

Bioconductor's SPIA package

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October 18, 2010

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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.

The KEGG data that was preprocessed for SPIA analysis was downloaded from KEGG's ftp repository on: 09/22/2010.

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 : Protein processing in end..  
Done pathway 14 : Lysosome..  
Done pathway 15 : mTOR signaling pathway..  
Done pathway 16 : Apoptosis..  
Done pathway 17 : Vascular smooth muscle co..  
Done pathway 18 : Wnt signaling pathway..  
Done pathway 19 : Dorso-ventral axis format..  
Done pathway 20 : Notch signaling pathway..  
Done pathway 21 : Hedgehog signaling pathwa..  
Done pathway 22 : TGF-beta signaling pathwa..  
Done pathway 23 : Axon guidance..  
Done pathway 24 : VEGF signaling pathway..  
Done pathway 25 : Focal adhesion..  
Done pathway 26 : ECM-receptor interaction..  
Done pathway 27 : Cell adhesion molecules (..  
Done pathway 28 : Adherens junction..  
Done pathway 29 : Tight junction..  
Done pathway 30 : Gap junction..  
Done pathway 31 : Complement and coagulatio..  
Done pathway 32 : Antigen processing and pr..  
Done pathway 33 : Toll-like receptor signal..  
Done pathway 34 : NOD-like receptor signali..  
Done pathway 35 : RIG-I-like receptor signa..  
Done pathway 36 : Cytosolic DNA-sensing pat..  
Done pathway 37 : Jak-STAT signaling pathwa..  
Done pathway 38 : Natural killer cell media..  
Done pathway 39 : T cell receptor signaling..  
Done pathway 40 : B cell receptor signaling..
```

Done pathway 41 : Fc epsilon RI signaling p..
Done pathway 42 : Fc gamma R-mediated phago..
Done pathway 43 : Leukocyte transendothelia..
Done pathway 44 : Intestinal immune network..
Done pathway 45 : Circadian rhythm - mammal..
Done pathway 46 : Long-term potentiation..
Done pathway 47 : Neurotrophin signaling pa..
Done pathway 48 : Long-term depression..
Done pathway 49 : Olfactory transduction..
Done pathway 50 : Taste transduction..
Done pathway 51 : Phototransduction..
Done pathway 52 : Regulation of actin cytos..
Done pathway 53 : Insulin signaling pathway..
Done pathway 54 : GnRH signaling pathway..
Done pathway 55 : Progesterone-mediated ooc..
Done pathway 56 : Melanogenesis..
Done pathway 57 : Adipocytokine signaling p..
Done pathway 58 : Type II diabetes mellitus..
Done pathway 59 : Type I diabetes mellitus..
Done pathway 60 : Maturity onset diabetes o..
Done pathway 61 : Aldosterone-regulated sod..
Done pathway 62 : Vasopressin-regulated wat..
Done pathway 63 : Salivary secretion..
Done pathway 64 : Gastric acid secretion..
Done pathway 65 : Alzheimer's disease..
Done pathway 66 : Parkinson's disease..
Done pathway 67 : Amyotrophic lateral scler..
Done pathway 68 : Huntington's disease..
Done pathway 69 : Prion diseases..
Done pathway 70 : Bacterial invasion of epi..
Done pathway 71 : Vibrio cholerae infection..
Done pathway 72 : Epithelial cell signaling..
Done pathway 73 : Pathogenic Escherichia co..
Done pathway 74 : Shigellosis..
Done pathway 75 : Leishmaniasis..
Done pathway 76 : Chagas disease..
Done pathway 77 : Malaria..
Done pathway 78 : Amoebiasis..
Done pathway 79 : Pathways in cancer..
Done pathway 80 : Colorectal cancer..
Done pathway 81 : Renal cell carcinoma..
Done pathway 82 : Pancreatic cancer..
Done pathway 83 : Endometrial cancer..
Done pathway 84 : Glioma..
Done pathway 85 : Prostate cancer..

Done pathway 86 : Thyroid cancer..
 Done pathway 87 : Basal cell carcinoma..
 Done pathway 88 : Melanoma..
 Done pathway 89 : Bladder cancer..
 Done pathway 90 : Chronic myeloid leukemia..
 Done pathway 91 : Acute myeloid leukemia..
 Done pathway 92 : Small cell lung cancer..
 Done pathway 93 : Non-small cell lung cance..
 Done pathway 94 : Asthma..
 Done pathway 95 : Autoimmune thyroid diseas..
 Done pathway 96 : Systemic lupus erythemato..
 Done pathway 97 : Allograft rejection..
 Done pathway 98 : Graft-versus-host disease..
 Done pathway 99 : Arrhythmogenic right vent..
 Done pathway 100 : Dilated cardiomyopathy..
 Done pathway 101 : 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	-12.026	7.22e-14	0.063000	1.55e-13	1.50e-11
2	Alzheimer'	05010	146	69	-6.240	1.33e-13	0.241000	1.03e-12	4.99e-11
3	Focal adhe	04510	177	63	100.415	1.09e-06	0.000005	1.47e-10	4.74e-09
4	Huntington	05016	164	65	-3.273	6.68e-09	0.179000	2.58e-08	6.25e-07
5	ECM-recept	04512	74	26	22.173	1.84e-03	0.000005	1.79e-07	3.47e-06
6	PPAR signa	03320	64	30	-3.099	1.30e-06	0.051000	1.16e-06	1.87e-05
7	Axon guida	04360	119	47	9.220	8.87e-07	0.341000	4.84e-06	6.71e-05
8	Small cell	05222	75	21	25.070	6.30e-02	0.003000	1.81e-03	2.19e-02
9	Wnt signal	04310	138	43	-8.449	1.36e-03	0.188000	2.38e-03	2.56e-02
10	Regulation	04810	192	56	15.464	1.66e-03	0.273000	3.95e-03	3.83e-02
11	Lysosome	04142	116	36	-0.753	3.45e-03	0.161000	4.72e-03	4.16e-02
12	MAPK signa	04010	245	69	5.732	1.47e-03	0.419000	5.16e-03	4.17e-02
13	Renal cell	05211	64	21	-7.963	1.15e-02	0.095000	8.56e-03	6.38e-02
14	Pathogenic	05130	48	13	17.180	1.52e-01	0.018000	1.89e-02	1.31e-01
15	Circadian	04710	16	8	-2.640	7.23e-03	0.410000	2.02e-02	1.31e-01

	pGFWER	Status
1	1.50e-11	Inhibited
2	9.99e-11	Inhibited
3	1.42e-08	Activated
4	2.50e-06	Inhibited
5	1.74e-05	Activated
6	1.12e-04	Inhibited
7	4.70e-04	Activated
8	1.75e-01	Activated
9	2.31e-01	Inhibited

```

10 3.83e-01 Activated
11 4.58e-01 Inhibited
12 5.00e-01 Activated
13 8.30e-01 Inhibited
14 1.00e+00 Activated
15 1.00e+00 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.

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. The Colorectal cancer pathway is shown in green.

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	-5.7374	0.000208	0.088	0.000218	0.0194	0.0194
2	Focal adhe	04510	198	16	-6.2100	0.000116	0.372	0.000478	0.0213	0.0425
3	Regulation	04810	210	14	9.4361	0.002001	0.064	0.001276	0.0325	0.1135
4	Viral myoc	05416	70	8	-1.6656	0.000635	0.288	0.001757	0.0325	0.1564
5	Neuroactiv	04080	271	18	-0.5104	0.000512	0.428	0.002066	0.0325	0.1839
6	Intestinal	04672	46	7	0.0000	0.000234	1.000	0.002193	0.0325	0.1952
7	Antigen pr	04612	76	7	1.4922	0.004739	0.088	0.003662	0.0466	0.3260
8	Leishmania	05140	68	8	0.0122	0.000522	0.992	0.004432	0.0493	0.3944
9	Complement	04610	67	7	4.5721	0.002325	0.344	0.006504	0.0587	0.5788
10	Graft-vers	05332	41	6	0.0000	0.000813	1.000	0.006597	0.0587	0.5871
11	Asthma	05310	29	5	0.0000	0.001038	1.000	0.008167	0.0613	0.7268
12	Type I dia	04940	43	6	0.0000	0.001053	1.000	0.008270	0.0613	0.7360
13	Notch sign	04330	46	4	7.5682	0.036603	0.032	0.009077	0.0621	0.8079
14	Wnt signal	04310	150	11	1.0119	0.002836	0.684	0.014055	0.0893	1.0000
15	Leukocyte	04670	116	9	-1.9287	0.004608	0.616	0.019484	0.1156	1.0000

Status

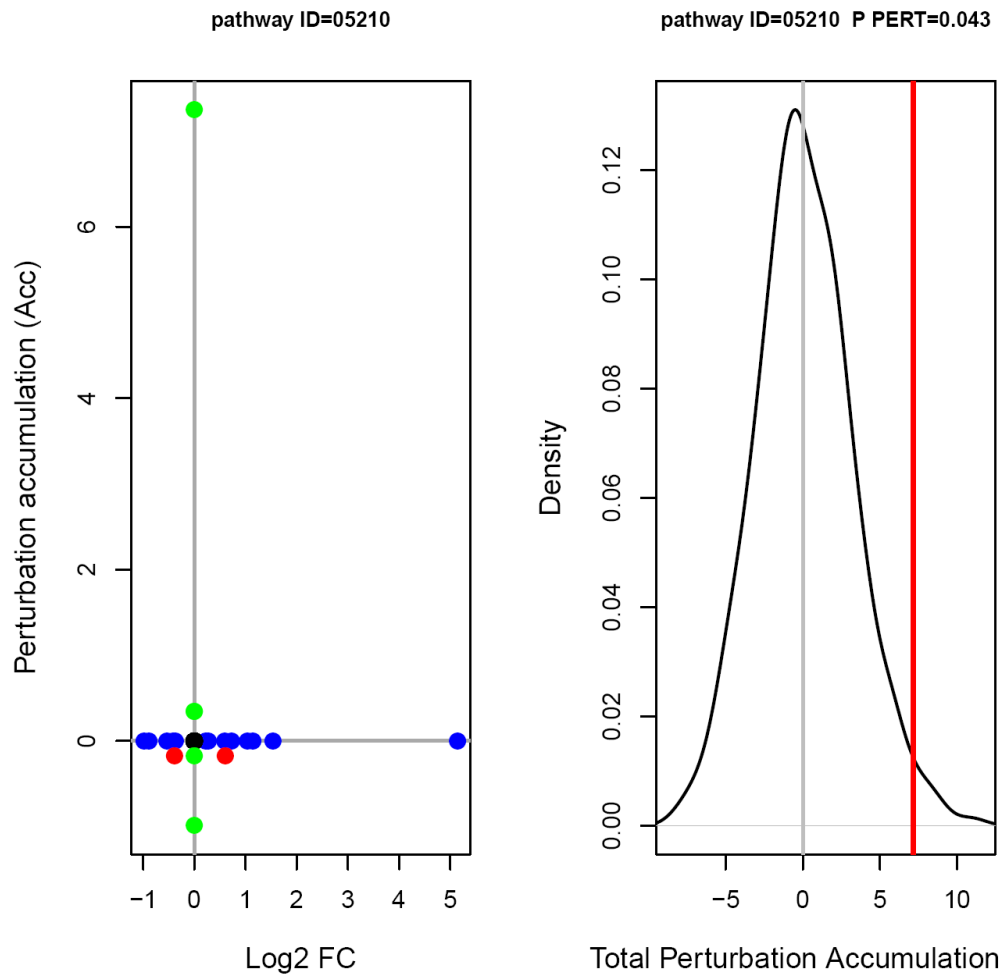


Figure 1: Perturbations plot for colorectal cancer pathway (KEGG ID hsa:05210) using the `colorectal_cancer` dataset. The perturbation of all genes in the pathway are shown as a function of their initial log2 fold changes (left panel). Non DE genes are assigned 0 log2 fold-change. The null distribution 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.

```

> plotP(res, threshold = 0.05)
> points(I(-log(pPERT)) ~ I(-log(pNDE)), data = res[res$ID == "05210",
+           ], col = "green", pch = 19, cex = 1.5)

```

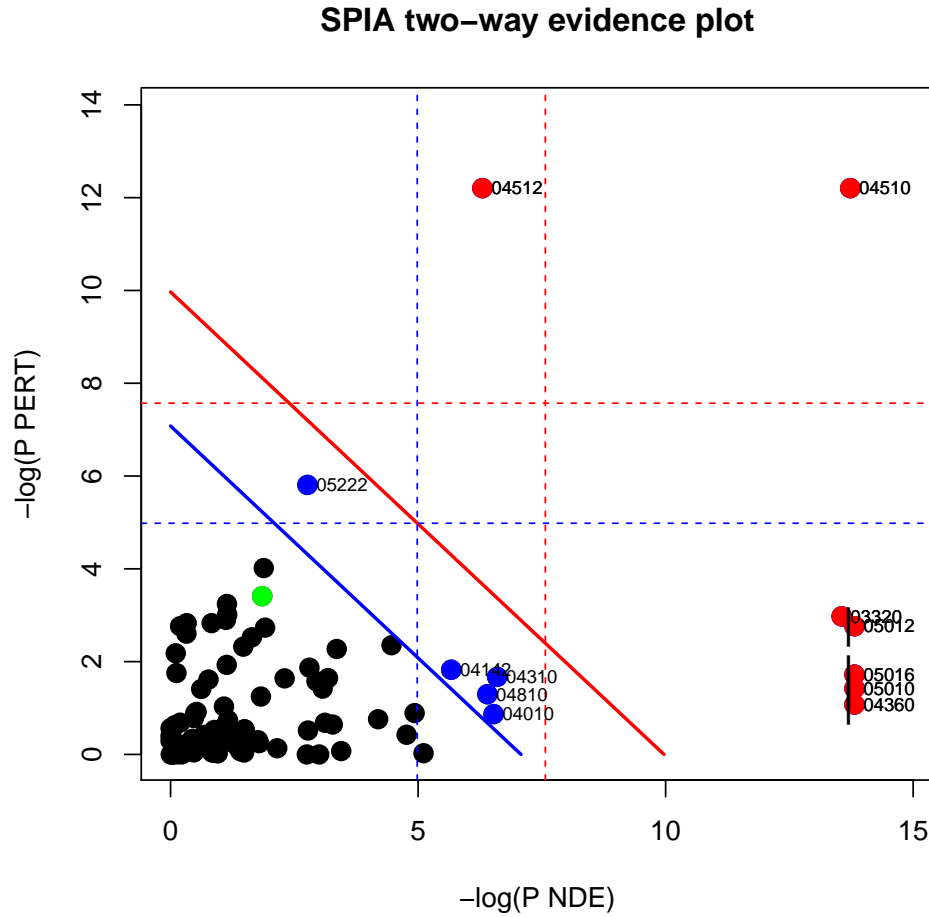


Figure 2: SPIA evidence plot for the colorectal cancer dataset. Each pathway is represented by one dot. The pathways at the right of the red oblique line are significant after Bonferroni correction of the global p-values, pG. The pathways at the right of the blue oblique line are significant after a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


```

1 Inhibited
2 Inhibited
3 Activated
4 Inhibited
5 Inhibited
6 Inhibited
7 Activated
8 Activated
9 Activated
10 Inhibited
11 Inhibited
12 Inhibited
13 Activated
14 Activated
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?hsa04540+3356+983+6714+5155+80310"
```

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"]][25:35, 30:40]
```

	5602	8312	8313	5900	387	5879	5880	5881	332	4609	595
369	0	0	0	0	0	0	0	0	0	0	0
5894	0	0	0	0	0	0	0	0	0	0	0
673	0	0	0	0	0	0	0	0	0	0	0
5599	0	0	0	0	1	1	1	1	0	0	0
5601	0	0	0	0	1	1	1	1	0	0	0
5602	0	0	0	0	1	1	1	1	0	0	0
8312	0	0	0	0	0	0	0	0	0	0	0
8313	0	0	0	0	0	0	0	0	0	0	0
5900	0	0	0	0	0	0	0	0	0	0	0
387	0	0	0	1	0	0	0	0	0	0	0
5879	0	0	0	1	0	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"`.

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

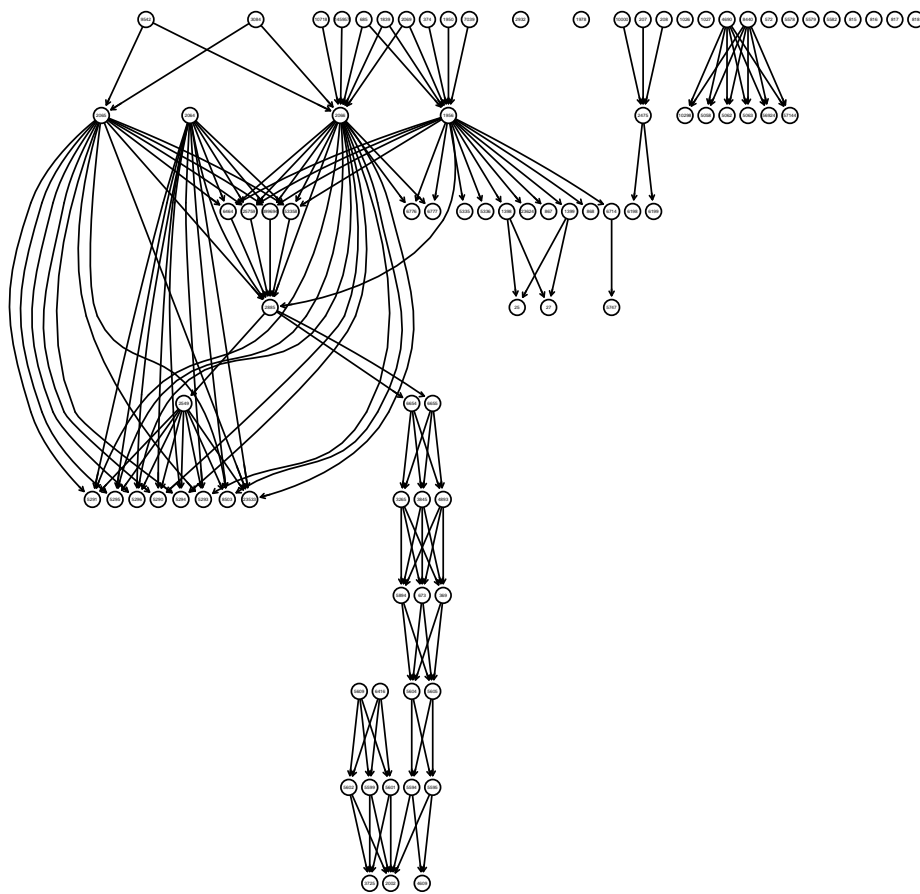


Figure 3: Display of the "activation" relations in the ErbB signaling pathway, based on the hsaSPIA data.

References

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