Microarray data analysis using BioConductor

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Outline

Bioconductor `http://www.bioconductor.org`

- Pre-process data
- Identify differential expression
- Network inference

Pre-process of data

- Entering data into Bioconductor
- Extraction of Cerevisiae probesets
- Exploratory data analysis
- Normalising Microarray data
- Probeset level expression to gene level expression
- Principal Component Analysis

Entering data into Bioconductor

```
library(affy)
fns2 = list.celfiles(path="data2", full.names=TRUE)
rawdata = ReadAffy(filenames=fns2)
print(rawdata)
```

Strain	0 hours	1 hour	2 hours	3 hours	4 hours
Mutant 1	yeast01.cel	yeast02.cel	yeast03.cel	yeast04.cel	yeast05.cel
Wild type 1	yeast06.cel	yeast07.cel	yeast08.cel	yeast09.cel	yeast10.cel
Mutant 2	yeast11.cel	yeast12.cel	yeast13.cel	yeast14.cel	yeast15.cel
Wild type 2	yeast16.cel	yeast17.cel	yeast18.cel	yeast19.cel	yeast20.cel
Mutant 3	yeast21.cel	yeast22.cel	yeast23.cel	yeast24.cel	yeast25.cel
Wild type 3	yeast26.cel	yeast27.cel	yeast28.cel	yeast29.cel	yeast30.cel

Mask file for Cerevisiae probesets

- Mask file to filter out pombe probesets
  `http://www.affymetrix.com/Auth/support/downloads/mask_files/s_cerevisiae.zip`

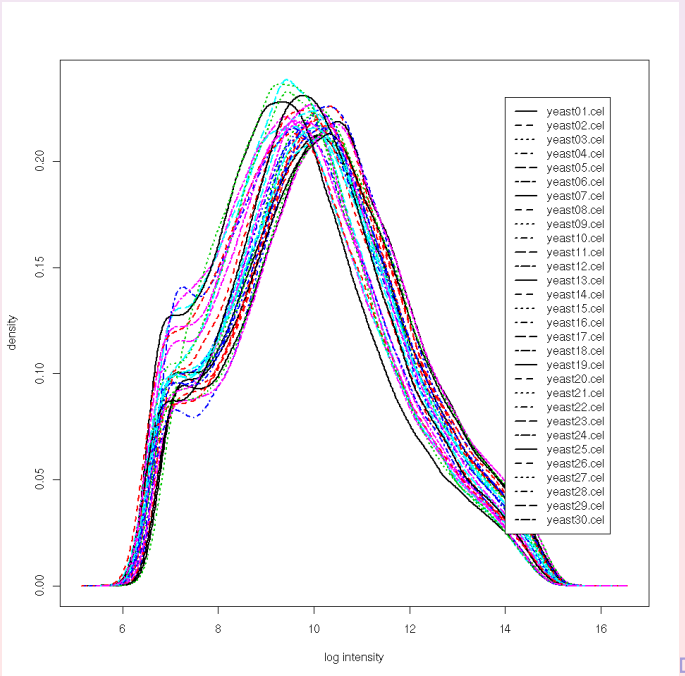
```
s_cerevisiae<-scan("s_cerevisiae.msk", skip=2, list("", ""))
pombe_filter_out<-s_cerevisiae[[1]]
```

- RemoveProbe [1]

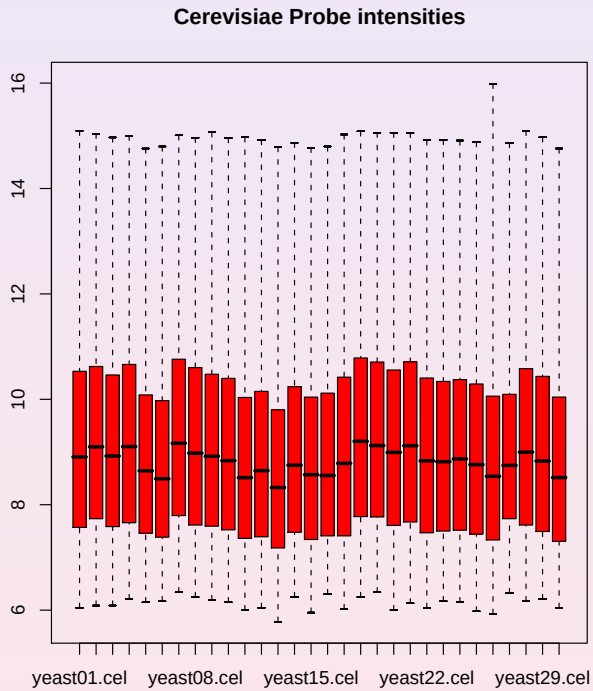
```
source("RemoveProbes.r")
library(yeast2probe)
cleancdf = cleancdfname("yeast2")
RemoveProbes(listOutProbes=NULL, pombe_filter_out,
                          "yeast2cdf","yeast2probe")
```

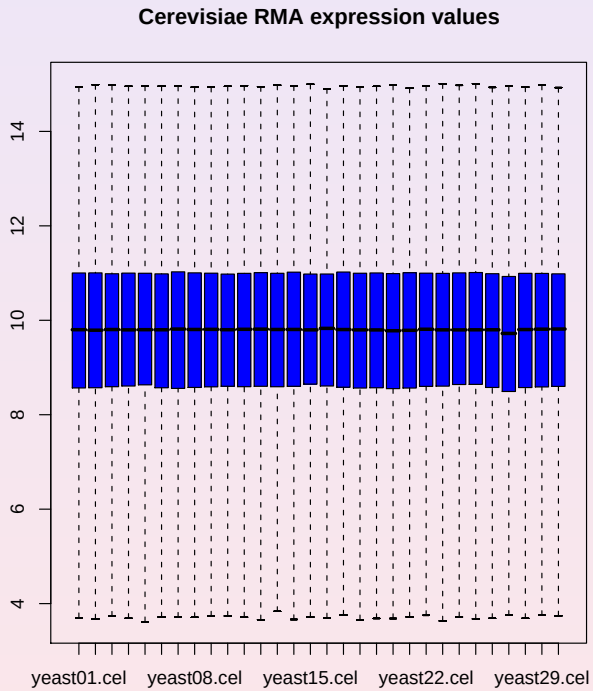
Exploratory data analysis

- Examining raw images
- Probe intensities
- MA plots
- RNA degradation

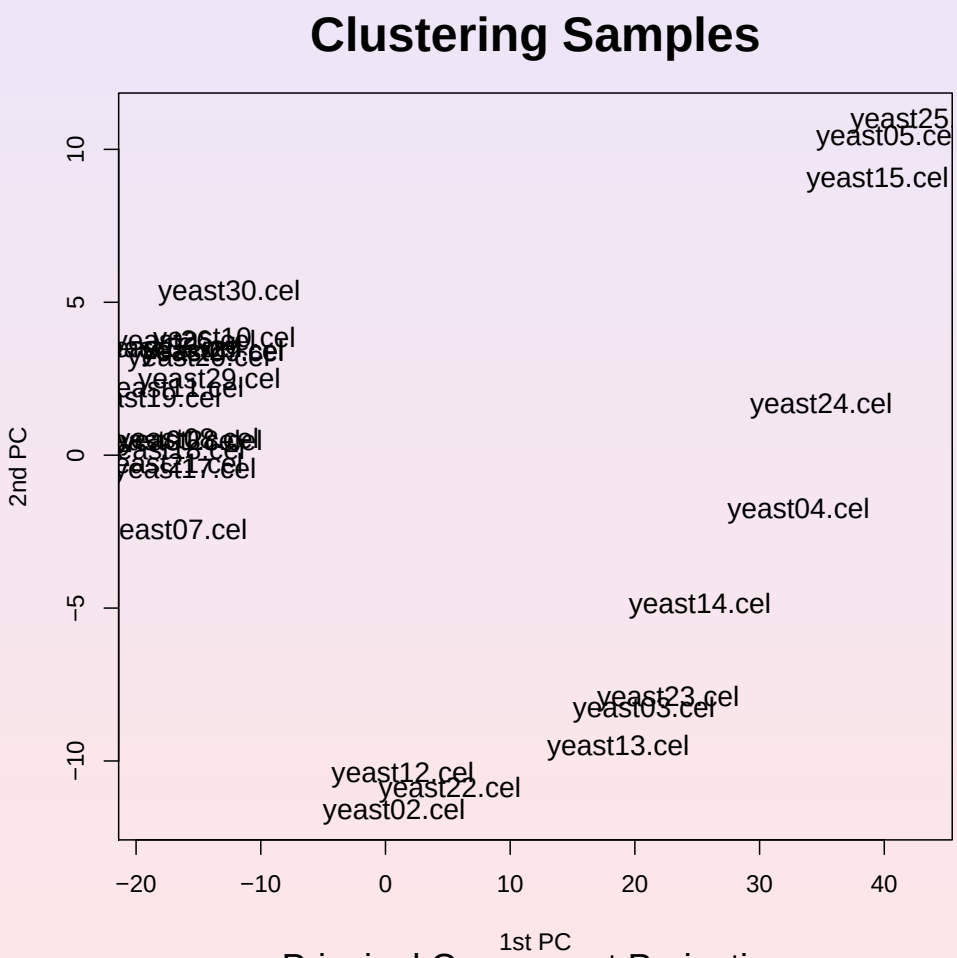


Pre-process of data: Normalisation





Principal Component Analysis [5]



Principal Component Projection

Model Fitting for Identifying Differential Expression

- Limma model [4]
  - Construct design matrix
  - Construct constrasts
- Up-regulated and down-regulated list
- Plot time course for top differential expression
- Volcano Plot for viewing p-value and fold-change
- Heatmap

Up and down regulated list

For identifying differential expression, combine the contrasts by comparing mutant type and wild type at time point 1,2,3 and 4.

```
#Model Fitting
fit<-lmFit(CerevisiaeProbeData, design)
mc<-makeContrasts('m1-w1','m2-w2','m3-w3','m4-w4',levels=design)
fit2<-contrasts.fit(fit, mc)
eb<-eBayes(fit2)
```

Probeset ID	Gene Symbol	T1	T2	T3	T4
ProbesetID 1	Gene 1	-1	-1	-1	-1
ProbesetID 2	Gene 2	1	1	1	1
ProbesetID 3	Gene 3	-1	-1	-1	-1

Table: Up and down regulated list[1]

[1] Not use real gene names here

Plot time course for top differential expression

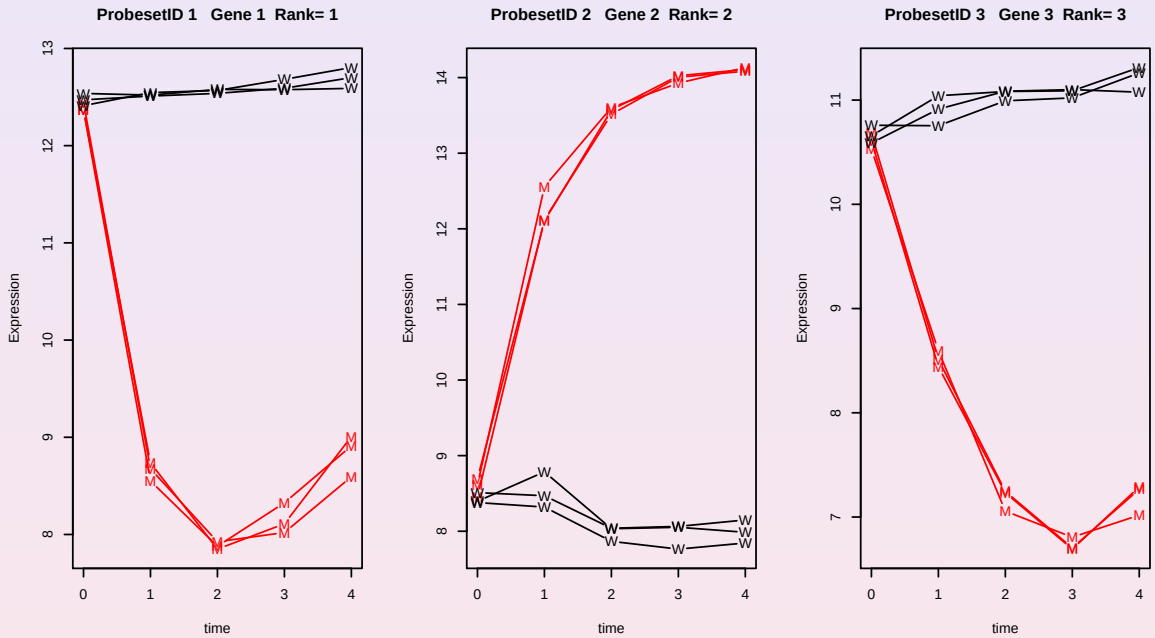


Figure: Time course expression for top 3 differentially expressed Yeast genes[2]

[2]Not use real gene names here

Volcano Plot for viewing p-value and fold-change

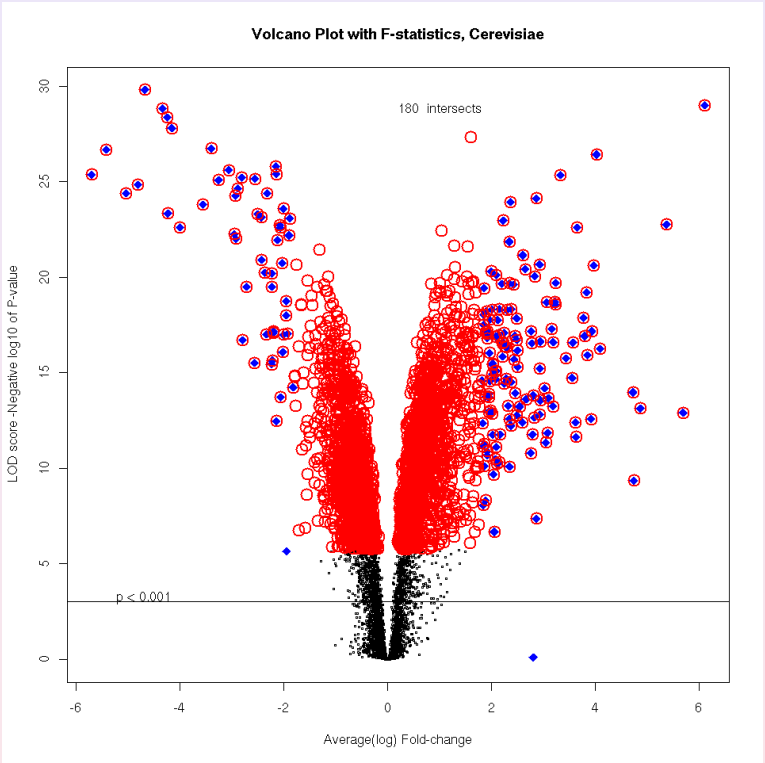
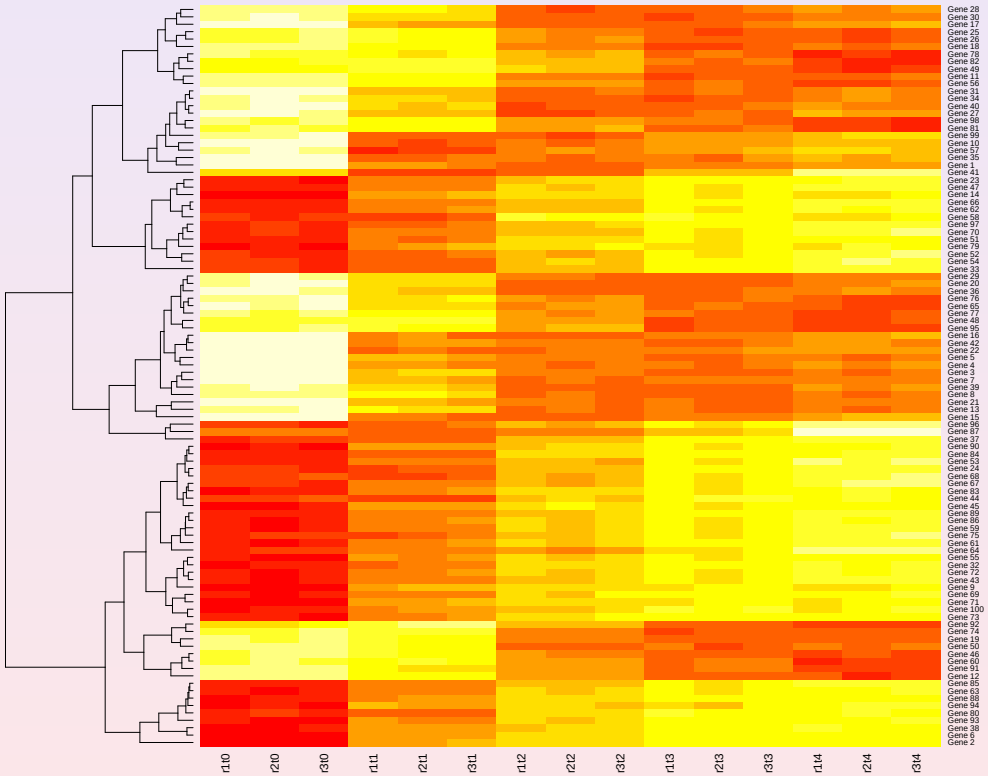


Figure: Bonferroni adjusted p-value less than 0.01 and fold-change larger than 3.5

Heatmap


r1t0
r2t0
r3t0
r1t1
r2t1
r3t1
r1t2
r2t2
r3t2
r1t3
r2t3
r3t3
r1t4
r2t4
r3t4

Network Inference

- GeneNet [2]: simple, quick, but not robust
- Metropolis Hastings for decomposable graphs (MH-d) [3]. Computationaly intensive but more stable
- Reversiable Jump MCMC for time course data

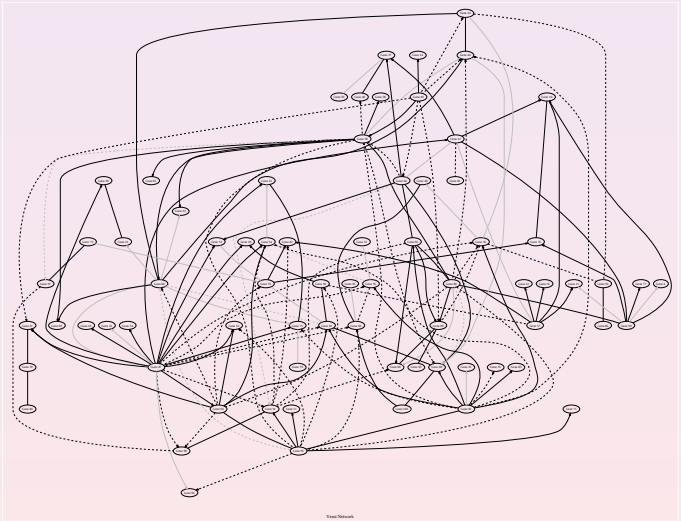


Figure: Inferred network by GeneNet package for top 100 differentially expressed Yeast genes

References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