

WGCNA – Average Expression Plot for B73

```
1  #!/usr/bin/Rscript --vanilla
2  rm(list=ls())
3
4  library(jpeg)
5  library(dplyr)
6  library(tidyr)
7  library(tibble)
8  library(stringr)
9  library(ggplot2)
10
11  library(argparse)
12
13  library(foreach)
14  library(iterators)
15  library(parallel)
16  library(doParallel)
17
18  library(WGCNA)
19  # library(KEGGREST)
20  # library(biomaRt)
21
22  set.seed(1)
23
24  # Enable WGCNA threads to speed up calculations
25  enableWGCNAThreads()
```

```
28 #####
29 # Constants/Variables
30 #####
31 selected_genotype <- "B73"
32 softPower <- 14
33 minModuleSize <- 5
34 mergeCutHeight <- 0.0000001
35
36
37 #####
38 # Output folder
39 #####
40 output_path <- file.path(
41   paste0(
42     "/home/ycth8/data/projects/2021_05_30_summer_WGCNA/Maize_proteomics_output/",
43     paste0("2021_06_10_", selected_genotype, "_step_by_step_network_construction")
44   )
45 )
46
47 if(!dir.exists(output_path)){
48   dir.create(output_path, showWarnings=FALSE, recursive=TRUE)
49   if(!dir.exists(output_path)){
50     quit(status=1)
51   }
52 }
```

```
55 #####
56 # Read in input file
57 #####
58
59 folder_path = file.path("/home/ycth8/data/projects/2021_05_30_summer_WGCNA/Maize_proteomics_output/")
60
61 datExpr = read.csv(
62   file = file.path(folder_path, "datExpr.csv"),
63   header = TRUE,
64   row.names = 1,
65   check.names = FALSE,
66   stringsAsFactors = FALSE
67 )
68
69 datExpr = datExpr[startsWith(rownames(datExpr), selected_genotype),]
```

```

72 #####
73 # Choose a set of soft-thresholding powers
74 #####
75
76 powers = 1:40
77 # Call the network topology analysis function
78 sft = pickSoftThreshold(datExpr, powerVector = powers, verbose = 5)
79
80 # Plot tree
81 cat(rep("\n", 2))
82 jpeg(filename = file.path(output_path, "softThreshold.jpeg"), width = 1920, height = 480)
83 par(mfrow = c(1,2))
84 cex1 = 0.9
85
86 # Scale-free topology fit index as a function of the soft-thresholding power
87 plot(sft$fitIndices[,1], -sign(sft$fitIndices[,3])*sft$fitIndices[,2],
88      xlab="Soft Threshold (power)",ylab="Scale Free Topology Model Fit, signed R^2",type="n",
89      main = paste("Scale independence"))
90 text(sft$fitIndices[,1], -sign(sft$fitIndices[,3])*sft$fitIndices[,2],
91      labels=powers,cex=cex1,col="red")
92 # this line corresponds to using an R^2 cut-off of h
93 abline(h=sft$fitIndices[sft$fitIndices$Power == softPower, "SFT.R.sq"], col="red")
94
95 # Mean connectivity as a function of the soft-thresholding power
96 plot(sft$fitIndices[,1], sft$fitIndices[,5],
97      xlab="Soft Threshold (power)",ylab="Mean Connectivity", type="n",
98      main = paste("Mean connectivity"))
99 text(sft$fitIndices[,1], sft$fitIndices[,5], labels=powers, cex=cex1,col="red")
100 abline(h=sft$fitIndices[sft$fitIndices$Power == softPower, "mean.k"], col="red")
101 dev.off()

```

```
108 #####
109 # Make a gene tree and identify modules
110 #####
111 adjacency = adjacency(datExpr, power = softPower)
112
113 # Turn adjacency into topological overlap
114 TOM = TOMsimilarity(adjacency)
115 disSTOM = 1-TOM
116
117 # Call the hierarchical clustering function
118 geneTree = hclust(as.dist(disSTOM), method = "average")
119
120 # Plot the resulting clustering tree (dendrogram)
121 cat(rep("\n", 2))
122 jpeg(filename = file.path(output_path, "geneClustering.jpeg"), width = 1920, height = 720)
123 plot(geneTree, xlab="", sub="", main = "Gene clustering on TOM-based dissimilarity",
124       labels = FALSE, hang = 0.04)
125 dev.off()
126
127 jpeg(filename = file.path(output_path, "geneClustering_with_gene_id.jpeg"), width = 1920, height = 720)
128 plot(geneTree, xlab="", sub="", main = "Gene clustering on TOM-based dissimilarity",
129       labels = colnames(datExpr), hang = 0.04)
130 dev.off()
```

```
137 dynamicMods = cutreeDynamic(
138     dendro = geneTree,
139     distM = dissTOM,
140     deepSplit = 2,
141     pamRespectsDendro = FALSE,
142     minClusterSize = minModuleSize
143 )
144 print(table(dynamicMods))
145
146
147 # Convert numeric labels into colors
148 dynamicColors = labels2colors(dynamicMods)
149
150 print(table(dynamicColors))
151
152 # Plot the dendrogram and colors underneath
153 cat(rep("\n", 2))
154 jpeg(filename = file.path(output_path, "networkConstruction_dynamic_colors.jpeg"), width = 1920, height = 720)
155 plotDendroAndColors(
156     geneTree,
157     dynamicColors,
158     "Dynamic Tree Cut",
159     dendroLabels = FALSE,
160     hang = 0.03,
161     addGuide = TRUE,
162     guideHang = 0.05,
163     main = "Gene dendrogram and module colors"
164 )
165 dev.off()
```

```
265 # Call an automatic merging function
266 merge = mergeCloseModules(datExpr, dynamicColors, cutHeight = mergeCutHeight, verbose = 3)
267
268 # The merged module colors
269 mergedColors = merge$colors
270
271 print(table(mergedColors))
272
273 # Eigengenes of the new merged modules:
274 mergedMEs = merge$newMEs
275
276 cat(rep("\n", 2))
277 jpeg(filename = file.path(output_path, "networkConstruction_dynamic_and_merged_colors.jpeg"), width = 1920, height = 7
278 plotDendroAndColors(geneTree, cbind(dynamicColors, mergedColors),
279                     c("Dynamic Tree Cut", "Merged dynamic"),
280                     dendroLabels = FALSE, hang = 0.03,
281                     addGuide = TRUE, guideHang = 0.05)
282 dev.off()
283
284 cat(rep("\n", 2))
285 jpeg(filename = file.path(output_path, "networkConstruction_merged_colors.jpeg"), width = 1920, height = 720)
286 plotDendroAndColors(geneTree, mergedColors,
287                     "Merged dynamic",
288                     dendroLabels = FALSE, hang = 0.03,
289                     addGuide = TRUE, guideHang = 0.05)
290 dev.off()
```



```

331 #####
332 # Organize merged average expression table and plot average expression
333 #####
334 MEList2 = moduleEigengenes(datExpr, colors = mergedColors)
335
336 # Create average expression table
337 averageExpr <- MEList2$averageExpr %>%
338   rownames_to_column(var = "Sample") %>%
339   pivot_longer(!Sample, names_to = "Module", values_to = "Measurement") %>%
340   separate(Sample, c("Sample", "Time")) %>%
341   as.data.frame(stringsAsFactors = FALSE)
342
343 averageExpr$Sample <- factor(averageExpr$Sample, levels = unique(averageExpr$Sample))
344
345 averageExpr$Module <- sub("^AE", "", averageExpr$Module)
346 averageExpr$Module <- factor(averageExpr$Module, levels = unique(averageExpr$Module))
347
348 write.csv(
349   x = averageExpr,
350   file = file.path(output_path, "mergedAverageExpr.csv"),
351   na = "",
352   quote = FALSE
353 )
354
355 # Plot average expression plot
356 p <- ggplot(data = averageExpr, mapping = aes(x = Time, y = Measurement, group=1)) +
357   geom_line() +
358   facet_grid(Module ~ Sample, scales = "free") +
359   labs(x = "Time Point", y = "Average Expression Value")

```

B73



