

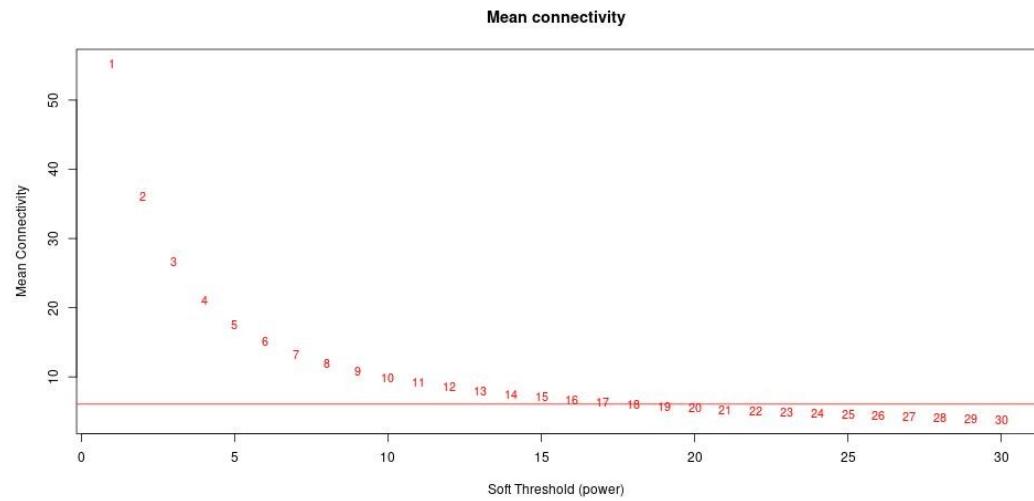
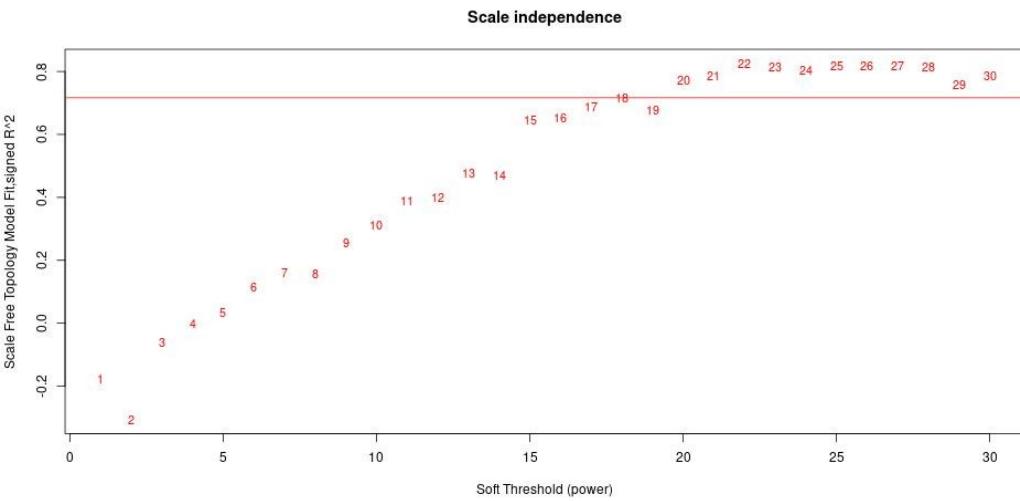
WGCNA – Auto Network Construction for O2

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

```
25 #####
26 # Constants/Variables
27 #####
28 selected_genotype <- "02"
29
30 softPower <- 18
31 minModuleSize <- 5
32
33 # Eigengenes clustering tree cutting threshold
34 MEDissThres <- 0.0000001
35
36
37 #####
38 # Output folder
39 #####
40 output_path <- file.path("/home/ycth8/data/projects/05_30_2021_summer_WGCNA/Maize_proteomics_output/2021_06_10_02_auto_network_construction")
41
42 if(!dir.exists(output_path)){
43   dir.create(output_path, showWarnings=FALSE, recursive=TRUE)
44   if(!dir.exists(output_path)){
45     quit(status=1)
46   }
47 }
```

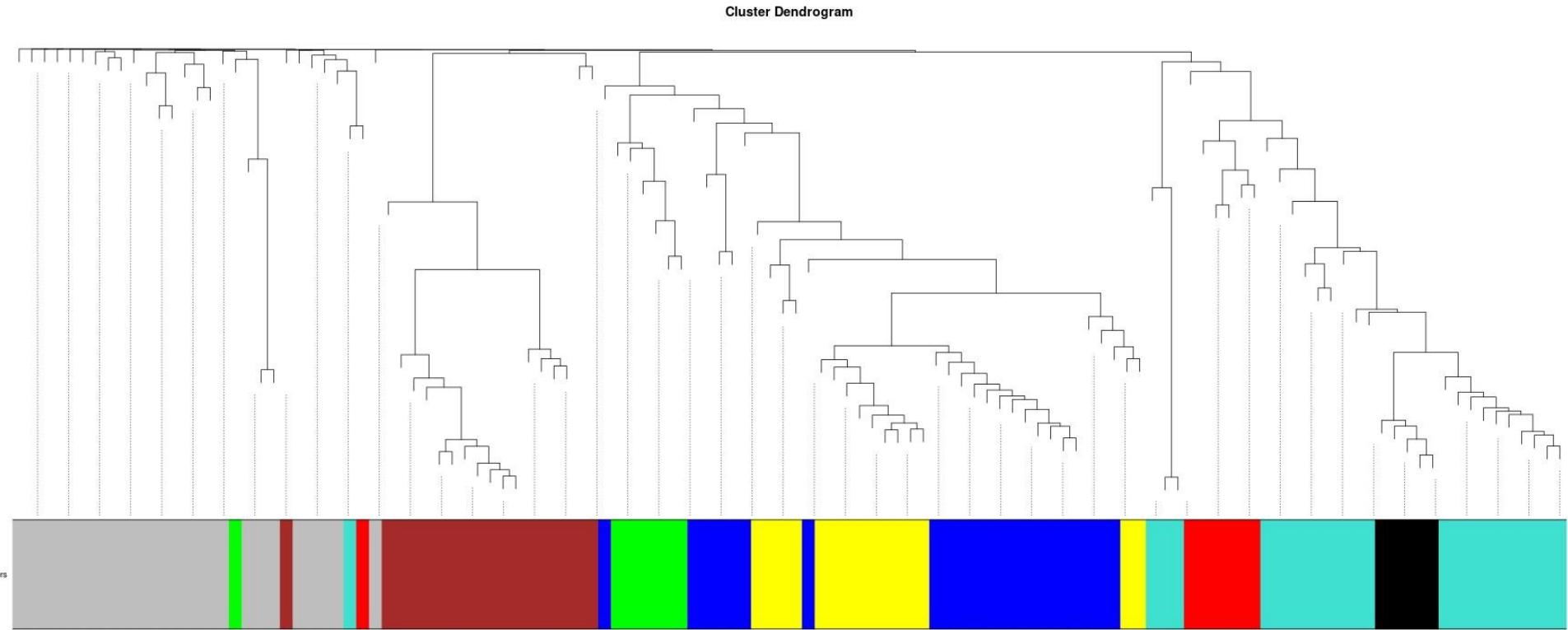
```
50 #####  
51 # Read in input file  
52 #####  
53  
54 folder_path = file.path("/home/ycth8/data/projects/05_30_2021_summer_WGCNA/Maize_proteomics_output")  
55  
56 datExpr = read.csv(  
57   file = file.path(folder_path, "datExpr.csv"),  
58   header = TRUE,  
59   row.names = 1,  
60   check.names = FALSE,  
61   stringsAsFactors = FALSE  
62 )  
63  
64 datExpr = datExpr[startsWith(rownames(datExpr), selected_genotype),]  
65
```

```
67 #####  
68 # Choose a set of soft-thresholding powers  
69 #####  
70  
71 powers = 1:30  
72 # Call the network topology analysis function  
73 sft = pickSoftThreshold(datExpr, powerVector = powers, verbose = 5)  
74  
75 # Plot tree  
76 cat(rep("\n", 2))  
77 jpeg(filename = file.path(output_path, "softThreshold.jpeg"), width = 1920, height = 480)  
78 par(mfrow = c(1,2))  
79 cex1 = 0.9  
80  
81 # Scale-free topology fit index as a function of the soft-thresholding power  
82 plot(sft$fitIndices[,1], -sign(sft$fitIndices[,3])*sft$fitIndices[,2],  
83       xlab="Soft Threshold (power)", ylab="Scale Free Topology Model Fit, signed R^2", type="n",  
84       main = paste("Scale independence"))  
85 text(sft$fitIndices[,1], -sign(sft$fitIndices[,3])*sft$fitIndices[,2],  
86       labels=powers, cex=cex1, col="red")  
87 # this line corresponds to using an R^2 cut-off of h  
88 abline(h=sft$fitIndices[sft$fitIndices$Power == softPower, "SFT.R.sq"], col="red")  
89  
90 # Mean connectivity as a function of the soft-thresholding power  
91 plot(sft$fitIndices[,1], sft$fitIndices[,5],  
92       xlab="Soft Threshold (power)", ylab="Mean Connectivity", type="n",  
93       main = paste("Mean connectivity"))  
94 text(sft$fitIndices[,1], sft$fitIndices[,5], labels=powers, cex=cex1, col="red")  
95 abline(h=sft$fitIndices[sft$fitIndices$Power == softPower, "mean.k."], col="red")  
96 dev.off()  
97  
98  
99 # Collect garbage  
100 collectGarbage()
```



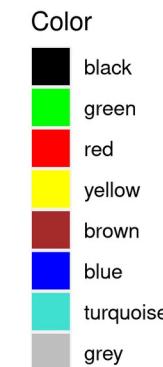
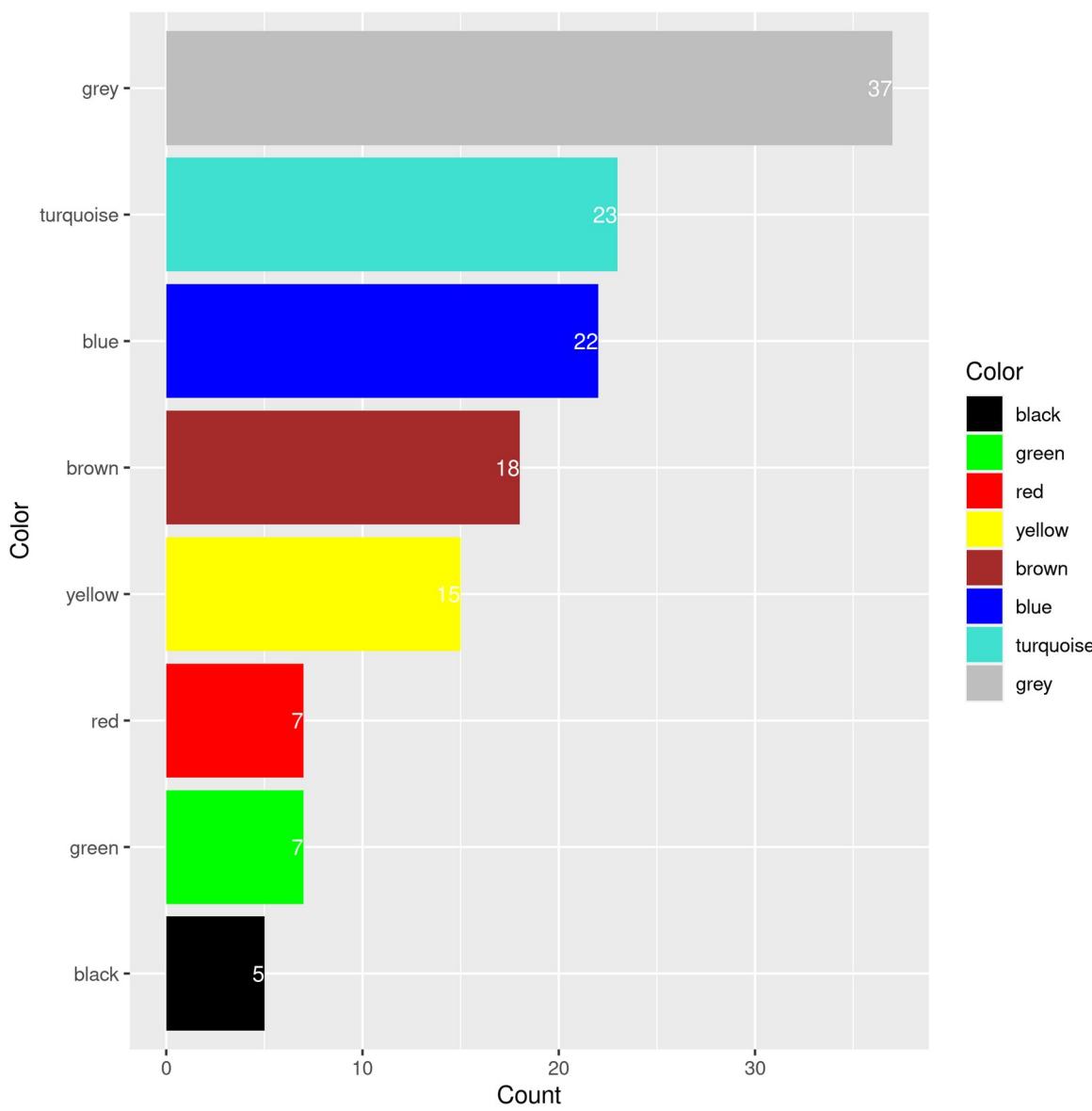
```
103 #####  
104 # Create blockwise modules  
105 #####  
106  
107 net = blockwiseModules(  
108     datExpr,  
109     power = softPower,  
110     TOMType = "unsigned",  
111     minModuleSize = minModuleSize,  
112     reassignThreshold = 0,  
113     mergeCutHeight = MEDissThres,  
114     numericLabels = TRUE,  
115     pamRespectsDendro = FALSE,  
116     saveTOMs = TRUE,  
117     saveTOMFileBase = file.path(output_path, "02MaizeTOM"),  
118     verbose = 3  
119 )  
120  
121 # The numbers on top are the color labels  
122 print(table(net$colors))  
123
```

```
125 #####  
126 # Plot the dendrogram and colors underneath  
127 #####  
128  
129 # Convert labels to colors for plotting  
130 mergedColors = labels2colors(net$colors)  
131  
132 cat(rep("\n", 2))  
133 jpeg(filename = file.path(output_path, "networkConstruction_merged_colors.jpeg"), width = 1920, height = 720)  
134 plotDendroAndColors(  
135   net$dendrograms[[1]],  
136   mergedColors[net$blockGenes[[1]]],  
137   "Module colors",  
138   dendroLabels = FALSE,  
139   hang = 0.03,  
140   addGuide = TRUE,  
141   guideHang = 0.05  
142 )  
143 dev.off()  
...
```



```
169 #####  
170 # Save genes and colors  
171 #####  
172 genes_colors_df <- data.frame(  
173     "Gene" = colnames(datExpr),  
174     "Color" = mergedColors,  
175     stringsAsFactors = FALSE  
176 )  
177  
178 write.csv(  
179     x = genes_colors_df,  
180     file = file.path(output_path, "genes_colors_df.csv"),  
181     na = "",  
182     quote = FALSE,  
183     row.names = FALSE  
184 )
```

```
187 genes_colors_summary_df <- genes_colors_df %>%
188   group_by(Color) %>%
189   summarize(Count = n()) %>%
190   arrange(Count) %>%
191   as.data.frame(stringsAsFactors = FALSE)
192
193 genes_colors_summary_df$Color <- factor(genes_colors_summary_df$Color, levels = unique(genes_colors_summary_df$Color))
194
195 p <- ggplot(data=genes_colors_summary_df, aes(x = Color, y=Count)) +
196   geom_bar(mapping = aes(fill = Color), stat="identity") +
197   geom_text(aes(label=Count), hjust=1, color="white", size=3.5) +
198   coord_flip() +
199   scale_fill_manual(values = levels(genes_colors_summary_df$Color))
200
201 ggsave(
202   filename = "genes_colors_summary.png",
203   plot = p,
204   path = output_path
205 )
```



```
146 #####  
147 # Save important variables as RData  
148 #####  
149  
150 moduleLabels = net$colors  
151 moduleColors = labels2colors(net$colors)  
152 MEs = net$MEs;  
153 geneTree = net$dendograms[[1]];  
154 save(  
155   MEs, moduleLabels, moduleColors, geneTree,  
156   file = file.path(output_path, "02-networkConstruction-auto.RData")  
157 )
```
