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/hippocampus/_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"),__
      \rightarrowby=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>
 ⇔hippocampus/",
                                    " 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$goodSamples,__
 →gsg$goodGenes]
        }
    # 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),__
 →method="average")
    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

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

```
prepare_data(setLabels)
plot_sample_clustering(setLabels)
prepare_traits()
figure_out_power_parameter()
Loading required package: limma
[1] 133
          2
[1] 109
          2
Warning message in
data.table::fread(paste0("../../../differential_analysis/hippocampus/", :
"Detected 242 column names but the data has 243 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] 22269
            242
$nSets
Γ1 2
$nGenes
[1] 22269
```

\$nSamples [1] 133 109 \$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 2009. pickSoftThreshold: calculating connectivity for given powers... ..working on genes 1 through 2009 of 22269 Warning message: "executing %dopar% sequentially: no parallel backend registered" ..working on genes 2010 through 4018 of 22269 ..working on genes 4019 through 6027 of 22269 ..working on genes 6028 through 8036 of 22269 ..working on genes 8037 through 10045 of 22269 ..working on genes 10046 through 12054 of 22269 ..working on genes 12055 through 14063 of 22269 ..working on genes 14064 through 16072 of 22269 ..working on genes 16073 through 18081 of 22269 ..working on genes 18082 through 20090 of 22269 ..working on genes 20091 through 22099 of 22269 ..working on genes 22100 through 22269 of 22269 Power SFT.R.sq slope truncated.R.sq mean.k. median.k. max.k. 1 4 0.204 - 9.300.913 1550.000 1540.000 1850.0 2 5 0.357 -8.88 0.931 828.000 821.000 1070.0 3 6 0.506 - 8.050.950 449.000 442.000 645.0 4 7 0.626 - 7.210.967 247.000 242.000 399.0 5 8 0.720 - 6.370.979 138.000 134.000 254.0 6 9 0.792 - 5.570.985 78.700 74.900 168.0 7 10 0.849 - 5.090.989 45.600 42.500 118.0 8 11 0.905 - 4.460.996 26.900 24.400 85.5 9 0.944 - 3.8914.200 64.2 12 0.996 16.100 10 13 0.963 - 3.600.995 9.890 8.320 52.6 11 0.976 - 3.300.995 6.190 4.940 44.2 14 12 38.0 15 0.980 - 3.040.994 3.960 2.970 13 0.974 - 2.830.985 2.600 33.3 16 1.810 14 17 0.979 - 2.630.989 1.740 1.110 29.6 15 18 0.978 - 2.460.987 1.200 0.689 26.5 16 19 0.971 - 2.330.982 0.841 0.432 23.8

0.606

0.273

21.6

0.985

17

20

0.973 - 2.20

pickSoftThreshold: will use block size 2009.

```
..working on genes 1 through 2009 of 22269
       ..working on genes 2010 through 4018 of 22269
       ..working on genes 4019 through 6027 of 22269
       ..working on genes 6028 through 8036 of 22269
       ..working on genes 8037 through 10045 of 22269
       ..working on genes 10046 through 12054 of 22269
       ..working on genes 12055 through 14063 of 22269
       ..working on genes 14064 through 16072 of 22269
       ..working on genes 16073 through 18081 of 22269
       ..working on genes 18082 through 20090 of 22269
       ..working on genes 20091 through 22099 of 22269
       ..working on genes 22100 through 22269 of 22269
       Power SFT.R.sq slope truncated.R.sq mean.k. median.k. max.k.
    1
                 0.196 - 9.45
                                      0.906 1580.00 1570.000 1900.0
    2
           5
                0.430 - 9.45
                                      0.927 851.00
                                                       842.000 1140.0
    3
           6
                0.613 - 8.27
                                      0.952 468.00
                                                       460.000 710.0
    4
           7
                0.743 - 7.14
                                      0.974 262.00
                                                       255.000 459.0
    5
           8
                0.814 -6.09
                                      0.985 149.00
                                                       143.000 307.0
    6
           9
                0.861 - 5.24
                                      0.991
                                              86.90
                                                        81.800 212.0
    7
          10
                0.895 - 4.53
                                      0.993
                                              51.50
                                                        47.400 151.0
    8
          11
                0.923 - 3.92
                                      0.994
                                               31.10
                                                        27.800 110.0
    9
          12
                0.945 - 3.51
                                      0.993
                                              19.20
                                                        16.500
                                                                 83.5
    10
                                      0.993
                                                                 69.5
          13
                0.946 - 3.39
                                               12.10
                                                         9.930
    11
          14
                0.956 - 3.19
                                      0.994
                                               7.78
                                                         6.040
                                                                 59.2
    12
          15
                0.962 - 3.00
                                      0.996
                                                5.12
                                                         3.720
                                                                 51.3
    13
                                                         2.320
          16
                0.964 - 2.82
                                      0.993
                                                3.45
                                                                 45.1
    14
          17
                0.971 - 2.64
                                      0.997
                                                2.37
                                                         1.460
                                                                 40.0
    15
                                                         0.929
                                                                 35.7
          18
                0.967 - 2.50
                                      0.992
                                                1.67
    16
          19
                0.972 - 2.35
                                      0.995
                                                1.20
                                                         0.598
                                                                 32.1
    17
          20
                0.973 - 2.23
                                      0.994
                                                0.88
                                                         0.388
                                                                 28.9
    [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..
```

pickSoftThreshold: calculating connectivity for given powers...

```
..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 12:36:13 EDT"
                system elapsed
    5286.299 1856.225 1440.769
      Session info
     setting value
     version R version 4.0.3 (2020-10-10)
     os
              Arch Linux
     system
              x86_64, linux-gnu
```

ui X11 language (EN)

collate en_US.UTF-8
ctype en_US.UTF-8
tz America/New_York

date 2021-07-12

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
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
                  1.0
                          2021-01-20 [1] CRAN (R 4.0.2)
IRdisplay
                          2021-05-11 [1] CRAN (R 4.0.3)
IRkernel
                  1.2
                          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
jsonlite
                  1.7.2
                          2020-12-09 [1] CRAN (R 4.0.2)
                          2021-04-24 [1] CRAN (R 4.0.3)
knitr
                  1.33
lattice
                  0.20-41 2020-04-02 [2] CRAN (R 4.0.3)
                          2019-12-19 [1] CRAN (R 4.0.2)
                  0.6 - 29
latticeExtra
                  1.0.0
                          2021-02-15 [1] CRAN (R 4.0.3)
lifecycle
               * 3.46.0
                          2020-10-27 [1] Bioconductor
limma
                          2020-11-17 [1] CRAN (R 4.0.2)
magrittr
                  2.0.1
Matrix
                  1.3 - 4
                          2021-06-01 [1] CRAN (R 4.0.3)
                  0.59.0
                          2021-06-01 [1] CRAN (R 4.0.3)
matrixStats
memoise
                  2.0.0
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WGCNA
               * 1.70-3
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- [1] /home/jbenja13/R/x86_64-pc-linux-gnu-library/4.0
 [2] /usr/lib/R/library