osativa)
rm(motifs_zmays)
```

To make it easier, let's define wrapper functions around `BioNERO::enrichment_analysis()` that perform the enrichment analysis for each species and peak.

```
# Perform SEA and add columns with info on class, mode,
# motif, and peak
enrichment <- function(genes, background, annot, mode = NULL,
    motif = NULL, peak = NULL) {
```

```
        if (is(annot, "data.frame")) {
            class <- names(annot)[2]
            enrich <- BioNERO::enrichment_analysis(genes, background,
                annot, column = class)
            if (!is.null(enrich)) {
                enrich$Class <- class
                enrich$Mode <- mode
                enrich$Motif <- motif
                if (!is.null(peak)) {
                    enrich$Peak <- peak
                }
            }

        } else {
            classes <- c("GOBP", "GOMF", "InterPro")
            enrich <- lapply(classes, function(x) {
                annot_df <- annot[[x]]
                col <- names(annot_df)[2]
                res <- BioNERO::enrichment_analysis(genes, background,
                    annot_df, column = col)
                if (!is.null(res)) {
                    res$Class <- x
                    res$Mode <- mode
                    res$Motif <- motif
                    if (!is.null(peak)) {
                      res$Peak <- peak
                    }
                }
                return(res)
            })
            enrich <- Reduce(rbind, enrich)
        }
        return(enrich)
    }


    # Perform SEA for each species, by peak and mode
    sea <- function(motifs, annot_list, tf_list, bg_targets, bg_tfs,
        species) {

        # Iterate through each species
        enrichment_res <- Reduce(rbind, lapply(species, function(x) {
            message("Working on species ", x)
            # Functional annotation and TFs
            annot <- annot_list[[x]]
            tf <- tf_list[[x]]

            # Background genes
            background_targets <- bg_targets[[x]]
            background_tfs <- bg_tfs[[x]]
```

Enrichment analysis for genes in GRN motifs

```
                peaks <- names(motifs[[x]])
                # Iterate through each peak
                bypeak <- Reduce(rbind, lapply(peaks, function(y) {
                    message("Peak: ", y)

                    events <- c("WGD", "SSD")
                    byevent <- Reduce(rbind, lapply(events, function(z) {
                        message("Mode: ", z)
                        # legend: x = species; y = peak; z = mode
                        motif_vec <- motifs[[x]][[y]][[z]]

                        # V motifs
                        v_genes <- unique(gsub("->.*", "", gsub(".*<-",
                          "", motif_vec$V)))
                        v_sea <- enrichment(v_genes, background_tfs,
                          tf, z, "V", y)

                        # Bifan motifs - TF
                        bifan_tf <- gsub("->.*", "", motif_vec$bifan)
                        bifan_tf <- unique(unlist(strsplit(bifan_tf,
                          ",")))
                        bifan_sea_tf <- NULL
                        if (length(bifan_tf) != 0) {
                          bifan_sea_tf <- enrichment(bifan_tf, background_tfs,
                            tf, z, "bifan", y)
                        }

                        # Bifan motifs - target
                        bifan_genes <- gsub(".*->", "", motif_vec$bifan)
                        bifan_genes <- unique(unlist(strsplit(bifan_genes,
                          ",")))
                        bifan_sea_tar <- NULL
                        if (length(bifan_genes) != 0) {
                          bifan_sea_tar <- enrichment(bifan_genes, background_targets,
                            annot, z, "bifan", y)
                        }

                        # Lambda
                        lambda_genes <- gsub("<-.*->", ",", motif_vec$lambda)
                        lambda_genes <- unique(unlist(strsplit(lambda_genes,
                          ",")))
                        lambda_sea <- NULL
                        if (length(lambda_genes) != 0) {
                          lambda_sea <- enrichment(lambda_genes, background_targets,
                            annot, z, "lambda", y)
                        }

                        # Delta
                        delta_genes <- gsub("<-.*->", ",", motif_vec$delta)
                        delta_genes <- unique(unlist(strsplit(delta_genes,
                          ",")))
```

```
                    delta_sea <- NULL
                    if (length(delta_genes) != 0) {
                      delta_sea <- enrichment(delta_genes, background_targets,
                        annot, z, "delta", y)
                    }

                    # Combine results in a single data frame
                    sea_final <- rbind(v_sea, bifan_sea_tf, bifan_sea_tar)
                    return(sea_final)
                }))

                return(byevent)
            }))
            if (!is.null(bypeak)) {
                bypeak$Species <- x
            }
            return(bypeak)
        }))
        return(enrichment_res)
    }

    # Broader SEA, not filtered by peak
    sea_global <- function(motifs, annot_list, tf_list, bg_targets,
        bg_tfs, species) {

        # Iterate through each species
        enrichment_res <- Reduce(rbind, lapply(species, function(x) {
            message("Working on species ", x)
            # Functional annotation and TFs
            annot <- annot_list[[x]]
            tf <- tf_list[[x]]

            # Background genes
            background_targets <- bg_targets[[x]]
            background_tfs <- bg_tfs[[x]]

            # Create a list of motifs by mode (WGD and SSD),
            # combining peaks
            peaks <- names(motifs[[x]])
            genes_wgd <- unlist(lapply(peaks, function(y) {
                motif_vec <- motifs[[x]][[y]]$WGD
                return(motif_vec)
            }), recursive = FALSE)
            genes_wgd <- sapply(unique(names(genes_wgd)), function(x) {
                return(unname(unlist(genes_wgd[names(genes_wgd) ==
                    x])))
            }, simplify = FALSE)

            genes_ssd <- unlist(lapply(peaks, function(y) {
                motif_vec <- motifs[[x]][[y]]$SSD
                return(motif_vec)
```

```
}), recursive = FALSE)
genes_ssd <- sapply(unique(names(genes_ssd)), function(x) {
    return(unname(unlist(genes_ssd[names(genes_ssd) ==
        x])))
}, simplify = FALSE)
mlist <- list(WGD = genes_wgd, SSD = genes_ssd)

# Iterate through `mlist` and perform SEA by mode
events <- c("WGD", "SSD")
byevent <- Reduce(rbind, lapply(events, function(z) {
    message("Mode: ", z)
    motif_vec <- mlist[[z]]

    # V motifs
    v_genes <- unique(gsub("->.*", "", gsub(".*<-", "",
        motif_vec$V)))
    v_sea <- enrichment(v_genes, background_tfs, tf,
        z, "V", y)

    # Bifan motifs - TF
    bifan_tf <- gsub("->.*", "", motif_vec$bifan)
    bifan_tf <- unique(unlist(strsplit(bifan_tf, ",")))
    bifan_sea_tf <- NULL
    if (length(bifan_tf) != 0) {
        bifan_sea_tf <- enrichment(bifan_tf, background_tfs,
          tf, z, "bifan", y)
    }

    # Bifan motifs - target
    bifan_genes <- gsub(".*->", "", motif_vec$bifan)
    bifan_genes <- unique(unlist(strsplit(bifan_genes,
        ",")))
    bifan_sea_tar <- NULL
    if (length(bifan_genes) != 0) {
        bifan_sea_tar <- enrichment(bifan_genes, background_targets,
          annot, z, "bifan", y)
    }

    # Lambda
    lambda_genes <- gsub("<-.*->", ",", motif_vec$lambda)
    lambda_genes <- unique(unlist(strsplit(lambda_genes,
        ",")))
    lambda_sea <- NULL
    if (length(lambda_genes) != 0) {
        lambda_sea <- enrichment(lambda_genes, background_targets,
          annot, z, "lambda", y)
    }

    # Delta
    delta_genes <- gsub("<-.*->", ",", motif_vec$delta)
    delta_genes <- unique(unlist(strsplit(delta_genes,
```

```
                ",")))
            delta_sea <- NULL
            if (length(delta_genes) != 0) {
                delta_sea <- enrichment(delta_genes, background_targets,
                  annot, z, "delta", y)
            }

            # Combine results in a single data frame
            sea_final <- rbind(v_sea, bifan_sea_tf, bifan_sea_tar)
            return(sea_final)
        }))

        return(byevent)
    }))

    if (!is.null(enrichment_res)) {
        enrichment_res$Species <- x
    }
    return(enrichment_res)
}
```

Now, let's perform the SEA for each species.

```
annot_list <- functional_annotation
tf_list <- tfs

# Performing SEA for each species
sea_ath <- sea(motifs, annot_list, tf_list, bg_targets, bg_tfs,
    "Athaliana")
sea_gma <- sea(motifs, annot_list, tf_list, bg_targets, bg_tfs,
    "Gmax")
sea_ptr <- sea(motifs, annot_list, tf_list, bg_targets, bg_tfs,
    "Ptrichocarpa")
sea_sly <- sea(motifs, annot_list, tf_list, bg_targets, bg_tfs,
    "Slycopersicum")
sea_vvi <- sea(motifs, annot_list, tf_list, bg_targets, bg_tfs,
    "Vvinifera")
sea_osa <- sea(motifs, annot_list, tf_list, bg_targets, bg_tfs,
    "Osativa")
sea_zma <- sea(motifs, annot_list, tf_list, bg_targets, bg_tfs,
    "Zmays")

sea_by_peak <- rbind(sea_ath, sea_gma, sea_sly, sea_vvi, sea_osa,
    sea_zma)
sea_by_peak$GeneID <- NULL
readr::write_tsv(sea_by_peak, file = here("products", "tables",
    "motif_functional_enrichment_by_peak.tsv"))
```

To conclude, let's perform another SEA, but not by peak. Here, we will only perform the SEA for WGD- and SSD- derived gene pairs in motifs.

```
# Perform global SEA
seag_ath <- sea_global(motifs, annot_list, tf_list, bg_targets,
    bg_tfs, "Athaliana")
seag_gma <- sea_global(motifs, annot_list, tf_list, bg_targets,
    bg_tfs, "Gmax")
seag_ptr <- sea_global(motifs, annot_list, tf_list, bg_targets,
    bg_tfs, "Ptrichocarpa")
seag_sly <- sea_global(motifs, annot_list, tf_list, bg_targets,
    bg_tfs, "Slycopersicum")
seag_vvi <- sea_global(motifs, annot_list, tf_list, bg_targets,
    bg_tfs, "Vvinifera")
seag_osa <- sea_global(motifs, annot_list, tf_list, bg_targets,
    bg_tfs, "Osativa")
seag_zma <- sea_global(motifs, annot_list, tf_list, bg_targets,
    bg_tfs, "Zmays")

# Combine results into a single data frame
sea_combined <- rbind(seag_ath %>%
    mutate(Species = "Athaliana"), seag_gma %>%
    mutate(Species = "Gmax"), seag_sly %>%
    mutate(Species = "Slycopersicum"), seag_vvi %>%
    mutate(Species = "Vvinifera"), seag_osa %>%
    mutate(Species = "Osativa"), seag_zma %>%
    mutate(Species = "Zmays"), seag_ptr %>%
    mutate(Species = "Ptrichocarpa"))
sea_combined$GeneID <- NULL
sea_combined$Peak <- NULL
readr::write_tsv(sea_combined, file = here("products", "tables",
    "motif_functional_enrichment_by_mode.tsv"))
```

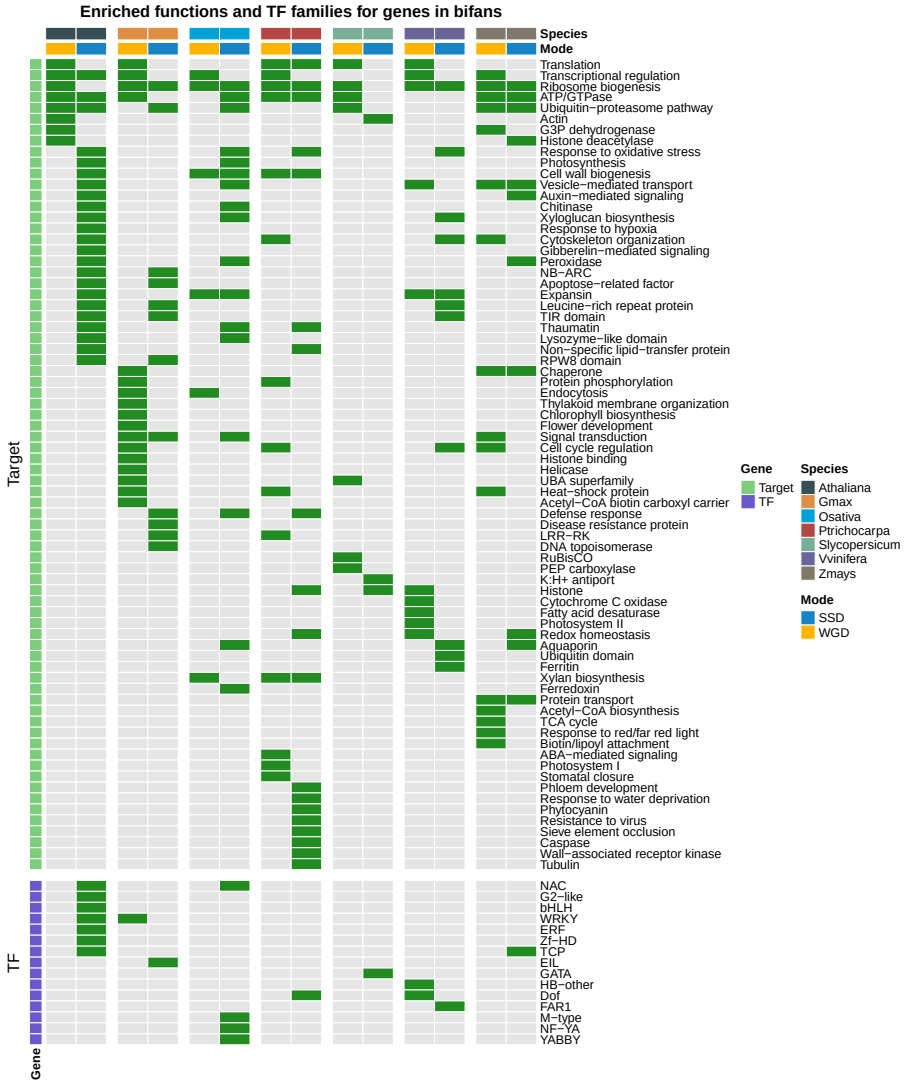
After manual inspection of the enrichment results, I created the table *summarized_functional_enrichment_of_motifs.csv*, which contains summarized enrichment results for visual exploration. One important thing to observe is that the only class of motifs with enriched functions/TF families was bifans.

To conclude, let's visualize enrichment results as a presence/absence heatmap.

```
# Load data
sum_enrich <- readr::read_csv(here("products", "tables", "summarized_functional_enrichment_of_motifs.csv"),
    show_col_types = FALSE)

# Turn long data frame to gene/absence matrix
pav <- sum_enrich %>%
    mutate(present = 1) %>%
    mutate(cols = str_c(Species, "_", Mode)) %>%
    select(Class, present, cols) %>%
    pivot_wider(names_from = cols, values_from = present) %>%
    column_to_rownames("Class") %>%
    as.matrix()
pav[is.na(pav)] <- 0

# Create row annotation, column annotation, and annotation
```

```
# colors Column annotation: species, mode
annotation_col <- data.frame(row.names = colnames(pav), Mode = gsub(".*_",
    "", colnames(pav)), Species = gsub("_.*", "", colnames(pav)))

## Row annotation: gene type (TF or target)
c <- sum_enrich %>%
    dplyr::select(Class, Motif) %>%
    distinct() %>%
    as.data.frame()

annotation_row <- data.frame(row.names = c$Class, Gene = c$Motif)
annotation_row$Gene <- gsub("Targets", "Target", annotation_row$Gene)

## Annotation colors
annotation_colors <- list(Mode = dup_palette, Species = c(Athaliana = "#374E55FF",
    Gmax = "#DF8F44FF", Osativa = "#00A1D5FF", Ptrichocarpa = "#B24745FF",
    Slycopersicum = "#79AF97FF", Vvinifera = "#6A6599FF", Zmays = "#80796BFF"),
    Gene = c(TF = "slateblue3", Target = "palegreen3"))

# Plot heatmap
p_heatmap <- ComplexHeatmap::pheatmap(pav, name = "Presence/absence",
    main = "Enriched functions and TF families for genes in bifans",
    cluster_rows = FALSE, cluster_cols = FALSE, annotation_col = annotation_col,
    annotation_row = annotation_row, labels_col = rep("", ncol(pav)),
    annotation_colors = annotation_colors, column_split = annotation_col$Species,
    row_split = annotation_row$Gene, row_gap = unit(3, "mm"),
    column_gap = unit(3, "mm"), color = c("grey90", "forestgreen"),
    border_color = "white", legend = FALSE)
p_heatmap
```

Enrichment analysis for genes in GRN motifs



By looking at the figure, we can see some patterns:

- SSD-derived genes in bifans are enriched in stress-related genes. These include genes involved in response to oxidative stress, response to hypoxia, and different classes of pathogenesis-related proteins, such as chitinases and lysozymes, peroxidases, and thaumatins. There are also leucine-rich repeat receptor kinases, TIR domains, and wall-associated kinases.
- Overall, only SSD-derived genes were enriched in particular TF families. Although WGD-derived tend to be retained more often than SSD-derived genes, WGD-derived genes in bifans were not enriched in any specific TF family.
- SSD-derived genes are enriched in stress-related transcription factor families, such as NAC, G2-like, bHLH, WRKY, and ERF. This is in line with previous observations that stress-related TFs tend to be duplicated by tandem duplications. This mechanism is likely adaptive, as genes in tandem tend to be co-regulated. Then, keeping stress-related genes in tandem arrays would ensure that they are coordinately activated.

- WGD-derived genes in bifans are enriched in processes such as translation, flower development, transcriptional regulation, histone modifications, photosynthesis-related processes (e.g., chlorophyll biosynthesis, thylakoid membrane organization, RuBisCO, and photosystem I formation), cell cycle regulation, and carbohydrate and lipid metabolism.

```
# Save heatmap as PDF
pdf(file = here("products", "plots", "heatmap_motif_functional_enrichment.pdf"),
    width = 10, height = 12)
p_heatmap
dev.off()
```

Session info

This document was created under the following conditions:

```
sessioninfo::session_info()
## - Session info -------------------------------------------------------------
##  setting  value
##  version  R version 4.2.1 (2022-06-23)
##  os       Ubuntu 20.04.4 LTS
##  system   x86_64, linux-gnu
##  ui       X11
##  language (EN)
##  collate  en_US.UTF-8
##  ctype    en_US.UTF-8
##  tz       Europe/Brussels
##  date     2022-08-12
##  pandoc   2.18 @ /usr/lib/rstudio/bin/quarto/bin/tools/ (via rmarkdown)
##
## - Packages -----------------------------------------------------------------
##  package              * version    date (UTC) lib source
##  abind                  1.4-5      2016-07-21 [1] CRAN (R 4.2.0)
##  annotate               1.74.0     2022-04-26 [1] Bioconductor
##  AnnotationDbi          1.58.0     2022-04-26 [1] Bioconductor
##  assertthat             0.2.1      2019-03-21 [1] CRAN (R 4.2.0)
##  backports              1.4.1      2021-12-13 [1] CRAN (R 4.2.0)
##  base64enc              0.1-3      2015-07-28 [1] CRAN (R 4.2.0)
##  Biobase                2.56.0     2022-04-26 [1] Bioconductor
##  BiocGenerics           0.42.0     2022-04-26 [1] Bioconductor
##  BiocManager            1.30.18    2022-05-18 [1] CRAN (R 4.2.0)
##  BiocParallel           1.30.3     2022-06-05 [1] Bioconductor
##  BiocStyle            * 2.25.0     2022-06-15 [1] Github (Bioconductor/BiocStyle@7150c28)
##  BioNERO              * 1.4.0      2022-04-26 [1] Bioconductor
##  Biostrings             2.64.0     2022-04-26 [1] Bioconductor
##  bit                    4.0.4      2020-08-04 [1] CRAN (R 4.2.0)
##  bit64                  4.0.5      2020-08-30 [1] CRAN (R 4.2.0)
##  bitops                 1.0-7      2021-04-24 [1] CRAN (R 4.2.0)
##  blob                   1.2.3      2022-04-10 [1] CRAN (R 4.2.0)
##  bookdown               0.27       2022-06-14 [1] CRAN (R 4.2.0)
##  broom                  0.8.0      2022-04-13 [1] CRAN (R 4.2.0)
```

```
##  cachem                1.0.6     2021-08-19 [1] CRAN (R 4.2.0)
##  car                   3.1-0     2022-06-15 [1] CRAN (R 4.2.0)
##  carData               3.0-5     2022-01-06 [1] CRAN (R 4.2.0)
##  cellranger            1.1.0     2016-07-27 [1] CRAN (R 4.2.0)
##  checkmate             2.1.0     2022-04-21 [1] CRAN (R 4.2.0)
##  circlize              0.4.15    2022-05-10 [1] CRAN (R 4.2.0)
##  cli                   3.3.0     2022-04-25 [1] CRAN (R 4.2.0)
##  clue                  0.3-61    2022-05-30 [1] CRAN (R 4.2.0)
##  cluster               2.1.3     2022-03-28 [1] CRAN (R 4.2.0)
##  coda                  0.19-4    2020-09-30 [1] CRAN (R 4.2.0)
##  codetools             0.2-18    2020-11-04 [1] CRAN (R 4.2.0)
##  colorspace            2.0-3     2022-02-21 [1] CRAN (R 4.2.0)
##  ComplexHeatmap      * 2.12.0    2022-04-26 [1] Bioconductor
##  crayon                1.5.1     2022-03-26 [1] CRAN (R 4.2.0)
##  data.table            1.14.2    2021-09-27 [1] CRAN (R 4.2.0)
##  DBI                   1.1.3     2022-06-18 [1] CRAN (R 4.2.0)
##  dbplyr                2.2.1     2022-06-27 [1] CRAN (R 4.2.1)
##  DelayedArray          0.22.0    2022-04-26 [1] Bioconductor
##  DESeq2                1.36.0    2022-04-26 [1] Bioconductor
##  digest                0.6.29    2021-12-01 [1] CRAN (R 4.2.0)
##  doParallel            1.0.17    2022-02-07 [1] CRAN (R 4.2.0)
##  dplyr               * 1.0.9     2022-04-28 [1] CRAN (R 4.2.0)
##  dynamicTreeCut        1.63-1    2016-03-11 [1] CRAN (R 4.2.0)
##  edgeR                 3.38.1    2022-05-15 [1] Bioconductor
##  ellipsis              0.3.2     2021-04-29 [1] CRAN (R 4.2.0)
##  evaluate              0.15      2022-02-18 [1] CRAN (R 4.2.0)
##  fansi                 1.0.3     2022-03-24 [1] CRAN (R 4.2.0)
##  fastcluster           1.2.3     2021-05-24 [1] CRAN (R 4.2.0)
##  fastmap               1.1.0     2021-01-25 [1] CRAN (R 4.2.0)
##  forcats             * 0.5.1     2021-01-27 [1] CRAN (R 4.2.0)
##  foreach               1.5.2     2022-02-02 [1] CRAN (R 4.2.0)
##  foreign               0.8-82    2022-01-13 [1] CRAN (R 4.2.0)
##  formatR               1.12      2022-03-31 [1] CRAN (R 4.2.0)
##  Formula               1.2-4     2020-10-16 [1] CRAN (R 4.2.0)
##  fs                    1.5.2     2021-12-08 [1] CRAN (R 4.2.0)
##  genefilter            1.78.0    2022-04-26 [1] Bioconductor
##  geneplotter           1.74.0    2022-04-26 [1] Bioconductor
##  generics              0.1.2     2022-01-31 [1] CRAN (R 4.2.0)
##  GENIE3                1.18.0    2022-04-26 [1] Bioconductor
##  GenomeInfoDb          1.32.2    2022-05-15 [1] Bioconductor
##  GenomeInfoDbData      1.2.8     2022-05-06 [1] Bioconductor
##  GenomicRanges         1.48.0    2022-04-26 [1] Bioconductor
##  GetoptLong            1.0.5     2020-12-15 [1] CRAN (R 4.2.0)
##  ggnetwork             0.5.10    2021-07-06 [1] CRAN (R 4.2.0)
##  ggnewscale            0.4.7     2022-03-25 [1] CRAN (R 4.2.0)
##  ggplot2             * 3.3.6     2022-05-03 [1] CRAN (R 4.2.0)
##  ggpubr                0.4.0     2020-06-27 [1] CRAN (R 4.2.0)
##  ggsignif              0.6.3     2021-09-09 [1] CRAN (R 4.2.0)
##  GlobalOptions         0.1.2     2020-06-10 [1] CRAN (R 4.2.0)
##  glue                  1.6.2     2022-02-24 [1] CRAN (R 4.2.0)
##  GO.db                 3.15.0    2022-05-06 [1] Bioconductor
```

```
##  gridExtra             2.3        2017-09-09 [1] CRAN (R 4.2.0)
##  gtable                0.3.0      2019-03-25 [1] CRAN (R 4.2.0)
##  haven                 2.5.0      2022-04-15 [1] CRAN (R 4.2.0)
##  here                * 1.0.1      2020-12-13 [1] CRAN (R 4.2.0)
##  Hmisc                 4.7-0      2022-04-19 [1] CRAN (R 4.2.0)
##  hms                   1.1.1      2021-09-26 [1] CRAN (R 4.2.0)
##  htmlTable             2.4.0      2022-01-04 [1] CRAN (R 4.2.0)
##  htmltools             0.5.2      2021-08-25 [1] CRAN (R 4.2.0)
##  htmlwidgets           1.5.4      2021-09-08 [1] CRAN (R 4.2.0)
##  httr                  1.4.3      2022-05-04 [1] CRAN (R 4.2.0)
##  igraph                1.3.2      2022-06-13 [1] CRAN (R 4.2.0)
##  impute                1.70.0     2022-04-26 [1] Bioconductor
##  intergraph            2.0-2      2016-12-05 [1] CRAN (R 4.2.0)
##  IRanges               2.30.0     2022-04-26 [1] Bioconductor
##  iterators             1.0.14     2022-02-05 [1] CRAN (R 4.2.0)
##  jpeg                  0.1-9      2021-07-24 [1] CRAN (R 4.2.0)
##  jsonlite              1.8.0      2022-02-22 [1] CRAN (R 4.2.0)
##  KEGGREST              1.36.2     2022-06-09 [1] Bioconductor
##  knitr                 1.39       2022-04-26 [1] CRAN (R 4.2.0)
##  lattice               0.20-45    2021-09-22 [1] CRAN (R 4.2.0)
##  latticeExtra          0.6-29     2019-12-19 [1] CRAN (R 4.2.0)
##  lifecycle             1.0.1      2021-09-24 [1] CRAN (R 4.2.0)
##  limma                 3.52.2     2022-06-19 [1] Bioconductor
##  locfit                1.5-9.5    2022-03-03 [1] CRAN (R 4.2.0)
##  lubridate             1.8.0      2021-10-07 [1] CRAN (R 4.2.0)
##  magick                2.7.3      2021-08-18 [1] CRAN (R 4.2.0)
##  magrene             * 0.99.0     2022-07-27 [1] Bioconductor
##  magrittr              2.0.3      2022-03-30 [1] CRAN (R 4.2.0)
##  Matrix                1.4-1      2022-03-23 [1] CRAN (R 4.2.0)
##  MatrixGenerics        1.8.1      2022-06-26 [1] Bioconductor
##  matrixStats           0.62.0     2022-04-19 [1] CRAN (R 4.2.0)
##  memoise               2.0.1      2021-11-26 [1] CRAN (R 4.2.0)
##  mgcv                  1.8-40     2022-03-29 [1] CRAN (R 4.2.0)
##  minet                 3.54.0     2022-04-26 [1] Bioconductor
##  modelr                0.1.8      2020-05-19 [1] CRAN (R 4.2.0)
##  munsell               0.5.0      2018-06-12 [1] CRAN (R 4.2.0)
##  NetRep                1.2.4      2020-10-07 [1] CRAN (R 4.2.0)
##  network               1.17.2     2022-05-21 [1] CRAN (R 4.2.0)
##  networkD3             0.4        2017-03-18 [1] CRAN (R 4.2.0)
##  nlme                  3.1-158    2022-06-15 [1] CRAN (R 4.2.0)
##  nnet                  7.3-17     2022-01-13 [1] CRAN (R 4.2.0)
##  pillar                1.7.0      2022-02-01 [1] CRAN (R 4.2.0)
##  pkgconfig             2.0.3      2019-09-22 [1] CRAN (R 4.2.0)
##  plyr                  1.8.7      2022-03-24 [1] CRAN (R 4.2.0)
##  png                   0.1-7      2013-12-03 [1] CRAN (R 4.2.0)
##  preprocessCore        1.58.0     2022-04-26 [1] Bioconductor
##  purrr               * 0.3.4      2020-04-17 [1] CRAN (R 4.2.0)
##  R6                    2.5.1      2021-08-19 [1] CRAN (R 4.2.0)
##  RColorBrewer          1.1-3      2022-04-03 [1] CRAN (R 4.2.0)
##  Rcpp                  1.0.8.3    2022-03-17 [1] CRAN (R 4.2.0)
##  RCurl                 1.98-1.7   2022-06-09 [1] CRAN (R 4.2.0)
```

```
##  readr                * 2.1.2      2022-01-30 [1] CRAN (R 4.2.0)
##  readxl                 1.4.0      2022-03-28 [1] CRAN (R 4.2.0)
##  reprex                 2.0.1      2021-08-05 [1] CRAN (R 4.2.0)
##  reshape2               1.4.4      2020-04-09 [1] CRAN (R 4.2.0)
##  RhpcBLASctl            0.21-247.1 2021-11-05 [1] CRAN (R 4.2.0)
##  rjson                  0.2.21     2022-01-09 [1] CRAN (R 4.2.0)
##  rlang                  1.0.3      2022-06-27 [1] CRAN (R 4.2.1)
##  rmarkdown              2.14       2022-04-25 [1] CRAN (R 4.2.0)
##  rpart                  4.1.16     2022-01-24 [1] CRAN (R 4.2.0)
##  rprojroot              2.0.3      2022-04-02 [1] CRAN (R 4.2.0)
##  RSQLite                2.2.14     2022-05-07 [1] CRAN (R 4.2.0)
##  rstatix                0.7.0      2021-02-13 [1] CRAN (R 4.2.0)
##  rstudioapi             0.13       2020-11-12 [1] CRAN (R 4.2.0)
##  rvest                  1.0.2      2021-10-16 [1] CRAN (R 4.2.0)
##  S4Vectors              0.34.0     2022-04-26 [1] Bioconductor
##  scales                 1.2.0      2022-04-13 [1] CRAN (R 4.2.0)
##  sessioninfo            1.2.2      2021-12-06 [1] CRAN (R 4.2.0)
##  shape                  1.4.6      2021-05-19 [1] CRAN (R 4.2.0)
##  statmod                1.4.36     2021-05-10 [1] CRAN (R 4.2.0)
##  statnet.common         4.6.0      2022-05-02 [1] CRAN (R 4.2.0)
##  stringi                1.7.6      2021-11-29 [1] CRAN (R 4.2.0)
##  stringr              * 1.4.0      2019-02-10 [1] CRAN (R 4.2.0)
##  SummarizedExperiment   1.26.1     2022-04-29 [1] Bioconductor
##  survival               3.3-1      2022-03-03 [1] CRAN (R 4.2.0)
##  sva                    3.44.0     2022-04-26 [1] Bioconductor
##  tibble               * 3.1.7      2022-05-03 [1] CRAN (R 4.2.0)
##  tidyr                * 1.2.0      2022-02-01 [1] CRAN (R 4.2.0)
##  tidyselect             1.1.2      2022-02-21 [1] CRAN (R 4.2.0)
##  tidyverse            * 1.3.1      2021-04-15 [1] CRAN (R 4.2.0)
##  tzdb                   0.3.0      2022-03-28 [1] CRAN (R 4.2.0)
##  utf8                   1.2.2      2021-07-24 [1] CRAN (R 4.2.0)
##  vctrs                  0.4.1      2022-04-13 [1] CRAN (R 4.2.0)
##  vroom                  1.5.7      2021-11-30 [1] CRAN (R 4.2.0)
##  WGCNA                  1.71       2022-04-22 [1] CRAN (R 4.2.0)
##  withr                  2.5.0      2022-03-03 [1] CRAN (R 4.2.0)
##  xfun                   0.31       2022-05-10 [1] CRAN (R 4.2.0)
##  XML                    3.99-0.10  2022-06-09 [1] CRAN (R 4.2.0)
##  xml2                   1.3.3      2021-11-30 [1] CRAN (R 4.2.0)
##  xtable                 1.8-4      2019-04-21 [1] CRAN (R 4.2.0)
##  XVector                0.36.0     2022-04-26 [1] Bioconductor
##  yaml                   2.3.5      2022-02-21 [1] CRAN (R 4.2.0)
##  zlibbioc               1.42.0     2022-04-26 [1] Bioconductor
##
##  [1] /home/faalm/R/x86_64-pc-linux-gnu-library/4.2
##  [2] /usr/local/lib/R/site-library
##  [3] /usr/lib/R/site-library
##  [4] /usr/lib/R/library
##
## ------------------------------------------------------------------------------
```