

Easily run a RNA-Seq analysis via RNASeqHelper

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2024-02-17

Abstract

Our work introduces an R package for interactive RNA-Seq analysis, enhancing DESeq2's robust statistical methods. It simplifies complex analyses, offering user-friendly features like heatmap and volcano plot generation. This package serves as both an interactive tool and an automated pipeline, enabling efficient exploration of RNA-Seq data without extensive programming knowledge. Its intuitive visualization and seamless integration with DESeq2 facilitate gene expression studies, fostering discoveries.

Package

RNASeqHelper 0.99.7

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Contents

1 RNASeqHelper

This vignette walks through an RNA-seq analysis using simulated datasets.

Let us first load the library

```
library(RNASeqHelper)
## Loading required package: SummarizedExperiment
## Loading required package: MatrixGenerics
## Loading required package: matrixStats
##
## Attaching package: 'MatrixGenerics'
## The following objects are masked from 'package:matrixStats':
##
##   colAlls, colAnyNAs, colAnys, colAvgPerRowSet, colCollapse,
##   colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
##   colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
##   colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
##   colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
##   colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
##   colWeightedMeans, colWeightedMedians, colWeightedSds,
##   colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgPerColSet,
##   rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
##   rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
##   rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
##   rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
##   rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
##   rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
##   rowWeightedSds, rowWeightedVars
## Loading required package: GenomicRanges
## Loading required package: stats4
## Loading required package: BiocGenerics
##
## Attaching package: 'BiocGenerics'
## The following objects are masked from 'package:stats':
##
##   IQR, mad, sd, var, xtabs
## The following objects are masked from 'package:base':
##
##   Filter, Find, Map, Position, Reduce, anyDuplicated, aperm, append,
##   as.data.frame, basename, cbind, colnames, dirname, do.call,
##   duplicated, eval, evalq, get, grep, grepl, intersect, is.unsorted,
##   lapply, mapply, match, mget, order, paste, pmax, pmax.int, pmin,
##   pmin.int, rank, rbind, rownames, sapply, setdiff, table, tapply,
##   union, unique, unsplit, which.max, which.min
## Loading required package: S4Vectors
##
## Attaching package: 'S4Vectors'
```

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```
## The following object is masked from 'package:utils':
##
##   findMatches
## The following objects are masked from 'package:base':
##
##   I, expand.grid, unname
## Loading required package: IRanges
## Loading required package: GenomeInfoDb
## Loading required package: Biobase
## Welcome to Bioconductor
##
##   Vignettes contain introductory material; view with
##   'browseVignettes()'. To cite Bioconductor, see
##   'citation("Biobase)", and for packages 'citation("pkgname)".
##
## Attaching package: 'Biobase'
## The following object is masked from 'package:MatrixGenerics':
##
##   rowMedians
## The following objects are masked from 'package:matrixStats':
##
##   anyMissing, rowMedians
## Loading required package: svglite
```

1.1 Input Matrices and Annotation

Every RNA-seq analysis begins with two datasets:

- The input matrix
- The phenotype data

1.1.0.1 Simulate A Dataset Here we create 9 RNA samples with slightly varying means and SDs, for 10000 genes.

```
num_genes <- 10000
mean_sd <- list(c(10, 10), c(10, 10), c(10, 10),
               c(20, 20), c(20, 20), c(30, 30),
               c(90, 90), c(90, 90), c(90, 90))

mat <- as.matrix(
  do.call(cbind,
    lapply(seq_along(mean_sd), function(i) {
      x <- mean_sd[[i]]
      tab <- matrix(as.integer(rnorm(num_genes, x[[1]], x[[2]])), ncol=1,
        dimnames=list(paste0("G", 1:num_genes), paste0("S", i)))
      tab <- tab - min(tab) ## no zeroes
      return(tab)
    })))

phenotype_data <- data.frame(sample = colnames(mat),
  condition = c(rep("green", 3), rep("red", 3), rep("blue", 3)),
```

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```
time = c(5, 5, 15, 10, 10, 15, 5, 10, 15))

message("First 6 lines of the matrix")
## First 6 lines of the matrix
head(mat)
##   S1 S2 S3 S4 S5 S6 S7 S8 S9
## G1 29 46 33 111 65 138 331 284 343
## G2 33 37 34 117 62 111 415 399 302
## G3 29 29 35 94 75 136 274 422 363
## G4 46 34 24 94 42 151 400 435 286
## G5 43 33 52 64 71 100 487 395 262
## G6 53 26 33 115 105 109 383 194 289
message("Phenotype Data")
## Phenotype Data
phenotype_data
##  sample condition time
## 1      S1      green   5
## 2      S2      green   5
## 3      S3      green  15
## 4      S4       red  10
## 5      S5       red  10
## 6      S6       red  15
## 7      S7      blue   5
## 8      S8      blue  10
## 9      S9      blue  15
```

So now we have a matrix of 9 samples and 10000 genes, and accompanying phenotype data for the samples.

1.1.0.2 Genes of Interest We also might want some gene plots for some genes of interest. We can either load some pre-defined lists via:

```
gois_list <- source(system.file("extdata", "gois.R", package="RNASeqHelper"),
                     local=TRUE)

gois_list
## $value
## $value$bra.down
## [1] "Msgn1" "Osr1" "Rspo3" "Fgf8" "Wnt3a"
##
## $value$eo.down
## [1] "Mesp1" "Foxa2" "Sox17" "Lhx1" "Cer1"
##
## $value$eo.down.repr
## [1] "Dmdx1" "Dpf3" "Foxa1" "Hey1" "Hhex" "Tcf7l2" "Tle2"
##
## $value$pp.up
## [1] "Lefty1" "Lefty2" "Nodal" "Wnt8a" "Fgf5" "Otx2" "Cldn6"
##
## $value$episc.up
## [1] "Nanog" "Pou5f1" "Sox2" "L1td1" "Utf1"
##
```

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```
## $value$ne.up
## [1] "Sox1" "Sox3" "Olig3" "Gbx2" "Pou3f1" "Msx3" "Slc7a3" "Zic1"
## [9] "Zic2" "Nkx1-2" "Epha2" "Efnb1" "Nkx6-2"
##
## $value$me.down
## [1] "Pdgfra" "Foxc1" "Foxc2" "Isl1" "Kdr" "Mixl1" "Hand1" "Myh6"
##
## $value$hox.genes
## [1] "Hoxa1" "Hoxa10" "Hoxa11" "Hoxa11os" "Hoxa13" "Hoxa2"
## [7] "Hoxa3" "Hoxa4" "Hoxa5" "Hoxa6" "Hoxa7" "Hoxa9"
## [13] "Hoxaas2" "Hoxaas3" "Hoxb1" "Hoxb13" "Hoxb2" "Hoxb3"
## [19] "Hoxb3os" "Hoxb4" "Hoxb5" "Hoxb5os" "Hoxb6" "Hoxb7"
## [25] "Hoxb8" "Hoxb9" "Hoxc10" "Hoxc11" "Hoxc12" "Hoxc13"
## [31] "Hoxc4" "Hoxc5" "Hoxc6" "Hoxc8" "Hoxc9" "Hoxd1"
## [37] "Hoxd10" "Hoxd11" "Hoxd12" "Hoxd13" "Hoxd3" "Hoxd3os1"
## [43] "Hoxd4" "Hoxd8" "Hoxd9"
##
## $value$main.genes
## [1] "T" "Eomes" "Wnt3" "Wnt3a" "Nodal" "Fgf8" "Mixl1" "EGFP"
##
## $value$ac.1
## [1] "Fgf8" "Eomes" "Foxa2" "Meis1" "Otx2" "Lhx1" "Mixl1"
## [8] "Cyp26a1" "T" "Cdx1" "Cdx2" "Axin2" "Rspo3" "Wnt3a"
##
## $value$ac.2
## [1] "Axin2" "Cdx1" "Cdx2" "Cyp26a1" "Dkk1" "Meis1" "Mesp1"
##
## $value$other1
## [1] "Foxc2" "Gsc" "Mixl1" "Foxc1" "Pdgfra" "Kdr"
## [7] "Tbx1" "Amot" "Eya1" "Prrx1" "Lhx1" "Tnnt1"
## [13] "Isl1" "Tbx20" "Myh7" "Myh6" "Eya2" "Tbx18"
## [19] "Snai1" "Snai2" "Hand1" "Hand2" "Mesp1" "Pax2"
## [25] "Mesp2" "Col2a1" "Myocd" "Pax3" "Wt1" "Dll3"
## [31] "Prickle1" "Msgn1" "Rspo3" "Osr1" "Twist1" "Twist2"
## [37] "Tbx6" "Meox1" "Gata6" "Gata4" "Foxa2" "Sox17"
## [43] "Lama1" "Tgfa" "Fn1" "Cer1" "Tgfb2" "Bmper"
## [49] "Chrd" "Lgr5" "Bmp2" "Fzd7" "Wnt3a" "Bmp7"
## [55] "Cfc1" "Dkk1" "Fgf1" "Tdgf1" "Wnt3" "Tgfb1"
## [61] "Nodal" "Dact1" "Bmp4" "Dll1" "Hey1" "Hhex"
## [67] "Id1" "Dmbx1" "Dpf3" "Sal1" "Foxa1" "Tcf7l2"
## [73] "Hesx1" "Tcf7l1" "Hdac7" "Otx2" "Fbn2" "Otx1"
##
## $value$other2
## [1] "Six3" "Fabp7" "Ntrk2" "Zic1" "Mab21l2" "Nefl" "Olig3"
## [8] "Pou4f2" "Nkx1-2" "Sox3" "Sema3c" "Epha2" "Zic3" "Efnb1"
## [15] "Radil" "Syt11" "Slc7a3" "Nes" "Zic5" "Snph" "Nfasc"
## [22] "Pou4f1" "Elavl3" "Nrcam" "Grin1" "Gfra3" "Phox2a" "Nkx6-2"
## [29] "Pax6" "Nsg2" "Msx3" "Ephb1" "Ncan" "Nova2" "Zic2"
## [36] "Grik3" "Epha1" "Bcl11a" "Hoxa2" "Tubb3" "Sox1" "Neurod1"
## [43] "Neurog1" "Stmn2" "Atxn1" "Cntn2" "Neurl1a" "Sema3c" "Gap43"
## [50] "Fgf5" "Tbx3" "Cldn6" "Pou3f1" "Lefty2" "Gbx2" "Nanog"
```

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```
## [57] "L1td1" "Wnt8a" "Pou5f1" "Lefty1" "Utf1" "Esrrb" "Sox2"
## [64] "Lin28a" "Dnmt3a" "CbX7" "Kdm2b" "Atf7ip2"
##
##
## $visible
## [1] FALSE
```

Or we can make our own. Note that the genes above are good defaults for real (Mouse) datasets, but will not work in the remainder of this vignette.

We will instead construct our own, following the schema of "name" -> vector of genes.

```
gois_list <- list(apple = paste0("G", 100:105),
                  banana = paste0("G", 810:819),
                  carrot = paste0("G", 256:260))

gois_list
## $apple
## [1] "G100" "G101" "G102" "G103" "G104" "G105"
##
## $banana
## [1] "G810" "G811" "G812" "G813" "G814" "G815" "G816" "G817" "G818" "G819"
##
## $carrot
## [1] "G256" "G257" "G258" "G259" "G260"
```

1.2 RNA-Seq Analysis (Exploratory)

Let us now perform an analysis.

It is possible to do the entire analysis with a single call to the `rnaseqhelper` function, but it's sometimes better to perform a more exploratory analysis.

1.2.0.1 Selecting for Informative Genes Not all of our genes will be useful for RNA-seq analysis, so we need to select for an informative subset using two parameters:

- `min_detect`, the minimum threshold of expression a gene must express in a sample before it can count as "detected".
- `min_occur`, the minimum occurrences of a "detected" gene across all samples.

Here we will use 3 and 2, selecting for genes that occur in at least 3 samples with a minimum expression of 2.

```
keep_genes <- high_quality_genes(mat, min_occur=3, min_detect=2,
                                out_dir=file.path(tempdir(), "0_genes"))
## Dropped: 0 genes (0%)
```

We are told that no genes were dropped.

We can inspect this ourselves by examining the output file "0_genes/smallestGroup3-detected2-keep_genes".

Usually we expect a few genes to be dropped, so let's increase the parameters:

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```
keep_genes <- high_quality_genes(mat, min_occur=8, min_detect=20,  
                                out_dir=file.path(tempdir(), "0_genes"))  
## Dropped: 77 genes (0%)
```

Here we see 87 genes are dropped, leaving us with 92% of the data still, and we can once again inspect this directly by looking at the “0_genes/smallestGroup9-detected10-keep_genes” file

1.2.0.2 Validating for Informative Genes We will now perform a preliminary DESeq2 analysis using our filtered genes and using the `phenotype_data` to select for sample groups.

The `condition` column in the `phenotype_data` is used to group are samples, and is an implicit declaration.

```
res <- run_deseq(mat, keep_genes, phenotype_data,  
                out_dir = file.path(tempdir(), "1_matrices_and_deseq"))  
## Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in  
## design formula are characters, converting to factors  
## estimating size factors  
## estimating dispersions  
## gene-wise dispersion estimates  
## mean-dispersion relationship  
## -- note: fitType='parametric', but the dispersion trend was not well captured by the  
##    function: y = a/x + b, and a local regression fit was automatically substituted.  
##    specify fitType='local' or 'mean' to avoid this message next time.  
## final dispersion estimates  
## fitting model and testing  
## -- note: fitType='parametric', but the dispersion trend was not well captured by the  
##    function: y = a/x + b, and a local regression fit was automatically substituted.  
##    specify fitType='local' or 'mean' to avoid this message next time.  
## using ntop=500 top features by variance  
## -- note: fitType='parametric', but the dispersion trend was not well captured by the  
##    function: y = a/x + b, and a local regression fit was automatically substituted.  
##    specify fitType='local' or 'mean' to avoid this message next time.  
## using ntop=500 top features by variance
```

Here tables and plots were written to the “1_matrices_and_deseq” folder:

- `deseq2obj.rds`, an RData object of the DESeq2 object, useful mostly for debugging.
- `phenotype_data.tsv`, the input phenotype data table.
- `input_matrix.tsv`, the input matrix table.
- `input_matrix.filt.tsv`, the above matrix, filtered for the genes kept.
- `input_matrix.filt.normalized.tsv`, the above filtered matrix, normalized by DESeq2.
- `input_matrix.filt.normalized.vst_corrected.tsv`, the filtered matrix with a Variance Stabilizing Transform to correct the data.
- `input_matrix.filt.normalized.vst_corrected_PCA.pdf`, a PCA of the normalized and transformed values.

What you should be seeing at this stage are samples of the same type clustered together. The transformed values might cluster better than the normalized values, so it is up to you which one of these you choose for downstream analysis.

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1.2.0.3 Heatmaps and Volcano Plots We will now perform the rest of the analysis, by searching for differentially expressed (DE) genes via contrasting sample groups against one another, and producing pairwise (sample group1 vs sample group2) kmeans heatmaps and global (all samples) kmeans heatmaps for a variety of k-values.

Let's contrast our green and blue samples against one another.

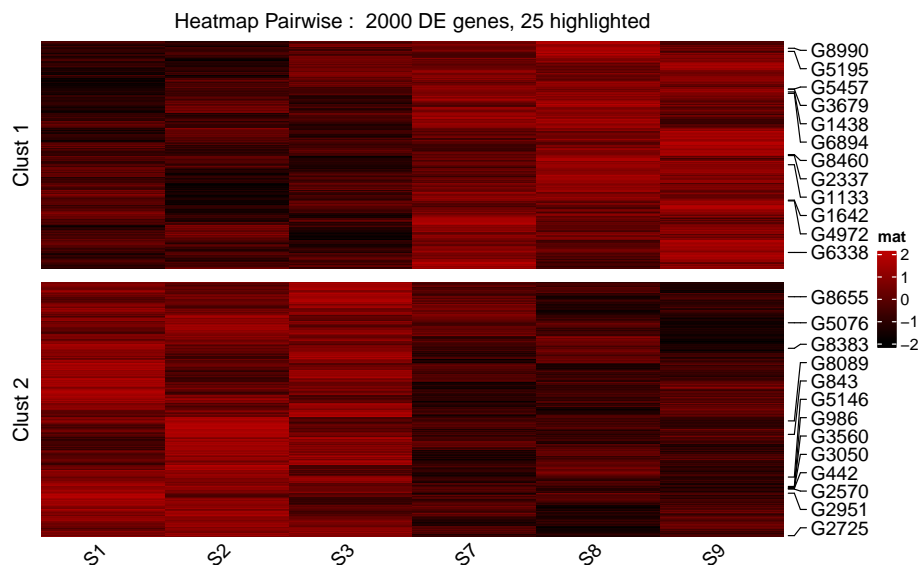
We will be using the output of the `run_deseq` function in the last step which we called `res`. We will also use our genes of interest.

```
pairwise_hmap_volcano(res[["ddsObj"]],
                      numer = "green", denom = "blue",
                      transformed_counts = assay(res[["vFalse"]]),
                      top_ngenes_tocluster = 2000,
                      top_ngenes_tohighlight = 25,
                      score_thresh = c(0.5, 0.9),
                      genes_of_interest = gois_list,
                      kmeans_list = c(2, 5),
                      out_dirprefix=file.path(tempdir(), "2_heatmaps"))

## Started Analysis: Sat Feb 17 17:38:57 2024
## Saved Volcano: /tmp/Rtmp1Iu96p/2_heatmaps/green-vs-blue/volcano-pairwise.svg
## Saved DESeq2 Results: /tmp/Rtmp1Iu96p/2_heatmaps/green-vs-blue/deseq2_results.tsv
## [Running k=2]
## - Heatmap on samples: S1,S2,S3,S7,S8,S9
## - Using normalized counts
## - Saved Norm: /tmp/Rtmp1Iu96p/2_heatmaps/green-vs-blue/kmeans2/k2_norm.tsv
## - Saved Scale: /tmp/Rtmp1Iu96p/2_heatmaps/green-vs-blue/kmeans2/k2_norm.tsv
## Condition per Time Ribbon Plot for score >=0.5
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - Plotting genes Norm
## Condition per Time Ribbon Plot for score >=0.5
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - Plotting genes Scaled
```


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```
##      - Plotting genes List
##      - apple (no genes found)
##      - banana
## Condition per Time Ribbon [Log10 Normalised Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon [Scaled Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
##      - carrot
## Condition per Time Ribbon [Log10 Normalised Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon [Scaled Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
##      - Using transformed counts too
```

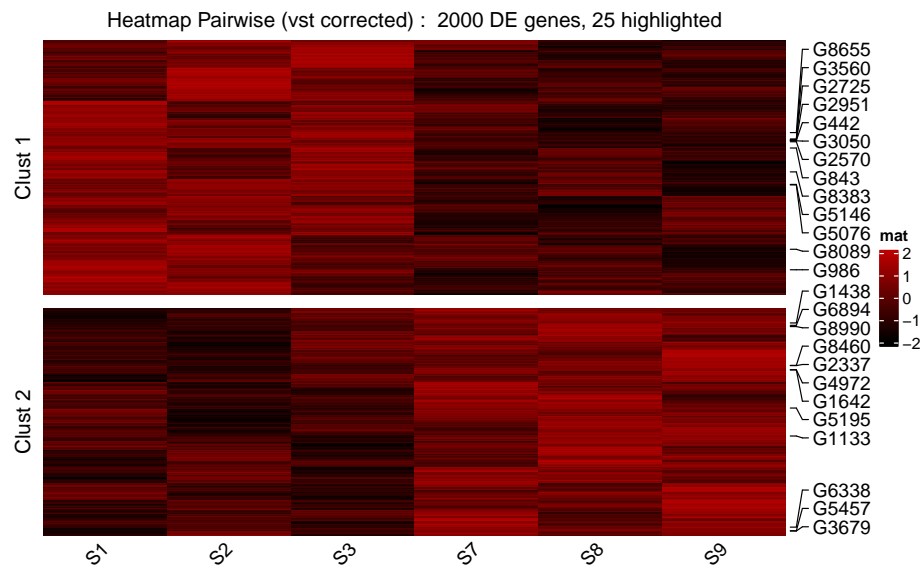


```
##      - Saved Norm: /tmp/Rtmp1Iu96p/2_heatmaps/green_vs_blue/kmeans2/k2_norm.tsv
##      - Saved Scale: /tmp/Rtmp1Iu96p/2_heatmaps/green_vs_blue/kmeans2/k2_norm.tsv
## Condition per Time Ribbon Plot for score >=0.5
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
```

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```
## Computation failed in `stat_summary()`  
## Caused by error in `get()`:  
## ! object 'mean_sdl' of mode 'function' was not found  
## - Plotting genes Norm  
## Condition per Time Ribbon Plot for score >=0.5  
## Warning: Computation failed in `stat_summary()`  
## Computation failed in `stat_summary()`  
## Caused by error in `get()`:  
## ! object 'mean_sdl' of mode 'function' was not found  
## Condition per Time Ribbon Plot for score >=0.9  
## Warning: Computation failed in `stat_summary()`  
## Computation failed in `stat_summary()`  
## Computation failed in `stat_summary()`  
## Computation failed in `stat_summary()`  
## Caused by error in `get()`:  
## ! object 'mean_sdl' of mode 'function' was not found  
## - Plotting genes Scaled  
## - Plotting genes List  
## - apple (no genes found)  
## - banana  
## Condition per Time Ribbon [Log10 Normalised Expression]  
## Warning: Computation failed in `stat_summary()`  
## Caused by error in `get()`:  
## ! object 'mean_sdl' of mode 'function' was not found  
## Condition per Time Ribbon [Scaled Expression]  
## Warning: Computation failed in `stat_summary()`  
## Caused by error in `get()`:  
## ! object 'mean_sdl' of mode 'function' was not found  
## - carrot  
## Condition per Time Ribbon [Log10 Normalised Expression]  
## Warning: Computation failed in `stat_summary()`  
## Caused by error in `get()`:  
## ! object 'mean_sdl' of mode 'function' was not found  
## Condition per Time Ribbon [Scaled Expression]  
## Warning: Computation failed in `stat_summary()`  
## Caused by error in `get()`:  
## ! object 'mean_sdl' of mode 'function' was not found  
## - Storing Results k2:/tmp/Rtmp1Iu96p/2_heatmaps/green_vs_blue/kmeans2/deseq2.results.cluster.k2.tsv  
## - Heatmap all samples in normalised matrix for clustering  
## - Using normalized counts
```

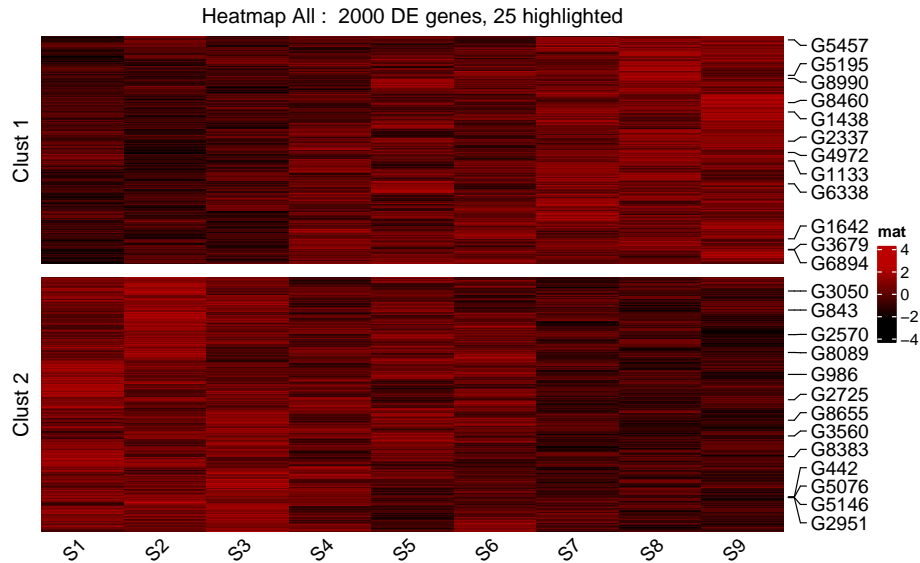
Easily run a RNA-Seq analysis via RNASeqHelper



```
## - Saved Norm: /tmp/Rtmp1Iu96p/2-heatmaps/green-vs-blue/kmeans2/k2_norm.tsv
## - Saved Scale: /tmp/Rtmp1Iu96p/2-heatmaps/green-vs-blue/kmeans2/k2_norm.tsv
## Condition per Time Ribbon Plot for score >=0.5
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - Plotting genes Norm
## Condition per Time Ribbon Plot for score >=0.5
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - Plotting genes Scaled
## - Plotting genes List
## - apple (no genes found)
## - banana
## Condition per Time Ribbon [Log10 Normalised Expression]
## Warning: Computation failed in `stat_summary()`
```

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```
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon [Scaled Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - carrot
## Condition per Time Ribbon [Log10 Normalised Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon [Scaled Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - Using transformed counts too
```

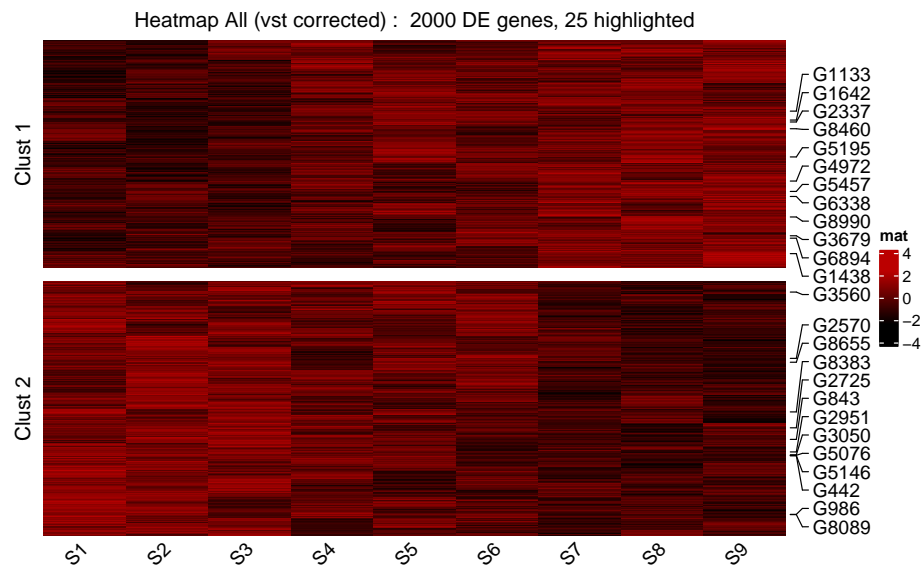


```
## - Saved Norm: /tmp/Rtmp1Iu96p/2_heatmaps/green_vs_blue/kmeans2/k2_norm.tsv
## - Saved Scale: /tmp/Rtmp1Iu96p/2_heatmaps/green_vs_blue/kmeans2/k2_norm.tsv
## Condition per Time Ribbon Plot for score >=0.5
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - Plotting genes Norm
## Condition per Time Ribbon Plot for score >=0.5
```

Easily run a RNA-Seq analysis via RNASeqHelper

```
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
##   - Plotting genes Scaled
##   - Plotting genes List
##   - apple (no genes found)
##   - banana
## Condition per Time Ribbon [Log10 Normalised Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon [Scaled Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
##   - carrot
## Condition per Time Ribbon [Log10 Normalised Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon [Scaled Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
##   - Storing Results k2:/tmp/Rtmp1Iu96p/2_heatmaps/green_vs_blue/kmeans2/deseq2.results.cluster.k2.tsv
## [Running k=5]
##   - Heatmap on samples: S1,S2,S3,S7,S8,S9
##   - Using normalized counts
```

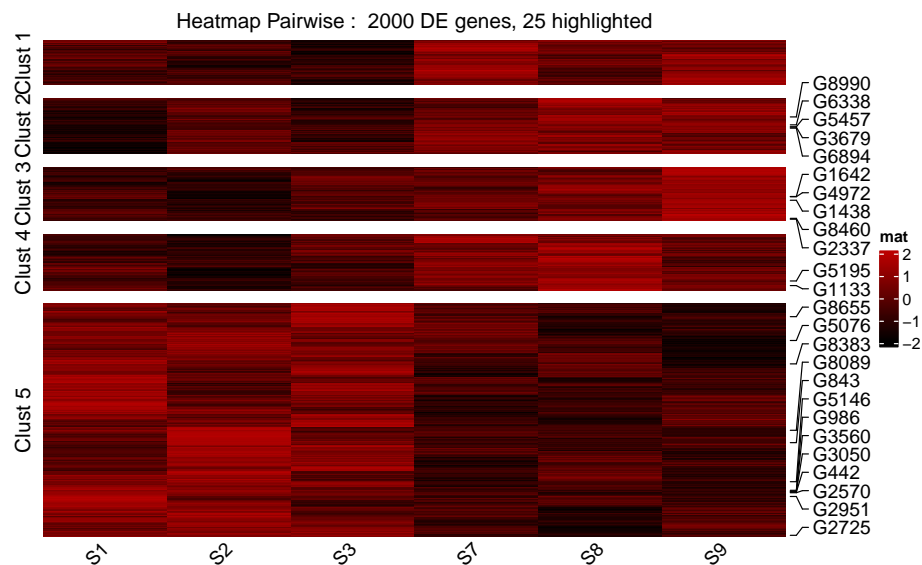
Easily run a RNA-Seq analysis via RNASeqHelper



```
## - Saved Norm: /tmp/Rtmp1Iu96p/2-heatmaps/green-vs-blue/kmeans5/k5_norm.tsv
## - Saved Scale: /tmp/Rtmp1Iu96p/2-heatmaps/green-vs-blue/kmeans5/k5_norm.tsv
## Condition per Time Ribbon Plot for score >=0.5
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - Plotting genes Norm
## Condition per Time Ribbon Plot for score >=0.5
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
```

Easily run a RNA-Seq analysis via RNASeqHelper

```
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
##   - Plotting genes Scaled
##   - Plotting genes List
##   - apple (no genes found)
##   - banana
## Condition per Time Ribbon [Log10 Normalised Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon [Scaled Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
##   - carrot
## Condition per Time Ribbon [Log10 Normalised Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon [Scaled Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
##   - Using transformed counts too
```



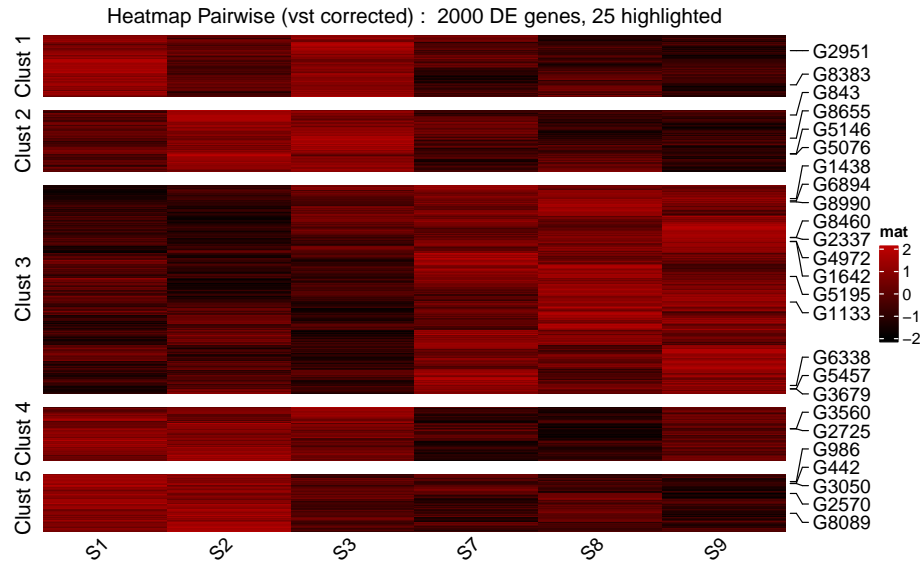
```
##   - Saved Norm: /tmp/Rtmp1Iu96p/2_heatmaps/green_vs_blue/kmeans5/k5_norm.tsv
##   - Saved Scale: /tmp/Rtmp1Iu96p/2_heatmaps/green_vs_blue/kmeans5/k5_norm.tsv
## Condition per Time Ribbon Plot for score >=0.5
## Warning: Computation failed in `stat_summary()`
```

Easily run a RNA-Seq analysis via RNASeqHelper

```
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - Plotting genes Norm
## Condition per Time Ribbon Plot for score >=0.5
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - Plotting genes Scaled
## - Plotting genes List
## - apple (no genes found)
## - banana
## Condition per Time Ribbon [Log10 Normalised Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon [Scaled Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - carrot
## Condition per Time Ribbon [Log10 Normalised Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
```


Easily run a RNA-Seq analysis via RNASeqHelper

```
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon [Scaled Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - Storing Results k5:/tmp/Rtmp1Iu96p/2_heatmaps/green_vs_blue/kmeans5/deseq2.results.cluster.k5.tsv
## - Heatmap all samples in normalised matrix for clustering
## - Using normalized counts
```

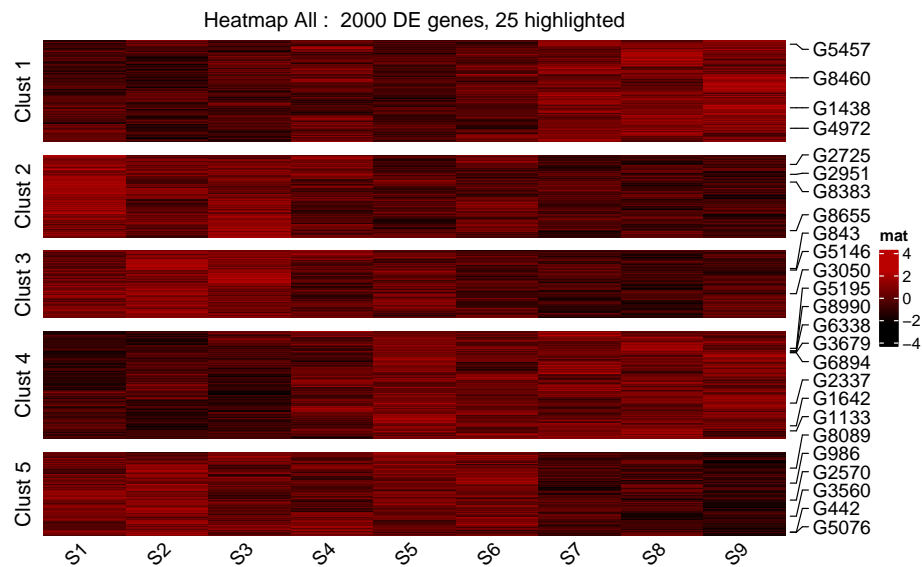


```
## - Saved Norm: /tmp/Rtmp1Iu96p/2_heatmaps/green_vs_blue/kmeans5/k5_norm.tsv
## - Saved Scale: /tmp/Rtmp1Iu96p/2_heatmaps/green_vs_blue/kmeans5/k5_norm.tsv
## Condition per Time Ribbon Plot for score >=0.5
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - Plotting genes Norm
## Condition per Time Ribbon Plot for score >=0.5
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
```

Easily run a RNA-Seq analysis via RNASeqHelper

```
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
##   - Plotting genes Scaled
##   - Plotting genes List
##   - apple (no genes found)
##   - banana
## Condition per Time Ribbon [Log10 Normalised Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon [Scaled Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
##   - carrot
## Condition per Time Ribbon [Log10 Normalised Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon [Scaled Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
##   - Using transformed counts too
```

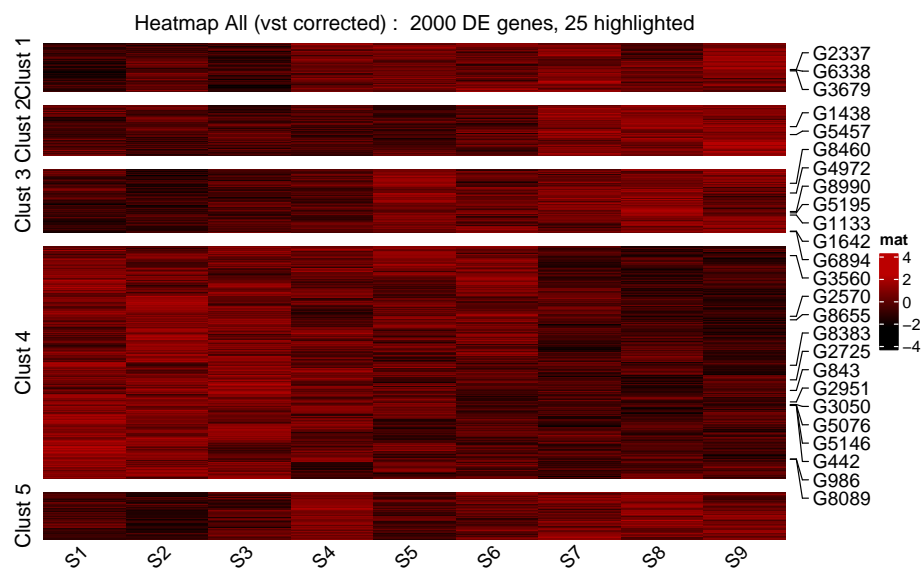
Easily run a RNA-Seq analysis via RNASeqHelper



```
## - Saved Norm: /tmp/Rtmp1Iu96p/2_heatmaps/green_vs_blue/kmeans5/k5_norm.tsv
## - Saved Scale: /tmp/Rtmp1Iu96p/2_heatmaps/green_vs_blue/kmeans5/k5_norm.tsv
## Condition per Time Ribbon Plot for score >=0.5
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - Plotting genes Norm
## Condition per Time Ribbon Plot for score >=0.5
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
```

Easily run a RNA-Seq analysis via RNASeqHelper

```
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
##   - Plotting genes Scaled
##   - Plotting genes List
##   - apple (no genes found)
##   - banana
## Condition per Time Ribbon [Log10 Normalised Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon [Scaled Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
##   - carrot
## Condition per Time Ribbon [Log10 Normalised Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Condition per Time Ribbon [Scaled Expression]
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
##   - Storing Results k5:/tmp/Rtmp1Iu96p/2_heatmaps/green_vs_blue/kmeans5/deseq2.results.cluster.k5.tsv
## Finished Analysis: Sat Feb 17 17:40:29 2024
```



The volcano plots are generated from the contrasts of our two sample groups “green” and “blue”. The DE genes from these contrasts are then used to cluster the samples pairwise and globally for different Kmeans k-values.

Easily run a RNA-Seq analysis via RNASeqHelper

The results of all can be found in the "~/myanalysis/2_heatmaps" folder.

1.3 RNA-Seq Analysis (Pipeline)

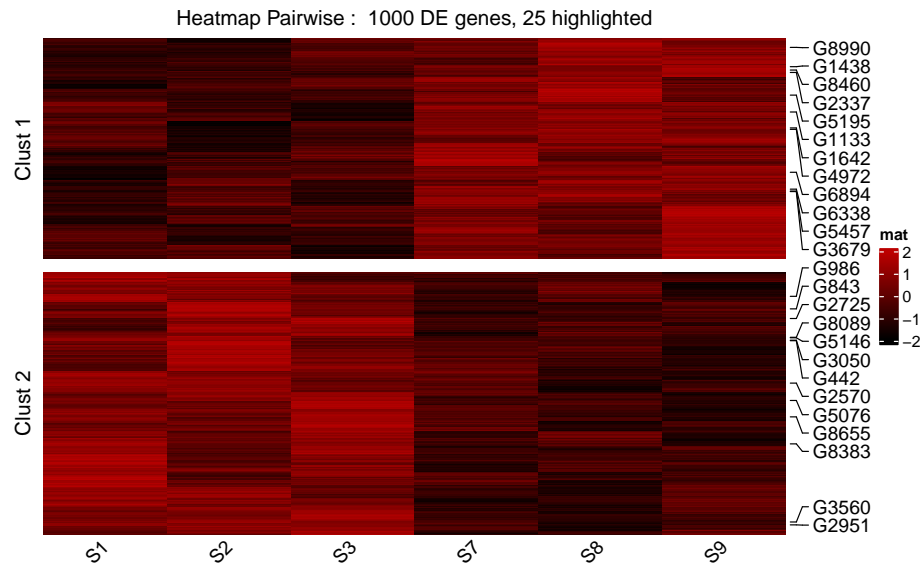
It is entirely possible to run the complete analysis above with a single function. Here we change some parameters to speed up the analysis.

```
rnaseqhelper(mat, phenotype_data, numer = "green", denom = "blue",
             out_dir=file.path(tempdir(), "second_analysis"),
             keep_params = list(min_occur=8, min_detect=20),
             heat_params = list(top_ngenes_tocluster = 1000,
                                top_ngenes_tohighlight = 25,
                                score_thresh = 0.9,
                                genes_of_interest = gois_list[["apple"]],
                                kmeans_list = 2))

## Dropped: 77 genes (0%)
## Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in
## design formula are characters, converting to factors
## estimating size factors
## estimating dispersions
## gene-wise dispersion estimates
## mean-dispersion relationship
## -- note: fitType='parametric', but the dispersion trend was not well captured by the
## function: y = a/x + b, and a local regression fit was automatically substituted.
## specify fitType='local' or 'mean' to avoid this message next time.
## final dispersion estimates
## fitting model and testing
## -- note: fitType='parametric', but the dispersion trend was not well captured by the
## function: y = a/x + b, and a local regression fit was automatically substituted.
## specify fitType='local' or 'mean' to avoid this message next time.
## using ntop=500 top features by variance
## -- note: fitType='parametric', but the dispersion trend was not well captured by the
## function: y = a/x + b, and a local regression fit was automatically substituted.
## specify fitType='local' or 'mean' to avoid this message next time.
## using ntop=500 top features by variance
## Started Analysis: Sat Feb 17 17:40:44 2024
## Saved Volcano: /tmp/Rtmp1Iu96p/second_analysis/2_heatmaps/green_vs_blue/volcano_pairwise.svg
## Saved DESeq2 Results: /tmp/Rtmp1Iu96p/second_analysis/2_heatmaps/green_vs_blue/deseq2_results.tsv
## [Running k=2]
## - Heatmap on samples: S1,S2,S3,S7,S8,S9
## - Using normalized counts
## - Saved Norm: /tmp/Rtmp1Iu96p/second_analysis/2_heatmaps/green_vs_blue/kmeans2/k2_norm.tsv
## - Saved Scale: /tmp/Rtmp1Iu96p/second_analysis/2_heatmaps/green_vs_blue/kmeans2/k2_norm.tsv
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
```

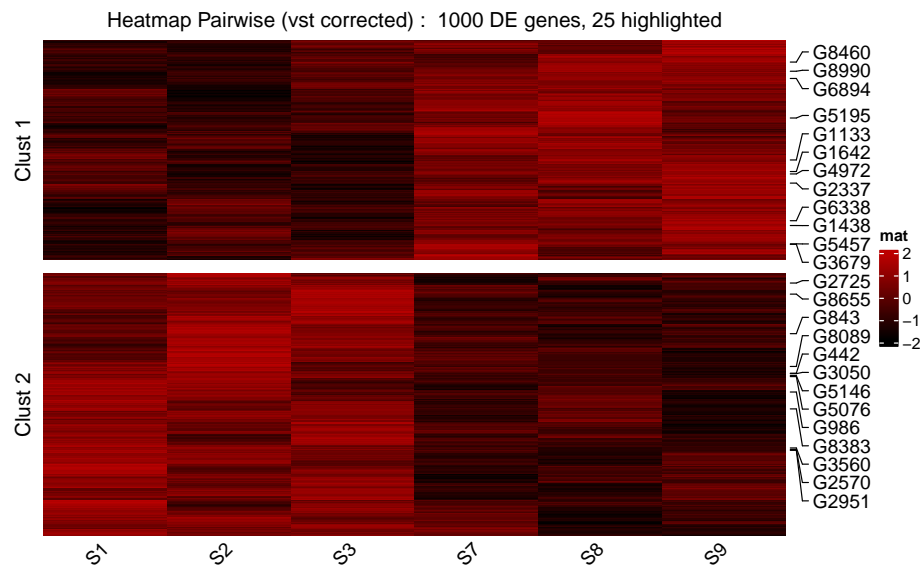
Easily run a RNA-Seq analysis via RNASeqHelper

```
## - Plotting genes Norm
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - Plotting genes Scaled
## - Plotting genes List
## - Using transformed counts too
```



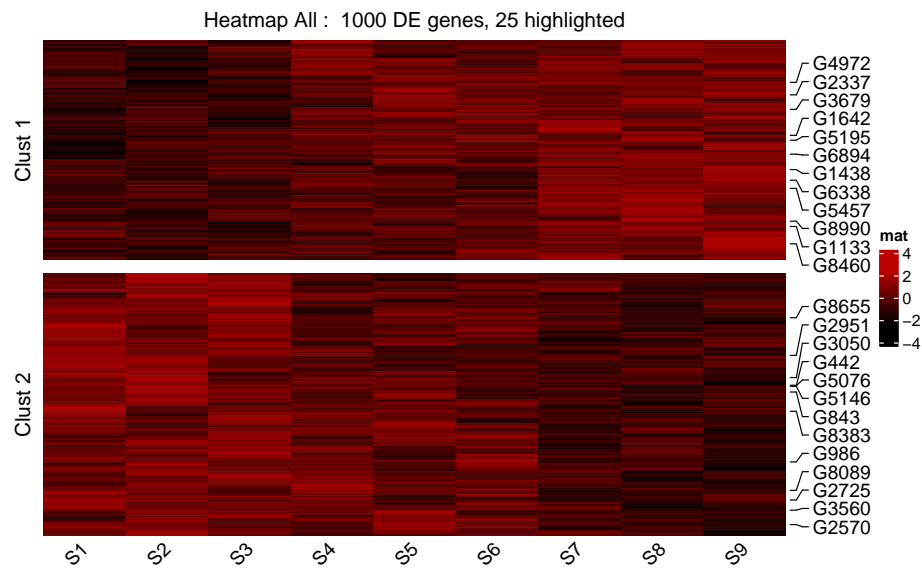
```
## - Saved Norm: /tmp/Rtmp1Iu96p/second_analysis/2_heatmaps/green_vs_blue/kmeans2/k2_norm.tsv
## - Saved Scale: /tmp/Rtmp1Iu96p/second_analysis/2_heatmaps/green_vs_blue/kmeans2/k2_norm.tsv
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - Plotting genes Norm
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - Plotting genes Scaled
## - Plotting genes List
## - Storing Results k2:/tmp/Rtmp1Iu96p/second_analysis/2_heatmaps/green_vs_blue/kmeans2/deseq2.results.csv
## - Heatmap all samples in normalised matrix for clustering
## - Using normalized counts
```

Easily run a RNA-Seq analysis via RNASeqHelper



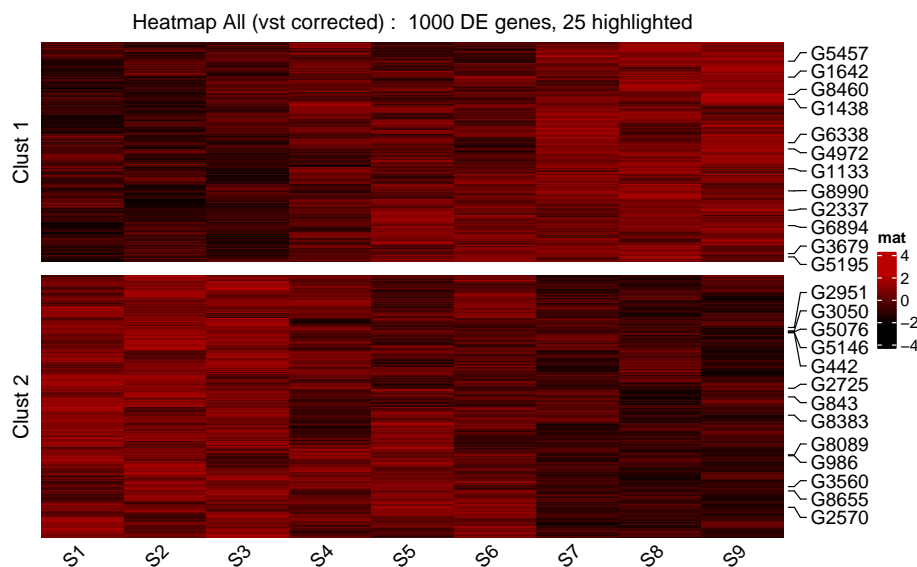
```
## - Saved Norm: /tmp/Rtmp1Iu96p/second_analysis/2_heatmaps/green-vs_blue/kmeans2/k2_norm.tsv
## - Saved Scale: /tmp/Rtmp1Iu96p/second_analysis/2_heatmaps/green-vs_blue/kmeans2/k2_norm.tsv
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - Plotting genes Norm
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - Plotting genes Scaled
## - Plotting genes List
## - Using transformed counts too
```

Easily run a RNA-Seq analysis via RNASeqHelper



```
## - Saved Norm: /tmp/Rtmp1Iu96p/second_analysis/2_heatmaps/green-vs-blue/kmeans2/k2_norm.tsv
## - Saved Scale: /tmp/Rtmp1Iu96p/second_analysis/2_heatmaps/green-vs-blue/kmeans2/k2_norm.tsv
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - Plotting genes Norm
## Condition per Time Ribbon Plot for score >=0.9
## Warning: Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Computation failed in `stat_summary()`
## Caused by error in `get()`:
## ! object 'mean_sdl' of mode 'function' was not found
## - Plotting genes Scaled
## - Plotting genes List
## - Storing Results k2:/tmp/Rtmp1Iu96p/second_analysis/2_heatmaps/green-vs-blue/kmeans2/deseq2.results.csv
## Finished Analysis: Sat Feb 17 17:41:04 2024
```


Easily run a RNA-Seq analysis via RNASeqHelper



1.4 SessionInfo

```
sessionInfo()
## R Under development (unstable) (2024-01-16 r85808)
## Platform: x86_64-pc-linux-gnu
## Running under: Ubuntu 22.04.3 LTS
##
## Matrix products: default
## BLAS: /home/biocbuild/bbs-3.19-bioc/R/lib/libRblas.so
## LAPACK: /usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.10.0
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
##  [3] LC_TIME=en_GB             LC_COLLATE=C
##  [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
##
## time zone: America/New_York
## tzcode source: system (glibc)
##
## attached base packages:
## [1] stats4      stats      graphics  grDevices  utils      datasets  methods
## [8] base
##
## other attached packages:
##  [1] RNASeqHelper_0.99.7      svglite_2.1.3
##  [3] SummarizedExperiment_1.33.3 Biobase_2.63.0
##  [5] GenomicRanges_1.55.3     GenomeInfoDb_1.39.6
##  [7] IRanges_2.37.1           S4Vectors_0.41.3
##  [9] BiocGenerics_0.49.1      MatrixGenerics_1.15.0
```

Easily run a RNA-Seq analysis via RNASeqHelper

```
## [11] matrixStats_1.2.0          BiocStyle_2.31.0
##
## loaded via a namespace (and not attached):
## [1] bitops_1.0-7                gridExtra_2.3             rlang_1.1.3
## [4] magrittr_2.0.3              clue_0.3-65               GetoptLong_1.0.5
## [7] compiler_4.4.0              png_0.1-8                 systemfonts_1.0.5
## [10] vctrs_0.6.5                 stringr_1.5.1             pkgconfig_2.0.3
## [13] shape_1.4.6                 crayon_1.5.2              fastmap_1.1.1
## [16] magick_2.8.2                backports_1.4.1           XVector_0.43.1
## [19] labeling_0.4.3              utf8_1.2.4                rmarkdown_2.25
## [22] tzdb_0.4.0                  ragg_1.2.7                bit_4.0.5
## [25] purrr_1.0.2                 xfun_0.42                 zlibbioc_1.49.0
## [28] DelayedArray_0.29.3         BiocParallel_1.37.0       parallel_4.4.0
## [31] cluster_2.1.6               R6_2.5.1                  stringi_1.8.3
## [34] RColorBrewer_1.1-3          rpart_4.1.23              Rcpp_1.0.12
## [37] bookdown_0.37               iterators_1.0.14           knitr_1.45
## [40] base64enc_0.1-3            readr_2.1.5               Matrix_1.6-5
## [43] nnet_7.3-19                 tidyselect_1.2.0          rstudioapi_0.15.0
## [46] abind_1.4-5                  yaml_2.3.8                doParallel_1.0.17
## [49] codetools_0.2-19           lattice_0.22-5            tibble_3.2.1
## [52] withr_3.0.0                 evaluate_0.23             foreign_0.8-86
## [55] archive_1.1.7               circlize_0.4.15           pillar_1.9.0
## [58] BiocManager_1.30.22        checkmate_2.3.1           foreach_1.5.2
## [61] generics_0.1.3             vroom_1.6.5               RCurl_1.98-1.14
## [64] hms_1.1.3                   ggplot2_3.4.4             munsell_0.5.0
## [67] scales_1.3.0                glue_1.7.0                pheatmap_1.0.12
## [70] Hmisc_5.1-1                 tools_4.4.0               data.table_1.15.0
## [73] locfit_1.5-9.8              Cairo_1.6-2               grid_4.4.0
## [76] tidyr_1.3.1                 colorspace_2.1-0          GenomeInfoDbData_1.2.11
## [79] patchwork_1.2.0             htmlTable_2.4.2           Formula_1.2-5
## [82] cli_3.6.2                   textshaping_0.3.7         fansi_1.0.6
## [85] S4Arrays_1.3.3              ComplexHeatmap_2.19.0     dplyr_1.1.4
## [88] gtable_0.3.4                DESeq2_1.43.2             digest_0.6.34
## [91] SparseArray_1.3.4           ggrepel_0.9.5             farver_2.1.1
## [94] rjson_0.2.21                htmlwidgets_1.6.4         htmltools_0.5.7
## [97] lifecycle_1.0.4             GlobalOptions_0.1.2       bit64_4.0.5
```