pcaGoPromoter version 1.0.0

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1 Introduction

This R package provides functions to ease the analysis of Affymetrix DNA micro arrays by principal component analysis with annotation by GO terms and possible transcription factors.

2 Requirements

R version 2.14.0 or higher

```
> source("http://bioconductor.org/biocLite.R")
> biocLite("pcaGoPromoter",dependencies=TRUE)
```

Rgraphviz from Bioconductor is needed to draw Gene Ontology tree. Note: Graphviz needs to be installed on the computer for Rgraphviz to work. See Rgraphviz README for installation.

3 Example

3.1 Load the library

```
> library("pcaGoPromoter")
```

3.2 Read in data set serumStimulation

```
> library("serumStimulation")
> data(serumStimulation)
```

The serumStimulation data set has been created from 13 CEL files - 5 controls, 5 serum stimulated with inhibitor and 3 serum stimulated without inhibitor. They are read with ReadAffy(), normalized with rma() and the expression data extracted with exprs(). All of these function are part of the affy package.

The arrays are most likely grouped in some sort of way. Create a factor vector to indicate the groups:

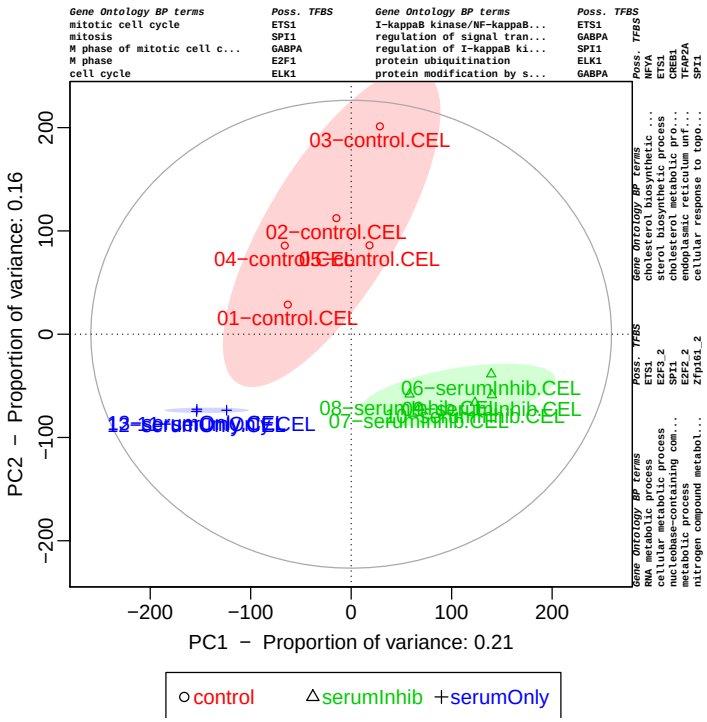
```
> groups <- as.factor( c( rep("control",5) , rep("serumInhib",5) ,
+                         rep("serumOnly",3) ) )
> groups

 [1] control    control    control    control    control    serumInhib
 [7] serumInhib serumInhib serumInhib serumInhib serumOnly  serumOnly
[13] serumOnly
Levels: control serumInhib serumOnly
```

3.3 Make PCA informative plot

This function "does-it-all". It will make a PCA plot and annotate the axis will GO terms and possible commmon transcription factors.

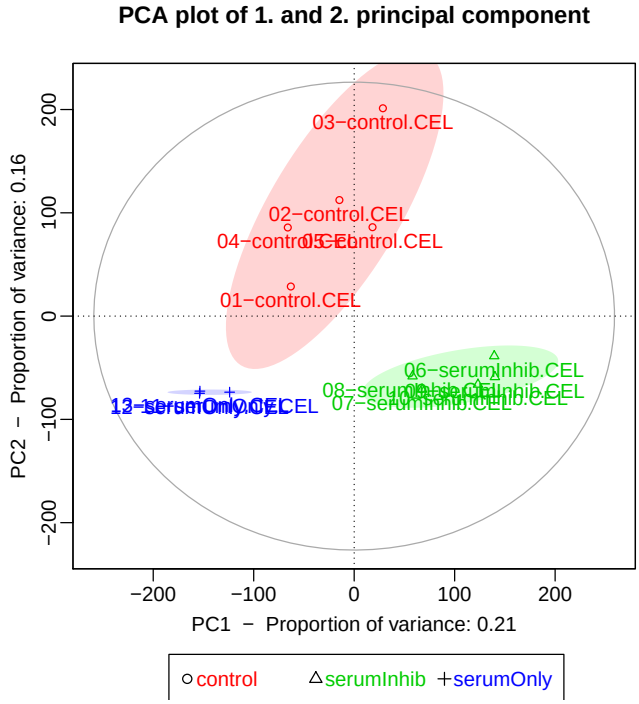
```
> pcaInfoPlot(serumStimulation,groups=groups)
```



3.4 Principal component analysis (PCA)

```
> pcaOutput <- pca(serumStimulation)
```

```
> plot(pcaOutput, groups=groups)
```



Proportion of variance is noted along the axis. In this case there are 3 groups in the data set - control, serumInhib and serumOnly. There is a clear separation of the groups along the 1. principal component (X-axis). The 2. principal component shown a difference between the controls and the serum stimulated.

3.5 Get loadings from PCA

We would like to have the first 1365 probe ids (2,5 %) from 2. principal component in the negative (serum stimulated) direction.

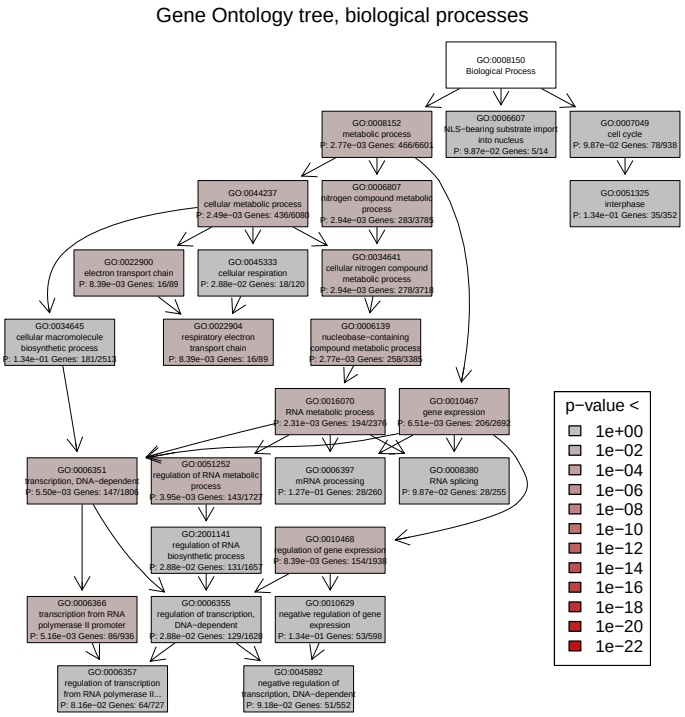
```
> loadsNegPC2 <- getRankedProbeIds( pcaOutput, pc=2, decreasing=FALSE )[1:1365]
```

3.6 Create Gene Ontology tree from loadings

Note: In this step you will be asked to install the necessary data packages.

```
> GOtreeOutput <- GOtree( input = loadsNegPC2)
```

```
> plot(GOtreeOutput,legendPosition = "bottomright")
```



Output to PDF file is advised. This can be done by coping output to a PDF file:

```
> dev.copy2pdf(file="GOtree.pdf")
```

Function 'GOtree()' also outputs a list of GO terms order by p-value.

```
> head(GOtreeOutput$sigGOs,n=10)
```

```
          GOid genesInTerm totalGenesInTerm     pValue
903  GO:0016070         194             2376 0.00230688
1651 GO:0044237         436             6080 0.00249049
246  GO:0006139         258             3385 0.00277233
656  GO:0008152         466             6601 0.00277233
424  GO:0006807         283             3785 0.00294415
1381 GO:0034641         278             3718 0.00294415
2050 GO:0051252         143             1727 0.00395398
296  GO:0006366          86              936 0.00516168
285  GO:0006351         147             1806 0.00550454
779  GO:0010467         206             2692 0.00651298
                                                GOterm
903                                RNA metabolic process
1651                          cellular metabolic process
246  nucleobase-containing compound metabolic process
656                                   metabolic process
424                 nitrogen compound metabolic process
1381     cellular nitrogen compound metabolic process
```

```
2050              regulation of RNA metabolic process
296     transcription from RNA polymerase II promoter
285                       transcription, DNA-dependent
779                                    gene expression
```

3.7 Get list of possible transcription factors

To get possible transcription factors, use function primo() function.

```
> TFtable <- primo( loadsNegPC2 )
> head(TFtable$overRepresented)
```

```
     id baseId pwmLength     gene      pValue
1  9326 MA0098         6     ETS1 1.79524e-08
2 10235 PB0113        17   E2F3_2 3.53616e-08
3  9308 MA0080         6     SPI1 3.05400e-05
4 10234 PB0112        17   E2F2_2 7.77734e-05
5 10321 PB0199        14 Zfp161_2 2.12513e-04
6 10217 PB0095        16 Zfp161_1 2.78272e-04
```

The output shows you which possible transcription factors (genes) the supplied probes have in common.

3.8 Get a list of probe ids for a specific transcription factor

```
> probeIds <- primoHits( loadsNegPC2 , id = 9343 )
> head(probeIds)
```

```
[1] "NM_001121"    "NM_016824"    "NM_001114380" "NM_002209"    "NM_003342"
[6] "NM_006403"
```