Eigengene Network Analysis:
Male-Female Mouse Liver Comparison
R Tutorial

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This is a self-contained R software tutorial that illustrates how to carry out an eigengene network analysis across two datasets. The two data sets correspond to gene expression measurements in the livers of male and female mice. This supplement describes the microarray data set. The R code allows to reproduce the Figures and tables reported in Langelder and Horvath (2007).

Some familiarity with the R software is desirable but the document is fairly self-contained. The data and biological implications are described in the following refence

- *Langfelder P, Horvath S (2007) Eigengene networks for studying the relationships between co-expression modules. BMC Bioinformatics*

To facilitate comparison with the original analysis, we use the microarray normalization procedures and gene selection procedures described in Ghazalpouret al (2006).

This tutorial and the data files can be found at the following webpage:
http://www.genetics.ucla.edu/labs/horvath/CoexpressionNetwork/EigengeneNetwork
More material on weighted network analysis can be found here
http://www.genetics.ucla.edu/labs/horvath/CoexpressionNetwork/

Microarray Data

The Agilent microarrays measured gene expression profiles in female mice of an F2 mouse intercross described in Ghazalpour et al 2006. The gene expression data were obtained from liver tissues of male and female mice.

The F2 mouse intercross data set (referred to as B × H cross) was obtained from liver tissues of female and male mice. The F2 intercross was based on the inbred strains C3H/HeJ and C57BL/6J (Ghazalpour et al. 2006; Wang et al. 2006).

Body weight and related physiologic (“clinical”) traits were measured for the mice.

B × H mice are ApoE null (ApoE –/–) and thus hyperlipidemic and were fed a high-fat diet

The B × H mice were sacrificed at 24 weeks.

BxH mouse data

For this analysis, we started with the 3421 genes (probesets) used in Ghazalpour et al 2006. The genes were the most connected genes among the 8000 most varying genes of the female liver dataset. We filtered out 6 genes that had zero variance in at least one of the four tissue datasets, leaving 3415 genes. For each of the data

sets (corresponding to the 4 tissues), the Pearson correlation matrix of the genes was calculated and turned into adjacencies by raising the absolute value to power beta=6. From the adjacency matrices, we calculated the TOM similarities which were then used to calculate the consensus dissimilarity.

The dissimilarity was used as input in average-linkage

hierarchical clustering. Branches of the resulting dendrogram were identified using the ``dynamic'' tree-cut algorithm used in Ghazalpour et al 2006. The maximum merging height for the cutting was set to 0.97, and minimum module size to 25. This procedure resulted in 12 initial consensus modules. To determine whether some of the initial consensus modules were too close, we calculated their eigengenes in each dataset, and formed their correlation matrices (one for each dataset). A ``minimum consensus similarity'' matrix was calculated as the minimum of the dataset eigengene correlation matrices; this matrix was turned into dissimilarity by subtracting it from one and used as input of average-linkage hierarchical clustering again. In the resulting dendrogram of consensus modules, branches with merging height less than 0.25 were identified and modules on these branches were merged. Such branches correspond to modules whose eigengenes have a correlation of 0.75 or higher, which we judge to be close enough to be merged. This module-merging procedure resulted in 11 final consensus modules that are described in the main text.

Animal husbandry and physiological trait measurements.

C57BL/6J apoE null (B6.apoE–/–) mice were purchased from the Jackson Laboratory (Bar Harbor, Maine, United States) and C3H/HeJ apoE null (C3H.apoE–/–) mice were bred by backcrossing B6.apoE–/– to C3H/HeJ for ten generations with selection at each generation for the targeted ApoE–/– alleles on Chromosome 7. All mice were fed ad libidum and maintained on a 12-h light/dark cycle. F2 mice were generated by crossing B6.apoE–/– with C3H.apoE–/– and subsequently intercrossing the F1 mice. F2 mice were fed Purina Chow (Ralston-Purina Co., St. Louis, Missouri, United States)

Physiologic traits

The original data Ghazalpour et al 2006 contain 26 traits, some of which are highly correlated. Here we consider 10 physiologic traits related to metabolic syndrome.

To select independent traits, we clustered the traits with dissimilarity given by correlation subtracted from one. Branches of the dendrogram correspond to groups of highly related traits; we chose a height cutoff of 0.35 (that is, correlation of 0.65) for the branch detection. For each branch, we selected a representative trait by taking the trait that is closest to the branch ``eigentrait", that is the first principal component of the trait matrix (we would like to emphasize that the selected traits are actually measured traits and are not composite measurements). This procedure resulted in 20 traits. For brevity we only report significance for traits for which there is at least one module whose (eigengene–trait) correlation p-value is 0.01 or less. This restriction leads to 6 potentially interesting traits which we used in the analysis of module significance used in the main text.

At the time of euthanasia, all mice were weighed and measured from the tip of the nose to the anus. Fat depots, plasma lipids (free fatty acids and triglycerides), plasma high-density liporprotein (HDL) cholesterol and total cholesterol, and plasma insulin levels were measured as previously described. Very low-density lipoprotein (VLDL)/LDL cholesterol levels were calculated by subtracting HDL cholesterol from total cholesterol levels. Plasma glucose concentrations were measured using a glucose kit (#315–100; Sigma, St. Louis, Missouri, United States). Plasma leptin, adiponectin, and MCP-1 levels were measured using mouse enzyme-linked immunoabsorbent (ELISA) kits (#MOB00, #MRP300, and MJE00; R&D Systems, Minneapolis, Minnesota, United States).

Microarray analysis.

RNA preparation and array hybridizations were performed at Rosetta Inpharmatics (Seattle, Washington, United States). The custom ink-jet microarrays used in this study (Agilent Technologies [Palo Alto, California, United States], previously described [3,45]) contain 2,186 control probes and 23,574 non-control oligonucleotides extracted from mouse Unigene clusters and combined with RefSeq sequences and RIKEN full-length clones. Mouse livers were homogenized and total RNA extracted using Trizol reagent (Invitrogen, Carlsbad, California, United States) according to manufacturer's protocol. Three g of total RNA was reverse transcribed and labeled with either Cy3 or Cy5 fluorochromes. Purified Cy3 or Cy5 complementary RNA was hybridized to at least two microarray slides with fluor reversal for 24 h in a hybridization chamber, washed, and scanned using a laser confocal scanner. Arrays were quantified on the basis of spot intensity relative to background, adjusted for experimental variation between arrays using average intensity over multiple channels, and fit to an error model to determine significance (type I error). Gene expression is reported as the ratio of the mean log10 intensity (mlratio) relative to the pool derived from 150 mice randomly selected from the F2 population.

Microarray data reduction.

In order to minimize noise in the gene expression dataset, several data-filtering steps were taken. First, preliminary evidence showed major differences in gene expression levels between sexes among the F2 mice used, and therefore only female mice were used for network construction. The construction and comparison of the male network will be reported elsewhere. Only those mice with complete phenotype, genotype, and array data were used. This gave a final experimental sample of 135 female mice used for network construction. To reduce the computational burden and to possibly enhance the signal in our data, we used only the 8,000 most-varying female liver genes in our preliminary network construction. For module detection, we limited our analysis to the 3,600 most-connected genes because our module construction method and visualization tools cannot handle larger datasets at this point. By definition, module genes are highly connected with the genes of their module (i.e., module genes tend to have relatively high connectivity). Thus, for the purpose of module detection, restricting the analysis to the most-connected genes should not lead to major information loss. Since the network nodes in our analysis correspond to genes as opposed to probesets, we eliminated multiple probes with similar expression patterns for the same gene. Specifically, the 3,600 genes were examined, and where appropriate, gene isoforms and genes containing duplicate probes were excluded by using only those with the highest expression among the redundant transcripts. This final filtering step yielded a count of 3,421 genes for the experimental network construction.

Weighted gene co-expression network construction.

Constructing a weighted co-expression network is critical for identifying modules and for defining the intramodular connectivity. In co-expression networks, nodes correspond to genes, and connection strengths are determined by the pairwise correlations between expression profiles. In contrast to unweighted networks, weighted networks use soft thresholding of the Pearson correlation matrix for determining the connection strengths between two genes. Soft thresholding of the Pearson correlation preserves the continuous nature of the gene co-expression information, and leads to results that are highly robust with respect to the weighted network construction method (Zhang and Horvath 2005).

The theory of the network construction algorithm is described in detail elsewhere Zhang and Horvath (2005). Briefly, a gene co-expression similarity measure (absolute value of the Pearson product moment correlation) was used to relate every pairwise gene–gene relationship. An adjacency matrix was then constructed using a “soft” power adjacency function aij = |cor(xi, xj)|^ where the absolute value of the Pearson correlation measures gene is the co-expression similarity, and aij represents the resulting adjacency that measures the connection strengths. The network connectivity (kall) of the ith gene is the sum of the connection strengths with the other genes. This summation performed over all genes in a particular module is the intramodular connectivity (kin). The network satisfies scale-free topology if the connectivity distribution of the nodes follows an inverse power law, (frequency of connectivity p(k) follows an approximate inverse power law in k, i.e., p(k) ~ k^{−). We chose a power of = 6 based on the scale-free topology criterion. This criterion says that the power parameter, , is the lowest integer such that the resulting network satisfies approximate scale-free topology (linear model fitting index R2 of the regression line between log(p(k)) and log(k) is larger than 0.8). This criterion uses the fact that gene co-expression networks have been found to satisfy approximate scale-free topology. Since we are using a weighted network as opposed to an unweighted network, the biological findings are highly robust with respect to the choice of this power. Many co-expression networks satisfy the scale-free property only approximately.

Consensus module detection

Consensus module detection is similar to the individual dataset module detection in that it uses hierarchical clustering of genes according to a measure of gene dissimilarity.
We use a gene-gene ``consensus" dissimilarity measure Dissim(i,j) as input of average linkage hierarchical clustering.
We define modules as branches of the tree. To cut-off branches we use a fixed height cut-off. Modules must contains a minimum number n0 of genes.
Module detection proceeds along the following steps.
(1) perform a hierarchical clustering using the consensus dissimilarity measure;
(2) cut the clustering tree at a fixed height cut-off;
(3) each cut branch with at least n0 genes is considered a separate module;
(4) all other genes are considered unassigned and are colored in ``grey".
The resulting modules will depend to some degree on the cut height and minimum size.

The analysis here is identical to the female four-tissue one described above; the only difference was that the maximum joining height for the consensus dendrogram was 0.995 to make our results easier to compare to Ghazalpour et al 2006. The minimum module size was dataset to 40.

Topological Overlap and Module Detection

A major goal of network analysis is to identify groups, or "modules", of densely interconnected genes. Such groups are often identified by searching for genes with similar patterns of connection strengths to other genes, or high "topological overlap". It is important to recognize that correlation and topological overlap are very different ways of describing the relationship between a pair of genes: while correlation considers each pair of genes in isolation, topological overlap considers each pair of genes in relation to all other genes in the network. More specifically, genes are said to have high topological overlap if they are both strongly connected to the same group of genes in the network (i.e. they share the same "neighborhood"). Topological overlap thus serves as a crucial filter to exclude spurious or isolated connections during network construction (Yip and Horvath 2007). To calculate the topological overlap for a pair of genes, their connection strengths with all other genes in the network are compared. By calculating the topological overlap for all pairs of genes in the network, modules can be identified.

The advantages and disadvantages of the topological overlap measure are reviewed in Yip and Horvath (2007) and Zhang and Horvath (2005).

Definition of the Eigengene

Denote by X the expression data of a given module (rows are genes, columns are microarray samples).

First, the gene expression data X are scaled so that each gene expression profile has mean 0 and variance 1. Next, the gene-expression data X are decomposed via singular value decomposition ($X=UDV^T$) and the value of the first module eigengene, V_1, represents the module eigengene. Specifically, V_1 corresponds to the largest singular value.

This definition is equivalent to defining the module eigengene as the first principal component of cor(t(X)), i.e. the correlation matrix of the gene expression data.

References

The microarray data and processing steps are described in

- *Ghazalpour A, Doss S, Zhang B, Wang S, Plaisier C, Castellanos R, Brozell A, Schadt EE, Drake TA, Lusis AJ, Horvath S (2006) "Integrating Genetic and Network Analysis to Characterize Genes Related to Mouse Weight". PLoS Genetics. Volume 2 | Issue 8 | AUGUST 2006*

The mouse cross is described in

- *Wang S, Yehya N, Schadt EE, Wang H, Drake TA, et al. (2006) Genetic and genomic analysis of a fat mass trait with complex inheritance reveals marked sex specificity. PLoS Genet 2:e15*

Weighted gene co-expression network analysis is described in

- *Bin Zhang and Steve Horvath (2005) "A General Framework for Weighted Gene Co-Expression Network Analysis", Statistical Applications in Genetics and Molecular Biology: Vol. 4: No. 1, Article 17.*

Other references

- *Yip A, Horvath S (2007) Gene network interconnectedness and the generalized topological overlap measure BMC Bioinformatics 2007, 8:22*

# Absolutely no warranty on the code. Please contact Peter Langfelder and Steve Horvath
#with suggestions.

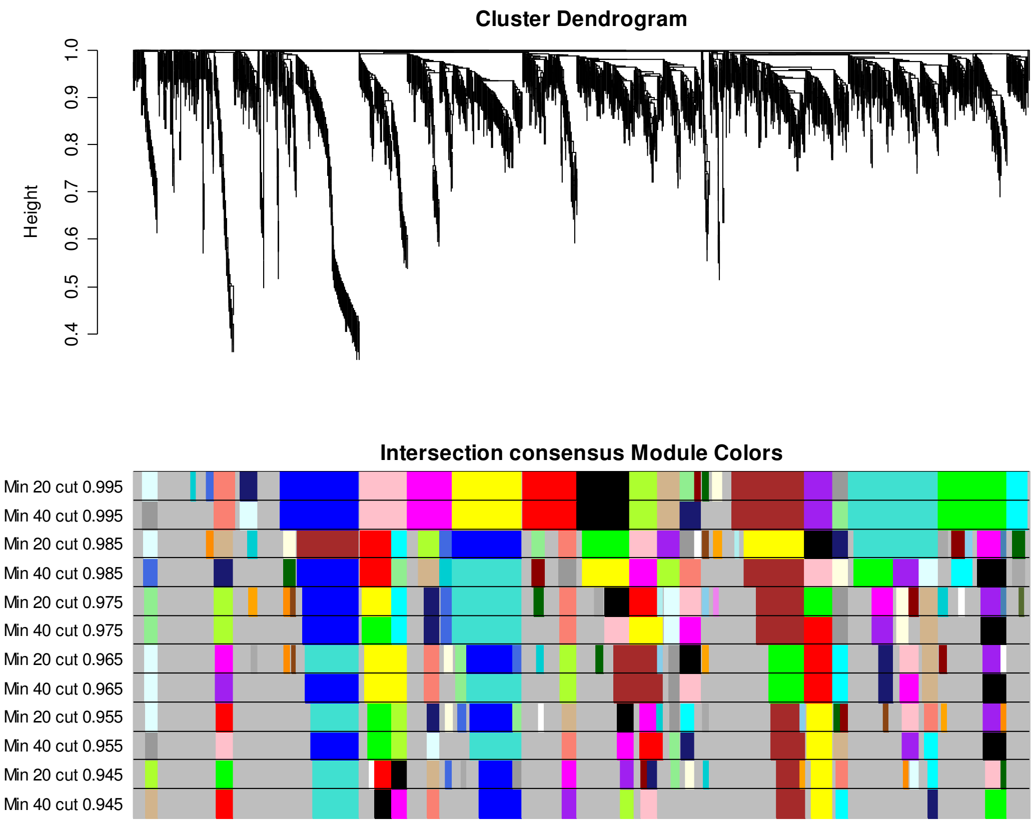
# Downloading the R software
# 1) Go to http://www.R-project.org, download R and install it on your computer
# After installing R, you need to install several additional R library packages:
# For example to install Hmisc, open R,
# go to menu "Packages\Install package(s) from CRAN",
# then choose Hmisc. R will automatically install the package.
# When asked "Delete downloaded files (y/N)? ", answer "y".
# Do the same for some of the other libraries mentioned below. But note that
# several libraries are already present in the software so there is no need to re-install them.

# Download the zip file containing:
# 1) R function file: "NetworkFunctions.txt", which contains several R functions
#   needed for Network Analysis.
# 2) The data files and this tutorial

# Unzip all the files into the same directory.
# The user should copy and paste the following script into the R session.
# Text after "#" is a comment and is automatically ignored by R.

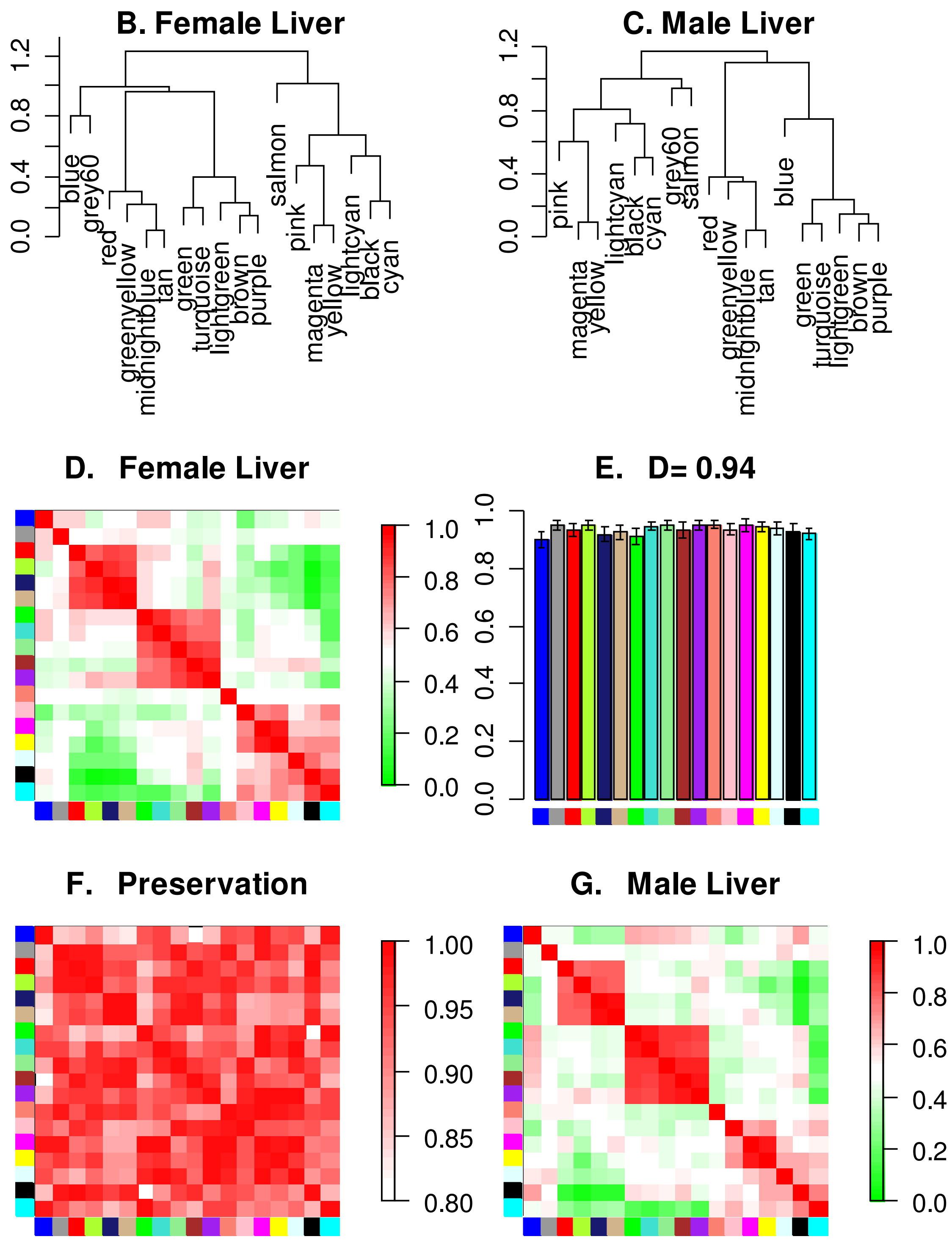
```
source("NetworkFunctions-Mouse.R");

set.seed(1);  #needed for .Random.seed to be defined

options(stringsAsFactors = FALSE);

# Read in the datasets

data =
read.table("cnew_liver_bxh_f2female_8000mvgenes_p3600_UNIQUE_tommodules.xls",
                 header=T, strip.white=T, comment.char="")

AllLiverColors = data$module;
```

```
data2 = read.csv("LiverMaleFromLiverFemale3600.csv",
                 header=T, strip.white=T, comment.char="")
data2_expr = data2[, c(9:(dim(data2)[2]))];

# Separate out auxiliary data

AuxData = data[,c(1:8,144:150)]
names(AuxData) = colnames(data)[c(1:8,144:150)]
ProbeNames = data[,1];
SampleNames = colnames(data)[9:143];

# General settings and parameters

No.Sets = 2;
Set.Labels = c("Female Liver", "Male Liver");
ModuleMinSize = 40;

# Put  the data into a standard "multi set" structure: vector of lists

ExprData = vector(mode = "list", length = No.Sets);
ExprData[[1]] = list(data = data.frame(t(data[,-c(1:8,144:150)])));
ExprData[[2]] = list(data = data.frame(t(data2_expr)));

names(ExprData[[1]]$data) = ProbeNames;
names(ExprData[[2]]$data) = data2[, 1];

ExprData = KeepCommonProbes(ExprData);

#Impute zeros into normalized ExprData for NAs

for (set in 1:No.Sets)
{
  ExprData[[set]]$data = scale(ExprData[[set]]$data);
  ExprData[[set]]$data[is.na(ExprData[[set]]$data)] = 0;
}

# Clean-up variables not needed anymore

rm(data, data2, data2_expr);
collect_garbage();

#  Read in the clinical trait data

trait_data = read.csv("ClinicalTraits.csv",
                      header=T, strip.white=T, comment.char="");

Gender = trait_data$sex;

AllTraits = trait_data[, -c(31, 16)];
AllTraits = AllTraits[, c(2, 11:36) ];

# Put the traits into a structure resembling the structure of expression data
# For each set only keep traits for the samples that also have expression data

Traits = vector(mode="list", length = No.Sets);
for (set in 1:No.Sets)
{
  SetSampleNames = data.frame(Names = row.names(ExprData[[set]]$data));
  SetTraits = merge(SetSampleNames, AllTraits, by.y = "Mice", by.x = "Names", all
= FALSE, sort = FALSE);
  Traits[[set]] = list(data = SetTraits[, -1]);
  row.names(Traits[[set]]$data) = SetTraits[,1 ];
}

rm(AllTraits); rm(SetTraits); rm(SetSampleNames); rm(trait_data);

collect_garbage();

# More general settings and parameters

OutFileBase = "Mouse-FeMaleConsensus-";
OutDir = ""
PlotDir = ""
FuncAnnoDir = ""
NetworkFile = "Mouse-FeMaleConsensus-Network.RData";
StandardCex = 1.4;

# Calculate standard weighted gene coexpression networks in all sets

Network = GetNetwork(ExprData = ExprData, DegreeCut = 0,
                     BranchHeightCutoff = 0.985, ModuleMinSize = 80, verbose =
4);

#save(Network, file=NetworkFile);
#load(file=NetworkFile);

LiverColors = AllLiverColors[Network$SelectedGenes];
#--------------------------------------------------------------------------------
# Calculate and cluster consensus gene dissimilarity

ConsBranchHeightCut = 0.95; ConsModMinSize = ModuleMinSize;
ConsModMinSize2 = 20;

Consensus = IntersectModules(Network = Network,
                             ConsBranchHeightCut = ConsBranchHeightCut,
ConsModMinSize = ConsModMinSize,
                             verbose = 4)

# Detect modules in the dissimilarity for several cut heights

No.Genes = sum(Network$SelectedGenes);
ConsCutoffs = seq(from = 0.945, to = 0.995, by = 0.01);
No.Cutoffs = length(ConsCutoffs);
ConsensusColor = array(dim = c(No.Genes, 2*No.Cutoffs));
#ConsensusColor2 = array(dim = c(No.Genes, No.Cutoffs));
for (cut in 1:No.Cutoffs)
{
  ConsensusColor[ ,2*cut-1] = labels2colors(cutreeDynamic(dendro =
Consensus$ClustTree,
                                     deepSplit = FALSE,
                                     cutHeight = ConsCutoffs[cut],
minClusterSize=ConsModMinSize,
                                     method ="tree"));
  ConsensusColor[ ,2*cut] = labels2colors(cutreeDynamic(dendro =
Consensus$ClustTree,
                                     deepSplit = FALSE,
                                     cutHeight = ConsCutoffs[cut],
minClusterSize=ConsModMinSize2,
                                     method ="tree"));
}

# Plot found module colors with the consensus dendrogram

SizeWindow(12,9);
par(mfrow = c(2,1));
par(mar=(c(2,7,2,2)+0.1));
plot(Consensus$ClustTree, labels = FALSE);
labels1 = paste("Min", ConsModMinSize, "cut", ConsCutoffs);
labels2 = paste("Min", ConsModMinSize2, "cut", ConsCutoffs);
labels = as.vector(rbind(labels1, labels2));
hclustplotn(Consensus$ClustTree, ConsensusColor,
            main=paste("Intersection consensus Module Colors"), RowLabels =
labels);
```



```
# We choose the cutoff height to be 0.995.

ChosenCut = 6;
Consensus$Colors = ConsensusColor[, 2*ChosenCut-1];

# Redo the set module detection using dynamic colors and the same parameters as
the consensus, so
# they are comparable.
```

```
ModuleMergeCut = 0.25;

MergedSetColors = Network$Colors;
for (set in 1:No.Sets)
{
  Network$Colors[, set] = labels2colors(cutreeDynamic(dendro =
Network$ClusterTree[[set]]$data,
                                     deepSplit = FALSE,
                                     cutHeight= ConsCutoffs[ChosenCut],
minClusterSize=ConsModMinSize,
                                     method = "tree"));
  MergedSetCols = MergeCloseModules(ExprData, Network, Network$Colors[, set],
CutHeight = ModuleMergeCut,
                                   OnlySet = set,
                                   OrderedPCs = NULL, verbose = 4, print.level =
0);
  MergedSetColors[, set] = MergedSetCols$Colors;
  collect_garbage();
}

# Calculate "raw" consensus MEs and plot them

PCs = NetworkModulePCs(ExprData, Network, UniversalModuleColors =
Consensus$Colors,
                       verbose=3)
OrderedPCs = OrderPCs(PCs, GreyLast=TRUE, GreyName = "MEgrey");

# Standard plot of differential eigengene network analysis

SizeWindow(7, 9);
par(cex = StandardCex/1.4);
PlotCorPCsAndDendros(OrderedPCs, Titles = Set.Labels, ColorLabels = TRUE,
IncludeSign = TRUE,
           IncludeGrey = FALSE, plotCPMeasure = FALSE,
           plotMeans = T, CPzlim = c(0.8,1), plotErrors = TRUE, marHeatmap =
c(1.6,2.7,1.6,6.0),
            marDendro = c(0.2,3,1.2,2), LetterSubPlots = TRUE, Letters =
"BCDEFGHIJKLMNOPQRSTUV",
            PlotDiagAdj = TRUE);
```


B. Female Liver

C. Male Liver

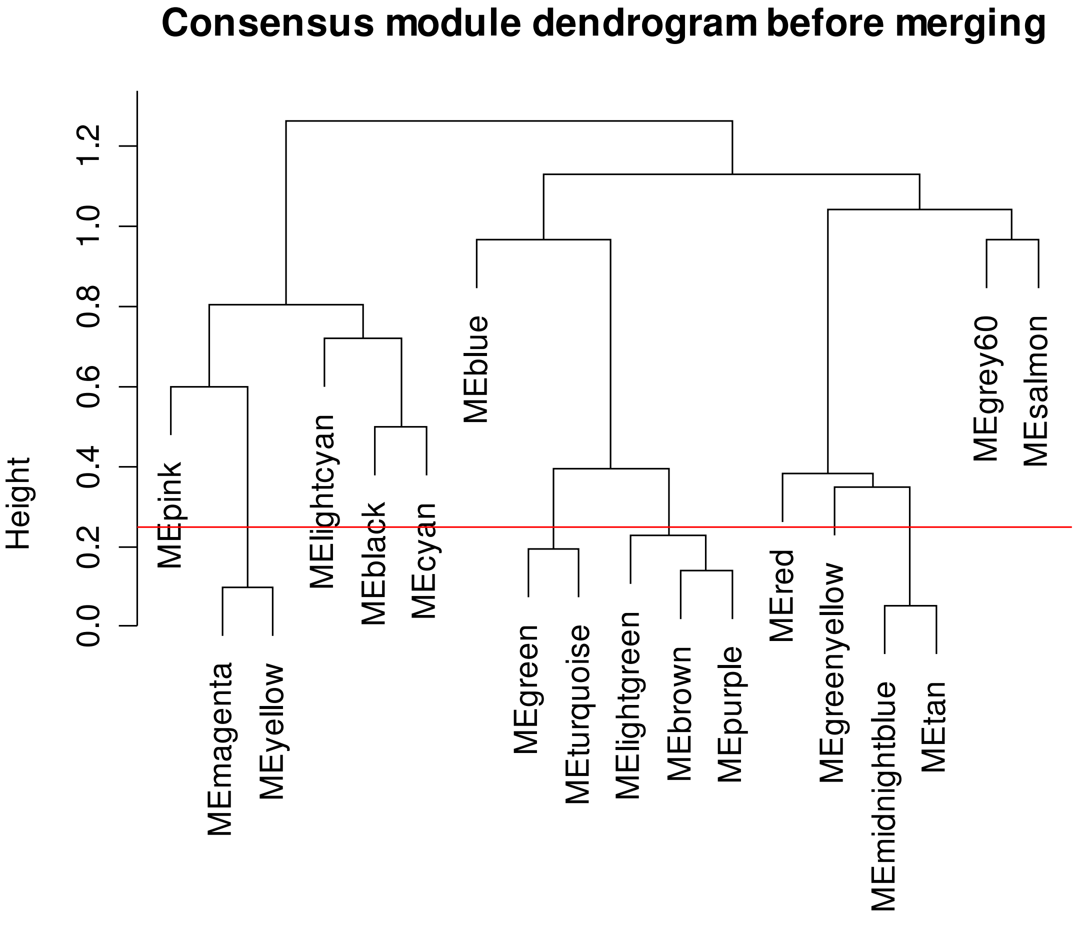
D. Female Liver

E. D= 0.94

F. Preservation

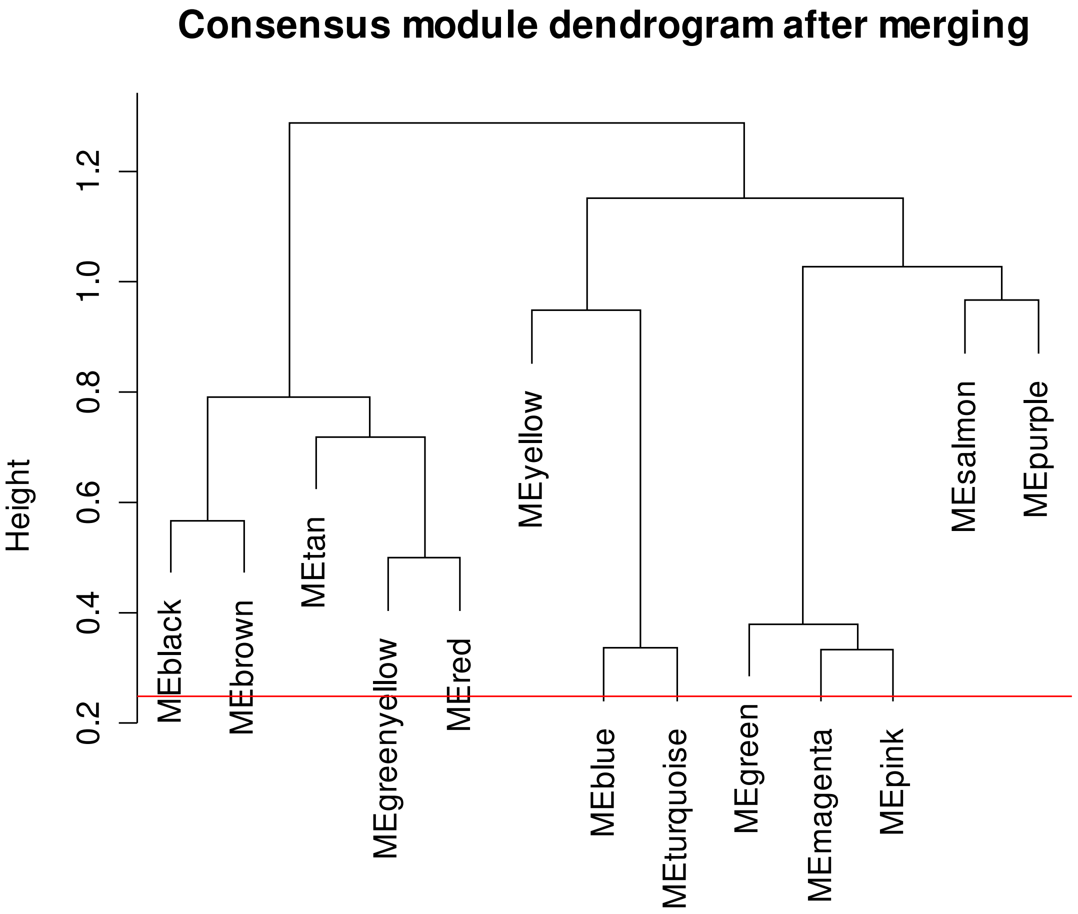
G. Male Liver

```
# Merging close modules:
# Cluster the found module eigengenes and merge ones that are too close to one
another _in both sets_.

MergedColors = MergeCloseModules(ExprData, Network, Consensus$Colors, CutHeight =
ModuleMergeCut,
                       OrderedPCs = OrderedPCs, IncludeGrey = FALSE, verbose = 4,
print.level = 0);

# Plot the detailed consensus dendrogram with the module clustering

SizeWindow(7,7);
par(mfrow = c(1,1));
par(cex = StandardCex/1.2);
plot(MergedColors$ClustTree, main = "Consensus module dendrogram before merging",
xlab = "", sub = "");
abline(ModuleMergeCut, 0, col="red");
```



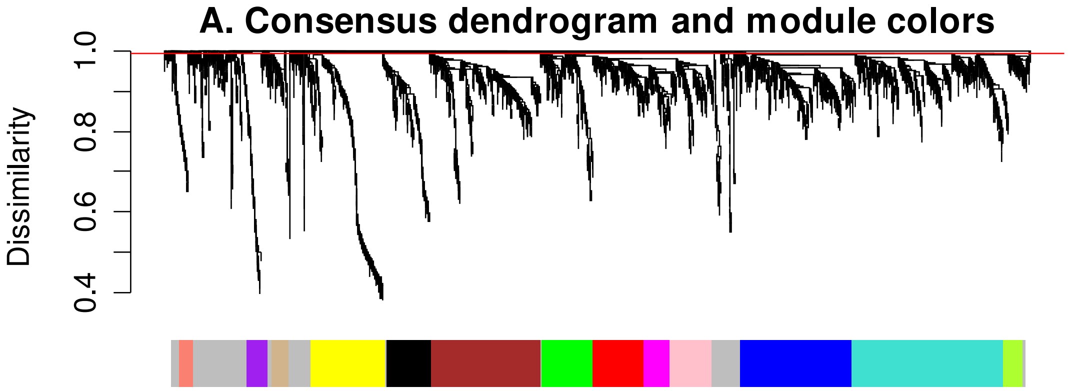
```
# Cluster again? Copy - paste this until there's no change.

MergedColors = MergeCloseModules(ExprData, Network, MergedColors$Colors,
CutHeight = ModuleMergeCut,
                       OrderedPCs = OrderedPCs, IncludeGrey = FALSE, verbose = 4,
print.level = 0);
```

```
SizeWindow(7,7);
par(mfrow = c(1,1));
par(cex = StandardCex/1.2);
plot(MergedColors$ClustTree, main = "Consensus module dendrogram after merging",
xlab = "", sub = "");
abline(ModuleMergeCut, 0, col="red");
```

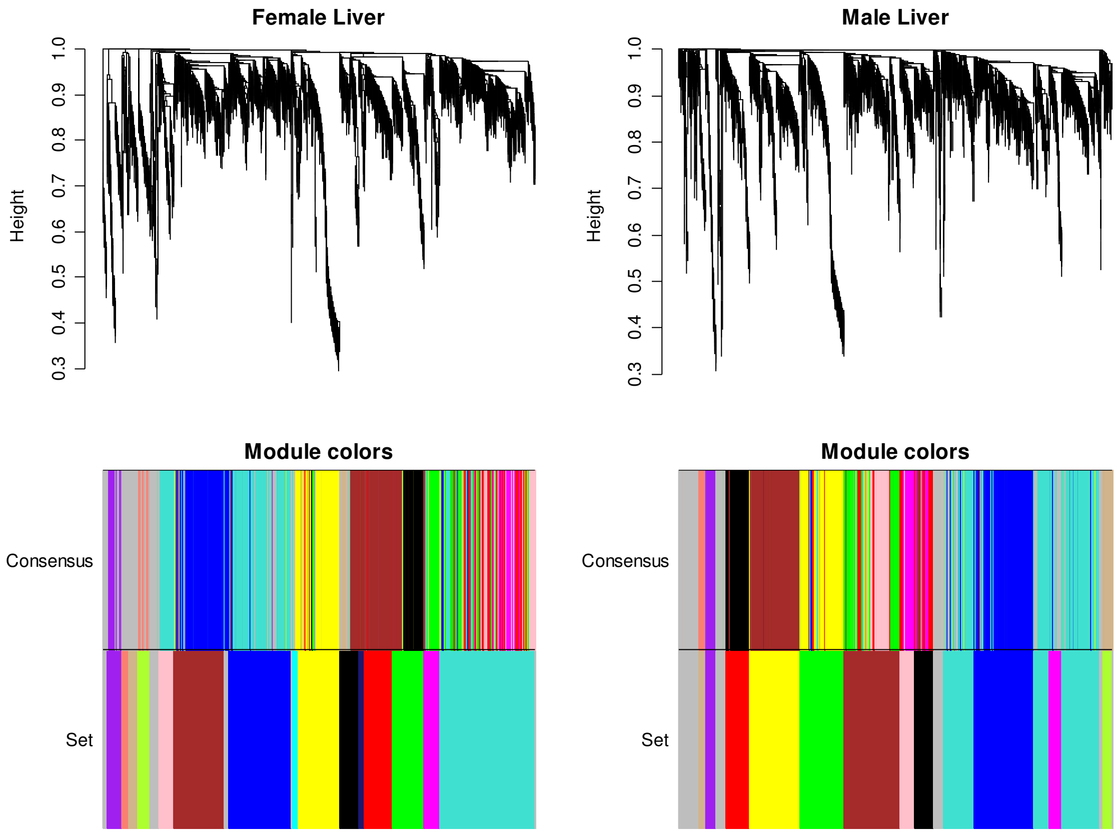


```
# Plot the new module membership vs. the consensus dendrogram for paper

SizeWindow(9,3);
lo = layout(matrix(c(1,2), 2, 1, byrow = TRUE), heights = c(0.85, 0.15));
layout.show(lo);
par(cex = StandardCex);

par(mar=c(0,5.2,1.4,1.4));
plot(Consensus$ClustTree, labels = FALSE, main = "A. Consensus dendrogram and
module colors", xlab="",
sub="", hang = 0.04, ylab = "Dissimilarity");
abline(ConsCutoffs[ChosenCut], 0, col="red");
```

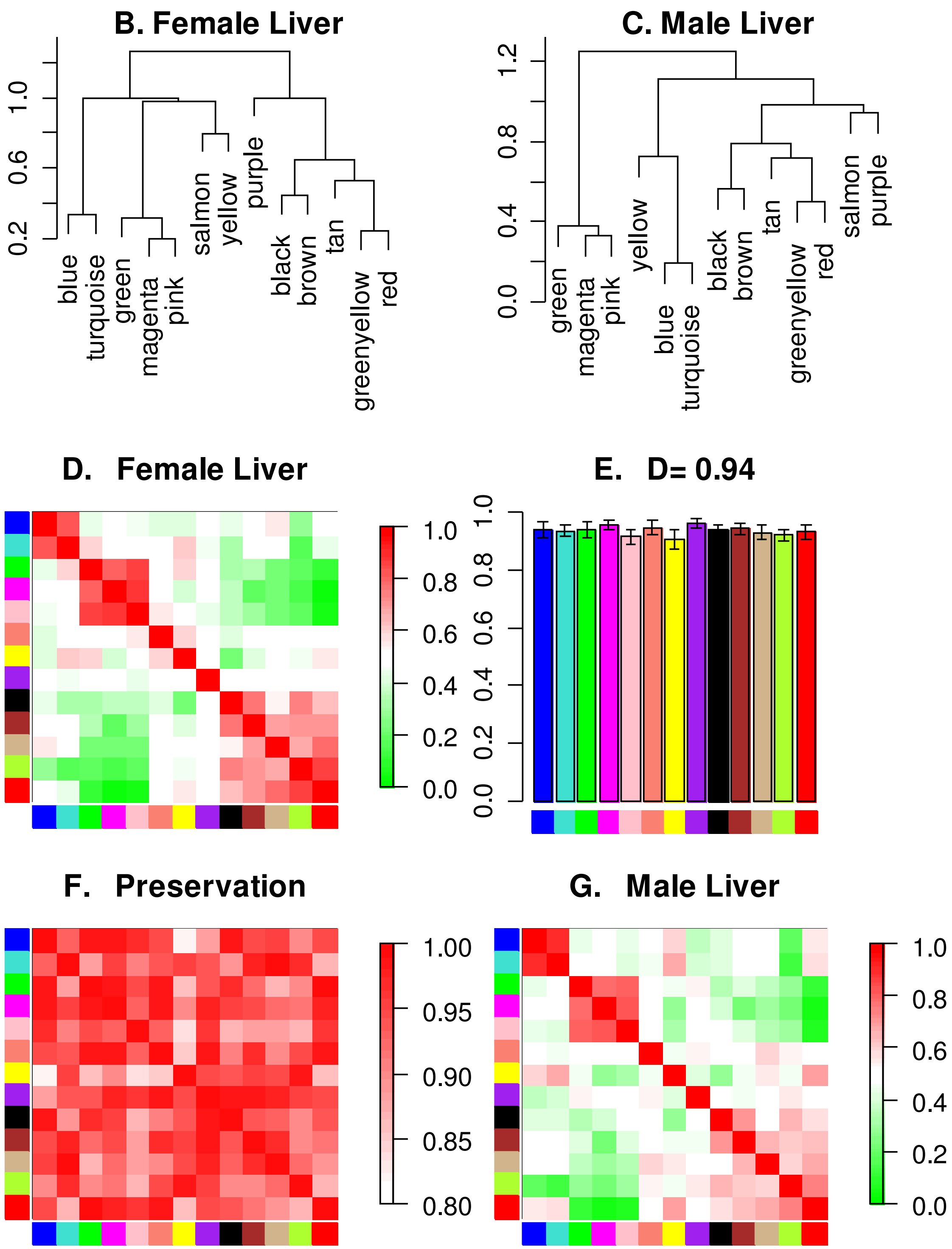
```
par(mar=c(0,5.2,0,1.4)+0.2);

hclustplot1(Consensus$ClustTree, MergedColors$Colors, title1="")
```



```
# Plot the new module membership vs. set dendrograms

SizeWindow(12,9);
par(mfcol=c(2,2));
par(mar=c(1.4,4,2,1.2)+0.1);
par(cex = StandardCex/1.4);
for (i in (1:No.Sets))
{
  plot(Network$ClusterTree[[i]]$data,labels=F,xlab="",main=Set.Labels[i],
          ylim=c(0,1), sub="")
  SCColor = cbind(MergedSetColors[, i], MergedColors$Colors);   # Compare it to
the set colors
  RowLabels = c("Set", "Consensus");
  hclustplotn(Network$ClusterTree[[i]]$data, SCColor, RowLabels = RowLabels,
cex.RowLabels = 1,
               main="Module colors")
}
```

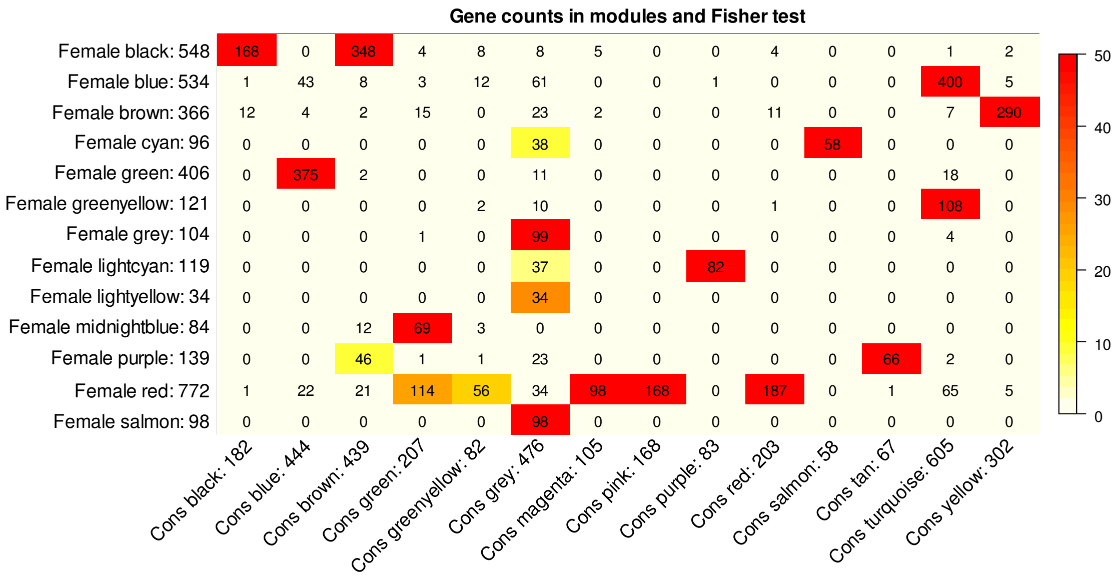


```
# Recalculate module principal components

PCs = NetworkModulePCs(ExprData, Network, UniversalModuleColors =
MergedColors$Colors,
                       verbose=3)
OrderedPCs = OrderPCs(PCs, GreyLast=TRUE, GreyName = "MEgrey");

# Remove the grey eigengene

for (set in 1:No.Sets)
{
   OrderedPCs[[set]]$data = OrderedPCs[[set]]$data[,
1:(ncol(OrderedPCs[[set]]$data)-1)];
}

# Plot for the paper: Standard plot of differential eigengene network analysis

SizeWindow(7, 9);
par(cex = StandardCex/1.4);
PlotCorPCsAndDendros(OrderedPCs, Titles = Set.Labels, ColorLabels = TRUE,
IncludeSign = TRUE,
           IncludeGrey = FALSE, plotCPMeasure = FALSE,
           plotMeans = T, CPzlim = c(0.8,1), plotErrors = TRUE, marHeatmap =
c(1.6,2.7,1.6,6.0),
            marDendro = c(0.2,3,1.2,2), LetterSubPlots = TRUE, Letters =
"BCDEFGHIJKLMNOPQRSTUV",
            PlotDiagAdj = TRUE);
```



```
#-----------------------------------------------------------------------------------
------
# Attempt to assign consensus and liver set modules to one another

#SetModColors = as.factor(Network$Colors[, 1]);
SetModColors = as.factor(LiverColors);
No.SetMods = nlevels(SetModColors);
```

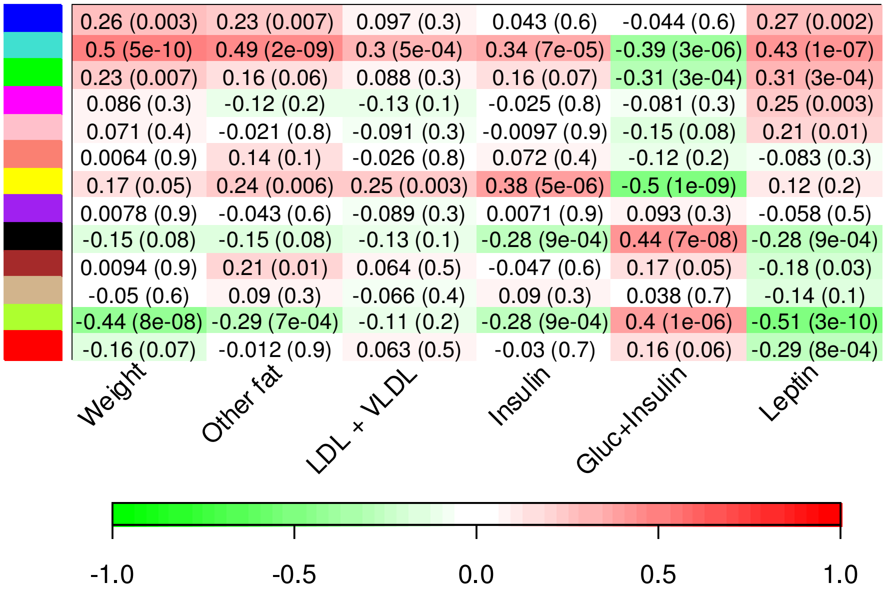
```
ConsModColors = as.factor(MergedColors$Colors);
No.ConsMods = nlevels(ConsModColors);

pTable = matrix(0, nrow = No.SetMods, ncol = No.ConsMods);
CountTbl = matrix(0, nrow = No.SetMods, ncol = No.ConsMods);

for (smod in 1:No.SetMods)
  for (cmod in 1:No.ConsMods)
  {
    SetMembers = (SetModColors == levels(SetModColors)[smod]);
    ConsMembers = (ConsModColors == levels(ConsModColors)[cmod]);
    pTable[smod, cmod] = -log10(fisher.test(SetMembers, ConsMembers, alternative
= "greater")$p.value);
    CountTbl[smod, cmod] = sum(SetModColors == levels(SetModColors)[smod] &
ConsModColors ==
                      levels(ConsModColors)[cmod])
  }

pTable[is.infinite(pTable)] = 1.3*max(pTable[is.finite(pTable)]);
pTable[pTable>50 ] = 50 ;

PercentageTbl = CountTbl;
for (smod in 1:No.SetMods)
  PercentageTbl[smod, ] = as.integer(PercentageTbl[smod, ]
/sum(PercentageTbl[smod, ]) * 100);


SetModTotals = as.vector(table(SetModColors));
ConsModTotals = as.vector(table(ConsModColors));

SizeWindow(10, 5);
par(mfrow=c(1,1));
par(cex = StandardCex/1.7);
par(mar=c(8,12 ,2,1)+0.3);

HeatmapWithTextLabels(Matrix = pTable,
                      xLabels = paste("Cons ", levels(ConsModColors), ": ",
                                      ConsModTotals, sep=""),
                      yLabels = paste("Female ", levels(SetModColors), ": ",
SetModTotals,
                                      sep=""),
                      NumMatrix = CountTbl,
                      InvertColors = TRUE, SetMargins = FALSE,
                      main = "Gene counts in modules and Fisher test",
                      cex.Num = 0.8, cex.lab = 1.0);
```



	Cons black: 182	Cons blue: 444	Cons brown: 439	Cons green: 207	Cons greenyellow: 82	Cons grey: 476	Cons magenta: 105	Cons pink: 168	Cons purple: 83	Cons red: 203	Cons salmon: 58	Cons tan: 67	Cons turquoise: 605	Cons yellow: 302
Female black: 548	168	0	348	4	8	8	5	0	0	4	0	0	1	2
Female blue: 534	1	43	8	3	12	61	0	0	1	0	0	0	400	5
Female brown: 366	12	4	2	15	0	23	2	0	0	11	0	0	7	290
Female cyan: 96	0	0	0	0	0	38	0	0	0	0	58	0	0	0
Female green: 406	0	375	2	0	0	11	0	0	0	0	0	0	18	0
Female greenyellow: 121	0	0	0	0	2	10	0	0	0	1	0	0	108	0
Female grey: 104	0	0	0	1	0	99	0	0	0	0	0	0	4	0
Female lightcyan: 119	0	0	0	0	0	37	0	0	82	0	0	0	0	0
Female lightyellow: 34	0	0	0	0	0	34	0	0	0	0	0	0	0	0
Female midnightblue: 84	0	0	12	69	3	0	0	0	0	0	0	0	0	0
Female purple: 139	0	0	46	1	1	23	0	0	0	0	0	66	2	0
Female red: 772	1	22	21	114	56	34	98	168	0	187	0	1	65	5
Female salmon: 98	0	0	0	0	0	98	0	0	0	0	0	0	0	0

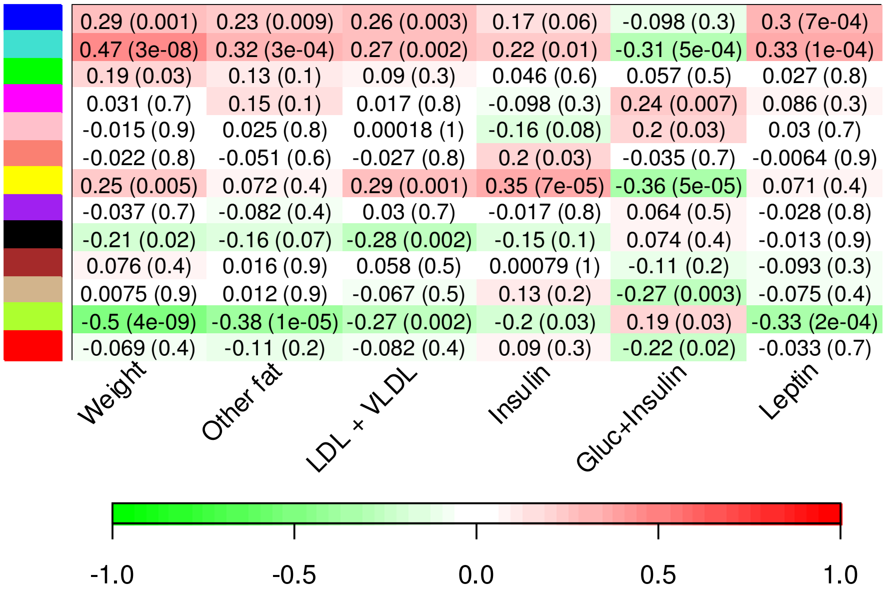
```
#-------------------------------------------------------------------------------
------
# Add traits; see whether there are any traits associated with any of the
consensus modules.

# If necessary, recalculate PCs

PCs = NetworkModulePCs(ExprData, Network, UniversalModuleColors =
MergedColors$Colors,
                       verbose=3)
OrderedPCs = OrderPCs(PCs, GreyLast=TRUE, GreyName = "MEgrey");

# Select interesting traits

SelTraits = SelectTraits(Traits, BranchCut = 0.35, Impute = TRUE,
                         SelectOnSignificance = TRUE, PCs = OrderedPCs,
                         SignifThres = 0.01, verbose=1);

No.SelTraits = SelTraits$No.SelectedTraits;
TraitSignif = vector(mode="list", length = No.Sets);
TraitCor = vector(mode="list", length = No.Sets);
No.Mods = dim(OrderedPCs[[1]]$data)[2]-1;

# Calculate module--trait significance

for (set in 1:No.Sets)
{
  TraitSignif[[set]] = list(data = matrix(0, nrow = No.Mods, ncol =
No.SelTraits));
  TraitCor[[set]] = list(data = matrix(0, nrow = No.Mods, ncol = No.SelTraits));
  for (mod in 1:No.Mods)
    for (trait in 1:No.SelTraits)
    {
      ct = cor.test(OrderedPCs[[set]]$data[, mod], SelTraits$Traits[[set]]$data[,
trait]);
      TraitSignif[[set]]$data[mod, trait] = ct$p.value;
      TraitCor[[set]]$data[mod, trait] = cor(OrderedPCs[[set]]$data[, mod],
                                             SelTraits$Traits[[set]]$data[,
trait]);
    }
}

# Plot the significance heatmap

minp = 1; maxp = 0;
for (set in 1:No.Sets)
{
  minp = min(minp, TraitSignif[[set]]$data);
  maxp = max(maxp, TraitSignif[[set]]$data);
}
# To make the plots mangeable: leave out the "BMD femurs only" trait

#TraitLabels = c("Weight", "Other fat", "LDL + VLDL", "Insulin",
#               "Gluc+Insulin", "Leptin", "BMD femurs only");
TraitLabels = c("Weight", "Other fat", "LDL + VLDL", "Insulin",
               "Gluc+Insulin", "Leptin");

for (set in 1:No.Sets)
{
  TraitSignif[[set]]$data = TraitSignif[[set]]$data[, -ncol
(TraitSignif[[set]]$data)];
  TraitCor[[set]]$data = TraitCor[[set]]$data[, -ncol(TraitCor[[set]]$data)];
}

# Make the plot for the paper

SizeWindow(4.6,7.0);
par(mfrow = c(2,1));
par(mar = c(8, 2,2.2,1));

Letters = "HIJKL";
letterInd = 1;
for (set in 1:No.Sets)
{
  par(cex = StandardCex/1.7);
  letter = paste(substring(Letters, set, set), ".", sep = "");
  NumMatrix = paste(signif(TraitCor[[set]]$data,2)," (",
signif(TraitSignif[[set]]$data,1), ")",  sep="");
  dim(NumMatrix) = dim(TraitCor[[set]]$data);
  # m = -log10(TraitSignif[[set]]$data);
  HeatmapWithTextLabels(Matrix = TraitCor[[set]]$data,
                        yLabels = names(OrderedPCs[[1]]$data)[c(1:No.Mods)],
                        xLabels = TraitLabels, NumMatrix = NumMatrix,
                        cex.Num = StandardCex/2.0, cex.lab = StandardCex/1.7,
                        InvertColors = FALSE, SetMargins = FALSE,
                        xColorLabels = FALSE, yColorLabels = TRUE,
                        main = paste(letter, Set.Labels[set],
                                     "module-trait correlation and p-value"),
                        color = GreenWhiteRed(50),
                        #zlim = c(-log10(maxp), -log10(minp)));
                        zlim = c(-1, 1), horizontal = TRUE, legend.mar = 5);
}
```

H. Female Liver module-trait correlation and p-value



	Weight	Other fat	LDL + VLDL	Insulin	Gluc+Insulin	Leptin
🟦	0.26 (0.003)	0.23 (0.007)	0.097 (0.3)	0.043 (0.6)	-0.044 (0.6)	0.27 (0.002)
■	0.5 (5e-10)	0.49 (2e-09)	0.3 (5e-04)	0.34 (7e-05)	-0.39 (3e-06)	0.43 (1e-07)
🟩	0.23 (0.007)	0.16 (0.06)	0.088 (0.3)	0.16 (0.07)	-0.31 (3e-04)	0.31 (3e-04)
■	0.086 (0.3)	-0.12 (0.2)	-0.13 (0.1)	-0.025 (0.8)	-0.081 (0.3)	0.25 (0.003)
■	0.071 (0.4)	-0.021 (0.8)	-0.091 (0.3)	-0.0097 (0.9)	-0.15 (0.08)	0.21 (0.01)
■	0.0064 (0.9)	0.14 (0.1)	-0.026 (0.8)	0.072 (0.4)	-0.12 (0.2)	-0.083 (0.3)
🟨	0.17 (0.05)	0.24 (0.006)	0.25 (0.003)	0.38 (5e-06)	-0.5 (1e-09)	0.12 (0.2)
🟪	0.0078 (0.9)	-0.043 (0.6)	-0.089 (0.3)	0.0071 (0.9)	0.093 (0.3)	-0.058 (0.5)
⬛	-0.15 (0.08)	-0.15 (0.08)	-0.13 (0.1)	-0.28 (9e-04)	0.44 (7e-08)	-0.28 (9e-04)
🟫	0.0094 (0.9)	0.21 (0.01)	0.064 (0.5)	-0.047 (0.6)	0.17 (0.05)	-0.18 (0.03)
■	-0.05 (0.6)	0.09 (0.3)	-0.066 (0.4)	0.09 (0.3)	0.038 (0.7)	-0.14 (0.1)
■	-0.44 (8e-08)	-0.29 (7e-04)	-0.11 (0.2)	-0.28 (9e-04)	0.4 (1e-06)	-0.51 (3e-10)
🟥	-0.16 (0.07)	-0.012 (0.9)	0.063 (0.5)	-0.03 (0.7)	0.16 (0.06)	-0.29 (8e-04)

I. Male Liver module-trait correlation and p-value



	Weight	Other fat	LDL + VLDL	Insulin	Gluc+Insulin	Leptin
🟦	0.29 (0.001)	0.23 (0.009)	0.26 (0.003)	0.17 (0.06)	-0.098 (0.3)	0.3 (7e-04)
■	0.47 (3e-08)	0.32 (3e-04)	0.27 (0.002)	0.22 (0.01)	-0.31 (5e-04)	0.33 (1e-04)
🟩	0.19 (0.03)	0.13 (0.1)	0.09 (0.3)	0.046 (0.6)	0.057 (0.5)	0.027 (0.8)
■	0.031 (0.7)	0.15 (0.1)	0.017 (0.8)	-0.098 (0.3)	0.24 (0.007)	0.086 (0.3)
■	-0.015 (0.9)	0.025 (0.8)	0.00018 (1)	-0.16 (0.08)	0.2 (0.03)	0.03 (0.7)
■	-0.022 (0.8)	-0.051 (0.6)	-0.027 (0.8)	0.2 (0.03)	-0.035 (0.7)	-0.0064 (0.9)
🟨	0.25 (0.005)	0.072 (0.4)	0.29 (0.001)	0.35 (7e-05)	-0.36 (5e-05)	0.071 (0.4)
🟪	-0.037 (0.7)	-0.082 (0.4)	0.03 (0.7)	-0.017 (0.8)	0.064 (0.5)	-0.028 (0.8)
⬛	-0.21 (0.02)	-0.16 (0.07)	-0.28 (0.002)	-0.15 (0.1)	0.074 (0.4)	-0.013 (0.9)
🟫	0.076 (0.4)	0.016 (0.9)	0.058 (0.5)	0.00079 (1)	-0.11 (0.2)	-0.093 (0.3)
■	0.0075 (0.9)	0.012 (0.9)	-0.067 (0.5)	0.13 (0.2)	-0.27 (0.003)	-0.075 (0.4)
■	-0.5 (4e-09)	-0.38 (1e-05)	-0.27 (0.002)	-0.2 (0.03)	0.19 (0.03)	-0.33 (2e-04)
🟥	-0.069 (0.4)	-0.11 (0.2)	-0.082 (0.4)	0.09 (0.3)	-0.22 (0.02)	-0.033 (0.7)