pcaGoPromoter version 1.8.0

Morten Hansen

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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:

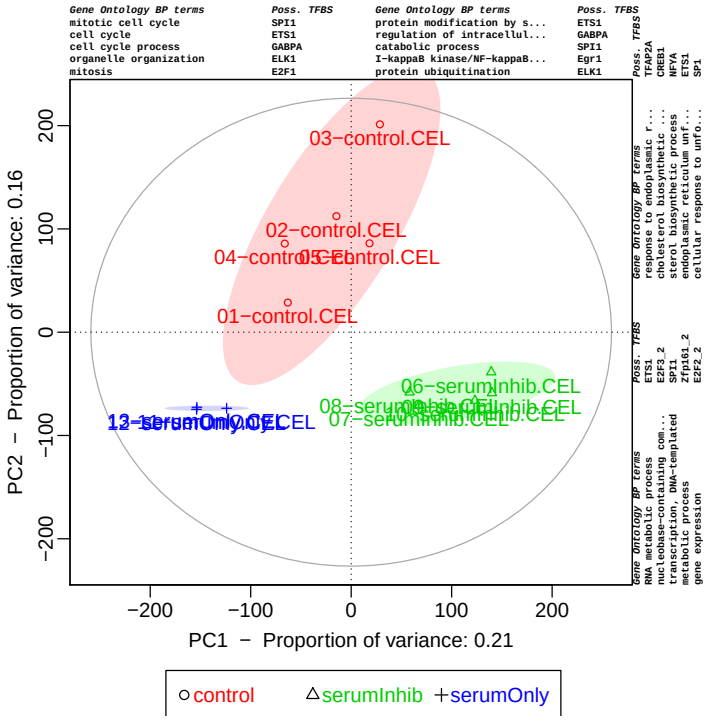
```
> groups <- as.factor( c( rep("control",5) , rep("serumInhib",5) ,
+                         rep("serumOnly",3) ) )
> groups

 [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.

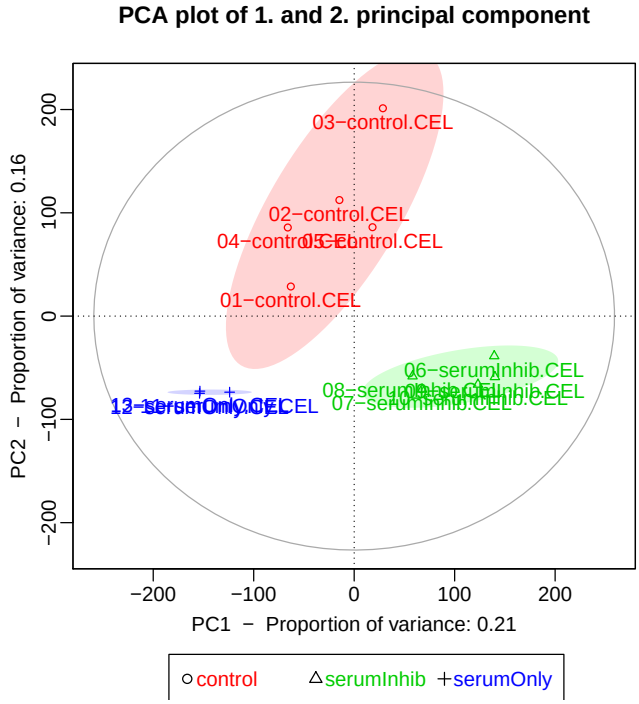
```
> pcaInfoPlot(serumStimulation,groups=groups)
```



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.

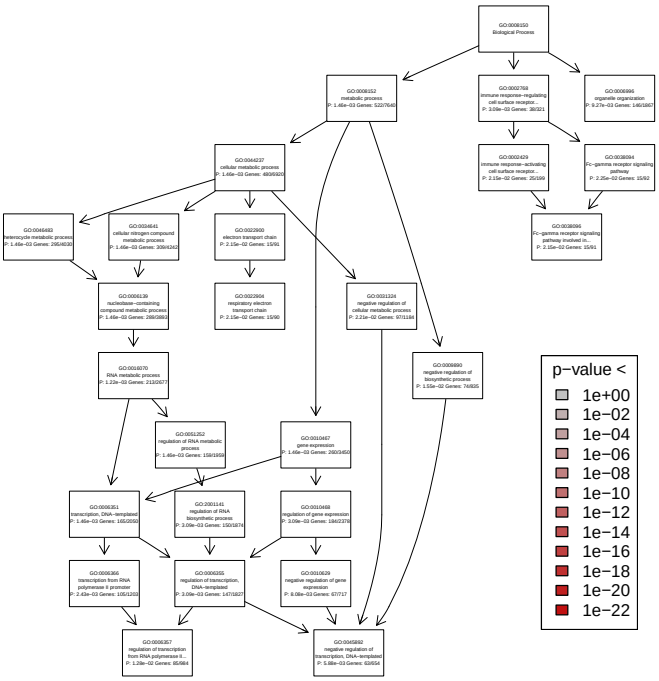
```
> loadsNegPC2 <- getRankedProbeIds( pcaOutput, pc=2, decreasing=FALSE )[1:1365]
```

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
984  GO:0016070         213             2677 0.00121903
276  GO:0006139         289             3893 0.00145807
317  GO:0006351         165             2050 0.00145807
701  GO:0008152         522             7640 0.00145807
836  GO:0010467         260             3450 0.00145807
1550 GO:0034641         309             4242 0.00145807
1888 GO:0044237         480             6920 0.00145807
2067 GO:0046483         295             4030 0.00145807
2349 GO:0051252         159             1959 0.00145807
329  GO:0006366         105             1203 0.00243122
                                                GOterm
984                              RNA metabolic process
276  nucleobase-containing compound metabolic process
317                      transcription, DNA-templated
701                                metabolic process
836                                  gene expression
1550     cellular nitrogen compound metabolic process
```

```
1888                       cellular metabolic process
2067                    heterocycle metabolic process
2349              regulation of RNA metabolic process
329     transcription from RNA polymerase II promoter
```

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 2.67513e-08
2 10235 PB0113        17   E2F3_2 1.03006e-07
3  9308 MA0080         6     SPI1 4.42403e-05
4 10321 PB0199        14 Zfp161_2 7.10571e-05
5 10234 PB0112        17   E2F2_2 9.37617e-05
6 10132 PB0010        14   Egr1_1 1.03372e-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"
```