main

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1 WGCNA

- @author = 'Apua Paquola'
- Edits by K.J. Benjamin
- Edits2 by Arthur S. Feltrin
 - New scale-free plots, export data to create network on cytoscape/igraph and format for jupyter notebook (05/2019)
 - Conversion from Rscript to jupyter notebook

Final edits by K.J. Benjamin for publication

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

1.1 Prepare Data and Traits Table

```
[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 >
    \to 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
}</pre>
```

```
[3]: prepare_data=function()
{
    suppressMessages(library(dplyr))
    # Load sample data
    load("../../../differential_analysis/caudate/_m/genes/voomSVA.RData")
    sample_table = v$design %>% as.data.frame %>% select(-Intercept) %>%
```

```
rename("Ancestry"="EA", "Sex"="Male") #%>% select(Ancestry, Sex, Age)
  # Load residualized expression
  vsd <- data.table::fread("../../differential_analysis/caudate/_m/genes/</pre>
 →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 = intersect(colnames(vsd), rownames(sample_table))
  vsd = vsd[,samples]
  sample_table = sample_table[samples,]
  # WGCNA data import
  suppressMessages(library(WGCNA))
  options(stringsAsFactors = FALSE)
  datExpr0 = t(vsd)
  # Remove offending genes and samples from the data
  gsg = goodSamplesGenes(datExpr0, verbose = 3);
  if (!gsg$allOK)
    datExpr0 = datExpr0[gsg$goodSamples, gsg$goodGenes]
  datExpr=datExpr0
  # Remove outliers
  datExpr = filter_outliers(datExpr0, z_threshold = 2.5)
  rm(datExpr0)
  # Clean data
  samples = intersect(rownames(datExpr), rownames(sample_table))
  sample table = sample table[samples,]
  datExpr = datExpr[samples,]
 print(dim(datExpr))
  save(datExpr, sample_table, file = '00.RData')
}
```

1.2 Create Sample Dendogram Based on Distance (h)

1.3 Calculate Scale-Free Topology

```
[5]: plot_power_parameter=function(datExpr, plot_filename)
         # Choose a set of soft-thresholding powers
        powers = seq(from = 1, to=30, by=1)
         # Call the network topology analysis function
        sft = pickSoftThreshold(datExpr, networkType = PARAM_NETWORK_TYPE,
                                powerVector = powers, verbose = 5)
         # Plot the results:
        pdf(file=plot_filename)
        par(mfcol = c(2,2));
        par(mar = c(4.2, 4.5, 2.2, 0.5), oma=c(0,0,2,0))
         cex1 = 0.7;
         # Scale-free topology fit index as a function of the
         # soft-thresholding power
        plot(sft$fitIndices[,1], -sign(sft$fitIndices[,3])*sft$fitIndices[,2],
             xlab="Soft Threshold (power)",
             ylab="Scale Free Topology Model Fit, signed R^2", type="n",
             main = paste("Scale independence"))
        text(sft$fitIndices[,1], -sign(sft$fitIndices[,3])*sft$fitIndices[,2],
             labels=powers,cex=cex1,col="blue");
         # this line corresponds to using an R^2 cut-off of h
        abline(h=0.80,col="red")
         # Mean connectivity as a function of the soft-thresholding power
        plot(sft$fitIndices[,1], sft$fitIndices[,5],
             xlab="Soft Threshold (power)", ylab="Mean Connectivity",
             type="n", main = paste("Mean connectivity"))
        text(sft$fitIndices[,1], sft$fitIndices[,5], labels=powers,__
      #####
```

```
plot(sft$fitIndices[,1], sft$fitIndices[,6],
         xlab="Soft Threshold (power)", ylab="Median Connectivity",
         type="n", main = paste("Median connectivity"))
   text(sft$fitIndices[,1], sft$fitIndices[,6], labels=powers,_
 ⇒cex=cex1,col="blue")
    #####
   plot(sft$fitIndices[,1], sft$fitIndices[,7],
         xlab="Soft Threshold (power)", ylab="Max Connectivity",
         type="n", main = paste("Max connectivity"))
   text(sft$fitIndices[,1], sft$fitIndices[,7], labels=powers,_
 ⇒cex=cex1,col="blue")
   dev.off()
    ####plot on jupyter
   par(mfcol = c(2,2));
   par(mar = c(4.2, 4.5, 2.2, 0.5), oma = c(0,0,2,0))
   cex1 = 0.7;
    # Scale-free topology fit index as a function of the
   # soft-thresholding power
   plot(sft$fitIndices[,1], -sign(sft$fitIndices[,3])*sft$fitIndices[,2],
         xlab="Soft Threshold (power)",
         ylab="Scale Free Topology Model Fit, signed R^2", type="n",
         main = paste("Scale independence"))
   text(sft$fitIndices[,1], -sign(sft$fitIndices[,3])*sft$fitIndices[,2],
         labels=powers,cex=cex1,col="blue");
    # this line corresponds to using an R^2 cut-off of h
   abline(h=0.80,col="red")
    # Mean connectivity as a function of the soft-thresholding power
   plot(sft$fitIndices[,1], sft$fitIndices[,5],
         xlab="Soft Threshold (power)", ylab="Mean Connectivity",
         type="n", main = paste("Mean connectivity"))
   text(sft$fitIndices[,1], sft$fitIndices[,5], labels=powers,__
⇔cex=cex1,col="blue")
    #####
   plot(sft$fitIndices[,1], sft$fitIndices[,6],
         xlab="Soft Threshold (power)", ylab="Median Connectivity",
         type="n", main = paste("Median connectivity"))
   text(sft$fitIndices[,1], sft$fitIndices[,6], labels=powers,__
⇔cex=cex1,col="blue")
    #####
   plot(sft$fitIndices[,1], sft$fitIndices[,7],
         xlab="Soft Threshold (power)",ylab="Max Connectivity", type="n",
         main = paste("Max connectivity"))
   text(sft$fitIndices[,1], sft$fitIndices[,7], labels=powers,_
⇒cex=cex1,col="blue")
}
```

```
[6]: figure_out_power_parameter=function()
{
    library(WGCNA)
    options(stringsAsFactors = FALSE);
    #enableWGCNAThreads(nThreads=16)
    lnames = load(file = '01.RData')
    plot_power_parameter(datExpr, 'power_parameter_selection.pdf')
}
```

1.4 Build the Network

```
[7]: construct_network=function()
      library(WGCNA)
       options(stringsAsFactors = FALSE);
       enableWGCNAThreads(nThreads=16)
       lnames = load(file = "01.RData")
       # softPower value from previous plot power_parameter_selection.pdf
       softPower = 9; #Based on the hippocampus
       # ALWAYS choose a value equal or above (better) 0.8
       cor <- WGCNA::cor</pre>
       net = blockwiseModules(datExpr, maxBlockSize=30000,
                              power = softPower, deepSplit = 3,
                              networkType = PARAM_NETWORK_TYPE,
                              TOMType = PARAM_NETWORK_TYPE,
                              numericLabels = TRUE, corType = "bicor",
                              saveTOMs = TRUE, mergeCutHeight = 0.1,
                              saveTOMFileBase = "TOM",
                              verbose = 3)
      moduleLabels = net$colors
       moduleColors = labels2colors(net$colors)
      MEs = net$MEs;
       geneTree = net$dendrograms[[1]];
       save(net, MEs, moduleLabels, moduleColors, geneTree, softPower, file = "02.
      →RData")
     #cyt = exportNetworkToCytoscape(modTOM,
```

1.5 Use Topology Overlap Matrix (TOM) to cluster the genes on the networks into different modules

```
[8]: plot_cluster_dendrogram=function()
{
          library(WGCNA)
          options(stringsAsFactors = FALSE);
```

1.6 Use Pearson Correlation to measure the correlation between each module eigenvalue (kME) and the various sample traits

```
[9]: correlate_with_traits=function()
         library(WGCNA)
         options(stringsAsFactors = FALSE)
         enableWGCNAThreads(nThreads=16)
         lnames = load(file = "01.RData")
         lnames = load(file = "02.RData")
         # Define numbers of genes and samples
         nGenes = ncol(datExpr);
         nSamples = nrow(datExpr);
         # Recalculate MEs with color labels
         MEs0 = moduleEigengenes(datExpr, moduleColors)$eigengenes
         MEs = orderMEs(MEs0)
         moduleTraitCor = cor(MEs, datTraits, use = "p");
         moduleTraitPvalue = corPvalueStudent(moduleTraitCor, nSamples);
         # Plot
         pdf(file="module_trait_relationships.pdf", height=16, width = 22)
         # Will display correlations and their p-values
         textMatrix = paste(signif(moduleTraitCor, 2), "\n(",
                            signif(moduleTraitPvalue, 1), ")", sep = "");
         dim(textMatrix) = dim(moduleTraitCor)
         par(mar = c(6, 8.5, 3, 3));
         # Display the correlation values within a heatmap plot
         labeledHeatmap(Matrix = moduleTraitCor,
                        xLabels = names(datTraits),
                        yLabels = names(MEs),
                        ySymbols = names(MEs),
                        colorLabels = FALSE,
                        naColor = "grey",
                        colors = blueWhiteRed(50),
```

```
textMatrix = textMatrix,
                   setStdMargins = FALSE,
                   cex.text = 0.9,
                   zlim = c(-1,1),
                   main = paste("Module kME-Trait Correlation"))
   dev.off()
    # Print output
   textMatrix = paste(signif(moduleTraitCor, 2), "\n(",
                       signif(moduleTraitPvalue, 1), ")", sep = "");
   dim(textMatrix) = dim(moduleTraitCor)
   par(mar = c(12, 6.5, 3, 0.5));
    # Display the correlation values within a heatmap plot
   labeledHeatmap(Matrix = moduleTraitCor, xLabels = names(datTraits),
                   yLabels = names(MEs), ySymbols = names(MEs),
                   colorLabels = FALSE, naColor = "grey",
                   colors = blueWhiteRed(50), textMatrix = textMatrix,
                   setStdMargins = FALSE, cex.text = 0.55, zlim = c(-1,1),
                   main = paste("Module kME-Trait Correlation"))
}
```

1.7 Export the main results

```
[10]: export_eigengene_tables = function()
          library(WGCNA)
          options(stringsAsFactors = FALSE)
          lnames = load(file = "01.RData")
          lnames = load(file = "02.RData")
          # Define numbers of genes and samples
          nGenes = ncol(datExpr)
          nSamples = nrow(datExpr)
          # Recalculate MEs with color labels
          MEs0 = moduleEigengenes(datExpr, moduleColors)$eigengenes
          rownames(MEs0) = rownames(datExpr)
          write.csv(MEs0, 'eigengenes.csv')
          # Write modules
          modules = data.frame(row.names=colnames(datExpr), module=moduleColors)
          write.csv(modules, 'modules.csv')
          save(datExpr,softPower,moduleColors, file = "cytoscapenetwork.Rdata")
      }
```

1.8 Run the functions and plot the results

```
[11]: prepare_data()
# 1 - Sample dendrogram and trait heatmap
prepare_traits()
# 2 - Scale Free Topology Model Fit
```

figure_out_power_parameter() Loading required package: limma Warning message in data.table::fread("../../differential_analysis/caudate/_m/ genes/residualized_expression.tsv"): "Detected 239 column names but the data has 240 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] 22374 239 Flagging genes and samples with too many missing values... [1] 230 22374 pickSoftThreshold: will use block size 1999. pickSoftThreshold: calculating connectivity for given powers... ..working on genes 1 through 1999 of 22374 Warning message: "executing %dopar% sequentially: no parallel backend registered" ..working on genes 2000 through 3998 of 22374 ..working on genes 3999 through 5997 of 22374 ..working on genes 5998 through 7996 of 22374 ..working on genes 7997 through 9995 of 22374 ..working on genes 9996 through 11994 of 22374 ..working on genes 11995 through 13993 of 22374 ..working on genes 13994 through 15992 of 22374 ..working on genes 15993 through 17991 of 22374 ..working on genes 17992 through 19990 of 22374 ..working on genes 19991 through 21989 of 22374 ..working on genes 21990 through 22374 of 22374 Power SFT.R.sq slope truncated.R.sq mean.k. median.k. max.k. 1 0.000349 -2.99 0.984 1.12e+04 1.12e+04 11500.0 1 2 2 0.005160 -4.45 0.951 5.71e+03 5.70e+03 6050.0 3 3 0.104000 -9.80 0.870 2.93e+03 2.92e+03 3280.0 4 4 0.436000 -12.10 0.872 1.53e+03 1.51e+03 1870.0 5 5 0.740000 -11.40 0.938 8.05e+02 7.89e+02 1130.0 6 6 0.888000 -9.24 0.977 4.30e+02 4.16e+02 720.0 7 7 0.944000 -7.34 0.991 2.33e+02 2.22e+02 481.0 8 -5.84 0.992 1.29e+02 1.19e+02 8 0.964000 336.0 9 9 0.971000 -4.720.991 7.24e+01 6.46e+01 245.0 10 10 0.977000 -3.890.988 4.15e+01 3.54e+01184.0 -3.330.983 2.44e+01 11 11 0.976000 1.96e+01 145.0

0.977 1.47e+01

0.984 9.11e+00

0.985 5.83e+00

0.979 3.85e+00 2.02e+00

1.09e+01

6.17e+00

3.51e+00

118.0

97.7

84.7

75.1

12

13

14

15

12 0.970000

13 0.979000

14 0.977000

15 0.965000 -2.20

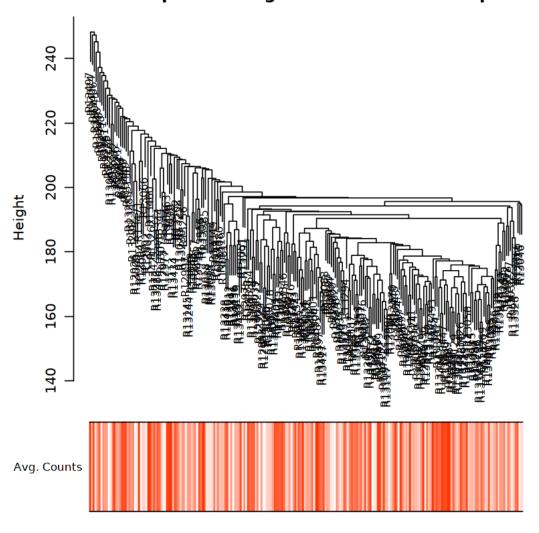
-2.93

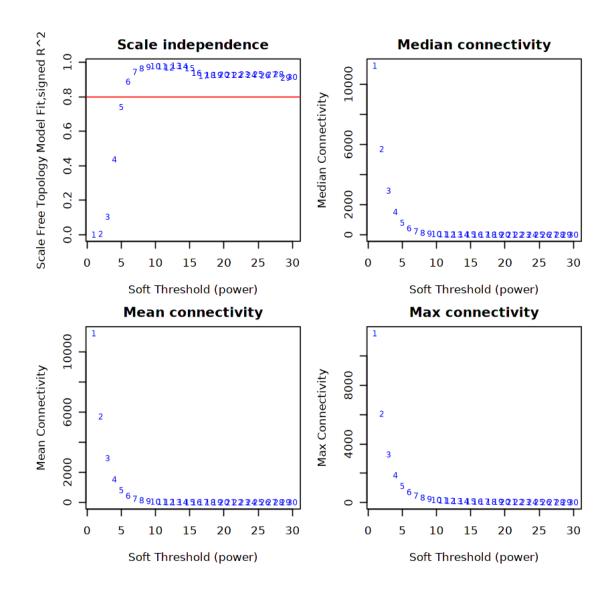
-2.58

-2.36

16 0.937000	-2.10	0.963 2.63e+00	1.17e+00	67.1
17 0.922000	-2.00	0.958 1.85e+00	6.87e-01	60.4
18 0.925000	-1.88	0.966 1.35e+00	4.07e-01	54.7
19 0.926000	-1.80	0.973 1.01e+00	2.44e-01	49.8
20 0.928000	-1.73	0.975 7.73e-01	1.47e-01	45.5
21 0.926000	-1.67	0.976 6.06e-01	8.96e-02	41.7
22 0.926000	-1.63	0.978 4.85e-01	5.50e-02	38.3
23 0.930000	-1.58	0.983 3.94e-01	3.41e-02	35.3
24 0.926000	-1.56	0.981 3.25e-01	2.13e-02	32.6
25 0.930000	-1.52	0.984 2.71e-01	1.34e-02	30.1
26 0.924000	-1.51	0.980 2.29e-01	8.53e-03	27.9
27 0.928000	-1.49	0.980 1.95e-01	5.46e-03	25.9
28 0.931000	-1.47	0.980 1.67e-01	3.50e-03	24.1
29 0.911000	-1.46	0.953 1.44e-01	2.26e-03	22.4
30 0.915000	-1.45	0.955 1.25e-01	1.47e-03	20.9
	17 0.922000 18 0.925000 19 0.926000 20 0.928000 21 0.926000 22 0.926000 23 0.930000 24 0.926000 25 0.930000 26 0.924000 27 0.928000 28 0.931000 29 0.911000	17 0.922000 -2.00 18 0.925000 -1.88 19 0.926000 -1.80 20 0.928000 -1.67 21 0.926000 -1.67 22 0.926000 -1.63 23 0.930000 -1.58 24 0.926000 -1.56 25 0.930000 -1.52 26 0.924000 -1.51 27 0.928000 -1.49 28 0.931000 -1.47 29 0.911000 -1.46	17 0.922000 -2.00 0.958 1.85e+00 18 0.925000 -1.88 0.966 1.35e+00 19 0.926000 -1.80 0.973 1.01e+00 20 0.928000 -1.73 0.975 7.73e-01 21 0.926000 -1.67 0.976 6.06e-01 22 0.926000 -1.63 0.978 4.85e-01 23 0.930000 -1.58 0.983 3.94e-01 24 0.926000 -1.56 0.981 3.25e-01 25 0.930000 -1.52 0.984 2.71e-01 26 0.924000 -1.51 0.980 2.29e-01 27 0.928000 -1.49 0.980 1.95e-01 28 0.931000 -1.47 0.980 1.67e-01 29 0.911000 -1.46 0.953 1.44e-01	17 0.922000 -2.00 0.958 1.85e+00 6.87e-01 18 0.925000 -1.88 0.966 1.35e+00 4.07e-01 19 0.926000 -1.80 0.973 1.01e+00 2.44e-01 20 0.928000 -1.73 0.975 7.73e-01 1.47e-01 21 0.926000 -1.67 0.976 6.06e-01 8.96e-02 22 0.926000 -1.63 0.978 4.85e-01 5.50e-02 23 0.930000 -1.58 0.983 3.94e-01 3.41e-02 24 0.926000 -1.56 0.981 3.25e-01 2.13e-02 25 0.930000 -1.52 0.984 2.71e-01 1.34e-02 26 0.924000 -1.51 0.980 2.29e-01 8.53e-03 27 0.928000 -1.49 0.980 1.95e-01 5.46e-03 28 0.931000 -1.47 0.980 1.67e-01 3.50e-03 29 0.911000 -1.46 0.953 1.44e-01 2.26e-03

Sample dendrogram and trait heatmap





[12]: construct_network()

Allowing parallel execution with up to 16 working processes.

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 16 parallel threads.

Fraction of slow calculations: 0.000000

..connectivity..

```
..matrix multiplication (system BLAS)..
   ..normalization..
   ..done.
  ..saving TOM for block 1 into file TOM-block.1.RData
...clustering...
...detecting modules...
...calculating module eigengenes...
...checking kME in modules...
    ..removing 1398 genes from module 1 because their KME is too low.
    ..removing 946 genes from module 2 because their KME is too low.
    ..removing 652 genes from module 3 because their KME is too low.
    ..removing 89 genes from module 4 because their KME is too low.
    ..removing 81 genes from module 5 because their KME is too low.
    ..removing 105 genes from module 6 because their KME is too low.
    ..removing 102 genes from module 7 because their KME is too low.
    ..removing 29 genes from module 8 because their KME is too low.
    ..removing 3 genes from module 9 because their KME is too low.
    ..removing 40 genes from module 10 because their KME is too low.
    .. removing 8 genes from module 11 because their KME is too low.
    ..removing 1 genes from module 12 because their KME is too low.
    ..removing 1 genes from module 13 because their KME is too low.
    ..removing 2 genes from module 14 because their KME is too low.
    ..removing 17 genes from module 15 because their KME is too low.
    ..removing 2 genes from module 17 because their KME is too low.
    ..removing 12 genes from module 18 because their KME is too low.
    ..removing 3 genes from module 19 because their KME is too low.
    ..removing 2 genes from module 22 because their KME is too low.
    ..removing 8 genes from module 27 because their KME is too low.
    ..removing 1 genes from module 29 because their KME is too low.
    ..removing 1 genes from module 32 because their KME is too low.
 ..reassigning 171 genes from module 1 to modules with higher KME.
 ..reassigning 78 genes from module 2 to modules with higher KME.
 ..reassigning 159 genes from module 3 to modules with higher KME.
 ..reassigning 30 genes from module 4 to modules with higher KME.
 ..reassigning 34 genes from module 5 to modules with higher KME.
 ..reassigning 21 genes from module 6 to modules with higher KME.
 ..reassigning 17 genes from module 7 to modules with higher KME.
 ..reassigning 5 genes from module 8 to modules with higher KME.
 ..reassigning 7 genes from module 9 to modules with higher KME.
 ..reassigning 1 genes from module 10 to modules with higher KME.
 ..reassigning 11 genes from module 11 to modules with higher KME.
 ..reassigning 3 genes from module 12 to modules with higher KME.
 ..reassigning 3 genes from module 13 to modules with higher KME.
 ..reassigning 1 genes from module 14 to modules with higher KME.
 ..reassigning 5 genes from module 15 to modules with higher KME.
 ..reassigning 2 genes from module 16 to modules with higher KME.
 ..reassigning 5 genes from module 17 to modules with higher KME.
 ..reassigning 4 genes from module 18 to modules with higher KME.
```

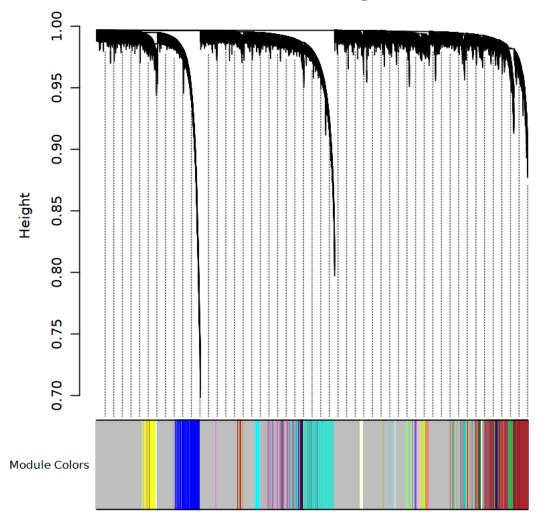
```
..reassigning 11 genes from module 20 to modules with higher KME.
..reassigning 5 genes from module 21 to modules with higher KME.
..reassigning 1 genes from module 22 to modules with higher KME.
..reassigning 2 genes from module 23 to modules with higher KME.
..reassigning 1 genes from module 24 to modules with higher KME.
..reassigning 2 genes from module 26 to modules with higher KME.
..reassigning 4 genes from module 29 to modules with higher KME.
..reassigning 2 genes from module 32 to modules with higher KME.
..merging modules that are too close..

mergeCloseModules: Merging modules whose distance is less than 0.1
Calculating new MEs...
```

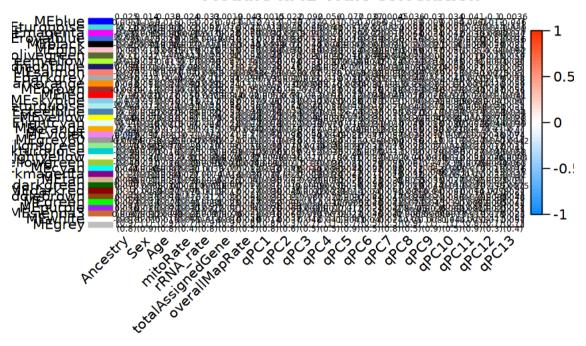
```
[13]: #3 - TOM Dendogram
plot_cluster_dendrogram()
#4 - Module Eigenvalue Correlation with sample's traits
correlate_with_traits()
```

Allowing parallel execution with up to 16 working processes. Allowing parallel execution with up to 16 working processes.

Cluster Dendrogram



Module kME-Trait Correlation



```
[14]: export_eigengene_tables()
```

1.9 Repreducibility Information

system elapsed

5541.270 1494.915 679.729

user

```
[15]: Sys.time()
    proc.time()
    options(width = 120)
    sessioninfo::session_info()

[1] "2021-07-12 11:05:23 EDT"
```

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

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561	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)	glue		1.4.2	2020-08-27	[1]	CRAN (R 4.0.2)	

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Hmisc		4.5-0	2021-02-28	[1]	CRAN (R 4.0.3)	
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htmltools		0.5.1.1	2021-01-22	[1]	CRAN (R 4.0.2)	
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iterators		1.0.13	2020-10-15	[1]	CRAN (R 4.0.2)	
		0.1-8.1	2019-10-24	[1]	CRAN (R 4.0.2)	
jpeg isoplito		1.7.2	2019-10-24	[1]	CRAN (R 4.0.2)	
jsonlite knitr		1.7.2	2020-12-09	[1]	CRAN (R 4.0.2) CRAN (R 4.0.3)	
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		0.6-29		[1]		
latticeExtra			2019-12-19	[1]		
lifecycle		1.0.0	2021-02-15	[1]		
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magrittr		2.0.1	2020-11-17	[1]	CRAN (R 4.0.2)	
Matrix		1.3-4	2021-06-01	[1]	CRAN (R 4.0.3)	
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RSQLite		2.2.7	2021-04-22	[1]	CRAN (R 4.0.3)	
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stringr		1.4.0	2019-02-10	[1]	CRAN (R 4.0.2)	
survival		3.2-7	2020-09-28	[2]	CRAN (R 4.0.3)	
tibble		3.1.2	2021-05-16	[1]	CRAN (R 4.0.3)	
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WGCNA	* 1.70-3	2021-02-28	[1] CRAN	(R 4.0.3)
withr	2.4.2	2021-04-18	[1] CRAN	(R 4.0.3)
xfun	0.24	2021-06-15	[1] CRAN	(R 4.0.3)

- [1] /home/jbenja13/R/x86_64-pc-linux-gnu-library/4.0 [2] /usr/lib/R/library