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:

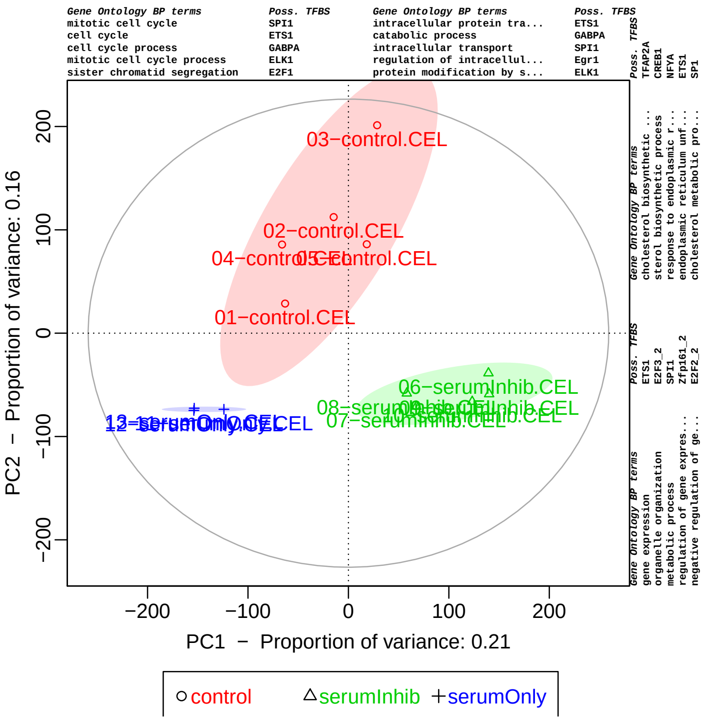
```
> groups <- as.factor( c( rep("control",5) , rep("serumInhib",5) ,
+                        rep("serumOnly",3) ) )
> groups
```

```
 [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.

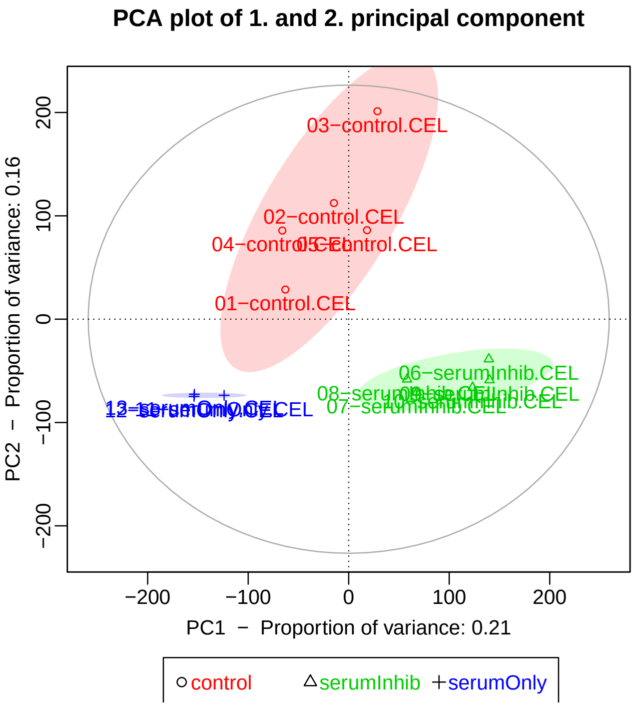
```
> pcaInfoPlot(serumStimulation,groups=groups)
```



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.

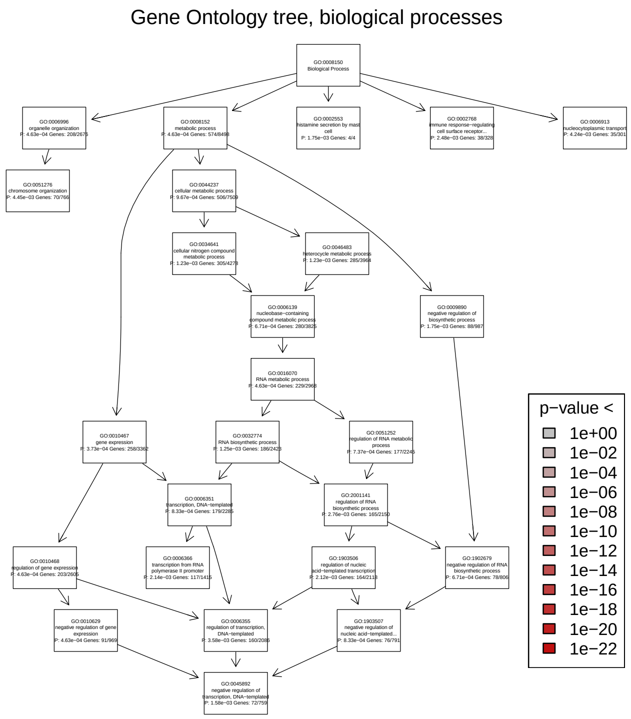
```
> loadsNegPC2 <- getRankedProbeIds( pcaOutput, pc=2, decreasing=FALSE )[1:1365]
```

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")
```


Gene Ontology tree, biological processes
GO:0008150 Biological Process
GO:0006996 organelle organization P: 4.63e−04 Genes: 208/2676
GO:0008152 metabolic process P: 4.63e−04 Genes: 574/8498
GO:0002553 histamine secretion by mast cell P: 1.75e−03 Genes: 4/4
GO:0002768 immune response−regulating cell surface receptor... P: 2.48e−03 Genes: 38/328
GO:0006913 nucleocytoplasmic transport P: 4.24e−03 Genes: 35/301
GO:0051276 chromosome organization P: 4.45e−03 Genes: 70/766
GO:0044237 cellular metabolic process P: 9.67e−04 Genes: 506/7509
GO:0034641 cellular nitrogen compound metabolic process P: 1.23e−03 Genes: 305/4278
GO:0046483 heterocycle metabolic process P: 1.23e−03 Genes: 285/3964
GO:0006139 nucleobase−containing compound metabolic process P: 6.71e−04 Genes: 280/3825
GO:0009890 negative regulation of biosynthetic process P: 1.75e−03 Genes: 88/987
GO:0016070 RNA metabolic process P: 4.63e−04 Genes: 229/2968
GO:0010467 gene expression P: 3.73e−04 Genes: 258/3362
GO:0032774 RNA biosynthetic process P: 1.25e−03 Genes: 186/2423
GO:0051252 regulation of RNA metabolic process P: 7.37e−04 Genes: 177/2245
GO:0006351 transcription, DNA−templated P: 8.33e−04 Genes: 179/2285
GO:2001141 regulation of RNA biosynthetic process P: 2.76e−03 Genes: 165/2150
GO:0010468 regulation of gene expression P: 4.63e−04 Genes: 203/2605
GO:0006366 transcription from RNA polymerase II promoter P: 2.14e−03 Genes: 117/1415
GO:1903506 regulation of nucleic acid−templated transcription P: 2.12e−03 Genes: 164/2118
GO:1902679 negative regulation of RNA biosynthetic process P: 6.71e−04 Genes: 78/806
GO:0010629 negative regulation of gene expression P: 4.63e−04 Genes: 91/969
GO:0006355 regulation of transcription, DNA−templated P: 3.58e−03 Genes: 160/2086
GO:1903507 negative regulation of nucleic acid−templated... P: 8.33e−04 Genes: 76/791
GO:0045892 negative regulation of transcription, DNA−templated P: 1.58e−03 Genes: 72/759
p−value <
1e+00
1e−02
1e−04
1e−06
1e−08
1e−10
1e−12
1e−14
1e−16
1e−18
1e−20
1e−22

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"
```