

Gene Network

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Loading

Load libraries

```
library(WGCNA)
```

```
## Loading required package: dynamicTreeCut
```

```
## Loading required package: fastcluster
```

```
##
```

```
## Attaching package: 'fastcluster'
```

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

```
##
```

```
##      hclust
```

```
##
```

```
##
```

```
## Attaching package: 'WGCNA'
```

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

```
##
```

```
##      cor
```

```
allowWGCNAThreads()
```

```
## Allowing multi-threading with up to 32 threads.
```

```
library(randomcoloR)
```

```
library(edgeR)
```

```
## Loading required package: limma
```

```
library(tidyverse)
```

```
## -- Attaching core tidyverse packages ----- tidyverse 2.0.0 --
## v dplyr      1.1.4      v readr      2.1.5
## v forcats    1.0.0      v stringr   1.5.1
## v ggplot2    3.5.1      v tibble    3.2.1
## v lubridate  1.9.4      v tidyr     1.3.1
## v purrr      1.0.4
```

```
## -- Conflicts ----- tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()     masks stats::lag()
## i Use the conflicted package (<http://conflicted.r-lib.org/>) to force all conflicts to become errors
```

```
library(dplyr)
library(grid)
library(gridExtra)
```

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

```
#devtools::install_github("kevinblighe/CorLevelPlot")
library(CorLevelPlot)
library(ggpubr)
library(plotly)
```

```
##
## Attaching package: 'plotly'
##
## The following object is masked from 'package:ggplot2':
##
##      last_plot
##
## The following object is masked from 'package:stats':
##
##      filter
##
## The following object is masked from 'package:graphics':
##
##      layout
```

```
library(factoextra)
```

```
## Welcome! Want to learn more? See two factoextra-related books at https://goo.gl/ve3WBa
```

```
library(umap)
library(heatmaply)
```

```
## Loading required package: viridis
## Loading required package: viridisLite
##
## =====
## Welcome to heatmaply version 1.5.0
##
## Type citation('heatmaply') for how to cite the package.
## Type ?heatmaply for the main documentation.
##
## The github page is: https://github.com/talgalili/heatmaply/
## Please submit your suggestions and bug-reports at: https://github.com/talgalili/heatmaply/issues
## You may ask questions at stackoverflow, use the r and heatmaply tags:
##   https://stackoverflow.com/questions/tagged/heatmaply
## =====
```

Load the data

Import the csv and metadata files

```
dataPath<-paste0("../data/wlen/data_wlen.csv")
metadataPath<-paste0("../data/metadata.txt")

dataNL<-read.delim(dataPath, row.names=1, stringsAsFactors=TRUE)
metadata<-read.delim(metadataPath, header=T, row.names=1, stringsAsFactors=TRUE)

colnames(metadata)<-c("specie", "quality", "tissue_abv", "rep", "location")
```

Data preparation

Get lengths vector

If the provided data contains the length of the genes, extract them to a vector

```
if ("Length" %in% colnames(dataNL)){
  length_vec<-dataNL$Length
}
```

Fix the data

Search for dimensional disparities

```
dim(dataNL)
```

```
## [1] 39756 566
```

```
dim(metadata)
```

```
## [1] 565 5
```

Match metadata rows to data columns

Match which samples appear in the data and metadata

```
dataMatcher<-function(data, metadata){
  options(warn=-1)

  cat("Data begins with:" , dim(data))
  cat("\nMetadata begins with:", dim(metadata))
  #Match data to metadata
  data <- data[, order(colnames(data))]
  metadata <- metadata[order(rownames(metadata)), ]

  cat("\nColumns data = Rows of metatdata?", all(rownames(metadata) == colnames(data)))
  #If TRUE, columns of data and rows of metadata are matched

  cat("\nRemove the excess from data")
  data<-data[,colnames(data) %in% rownames(metadata)] #remove data not present in metadata
  cat("\nData end with:" , dim(data))

  cat("\nRemove the excess from metadata")
  metadata<-metadata[rownames(metadata) %in% colnames(data),] #remove metadata not present in data
  cat("\nMetadata end with:" , dim(metadata), "\n")

  options(warn=0)
  return(list(data, metadata))
}

jointData<-dataMatcher(dataNL, metadata)
```

```
## Data begins with: 39756 566
## Metadata begins with: 565 5
## Columns data = Rows of metatdata? FALSE
## Remove the excess from data
## Data end with: 39756 565
## Remove the excess from metadata
## Metadata end with: 565 5
```

```
dataNL<-jointData[[1]]
metadata<-jointData[[2]]
```

Metadata levels

```
#Should be all 0 due to preprocessing filtering
levels(as.factor(metadata$quality))
```

```
## [1] "0"
```

```
#Mapped abbreviations
levels(metadata$tissue_abv)
```

```
## [1] "Cob"      "CobIm"    "CobPp"    "Coleo"    "FE1In"    "Le1"
## [7] "Le3Bl"    "Le3Sh"    "Le5"      "Le5El"    "Le5M"     "Le5Mer"
## [13] "Le8"      "LeFl"     "Msphy"    "RoCr"     "RoHaZ"    "RoPr"
## [19] "RoPrEl"   "RoPrMers" "RoSe"     "Se10"     "Se15"     "Se20"
## [25] "Se25"     "Se30"     "Se40M"    "Silk"     "TaIm"     "TaMe"
```

```
#reps 1,2,3 and 4, is there an imbalance?
table(metadata$rep)
```

```
##
## 1 2 3 4
## 145 144 139 137
```

```
levels(metadata$location)
```

```
## [1] "cob"                "coleoptile"
## [3] "crown root"         "first elongated internode"
## [5] "flag leaf"          "immature cob"
## [7] "immature tassel"    "Leaf 1"
## [9] "Leaf 3 blade"       "Leaf 3 sheath"
## [11] "Leaf 5"             "Leaf 5 elongation zone"
## [13] "Leaf 5 mature"      "Leaf 5 meristem"
## [15] "Leaf 8"             "mature seed 40 dap"
## [17] "meotic tassel"      "mesophyll"
## [19] "prepollinated cob"  "primary root"
## [21] "primary root elongation zone" "primary root meristematic zone"
## [23] "root hair zone"     "Seed 10 dap"
## [25] "Seed 15 dap"        "Seed 20 dap"
## [27] "Seed 25 dap"        "Seed 30 dap"
## [29] "seminal root"       "silk"
```

```
#Different total amounts of each tissue replicate would indicate that one of the species does not present
table(metadata$location)
```

```
##
##                cob                coleoptile
##                20                20
##      crown root  first elongated internode
##                20                20
##      flag leaf   immature cob
##                20                19
##      immature tassel      Leaf 1
##                18                20
##      Leaf 3 blade      Leaf 3 sheath
##                20                20
##      Leaf 5           Leaf 5 elongation zone
```

```
##                20                20
##          Leaf 5 mature          Leaf 5 meristem
##                20                20
##          Leaf 8          mature seed 40 dap
##                20                14
##          meotic tassel          mesophyll
##                19                20
##          prepollinated cob          primary root
##                18                20
## primary root elongation zone primary root meristematic zone
##                20                20
##          root hair zone          Seed 10 dap
##                20                15
##          Seed 15 dap          Seed 20 dap
##                16                17
##          Seed 25 dap          Seed 30 dap
##                15                15
##          seminal root          silk
##                20                19
```

```
#there are different total numbers of tissue replicates
#Solve it by using 1 replicate per tissue (mean of existing replicates)
```

Outlier check

Check for outlier genes

```
outDetect<-goodSamplesGenes(t(dataNL))
```

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

```
table(outDetect$goodGenes) #False genes are outliers
```

```
##
## FALSE  TRUE
##   171 39585
```

```
table(outDetect$goodSamples) #All samples are True = no outliers
```

```
##
## TRUE
##   565
```

```
dataNL<-dataNL[outDetect$goodGenes==TRUE,] #remove outliers
```

```
if (exists("length_vec")){ #only if it exists
  length_vec<-length_vec[outDetect$goodGenes==TRUE] #if length_vec exists remove outliers from there as
}
```

Replicate joining

```
#add the name of the species to the replicate, to be able to differentiate it
```

```
#save location
```

```
metadata$org_location<-as.factor(metadata$location)
```

```
#create specialized location
```

```
metadata$location<-paste(metadata$specie, metadata$location, sep="_")
```

```
metadata$location<-as.factor(metadata$location)
```

```
rsumer<-function(data, metadata, tissue_name){ #calculates the mean of all columns that belong to a location
```

```
  loc_mdata<-metadata[metadata$location == tissue_name, ] #filter metadata tissue (get metadata of location)
```

```
  data<-data[,colnames(data) %in% rownames(loc_mdata)] #get data of location only, based on metadata
```

```
  if (1<ncol(data.frame(data))){ #If there's only 1 replicate, don't try to do the mean (it gives error)
```

```
    data<-rowMeans(data) #calculate mean for each gene out of the locations(replicates)
```

```
  }
```

```
  return(as.data.frame(data))
```

```
}
```

```
tissue_data<-levels(metadata$location) #get list of tissue names
```

```
d_joint<-sapply(tissue_data, function(tissue_name) rsumer(dataNL, metadata, tissue_name)) #returns an array
```

```
repl_data<-as.data.frame(d_joint) #data joint by replicate
```

```
colnames(repl_data) = gsub(pattern = "*.data", replacement = "", x = tolower(colnames(repl_data))) #get
```

```
rownames(repl_data)<-rownames(dataNL) #rename rows to be genes again
```

Create replicate metadata

```
#Create a column for the location+species
```

```
repl_meta<-as.data.frame(colnames(repl_data))
```

```
colnames(repl_meta)<-c("location")
```

```
#Split the created column and add 2 columns to repl_meta, one for the species and one for the tissue
```

```
temp_meta<-data.frame(t(data.frame(strsplit(as.character(repl_meta$location), "_"))))
```

```
repl_meta<-data.frame(repl_meta$location, temp_meta$X1, temp_meta$X2)
```

```
colnames(repl_meta)<-c("location", "species", "org_location")
```

Data diagnostic

Plot the raw data

Create the plot functions and plot information

```

multiColHist<-function(data, location, color_var){ #gets the data table, the concrete column(tissue) and
  loc_var<-data[[location]] #get the tissue data
  p<-ggplot(data, aes(x=loc_var))+
    geom_histogram(bins = 30, fill=color_var, color="black")+
    xlab("")+ylab("")+theme_minimal() #plot a colored histogram for all genes of X tissue
  return(p)
}

#Create a vector to select 2 examples of each line
repl_selection<-seq(1,ncol(repl_data), by=round(ncol(repl_data)/10))

colorList<-distinctColorPalette(length(repl_selection)) #make a list with a color for each tissue

#Create a legend that realates each color to a tissue

#use the repl_meta dataframe for creating the plot, as it contains all tissue(location) names
legend_plot<-ggplot(repl_meta[repl_selection,], aes(x=1, y=location, color=location))+
  geom_point()+
  scale_color_manual(values=colorList)+
  guides(color=guide_legend(ncol=1))+
  theme_void()+labs(color="Tissue")+
  theme(legend.title=element_text(size=18),
        legend.text=element_text(size=15)) #plot used only to get the legend that associates colors with tissues

legend_var<-get_legend(legend_plot) #place the legend into a variable

```

Create the plot list

```

RawHistList<-list() #to store the plots

for (i in 1:nrow(repl_meta[repl_selection,])){
  color_var<-colorList[i]
  loc<-colnames(repl_data)[i]

  temp_plot<-multiColHist(repl_data, loc, color_var)
  RawHistList[[i]]<-temp_plot
} #iterates over each tissue, creates a plot with a distinct color for it and stores it in a list

```

Joins the plot and legend to create a plot of the total raw data distribution

```

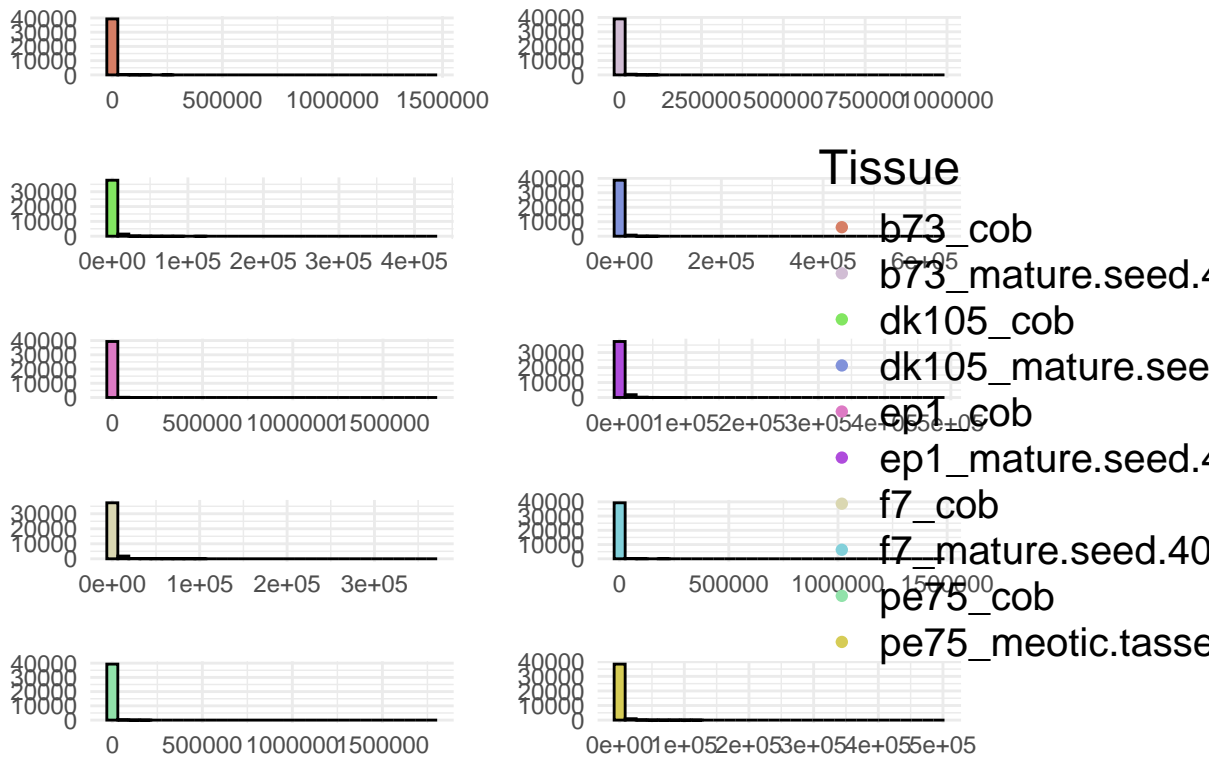
plotTitle<-paste0("Raw data distribution")

raw_Ptab<-arrangeGrob(grobs=RawHistList, ncol=ceiling(nrow(repl_meta[repl_selection,])/6)) #creates a table of plots

#png("./DistrPlots/Raw_distPlot.png", width=1600, height=800) #Raw_distPlot.png
grid.arrange(raw_Ptab, legend_var, widths = c(10, 2.3), ncol=2, top=textGrob(plotTitle, gp=gpar(fontsize=14)))

```


Raw data distribution



```
#dev.off()
```

Normalization

We can't use VST methods as we have 1 replicate of each type, it would be unreliable, we will use cpm and rpkm

CPM

```
#edgeR object
dge<-DGEList(repl_data)

#Calculate normalization factors
dge<-calcNormFactors(dge)
#Get normalized counts
Nrepl_data<-cpm(dge, log=TRUE)

NormType<-"CPM"
```

```
#Filter low expression genes
keep<-apply(Nrepl_data, 1, max)>=0 #keep genes where the counts for at least one replicate are of at le
Nrepl_data<-Nrepl_data[keep,]

#Nrepl to data frame
Nrepl_data<-as.data.frame(Nrepl_data) #eventually transpose
```

Low expression removal

Plot CPM normalized data Create the plot list

```
NormHistList<-list() #to store the plots

for (i in 1:nrow(repl_meta[repl_selection,])){
  color_var<-colorList[i]
  loc<-colnames(repl_data)[i]

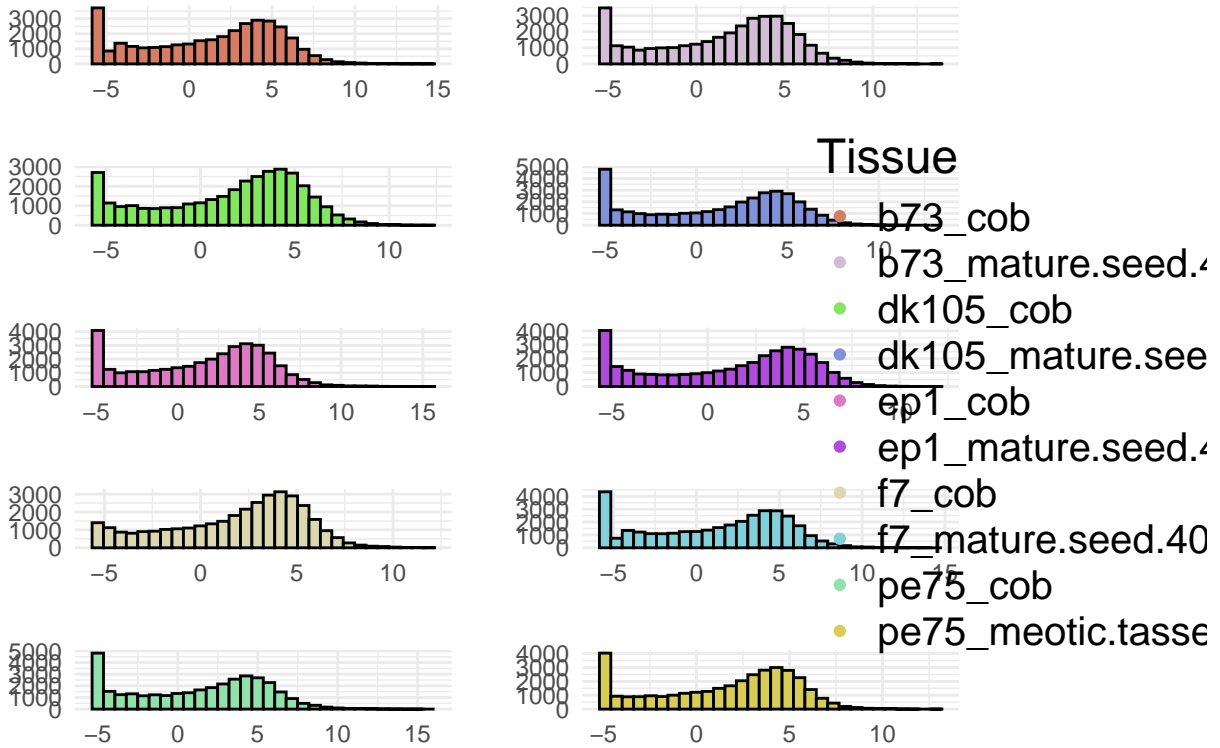
  temp_plot<-multiColHist(Nrepl_data, loc, color_var)
  NormHistList[[i]]<-temp_plot
} #iterates over each tissue, creates a plot with a distinct color for it and stores it in a list
```

Joins the plot and legend to create a plot of the total normalized data distribution

```
plotTitle<-paste0("Normalized data distribution using ", NormType)

norm_Ptab<-arrangeGrob(grobs=NormHistList, ncol=ceiling(nrow(repl_meta[repl_selection,])/6)) #creates a
#png(paste0("./DistrPlots/norm", NormType, "_distPlot.png"), width=1600, height=800) #normCPM_distPlot
grid.arrange(norm_Ptab, legend_var, widths = c(10, 2.3), ncol=2, top=textGrob(plotTitle, gp=gpar(fontsi
```

Normalized data distribution using CPM



```
#dev.off()
```

RPKM

Only if we have the gene lengths

```
if (exists("length_vec")){ #if we have lengths
  length_vec<-data.frame(Length=length_vec) #convert to dataframe

  dge <- DGEList(repl_data,genes=length_vec) #use edgeR for normalization

  dge <- calcNormFactors(dge)
  Nrepl_data <- rpkm(dge, log=TRUE)

  Nrepl_data<-as.data.frame(Nrepl_data)

  NormType<-"RPKM"
}
```

```
#Filter low expression genes
keep<-apply(Nrepl_data, 1, max)>=0 #keep genes where the counts for at least one replicate are of at least 1
Nrepl_data<-Nrepl_data[keep,]
```

```
#Nrepl to data frame
Nrepl_data<-as.data.frame(Nrepl_data) #eventually transpose

#More genes kept after normalization and filtering than cpm
```

Low expression removal

Plot RPKM normalized data Create the plot list

```
NormHistList<-list() #to store the plots

for (i in 1:nrow(repl_meta[repl_selection,])){
  color_var<-colorList[i]
  loc<-colnames(repl_data)[i]

  temp_plot<-multiColHist(Nrepl_data, loc, color_var)
  NormHistList[[i]]<-temp_plot
} #iterates over each tissue, creates a plot with a distinct color for it and stores it in a list
```

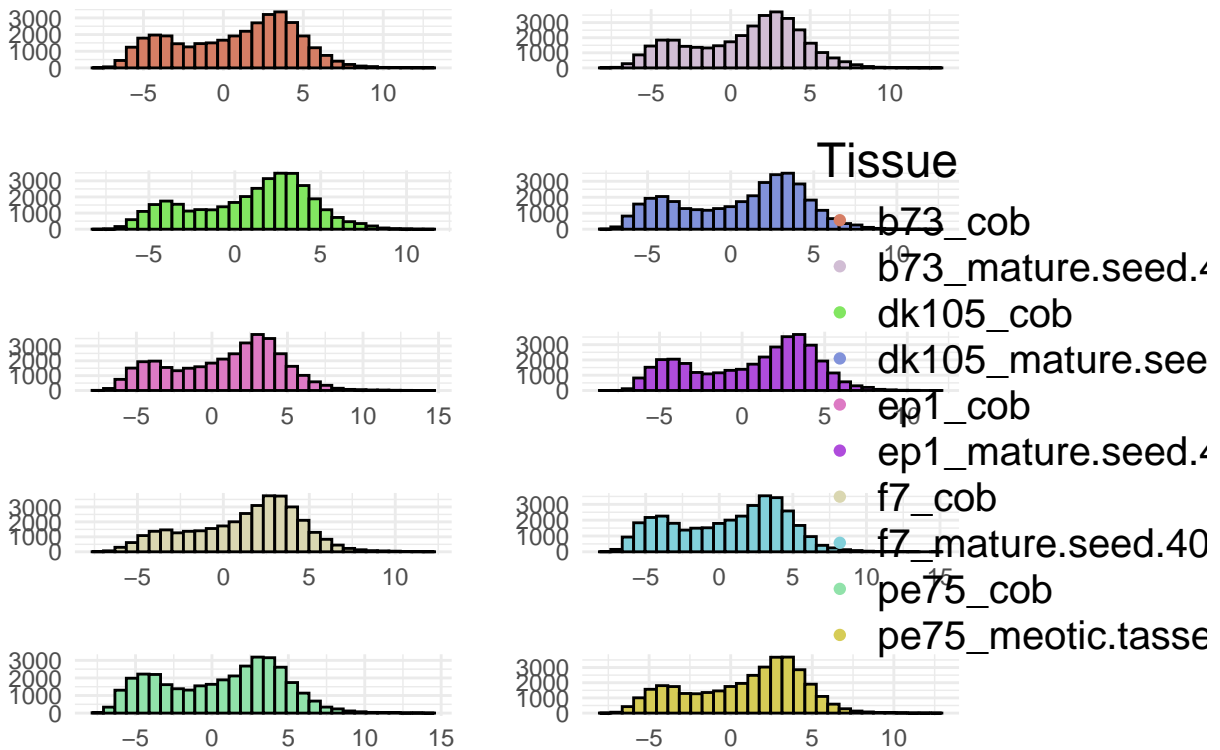
Joins the plot and legend to create a plot of the total normalized data distribution

```
plotTitle<-paste0("Normalized data distribution using ", NormType)

norm_Ptab<-arrangeGrob(grobs=NormHistList, ncol=ceiling(nrow(repl_meta[repl_selection,])/6)) #creates a

#png(paste0("./DistrPlots/norm", NormType, "_distPlot.png"), width=1600, height=800) #normRPKM_distPlot
grid.arrange(norm_Ptab, legend_var, widths = c(10, 2.3), ncol=2, top=textGrob(plotTitle, gp=gpar(fonts
```

Normalized data distribution using RPKM



```
#dev.off()
```

Expression data analysis

PCA

```
#set genes as columns and replicates by species as rows
repl_dvData<-as.data.frame(t(repl_data))
Nrepl_dvData<-as.data.frame(t(Nrepl_data))
```

Run a PCA

```
set.seed(42)
```

```
#Normalized
```

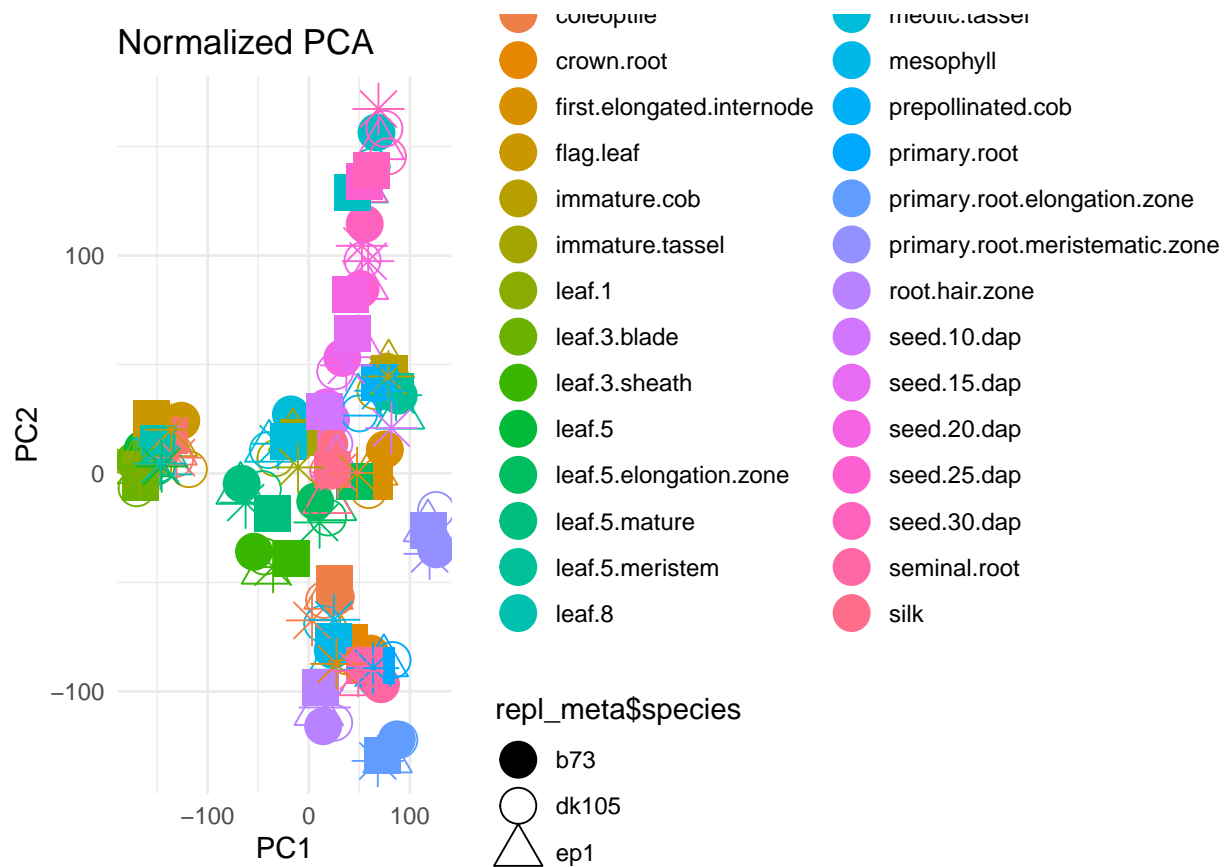
```
Nrepl_dvData_sc<-scale(Nrepl_dvData)
```

```
N_pca<-prcomp(Nrepl_dvData_sc, scale=T)
```

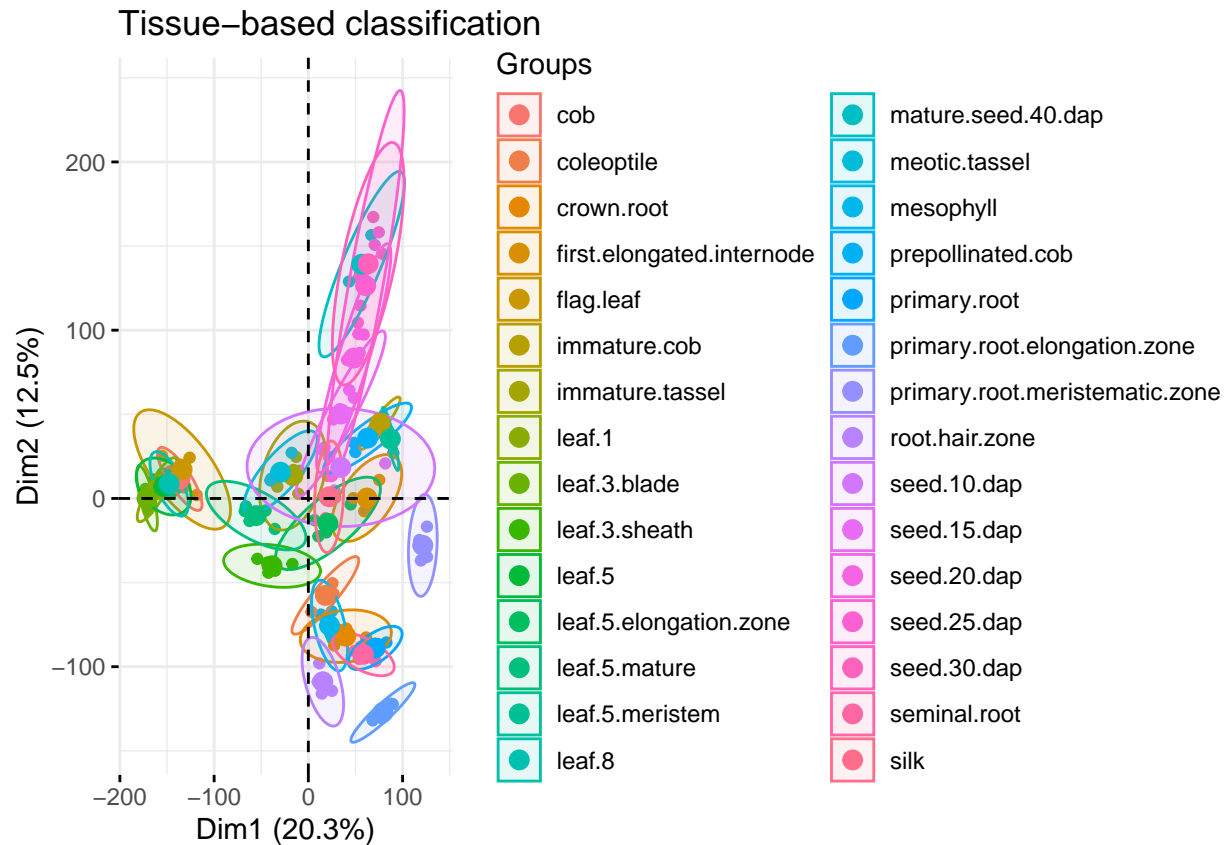
```
#Normalized
```

```
ggplot(N_pca$x, aes(x=PC1, y=PC2, color=repl_meta$org_location, shape=repl_meta$species))+
  geom_point(size=6)+
```

```
scale_shape_manual(values = c(19, 1, 2, 15, 8))+
#scale_size_manual(values = c(2, 6))+
#scale_color_brewer(palette="Dark2")+
labs(title="Normalized PCA")+
theme_minimal()
```



```
#Tissue clusters
fviz_pca_ind(N_pca, geom.ind = "point",
             habillage = repl_meta$org_location, addEllipses = TRUE, pointshape = 19,
             title="Tissue-based classification")
```



Umap

Create a function to streamline the plotting of UMAP

```
dataPlotter<-function(umap_data, metadata){
  plt<-ggplot(as.data.frame(umap_data$layout), aes(x=umap_data$layout[,1], y=umap_data$layout[,2], shape=
    geom_point(size=6)+
    scale_shape_manual(values = c(19, 1, 2, 15, 8))+
    labs(x="x", y="y", shape="Species", color="Tissue")+
    theme_minimal()
  return(plt)
}
```

Normalized UMAP

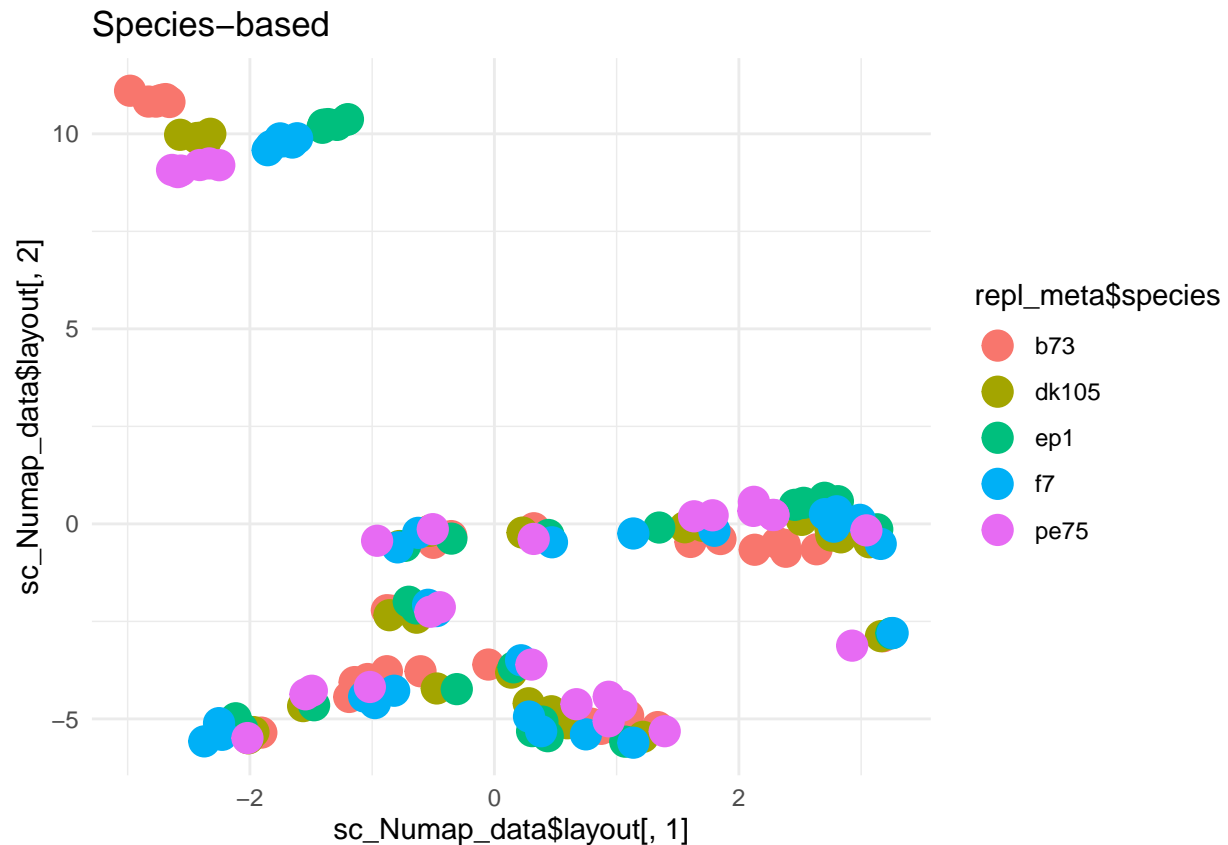
```
sc_Numap_data<-umap(Nrepl_dvData_sc)
ggplotly(dataPlotter(sc_Numap_data, repl_meta)+ggtitle("Normalized-scaled data Umap"))
```

PhantomJS not found. You can install it with `webshot::install_phantomjs()`. If it is installed, please

Alternatives for visualizing only 1 characteristic at a time

```
ggplotly(ggplot(as.data.frame(sc_Numap_data$layout), aes(x=sc_Numap_data$layout[,1], y=sc_Numap_data$layout[,2]),
  geom_point(size=5)+
  scale_shape_manual(values = c(19, 1, 2, 15, 8))+
  theme_minimal()+ggtitle("Tissue-based"))
```

```
ggplot(as.data.frame(sc_Numap_data$layout), aes(x=sc_Numap_data$layout[,1], y=sc_Numap_data$layout[,2]),
  geom_point(size=5)+
  scale_shape_manual(values = c(19, 1, 2, 15, 8))+
  theme_minimal()+ggtitle("Species-based"))
```



Final UMAP

```
FinalUmap<-umap(Nrepl_dvData_sc, n_neighbors=29, n_epochs=450, min_dist=0.1) #nneighbor:22, 29, 35
ggplotly(dataPlotter(FinalUmap, repl_meta)+ggtitle("Final UMAP with cleaned data"))
```

Heatmap


```

#Convert to matrix
expression_matrix<-as.matrix(Nrepl_data)

sample_correlations<-cor(expression_matrix)

heatmaply(sample_correlations, clustering_method = "complete",
           colors = blueWhiteRed(256),
           #grid_color = "black",
           grid_gap = 0.001)

```

Network construction

Power choosing

Get list of powers and do the calculations

```

Nrepl_data<-t(Nrepl_data)

power <- c(c(1:15), seq(from = 17, to = 50, by = 2)) #more detailed in lower values

#Network topology analysis
sft <- pickSoftThreshold(Nrepl_data,
                        powerVector = power,
                        networkType = "signed",
                        verbose = 5)

```

```

## pickSoftThreshold: will use block size 1255.
## pickSoftThreshold: calculating connectivity for given powers...
## ..working on genes 1 through 1255 of 35634
## ..working on genes 1256 through 2510 of 35634
## ..working on genes 2511 through 3765 of 35634
## ..working on genes 3766 through 5020 of 35634
## ..working on genes 5021 through 6275 of 35634
## ..working on genes 6276 through 7530 of 35634
## ..working on genes 7531 through 8785 of 35634
## ..working on genes 8786 through 10040 of 35634
## ..working on genes 10041 through 11295 of 35634
## ..working on genes 11296 through 12550 of 35634
## ..working on genes 12551 through 13805 of 35634
## ..working on genes 13806 through 15060 of 35634
## ..working on genes 15061 through 16315 of 35634
## ..working on genes 16316 through 17570 of 35634
## ..working on genes 17571 through 18825 of 35634
## ..working on genes 18826 through 20080 of 35634
## ..working on genes 20081 through 21335 of 35634
## ..working on genes 21336 through 22590 of 35634
## ..working on genes 22591 through 23845 of 35634

```

```
## ..working on genes 23846 through 25100 of 35634
## ..working on genes 25101 through 26355 of 35634
## ..working on genes 26356 through 27610 of 35634
## ..working on genes 27611 through 28865 of 35634
## ..working on genes 28866 through 30120 of 35634
## ..working on genes 30121 through 31375 of 35634
## ..working on genes 31376 through 32630 of 35634
## ..working on genes 32631 through 33885 of 35634
## ..working on genes 33886 through 35140 of 35634
## ..working on genes 35141 through 35634 of 35634
## Power SFT.R.sq slope truncated.R.sq mean.k. median.k. max.k.
## 1 1 0.06750 19.200 0.942 17900.00 1.79e+04 18600
## 2 2 0.06930 7.790 0.964 9710.00 9.70e+03 10900
## 3 3 0.00385 -0.647 0.889 5630.00 5.59e+03 7170
## 4 4 0.10900 -1.770 0.868 3450.00 3.38e+03 5070
## 5 5 0.17100 -1.450 0.863 2220.00 2.13e+03 3800
## 6 6 0.28800 -1.520 0.875 1490.00 1.40e+03 3000
## 7 7 0.41400 -1.590 0.889 1030.00 9.44e+02 2440
## 8 8 0.53600 -1.630 0.909 743.00 6.54e+02 2040
## 9 9 0.63300 -1.690 0.922 549.00 4.66e+02 1730
## 10 10 0.72500 -1.740 0.944 417.00 3.40e+02 1500
## 11 11 0.78200 -1.800 0.954 323.00 2.52e+02 1320
## 12 12 0.81500 -1.840 0.959 255.00 1.90e+02 1180
## 13 13 0.83400 -1.870 0.959 205.00 1.46e+02 1060
## 14 14 0.85500 -1.880 0.965 167.00 1.14e+02 954
## 15 15 0.86700 -1.890 0.967 138.00 9.01e+01 868
## 16 17 0.88700 -1.890 0.970 97.20 5.80e+01 728
## 17 19 0.89300 -1.890 0.971 71.10 3.87e+01 626
## 18 21 0.89500 -1.890 0.972 53.70 2.63e+01 545
## 19 23 0.89700 -1.870 0.973 41.60 1.83e+01 478
## 20 25 0.89800 -1.850 0.974 33.00 1.28e+01 424
## 21 27 0.89300 -1.840 0.973 26.60 9.12e+00 377
## 22 29 0.89000 -1.820 0.972 21.80 6.58e+00 338
## 23 31 0.88800 -1.800 0.973 18.10 4.82e+00 304
## 24 33 0.88800 -1.770 0.974 15.30 3.56e+00 275
## 25 35 0.88400 -1.750 0.972 13.00 2.68e+00 249
## 26 37 0.87800 -1.730 0.969 11.20 2.06e+00 226
## 27 39 0.87500 -1.710 0.966 9.71 1.60e+00 206
## 28 41 0.86600 -1.700 0.960 8.49 1.26e+00 189
## 29 43 0.86300 -1.680 0.957 7.48 1.04e+00 173
## 30 45 0.85900 -1.660 0.951 6.63 8.78e-01 158
## 31 47 0.85700 -1.640 0.946 5.91 7.08e-01 146
## 32 49 0.85700 -1.610 0.938 5.30 5.69e-01 134
```

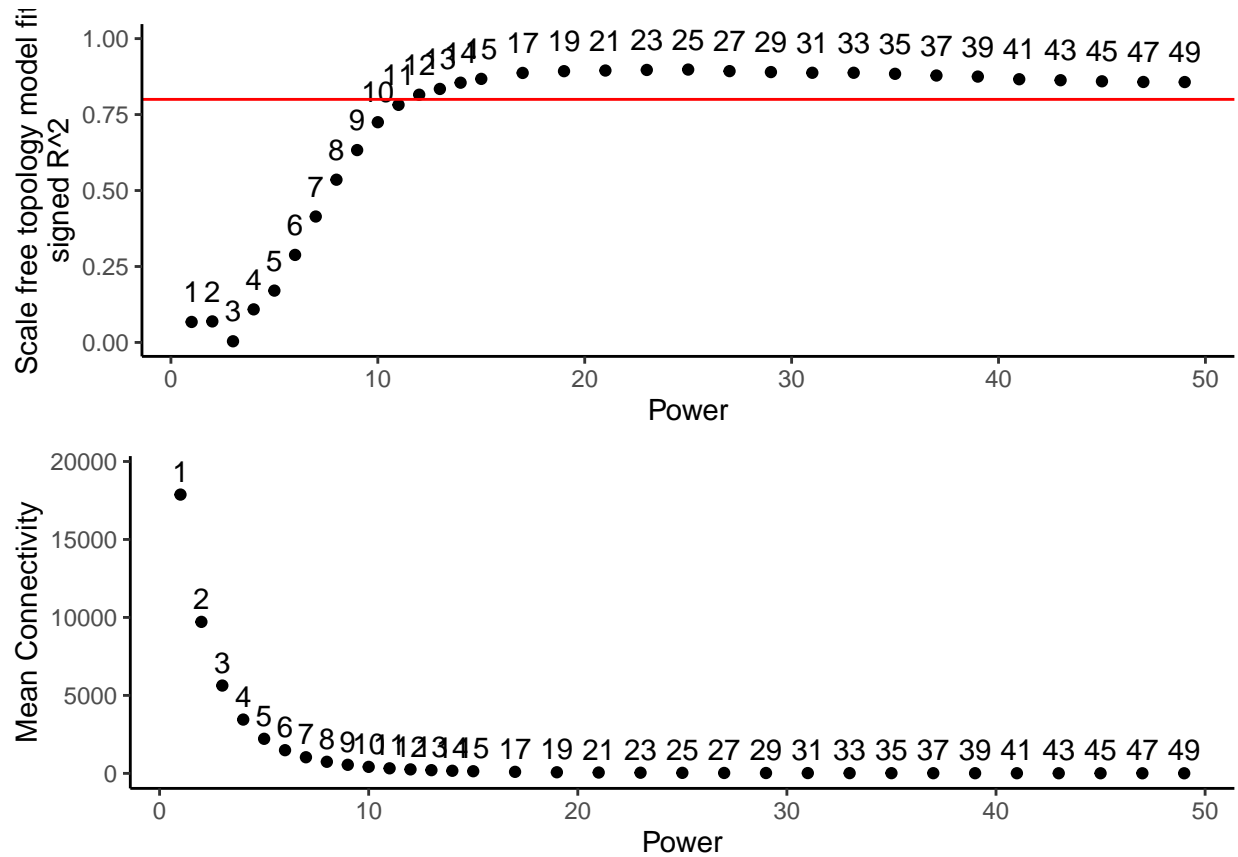
Plot and choose the best possible outcome

```
sftIn<-sft$fitIndices

p1<-ggplot(sftIn, 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\nsigned R^2') +
  theme_classic()
```

```
p2<-ggplot(sftIn, aes(Power, mean.k., label = Power)) +
  geom_point() +
  geom_text(nudge_y = 1500) +
  labs(x = 'Power', y = 'Mean Connectivity') +
  theme_classic()

grid.arrange(p1, p2, nrow=2)
```



```
print(sft$powerEstimate)
```

```
## [1] 14
```

```
#Use automatic calculation
if (is.na(sft$powerEstimate)){
  softPw<-30 #if the soft power is NA, set to 30, else take smallest possible
}else{
  softPw <- min(sft$powerEstimate, 30)
}
```

Block building

```

temp_cor<-cor
cor<-WGCNA::cor

ModNetwork<-blockwiseModules(Nrepl_data,
                             nThreads = 32, #16
                             maxBlockSize = 64000, #directly related to memory, if maxBlockSize<total genes, multip
                             deepSplit = 4,
                             TOMType = "unsigned", #unsigned?
                             power = softPw,
                             mergeCutHeight = 0.3, #0.3->4799 #0.8->4799 (low module granularity) #0.1->4799 (high gran
                             minModuleSize = 20,
                             numericLabels = FALSE,
                             pamRespectsDendro = FALSE,
                             minKMEtoStay = 0.3, #0.3->4799 #0.8->7781 #<0.3 stays the same
                             randomSeed = 42,
                             verbose = 4)

```

```

## 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 32 parallel threads.
## Fraction of slow calculations: 0.000000
## ..connectivity..
## ..matrix multiplication (system BLAS)..
## ..normalization..
## ..done.
## ...clustering..
## ...detecting modules..
## ..done.
## ...calculating module eigengenes..
## moduleEigengenes: Calculating 193 module eigengenes in given set.
## ...checking kME in modules..
## ..removing 1 genes from module 6 because their KME is too low.
## ..removing 1 genes from module 12 because their KME is too low.
## ..removing 1 genes from module 132 because their KME is too low.
## ..reassigning 604 genes from module 1 to modules with higher KME.
## ..reassigning 494 genes from module 2 to modules with higher KME.
## ..reassigning 88 genes from module 3 to modules with higher KME.
## ..reassigning 158 genes from module 4 to modules with higher KME.
## ..reassigning 238 genes from module 5 to modules with higher KME.
## ..reassigning 25 genes from module 6 to modules with higher KME.
## ..reassigning 140 genes from module 7 to modules with higher KME.
## ..reassigning 124 genes from module 8 to modules with higher KME.
## ..reassigning 210 genes from module 9 to modules with higher KME.
## ..reassigning 133 genes from module 10 to modules with higher KME.
## ..reassigning 87 genes from module 11 to modules with higher KME.
## ..reassigning 19 genes from module 12 to modules with higher KME.
## ..reassigning 118 genes from module 13 to modules with higher KME.
## ..reassigning 24 genes from module 14 to modules with higher KME.
## ..reassigning 90 genes from module 15 to modules with higher KME.
## ..reassigning 130 genes from module 16 to modules with higher KME.

```

[illegible]

[illegible]

```

## ..reassigning 4 genes from module 129 to modules with higher KME.
## ..reassigning 4 genes from module 130 to modules with higher KME.
## ..reassigning 2 genes from module 131 to modules with higher KME.
## ..reassigning 1 genes from module 132 to modules with higher KME.
## ..reassigning 3 genes from module 133 to modules with higher KME.
## ..reassigning 1 genes from module 134 to modules with higher KME.
## ..reassigning 4 genes from module 135 to modules with higher KME.
## ..reassigning 2 genes from module 136 to modules with higher KME.
## ..reassigning 1 genes from module 139 to modules with higher KME.
## ..reassigning 1 genes from module 140 to modules with higher KME.
## ..reassigning 4 genes from module 142 to modules with higher KME.
## ..reassigning 9 genes from module 143 to modules with higher KME.
## ..reassigning 2 genes from module 144 to modules with higher KME.
## ..reassigning 2 genes from module 146 to modules with higher KME.
## ..reassigning 8 genes from module 148 to modules with higher KME.
## ..reassigning 1 genes from module 149 to modules with higher KME.
## ..reassigning 3 genes from module 150 to modules with higher KME.
## ..reassigning 1 genes from module 151 to modules with higher KME.
## ..reassigning 1 genes from module 156 to modules with higher KME.
## ..reassigning 1 genes from module 157 to modules with higher KME.
## ..reassigning 3 genes from module 158 to modules with higher KME.
## ..reassigning 7 genes from module 162 to modules with higher KME.
## ..reassigning 3 genes from module 163 to modules with higher KME.
## ..reassigning 1 genes from module 164 to modules with higher KME.
## ..reassigning 1 genes from module 165 to modules with higher KME.
## ..reassigning 1 genes from module 172 to modules with higher KME.
## ..reassigning 1 genes from module 174 to modules with higher KME.
## ..reassigning 4 genes from module 175 to modules with higher KME.
## ..reassigning 3 genes from module 178 to modules with higher KME.
## ..reassigning 3 genes from module 181 to modules with higher KME.
## ..reassigning 1 genes from module 185 to modules with higher KME.
## ..reassigning 1 genes from module 187 to modules with higher KME.
## ..reassigning 2 genes from module 188 to modules with higher KME.
## ..merging modules that are too close..
##     mergeCloseModules: Merging modules whose distance is less than 0.3
##     multiSetMEs: Calculating module MEs.
##         Working on set 1 ...
##     multiSetMEs: Calculating module MEs.
##         Working on set 1 ...
##     multiSetMEs: Calculating module MEs.
##         Working on set 1 ...
##     multiSetMEs: Calculating module MEs.
##         Working on set 1 ...
##     multiSetMEs: Calculating module MEs.
##         Working on set 1 ...
##     Calculating new MEs...
##     multiSetMEs: Calculating module MEs.
##         Working on set 1 ...

```

```
cor<-temp_cor
```

Modules and eigengenes

Eigengenes summarize gene expression for a module(cluster of highly co-expressed genes(detected by similar gene expression patterns)) using PC1. Group up genes into 1 value

```
module_eigengenes<-ModNetwork$MEs
```

```
#get number of genes for each module  
table(ModNetwork$colors)
```

```
##  
##          black          blue          brown          cyan          darkgreen  
##          1266          4671          3637          537           282  
##          darkgrey      darkmagenta darkolivegreen      darkorange      darkred  
##          271           64           76           261           301  
##          darkturquoise      green      greenyellow      grey           grey60  
##          271           2027          923           3978          409  
##          lightcyan      lightgreen      lightyellow      magenta      midnightblue  
##          454           356           350           1062          515  
##          orange      paleturquoise          pink          plum1           purple  
##          268           118           1120          28           990  
##          red          royalblue      saddlebrown      salmon          sienna3  
##          1361          344           208           580           44  
##          skyblue      skyblue3      steelblue      tan           turquoise  
##          236           32           153           734           4926  
##          violet          white          yellow      yellowgreen  
##          83           238           2423          37
```

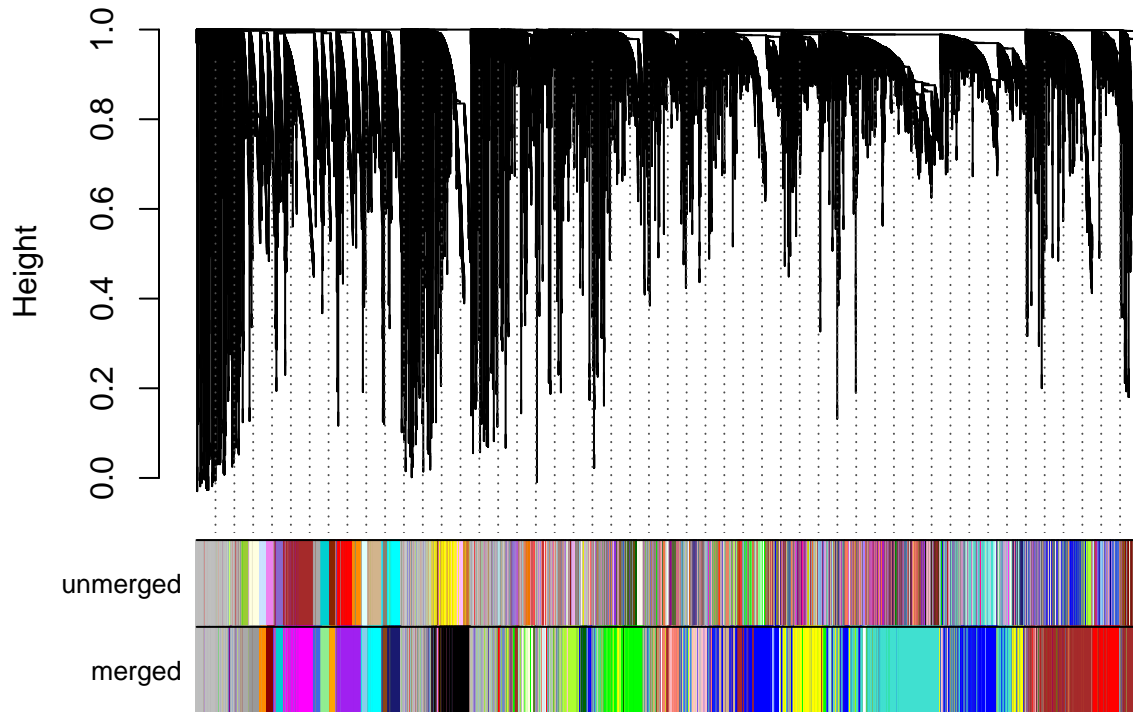
```
dim(Nrepl_data) #get total number of genes from the dimensions
```

```
## [1] 149 35634
```

```
#Plot the module colors before and after merging underneath
```

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


Cluster Dendrogram



Relate modules to traits

Binarize metadata

Binarize the location

```
levels(as.factor(repl_meta$location))
```

```
## [1] "b73_cob"
## [2] "b73_coleoptile"
## [3] "b73_crown.root"
## [4] "b73_first.elongated.internode"
## [5] "b73_flag.leaf"
## [6] "b73_immature.cob"
## [7] "b73_immature.tassel"
## [8] "b73_leaf.1"
## [9] "b73_leaf.3.blade"
## [10] "b73_leaf.3.sheath"
## [11] "b73_leaf.5"
## [12] "b73_leaf.5.elongation.zone"
## [13] "b73_leaf.5.mature"
## [14] "b73_leaf.5.meristem"
## [15] "b73_leaf.8"
## [16] "b73_mature.seed.40.dap"
```

```

## [17] "b73_meotic.tassel"
## [18] "b73_mesophyll"
## [19] "b73_prepollinated.cob"
## [20] "b73_primary.root"
## [21] "b73_primary.root.elongation.zone"
## [22] "b73_primary.root.meristematic.zone"
## [23] "b73_root.hair.zone"
## [24] "b73_seed.10.dap"
## [25] "b73_seed.15.dap"
## [26] "b73_seed.20.dap"
## [27] "b73_seed.25.dap"
## [28] "b73_seed.30.dap"
## [29] "b73_seminal.root"
## [30] "b73_silk"
## [31] "dk105_cob"
## [32] "dk105_coleoptile"
## [33] "dk105_crown.root"
## [34] "dk105_first.elongated.internode"
## [35] "dk105_flag.leaf"
## [36] "dk105_immature.cob"
## [37] "dk105_immature.tassel"
## [38] "dk105_leaf.1"
## [39] "dk105_leaf.3.blade"
## [40] "dk105_leaf.3.sheath"
## [41] "dk105_leaf.5"
## [42] "dk105_leaf.5.elongation.zone"
## [43] "dk105_leaf.5.mature"
## [44] "dk105_leaf.5.meristem"
## [45] "dk105_leaf.8"
## [46] "dk105_mature.seed.40.dap"
## [47] "dk105_meotic.tassel"
## [48] "dk105_mesophyll"
## [49] "dk105_prepollinated.cob"
## [50] "dk105_primary.root"
## [51] "dk105_primary.root.elongation.zone"
## [52] "dk105_primary.root.meristematic.zone"
## [53] "dk105_root.hair.zone"
## [54] "dk105_seed.10.dap"
## [55] "dk105_seed.15.dap"
## [56] "dk105_seed.20.dap"
## [57] "dk105_seed.25.dap"
## [58] "dk105_seed.30.dap"
## [59] "dk105_seminal.root"
## [60] "dk105_silk"
## [61] "ep1_cob"
## [62] "ep1_coleoptile"
## [63] "ep1_crown.root"
## [64] "ep1_first.elongated.internode"
## [65] "ep1_flag.leaf"
## [66] "ep1_immature.cob"
## [67] "ep1_immature.tassel"
## [68] "ep1_leaf.1"
## [69] "ep1_leaf.3.blade"
## [70] "ep1_leaf.3.sheath"

```

```

## [71] "ep1_leaf.5"
## [72] "ep1_leaf.5.elongation.zone"
## [73] "ep1_leaf.5.mature"
## [74] "ep1_leaf.5.meristem"
## [75] "ep1_leaf.8"
## [76] "ep1_mature.seed.40.dap"
## [77] "ep1_meotic.tassel"
## [78] "ep1_mesophyll"
## [79] "ep1_prepollinated.cob"
## [80] "ep1_primary.root"
## [81] "ep1_primary.root.elongation.zone"
## [82] "ep1_primary.root.meristematic.zone"
## [83] "ep1_root.hair.zone"
## [84] "ep1_seed.10.dap"
## [85] "ep1_seed.15.dap"
## [86] "ep1_seed.20.dap"
## [87] "ep1_seed.25.dap"
## [88] "ep1_seed.30.dap"
## [89] "ep1_seminal.root"
## [90] "ep1_silk"
## [91] "f7_cob"
## [92] "f7_coleoptile"
## [93] "f7_crown.root"
## [94] "f7_first.elongated.internode"
## [95] "f7_flag.leaf"
## [96] "f7_immature.cob"
## [97] "f7_immature.tassel"
## [98] "f7_leaf.1"
## [99] "f7_leaf.3.blade"
## [100] "f7_leaf.3.sheath"
## [101] "f7_leaf.5"
## [102] "f7_leaf.5.elongation.zone"
## [103] "f7_leaf.5.mature"
## [104] "f7_leaf.5.meristem"
## [105] "f7_leaf.8"
## [106] "f7_mature.seed.40.dap"
## [107] "f7_meotic.tassel"
## [108] "f7_mesophyll"
## [109] "f7_prepollinated.cob"
## [110] "f7_primary.root"
## [111] "f7_primary.root.elongation.zone"
## [112] "f7_primary.root.meristematic.zone"
## [113] "f7_root.hair.zone"
## [114] "f7_seed.10.dap"
## [115] "f7_seed.15.dap"
## [116] "f7_seed.20.dap"
## [117] "f7_seed.25.dap"
## [118] "f7_seed.30.dap"
## [119] "f7_seminal.root"
## [120] "f7_silk"
## [121] "pe75_cob"
## [122] "pe75_coleoptile"
## [123] "pe75_crown.root"
## [124] "pe75_first.elongated.internode"

```

```
## [125] "pe75_flag.leaf"
## [126] "pe75_immature.cob"
## [127] "pe75_immature.tassel"
## [128] "pe75_leaf.1"
## [129] "pe75_leaf.3.blade"
## [130] "pe75_leaf.3.sheath"
## [131] "pe75_leaf.5"
## [132] "pe75_leaf.5.elongation.zone"
## [133] "pe75_leaf.5.mature"
## [134] "pe75_leaf.5.meristem"
## [135] "pe75_leaf.8"
## [136] "pe75_meotic.tassel"
## [137] "pe75_mesophyll"
## [138] "pe75_prepollinated.cob"
## [139] "pe75_primary.root"
## [140] "pe75_primary.root.elongation.zone"
## [141] "pe75_primary.root.meristematic.zone"
## [142] "pe75_root.hair.zone"
## [143] "pe75_seed.10.dap"
## [144] "pe75_seed.15.dap"
## [145] "pe75_seed.20.dap"
## [146] "pe75_seed.25.dap"
## [147] "pe75_seed.30.dap"
## [148] "pe75_seminal.root"
## [149] "pe75_silk"
```

```
###
```

#Create a relations matrix for the replicates of each species, where both columns and rows are the repl

```
bin_metadata <- data.frame(
  cob_b73=as.integer(repl_meta$location=="b73_cob"),
  coleoptile_b73=as.integer(repl_meta$location=="b73_coleoptile"),
  crown_root_b73=as.integer(repl_meta$location=="b73_crown.root"),
  first_elongated_internode_b73=as.integer(repl_meta$location=="b73_first.elongated.internode"),
  flag_leaf_b73=as.integer(repl_meta$location=="b73_flag.leaf"),
  immature_cob_b73=as.integer(repl_meta$location=="b73_immature.cob"),
  immature_tassel_b73=as.integer(repl_meta$location=="b73_immature.tassel"),
  leaf_1_b73=as.integer(repl_meta$location=="b73_leaf.1"),
  leaf_3_blade_b73=as.integer(repl_meta$location=="b73_leaf.3.blade"),
  leaf_3_sheath_b73=as.integer(repl_meta$location=="b73_leaf.3.sheath"),
  leaf_5_b73=as.integer(repl_meta$location=="b73_leaf.5"),
  leaf_5_elongation_zone_b73=as.integer(repl_meta$location=="b73_leaf.5.elongation.zone"),
  leaf_5_mature_b73=as.integer(repl_meta$location=="b73_leaf.5.mature"),
  leaf_5_meristem_b73=as.integer(repl_meta$location=="b73_leaf.5.meristem"),
  leaf_8_b73=as.integer(repl_meta$location=="b73_leaf.8"),
  mature_seed_40_dap_b73=as.integer(repl_meta$location=="b73_mature.seed.40.dap"),
  meotic_tassel_b73=as.integer(repl_meta$location=="b73_meotic.tassel"),
  mesophyll_b73=as.integer(repl_meta$location=="b73_mesophyll"),
  prepollinated_cob_b73=as.integer(repl_meta$location=="b73_prepollinated.cob"),
  primary_root_b73=as.integer(repl_meta$location=="b73_primary.root"),
  primary_root_elongation_zone_b73=as.integer(repl_meta$location=="b73_primary.root.elongation.zone"),
  primary_root_meristematic_zone_b73=as.integer(repl_meta$location=="b73_primary.root.meristematic.zone"),
  root_hair_zone_b73=as.integer(repl_meta$location=="b73_root.hair.zone"),
  seed_10_dap_b73=as.integer(repl_meta$location=="b73_seed.10.dap"),
  seed_15_dap_b73=as.integer(repl_meta$location=="b73_seed.15.dap"),
```

```

seed_20_dap_b73=as.integer(repl_meta$location=="b73_seed.20.dap"),
seed_25_dap_b73=as.integer(repl_meta$location=="b73_seed.25.dap"),
seed_30_dap_b73=as.integer(repl_meta$location=="b73_seed.30.dap"),
seminal_root_b73=as.integer(repl_meta$location=="b73_seminal.root"),
silk_b73=as.integer(repl_meta$location=="b73_silk"),

cob_dk105=as.integer(repl_meta$location=="dk105_cob"),
coleoptile_dk105=as.integer(repl_meta$location=="dk105_coleoptile"),
crown_root_dk105=as.integer(repl_meta$location=="dk105_crown.root"),
first_elongated_internode_dk105=as.integer(repl_meta$location=="dk105_first.elongated.internode"),
flag_leaf_dk105=as.integer(repl_meta$location=="dk105_flag.leaf"),
immature_cob_dk105=as.integer(repl_meta$location=="dk105_immature.cob"),
immature_tassel_dk105=as.integer(repl_meta$location=="dk105_immature.tassel"),
leaf_1_dk105=as.integer(repl_meta$location=="dk105_leaf.1"),
leaf_3_blade_dk105=as.integer(repl_meta$location=="dk105_leaf.3.blade"),
leaf_3_sheath_dk105=as.integer(repl_meta$location=="dk105_leaf.3.sheath"),
leaf_5_dk105=as.integer(repl_meta$location=="dk105_leaf.5"),
leaf_5_elongation_zone_dk105=as.integer(repl_meta$location=="dk105_leaf.5.elongation.zone"),
leaf_5_mature_dk105=as.integer(repl_meta$location=="dk105_leaf.5.mature"),
leaf_5_meristem_dk105=as.integer(repl_meta$location=="dk105_leaf.5.meristem"),
leaf_8_dk105=as.integer(repl_meta$location=="dk105_leaf.8"),
mature_seed_40_dap_dk105=as.integer(repl_meta$location=="dk105_mature.seed.40.dap"),
meotic_tassel_dk105=as.integer(repl_meta$location=="dk105_meotic.tassel"),
mesophyll_dk105=as.integer(repl_meta$location=="dk105_mesophyll"),
prepollinated_cob_dk105=as.integer(repl_meta$location=="dk105_prepollinated.cob"),
primary_root_dk105=as.integer(repl_meta$location=="dk105_primary.root"),
primary_root_elongation_zone_dk105=as.integer(repl_meta$location=="dk105_primary.root.elongation.zone"),
primary_root_meristematic_zone_dk105=as.integer(repl_meta$location=="dk105_primary.root.meristematic."),
root_hair_zone_dk105=as.integer(repl_meta$location=="dk105_root.hair.zone"),
seed_10_dap_dk105=as.integer(repl_meta$location=="dk105_seed.10.dap"),
seed_15_dap_dk105=as.integer(repl_meta$location=="dk105_seed.15.dap"),
seed_20_dap_dk105=as.integer(repl_meta$location=="dk105_seed.20.dap"),
seed_25_dap_dk105=as.integer(repl_meta$location=="dk105_seed.25.dap"),
seed_30_dap_dk105=as.integer(repl_meta$location=="dk105_seed.30.dap"),
seminal_root_dk105=as.integer(repl_meta$location=="dk105_seminal.root"),
silk_dk105=as.integer(repl_meta$location=="dk105_silk"),

cob_ep1=as.integer(repl_meta$location=="ep1_cob"),
coleoptile_ep1=as.integer(repl_meta$location=="ep1_coleoptile"),
crown_root_ep1=as.integer(repl_meta$location=="ep1_crown.root"),
first_elongated_internode_ep1=as.integer(repl_meta$location=="ep1_first.elongated.internode"),
flag_leaf_ep1=as.integer(repl_meta$location=="ep1_flag.leaf"),
immature_cob_ep1=as.integer(repl_meta$location=="ep1_immature.cob"),
immature_tassel_ep1=as.integer(repl_meta$location=="ep1_immature.tassel"),
leaf_1_ep1=as.integer(repl_meta$location=="ep1_leaf.1"),
leaf_3_blade_ep1=as.integer(repl_meta$location=="ep1_leaf.3.blade"),
leaf_3_sheath_ep1=as.integer(repl_meta$location=="ep1_leaf.3.sheath"),
leaf_5_ep1=as.integer(repl_meta$location=="ep1_leaf.5"),
leaf_5_elongation_zone_ep1=as.integer(repl_meta$location=="ep1_leaf.5.elongation.zone"),
leaf_5_mature_ep1=as.integer(repl_meta$location=="ep1_leaf.5.mature"),
leaf_5_meristem_ep1=as.integer(repl_meta$location=="ep1_leaf.5.meristem"),
leaf_8_ep1=as.integer(repl_meta$location=="ep1_leaf.8"),
mature_seed_40_dap_ep1=as.integer(repl_meta$location=="ep1_mature.seed.40.dap"),

```

```

meotic_tassel_ep1=as.integer(repl_meta$location=="ep1_meotic.tassel"),
mesophyll_ep1=as.integer(repl_meta$location=="ep1_mesophyll"),
prepollinated_cob_ep1=as.integer(repl_meta$location=="ep1_prepollinated.cob"),
primary_root_ep1=as.integer(repl_meta$location=="ep1_primary.root"),
primary_root_elongation_zone_ep1=as.integer(repl_meta$location=="ep1_primary.root.elongation.zone"),
primary_root_meristematic_zone_ep1=as.integer(repl_meta$location=="ep1_primary.root.meristematic.zone"),
root_hair_zone_ep1=as.integer(repl_meta$location=="ep1_root.hair.zone"),
seed_10_dap_ep1=as.integer(repl_meta$location=="ep1_seed.10.dap"),
seed_15_dap_ep1=as.integer(repl_meta$location=="ep1_seed.15.dap"),
seed_20_dap_ep1=as.integer(repl_meta$location=="ep1_seed.20.dap"),
seed_25_dap_ep1=as.integer(repl_meta$location=="ep1_seed.25.dap"),
seed_30_dap_ep1=as.integer(repl_meta$location=="ep1_seed.30.dap"),
seminal_root_ep1=as.integer(repl_meta$location=="ep1_seminal.root"),
silk_ep1=as.integer(repl_meta$location=="ep1_silk"),

cob_f7=as.integer(repl_meta$location=="f7_cob"),
coleoptile_f7=as.integer(repl_meta$location=="f7_coleoptile"),
crown_root_f7=as.integer(repl_meta$location=="f7_crown.root"),
first_elongated_internode_f7=as.integer(repl_meta$location=="f7_first.elongated.internode"),
flag_leaf_f7=as.integer(repl_meta$location=="f7_flag.leaf"),
immature_cob_f7=as.integer(repl_meta$location=="f7_immature.cob"),
immature_tassel_f7=as.integer(repl_meta$location=="f7_immature.tassel"),
leaf_1_f7=as.integer(repl_meta$location=="f7_leaf.1"),
leaf_3_blade_f7=as.integer(repl_meta$location=="f7_leaf.3.blade"),
leaf_3_sheath_f7=as.integer(repl_meta$location=="f7_leaf.3.sheath"),
leaf_5_f7=as.integer(repl_meta$location=="f7_leaf.5"),
leaf_5_elongation_zone_f7=as.integer(repl_meta$location=="f7_leaf.5.elongation.zone"),
leaf_5_mature_f7=as.integer(repl_meta$location=="f7_leaf.5.mature"),
leaf_5_meristem_f7=as.integer(repl_meta$location=="f7_leaf.5.meristem"),
leaf_8_f7=as.integer(repl_meta$location=="f7_leaf.8"),
mature_seed_40_dap_f7=as.integer(repl_meta$location=="f7_mature.seed.40.dap"),
meotic_tassel_f7=as.integer(repl_meta$location=="f7_meotic.tassel"),
mesophyll_f7=as.integer(repl_meta$location=="f7_mesophyll"),
prepollinated_cob_f7=as.integer(repl_meta$location=="f7_prepollinated.cob"),
primary_root_f7=as.integer(repl_meta$location=="f7_primary.root"),
primary_root_elongation_zone_f7=as.integer(repl_meta$location=="f7_primary.root.elongation.zone"),
primary_root_meristematic_zone_f7=as.integer(repl_meta$location=="f7_primary.root.meristematic.zone"),
root_hair_zone_f7=as.integer(repl_meta$location=="f7_root.hair.zone"),
seed_10_dap_f7=as.integer(repl_meta$location=="f7_seed.10.dap"),
seed_15_dap_f7=as.integer(repl_meta$location=="f7_seed.15.dap"),
seed_20_dap_f7=as.integer(repl_meta$location=="f7_seed.20.dap"),
seed_25_dap_f7=as.integer(repl_meta$location=="f7_seed.25.dap"),
seed_30_dap_f7=as.integer(repl_meta$location=="f7_seed.30.dap"),
seminal_root_f7=as.integer(repl_meta$location=="f7_seminal.root"),
silk_f7=as.integer(repl_meta$location=="f7_silk"),

cob_pe75=as.integer(repl_meta$location=="pe75_cob"),
coleoptile_pe75=as.integer(repl_meta$location=="pe75_coleoptile"),
crown_root_pe75=as.integer(repl_meta$location=="pe75_crown.root"),
first_elongated_internode_pe75=as.integer(repl_meta$location=="pe75_first.elongated.internode"),
flag_leaf_pe75=as.integer(repl_meta$location=="pe75_flag.leaf"),
immature_cob_pe75=as.integer(repl_meta$location=="pe75_immature.cob"),
immature_tassel_pe75=as.integer(repl_meta$location=="pe75_immature.tassel"),

```



```

leaf_1_pe75=as.integer(repl_meta$location=="pe75_leaf.1"),
leaf_3_blade_pe75=as.integer(repl_meta$location=="pe75_leaf.3.blade"),
leaf_3_sheath_pe75=as.integer(repl_meta$location=="pe75_leaf.3.sheath"),
leaf_5_pe75=as.integer(repl_meta$location=="pe75_leaf.5"),
leaf_5_elongation_zone_pe75=as.integer(repl_meta$location=="pe75_leaf.5.elongation.zone"),
leaf_5_mature_pe75=as.integer(repl_meta$location=="pe75_leaf.5.mature"),
leaf_5_meristem_pe75=as.integer(repl_meta$location=="pe75_leaf.5.meristem"),
leaf_8_pe75=as.integer(repl_meta$location=="pe75_leaf.8"),
mature_seed_40_dap_pe75=as.integer(repl_meta$location=="pe75_mature.seed.40.dap"),
meotic_tassel_pe75=as.integer(repl_meta$location=="pe75_meotic.tassel"),
mesophyll_pe75=as.integer(repl_meta$location=="pe75_mesophyll"),
prepollinated_cob_pe75=as.integer(repl_meta$location=="pe75_prepollinated.cob"),
primary_root_pe75=as.integer(repl_meta$location=="pe75_primary.root"),
primary_root_elongation_zone_pe75=as.integer(repl_meta$location=="pe75_primary.root.elongation.zone"),
primary_root_meristematic_zone_pe75=as.integer(repl_meta$location=="pe75_primary.root.meristematic.zone"),
root_hair_zone_pe75=as.integer(repl_meta$location=="pe75_root.hair.zone"),
seed_10_dap_pe75=as.integer(repl_meta$location=="pe75_seed.10.dap"),
seed_15_dap_pe75=as.integer(repl_meta$location=="pe75_seed.15.dap"),
seed_20_dap_pe75=as.integer(repl_meta$location=="pe75_seed.20.dap"),
seed_25_dap_pe75=as.integer(repl_meta$location=="pe75_seed.25.dap"),
seed_30_dap_pe75=as.integer(repl_meta$location=="pe75_seed.30.dap"),
seminal_root_pe75=as.integer(repl_meta$location=="pe75_seminal.root"),
silk_pe75=as.integer(repl_meta$location=="pe75_silk")
)

rownames(bin_metadata)<-rownames(Nrepl_data)

bin_metadata<-bin_metadata[,colSums(bin_metadata)>=1]
#if any tissue is not present in sample, it will be introduced in binarization, as a full 0 column, this

```

Correlation

```

nTissues <- nrow(Nrepl_data)
nGenes <- ncol(Nrepl_data)

ModTrait_cor<-cor(module_eigengenes, bin_metadata, use="p")
module.trait.corr.pvals <- corPvalueStudent(ModTrait_cor, nTissues)

```

Coexpression visualization

The modules will present a good visualization of the effect by specie.

```

heatmap.data<-merge(module_eigengenes, bin_metadata, by='row.names')

head(heatmap.data)

```

```

##           Row.names MEturquoise      MEwhite MEMagenta MEroyalblue
## 1           b73_cob  0.12680912  0.048667513 0.1723349  0.09896556
## 2       b73_coleoptile -0.03241655 -0.081491197 0.1454164  0.09412204

```

```

## 3          b73_crown.root -0.07565977 -0.090569758 0.1484701 0.08795427
## 4 b73_first.elongated.internode -0.03361611 -0.014457320 0.1674822 0.10306157
## 5          b73_flag.leaf 0.13579885 0.098228903 0.1784036 0.10584563
## 6          b73_immature.cob -0.04184954 -0.008878031 0.1784448 0.11510059
##      MElightcyan      MEred MEmidnightblue MESaddlebrown MEdarkgreen      MEblue
## 1 -0.007377188 0.08298471 -0.04673899 -0.07006608 -0.08069883 -0.11411621
## 2 -0.030236499 -0.03406939 -0.04983032 -0.06227923 0.11153939 0.01054342
## 3 0.028071613 -0.01250192 -0.05142081 -0.06303276 -0.10220547 0.04053513
## 4 0.117858280 0.03847302 -0.05390522 -0.07319145 0.03220624 0.10429605
## 5 0.007325352 0.05646976 -0.04927946 -0.07391966 -0.03848502 -0.08348106
## 6 0.160765917 0.08786477 -0.05718683 -0.08131539 0.07832549 0.11127916
##      MEyellowgreen Mesienna3      MEbrown MEdarkmagenta      MEcyan      MESkyblue
## 1 -0.12057913 -0.04020046 -0.03171364 -0.00761081 -0.05300685 -0.07542256
## 2 -0.07385969 -0.03405919 -0.05252301 -0.02319043 -0.03936977 -0.06602023
## 3 -0.04821862 -0.03173551 -0.04516864 -0.02592365 -0.04479200 -0.06621806
## 4 0.02990732 -0.03485419 -0.01870375 -0.02869022 -0.05046367 -0.07396093
## 5 -0.10371468 -0.03914557 -0.02695942 -0.01993510 -0.05263417 -0.07842008
## 6 0.08755560 -0.01055541 0.01357462 -0.02559522 -0.05590582 -0.08336653
##      MEsteelblue MElightgreen      MEorange      MEpurple MEdarkgrey MEdarkturquoise
## 1 -0.1005513 -0.11099031 -0.07679406 -0.06141240 -0.07667644 -0.10347059
## 2 -0.1527551 -0.09835241 -0.06143744 -0.05879291 -0.06930740 -0.09870614
## 3 -0.1575741 -0.10198875 -0.06706984 -0.05722312 -0.06789722 -0.10007380
## 4 -0.1395248 -0.11073523 -0.07824363 -0.06651108 -0.06990004 -0.11227703
## 5 -0.1075550 -0.11477376 -0.07881772 -0.06288595 -0.08089353 -0.11043307
## 6 -0.1352101 -0.11587063 -0.08594914 -0.07201833 -0.07751393 -0.11988942
##      MEgrey60 MEdarkorange      MEdarkred      MEsalmon      MEyellow      MEgreen
## 1 -0.05056942 -0.10665036 -0.07155753 -0.05993172 -0.15704307 -0.05558605
## 2 -0.04391956 -0.09088958 -0.06275483 0.05870567 0.07204794 0.03651010
## 3 -0.04388569 -0.09471405 -0.06463344 0.08807399 0.07866142 0.14887680
## 4 -0.04430253 -0.09731471 -0.06943144 0.07555833 0.06477265 -0.05400317
## 5 -0.05308113 -0.11114401 -0.07336458 -0.07769095 -0.11181427 -0.05407080
## 6 -0.04675427 -0.10898754 -0.07487255 0.03720614 0.03354497 -0.08438041
##      MEpaleturquoise MEdarkolivegreen MElightyellow      MEtan MEGreenyellow
## 1 -0.10048775 -0.099071017 -0.04187471 0.09048139 0.05585118
## 2 0.05128207 0.002763192 -0.02259789 0.02979209 0.06519197
## 3 0.08034716 0.047226796 0.11178989 0.03940434 0.08272507
## 4 0.01384662 -0.088556583 -0.08449001 -0.06741027 -0.13539507
## 5 -0.08150179 -0.082491412 -0.01416011 0.07775455 0.01005500
## 6 -0.05010075 -0.123028958 -0.11782745 -0.11807019 -0.14847475
##      MEpink MESkyblue3      MEplum1      MEblack      MEviolet      MEgrey
## 1 0.10063595 -0.02716172 0.0203108726 0.02006331 -0.01686075 0.01977880
## 2 0.01364427 -0.01228137 -0.0023294317 -0.03172503 -0.02620461 0.05630691
## 3 -0.00275038 -0.01024970 -0.0068957787 -0.03025022 0.07792427 0.07110698
## 4 -0.13995734 -0.02308838 -0.0001690687 -0.03461621 -0.06792489 -0.13372441
## 5 0.06532183 -0.02065277 0.0337498775 0.05083805 0.01097806 -0.01281604
## 6 -0.14462053 -0.01488177 -0.0103425760 -0.02967007 -0.06626329 -0.17178893
##      cob_b73 coleoptile_b73 crown_root_b73 first_elongated_internode_b73
## 1 1 0 0 0
## 2 0 1 0 0
## 3 0 0 1 0
## 4 0 0 0 1
## 5 0 0 0 0
## 6 0 0 0 0
##      flag_leaf_b73 immature_cob_b73 immature_tassel_b73 leaf_1_b73

```


## 1	0	0	0	0	
## 2	0	0	0	0	
## 3	0	0	0	0	
## 4	0	0	0	0	
## 5	1	0	0	0	
## 6	0	1	0	0	
##	leaf_3_blade_b73	leaf_3_sheath_b73	leaf_5_b73	leaf_5_elongation_zone_b73	
## 1	0	0	0	0	
## 2	0	0	0	0	
## 3	0	0	0	0	
## 4	0	0	0	0	
## 5	0	0	0	0	
## 6	0	0	0	0	
##	leaf_5_mature_b73	leaf_5_meristem_b73	leaf_8_b73	mature_seed_40_dap_b73	
## 1	0	0	0	0	
## 2	0	0	0	0	
## 3	0	0	0	0	
## 4	0	0	0	0	
## 5	0	0	0	0	
## 6	0	0	0	0	
##	meotic_tassel_b73	mesophyll_b73	prepollinated_cob_b73	primary_root_b73	
## 1	0	0	0	0	
## 2	0	0	0	0	
## 3	0	0	0	0	
## 4	0	0	0	0	
## 5	0	0	0	0	
## 6	0	0	0	0	
##	primary_root_elongation_zone_b73	primary_root_meristematic_zone_b73			
## 1		0		0	
## 2		0		0	
## 3		0		0	
## 4		0		0	
## 5		0		0	
## 6		0		0	
##	root_hair_zone_b73	seed_10_dap_b73	seed_15_dap_b73	seed_20_dap_b73	
## 1	0	0	0	0	
## 2	0	0	0	0	
## 3	0	0	0	0	
## 4	0	0	0	0	
## 5	0	0	0	0	
## 6	0	0	0	0	
##	seed_25_dap_b73	seed_30_dap_b73	seminal_root_b73	silk_b73	cob_dk105
## 1	0	0	0	0	0
## 2	0	0	0	0	0
## 3	0	0	0	0	0
## 4	0	0	0	0	0
## 5	0	0	0	0	0
## 6	0	0	0	0	0
##	coleoptile_dk105	crown_root_dk105	first_elongated_internode_dk105		
## 1	0	0		0	
## 2	0	0		0	
## 3	0	0		0	
## 4	0	0		0	
## 5	0	0		0	

```

## 6          0          0          0
## flag_leaf_dk105 immature_cob_dk105 immature_tassel_dk105 leaf_1_dk105
## 1          0          0          0          0
## 2          0          0          0          0
## 3          0          0          0          0
## 4          0          0          0          0
## 5          0          0          0          0
## 6          0          0          0          0
## leaf_3_blade_dk105 leaf_3_sheath_dk105 leaf_5_dk105
## 1          0          0          0
## 2          0          0          0
## 3          0          0          0
## 4          0          0          0
## 5          0          0          0
## 6          0          0          0
## leaf_5_elongation_zone_dk105 leaf_5_mature_dk105 leaf_5_meristem_dk105
## 1          0          0          0
## 2          0          0          0
## 3          0          0          0
## 4          0          0          0
## 5          0          0          0
## 6          0          0          0
## leaf_8_dk105 mature_seed_40_dap_dk105 meotic_tassel_dk105 mesophyll_dk105
## 1          0          0          0          0
## 2          0          0          0          0
## 3          0          0          0          0
## 4          0          0          0          0
## 5          0          0          0          0
## 6          0          0          0          0
## prepollinated_cob_dk105 primary_root_dk105 primary_root_elongation_zone_dk105
## 1          0          0          0
## 2          0          0          0
## 3          0          0          0
## 4          0          0          0
## 5          0          0          0
## 6          0          0          0
## primary_root_meristematic_zone_dk105 root_hair_zone_dk105 seed_10_dap_dk105
## 1          0          0          0
## 2          0          0          0
## 3          0          0          0
## 4          0          0          0
## 5          0          0          0
## 6          0          0          0
## seed_15_dap_dk105 seed_20_dap_dk105 seed_25_dap_dk105 seed_30_dap_dk105
## 1          0          0          0          0
## 2          0          0          0          0
## 3          0          0          0          0
## 4          0          0          0          0
## 5          0          0          0          0
## 6          0          0          0          0
## seminal_root_dk105 silk_dk105 cob_ep1 coleoptile_ep1 crown_root_ep1
## 1          0          0          0          0          0
## 2          0          0          0          0          0
## 3          0          0          0          0          0

```

## 4	0	0	0	0	0
## 5	0	0	0	0	0
## 6	0	0	0	0	0
##	first_elongated_internode_ep1	flag_leaf_ep1	immature_cob_ep1		
## 1		0	0	0	
## 2		0	0	0	
## 3		0	0	0	
## 4		0	0	0	
## 5		0	0	0	
## 6		0	0	0	
##	immature_tassel_ep1	leaf_1_ep1	leaf_3_blade_ep1	leaf_3_sheath_ep1	leaf_5_ep1
## 1	0	0	0	0	0
## 2	0	0	0	0	0
## 3	0	0	0	0	0
## 4	0	0	0	0	0
## 5	0	0	0	0	0
## 6	0	0	0	0	0
##	leaf_5_elongation_zone_ep1	leaf_5_mature_ep1	leaf_5_meristem_ep1	leaf_8_ep1	
## 1	0	0	0	0	
## 2	0	0	0	0	
## 3	0	0	0	0	
## 4	0	0	0	0	
## 5	0	0	0	0	
## 6	0	0	0	0	
##	mature_seed_40_dap_ep1	meiotic_tassel_ep1	mesophyll_ep1	prepollinated_cob_ep1	
## 1	0	0	0	0	
## 2	0	0	0	0	
## 3	0	0	0	0	
## 4	0	0	0	0	
## 5	0	0	0	0	
## 6	0	0	0	0	
##	primary_root_ep1	primary_root_elongation_zone_ep1			
## 1	0	0			
## 2	0	0			
## 3	0	0			
## 4	0	0			
## 5	0	0			
## 6	0	0			
##	primary_root_meristematic_zone_ep1	root_hair_zone_ep1	seed_10_dap_ep1		
## 1	0	0	0		
## 2	0	0	0		
## 3	0	0	0		
## 4	0	0	0		
## 5	0	0	0		
## 6	0	0	0		
##	seed_15_dap_ep1	seed_20_dap_ep1	seed_25_dap_ep1	seed_30_dap_ep1	
## 1	0	0	0	0	
## 2	0	0	0	0	
## 3	0	0	0	0	
## 4	0	0	0	0	
## 5	0	0	0	0	
## 6	0	0	0	0	
##	seminal_root_ep1	silk_ep1	cob_f7	coleoptile_f7	crown_root_f7
## 1	0	0	0	0	0

## 2	0	0	0	0	0
## 3	0	0	0	0	0
## 4	0	0	0	0	0
## 5	0	0	0	0	0
## 6	0	0	0	0	0
##	first_elongated_internode_f7	flag_leaf_f7	immature_cob_f7	immature_tassel_f7	
## 1		0	0	0	0
## 2		0	0	0	0
## 3		0	0	0	0
## 4		0	0	0	0
## 5		0	0	0	0
## 6		0	0	0	0
##	leaf_1_f7	leaf_3_blade_f7	leaf_3_sheath_f7	leaf_5_f7	
## 1	0	0	0	0	
## 2	0	0	0	0	
## 3	0	0	0	0	
## 4	0	0	0	0	
## 5	0	0	0	0	
## 6	0	0	0	0	
##	leaf_5_elongation_zone_f7	leaf_5_mature_f7	leaf_5_meristem_f7	leaf_8_f7	
## 1		0	0	0	0
## 2		0	0	0	0
## 3		0	0	0	0
## 4		0	0	0	0
## 5		0	0	0	0
## 6		0	0	0	0
##	mature_seed_40_dap_f7	meotic_tassel_f7	mesophyll_f7	prepollinated_cob_f7	
## 1		0	0	0	0
## 2		0	0	0	0
## 3		0	0	0	0
## 4		0	0	0	0
## 5		0	0	0	0
## 6		0	0	0	0
##	primary_root_f7	primary_root_elongation_zone_f7			
## 1	0		0		
## 2	0		0		
## 3	0		0		
## 4	0		0		
## 5	0		0		
## 6	0		0		
##	primary_root_meristematic_zone_f7	root_hair_zone_f7	seed_10_dap_f7		
## 1		0	0	0	
## 2		0	0	0	
## 3		0	0	0	
## 4		0	0	0	
## 5		0	0	0	
## 6		0	0	0	
##	seed_15_dap_f7	seed_20_dap_f7	seed_25_dap_f7	seed_30_dap_f7	seminal_root_f7
## 1	0	0	0	0	0
## 2	0	0	0	0	0
## 3	0	0	0	0	0
## 4	0	0	0	0	0
## 5	0	0	0	0	0
## 6	0	0	0	0	0

```

##  silk_f7  cob_pe75  coleoptile_pe75  crown_root_pe75
##  1      0      0      0      0
##  2      0      0      0      0
##  3      0      0      0      0
##  4      0      0      0      0
##  5      0      0      0      0
##  6      0      0      0      0
##  first_elongated_internode_pe75  flag_leaf_pe75  immature_cob_pe75
##  1      0      0      0
##  2      0      0      0
##  3      0      0      0
##  4      0      0      0
##  5      0      0      0
##  6      0      0      0
##  immature_tassel_pe75  leaf_1_pe75  leaf_3_blade_pe75  leaf_3_sheath_pe75
##  1      0      0      0      0
##  2      0      0      0      0
##  3      0      0      0      0
##  4      0      0      0      0
##  5      0      0      0      0
##  6      0      0      0      0
##  leaf_5_pe75  leaf_5_elongation_zone_pe75  leaf_5_mature_pe75
##  1      0      0      0
##  2      0      0      0
##  3      0      0      0
##  4      0      0      0
##  5      0      0      0
##  6      0      0      0
##  leaf_5_meristem_pe75  leaf_8_pe75  meiotic_tassel_pe75  mesophyll_pe75
##  1      0      0      0      0
##  2      0      0      0      0
##  3      0      0      0      0
##  4      0      0      0      0
##  5      0      0      0      0
##  6      0      0      0      0
##  prepollinated_cob_pe75  primary_root_pe75  primary_root_elongation_zone_pe75
##  1      0      0      0
##  2      0      0      0
##  3      0      0      0
##  4      0      0      0
##  5      0      0      0
##  6      0      0      0
##  primary_root_meristematic_zone_pe75  root_hair_zone_pe75  seed_10_dap_pe75
##  1      0      0      0
##  2      0      0      0
##  3      0      0      0
##  4      0      0      0
##  5      0      0      0
##  6      0      0      0
##  seed_15_dap_pe75  seed_20_dap_pe75  seed_25_dap_pe75  seed_30_dap_pe75
##  1      0      0      0      0
##  2      0      0      0      0
##  3      0      0      0      0
##  4      0      0      0      0

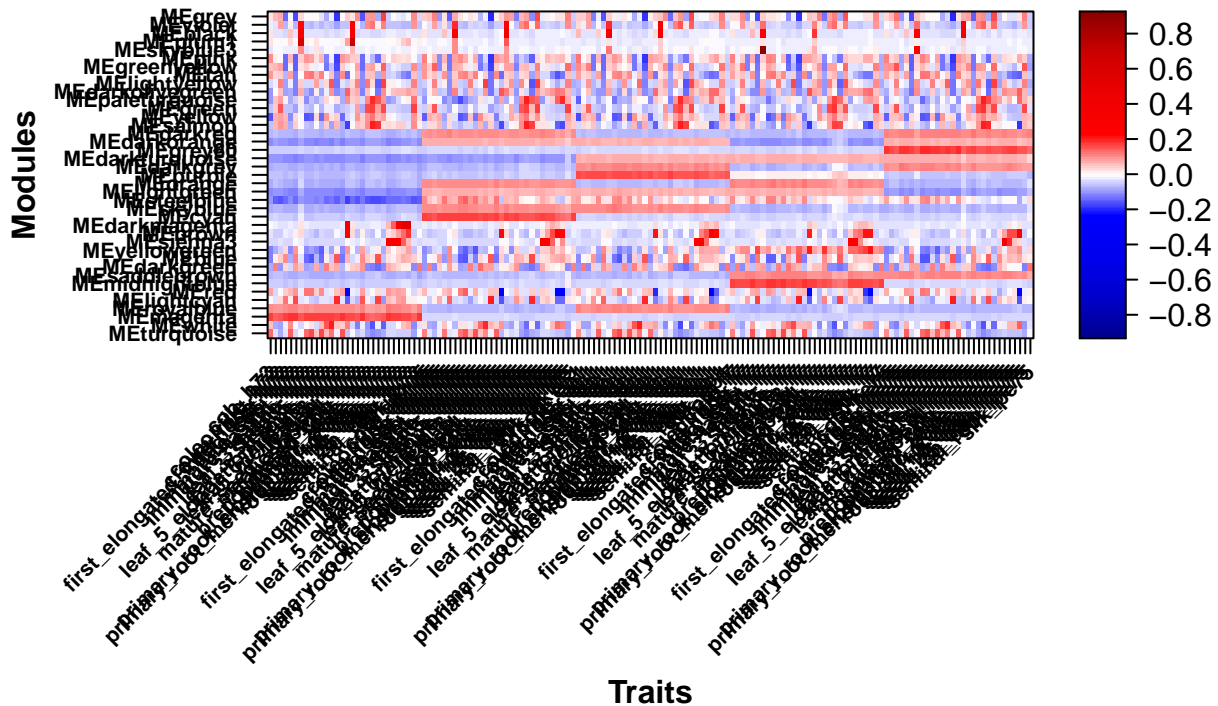
```

```
## 5      0      0      0      0
## 6      0      0      0      0
##  seminal_root_pe75 silk_pe75
## 1      0      0
## 2      0      0
## 3      0      0
## 4      0      0
## 5      0      0
## 6      0      0

rownames(heatmap.data)<-heatmap.data$Row.names
heatmap.data$Row.names<-NULL

plotTitle<-paste0("Correlation plot by species using ", NormType)
#png(paste0("./CorPlots/sp_", NormType, "_corplot.png"), width=1600, height=800) #sp_CPM_corplot
CorLevelPlot(heatmap.data,
  x = names(bin_metadata),
  y = names(module_eigengenes),
  titleX="Traits", titleY="Modules", main=plotTitle,
  rotLabX = 45, rotTitleY = 90,
  cexCorval = 0, cexLabY = 0.7, cexLabX = 0.7
)
```

Correlation plot by species using RPKM



```
#dev.off()
```

Resorted Coexpression Visualization

Sort the traits by tissue name for a better visualization of modules that affect a group of tissues

```
s_bin_metadata<-bin_metadata
s_bin_metadata <- s_bin_metadata[, sort(names(s_bin_metadata))]
s_bin_metadata <- s_bin_metadata[order(rownames(s_bin_metadata)), ]

heatmap.data <- merge(module_eigengenes, s_bin_metadata, by = 'row.names')

head(heatmap.data)
```

```
##           Row.names MEturquoise      MEwhite MEmagenta MERoyalblue
## 1           b73_cob  0.12680912  0.048667513 0.1723349 0.09896556
## 2           b73_coleoptile -0.03241655 -0.081491197 0.1454164 0.09412204
## 3           b73_crown.root -0.07565977 -0.090569758 0.1484701 0.08795427
## 4 b73_first.elongated.internode -0.03361611 -0.014457320 0.1674822 0.10306157
## 5           b73_flag.leaf  0.13579885  0.098228903 0.1784036 0.10584563
## 6           b73_immature.cob -0.04184954 -0.008878031 0.1784448 0.11510059
##      MELightcyan      MERed MEMidnightblue MESaddlebrown MEdarkgreen      MEblue
## 1 -0.007377188  0.08298471  -0.04673899  -0.07006608 -0.08069883 -0.11411621
## 2 -0.030236499 -0.03406939  -0.04983032  -0.06227923  0.11153939  0.01054342
## 3  0.028071613 -0.01250192  -0.05142081  -0.06303276 -0.10220547  0.04053513
## 4  0.117858280  0.03847302  -0.05390522  -0.07319145  0.03220624  0.10429605
## 5  0.007325352  0.05646976  -0.04927946  -0.07391966 -0.03848502 -0.08348106
## 6  0.160765917  0.08786477  -0.05718683  -0.08131539  0.07832549  0.11127916
##      MEyellowgreen      MESienna3      MEbrown MEdarkmagenta      MEcyan      MESkyblue
## 1  -0.12057913 -0.04020046 -0.03171364  -0.00761081 -0.05300685 -0.07542256
## 2  -0.07385969 -0.03405919 -0.05252301  -0.02319043 -0.03936977 -0.06602023
## 3  -0.04821862 -0.03173551 -0.04516864  -0.02592365 -0.04479200 -0.06621806
## 4   0.02990732 -0.03485419 -0.01870375  -0.02869022 -0.05046367 -0.07396093
## 5  -0.10371468 -0.03914557 -0.02695942  -0.01993510 -0.05263417 -0.07842008
## 6   0.08755560 -0.01055541  0.01357462  -0.02559522 -0.05590582 -0.08336653
##      MEsteelblue MELightgreen      MEorange      MEpurple MEdarkgrey MEdarkturbquoise
## 1  -0.1005513  -0.11099031 -0.07679406 -0.06141240 -0.07667644  -0.10347059
## 2  -0.1527551  -0.09835241 -0.06143744 -0.05879291 -0.06930740  -0.09870614
## 3  -0.1575741  -0.10198875 -0.06706984 -0.05722312 -0.06789722  -0.10007380
## 4  -0.1395248  -0.11073523 -0.07824363 -0.06651108 -0.06990004  -0.11227703
## 5  -0.1075550  -0.11477376 -0.07881772 -0.06288595 -0.08089353  -0.11043307
## 6  -0.1352101  -0.11587063 -0.08594914 -0.07201833 -0.07751393  -0.11988942
##      MEgrey60 MEdarkorange      MEdarkred      ESalmon      MEyellow      MEgreen
## 1 -0.05056942 -0.10665036 -0.07155753 -0.05993172 -0.15704307 -0.05558605
## 2 -0.04391956 -0.09088958 -0.06275483  0.05870567  0.07204794  0.03651010
## 3 -0.04388569 -0.09471405 -0.06463344  0.08807399  0.07866142  0.14887680
## 4 -0.04430253 -0.09731471 -0.06943144  0.07555833  0.06477265 -0.05400317
## 5 -0.05308113 -0.11114401 -0.07336458 -0.07769095 -0.11181427 -0.05407080
## 6 -0.04675427 -0.10898754 -0.07487255  0.03720614  0.03354497 -0.08438041
##      MEpaleturbquoise MEdarkolivegreen MELightyellow      MEtan MEGreenyellow
## 1  -0.10048775  -0.099071017  -0.04187471  0.09048139  0.05585118
## 2   0.05128207   0.002763192  -0.02259789  0.02979209  0.06519197
## 3   0.08034716   0.047226796  0.11178989  0.03940434  0.08272507
## 4   0.01384662  -0.088556583  -0.08449001 -0.06741027 -0.13539507
## 5  -0.08150179  -0.082491412  -0.01416011  0.07775455  0.01005500
```

```

## 6      -0.05010075      -0.123028958      -0.11782745 -0.11807019      -0.14847475
##      MEpink  MEskyblue3      MEplum1      MEblack      MEviolet      MEgrey
## 1  0.10063595 -0.02716172  0.0203108726  0.02006331 -0.01686075  0.01977880
## 2  0.01364427 -0.01228137 -0.0023294317 -0.03172503 -0.02620461  0.05630691
## 3 -0.00275038 -0.01024970 -0.0068957787 -0.03025022  0.07792427  0.07110698
## 4 -0.13995734 -0.02308838 -0.0001690687 -0.03461621 -0.06792489 -0.13372441
## 5  0.06532183 -0.02065277  0.0337498775  0.05083805  0.01097806 -0.01281604
## 6 -0.14462053 -0.01488177 -0.0103425760 -0.02967007 -0.06626329 -0.17178893
##  cob_b73 cob_dk105 cob_ep1 cob_f7 cob_pe75 coleoptile_b73 coleoptile_dk105
## 1      1      0      0      0      0      0      0
## 2      0      0      0      0      0      1      0
## 3      0      0      0      0      0      0      0
## 4      0      0      0      0      0      0      0
## 5      0      0      0      0      0      0      0
## 6      0      0      0      0      0      0      0
##  coleoptile_ep1 coleoptile_f7 coleoptile_pe75 crown_root_b73 crown_root_dk105
## 1      0      0      0      0      0      0
## 2      0      0      0      0      0      0
## 3      0      0      0      1      0      0
## 4      0      0      0      0      0      0
## 5      0      0      0      0      0      0
## 6      0      0      0      0      0      0
##  crown_root_ep1 crown_root_f7 crown_root_pe75 first_elongated_internode_b73
## 1      0      0      0      0
## 2      0      0      0      0
## 3      0      0      0      0
## 4      0      0      0      1
## 5      0      0      0      0
## 6      0      0      0      0
##  first_elongated_internode_dk105 first_elongated_internode_ep1
## 1      0      0
## 2      0      0
## 3      0      0
## 4      0      0
## 5      0      0
## 6      0      0
##  first_elongated_internode_f7 first_elongated_internode_pe75 flag_leaf_b73
## 1      0      0      0
## 2      0      0      0
## 3      0      0      0
## 4      0      0      0
## 5      0      0      1
## 6      0      0      0
##  flag_leaf_dk105 flag_leaf_ep1 flag_leaf_f7 flag_leaf_pe75 immature_cob_b73
## 1      0      0      0      0      0
## 2      0      0      0      0      0
## 3      0      0      0      0      0
## 4      0      0      0      0      0
## 5      0      0      0      0      0
## 6      0      0      0      0      1
##  immature_cob_dk105 immature_cob_ep1 immature_cob_f7 immature_cob_pe75
## 1      0      0      0      0
## 2      0      0      0      0
## 3      0      0      0      0

```



```

## 4          0          0          0          0
## 5          0          0          0          0
## 6          0          0          0          0
##   immature_tassel_b73 immature_tassel_dk105 immature_tassel_ep1
## 1          0          0          0
## 2          0          0          0
## 3          0          0          0
## 4          0          0          0
## 5          0          0          0
## 6          0          0          0
##   immature_tassel_f7 immature_tassel_pe75 leaf_1_b73 leaf_1_dk105 leaf_1_ep1
## 1          0          0          0          0          0
## 2          0          0          0          0          0
## 3          0          0          0          0          0
## 4          0          0          0          0          0
## 5          0          0          0          0          0
## 6          0          0          0          0          0
##   leaf_1_f7 leaf_1_pe75 leaf_3_blade_b73 leaf_3_blade_dk105 leaf_3_blade_ep1
## 1          0          0          0          0          0
## 2          0          0          0          0          0
## 3          0          0          0          0          0
## 4          0          0          0          0          0
## 5          0          0          0          0          0
## 6          0          0          0          0          0
##   leaf_3_blade_f7 leaf_3_blade_pe75 leaf_3_sheath_b73 leaf_3_sheath_dk105
## 1          0          0          0          0
## 2          0          0          0          0
## 3          0          0          0          0
## 4          0          0          0          0
## 5          0          0          0          0
## 6          0          0          0          0
##   leaf_3_sheath_ep1 leaf_3_sheath_f7 leaf_3_sheath_pe75 leaf_5_b73 leaf_5_dk105
## 1          0          0          0          0          0
## 2          0          0          0          0          0
## 3          0          0          0          0          0
## 4          0          0          0          0          0
## 5          0          0          0          0          0
## 6          0          0          0          0          0
##   leaf_5_elongation_zone_b73 leaf_5_elongation_zone_dk105
## 1          0          0
## 2          0          0
## 3          0          0
## 4          0          0
## 5          0          0
## 6          0          0
##   leaf_5_elongation_zone_ep1 leaf_5_elongation_zone_f7
## 1          0          0
## 2          0          0
## 3          0          0
## 4          0          0
## 5          0          0
## 6          0          0
##   leaf_5_elongation_zone_pe75 leaf_5_ep1 leaf_5_f7 leaf_5_mature_b73
## 1          0          0          0          0

```

```

## 2          0          0          0          0
## 3          0          0          0          0
## 4          0          0          0          0
## 5          0          0          0          0
## 6          0          0          0          0
## leaf_5_mature_dk105 leaf_5_mature_ep1 leaf_5_mature_f7 leaf_5_mature_pe75
## 1          0          0          0          0
## 2          0          0          0          0
## 3          0          0          0          0
## 4          0          0          0          0
## 5          0          0          0          0
## 6          0          0          0          0
## leaf_5_meristem_b73 leaf_5_meristem_dk105 leaf_5_meristem_ep1
## 1          0          0          0
## 2          0          0          0
## 3          0          0          0
## 4          0          0          0
## 5          0          0          0
## 6          0          0          0
## leaf_5_meristem_f7 leaf_5_meristem_pe75 leaf_5_pe75 leaf_8_b73 leaf_8_dk105
## 1          0          0          0          0          0
## 2          0          0          0          0          0
## 3          0          0          0          0          0
## 4          0          0          0          0          0
## 5          0          0          0          0          0
## 6          0          0          0          0          0
## leaf_8_ep1 leaf_8_f7 leaf_8_pe75 mature_seed_40_dap_b73
## 1          0          0          0          0
## 2          0          0          0          0
## 3          0          0          0          0
## 4          0          0          0          0
## 5          0          0          0          0
## 6          0          0          0          0
## mature_seed_40_dap_dk105 mature_seed_40_dap_ep1 mature_seed_40_dap_f7
## 1          0          0          0
## 2          0          0          0
## 3          0          0          0
## 4          0          0          0
## 5          0          0          0
## 6          0          0          0
## meiotic_tassel_b73 meiotic_tassel_dk105 meiotic_tassel_ep1 meiotic_tassel_f7
## 1          0          0          0          0
## 2          0          0          0          0
## 3          0          0          0          0
## 4          0          0          0          0
## 5          0          0          0          0
## 6          0          0          0          0
## meiotic_tassel_pe75 mesophyll_b73 mesophyll_dk105 mesophyll_ep1 mesophyll_f7
## 1          0          0          0          0          0
## 2          0          0          0          0          0
## 3          0          0          0          0          0
## 4          0          0          0          0          0
## 5          0          0          0          0          0
## 6          0          0          0          0          0

```

```

## mesophyll_pe75 prepollinated_cob_b73 prepollinated_cob_dk105
## 1      0      0      0
## 2      0      0      0
## 3      0      0      0
## 4      0      0      0
## 5      0      0      0
## 6      0      0      0
## prepollinated_cob_ep1 prepollinated_cob_f7 prepollinated_cob_pe75
## 1      0      0      0
## 2      0      0      0
## 3      0      0      0
## 4      0      0      0
## 5      0      0      0
## 6      0      0      0
## primary_root_b73 primary_root_dk105 primary_root_elongation_zone_b73
## 1      0      0      0
## 2      0      0      0
## 3      0      0      0
## 4      0      0      0
## 5      0      0      0
## 6      0      0      0
## primary_root_elongation_zone_dk105 primary_root_elongation_zone_ep1
## 1      0      0
## 2      0      0
## 3      0      0
## 4      0      0
## 5      0      0
## 6      0      0
## primary_root_elongation_zone_f7 primary_root_elongation_zone_pe75
## 1      0      0
## 2      0      0
## 3      0      0
## 4      0      0
## 5      0      0
## 6      0      0
## primary_root_ep1 primary_root_f7 primary_root_meristematic_zone_b73
## 1      0      0      0
## 2      0      0      0
## 3      0      0      0
## 4      0      0      0
## 5      0      0      0
## 6      0      0      0
## primary_root_meristematic_zone_dk105 primary_root_meristematic_zone_ep1
## 1      0      0
## 2      0      0
## 3      0      0
## 4      0      0
## 5      0      0
## 6      0      0
## primary_root_meristematic_zone_f7 primary_root_meristematic_zone_pe75
## 1      0      0
## 2      0      0
## 3      0      0
## 4      0      0

```

```

## 5          0          0
## 6          0          0
## primary_root_pe75 root_hair_zone_b73 root_hair_zone_dk105 root_hair_zone_ep1
## 1          0          0          0          0
## 2          0          0          0          0
## 3          0          0          0          0
## 4          0          0          0          0
## 5          0          0          0          0
## 6          0          0          0          0
## root_hair_zone_f7 root_hair_zone_pe75 seed_10_dap_b73 seed_10_dap_dk105
## 1          0          0          0          0
## 2          0          0          0          0
## 3          0          0          0          0
## 4          0          0          0          0
## 5          0          0          0          0
## 6          0          0          0          0
## seed_10_dap_ep1 seed_10_dap_f7 seed_10_dap_pe75 seed_15_dap_b73
## 1          0          0          0          0
## 2          0          0          0          0
## 3          0          0          0          0
## 4          0          0          0          0
## 5          0          0          0          0
## 6          0          0          0          0
## seed_15_dap_dk105 seed_15_dap_ep1 seed_15_dap_f7 seed_15_dap_pe75
## 1          0          0          0          0
## 2          0          0          0          0
## 3          0          0          0          0
## 4          0          0          0          0
## 5          0          0          0          0
## 6          0          0          0          0
## seed_20_dap_b73 seed_20_dap_dk105 seed_20_dap_ep1 seed_20_dap_f7
## 1          0          0          0          0
## 2          0          0          0          0
## 3          0          0          0          0
## 4          0          0          0          0
## 5          0          0          0          0
## 6          0          0          0          0
## seed_20_dap_pe75 seed_25_dap_b73 seed_25_dap_dk105 seed_25_dap_ep1
## 1          0          0          0          0
## 2          0          0          0          0
## 3          0          0          0          0
## 4          0          0          0          0
## 5          0          0          0          0
## 6          0          0          0          0
## seed_25_dap_f7 seed_25_dap_pe75 seed_30_dap_b73 seed_30_dap_dk105
## 1          0          0          0          0
## 2          0          0          0          0
## 3          0          0          0          0
## 4          0          0          0          0
## 5          0          0          0          0
## 6          0          0          0          0
## seed_30_dap_ep1 seed_30_dap_f7 seed_30_dap_pe75 seminal_root_b73
## 1          0          0          0          0
## 2          0          0          0          0

```

```

## 3      0      0      0      0
## 4      0      0      0      0
## 5      0      0      0      0
## 6      0      0      0      0
##   seminal_root_dk105 seminal_root_ep1 seminal_root_f7 seminal_root_pe75
## 1      0      0      0      0
## 2      0      0      0      0
## 3      0      0      0      0
## 4      0      0      0      0
## 5      0      0      0      0
## 6      0      0      0      0
##   silk_b73 silk_dk105 silk_ep1 silk_f7 silk_pe75
## 1      0      0      0      0      0
## 2      0      0      0      0      0
## 3      0      0      0      0      0
## 4      0      0      0      0      0
## 5      0      0      0      0      0
## 6      0      0      0      0      0

```

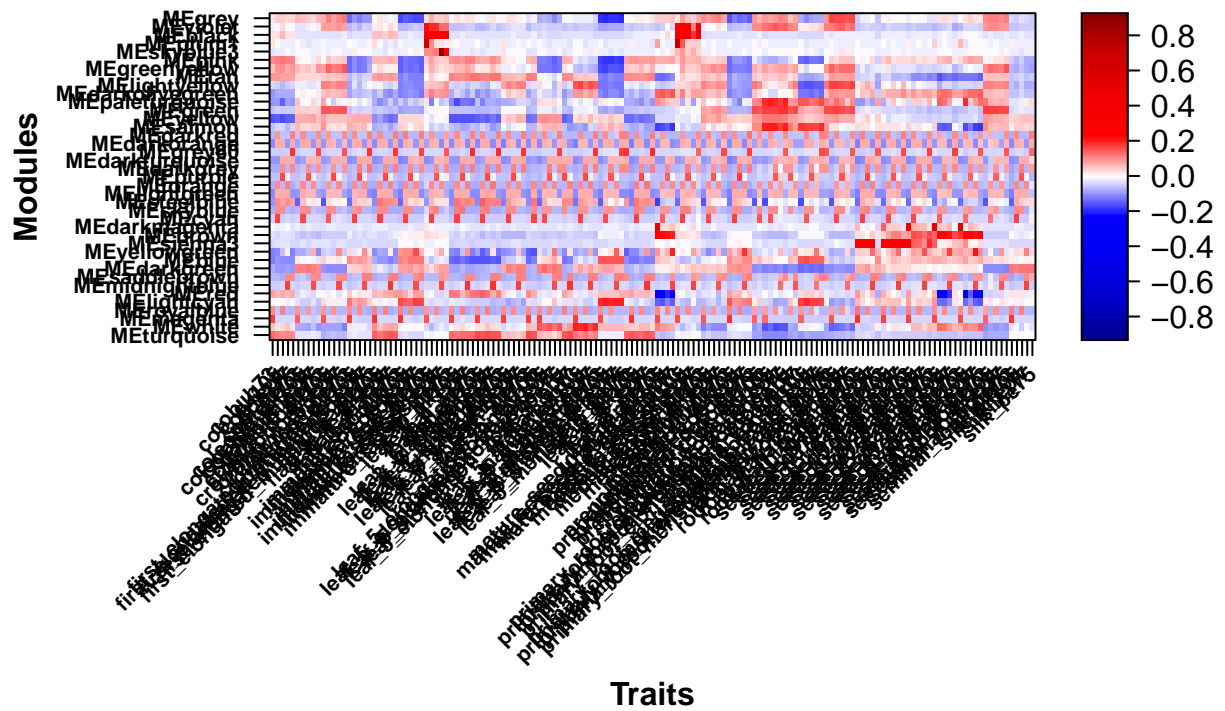
```

rownames(heatmap.data)<-heatmap.data$Row.names
heatmap.data$Row.names<-NULL

plotTitle<-paste0("Correlation plot by tissue using ", NormType)
#png(paste0("./CorPlots/ti_", NormType, "_corplot.png"), width=1600, height=800) #ti_CPM_corplot
CorLevelPlot(heatmap.data,
  x = names(s_bin_metadata),
  y = names(module_eigengenes),
  titleX="Traits", titleY="Modules", main=plotTitle,
  rotLabX = 45, rotTitleY = 90,
  cexCorval = 0, cexLabY = 0.7, cexLabX = 0.7
)

```

Correlation plot by tissue using RPKM



```
#dev.off()
```