pcaGoPromoter version 1.12.0

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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:

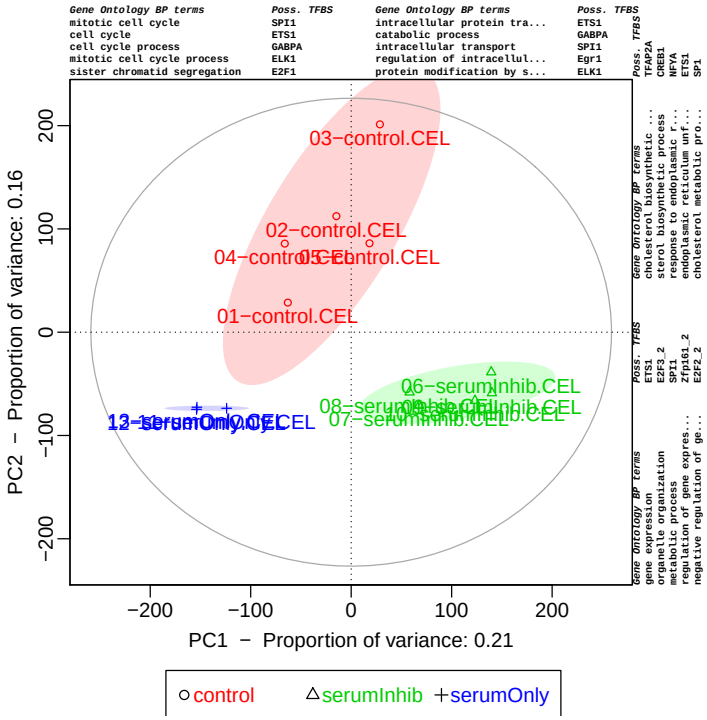
```
> groups <- as.factor( c( rep("control",5) , rep("serumInhib",5) ,
+                         rep("serumOnly",3) ) )
> groups
```

```
 [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.

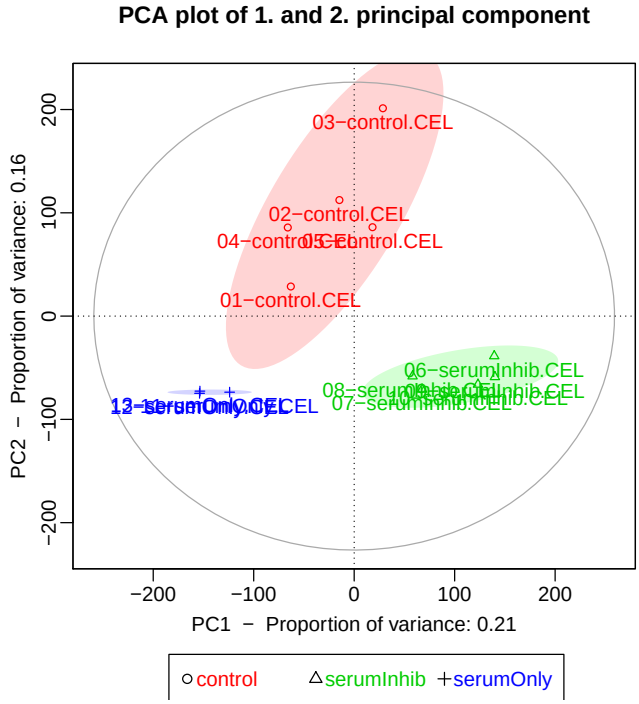
```
> pcaInfoPlot(serumStimulation,groups=groups)
```



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.

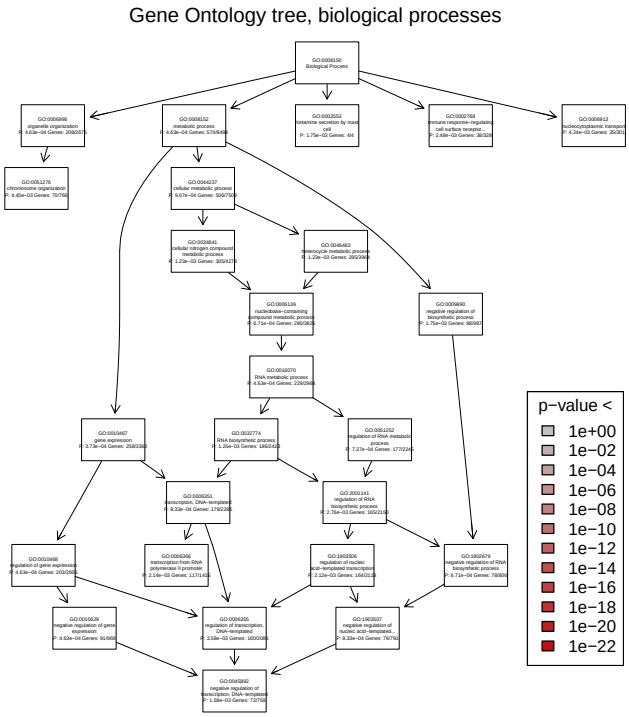
```
> loadsNegPC2 <- getRankedProbeIds( pcaOutput, pc=2, decreasing=FALSE )[1:1365]
```

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
890  GO:0010467         258             3362 0.000372599
578  GO:0006996         208             2676 0.000462888
748  GO:0008152         574             8498 0.000462888
891  GO:0010468         203             2605 0.000462888
917  GO:0010629          91              969 0.000462888
1041 GO:0016070         229             2968 0.000462888
308  GO:0006139         280             3825 0.000671210
3249 GO:1902679          78              806 0.000671210
2551 GO:0051252         177             2245 0.000737072
353  GO:0006351         179             2285 0.000833041
                                        GOterm
890                                gene expression
578                          organelle organization
748                              metabolic process
891                       regulation of gene expression
917              negative regulation of gene expression
1041                            RNA metabolic process
```

```
308  nucleobase-containing compound metabolic process
3249  negative regulation of RNA biosynthetic process
2551              regulation of RNA metabolic process
353                      transcription, DNA-templated
```

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 2.30355e-08
2 10235 PB0113        17   E2F3_2 1.08742e-07
3  9308 MA0080         6     SPI1 3.92539e-05
4 10321 PB0199        14 Zfp161_2 7.41396e-05
5 10234 PB0112        17   E2F2_2 9.72520e-05
6 10132 PB0010        14   Egr1_1 1.08150e-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"
```