

WGCNA Analysis of scRNA-seq Data

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1. Read the merged Data

```
## [1] 885 4

## Allowing multi-threading with up to 8 threads.

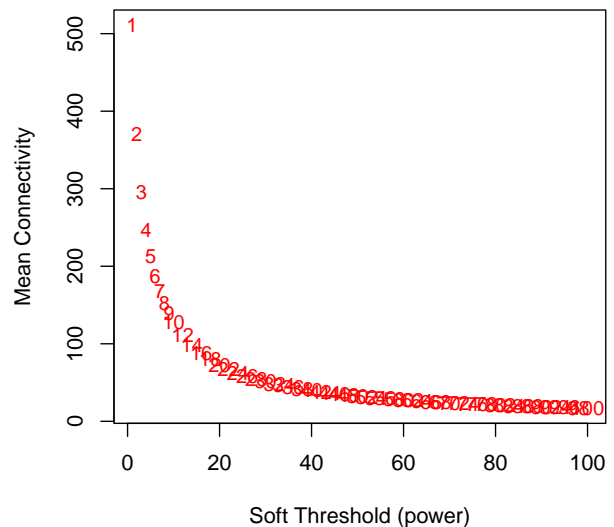
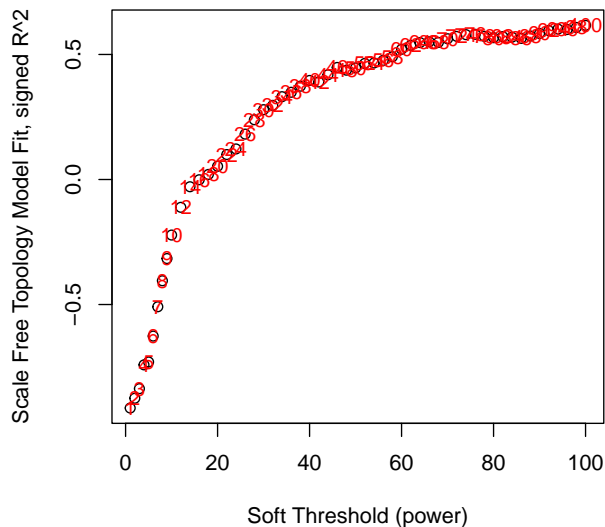
## pickSoftThreshold: will use block size 885.
## pickSoftThreshold: calculating connectivity for given powers...
## ..working on genes 1 through 885 of 885

## Power SFT.R.sq slope truncated.R.sq mean.k. median.k. max.k.
## 1 1 0.914000 4.0800 0.913 511.0 527.0 614.0
## 2 2 0.875000 2.0700 0.876 371.0 383.0 494.0
## 3 3 0.837000 1.3300 0.829 295.0 304.0 421.0
## 4 4 0.741000 0.9390 0.702 247.0 251.0 372.0
## 5 5 0.730000 0.6950 0.702 213.0 213.0 335.0
## 6 6 0.626000 0.5380 0.595 187.0 185.0 306.0
## 7 7 0.509000 0.4180 0.468 168.0 164.0 283.0
## 8 8 0.405000 0.3280 0.358 152.0 146.0 264.0
## 9 9 0.316000 0.2590 0.320 139.0 132.0 247.0
## 10 10 0.222000 0.2060 0.228 128.0 120.0 233.0
## 11 12 0.111000 0.1240 0.262 111.0 102.0 209.0
## 12 14 0.029000 0.0576 0.273 98.2 89.3 190.0
## 13 16 0.000998 0.0101 0.357 88.0 79.2 175.0
## 14 18 0.020200 -0.0452 0.439 79.8 71.8 162.0
## 15 20 0.052800 -0.0763 0.474 73.0 65.6 151.0
## 16 22 0.099700 -0.1030 0.567 67.3 60.7 141.0
## 17 24 0.122000 -0.1190 0.619 62.5 56.4 133.0
## 18 26 0.182000 -0.1450 0.657 58.3 52.3 126.0
## 19 28 0.239000 -0.1760 0.629 54.6 48.7 119.0
## 20 30 0.279000 -0.1950 0.672 51.4 45.8 114.0
## 21 32 0.296000 -0.2160 0.650 48.6 43.2 108.0
## 22 34 0.331000 -0.2290 0.707 46.0 40.9 104.0
## 23 36 0.349000 -0.2390 0.726 43.7 38.8 99.2
## 24 38 0.373000 -0.2570 0.722 41.7 36.8 95.2
## 25 40 0.396000 -0.2690 0.752 39.8 35.1 91.6
## 26 42 0.390000 -0.2750 0.739 38.1 33.5 88.2
## 27 44 0.419000 -0.2850 0.800 36.5 31.9 85.1
## 28 46 0.448000 -0.2990 0.812 35.1 30.5 82.2
## 29 48 0.437000 -0.2990 0.828 33.7 29.4 79.5
## 30 50 0.446000 -0.3030 0.842 32.5 28.3 77.0
## 31 52 0.464000 -0.3110 0.863 31.4 27.3 74.6
## 32 54 0.466000 -0.3140 0.860 30.3 26.2 72.4
## 33 56 0.476000 -0.3260 0.824 29.3 25.3 70.3
## 34 58 0.491000 -0.3340 0.841 28.4 24.6 68.3
## 35 60 0.521000 -0.3510 0.852 27.5 23.8 66.4
## 36 62 0.537000 -0.3560 0.845 26.7 23.2 64.6
## 37 64 0.548000 -0.3630 0.838 25.9 22.4 63.0
## 38 66 0.551000 -0.3640 0.841 25.2 21.8 61.4
## 39 68 0.548000 -0.3710 0.819 24.5 21.2 59.8
## 40 70 0.563000 -0.3770 0.821 23.8 20.6 58.4
## 41 72 0.572000 -0.3820 0.816 23.2 20.0 57.0
## 42 74 0.579000 -0.3850 0.819 22.7 19.5 55.7
## 43 76 0.581000 -0.3950 0.800 22.1 19.1 54.4
## 44 78 0.572000 -0.4000 0.775 21.6 18.7 53.2
## 45 80 0.567000 -0.4010 0.760 21.1 18.3 52.0
```

| | | | | | | | |
|-------|-----|----------|---------|-------|------|------|------|
| ## 46 | 82 | 0.571000 | -0.4020 | 0.757 | 20.6 | 17.9 | 50.9 |
| ## 47 | 84 | 0.569000 | -0.4060 | 0.743 | 20.1 | 17.5 | 49.9 |
| ## 48 | 86 | 0.564000 | -0.4080 | 0.725 | 19.7 | 17.2 | 48.8 |
| ## 49 | 88 | 0.574000 | -0.4090 | 0.733 | 19.3 | 16.8 | 47.9 |
| ## 50 | 90 | 0.584000 | -0.4120 | 0.743 | 18.9 | 16.5 | 46.9 |
| ## 51 | 92 | 0.596000 | -0.4180 | 0.745 | 18.5 | 16.2 | 46.1 |
| ## 52 | 94 | 0.602000 | -0.4170 | 0.750 | 18.1 | 15.8 | 45.2 |
| ## 53 | 96 | 0.600000 | -0.4190 | 0.745 | 17.8 | 15.5 | 44.4 |
| ## 54 | 98 | 0.608000 | -0.4210 | 0.752 | 17.4 | 15.2 | 43.6 |
| ## 55 | 100 | 0.617000 | -0.4210 | 0.770 | 17.1 | 14.9 | 42.8 |

Scale independence

Mean connectivity



```
## Calculating module eigengenes block-wise from all genes
## Flagging genes and samples with too many missing values...
## ..step 1
## ..Working on block 1 .
## TOM calculation: adjacency..
## ..will use 8 parallel threads.
## Fraction of slow calculations: 0.000000
## ..connectivity..
## ..matrix multiplication (system BLAS)..
## ..normalization..
## ..done.
## ..saving TOM for block 1 into file ER-block.1.RData
## ....clustering..
## ....detecting modules..
## ....calculating module eigengenes..
## ....checking kME in modules..
## ..removing 1 genes from module 3 because their KME is too low.
## ..removing 3 genes from module 4 because their KME is too low.
## ..removing 1 genes from module 9 because their KME is too low.
## ..merging modules that are too close..
## mergeCloseModules: Merging modules whose distance is less than 0.15
## Calculating new MEs...

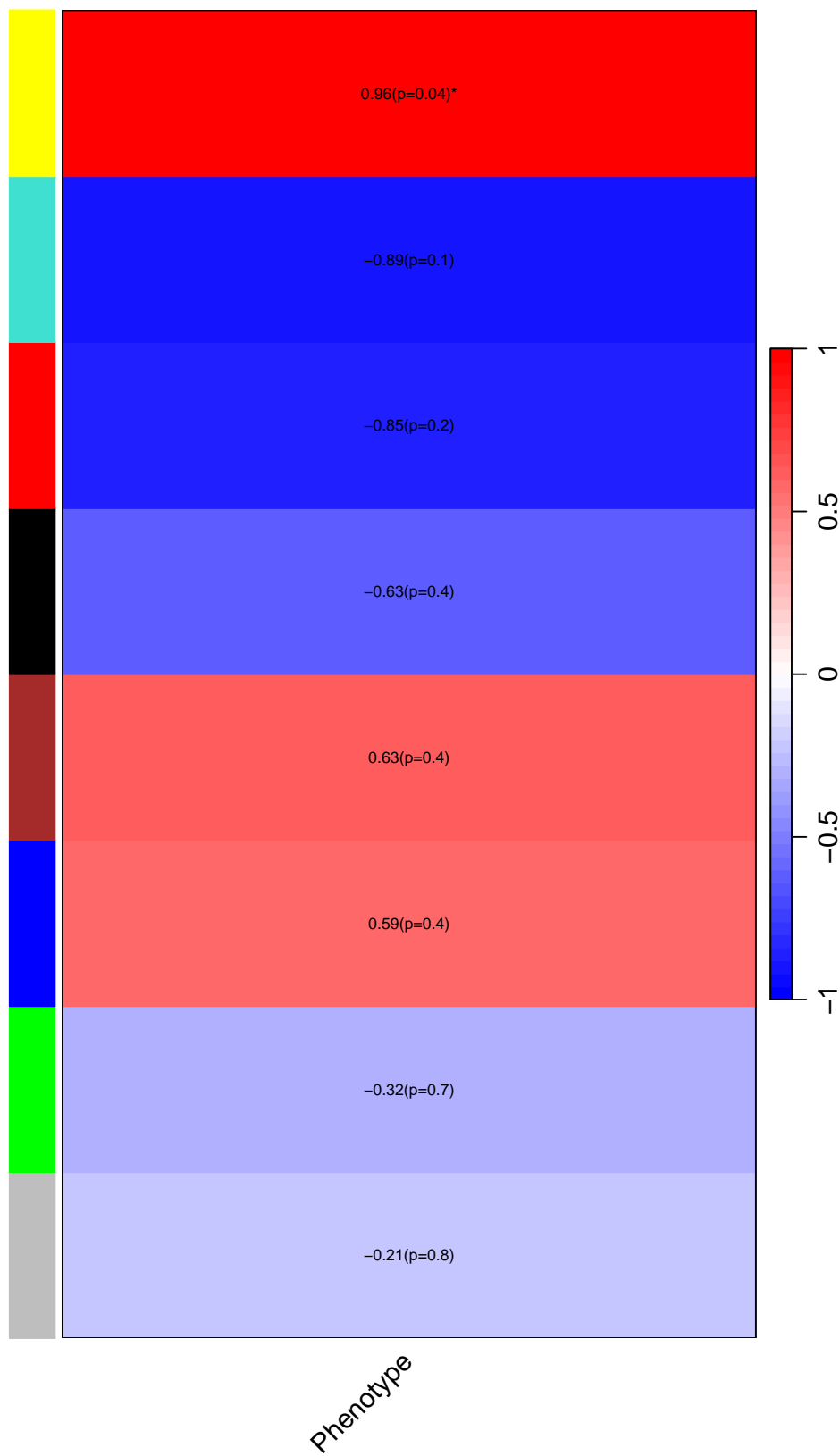
## mergedColors
## black blue brown green grey red turquoise yellow
```

```

##      75      114      103      81      5      76      345      86
## pdf
## 2
## pdf
## 2
## mergedColors
##      black      blue      brown      green      grey      red turquoise      yellow
##      75      114      103      81      5      76      345      86
## [1] 8

```

Module–Trait Correlations (cor, p-value, stars)



```
## pdf
## 2

## pdf
## 2

## [1] "Processing module: MEyellow"
## [1] "Processing module: MEturquoise"
```

Session Information

```
## R version 4.4.0 (2024-04-24)
## Platform: aarch64-apple-darwin20
## Running under: macOS 15.5
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.4-arm64/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.4-arm64/Resources/lib/libRlapack.dylib; LAPACK v
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## time zone: America/New_York
## tzcode source: internal
##
## attached base packages:
## [1] stats4      stats      graphics  grDevices  utils      datasets  methods
## [8] base
##
## other attached packages:
## [1] extrafont_0.19          gprofiler2_0.2.3
## [3] WGCNA_1.73              fastcluster_1.3.0
## [5] dynamicTreeCut_1.63-1  kableExtra_1.4.0
## [7] patchwork_1.3.0         pheatmap_1.0.12
## [9] data.table_1.17.4       harmony_1.2.3
## [11] Rcpp_1.0.14             conflicted_1.2.0
## [13] openxlsx_4.2.8          Seurat_5.3.0
## [15] SeuratObject_5.1.0      sp_2.2-0
## [17] AnnotationHub_3.12.0    BiocFileCache_2.12.0
## [19] dbplyr_2.5.0            simspec_0.0.0.9000
## [21] cowplot_1.1.3           EnsDb.Hsapiens.v86_2.99.0
## [23] ensemblDb_2.28.1        AnnotationFilter_1.28.0
## [25] GenomicFeatures_1.56.0  AnnotationDbi_1.66.0
## [27] Biobase_2.64.0          Signac_1.14.0
## [29] rtracklayer_1.64.0      GenomicRanges_1.56.2
## [31] GenomeInfoDb_1.40.1     IRanges_2.38.1
## [33] S4Vectors_0.42.1        BiocGenerics_0.50.0
## [35] knitr_1.50              lubridate_1.9.4
## [37] forcats_1.0.0           stringr_1.5.1
## [39] dplyr_1.1.4             purrr_1.0.4
## [41] readr_2.1.5             tidyr_1.3.1
## [43] tibble_3.2.1            ggplot2_3.5.2
## [45] tidyverse_2.0.0
##
## loaded via a namespace (and not attached):
## [1] ProtGenerics_1.36.0      matrixStats_1.5.0
## [3] spatstat.sparse_3.1-0    bitops_1.0-9
## [5] httr_1.4.7              RColorBrewer_1.1-3
## [7] doParallel_1.0.17        backports_1.5.0
## [9] tools_4.4.0             sctransform_0.4.2
## [11] R6_2.6.1                lazyeval_0.2.2
## [13] uwot_0.2.3              withr_3.0.2
## [15] gridExtra_2.3           preprocessCore_1.66.0
```

| | | |
|----------|-----------------------------|-------------------------|
| ## [17] | progressr_0.15.1 | cli_3.6.5 |
| ## [19] | textshaping_1.0.1 | spatstat.explore_3.4-3 |
| ## [21] | fastDummies_1.7.5 | labeling_0.4.3 |
| ## [23] | spatstat.data_3.1-6 | ggribes_0.5.6 |
| ## [25] | pbapply_1.7-2 | Rsamtools_2.20.0 |
| ## [27] | systemfonts_1.2.3 | foreign_0.8-90 |
| ## [29] | svglite_2.2.1 | dichromat_2.0-0.1 |
| ## [31] | parallelly_1.44.0 | impute_1.78.0 |
| ## [33] | rstudioapi_0.17.1 | RSQLite_2.3.11 |
| ## [35] | generics_0.1.4 | BiocIO_1.14.0 |
| ## [37] | ica_1.0-3 | spatstat.random_3.4-1 |
| ## [39] | zip_2.3.2 | G0.db_3.19.1 |
| ## [41] | Matrix_1.7-3 | abind_1.4-8 |
| ## [43] | lifecycle_1.0.4 | yaml_2.3.10 |
| ## [45] | SummarizedExperiment_1.34.0 | SparseArray_1.4.8 |
| ## [47] | Rtsne_0.17 | grid_4.4.0 |
| ## [49] | blob_1.2.4 | promises_1.3.3 |
| ## [51] | crayon_1.5.3 | miniUI_0.1.2 |
| ## [53] | lattice_0.22-7 | KEGGREST_1.44.1 |
| ## [55] | pillar_1.10.2 | rjson_0.2.23 |
| ## [57] | future.apply_1.11.3 | codetools_0.2-20 |
| ## [59] | fastmatch_1.1-6 | glue_1.8.0 |
| ## [61] | spatstat.univar_3.1-3 | vctrs_0.6.5 |
| ## [63] | png_0.1-8 | spam_2.11-1 |
| ## [65] | gtable_0.3.6 | cachem_1.1.0 |
| ## [67] | xfun_0.52 | S4Arrays_1.4.1 |
| ## [69] | mime_0.13 | survival_3.8-3 |
| ## [71] | RcppRoll_0.3.1 | iterators_1.0.14 |
| ## [73] | tinytex_0.57 | fitdistrplus_1.2-2 |
| ## [75] | ROCR_1.0-11 | nlme_3.1-168 |
| ## [77] | bit64_4.6.0-1 | filelock_1.0.3 |
| ## [79] | RcppAnnoy_0.0.22 | rprojroot_2.0.4 |
| ## [81] | irlba_2.3.5.1 | rpart_4.1.24 |
| ## [83] | KernSmooth_2.23-26 | colorspace_2.1-1 |
| ## [85] | DBI_1.2.3 | Hmisc_5.2-3 |
| ## [87] | nnet_7.3-20 | tidyselect_1.2.1 |
| ## [89] | extrafontdb_1.0 | bit_4.6.0 |
| ## [91] | compiler_4.4.0 | curl_6.2.3 |
| ## [93] | htmlTable_2.4.3 | xml2_1.3.8 |
| ## [95] | DelayedArray_0.30.1 | plotly_4.10.4 |
| ## [97] | checkmate_2.3.2 | scales_1.4.0 |
| ## [99] | lmtest_0.9-40 | rappdirs_0.3.3 |
| ## [101] | digest_0.6.37 | goftest_1.2-3 |
| ## [103] | spatstat.utils_3.1-4 | rmarkdown_2.29 |
| ## [105] | XVector_0.44.0 | htmltools_0.5.8.1 |
| ## [107] | pkgconfig_2.0.3 | base64enc_0.1-3 |
| ## [109] | MatrixGenerics_1.16.0 | fastmap_1.2.0 |
| ## [111] | rlang_1.1.6 | htmlwidgets_1.6.4 |
| ## [113] | UCSC.utils_1.0.0 | shiny_1.10.0 |
| ## [115] | farver_2.1.2 | zoo_1.8-14 |
| ## [117] | jsonlite_2.0.0 | BiocParallel_1.38.0 |
| ## [119] | RCurl_1.98-1.17 | magrittr_2.0.3 |
| ## [121] | Formula_1.2-5 | GenomeInfoDbData_1.2.12 |
| ## [123] | dotCall64_1.2 | reticulate_1.42.0 |

| | |
|-----------------------------|--------------------------|
| ## [125] stringi_1.8.7 | zlibbioc_1.50.0 |
| ## [127] MASS_7.3-65 | plyr_1.8.9 |
| ## [129] parallel_4.4.0 | listenv_0.9.1 |
| ## [131] ggrepel_0.9.6 | deldir_2.0-4 |
| ## [133] Biostrings_2.72.1 | splines_4.4.0 |
| ## [135] tensor_1.5 | hms_1.1.3 |
| ## [137] igraph_2.1.4 | spatstat.geom_3.4-1 |
| ## [139] RcppHNSW_0.6.0 | reshape2_1.4.4 |
| ## [141] BiocVersion_3.19.1 | XML_3.99-0.18 |
| ## [143] evaluate_1.0.3 | BiocManager_1.30.25 |
| ## [145] tzdb_0.5.0 | foreach_1.5.2 |
| ## [147] httpuv_1.6.16 | Rttf2pt1_1.3.12 |
| ## [149] RANN_2.6.2 | polyclip_1.10-7 |
| ## [151] future_1.49.0 | scattermore_1.2 |
| ## [153] xtable_1.8-4 | restfulr_0.0.15 |
| ## [155] RSpectra_0.16-2 | later_1.4.2 |
| ## [157] ragg_1.4.0 | viridisLite_0.4.2 |
| ## [159] memoise_2.0.1 | GenomicAlignments_1.40.0 |
| ## [161] cluster_2.1.8.1 | timechange_0.3.0 |
| ## [163] globals_0.18.0 | here_1.0.1 |