

# Bioconductor's SPIA package

Adi L. Tarca<sup>1,2,3</sup>, Purvesh Khatri<sup>1</sup> and Sorin Draghici<sup>1</sup>

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<sup>1</sup>Department of Computer Science, Wayne State University

<sup>2</sup>Bioinformatics and Computational Biology Unit of the NIH Perinatology Research Branch

<sup>3</sup>Center for Molecular Medicine and Genetics, Wayne State University

## 1 Overview

This package implements the Signaling Pathway Impact Analysis (SPIA) algorithm described in Tarca et al. (2009), Khatri et al. (2007) and Draghici et al. (2007). SPIA uses the information from a set of differentially expressed genes and their fold changes, as well as pathways topology in order to assess the significance of the pathways in the condition under the study. The current version of SPIA algorithm uses KEGG signaling pathway data. SPIA ready KEGG pathway data for homo sapiens is included in the package and also available at

<http://bioinformaticsprb.med.wayne.edu/SPIA/>.

The pathways included for each organism are those containing only directed relations between genes/proteins and no reactions.

## 2 Pathway analysis with SPIA package

This document provides basic introduction on how to use the SPIA package. For extended description of the methods used by this package please consult these references: Tarca et al. (2009); Khatri et al. (2007); Draghici et al. (2007).

We demonstrate the functionality of this package using a colorectal cancer dataset obtained using Affymetrix GeneChip technology and available through GEO (GSE4107). The experiment contains 10 normal samples and 12 colorectal cancer samples and is described by Hong et al. (2007). RMA preprocessing of the raw data was performed using the `affy` package, and a two group moderated t-test was applied using the `limma` package. The data frame obtained as an end result from the function `topTable` in `limma` is used as starting point for preparing the input data for SPIA. This data frame called `top` was made available in the `colorectalcancer` dataset included in the SPIA package:

```
> library(SPIA)
> data(colorectalcancer)
```

```
> options(digits = 3)
> head(top)
```

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
10738	201289_at	5.96	6.23	23.9	1.79e-17	9.78e-13	25.4
18604	209189_at	5.14	7.49	17.4	1.56e-14	2.84e-10	21.0
11143	201694_s_at	4.15	7.04	16.5	5.15e-14	7.04e-10	20.1
10490	201041_s_at	2.43	9.59	14.1	1.29e-12	1.41e-08	17.7
10913	201464_x_at	1.53	8.22	11.0	1.69e-10	1.15e-06	13.6
11463	202014_at	1.43	5.33	10.5	4.27e-10	2.42e-06	12.8

For SPIA to work, we need a vector with log2 fold changes between the two groups for all the genes considered to be differentially expressed. The names of this vector must be Entrez gene IDs. The following lines will add one additional column in the `top` data frame annotating each affymetrix probeset to an Entrez ID. Since there may be several probesets for the same Entrez ID, there are two easy ways to obtain one log fold change per gene. The first option is to use the fold change of the most significant probeset for each gene, while the second option is to average the log fold-changes of all probesets of the same gene. In the example below we used the former approach. The genes in this example are called differentially expressed provided that their FDR p-value is less than 0.05. The following lines start with the `top` data frame and produce two vectors that are required as input by `spia` function:

```
> library(hgu133plus2.db)
> x <- hgu133plus2ENTREZID
> top$ENTREZ <- unlist(as.list(x[top$ID]))
> top <- top[!is.na(top$ENTREZ), ]
> top <- top[!duplicated(top$ENTREZ), ]
> tg1 <- top[top$adj.P.Val < 0.05, ]
> DE_Colorectal = tg1$logFC
> names(DE_Colorectal) <- as.vector(tg1$ENTREZ)
> ALL_Colorectal = top$ENTREZ
```

The `DE_Colorectal` is a vector containing the log2 fold changes of the genes found to be differentially expressed between cancer and normal samples, and `ALL_Colorectal` is a vector with the Entrez IDs of all genes profiled on the microarray. The names of the `DE_Colorectal` are the Entrez gene IDs corresponding to the computed log fold-changes.

```
> DE_Colorectal[1:10]
```

3491	2353	1958	1843	3725	23645	9510	84869	7432	1490
5.96	5.14	4.15	2.43	1.53	1.43	3.94	-1.15	4.72	3.45

```
> ALL_Colorectal[1:10]
```

```
[1] "3491" "2353" "1958" "1843" "3725" "23645" "9510" "84869" "7432"
[10] "1490"
```

The SPIA algorithm takes as input the two vectors above and produces a table of pathways ranked from the most to the least significant. This can be achieved by calling the `spia` function as follows:

```
> res = spia(de = DE_Colorectal, all = ALL_Colorectal, organism = "hsa",  
+           nB = 2000, plots = FALSE, beta = NULL)
```

```
Done pathway 1 : PPAR signaling pathway..  
Done pathway 2 : MAPK signaling pathway..  
Done pathway 3 : ErbB signaling pathway..  
Done pathway 4 : Calcium signaling pathway..  
Done pathway 5 : Cytokine-cytokine recepto..  
Done pathway 6 : Chemokine signaling pathw..  
Done pathway 7 : Neuroactive ligand-recept..  
Done pathway 8 : Cell cycle..  
Done pathway 9 : Oocyte meiosis..  
Done pathway 10 : p53 signaling pathway..  
Done pathway 11 : SNARE interactions in ves..  
Done pathway 12 : Regulation of autophagy..  
Done pathway 13 : Lysosome..  
Done pathway 14 : mTOR signaling pathway..  
Done pathway 15 : Apoptosis..  
Done pathway 16 : Vascular smooth muscle co..  
Done pathway 17 : Wnt signaling pathway..  
Done pathway 18 : Dorso-ventral axis format..  
Done pathway 19 : Notch signaling pathway..  
Done pathway 20 : Hedgehog signaling pathwa..  
Done pathway 21 : TGF-beta signaling pathwa..  
Done pathway 22 : Axon guidance..  
Done pathway 23 : VEGF signaling pathway..  
Done pathway 24 : Focal adhesion..  
Done pathway 25 : ECM-receptor interaction..  
Done pathway 26 : Cell adhesion molecules (..  
Done pathway 27 : Adherens junction..  
Done pathway 28 : Tight junction..  
Done pathway 29 : Gap junction..  
Done pathway 30 : Complement and coagulatio..  
Done pathway 31 : Antigen processing and pr..  
Done pathway 32 : Toll-like receptor signal..  
Done pathway 33 : NOD-like receptor signali..  
Done pathway 34 : RIG-I-like receptor signa..  
Done pathway 35 : Cytosolic DNA-sensing pat..  
Done pathway 36 : Jak-STAT signaling pathwa..  
Done pathway 37 : Natural killer cell media..  
Done pathway 38 : T cell receptor signaling..  
Done pathway 39 : B cell receptor signaling..  
Done pathway 40 : Fc epsilon RI signaling p..
```

Done pathway 41 : Fc gamma R-mediated phago..  
Done pathway 42 : Leukocyte transendothelia..  
Done pathway 43 : Intestinal immune network..  
Done pathway 44 : Circadian rhythm - mammal..  
Done pathway 45 : Long-term potentiation..  
Done pathway 46 : Neurotrophin signaling pa..  
Done pathway 47 : Long-term depression..  
Done pathway 48 : Olfactory transduction..  
Done pathway 49 : Taste transduction..  
Done pathway 50 : Regulation of actin cytos..  
Done pathway 51 : Insulin signaling pathway..  
Done pathway 52 : GnRH signaling pathway..  
Done pathway 53 : Progesterone-mediated ooc..  
Done pathway 54 : Melanogenesis..  
Done pathway 55 : Adipocytokine signaling p..  
Done pathway 56 : Type II diabetes mellitus..  
Done pathway 57 : Type I diabetes mellitus..  
Done pathway 58 : Maturity onset diabetes o..  
Done pathway 59 : Aldosterone-regulated sod..  
Done pathway 60 : Vasopressin-regulated wat..  
Done pathway 61 : Alzheimer's disease..  
Done pathway 62 : Parkinson's disease..  
Done pathway 63 : Amyotrophic lateral scler..  
Done pathway 64 : Huntington's disease..  
Done pathway 65 : Prion diseases..  
Done pathway 66 : Vibrio cholerae infection..  
Done pathway 67 : Epithelial cell signaling..  
Done pathway 68 : Pathogenic Escherichia co..  
Done pathway 69 : Pathways in cancer..  
Done pathway 70 : Colorectal cancer..  
Done pathway 71 : Renal cell carcinoma..  
Done pathway 72 : Pancreatic cancer..  
Done pathway 73 : Endometrial cancer..  
Done pathway 74 : Glioma..  
Done pathway 75 : Prostate cancer..  
Done pathway 76 : Thyroid cancer..  
Done pathway 77 : Basal cell carcinoma..  
Done pathway 78 : Melanoma..  
Done pathway 79 : Bladder cancer..  
Done pathway 80 : Chronic myeloid leukemia..  
Done pathway 81 : Acute myeloid leukemia..  
Done pathway 82 : Small cell lung cancer..  
Done pathway 83 : Non-small cell lung cance..  
Done pathway 84 : Asthma..  
Done pathway 85 : Autoimmune thyroid diseas..

```

Done pathway 86 : Systemic lupus erythemato..
Done pathway 87 : Allograft rejection..
Done pathway 88 : Graft-versus-host disease..
Done pathway 89 : Arrhythmogenic right vent..
Done pathway 90 : Dilated cardiomyopathy..
Done pathway 91 : Viral myocarditis..

```

```

> res$Name = substr(res$Name, 1, 10)
> res[1:15, -12]

```

	Name	ID	pSize	NDE	tA	pNDE	pPERT	pG	pGFdr
1	Parkinson'	05012	106	56	-12.121	7.05e-14	0.079000	1.88e-13	1.64e-11
2	Alzheimer'	05010	146	69	-6.216	1.30e-13	0.219000	9.13e-13	3.97e-11
3	Focal adhe	04510	177	63	100.717	1.07e-06	0.000005	1.44e-10	4.17e-09
4	Huntington	05016	165	65	-3.150	8.68e-09	0.187000	3.45e-08	7.50e-07
5	ECM-recept	04512	74	26	22.002	1.82e-03	0.000005	1.78e-07	3.09e-06
6	PPAR signa	03320	64	30	-3.127	1.28e-06	0.040000	9.11e-07	1.32e-05
7	Axon guida	04360	119	47	8.919	8.73e-07	0.335000	4.69e-06	5.83e-05
8	Circadian	04710	8	6	-3.081	1.26e-03	0.143000	1.74e-03	1.89e-02
9	Wnt signal	04310	137	43	-8.343	1.15e-03	0.204000	2.19e-03	2.03e-02
10	Small cell	05222	75	21	25.226	6.27e-02	0.004000	2.33e-03	2.03e-02
11	Colorectal	05210	78	26	7.241	4.17e-03	0.067000	2.57e-03	2.03e-02
12	MAPK signa	04010	247	71	3.851	6.77e-04	0.597000	3.56e-03	2.54e-02
13	Regulation	04810	192	56	15.022	1.64e-03	0.264000	3.79e-03	2.54e-02
14	Lysosome	04142	117	36	-0.753	4.00e-03	0.157000	5.26e-03	3.27e-02
15	Renal cell	05211	64	21	-7.742	1.15e-02	0.105000	9.29e-03	5.39e-02

	pGFWER	Status
1	1.64e-11	Inhibited
2	7.95e-11	Inhibited
3	1.25e-08	Activated
4	3.00e-06	Inhibited
5	1.55e-05	Activated
6	7.92e-05	Inhibited
7	4.08e-04	Activated
8	1.51e-01	Inhibited
9	1.91e-01	Inhibited
10	2.03e-01	Activated
11	2.23e-01	Activated
12	3.10e-01	Activated
13	3.30e-01	Activated
14	4.57e-01	Inhibited
15	8.08e-01	Inhibited

If the `plots` argument is set to `TRUE` in the function call above, a plot like the one shown in Figure 1 is produced for each pathway on which there are differentially expressed genes. These plots are saved in a pdf file in the current directory.

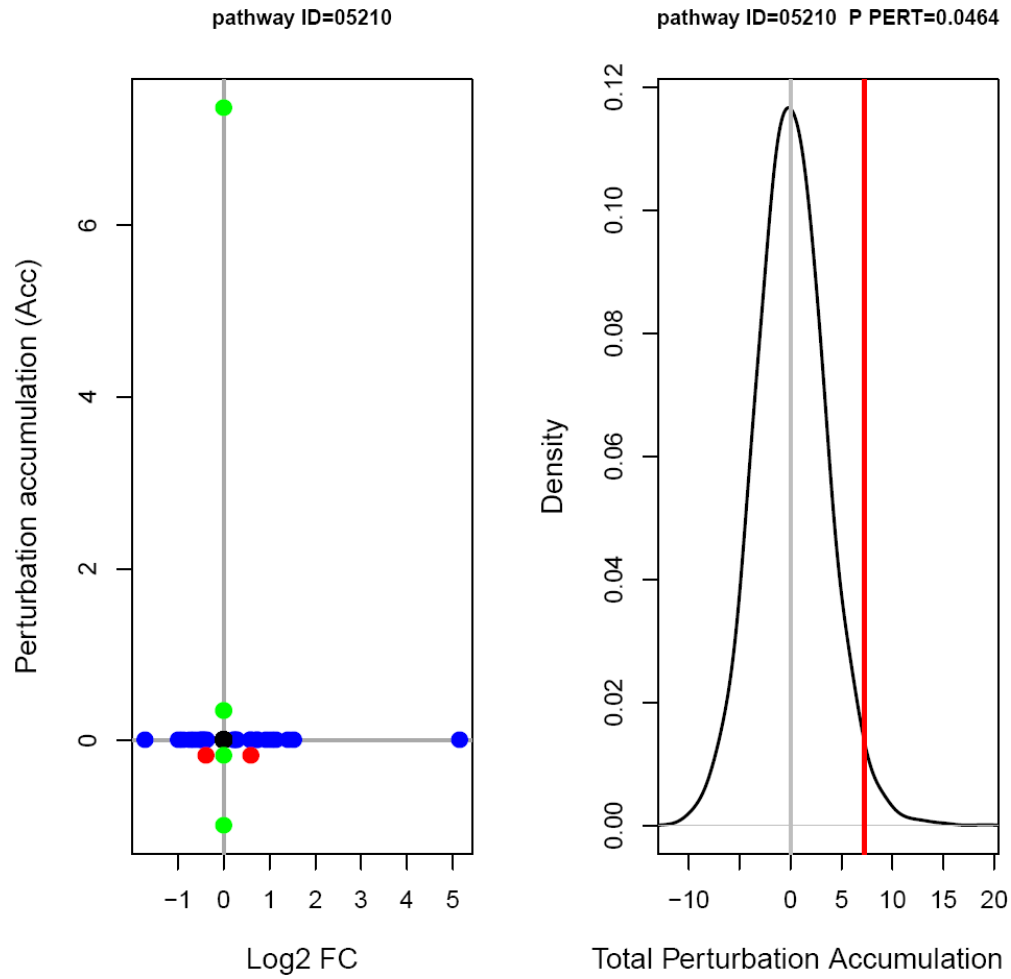


Figure 1: Perturbations plot for colorectal cancer pathway (KEGG ID hsa:05210) using the `colorectal_cancer` dataset. The perturbation of all genes in the pathway are shown as a function of their initial log2 fold changes (left panel). Non DE genes are assigned 0 log2 fold-change. The null distribution of the net accumulated perturbations is also given (right panel). The observed net accumulation  $tA$  with the real data is shown as a red vertical line.

An overall picture of the pathways significance according to both the over-representation evidence and perturbations based evidence can be obtained with the function `plotP` and shown in Figure 2.

```
> plotP(res, threshold = 0.05)
```

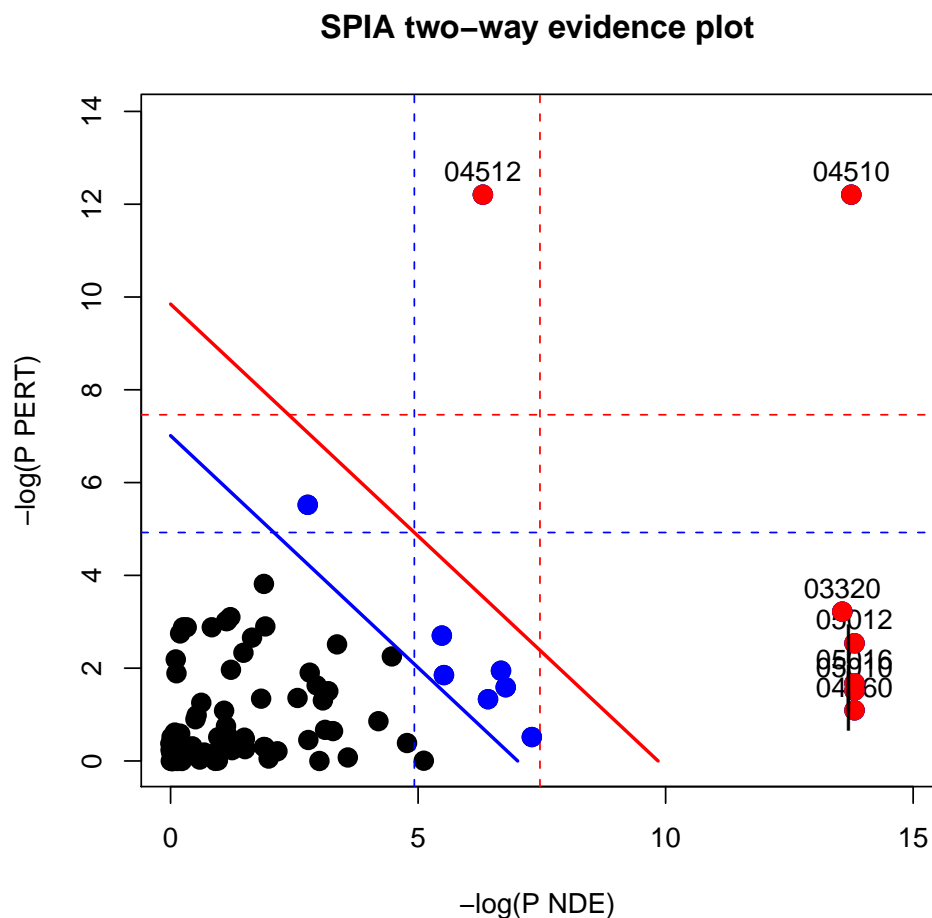


Figure 2: SPIA evidence plot for the colorectal cancer dataset. Each pathway is represented by one dot. The pathways at the right of the red oblique line are significant after Bonferroni correction of the global p-values,  $pG$ . The pathways at the right of the blue oblique line are significant after a FDR correction of the global p-values,  $pG$ .

In this plot, the horizontal axis represents the p-value (minus log of) corresponding to the probability of obtaining at least the observed number of genes (NDE) on the given pathway just by chance. The vertical axis represents the p-value (minus log of) corresponding to the probability of obtaining the observed total accumulation (tA) or more extreme on the given pathway just by chance. The computation of pPERT is described in Tarca et al. (2009). In Figure 2 each pathway is shown as a bullet point, and those significant at 5% (set by the `threshold` argument in `plotP`) after Bonferroni correction are shown in red.

SPIA algorithm is illustrated also using the Vessels dataset:

```
> data(Vessels)
> res <- spia(de = DE_Vessels, all = ALL_Vessels, organism = "hsa",
+           nB = 500, plots = FALSE, beta = NULL, verbose = FALSE)
> res$Name = substr(res$Name, 1, 10)
> res[1:15, -12]
```

	Name	ID	pSize	NDE	tA	pNDE	pPERT	pG	pGFdr	pGFWER
1	Axon guida	04360	128	12	-5.73	0.000208	0.128	0.000307	0.0191	0.0246
2	Focal adhe	04510	198	16	-5.99	0.000116	0.372	0.000478	0.0191	0.0382
3	Regulation	04810	210	14	9.42	0.002001	0.052	0.001058	0.0216	0.0846
4	Neuroactiv	04080	255	18	-0.51	0.000247	0.432	0.001081	0.0216	0.0865
5	Viral myoc	05416	70	8	-1.67	0.000635	0.292	0.001779	0.0285	0.1423
6	Intestinal	04672	46	7	0.00	0.000234	1.000	0.002193	0.0292	0.1754
7	Notch sign	04330	46	4	7.58	0.036603	0.020	0.006017	0.0586	0.4814
8	Complement	04610	67	7	4.59	0.002325	0.332	0.006304	0.0586	0.5043
9	Graft-vers	05332	41	6	0.00	0.000813	1.000	0.006597	0.0586	0.5278
10	Asthma	05310	29	5	0.00	0.001038	1.000	0.008167	0.0601	0.6533
11	Type I dia	04940	43	6	0.00	0.001053	1.000	0.008270	0.0601	0.6616
12	Wnt signal	04310	150	11	1.03	0.002836	0.632	0.013128	0.0856	1.0000
13	Antigen pr	04612	88	7	1.49	0.010418	0.184	0.013911	0.0856	1.0000
14	Leukocyte	04670	116	9	-1.65	0.004608	0.664	0.020773	0.1146	1.0000
15	Allograft	05330	37	5	0.00	0.003183	1.000	0.021483	0.1146	1.0000

```
Status
1 Inhibited
2 Inhibited
3 Activated
4 Inhibited
5 Inhibited
6 Inhibited
7 Activated
8 Activated
9 Inhibited
10 Inhibited
11 Inhibited
12 Activated
13 Activated
14 Inhibited
15 Inhibited
```

The pathway image as provided by KEGG having the differentially expressed genes highlighted in red can be obtained by pasting in a web browser the links available in the KEGGLINK column of the data frame produced by the function spia. For example,

```
> res[, "KEGGLINK"][20]
```



[1] "[http://www.genome.jp/dbget-bin/show\\_pathway?hsa05320+3108+3109+3111+3113+3122](http://www.genome.jp/dbget-bin/show_pathway?hsa05320+3108+3109+3111+3113+3122)"

is the link that would display the image of the 20th pathway in the res dataframe above.

Note that the results for these datasets may differ from the ones described in Tarca et al. (2009) since

a) the pathways database used herein was updated and b) the default beta values were changed.

The directed adjacency matrices of the graphs describing the different types of relations between genes/proteins (such as activation or repression) used by SPIA are available in the `extdata/hsaSPIA.RData` file for the homo sapiens organism. The types of relations considered by SPIA and the default weight (beta coefficient) given to them are:

```
> rel <- c("activation", "compound", "binding/association", "expression",
+         "inhibition", "activation_phosphorylation", "phosphorylation",
+         "indirect", "inhibition_phosphorylation", "dephosphorylation_inhibition",
+         "dissociation", "dephosphorylation", "activation_dephosphorylation",
+         "state", "activation_indirect", "inhibition_ubiquination",
+         "ubiquination", "expression_indirect", "indirect_inhibition",
+         "repression", "binding/association_phosphorylation", "dissociation_phosphorylation",
+         "indirect_phosphorylation")
> beta = c(1, 0, 0, 1, -1, 1, 0, 0, -1, -1, 0, 0, 1, 0, 1, -1,
+         0, 1, -1, -1, 0, 0, 0)
> names(beta) <- rel
> cbind(beta)
```

	beta
activation	1
compound	0
binding/association	0
expression	1
inhibition	-1
activation_phosphorylation	1
phosphorylation	0
indirect	0
inhibition_phosphorylation	-1
dephosphorylation_inhibition	-1
dissociation	0
dephosphorylation	0
activation_dephosphorylation	1
state	0
activation_indirect	1
inhibition_ubiquination	-1
ubiquination	0
expression_indirect	1
indirect_inhibition	-1
repression	-1
binding/association_phosphorylation	0
dissociation_phosphorylation	0
indirect_phosphorylation	0

A 0 value for a given relation type results in discarding those type of relations from the analysis for all pathways. The default values of **beta** can be changed by the user at any time by setting the **beta** argument of the **spia** function call.

Other organisms' KEGG pathway data can be downloaded from <http://bioinformaticsprb.med.wayne.edu/SPIA> as a "[org]SPIA.RData" file and copied into the **extdata** directory of the SPIA package, and therefore make it available to the function **spia**.

The user has the ability to generate his own gene/protein relation data and put it in a list format as the one shown in the **hsaSPIA.RData** file. In this file, each pathway data is included in a list:

```
> load(file = paste(system.file("extdata/hsaSPIA.RData", package = "SPIA")))
> names(path.info[["05210"]])
```

```
[1] "activation"                "compound"
[3] "binding/association"       "expression"
[5] "inhibition"                "activation_phosphorylation"
[7] "phosphorylation"           "indirect"
[9] "inhibition_phosphorylation" "dephosphorylation_inhibition"
[11] "dissociation"              "dephosphorylation"
[13] "activation_dephosphorylation" "state"
[15] "activation_indirect"        "inhibition_ubiquination"
[17] "ubiquination"              "expression_indirect"
[19] "indirect_inhibition"        "repression"
[21] "binding/association_phosphorylation" "dissociation_phosphorylation"
[23] "indirect_phosphorylation"   "nodes"
[25] "title"                     "NumberOfReactions"
```

```
> path.info[["05210"]][["activation"]][48:60, 55:60]
```

	8313	5900	5879	5880	5881	332
369	0	0	0	0	0	0
5894	0	0	0	0	0	0
673	0	0	0	0	0	0
5599	0	0	1	1	1	0
5601	0	0	1	1	1	0
5602	0	0	1	1	1	0
8312	0	0	0	0	0	0
8313	0	0	0	0	0	0
5900	0	0	0	0	0	0
5879	0	1	0	0	0	0
5880	0	1	0	0	0	0
5881	0	1	0	0	0	0
332	0	0	0	0	0	0

In the matrix above, only 0 and 1 values are allowed. 1 means the gene/protein given by the column has a relation of type "activation" with the gene/protein given by the row of the matrix.

Using other R packages such as **graph** and **Rgraphviz** one can visualize the richness of gene/protein relations of each type in each pathway. Firstly we load the required packages and create a function that can be used to plot as a graph each type of relation of any pathway, as used by SPIA.

```

> library(graph)
> library(Rgraphviz)
> plotG <- function(B) {
+   nnms <- NULL
+   colls <- NULL
+   mynodes <- colnames(B)
+   L <- list()
+   n <- dim(B)[1]
+   for (i in 1:n) {
+     L[i] <- list(edges = rownames(B)[abs(B[, i]) > 0])
+     if (sum(B[, i] != 0) > 0) {
+       nnms <- c(nnms, paste(colnames(B)[i], rownames(B)[B[,
+         i] != 0], sep = "~"))
+     }
+   }
+   names(L) <- rownames(B)
+   g <- new("graphNEL", nodes = mynodes, edgeL = L, edgemode = "directed")
+   plot(g)
+ }

```

We plot then the "activation" relations in the ErbB signaling pathway, based on the `hsaSPIA` data. For more details on how to use the main function in this package use `"?spia"`.

## References

- S. Draghici, P. Khatri, A. Tarca, K. Amin, A. Done, C. Voichita, C. Georgescu, and R. Romero. A systems biology approach for pathway level analysis. *Genome Research*, 17, 2007.
- Y. Hong, K. S. Ho, K. W. Eu, and P. Y. Cheah. A susceptibility gene set for early onset colorectal cancer that integrates diverse signaling pathways: implication for tumorigenesis. *Clin Cancer Res*, 13(4):1107–14, 2007.
- P. Khatri, S. Draghici, A. L. Tarca, S. S. Hassan, and R. Romero. A system biology approach for the steady-state analysis of gene signaling networks. In *12th Iberoamerican Congress on Pattern Recognition*, Valparaiso, Chile, November 13-16 2007.
- A. L. Tarca, S. Draghici, P. Khatri, S. Hassan, P. Mital, J. Kim, C. Kim, J. P. Kusanovic, and R. Romero. A signaling pathway impact analysis for microarray experiments. *Bioinformatics*, 25:75–82, 2009.

```
> plotG(path.info[["04012"]][["activation"]])
```

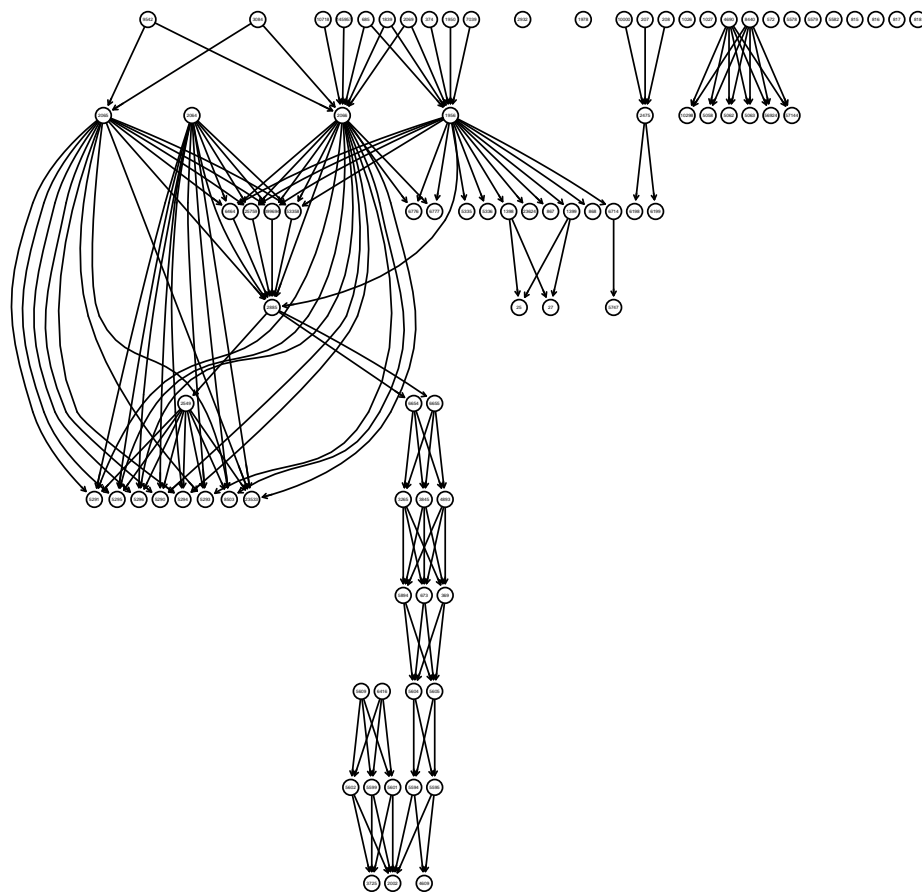


Figure 3: Display of the "activation" relations in the ErbB signaling pathway, based on the hsaSPIA data.