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/dlpfc/_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/dlpfc/",</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$goodSamples,__
 →gsg$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,
                                                          П
→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", __
\rightarrowylim = 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){</pre>
         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")
     }
     plot_cluster_dendrogram <- function(){</pre>
```

```
[5]: consensus_eigengene_network <- function(){</pre>
         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 \square
      \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

[1] 123 [1] 88 2

```
[6]: setLabels = c("AA DLPFC", "EA DLPFC")
    prepare_data(setLabels)
    plot_sample_clustering(setLabels)
    prepare_traits()
    figure_out_power_parameter()
```

Loading required package: limma

```
Warning message in data.table::fread(paste0("../../../differential_analysis/dlpfc/", : "Detected 211 column names but the data has 212 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] 22398 211

$nSets
[1] 2

$nGenes
[1] 22398
```

[1] 123 88

\$structureOK

```
[1] TRUE
Flagging genes and samples with too many missing values...
   ..bad gene count: 0, bad sample counts: 0, 0
png: 2
pickSoftThreshold: will use block size 1997.
pickSoftThreshold: calculating connectivity for given powers...
   ..working on genes 1 through 1997 of 22398
Warning message:
"executing %dopar% sequentially: no parallel backend registered"
   ..working on genes 1998 through 3994 of 22398
   ..working on genes 3995 through 5991 of 22398
   ..working on genes 5992 through 7988 of 22398
   ..working on genes 7989 through 9985 of 22398
   ..working on genes 9986 through 11982 of 22398
   ..working on genes 11983 through 13979 of 22398
   ..working on genes 13980 through 15976 of 22398
   ..working on genes 15977 through 17973 of 22398
   ..working on genes 17974 through 19970 of 22398
   ..working on genes 19971 through 21967 of 22398
   ..working on genes 21968 through 22398 of 22398
  Power SFT.R.sq slope truncated.R.sq mean.k. median.k. max.k.
1
       4
            0.163 - 5.17
                                  0.831 1610.00 1580.000 2020.0
2
       5
            0.498 - 6.29
                                  0.889 875.00
                                                  852.000 1270.0
3
            0.771 - 6.07
                                  0.952 486.00
                                                  465.000 858.0
4
       7
                                  0.980 275.00
                                                  258.000 612.0
            0.901 - 5.32
5
       8
            0.949 - 4.56
                                  0.985 160.00
                                                  145.000 458.0
6
       9
            0.967 - 3.92
                                  0.981
                                          94.60
                                                   83.000 356.0
7
      10
                                                   48.000 289.0
            0.975 - 3.45
                                  0.981
                                          57.50
8
      11
            0.979 - 3.06
                                  0.979
                                                   28.100 241.0
                                          35.80
                                                   16.700 205.0
9
      12
            0.979 - 2.76
                                  0.976
                                          22.90
10
      13
            0.979 - 2.53
                                  0.975
                                          15.00
                                                   10.000 178.0
11
            0.977 - 2.34
                                  0.973
                                          10.10
                                                    6.090 156.0
      14
12
                                                    3.740 138.0
      15
            0.977 - 2.17
                                  0.972
                                           7.01
13
      16
            0.976 - 2.05
                                  0.971
                                           4.98
                                                    2.330 123.0
14
            0.977 - 1.93
                                           3.63
                                                    1.460 110.0
      17
                                  0.973
15
                                                    0.930
      18
            0.979 - 1.84
                                  0.976
                                           2.71
                                                             99.1
16
      19
            0.977 - 1.76
                                  0.974
                                           2.07
                                                    0.597
                                                             89.7
17
      20
            0.972 - 1.70
                                  0.969
                                           1.61
                                                    0.387
                                                             81.6
pickSoftThreshold: will use block size 1997.
```

pickSoftThreshold: calculating connectivity for given powers...

- ..working on genes 1 through 1997 of 22398
- ..working on genes 1998 through 3994 of 22398

```
..working on genes 3995 through 5991 of 22398
       ..working on genes 5992 through 7988 of 22398
       ..working on genes 7989 through 9985 of 22398
       ..working on genes 9986 through 11982 of 22398
       ..working on genes 11983 through 13979 of 22398
       ..working on genes 13980 through 15976 of 22398
       ..working on genes 15977 through 17973 of 22398
       ..working on genes 17974 through 19970 of 22398
       ..working on genes 19971 through 21967 of 22398
       ..working on genes 21968 through 22398 of 22398
       Power SFT.R.sq slope truncated.R.sq mean.k. median.k. max.k.
                0.572 - 7.84
                                      0.911 1680.00 1640.000 2250.0
    1
    2
           5
                0.741 - 6.06
                                      0.941 936.00
                                                       905.000 1460.0
    3
                0.842 - 5.00
                                      0.965 536.00
                                                       508.000 1010.0
    4
           7
                0.900 - 4.16
                                      0.979 315.00
                                                       291.000 721.0
    5
                0.929 - 3.60
                                      0.984 190.00
                                                       169.000 539.0
    6
           9
                0.940 - 3.24
                                      0.985 117.00
                                                       100.000 421.0
    7
          10
                0.940 - 2.98
                                      0.982
                                              74.60
                                                        60.400 340.0
    8
                0.946 - 2.77
                                      0.985
                                                        36.800 284.0
          11
                                              48.60
    9
          12
                0.952 - 2.59
                                      0.988
                                              32.50
                                                        22.800 241.0
                                      0.990
    10
          13
                0.956 - 2.43
                                              22.30
                                                        14.200 208.0
    11
          14
                0.946 - 2.33
                                      0.983
                                               15.60
                                                         9.040 182.0
    12
                0.943 - 2.23
                                      0.982
                                              11.20
                                                         5.790 161.0
    13
                0.941 - 2.14
                                      0.980
                                                         3.760 143.0
          16
                                               8.26
    14
          17
                0.938 - 2.05
                                      0.979
                                               6.20
                                                         2.460 128.0
    15
                                                         1.640 116.0
                0.940 - 1.97
                                      0.982
                                                4.74
          18
                                                         1.100 105.0
    16
          19
                0.943 - 1.90
                                      0.984
                                                3.69
    17
          20
                0.944 - 1.84
                                      0.985
                                               2.92
                                                         0.741
                                                                 95.1
    [1] 7
    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 12:11:58 EDT"
        user
               system elapsed
    5416.852 1581.629 1381.389
      Session info
     setting value
     version R version 4.0.3 (2020-10-10)
     os
              Arch Linux
              x86_64, linux-gnu
     system
              X11
     ui
     language (EN)
     collate en_US.UTF-8
```

en_US.UTF-8 ctype America/New_York 2021-07-12 tz

date

Packages

1 ackages					
package	*	version			source
AnnotationDbi		1.52.0	2020-10-27		Bioconductor
assertthat		0.2.1	2019-03-21	[1]	
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]	
IRanges		2.24.1	2020-12-12	[1]	Bioconductor

```
IRdisplay
                  1.0
                          2021-01-20 [1] CRAN (R 4.0.2)
                          2021-05-11 [1] CRAN (R 4.0.3)
IRkernel
                  1.2
iterators
                 1.0.13
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^{[1] /}home/jbenja13/R/x86_64-pc-linux-gnu-library/4.0

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