WGCNA Analysis

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Call libraries

```
library(WGCNA)
```

```
## Loading required package: dynamicTreeCut

## Loading required package: fastcluster

##
## Attaching package: 'fastcluster'

## The following object is masked from 'package:stats':
##
##     hclust

## Warning: replacing previous import 'utils::findMatches' by
## 'S4Vectors::findMatches' when loading 'AnnotationDbi'

##

##
## Attaching package: 'WGCNA'

## The following object is masked from 'package:stats':
##
##     cor
```

```
library(DESeq2)
```

```
## Loading required package: S4Vectors

## Loading required package: stats4

## Loading required package: BiocGenerics

##
## Attaching package: 'BiocGenerics'
```

```
## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs

## The following objects are masked from 'package:base':
##
##     anyDuplicated, append, as.data.frame, basename, cbind, colnames,
##     dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
##     grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
##     order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
##     rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
##     union, unique, unsplit, which.max, which.min

##
## Attaching package: 'S4Vectors'

## The following object is masked from 'package:utils':
##
##     findMatches

## The following objects are masked from 'package:base':
##
##     expand.grid, I, unname

## Loading required package: IRanges

##
## Attaching package: 'IRanges'

## The following object is masked from 'package:grDevices':
##
##     windows

## Loading required package: GenomicRanges

## Loading required package: GenomeInfoDb

## Loading required package: SummarizedExperiment

## Loading required package: MatrixGenerics

## Loading required package: matrixStats

##
## Attaching package: 'MatrixGenerics'

## The following objects are masked from 'package:matrixStats':
##
##     colAlls, colAnyNAs, colAnys, colAvgsPerRowSet, colCollapse,
##     colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
```

```
##     colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
##     colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
##     colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
##     colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
##     colWeightedMeans, colWeightedMedians, colWeightedSds,
##     colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
##     rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
##     rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
##     rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
##     rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
##     rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
##     rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
##     rowWeightedSds, rowWeightedVars

## Loading required package: Biobase

## Welcome to Bioconductor
##
##     Vignettes contain introductory material; view with
##     'browseVignettes()'. To cite Bioconductor, see
##     'citation("Biobase")', and for packages 'citation("pkgname")'.

##
## Attaching package: 'Biobase'

## The following object is masked from 'package:MatrixGenerics':
##
##     rowMedians

## The following objects are masked from 'package:matrixStats':
##
##     anyMissing, rowMedians
```

```
library(tidyverse)
```

```
## -- Attaching packages --------------------------------------- tidyverse 1.3.1 --

## v ggplot2 3.4.2     v purrr   1.0.1
## v tibble  3.2.1     v dplyr   1.1.1
## v tidyr   1.3.0     v stringr 1.5.0
## v readr   2.1.2     v forcats 1.0.0

## -- Conflicts ------------------------------------------ tidyverse_conflicts() --
## x dplyr::collapse()   masks IRanges::collapse()
## x dplyr::combine()    masks Biobase::combine(), BiocGenerics::combine()
## x dplyr::count()      masks matrixStats::count()
## x dplyr::desc()       masks IRanges::desc()
## x tidyr::expand()     masks S4Vectors::expand()
## x dplyr::filter()     masks stats::filter()
## x dplyr::first()      masks S4Vectors::first()
## x dplyr::lag()        masks stats::lag()
```

```
## x ggplot2::Position() masks BiocGenerics::Position(), base::Position()
## x purrr::reduce()     masks GenomicRanges::reduce(), IRanges::reduce()
## x dplyr::rename()     masks S4Vectors::rename()
## x dplyr::slice()      masks IRanges::slice()
```

```
library(CorLevelPlot)
library(gridExtra)
```

```
##
## Attaching package: 'gridExtra'

## The following object is masked from 'package:dplyr':
##
##     combine

## The following object is masked from 'package:Biobase':
##
##     combine

## The following object is masked from 'package:BiocGenerics':
##
##     combine
```

```
library(magrittr)
```

```
##
## Attaching package: 'magrittr'

## The following object is masked from 'package:purrr':
##
##     set_names

## The following object is masked from 'package:tidyr':
##
##     extract
```

```
library(ggplot2)

allowWGCNAThreads()
```

```
## Allowing multi-threading with up to 8 threads.
```

```
setwd('E:/WGCNA')
```

1. Read data

```
data<- read.csv('Counts.csv', row.names = 1)
Sample_info<- read.csv('Sample_info.csv', row.names = 1)
```

2. Quality control

```
gg<- goodSamplesGenes(t(data))
```

Step1: Detect outlier genes with WGCNA package

```
##  Flagging genes and samples with too many missing values...
##   ..step 1
##   ..step 2
```

```
summary(gg)
```

```
##             Length Class  Mode
## goodGenes   28089  -none- logical
## goodSamples    52  -none- logical
## allOK           1  -none- logical
```

```
gg$allOK
```

```
## [1] FALSE
```

Since it indicates tat all genes are not good genes let's quantify the outlier genes

```
table(gg$goodGenes)
```

We'll do it for samples as well

```
##
## FALSE  TRUE
##  3576 24513
```

```
table(gg$goodSamples)
```

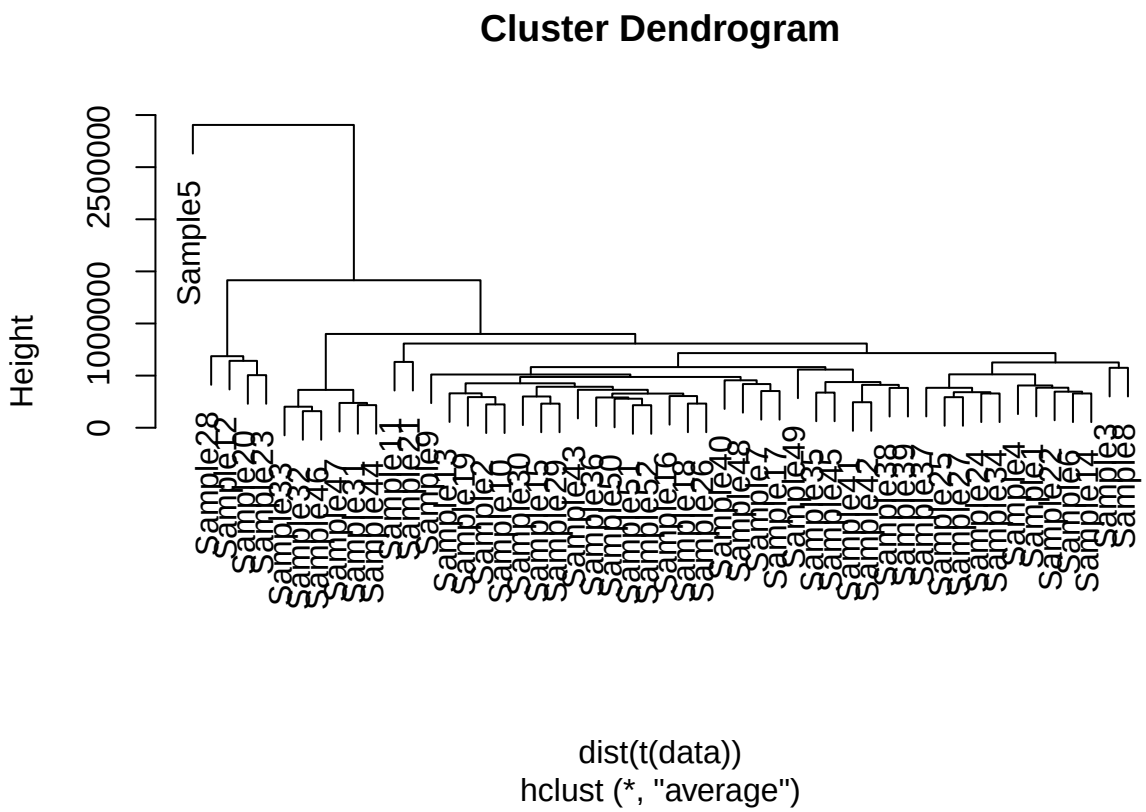
```
##
## TRUE
##   52
```

```
data<- data[gg$goodGenes=='TRUE',]
```

Around 3500 outlier genes but no outlier samples. Let's remove those genes

Step2: Identify outlier samples with hierachical clustering

```
htree <- hclust(dist(t(data)), method = "average")
plot(htree)
```



Step3: Identify outlier samples with PCA

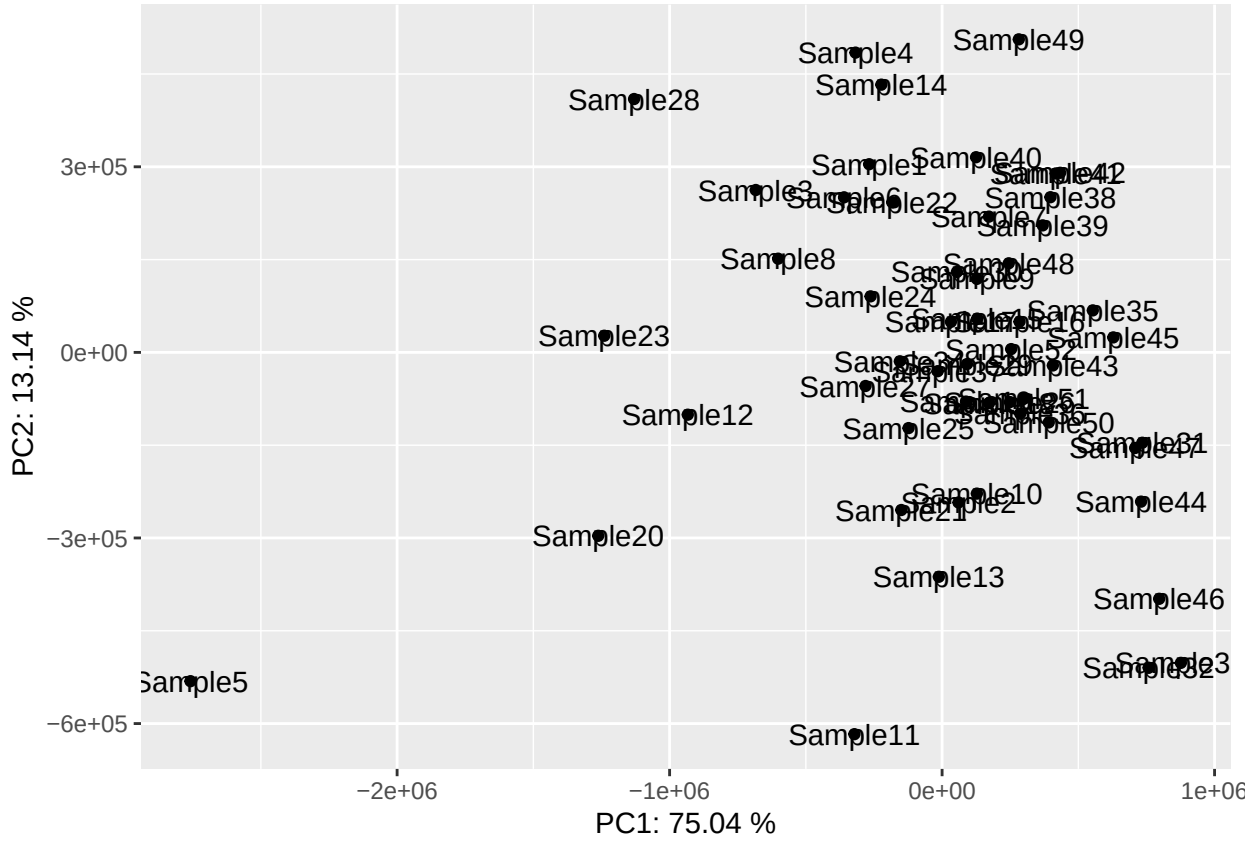
```
pca <- prcomp(t(data))
pca.dat <- pca$x

pca.var <- pca$sdev^2
pca.var.percent <- round(pca.var/sum(pca.var)*100, digits = 2)

pca.dat <- as.data.frame(pca.dat)

ggplot(pca.dat, aes(PC1, PC2)) +
  geom_point() +
  geom_text(label = rownames(pca.dat)) +
  labs(x = paste0('PC1: ', pca.var.percent[1], ' %'),

       y = paste0('PC2: ', pca.var.percent[2], ' %'))
```




```
#N.B. We need to remove batch effect if there's any at this stage before we proceed further

#In both these methods we found Sample5 is distantly related
#So we'll exclude them
discard_samples<- c('Sample5')
data.final<- data[,!colnames(data) %in% discard_samples]
Sample_info_final<- Sample_info[!row.names(Sample_info) %in% discard_samples,]
```

4. Perform VST normalization with DESeq2 since it's a count matrix

N.B. FPKM or RPKM matrices need to be log transformed ####Before performing normalization make sure if the rownames in matrix and colnames in #### Sample info are identical

```
all(rownames(Sample_info_final)%in%colnames(data.final)) #If present
```

```
## [1] TRUE
```

```
all(rownames(Sample_info_final)==colnames(data.final)) #If present in same order
```

```
## [1] TRUE
```

Create deseq2 object

```
dds <- DESeqDataSetFromMatrix(countData = data.final,
                              colData = Sample_info_final,
                              design = ~ 1) #Specifying no design
nrow(dds)
```

```
## [1] 24513
```

Remove samples that have <15 counts in more than 75% of the samples

Recommended by WGCNA

However, the downstream analysis with higher # of genes will depend largely on

```
keep <- dds[rowSums(counts(dds) >= 15)>=39,] #75% of 52 samples is 52*0.75=39
nrow(keep)
```

the available ram of PC. So, we'll consider gene numbers based on our PC capacity.

```
## [1] 12226
```

Perform VST transformation

```
dds.vst<- vst(keep)
dds.vst<- dds.vst[1:5000,] #We reduced the # of genes to 8000 because of low RAM
nrow(dds.vst)
```

```
## [1] 5000
```

Get normalized counts

```
norm.counts <- assay(dds.vst) %>%
  t()
```

5. Constructing network

Set softhreshold power

```
power <- c(c(1:10), seq(from = 12, to = 50, by = 2))
```

Set the network topology analysis function

```
sft <- pickSoftThreshold(norm.counts,
                         powerVector = power,
                         networkType = "signed",
                         verbose = 5)
```

```
## pickSoftThreshold: will use block size 5000.
##  pickSoftThreshold: calculating connectivity for given powers...
##    ..working on genes 1 through 5000 of 5000
##    Power SFT.R.sq slope truncated.R.sq  mean.k. median.k.  max.k.
## 1      1   0.1310 32.20          0.974 2.50e+03  2.50e+03 2580.00
## 2      2   0.0456 -5.56          0.951 1.34e+03  1.33e+03 1470.00
## 3      3   0.2070 -5.36          0.962 7.52e+02  7.47e+02  931.00
## 4      4   0.3250 -4.12          0.966 4.43e+02  4.36e+02  627.00
## 5      5   0.3900 -3.25          0.960 2.71e+02  2.64e+02  443.00
## 6      6   0.4500 -2.67          0.962 1.72e+02  1.64e+02  324.00
## 7      7   0.5190 -2.36          0.966 1.12e+02  1.05e+02  244.00
## 8      8   0.5990 -2.33          0.975 7.56e+01  6.88e+01  191.00
## 9      9   0.6550 -2.37          0.977 5.21e+01  4.59e+01  153.00
## 10    10   0.7020 -2.35          0.980 3.67e+01  3.12e+01  124.00
## 11    12   0.7560 -2.39          0.983 1.92e+01  1.51e+01   85.80
## 12    14   0.7910 -2.47          0.982 1.08e+01  7.73e+00   61.50
## 13    16   0.8010 -2.47          0.965 6.33e+00  4.13e+00   45.40
## 14    18   0.8090 -2.55          0.977 3.89e+00  2.27e+00   34.20
## 15    20   0.8280 -2.47          0.982 2.48e+00  1.28e+00   26.30
## 16    22   0.8380 -2.42          0.975 1.63e+00  7.48e-01   20.50
## 17    24   0.8280 -2.41          0.948 1.10e+00  4.44e-01   16.20
## 18    26   0.8410 -2.35          0.949 7.63e-01  2.70e-01   13.00
## 19    28   0.8500 -2.29          0.947 5.39e-01  1.66e-01   10.50
## 20    30   0.3950 -2.99          0.321 3.87e-01  1.04e-01    8.52
## 21    32   0.3950 -2.91          0.321 2.83e-01  6.61e-02    6.99
## 22    34   0.4190 -2.90          0.426 2.10e-01  4.29e-02    5.78
## 23    36   0.4150 -2.82          0.407 1.58e-01  2.79e-02    4.81
## 24    38   0.9330 -1.95          0.994 1.20e-01  1.83e-02    4.03
## 25    40   0.9280 -1.90          0.962 9.27e-02  1.23e-02    3.39
## 26    42   0.9340 -1.88          0.956 7.23e-02  8.30e-03    3.11
## 27    44   0.9460 -1.86          0.957 5.69e-02  5.56e-03    2.92
## 28    46   0.9470 -1.85          0.950 4.52e-02  3.73e-03    2.74
## 29    48   0.9400 -1.82          0.932 3.63e-02  2.54e-03    2.58
## 30    50   0.4160 -2.32          0.276 2.94e-02  1.75e-03    2.43
```

N.B. unsigned -> nodes with positive & negative correlation are treated equally

```
sft.data <- sft$fitIndices
```

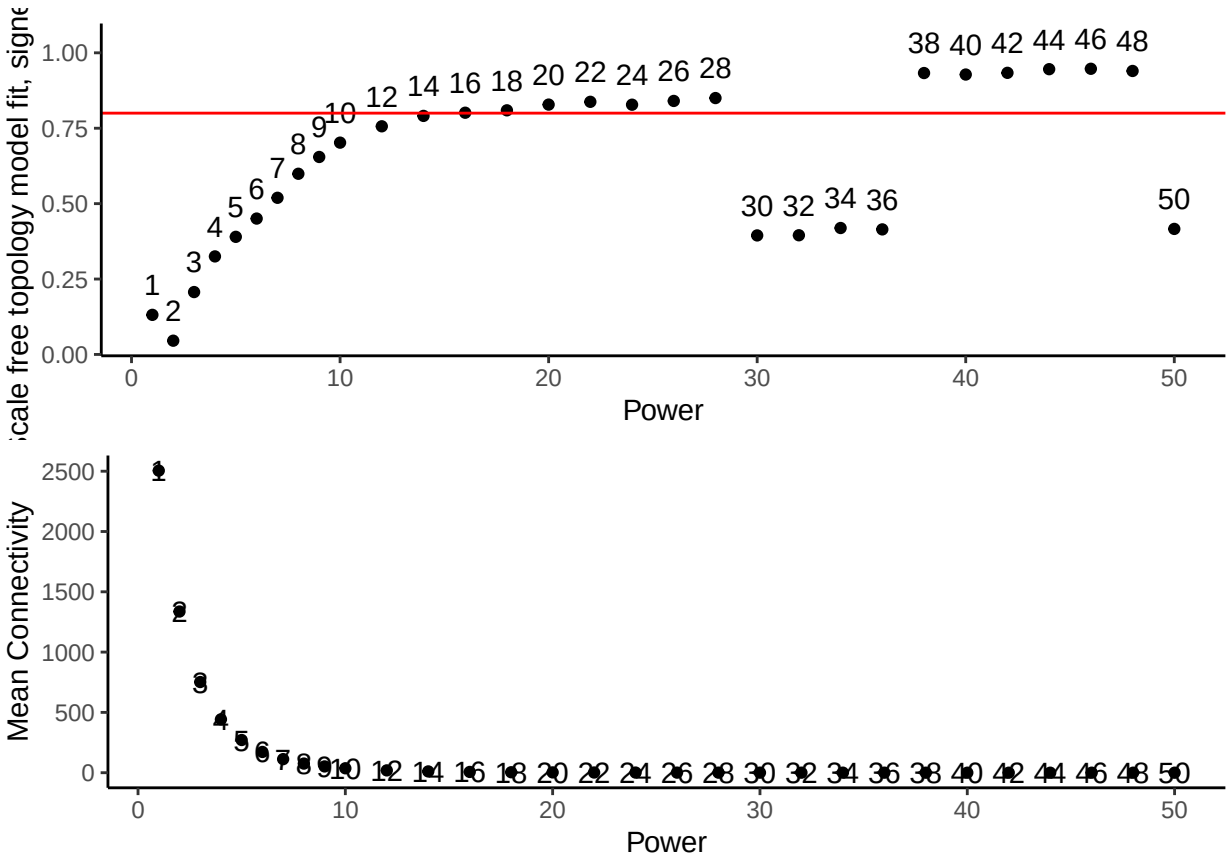
N.B. signed -> nodes with negative correlation are considered *unconnected*, treated as zero

visualization to pick power

```
Plot1 <- ggplot(sft.data, aes(Power, SFT.R.sq, label = Power)) +
  geom_point() +
  geom_text(nudge_y = 0.1) +
  geom_hline(yintercept = 0.8, color = 'red') +
  labs(x = 'Power', y = 'Scale free topology model fit, signed R^2') +
  theme_classic()


Plot2 <- ggplot(sft.data, aes(Power, mean.k., label = Power)) +
  geom_point() +
  geom_text(nudge_y = 0.1) +
  labs(x = 'Power', y = 'Mean Connectivity') +
  theme_classic()


grid.arrange(Plot1, Plot2, nrow = 2)
```



Convert matrix to numeric and assign power

```
norm.counts[] <- sapply(norm.counts, as.numeric)
soft_power <- 14
```

```
temp_cor <- cor
cor <- WGCNA::cor
```

Estimate w.r.t blocksize memory depends on available ram of PC

```
bwnet <- blockwiseModules(norm.counts,
                          maxBlockSize = 5000,
                          TOMType = "signed",
                          power = soft_power,
                          mergeCutHeight = 0.25,
                          numericLabels = FALSE,
                          randomSeed = 1234,
                          verbose = 3)
```

```
##  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 not use multithreading.
##      Fraction of slow calculations: 0.000000
##     ..connectivity..
##     ..matrix multiplication (system BLAS)..
##     ..normalization..
##     ..done.
##  ....clustering..
##  ....detecting modules..
##  ....calculating module eigengenes..
##  ....checking kME in modules..
##   ..reassigning 1 genes from module 3 to modules with higher KME.
##  ..merging modules that are too close..
##      mergeCloseModules: Merging modules whose distance is less than 0.25
##        Calculating new MEs...
```

```
cor <- temp_cor
```

6. Identify Module Eigengenes

```
module_eigengenes <- bwnet$MEs
head(module_eigengenes)
```

```
##         MEturquoise     MEbrown      MEgreen     MEblack        MEred
## Sample1 -0.10375879  0.21685398  0.020542538 -0.09222291 -0.100192258
## Sample2 -0.01255866  0.12839304  0.032163029 -0.07031597 -0.221029610
## Sample3  0.04519388  0.03717920  0.066635498 -0.05908388  0.017316170
## Sample4  0.06835863 -0.08730094 -0.003943952 -0.06046904  0.074580135
## Sample6  0.15559583 -0.04275675  0.087524806 -0.07252025  0.041854527
## Sample7  0.05497992  0.13879174 -0.144092037 -0.04763668 -0.004967255
```

```
##               MEblue     MEyellow        MEgrey
## Sample1 -0.06997022 -0.167852659 -0.0823958709
## Sample2 -0.12854895 -0.149656308  0.0066448007
## Sample3 -0.02317673 -0.073541884 -0.0006775664
## Sample4 -0.06173939 -0.047079887  0.0677114636
## Sample6 -0.14128791 -0.004358101  0.1408660417
## Sample7 -0.13322219 -0.090824750  0.0951961513
```

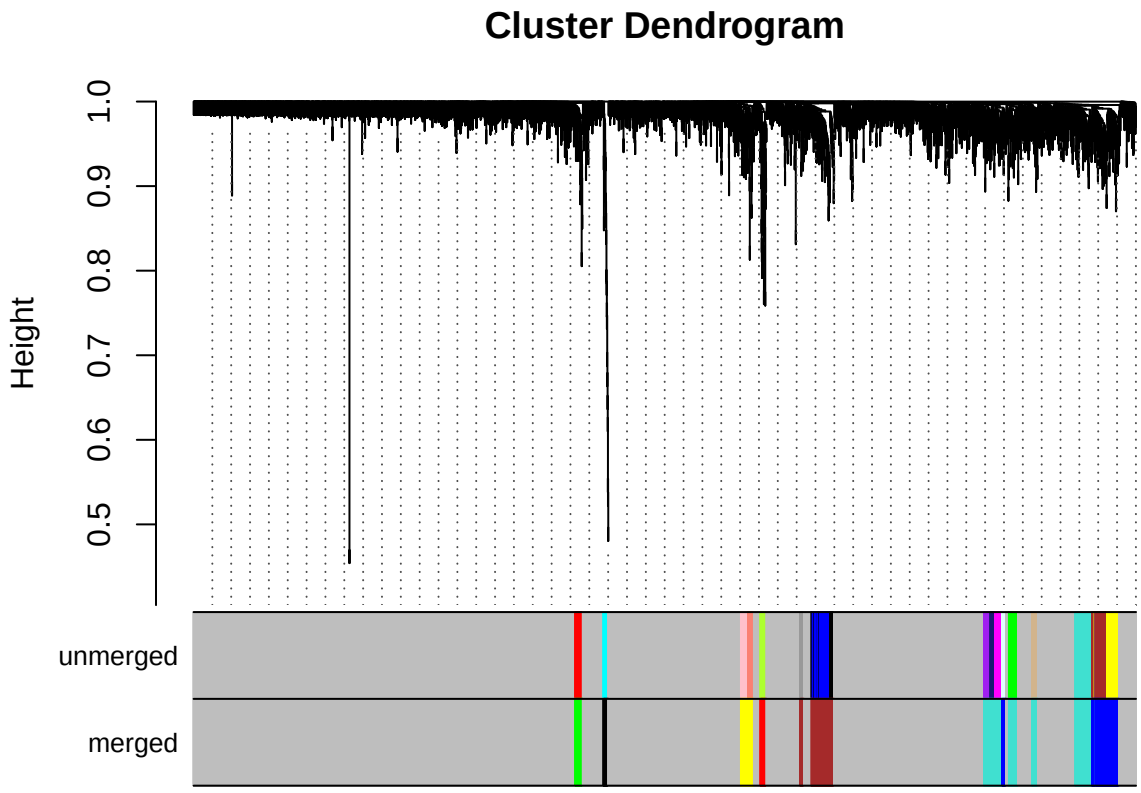
Check the number of genes for each module

```
table(bwnet$colors)
```

```
##
##     black      blue     brown     green      grey       red turquoise    yellow
##        26       164       141        39      4262        32       267        69
```

Plot the dendrogram and the module colors before and after merging underneath

```
plotDendroAndColors(bwnet$dendrograms[[1]], cbind(bwnet$unmergedColors, bwnet$colors),
                    c("unmerged", "merged"),
                    dendroLabels = FALSE,
                    addGuide = TRUE,
                    hang= 0.03,
                    guideHang = 0.05)
```



Grey module = all genes that doesn't fall into other modules were assigned to the grey module

7. Extract genes and their associated color modules and save them

```
mergedColors<- labels2colors(bwnet$colors)
module_df <- data.frame(
  gene_id <- names(bwnet$colors),
  colors <- labels2colors(bwnet$colors)
)

module_df[1:5,]
```

```
##   gene_id....names.bwnet.colors. colors....labels2colors.bwnet.colors.
## 1                ENSG00000000419                                  green
## 2                ENSG00000000457                                  green
## 3                ENSG00000000460                                  green
## 4                ENSG00000000938                                  green
## 5                ENSG00000001036                                  green
```

```
write_delim(module_df,
            file = "gene_modules.txt",
            delim = "\t")
```

8. Merging and clustering modules from eigengenes

Calculate eigengenes

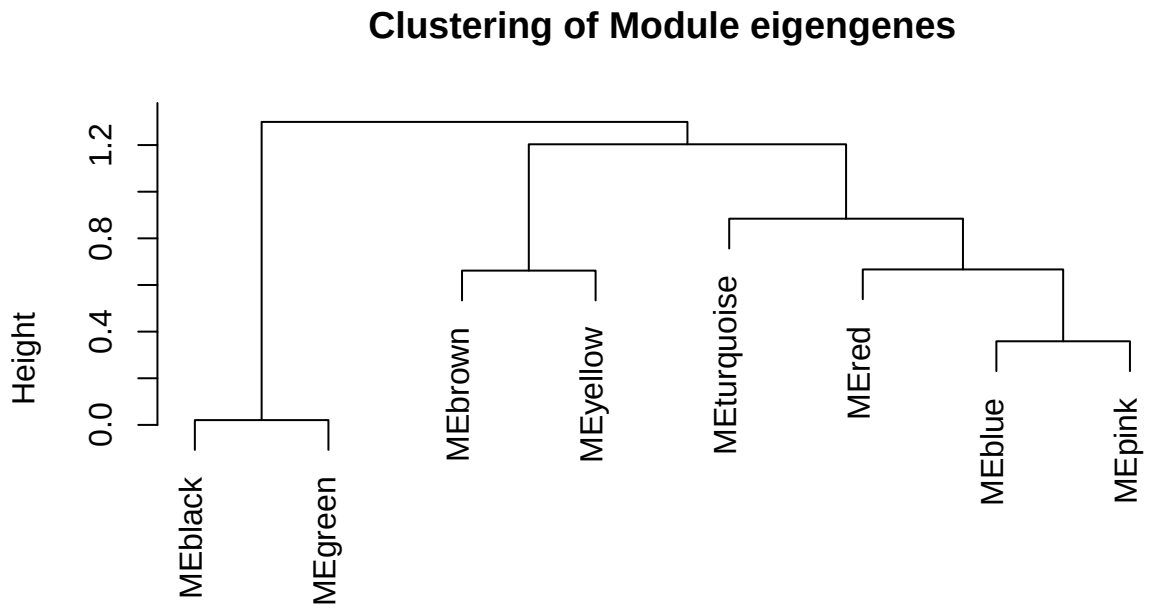
```
MEList <- moduleEigengenes(norm.counts, colors = mergedColors)
MEs <- MEList$eigengenes
```

Calculate dissimilarity of module eigengenes

```
MEDiss <- 1-cor(MEs)
METree <- hclust(as.dist(MEDiss), method = "average")
```

Plot the result

```
plot(METree, main = "Clustering of Module eigengenes",
     xlab = "", sub = "")
```



9. Show the correlation between modules and each sample

Get Module Eigengenes per cluster

```
MEs0 <- moduleEigengenes(norm.counts, mergedColors)$eigengenes
```

Reorder modules so similar modules are next to each other

```
MEs0 <- orderMEs(MEs0)
module_order <- names(MEs0) %>% gsub("ME","", .)
```

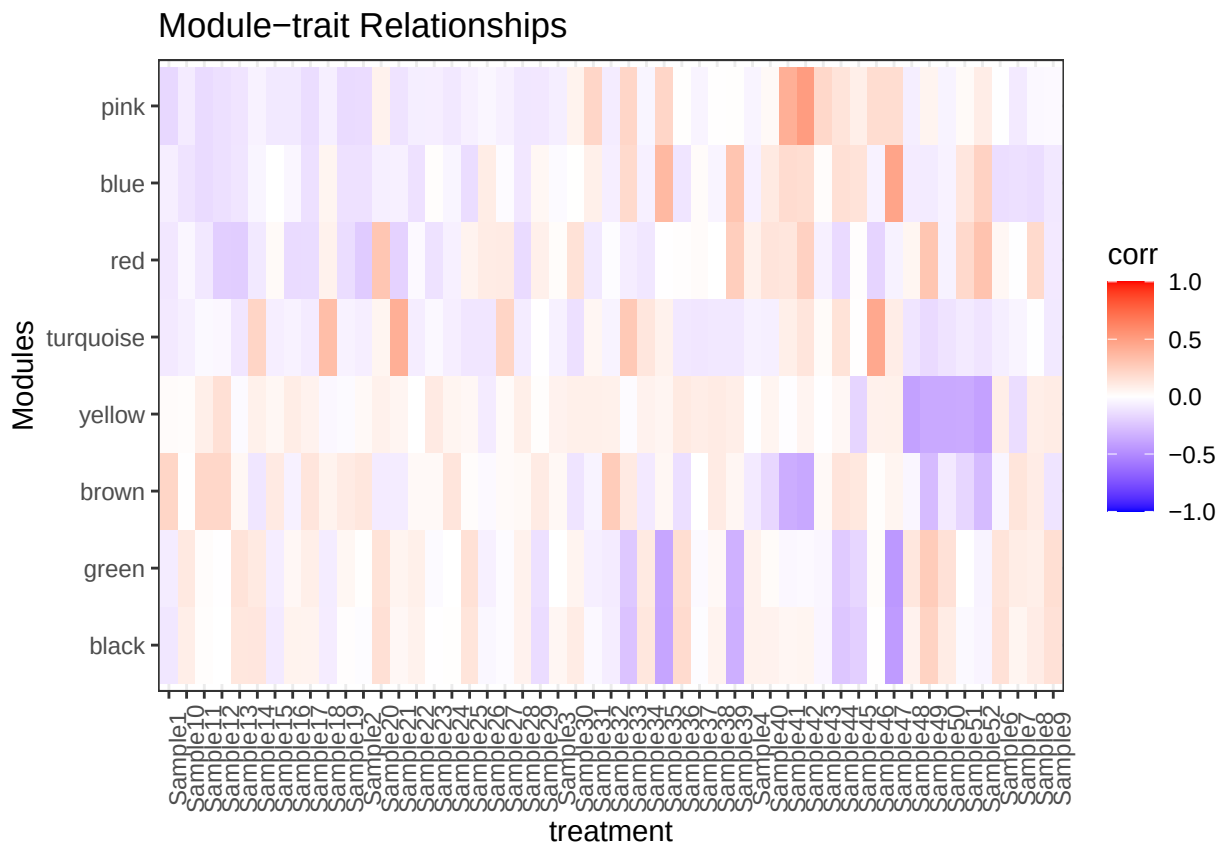
Add the treatment names

```
MEs0$treatment <- row.names(MEs0)
```

Tidy & plot data

```
mME = MEs0 %>%
  pivot_longer(-treatment) %>%
  mutate(
    name = gsub("ME", "", name),
    name = factor(name, levels = module_order)
  )

mME %>% ggplot(., aes(x=treatment, y=name, fill=value)) +
  geom_tile() +
  theme_bw() +
  scale_fill_gradient2(
    low = "blue",
    high = "red",
    mid = "white",
    midpoint = 0,
    limit = c(-1,1)) +
  theme(axis.text.x = element_text(angle=90)) +
  labs(title = "Module-trait Relationships", y = "Modules", fill="corr")
```



10. Showing gene expression level in specific modules

```
modules_of_interest = c("green", "turquoise", "red")
```

Pull out list of genes in that module

```
submod = module_df %>%
  subset(colors %in% modules_of_interest)

row.names(module_df) = module_df$gene_id
```

Get normalized expression for those genes

```
norm.counts[1:5,1:10]
```

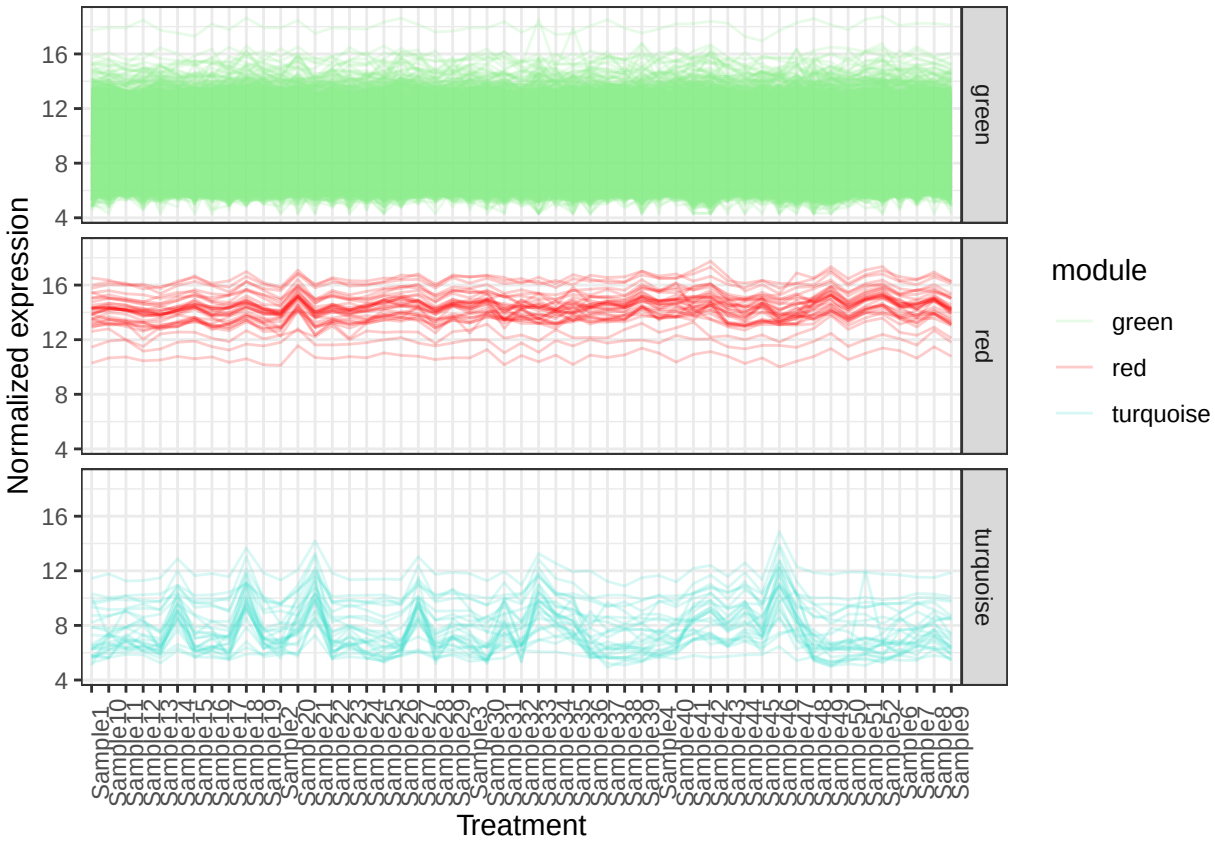
```
##         ENSG00000000419 ENSG00000000457 ENSG00000000460 ENSG00000000938
## Sample1        7.817808        9.010102        8.278295        13.14962
## Sample2        7.860089        9.828239        8.520372        13.56801
## Sample3        8.152240        8.428289        8.494442        11.96361
```

```
## Sample4        8.430191        9.276900        8.532905        12.65407
## Sample6        8.316706        8.996154        8.293994        12.79312
##         ENSG00000001036 ENSG00000001084 ENSG00000001167 ENSG00000001460
## Sample1        7.925833        9.816597        9.853574        7.460652
## Sample2        8.382598        9.414906        9.628366        7.404398
## Sample3        8.657241        9.982769        9.601348        6.924517
## Sample4        8.300237       10.903511       10.299930        7.517321
## Sample6        8.890079       10.308196        9.615493        7.567784
##         ENSG00000001461 ENSG00000001497
## Sample1        9.763363       10.061901
## Sample2       10.019682        9.610658
## Sample3        9.858863       10.066467
## Sample4        9.777715       10.017410
## Sample6        9.932600       10.083488
```

```r
norm.exprs<- data.frame(t(norm.counts))
norm.exprs$gene_id<- row.names(norm.exprs)
norm.exprs<- norm.exprs %>% select(gene_id,everything())
subexpr <- norm.exprs[submod$gene_id,]

submod_df <- data.frame(subexpr) %>%
  mutate(
    gene_id = row.names(.)
  ) %>%
  pivot_longer(-gene_id) %>%
  mutate(
    module = module_df[gene_id,]$colors
  )

submod_df %>% ggplot(., aes(x=name, y=value, group=gene_id, colour=module)) +
  geom_line(alpha=0.2) +
  theme_bw() +
  theme(
    axis.text.x = element_text(angle = 90)
  ) +
  facet_grid(rows = vars(module)) +
  labs(x = "Treatment",
       y = "Normalized expression") +
  scale_color_manual(values = c("lightgreen","red","turquoise"))
```



11. Finding the association between module and traits ### Prepare and manipulate data for association analysis ### Converting categorical variable into binary group variables

```
type <- Sample_info_final %>%
        mutate(type = ifelse(grepl('Lung cancer', Group), 1, 0)) %>%
        select(4)
```

```
Sample_info_final$Status <- factor(Sample_info_final$Status,
                                   levels = c("None", "Newly diagnosed",
                                   "Post Chemotherapy", "On Doxorubicin"))
```

```
Status<- binarizeCategoricalColumns(Sample_info_final$Status,
                                    includePairwise = FALSE,
                                    includeLevelVsAll = TRUE,
                                    minCount = 1)


Status.final<- cbind(type, Status)
```

Define numbers of genes and samples and draw correlation

```
nSamples <- nrow(norm.counts)
nGenes <- ncol(norm.counts)
status.module.corr <- cor(module_eigengenes, Status.final, use = 'p')
```

```
status.module.corr.pvals <- corPvalueStudent(status.module.corr , nSamples)
nrow(module_eigengenes)
```

```
## [1] 51
```

Visualize module-trait association as a heatmap

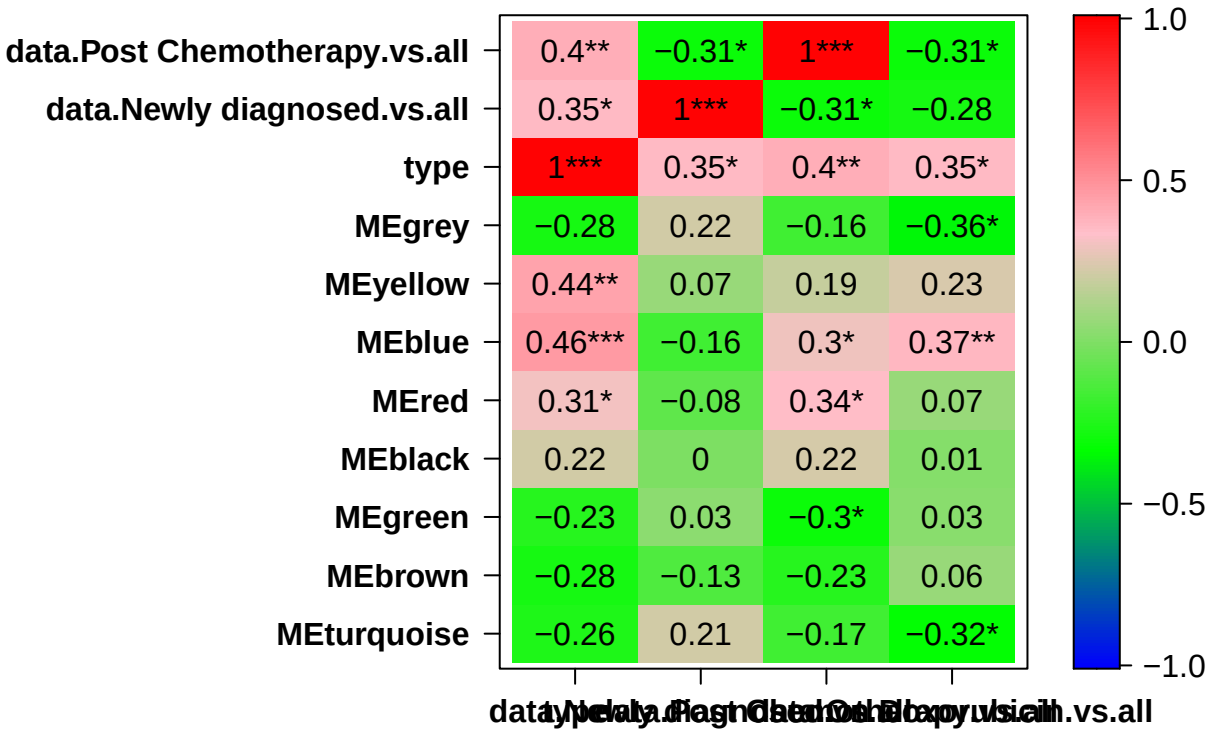
```
heatmap.data <- merge(module_eigengenes, Status.final, by = 'row.names')

head(heatmap.data)
```

```
##   Row.names   MEturquoise     MEbrown     MEgreen     MEblack       MEred
## 1   Sample1 -0.1037587873  0.21685398  0.02054254 -0.09222291 -0.10019226
## 2  Sample10  0.0895341492  0.00293838  0.01367637 -0.06928702 -0.03836467
## 3  Sample11  0.0099912350  0.20925609  0.08402187 -0.02660053 -0.09621342
## 4  Sample12  0.0006960986  0.20967381  0.16100147 -0.03369510 -0.20749830
## 5  Sample13  0.1272433032  0.04066019 -0.02093506 -0.10491016 -0.21409801
## 6  Sample14  0.1288343520 -0.10802289  0.07607466  0.22150429 -0.09486251
##        MEblue    MEyellow       MEgrey type data.Newly diagnosed.vs.all
## 1 -0.06997022 -0.16785266 -0.082395871    0                           0
## 2 -0.11979305 -0.08319146  0.116674013    0                           0
## 3 -0.15188447 -0.15462433  0.014037255    0                           0
## 4 -0.13145467 -0.13190268 -0.003316252    0                           0
## 5 -0.10896400 -0.11249850  0.148243795    0                           0
## 6 -0.04248159 -0.05396714  0.110840769    0                           0
##   data.Post Chemotherapy.vs.all data.On Doxorubicin.vs.all
## 1                             0                         0
## 2                             0                         0
## 3                             0                         0
## 4                             0                         0
## 5                             0                         0
## 6                             0                         0
```

```
heatmap.data <- heatmap.data %>%
   column_to_rownames(var = 'Row.names')

CorLevelPlot(heatmap.data,
             x = names(heatmap.data)[9:12],
             y = names(heatmap.data)[1:11],
             col = c("blue1", "green", "pink", "red"))
```




```
module.gene.mapping <- as.data.frame(bwnet$colors)
module.gene.mapping %>%
  filter(`bwnet$colors` == 'blue') %>%
  rownames()
```

```
##   [1] "ENSG00000005483" "ENSG00000006607" "ENSG00000010072" "ENSG00000010165"
##   [5] "ENSG00000029363" "ENSG00000035141" "ENSG00000043093" "ENSG00000044574"
##   [9] "ENSG00000048649" "ENSG00000051382" "ENSG00000051620" "ENSG00000053254"
##  [13] "ENSG00000053770" "ENSG00000055070" "ENSG00000056586" "ENSG00000059769"
##  [17] "ENSG00000067064" "ENSG00000067082" "ENSG00000069345" "ENSG00000069956"
##  [21] "ENSG00000070831" "ENSG00000072415" "ENSG00000075568" "ENSG00000076053"
##  [25] "ENSG00000076248" "ENSG00000077152" "ENSG00000079332" "ENSG00000080371"
##  [29] "ENSG00000080824" "ENSG00000081320" "ENSG00000084463" "ENSG00000087074"
##  [33] "ENSG00000088682" "ENSG00000089597" "ENSG00000090432" "ENSG00000090863"
##  [37] "ENSG00000091527" "ENSG00000092094" "ENSG00000092871" "ENSG00000095002"
##  [41] "ENSG00000095370" "ENSG00000095787" "ENSG00000099246" "ENSG00000099797"
##  [45] "ENSG00000100221" "ENSG00000100354" "ENSG00000100393" "ENSG00000100395"
##  [49] "ENSG00000100401" "ENSG00000100532" "ENSG00000100603" "ENSG00000100614"
##  [53] "ENSG00000100982" "ENSG00000101166" "ENSG00000101337" "ENSG00000101367"
##  [57] "ENSG00000103061" "ENSG00000103415" "ENSG00000103429" "ENSG00000104164"
##  [61] "ENSG00000104312" "ENSG00000104889" "ENSG00000105835" "ENSG00000105856"
##  [65] "ENSG00000106245" "ENSG00000106263" "ENSG00000106615" "ENSG00000106682"
##  [69] "ENSG00000107560" "ENSG00000107897" "ENSG00000107937" "ENSG00000108061"
##  [73] "ENSG00000108106" "ENSG00000108829" "ENSG00000108960" "ENSG00000109332"
##  [77] "ENSG00000110696" "ENSG00000111802" "ENSG00000112081" "ENSG00000112118"
##  [81] "ENSG00000112149" "ENSG00000112245" "ENSG00000113163" "ENSG00000113575"
```

```
##  [85] "ENSG00000113734" "ENSG00000113811" "ENSG00000114125" "ENSG00000114796"
##  [89] "ENSG00000114988" "ENSG00000115165" "ENSG00000115520" "ENSG00000115840"
##  [93] "ENSG00000116455" "ENSG00000116731" "ENSG00000116747" "ENSG00000116752"
##  [97] "ENSG00000116830" "ENSG00000116906" "ENSG00000117036" "ENSG00000117505"
## [101] "ENSG00000117614" "ENSG00000118894" "ENSG00000119392" "ENSG00000119559"
## [105] "ENSG00000119725" "ENSG00000119801" "ENSG00000119899" "ENSG00000119929"
## [109] "ENSG00000119950" "ENSG00000119953" "ENSG00000120690" "ENSG00000120705"
## [113] "ENSG00000120709" "ENSG00000120727" "ENSG00000121578" "ENSG00000121864"
## [117] "ENSG00000122068" "ENSG00000122257" "ENSG00000122882" "ENSG00000123091"
## [121] "ENSG00000123505" "ENSG00000123728" "ENSG00000124209" "ENSG00000124380"
## [125] "ENSG00000125835" "ENSG00000126524" "ENSG00000126945" "ENSG00000128245"
## [129] "ENSG00000128590" "ENSG00000128881" "ENSG00000128989" "ENSG00000129235"
## [133] "ENSG00000129315" "ENSG00000129691" "ENSG00000130311" "ENSG00000130522"
## [137] "ENSG00000130803" "ENSG00000130844" "ENSG00000131263" "ENSG00000131323"
## [141] "ENSG00000131381" "ENSG00000132507" "ENSG00000132603" "ENSG00000132661"
## [145] "ENSG00000132823" "ENSG00000133026" "ENSG00000133606" "ENSG00000133773"
## [149] "ENSG00000134375" "ENSG00000134644" "ENSG00000134686" "ENSG00000134970"
## [153] "ENSG00000135334" "ENSG00000136527" "ENSG00000136819" "ENSG00000137075"
## [157] "ENSG00000137502" "ENSG00000137575" "ENSG00000137876" "ENSG00000137947"
## [161] "ENSG00000138032" "ENSG00000138069" "ENSG00000138166" "ENSG00000138433"
```

12. Identifying significant genes against different features of data

###Calculating the module membership and the associated p-values #### The module membership/intramodular connectivity is calculated as the correlation of the eigengene and the gene expression profile. #### This quantifies the similarity of all genes on the array to every module.

```
module.membership.measure <- cor(module_eigengenes, norm.counts, use = 'p')
module.membership.measure.pvals <- corPvalueStudent(module.membership.measure, nSamples)

module.membership.measure.pvals[1:5,1:10]
```

```
##             ENSG00000000419 ENSG00000000457 ENSG00000000460 ENSG00000000938
## MEturquoise      0.08179877    3.935824e-06    7.873861e-01      0.16687163
## MEbrown          0.01031164    3.801793e-01    1.015938e-01      0.02125898
## MEgreen          0.27024680    2.434798e-02    4.255289e-05      0.07970018
## MEblack          0.95418281    7.291502e-01    3.064485e-01      0.46636890
## MEred            0.06741831    1.364833e-01    6.076142e-02      0.03317124
##             ENSG00000001036 ENSG00000001084 ENSG00000001167 ENSG00000001460
## MEturquoise    1.066509e-05    1.211570e-02    0.1373691154      0.03960014
## MEbrown        1.158562e-02    7.932893e-01    0.0255369951      0.02068358
## MEgreen        3.976450e-02    2.249371e-07    0.0006352417      0.77101215
## MEblack        3.100530e-01    6.265870e-02    0.2118802323      0.83164735
## MEred          7.442618e-01    8.543609e-02    0.4044932254      0.89784399
##             ENSG00000001461 ENSG00000001497
## MEturquoise    2.468861e-05    0.0001716473
## MEbrown        8.252186e-01    0.7602053664
## MEgreen        4.901483e-01    0.1947561939
## MEblack        5.127292e-04    0.7311565906
## MEred          6.122777e-01    0.8786558002
```

Calculating the gene significance and associated p-values against a particular trait

```
gene.signf.corr <- cor(norm.counts, Status.final$'data.Post Chemotherapy.vs.all', use = 'p')
gene.signf.corr.pvals <- corPvalueStudent(gene.signf.corr, nSamples)
```

Sub-setting based on significant association: X1:corr, X2:pval

```
Sig_chemo<-data.frame(cbind(gene.signf.corr, gene.signf.corr.pvals))
Sig_chemo_final<- filter(Sig_chemo, Sig_chemo$X1> 0.3 | Sig_chemo$X1< -0.3
                         & Sig_chemo$X2<0.05)

write.csv(Sig_chemo_final, 'sig_genes_chemo.csv')
```