Tutorial for the WGCNA package for R
II. Consensus network analysis of liver expression data, female and male mice

5. Comparing eigengene networks in male and female mice

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February 13, 2016

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0 Preliminaries: setting up the R session

Here we assume that a new R session has just been started. We load the WGCNA package, set up basic parameters and load data saved in the parts 1 and 2 of the tutorial.

```r
# Display the current working directory
getwd();
# If necessary, change the path below to the directory where the data files are stored.
# "." means current directory. On Windows use a forward slash / instead of the usual \.
workingDir = ".";
setwd(workingDir);
# Load the WGCNA package
library(WGCNA)
# The following setting is important, do not omit.
options(stringsAsFactors = FALSE);
# Basic settings: we work with two data sets
nSets = 2;
# For easier labeling of plots, create a vector holding descriptive names of the two sets.
setLabels = c("Female liver", "Male liver")
shortLabels = c("Female", "Male")
# Load the data saved in the first part
lnames = load(file = "Consensus-dataInput.RData");
#The variable lnames contains the names of loaded variables.
lnames
# Load the results of network analysis, tutorial part 2.a
lnames = load(file = "Consensus-NetworkConstruction-auto.RData");
lnames
```

We have loaded the variables `multiExpr` and `Traits` containing the expression and trait data, respectively. Further, expression data dimensions are stored in `nGenes` and `nSamples`. The consensus network analysis results are represented by the variables `consMEs`, `moduleLabels`, `moduleColors`, and `consTree`.

5 Comparing eigengene networks in male and female mice

In this section we compare the consensus eigengene networks in the female and male data sets (such comparison is often called differential analysis). Consensus eigengene networks capture the relationships among consensus modules; the relationships are quantified by eigengene correlations. We first extend the consensus eigengenes by adding the body weight clinical trait as an additional "eigengene":

```
# Create a variable weight that will hold just the body weight of mice in both sets
weight = vector(mode = "list", length = nSets);
for (set in 1:nSets)
{
  weight[[set]] = list(data = as.data.frame(Traits[[set]]$data$weight_g));
  names(weight[[set]]$data) = "weight"
}
# Recalculate consMEs to give them color names
consMEsC = multiSetMEs(multiExpr, universalColors = moduleColors);
# We add the weight trait to the eigengenes and order them by consesus hierarchical clustering:
MET = consensusOrderMEs(addTraitToMEs(consMEsC, weight));
```

We now call the function `plotEigengeneNetworks` that performs the differential analysis:

```
sizeGrWindow(8,10);
#pdf(file = "Plots/EigengeneNetworks.pdf", width= 8, height = 10);
par(cex = 0.9)
plotEigengeneNetworks(MET, setLabels, marDendro = c(0,2,2,1), marHeatmap = c(3,3,2,1),
                      zlimPreservation = c(0.5, 1), xLabelsAngle = 90)
dev.off();
```

The plot is shown in Fig. 1. The plot shows the eigengene dendrograms and eigengene network heatmaps, as well as two plots of network preservation between the two sets, namely a heatmap plot of the preservation adjacency [1] and a barplot of mean preservation of relationships for each eigengene. The overall preservation of the two eigengene networks is 0.94, which is quite high. A visual inspection of the female and male network heatmap plots indicates that the inter-module relationships in the two data sets are indeed very similar. The red blocks along the diagonal in the network heatmaps indicate meta-modules, groups of correlated eigengenes, and these are largely preserved between the two sets. The preservation heatmap and barplots indicate that most relationships are very highly preserved. The message here is that inter-module relationships are strongly preserved across similar data sets and encode biologically meaningful information.

References

[1] P. Langfelder and S. Horvath. Eigengene networks for studying the relationships between co-expression modules. *BMC Systems Biology*, 1:54, 2007.
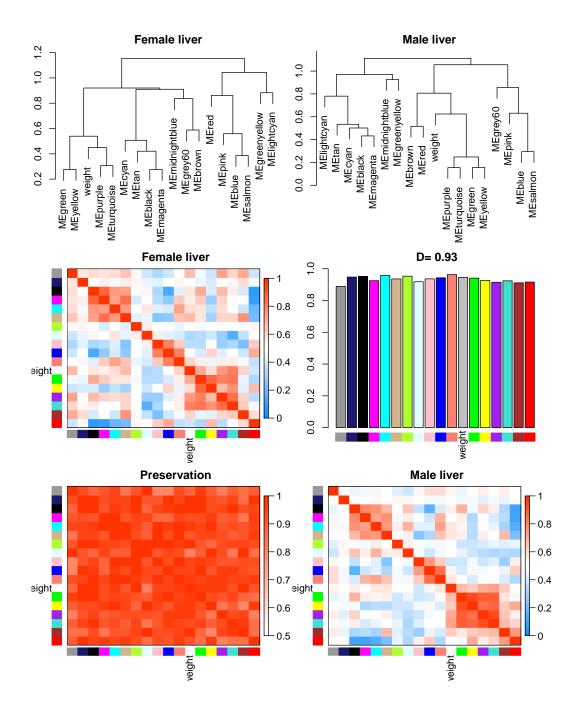
Figure 1: Summary plot of consensus eigengene networks and their differential analysis. The top two panels show the dendrograms (clustering trees) of the consensus module eigengenes in the two sets indicated in the titles. Below, the eigengene networks in the two sets are shown as heatmaps labeled Female liver and Male liver. In the heatmaps, red denotes high adjacency (positive correlation) and green denotes low adjacency (negative correlation). The Preservation heatmap shows the preservation network, defined as one minus the absolute difference of the eigengene networks in the two data sets. The barplot shows the mean preservation of adjacency for each of the eigengenes to all other eigengenes; in other words, the barplot depicts the column means of the preservation heatmap.