SCENIC

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Contents

Loading packages

```
suppressMessages(library(dplyr))
suppressMessages(library(Seurat))
suppressMessages(library(patchwork))
suppressMessages(library(ggplot2))
suppressMessages(library(SCENIC))
suppressMessages(library(ComplexHeatmap))
suppressMessages(library(AUCell))
suppressMessages(library(stringr))
```

Loading data

```
cells.combined <- readRDS("../8.2-Scoring_Ly6G_Macs/cells.combined_Part2.rds")
```

Prepare cellinfo and Expression matrix

```
exprMat <- cells.combined@assays$RNA@data
cellInfo <- data.frame(seuratCluster=Idents(cells.combined))
#cellInfo$Merge_annotation <- cells.combined$Merge_annotation
cellInfo$nGene <- colSums(exprMat>0)

dir.create ("int")
saveRDS(exprMat, file = "int/exprMat.Rds")
saveRDS(cellInfo, file = "int/cellInfo.Rds")
```

Download Species-specific databases

In addition to the R-packages, you will need to download the species-specific databases for RcisTarget (the motif rankings). By default, SCENIC uses the databases that score the motifs in the promoter of the genes (up to 500bp upstream the TSS), and in the 20kb around the TSS (+/-10kbp).

```
dbFiles <-
c("https://resources.aertslab.org/cistarget/databases/old/homo_sapiens/hg19/refseq_r45/mc9nr/gene_based/
"https://resources.aertslab.org/cistarget/databases/old/homo_sapiens/hg19/refseq_r45/mc9nr/gene_based/hg

dir.create("cisTarget_databases");
setwd("cisTarget_databases")
for(featherURL in dbFiles)
{
  download.file(featherURL, destfile=basename(featherURL)) # saved in current dir
}
```

Initialise Scenic

```
exprMat <- readRDS("int/exprMat.Rds")
cellInfo <- readRDS("int/cellInfo.Rds")

data(list="motifAnnotations_hgnc_v9", package="RcisTarget")
motifAnnotations_hgnc <- motifAnnotations_hgnc_v9

org <- "hgnc"
dbDir <- "cisTarget_databases"
dbDir <- path.expand(dbDir)
myDatasetTitle <- "SCENIC Analysis"
data(defaultDbNames)
dbs <- defaultDbNames[[org]]
scenicOptions <- initializeScenic(org=org, dbDir=dbDir, dbs=dbs, nCores=25)
```

Gene filtering

1. Filter by the total number of reads per gene. Keeps only the genes with at least 6 UMI counts across all samples.
2. Filter by the number of cells in which the gene is detected.

```
exprMat <- as.matrix(exprMat) # need a regulat matrix
genesKept <- geneFiltering(exprMat, scenicOptions=scenicOptions,
                           minCountsPerGene=3*.01*ncol(exprMat),
                           minSamples=ncol(exprMat)*.01)

dim(exprMat)
exprMat_filtered <- exprMat[genesKept, ]
dim(exprMat_filtered)
```

Correlation

```
runCorrelation(exprMat_filtered, scenicOptions)
```

Genie3

This step is time consuming

```
runGenie3(exprMat_filtered, scenicOptions)
```

Build and score the GRN

```
scenicOptions@settings$verbose <- TRUE
scenicOptions@settings$nCores <- 10
scenicOptions@settings$seed <- 123

runSCENIC_1_coexNetwork2modules(scenicOptions)
runSCENIC_2_createRegulons(scenicOptions)
runSCENIC_3_scoreCells(scenicOptions, exprMat_filtered)
```

Plotting the results

Viewing markers based on specificity score

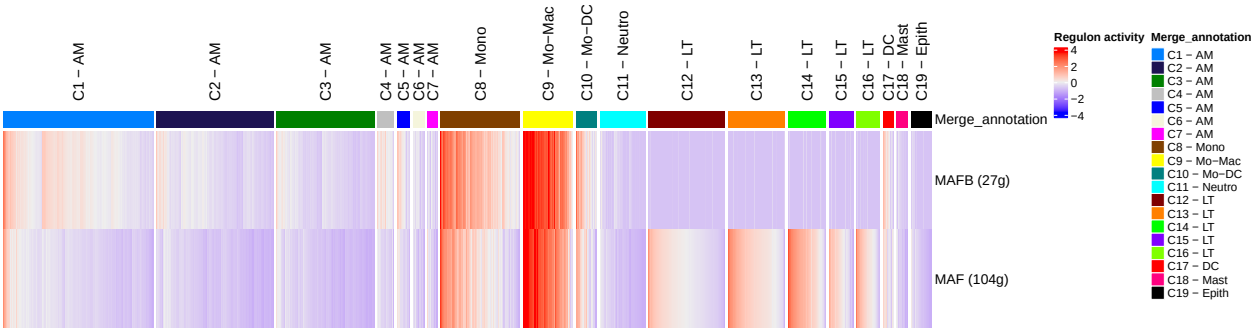
```
regulonAUC <- loadInt(scenicOptions, "aucell_regulonAUC") # require file
int/3.4_regulonAUC.Rds

rss <- calcRSS(AUC=getAUC(regulonAUC), cellAnnotation=cellInfo[colnames(regulonAUC),
"seuratCluster"])

rssPlot <- plotRSS(rss)
plotly::ggplotly(rssPlot$plot)
```

Plotting cMaf et Mafb

```
regulonAUC <- readRDS("int/3.4_regulonAUC.Rds")

names <- c("MAFB (27g)", "MAF (104g)")
regulonAUC.mat <- regulonAUC@assays@data@listData$AUC
```

```
Subset_regulonActivity <-regulonAUC.mat[names,]


regulonActivity_byCellType_Scaled <- t(scale(t(Subset_regulonActivity), center = T,
scale=T))

color_clusters <- c("#0080FF", "#1d1352", "#008000", "#C0C0C0", "#0000FF", "beige",
"#FF00FF", "#804000", "#FFFF00", "#008080", "#00FFFF", "#800000", "#FF8000", "#00FF00",
"#8000FF", "#80FF00", "#FF0000", "#FF0080", "#000000")

df <- as.data.frame(cells.combined$Merge_annotation)
colnames(df) <- "Merge_annotation"

color_df <- list(Merge_annotation = c("C1 - AM" = "#0080FF",
                                "C2 - AM" = "#1d1352",
                                "C3 - AM" = "#008000",
                                "C4 - AM" = "#C0C0C0",
                                "C5 - AM" = "#0000FF",
                                "C6 - AM" = "beige",
                                "C7 - AM" = "#FF00FF",
                                "C8 - Mono" = "#804000",
                                "C9 - Mo-Mac" = "#FFFF00",
                                "C10 - Mo-DC" = "#008080",
                                "C11 - Neutro" = "#00FFFF",
                                "C12 - LT" = "#800000",
                                "C13 - LT" = "#FF8000",
                                "C14 - LT" = "#00FF00",
                                "C15 - LT" = "#8000FF",
                                "C16 - LT" = "#80FF00",
                                "C17 - DC" = "#FF0000",
                                "C18 - Mast" = "#FF0080",
                                "C19 - Epith" = "#000000"))

Heatmap <- Heatmap(regulonActivity_byCellType_Scaled, name="Regulon activity",
show_column_names = FALSE,
        column_split = factor(cells.combined$Merge_annotation),
        cluster_column_slices = F,
        cluster_rows = F,
        top_annotation = HeatmapAnnotation(df = df, col = color_df),
        show_column_dend = F,
        column_title_rot = 90)

#tidyHeatmap::save_pdf(Heatmap,"Scenic_cMAFf&MAFB.pdf", width = 40, height = 10, units =
"cm")
```

```
Heatmap
```




```
sessionInfo()
```

```
## R version 4.3.3 (2024-02-29)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 22.04.4 LTS
##
## Matrix products: default
## BLAS:   /usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3
## LAPACK: /usr/lib/x86_64-linux-gnu/openblas-pthread/libopenblasp-r0.3.20.so;  LAPACK version 3.10.0
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=fr_BE.UTF-8        LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=fr_BE.UTF-8    LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=fr_BE.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=fr_BE.UTF-8 LC_IDENTIFICATION=C
##
## time zone: Europe/Brussels
## tzcode source: system (glibc)
##
## attached base packages:
## [1] grid      stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
## [1] stringr_1.5.0         AUCell_1.22.0         ComplexHeatmap_2.16.0
## [4] SCENIC_1.3.1          ggplot2_3.4.2         patchwork_1.1.2
## [7] SeuratObject_4.1.3    Seurat_4.3.0          dplyr_1.1.2
##
## loaded via a namespace (and not attached):
##   [1] RcppAnnoy_0.0.21            splines_4.3.3
##   [3] later_1.3.1                 bitops_1.0-7
##   [5] tibble_3.2.1                R.oo_1.25.0
##   [7] polyclip_1.10-4             graph_1.78.0
##   [9] XML_3.99-0.14               lifecycle_1.0.3
##  [11] doParallel_1.0.17           globals_0.16.2
##  [13] lattice_0.22-5              MASS_7.3-60
##  [15] magrittr_2.0.3              plotly_4.10.2
##  [17] rmarkdown_2.23              yaml_2.3.7
##  [19] httpuv_1.6.11               sctransform_0.3.5
##  [21] spam_2.9-1                  sp_2.0-0
##  [23] spatstat.sparse_3.0-2       reticulate_1.30
```

```
##  [25] cowplot_1.1.1              pbapply_1.7-2
##  [27] DBI_1.1.3                  RColorBrewer_1.1-3
##  [29] abind_1.4-5                zlibbioc_1.46.0
##  [31] Rtsne_0.16                 GenomicRanges_1.52.0
##  [33] purrr_1.0.1                R.utils_2.12.2
##  [35] BiocGenerics_0.46.0        RCurl_1.98-1.12
##  [37] circlize_0.4.15            GenomeInfoDbData_1.2.10
##  [39] IRanges_2.34.0             S4Vectors_0.38.1
##  [41] ggrepel_0.9.3              irlba_2.3.5.1
##  [43] listenv_0.9.0              spatstat.utils_3.0-3
##  [45] goftest_1.2-3              spatstat.random_3.1-5
##  [47] annotate_1.78.0            fitdistrplus_1.1-11
##  [49] parallelly_1.36.0          DelayedMatrixStats_1.22.1
##  [51] leiden_0.4.3               codetools_0.2-19
##  [53] DelayedArray_0.26.3        shape_1.4.6
##  [55] tidyselect_1.2.0           matrixStats_1.0.0
##  [57] stats4_4.3.3               spatstat.explore_3.2-1
##  [59] jsonlite_1.8.7             GetoptLong_1.0.5
##  [61] ellipsis_0.3.2             progressr_0.13.0
##  [63] iterators_1.0.14           ggridges_0.5.4
##  [65] survival_3.5-8             foreach_1.5.2
##  [67] tools_4.3.3                ica_1.0-3
##  [69] Rcpp_1.0.11                glue_1.6.2
##  [71] gridExtra_2.3              xfun_0.39
##  [73] MatrixGenerics_1.12.2      GenomeInfoDb_1.36.0
##  [75] withr_2.5.0                fastmap_1.1.1
##  [77] fansi_1.0.4                digest_0.6.33
##  [79] R6_2.5.1                   mime_0.12
##  [81] colorspace_2.1-0           scattermore_1.2
##  [83] tensor_1.5                 spatstat.data_3.0-1
##  [85] RSQLite_2.3.1              R.methodsS3_1.8.2
##  [87] utf8_1.2.3                 tidyr_1.3.0
##  [89] generics_0.1.3             data.table_1.14.8
##  [91] httr_1.4.6                 htmlwidgets_1.6.2
##  [93] S4Arrays_1.0.4             uwot_0.1.16
##  [95] pkgconfig_2.0.3            gtable_0.3.3
##  [97] blob_1.2.4                 lmtest_0.9-40
##  [99] XVector_0.40.0             htmltools_0.5.5
## [101] dotCall64_1.0-2            clue_0.3-64
## [103] GSEABase_1.62.0            scales_1.2.1
## [105] Biobase_2.60.0             png_0.1-8
## [107] knitr_1.43                 rstudioapi_0.14
## [109] rjson_0.2.21               reshape2_1.4.4
## [111] nlme_3.1-163               GlobalOptions_0.1.2
## [113] zoo_1.8-12                 cachem_1.0.8
## [115] KernSmooth_2.23-22         parallel_4.3.3
## [117] miniUI_0.1.1.1             AnnotationDbi_1.62.1
## [119] pillar_1.9.0               vctrs_0.6.3
## [121] RANN_2.6.1                 promises_1.2.0.1
## [123] xtable_1.8-4               cluster_2.1.6
## [125] evaluate_0.21              magick_2.7.5
## [127] cli_3.6.1                  compiler_4.3.3
## [129] rlang_1.1.1                crayon_1.5.2
## [131] future.apply_1.11.0        plyr_1.8.8
```

```
## [133] stringi_1.7.12              viridisLite_0.4.2
## [135] deldir_1.0-9                munsell_0.5.0
## [137] Biostrings_2.68.1           lazyeval_0.2.2
## [139] spatstat.geom_3.2-4         Matrix_1.6-1
## [141] sparseMatrixStats_1.12.0    bit64_4.0.5
## [143] future_1.33.0               KEGGREST_1.40.0
## [145] shiny_1.7.4.1               highr_0.10
## [147] SummarizedExperiment_1.30.2 ROCR_1.0-11
## [149] igraph_1.5.0.1              memoise_2.0.1
## [151] bit_4.0.5
```