Weighted Gene Co-expression Network Analysis (WGCNA)
R Tutorial, Part C
Breast Cancer Microarray Data.
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Summary
In our R tutorial GBMTutorial2.doc we showed how global gene expression profiling on RNA from 130 glioblastoma patient samples (dataset 1, n=55,15 and dataset 2, n=65) resulted in the identification of groups of highly coexpressed genes (modules). To determine whether these modules are common to multiple cancers, we present here the analysis of a publicly available breast cancer dataset (van’t Veer et al). This dataset was sufficiently large and contained gene expression data from a different microarray platform allowing for array platform independent conclusions. We demonstrate that the expression of intramodular hub genes inversely correlates with cancer recurrence in the breast cancer data

This tutorial describes how to carry out a gene co-expression network analysis with our custom made R functions. We show how construct weighted networks using soft thresholding.

We provide the statistical code used for generating the weighted gene co-expression network results in breast cancer. Thus, the reader be able to reproduce all of our findings.

This document also serves as a tutorial to weighted gene co-expression network analysis. Some familiarity with the R software is desirable but the document is fairly self-contained.

This tutorial and the data files can be found at the following webpage:
http://www.genetics.ucla.edu/labs/horvath/CoexpressionNetwork/ASPMgene

More material on weighted network analysis can be found here
http://www.genetics.ucla.edu/labs/horvath/CoexpressionNetwork/

The data and biological implications are described in


To cite the statistical methods please use

Data

We used the published data set from the breast cancer study by van ‘t Veer et al. (2002). We eliminated BRCA positive patients from the analysis since our interest was in investigating patients with BRCA negative risk profile. This resulted in 78 primary breast cancer patients. When carrying out an unsupervised clustering analysis involving the 5000 most varying genes, we found that sample 54 in the original data set was an array outlier. Since network analysis is susceptible to such outliers, we removed it from the analysis and ended up with 77 array samples (patients). As binary clinical outcome we considered cancer recurrence within 5 years. 34 patients developed distant metastases within 5 years, and 44 remained disease-free after a period of at least 5 years.

From each patient, 5 µg total RNA was isolated from snap-frozen tumour material and used to derive complementary RNA (cRNA). A reference cRNA pool was made by pooling equal amounts of cRNA from each of the sporadic carcinomas. Two hybridizations were carried out for each tumour using a fluorescent dye reversal technique on microarrays containing approximately 25,000 human genes synthesized by inkjet technology. Fluorescence intensities of scanned images were quantified, normalized and corrected to yield the transcript abundance of a gene as an intensity ratio with respect to that of the signal of the reference pool. We used the log10ratios provided by the original study as gene expression index. This is why this patient was dropped from our analysis.

Probe sets that were common to both array platforms (GBM data and breast cancer) were mapped, and Pearson correlations for all gene pairs found in glioblastoma were recalculated in the breast cancer dataset. To determine which glioblastoma modules were preserved in the breast cancer data, we assigned the glioblastoma module colors to the genes in the hierarchical clustering tree of the breast cancer data.

References


# R CODE
#copy and past the following code into the R session
# Please adapt the paths in the following. Make sure to use / instead of \nsetwd("C:/Documents and Settings/shorvath/My Documents/ADAG/PaulMischel/GBMnetworkpaper/Webpage/BreastCancerTutorial2")
source("C:/Documents and Settings/shorvath/My Documents/RFunctions/NetworkFunctions.txt")

#Memory
# check the maximum memory that can be allocated
memory.size(TRUE)/1024
# increase the available memory
memory.limit(size=4000)

sum1=function(x) sum(x,na.rm=T)

#Quote:
#“When popular opinion is nearly unanimous, contrary thinking tends to be most profitable. The reason is that once the crowd takes a position, it creates a short-term, self-fulfilling prophecy. But when a change occurs, everyone seems to change his mind at once.”
The Crowd - Gustave Le Bon
Mapping Affymetrix U133a arrays (GBM) data into Rosetta Arrays (breast cancer)

First we show how me mapped the 8000 most varying probes in the GBM samples (Affymetrix U133a) into the breast cancer data (Rosetta arrays)

# This data sets contains the breast cancer expression data
# and corresponding clinical traits
dat1=read.csv("BreastArrayDataCombined.csv",header=T)

datGenes=dat1[-c(1:5),]
datClinicalBreast=dat1[1:5,]
# There are 2 types of gene expression indices: intensity and ratio
# We prefer the ratio...
IndexIntensity=seq(from=2,to=233,by=3)
IndexRatio=seq(from=3,to=234,by=3)

names(datGenes[, IndexIntensity])
names(datGenes[, IndexRatio])

# This file contains gene information on the
datGBM=read.csv("GBM8000Summarydat55dat65.csv")

name1=row.names(dat1)
# these tables will allow us to translate U133A Affymetrix probe set IDs into Rosetta IDs
datAffy= read.csv("AffyChip.csv")
datRosetta=read.csv("RosettaChip.csv")

table(is.element(datGenes$Systematic_name, datRosetta$NAME))
table(is.element(datAffy$MEGID, datRosetta$MEGID))
table(is.element(datAffy$MEGACCESSOR, datRosetta$MEGACCESSOR))

table(is.element(datGBM$gbm133a,datAffy$NAME))

# Step 1: merge the GBM data with the folw datAffy, so that we get the MEG_IDs
datmerge=merge(datGBM, datAffy , by.x="gbm133a", by.y= "NAME")
dim(datGBM)
dim(datmerge)
table(is.element(datmerge$MEGACCESSOR, datRosetta$MEGACCESSOR))
# Step 2: merge the datmerge with datRosetta by MEG_ACCESSOR so that
# we get the Rosetta$NAME for each gene
datmerge=merge(datmerge, datRosetta, by.x="MEGACCESSOR",
by.y="MEGACCESSOR", all=FALSE)
dim(datmerge)
# The reason why we end up with a number different from 6569 rows is that the entries of
# MEGACCESSOR are not unique, i.e. some are repeated. But this does
# not lead to major trouble as seen below

#Step 3 merge datmerge with the breast cancer data
datmerge=merge(datmerge, datGenes, by.x="NAME", by.y="Systematic_name")

table(datmerge$colordata1)

blue   brown  green  grey  turquoise  yellow
 660   180   145  5747   1438    156

#Note that the number of genes per module is very close to that in the original data (see
#below) especially for the brown module.
table(datGBM$colordata1)

blue   brown  green  grey  turquoise  yellow
 618   167   140  5574   1352    149

names(datmerge)
# Since we focus on the ratio measurement for expression, we define
IndexRatio=seq(from=24,to=255,by=3)
names(datmerge[,IndexRatio])

# Whole Network Analysis
datExpr=data.frame( t(datmerge[,IndexRatio]))
names(datExpr)=datmerge[,1]

names(datClinicalBreast)
IndexRatio2=seq(from=3,to=234,by=3)
datClinicalBreast2=datClinicalBreast[,IndexRatio2]
table(names(datClinicalBreast2)==dimnames(datExpr)[[1]])
The following shows that sample 54 is an outlier that will be removed below.

```
h1=hclust(dist(datExpr), method="average")
plot(h1)
```

Cluster Dendrogram

```
# Note that array number 54 appears to be an outlier. To be safe we remove it from the analysis. Since dropping this array is done without reference to the clinical outcome, this does not bias our result.
```

```
#Quote:
#Until the day when God shall deign to reveal the future to man,
#all human wisdom is summed up in these two words,--'Wait and hope'.
#Alexandre Dumas, The Count of Monte Cristo
```
The following plot shows a close relationship between recurrence time and status.

```
rm(datExpr)
rm(dat1)
rm(datGBM)
collect_garbage()
```
# Now we define the connectivity (degree) in the breast network
# We use the following power for the power adjacency function.
beta1=6
collect_garbage()

DegreeBreast= SoftConnectivity(datExpr2,power=beta1)
collect_garbage()

ScaleFreePlot1(DegreeBreast,AF1="Check Scale Free Topology in Breast CA")

# Quote
#As an adolescent I aspired to lasting fame, I craved factual certainty, and I thirsted for a
meaningful vision of human life - so I became a scientist. This is like becoming an archbishop so
you can meet girls.
- M. Cartmill
# This code allows one to restrict the analysis to the most connected genes, which may speed up calculations when it comes to module detection.

```r
DegCut = 3000  # number of most connected genes that will be considered
DegreeRank = rank(-DegreeBreast)
restDegree = DegreeRank <= DegCut
# thus our module detection uses the following number of genes
sum(restDegree)
```

# The following code computes the topological overlap matrix based on the adjacency matrix.

```r
dissGTOM1 = TOMdist1(abs(cor(datExpr2[,restDegree], use="p"))^(beta1))
collect_garbage()
```

# Now we carry out hierarchical clustering with the TOM matrix.

```r
hierGTOM1 <- hclust(as.dist(dissGTOM1), method="average");
```

# This vector contains the colors of the GBM brain cancer network

```r
colorGBM = datmerge$color[restDegree]
```

```r
#par(mfrow=c(2,1), mar=c(2,2,2,1))
par(mfrow=c(2,1))
plot(hierGTOM1, main="Breast Cancer data set, n=77", labels=F, xlab="", sub="");
hclustplot1(hierGTOM1, colorGBM, title1="Colored by GBM modules")
```

<breast cancer data set, n=77>

<colored by GBM modules>

# This corresponds to **Figure 1c** in the article.

# Since the blue and the brown colors roughly group together, we find visual evidence that the blue and brown module are preserved in the breast cancer data set.
# Analysis of the brown (mitosis) module

```r
datExprbrown = datExpr2[,datmerge$colordata1 == "brown"]
```

# To ensure that the entries of datExprbrown are considered as numeric, we have to run
# the following code.
for (i in c(1:dim(datExprbrown)[[2]])) {
  datExprbrown[,i] = as.numeric(as.character(datExprbrown[,i]))
}

# This is the intramodular connectivity in the brown module
```
kbrownBreast = SoftConnectivity(datExprbrown, beta1)
```

# The following function determines the gene significance of a gene expression profile
# based on its association with the breast cancer recurrence time.
# Specifically, we first compute the p-value of the Spearman correlation between
# recurrence time and a gene expression profile.
# Then the gene significance is defined as the minus log10 of the p-value.
# Roughly speaking, this measure counts the zeroes in the p-value.
# If a gene has fewer than 6 measurements, its gene significance is set to missing
if (exists("corTime") ) rm(corTime);
```
corTime = function(x) {
  if (sum(!is.na(x) )<5 ) out1 = NA else out1 = cor.test(x, RecurrenceFreeTime, use="p", method="s" )$p.value
  -log10(out1)}
```

# The the gene significance for the brown module genes is given by
```
GSBREAST = as.vector(apply( datExprbrown, 2, corTime))
```

par(mfrow=c(1,2))

# Figure 2c
scatterplot1(kbrownBreast, datmerge$kBrowndata1[datmerge$colordata1 == "brown"], xlab1="Connectivity (k)-Breast CA", ylab1="Connectivity (k)-GBM", col1="brown")

# This is Figure 2d in our article.
scatterplot1(kbrownBreast, abs(GSBREAST), ylab1="Recurrence Association: |Z|", xlab1="Connectivity (k)-Breast CA", col1="brown")

# Quote:
# Don't let your shame degenerate into self pity! From a British Lady

Quote:
"If you've got it, flaunt it!" – Max Bialystock. From the Producers
# The following figures are also interesting

```r
datmerge$kBrownBreast2[datmerge$colordata1 == "brown"], xlab1="K.intra, Breast Cancer", ylab1="K.intra Brain Cancer 65", col1="brown")
scatterplot1(-log10(datmerge$pCoxdata1[datmerge$colordata1 == "brown")]
abs(GSBRST), ylab1="Breast CA RecurAssociation"
  , xlab1="GBM prognostic significance", col1="brown")
```

![Scatterplots](image)

```
cor = 0.54 p = 3.9e-15
cor = 0.65 p = <10^-20
```

# Note that intramodular connectivity is approximately preserved between the 2 data set.
# Similarly gene significance is preserved. This robustness does not hold across the entire
# gene set. Instead it is crucial to focus on the brown module.

**Quote:**

“Go back to the data!”

Key insight in the episode “Vector” by the math genius of the TV Series “Numbers”.

Weighted gene co-expression network analysis (WGCNA) is all about letting the data
speak for themselves. It does not assume prior pathway information but constructs
modules in an unsupervised fashion. It relates a handful of modules to the clinical trait to
find clinically interesting modules. By making modules (and equivalently their hub
genes) the focus of the analysis, it avoids the pitfalls of multiple testing. It uses
intramodular connectivity along with gene significance to screen for significant hub
genes. WGCNA can be considered as a biologically motivated data reduction scheme.

**THE END**