main

July 12, 2021

1 Building consensus network with WGCNA

```
[1]: PARAM_NETWORK_TYPE = 'signed'
```

1.1 Functions

```
[2]: filter_outliers = function(expression, z_threshold = 2.5)
          # Input: an expression matrix
          # Output: an expression matrix with outliers removed
          # Remove samples with z normalized total distance from other samples >\sqcup
      \rightarrow z threshold
          sample_distance = dist(expression)
          dist_z = scale(colSums(as.matrix(sample_distance)))
          stopifnot(all(rownames(dist_z) == rownames(expression)))
          keepSamples = dist_z < z_threshold
          new_expression = expression[keepSamples,]
          new_expression
     }
     prepare_data=function(setLabels)
         suppressMessages(library(dplyr))
         # Load sample data
         load("../../../differential_analysis/dentateGyrus/_m/genes/voomSVA.
      →RData")
         phenotypes = v$targets %>% as.data.frame %>% select(RNum, Race)
         sample_table0 = v$design %>% as.data.frame %>% select(-Intercept) %>%
             rename("Ancestry"="EA", "Sex"="Male")
         sample_table = phenotypes %>%
             inner_join(tibble::rownames_to_column(sample_table0, "RNum"),_
      →by=c("RNum")) %>%
             mutate("V1"=RNum) %>% tibble::column_to_rownames("V1")
         ## Filter by ancestry
         aa_samples = phenotypes %>% filter(Race == "AA")
```

```
ea_samples = phenotypes %>% filter(Race == "CAUC")
  print(dim(aa_samples))
  print(dim(ea_samples))
   # Load residualized expression
  vsd <- data.table::fread(paste0("../../../differential_analysis/</pre>
" m/genes/residualized expression.tsv")) %>%
      replace(is.na(.), "") %>% tibble::column_to_rownames("V1")
  print(dim(vsd))
   # Keep only the columns and rows that are present in
   # both the sample table and vsd file
   samples_aa = intersect(colnames(vsd), rownames(aa_samples))
   samples_ea = intersect(colnames(vsd), rownames(ea_samples))
  vsd_aa = vsd[,samples_aa]
  vsd_ea = vsd[,samples_ea]
   # WGCNA data import
  suppressMessages(library(WGCNA))
  nSets = 2; shortLabels = c("AA", "EA")
  multiExpr0 = vector(mode="list", length=nSets)
  multiExpr0[[1]] = list(data=as.data.frame(t(vsd_aa)))
  names(multiExpr0[[1]]$data) = rownames(vsd aa)
  rownames(multiExpr0[[1]]$data) = colnames(vsd aa)
  multiExpr0[[2]] = list(data=as.data.frame(t(vsd_ea)))
  names(multiExpr0[[2]]$data) = rownames(vsd_ea)
  rownames(multiExpr0[[2]]$data) = colnames(vsd_ea)
  exprSize = checkSets(multiExpr0)
  print(exprSize)
   # Remove offending genes and samples from the data
  gsg = goodSamplesGenesMS(multiExpr0, verbose = 3);
  if (!gsg$allOK)
  {
      for(set in 1:exprSize$nSets){
          multiExpr0[[set]]$data = multiExpr0[[set]]$data[gsg$goodSamples,__
}
  }
   # Secondary sample filtering
  for(set in 1:exprSize$nSets){
      multiExpr0[[set]]$data = filter_outliers(multiExpr0[[set]]$data, 2.5)
  }
  multiExpr <- multiExpr0</pre>
   exprSize = checkSets(multiExpr)
  samples_aa = intersect(rownames(multiExpr[[1]]$data), rownames(aa_samples))
  samples_ea = intersect(rownames(multiExpr[[2]]$data), rownames(ea_samples))
  samples = c(samples_aa, samples_ea)
   sample_table = sample_table[samples,]
   save(multiExpr, exprSize, sample_table, shortLabels, file = '00.RData')
```

```
}
plot_sample_clustering <- function(setLabels){</pre>
   lnames = load('00.RData')
    sampleTrees = list()
   for(set in 1:exprSize$nSets){
        sampleTrees[[set]] = hclust(dist(multiExpr[[set]]$data),__
 pdf(file='sample_clustering.pdf', height=12, width=12)
   par(mfrow=c(2,1))
   par(mar=c(0,4,2,0))
   for(set in 1:exprSize$nSets){
       plot(sampleTrees[[set]],
            main=paste("Sample clustering on all genes in ", setLabels[set]),
            xlab="", sub="", cex=0.7)
   dev.off()
}
```

```
[3]: prepare_traits = function()
         lnames = load('00.RData')
         Traits <- vector(mode="list", length=exprSize$nSets)</pre>
         # Associate traits with samples
         for(set in 1:exprSize$nSets){
             setSamples = rownames(multiExpr[[set]]$data)
             traitRows = match(setSamples, sample_table$RNum)
             Traits[[set]] = list(data=sample_table[traitRows, c(-1, -2)])
             rownames(Traits[[set]]$data) = sample_table[traitRows, 1]
         nGenes = exprSize$nGenes
         nSamples = exprSize$nSamples
         save(multiExpr, exprSize, sample_table, shortLabels,
              Traits, nGenes, nSamples, file = "01.RData")
     }
     plot_power_parameter <- function(nSets, multiExpr, RsquaredCut = 0.85){</pre>
         # Choose a set of soft-thresholding powers
         powers = seq(from = 4, to=20, by=1)
         # Initialize a list to hold the results of scale-free analysis
         powerTables = vector(mode = "list", length = nSets)
         softPowerTables = vector(mode = "list", length = nSets)
         # Call the network topology analysis function for each set in turn
         for (set in 1:nSets){
```

```
powerTables[[set]] = list(data =
→pickSoftThreshold(multiExpr[[set]]$data,
                                                           powerVector=powers,__
\rightarrowverbose = 2,
                                                          Ш
→networkType=PARAM_NETWORK_TYPE) [[2]])
       # Calculated softpower from fitted values
       cond = powerTables[[set]]$data$`SFT.R.sq` > RsquaredCut
       softPowerTables[[set]] = min(powerTables[[set]]$data[cond, "Power"])
   softpower = max(unlist(softPowerTables))
   print(softpower)
   # Plot the results:
   colors = c("black", "red")
   # Will plot these columns of the returned scale free analysis tables
   plotCols = c(2,5,6,7)
   colNames = c("Scale Free Topology Model Fit", "Mean connectivity",
                "Median connectivity", "Max connectivity")
   # Get the minima and maxima of the plotted points
   ylim = matrix(NA, nrow = 2, ncol = 4)
   for (set in 1:nSets){
       for (col in 1:length(plotCols)){
           ylim[1, col] = min(ylim[1, col],
                              powerTables[[set]]$data[, plotCols[col]],
                              na.rm = TRUE)
           ylim[2, col] = max(ylim[2, col],
                              powerTables[[set]]$data[, plotCols[col]],
                              na.rm = TRUE)
       }
   }
   # Plot the quantities in the chosen columns vs. the soft thresholding power
   sizeGrWindow(8, 6)
   pdf(file = "power_parameter_selection.pdf", wi = 8, he = 6)
   par(mfcol = c(2,2))
   par(mar = c(4.2, 4.2, 2.2, 0.5))
   cex1 = 0.7
   for (col in 1:length(plotCols)) for (set in 1:nSets){
       if (set==1){
           plot(powerTables[[set]]$data[,1],__
→-sign(powerTables[[set]]$data[,3])*powerTables[[set]]$data[,2],
                xlab="Soft Threshold (power)", ylab=colNames[col], type="n", u
→ylim = ylim[, col],
                main = colNames[col])
           addGrid()
       }
       if (col==1){
```

```
text(powerTables[[set]]$data[,1],__
 →-sign(powerTables[[set]]$data[,3])*powerTables[[set]]$data[,2],
                 labels=powers,cex=cex1,col=colors[set])
        } else {
            text(powerTables[[set]]$data[,1],__
 →powerTables[[set]]$data[,plotCols[col]],
                 labels=powers,cex=cex1,col=colors[set])
        }
        if (col==1){
            legend("bottomright", legend = setLabels, col = colors, pch = 20)
        } else {
            legend("topright", legend = setLabels, col = colors, pch = 20)
        }
    }
    dev.off()
}
figure_out_power_parameter <- function()</pre>
{
    suppressMessages(library(WGCNA))
    #enableWGCNAThreads()
    lnames = load('01.RData')
    nSets = exprSize$nSets
    plot_power_parameter(nSets, multiExpr, 0.85)
}
```

```
[4]: construct network <- function(softPower){
         suppressMessages(library(WGCNA))
         enableWGCNAThreads()
         lnames = load("01.RData")
         # softPower value from previous plot power_parameter_selection.pdf
         cor <- WGCNA::cor</pre>
         net = blockwiseConsensusModules(multiExpr, maxBlockSize=30000,
                                          power=softPower, minModuleSize=30,
                                          deepSplit=2, pamRespectsDendro=FALSE,
                                          mergeCutHeight=0.25, numericLabels=TRUE,
                                          minKMEtoStay=0, corType="bicor",
                                          saveTOMFileBase="TOM", saveTOMs=TRUE,
                                          networkType=PARAM_NETWORK_TYPE,
                                          TOMType=PARAM_NETWORK_TYPE, verbose=3)
         consMEs = net$multiMEs
         moduleLabels = net$colors
         moduleColors = labels2colors(moduleLabels)
         consTree = net$dendrograms[[1]]
         save(net, consMEs, moduleLabels, moduleColors, consTree, file="02.RData")
     }
```

```
[5]: consensus_eigengene_network <- function(){
         suppressMessages(library(WGCNA))
         lnames = load(file = "01.RData")
         lnames = load(file = "02.RData")
         nSets = exprSize$nSets
         # Create a variable weight that will hold just the body weight of mice in
      \rightarrowboth sets
         ancestry = vector(mode = "list", length = nSets);
         for (set in 1:nSets){
             ancestry[[set]] = list(data = as.data.
      →frame(Traits[[set]]$data$Ancestry))
             names(ancestry[[set]]$data) = "ancestry"
         }
         # Recalculate consMEs to give them color names
         consMEsC = multiSetMEs(multiExpr, universalColors = moduleColors)
         # Plot eigengene network
         sizeGrWindow(8,10)
         pdf(file = "eigengene_networks.pdf", width=8, height=10)
         par(cex = 0.9)
         plotEigengeneNetworks(consMEsC, setLabels, marDendro=c(0,2,2,1),
                                marHeatmap=c(3,3,2,1), xLabelsAngle=0,
                                zlimPreservation=c(0.5, 1))
         dev.off()
         # We add the weight trait to the eigengenes and order them by consesus_{\sqcup}
      \rightarrow hierarchical clustering:
         MET = consensusOrderMEs(addTraitToMEs(consMEsC, ancestry))
         # Plot eigengene network
         sizeGrWindow(8,10)
         pdf(file = "eigengene_networks_ancestry.pdf", width=8, height=10)
         par(cex = 0.9)
         plotEigengeneNetworks(MET, setLabels, marDendro=c(0,2,2,1),
                                marHeatmap=c(3,3,2,1), xLabelsAngle=0,
                                zlimPreservation=c(0.5, 1)
```

```
dev.off()
    save(MET, consMEsC, ancestry, file="03.RData")
}
export_eigengene_tables = function(){
    suppressMessages(library(WGCNA))
    lnames = load(file = "01.RData")
    lnames = load(file = "02.RData")
    lnames = load(file = "03.RData")
    nSets = exprSize$nSets
    ## Export eigengene tables
    for(set in 1:nSets){
        write.csv(consMEsC[[set]]$data,
                  paste0('eigengenes_',shortLabels[[set]],'.csv'))
    }
    # Write modules
    modules = data.frame(row.names=colnames(multiExpr[[1]]$data),
                         module=moduleColors)
    write.csv(modules, 'modules.csv')
}
```

1.2 Main

```
prepare_data(setLabels)
plot_sample_clustering(setLabels)
prepare_traits()
figure_out_power_parameter()
Loading required package: limma
[1] 47 2
[1] 43 2
Warning message in
data.table::fread(paste0("../../../differential_analysis/dentateGyrus/", :
"Detected 90 column names but the data has 91 columns (i.e. invalid file). Added
1 extra default column name for the first column which is guessed to be row
names or an index. Use setnames() afterwards if this guess is not correct, or
fix the file write command that created the file to create a valid file."
[1] 21140
             90
$nSets
Γ1 2
$nGenes
[1] 21140
```

[6]: setLabels = c("AA Dentate Gyrus", "EA Dentate Gyrus")

\$nSamples [1] 47 43 \$structureOK [1] TRUE Flagging genes and samples with too many missing values... ..step 1 ..bad gene count: 0, bad sample counts: 0, 0 **png:** 2 pickSoftThreshold: will use block size 2116. pickSoftThreshold: calculating connectivity for given powers... ..working on genes 1 through 2116 of 21140 Warning message: "executing %dopar% sequentially: no parallel backend registered" ..working on genes 2117 through 4232 of 21140 ..working on genes 4233 through 6348 of 21140 ..working on genes 6349 through 8464 of 21140 ..working on genes 8465 through 10580 of 21140 ..working on genes 10581 through 12696 of 21140 ..working on genes 12697 through 14812 of 21140 ..working on genes 14813 through 16928 of 21140 ..working on genes 16929 through 19044 of 21140 ..working on genes 19045 through 21140 of 21140 Power SFT.R.sq slope truncated.R.sq mean.k. median.k. max.k. 0.475 - 7.710.915 1680.00 1 1650.00 2300.0 2 5 0.582 - 6.080.928 967.00 945.00 1530.0 3 6 0.689 - 5.210.953 573.00 554.00 1060.0 4 7 0.769 - 4.620.972 349.00 332.00 759.0 5 8 0.827 - 4.190.985 218.00 203.00 561.0 6 9 0.990 139.00 127.00 425.0 0.866 - 3.837 10 0.891 - 3.590.994 90.50 80.30 329.0 8 11 0.912 - 3.350.995 60.20 51.80 259.0 9 12 0.928 - 3.160.996 40.80 33.90 208.0 10 13 0.935 - 3.050.997 28.20 22.50 170.0 15.10 141.0 11 14 0.940 - 2.950.997 19.80 12 15 0.946 - 2.850.997 14.10 10.20 119.0 13 0.941 - 2.800.995 10.20 7.03 101.0 16 14 17 7.50 4.88 87.0 0.941 - 2.740.995 15 0.945 - 2.660.996 5.58 3.42 75.2 18 16 2.42 19 0.942 - 2.600.994 4.20 65.5 17 20 0.914 - 2.620.981 3.20 1.72 57.4

pickSoftThreshold: calculating connectivity for given powers...
..working on genes 1 through 2116 of 21140

pickSoftThreshold: will use block size 2116.

```
..working on genes 2117 through 4232 of 21140
       ..working on genes 4233 through 6348 of 21140
       ..working on genes 6349 through 8464 of 21140
       ..working on genes 8465 through 10580 of 21140
       ..working on genes 10581 through 12696 of 21140
       ..working on genes 12697 through 14812 of 21140
       ..working on genes 14813 through 16928 of 21140
       ..working on genes 16929 through 19044 of 21140
       ..working on genes 19045 through 21140 of 21140
       Power SFT.R.sq slope truncated.R.sq mean.k. median.k. max.k.
           4
               0.0315 -2.05
                                      0.939 1700.00
                                                       1690.00 2250.0
    1
    2
           5
               0.2720 - 4.29
                                      0.953 986.00
                                                        969.00 1490.0
    3
               0.4590 - 4.38
                                      0.963 588.00
                                                        571.00 1040.0
    4
           7
               0.6210 - 4.35
                                      0.977 360.00
                                                        344.00 748.0
    5
               0.7250 - 4.09
                                      0.987 225.00
                                                        212.00 557.0
    6
           9
               0.7970 - 3.89
                                      0.991 145.00
                                                        133.00 427.0
    7
          10
               0.8390 - 3.79
                                      0.990
                                              94.60
                                                         84.80 337.0
    8
               0.8640 - 3.68
                                      0.996
                                              63.20
                                                         54.90 270.0
          11
    9
          12
               0.8890 - 3.51
                                      0.997
                                                         36.20 221.0
                                              43.10
    10
          13
               0.9060 - 3.37
                                      0.998
                                              29.80
                                                         24.10 183.0
    11
          14
               0.9110 - 3.26
                                      0.994
                                              21.00
                                                         16.30 154.0
                                                         11.10 131.0
    12
          15
               0.9160 - 3.16
                                      0.990
                                               15.00
    13
          16
               0.9190 - 3.09
                                      0.995
                                              10.90
                                                         7.68 112.0
    14
          17
               0.9280 - 2.98
                                      0.996
                                                          5.36
                                                                 97.0
                                               8.04
    15
          18
               0.9190 - 2.92
                                      0.989
                                               6.00
                                                          3.77
                                                                 84.5
                                                          2.68
                                                                 74.0
    16
          19
               0.9240 - 2.83
                                      0.989
                                               4.53
    17
          20
               0.9250 - 2.76
                                      0.985
                                                                 65.3
                                               3.46
                                                          1.92
    [1] 11
    png: 2
[7]: softpower = 11 ## Based on Dentate Gyrus and Hippocampus
     construct network(softpower)
     plot_cluster_dendrogram()
    Allowing parallel execution with up to 63 working processes.
     Calculating consensus modules and module eigengenes block-wise from all genes
     Calculating topological overlaps block-wise from all genes
       Flagging genes and samples with too many missing values...
        ..step 1
     ...Working on set 1
        TOM calculation: adjacency..
        ..will use 63 parallel threads.
         Fraction of slow calculations: 0.000000
        ..connectivity..
        ..matrix multiplication (system BLAS)..
        ..normalization..
        ..done.
     ...Working on set 2
```

```
TOM calculation: adjacency..
        ..will use 63 parallel threads.
         Fraction of slow calculations: 0.000000
         ..connectivity..
         ..matrix multiplication (system BLAS)..
         ..normalization..
         ..done.
     ..Working on block 1 .
     ...Working on set 1
     ...Working on set 2
     ...Calculating consensus network
     ..Working on block 1 .
     ...clustering and detecting modules...
     ...calculating eigengenes...
     ...checking consensus modules for statistical meaningfulness..
     ...checking for genes that should be reassigned...
     ..merging consensus modules that are too close..
         mergeCloseModules: Merging modules whose distance is less than 0.25
           Calculating new MEs...
    png: 2
[8]: consensus_eigengene_network()
     export_eigengene_tables()
     multiSetMEs: Calculating module MEs.
       Working on set 1 ...
       Working on set 2 ...
    1.3 Repreducibility Information
[9]: Sys.time()
     proc.time()
     options(width = 120)
     sessioninfo::session_info()
    [1] "2021-07-12 10:35:54 EDT"
        user
               system elapsed
    4248.222 1535.694 1218.664
      Session info
     setting value
     version R version 4.0.3 (2020-10-10)
              Arch Linux
              x86_64, linux-gnu
     system
     ui
              X11
     language (EN)
     collate en_US.UTF-8
              en_US.UTF-8
     ctype
```

tz America/New_York date 2021-07-12

Packages

Packages					
package	*	version	date	lib	source
AnnotationDbi		1.52.0	2020-10-27	[1]	Bioconductor
assertthat		0.2.1	2019-03-21	[1]	CRAN (R 4.0.2)
backports		1.2.1	2020-12-09	[1]	CRAN (R 4.0.2)
base64enc		0.1-3	2015-07-28	[1]	CRAN (R 4.0.2)
Biobase		2.50.0	2020-10-27	[1]	Bioconductor
BiocGenerics		0.36.1	2021-04-16	[1]	Bioconductor
bit		4.0.4	2020-08-04	[1]	CRAN (R 4.0.2)
bit64		4.0.5	2020-08-30	[1]	CRAN (R 4.0.2)
blob		1.2.1	2020-01-20	[1]	CRAN (R 4.0.2)
cachem		1.0.5	2021-05-15	[1]	CRAN (R 4.0.3)
checkmate		2.0.0	2020-02-06	[1]	CRAN (R 4.0.2)
cli		3.0.0	2021-06-30	[1]	CRAN (R 4.0.3)
cluster		2.1.0	2019-06-19	[2]	CRAN (R 4.0.3)
codetools		0.2-16	2018-12-24	[2]	CRAN (R 4.0.3)
colorspace		2.0-2	2021-06-24	[1]	CRAN (R 4.0.3)
crayon		1.4.1	2021-02-08	[1]	CRAN (R 4.0.3)
data.table		1.14.0	2021-02-21	[1]	CRAN (R 4.0.3)
DBI		1.1.1	2021-01-15	[1]	CRAN (R 4.0.2)
digest		0.6.27	2020-10-24	[1]	CRAN (R 4.0.2)
doParallel		1.0.16	2020-10-16	[1]	CRAN (R 4.0.3)
dplyr	*	1.0.7	2021-06-18	[1]	CRAN (R 4.0.3)
${\tt dynamicTreeCut}$	*	1.63-1	2016-03-11	[1]	CRAN (R 4.0.3)
ellipsis		0.3.2	2021-04-29	[1]	CRAN (R 4.0.3)
evaluate		0.14	2019-05-28	[1]	CRAN (R 4.0.2)
fansi		0.5.0	2021-05-25	[1]	CRAN (R 4.0.3)
fastcluster	*	1.2.3	2021-05-24	[1]	CRAN (R 4.0.3)
fastmap		1.1.0	2021-01-25	[1]	CRAN (R 4.0.2)
foreach		1.5.1	2020-10-15	[1]	CRAN (R 4.0.2)
foreign		0.8-80	2020-05-24	[2]	CRAN (R 4.0.3)
Formula		1.2-4	2020-10-16	[1]	CRAN (R 4.0.2)
generics		0.1.0	2020-10-31	[1]	CRAN (R 4.0.2)
ggplot2		3.3.5	2021-06-25	[1]	CRAN (R 4.0.3)
glue		1.4.2	2020-08-27	[1]	CRAN (R 4.0.2)
GO.db		3.12.1	2021-04-08	[1]	Bioconductor
${ t gridExtra}$		2.3	2017-09-09	[1]	CRAN (R 4.0.2)
gtable		0.3.0	2019-03-25	[1]	CRAN (R 4.0.2)
Hmisc		4.5-0	2021-02-28	[1]	CRAN (R 4.0.3)
htmlTable		2.2.1	2021-05-18	[1]	CRAN (R 4.0.3)
htmltools		0.5.1.1	2021-01-22	[1]	CRAN (R 4.0.2)
htmlwidgets		1.5.3	2020-12-10	[1]	CRAN (R 4.0.2)
impute		1.64.0	2020-10-27	[1]	Bioconductor
IRanges		2.24.1	2020-12-12	[1]	Bioconductor
IRdisplay		1.0	2021-01-20	[1]	CRAN (R 4.0.2)

```
IRkernel
                  1.2
                          2021-05-11 [1] CRAN (R 4.0.3)
                          2020-10-15 [1] CRAN (R 4.0.2)
iterators
                 1.0.13
                 0.1-8.1 2019-10-24 [1] CRAN (R 4.0.2)
jpeg
                 1.7.2
                          2020-12-09 [1] CRAN (R 4.0.2)
jsonlite
                 1.33
                          2021-04-24 [1] CRAN (R 4.0.3)
knitr
                 0.20-41 2020-04-02 [2] CRAN (R 4.0.3)
lattice
latticeExtra
                 0.6 - 29
                          2019-12-19 [1] CRAN (R 4.0.2)
lifecycle
                  1.0.0
                          2021-02-15 [1] CRAN (R 4.0.3)
               * 3.46.0
                          2020-10-27 [1] Bioconductor
limma
magrittr
                 2.0.1
                          2020-11-17 [1] CRAN (R 4.0.2)
                          2021-06-01 [1] CRAN (R 4.0.3)
Matrix
                  1.3 - 4
                 0.59.0
                          2021-06-01 [1] CRAN (R 4.0.3)
matrixStats
                          2021-01-26 [1] CRAN (R 4.0.2)
                 2.0.0
memoise
                          2018-06-12 [1] CRAN (R 4.0.2)
munsell
                 0.5.0
nnet
                 7.3-14
                          2020-04-26 [2] CRAN (R 4.0.3)
                 0.3 - 5
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^{[1] /}home/jbenja13/R/x86_64-pc-linux-gnu-library/4.0

^{[2] /}usr/lib/R/library