

# HiPathia

## Models of signaling pathway activity

Marta R. Hidalgo  
Unidad de Bioinformática y Bioestadística

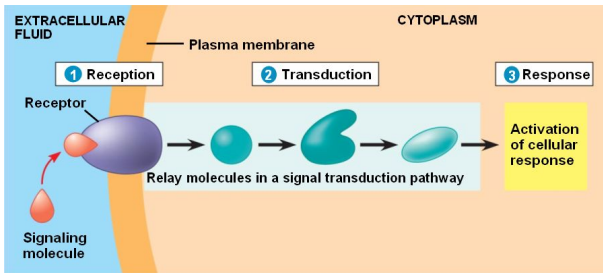


# WODA

WEB-BASED OMICS DATA ANALYSIS



# Signaling pathways



## Chemical signals

- Hormones
- Neurotransmitters
- Growth factors
- Cytokines
- Drugs

## Activation & Inhibition

- Phosphorilation
- Dephosphorilation
- Glycosylation
- Ubiquitination
- Methylation

## Cellular Function

- Apoptosis
- Survival
- Growth
- Migration
- Proliferation

---

# Pathway Analysis

Methods

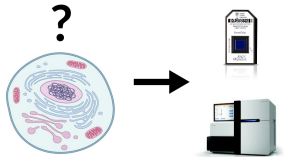
# From cell to pathways

---

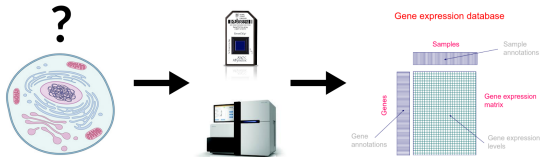


# From cell to pathways

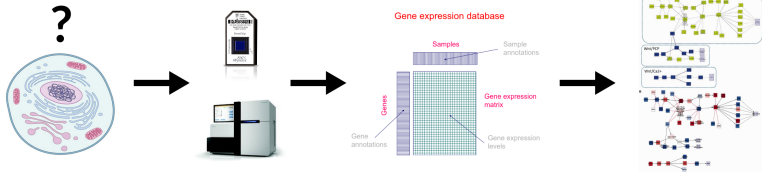
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# From cell to pathways



# From cell to pathways



# Pathway methods

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- **DEGraph**: Based on DE
- **Clipper**: 2 test method
- **SPIA**: Impact factor
- **Sub-SPIA**: Find subnetwork by DE and apply SPIA
- **HiPathia**: Computes signal for each sample

*The Annals of Applied Statistics*  
2012, Vol. 6, No. 2, 561–600  
DOI: 10.1214/11-AOS128  
© Institute of Mathematical Statistics, 2012

**MORE POWER VIA GRAPH-STRUCTURED TESTS FOR  
DIFFERENTIAL EXPRESSION OF GENE NETWORKS**

BY LAURENT JACOB, PIERRE NEUVIAL AND SANDRINE DUDOIT



# Pathway methods

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*Published online 21 September 2012*

*Nucleic Acids Research, 2013, Vol. 41, No. 1 e19  
doi:10.1093/nar/gks866*

## **Along signal paths: an empirical gene set approach exploiting pathway topology**

**Paolo Martini<sup>1</sup>, Gabriele Sales<sup>2</sup>, M. Sofia Massa<sup>3</sup>, Monica Chiogna<sup>4</sup> and  
Chiara Romualdi<sup>2,\*</sup>**

# Pathway methods

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**BIOINFORMATICS ORIGINAL PAPER**

Vol. 25 no. 1 2009, pages 75–82  
doi:10.1093/bioinformatics/btn577

*Systems biology*

## **A novel signaling pathway impact analysis**

Adi Laurentiu Tarca<sup>1,2</sup>, Sorin Draghici<sup>1,\*</sup>, Purvesh Khatri<sup>1</sup>, Sonia S. Hassan<sup>2</sup>,  
Pooja Mittal<sup>2</sup>, Jung-sun Kim<sup>2</sup>, Chong Jai Kim<sup>2</sup>, Juan Pedro Kusanovic<sup>2</sup>  
and Roberto Romero<sup>2</sup>

# Pathway methods

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[www.impactjournals.com/oncotarget/](http://www.impactjournals.com/oncotarget/)

Oncotarget, 2017, Vol. 8, (No. 3), pp: 5160-5178

Research Paper

**High throughput estimation of functional cell activities reveals disease mechanisms and predicts relevant clinical outcomes**

Marta R. Hidalgo<sup>1</sup>, Cankut Cubuk<sup>1</sup>, Alicia Amadoz<sup>1,2</sup>, Francisco Salavert<sup>1,3</sup>, José Carbonell-Caballero<sup>1</sup>, Joaquin Dopazo<sup>1,2,3</sup>

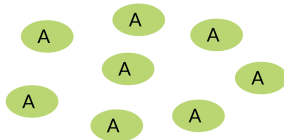
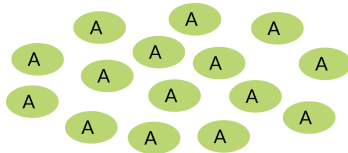
---

# Pathway Analysis

HiPathia

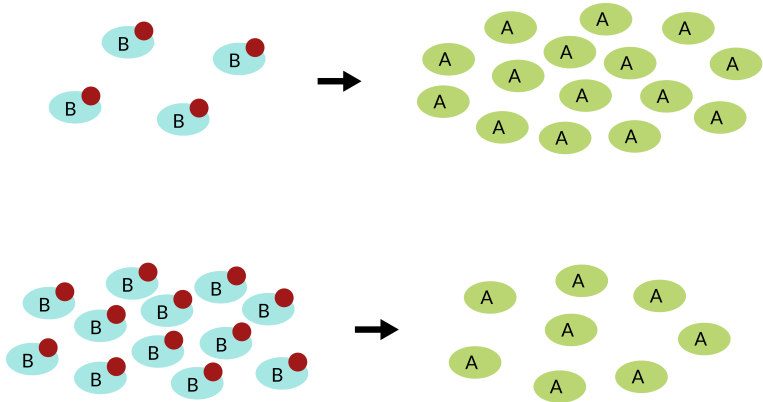
# Intuitive idea

---



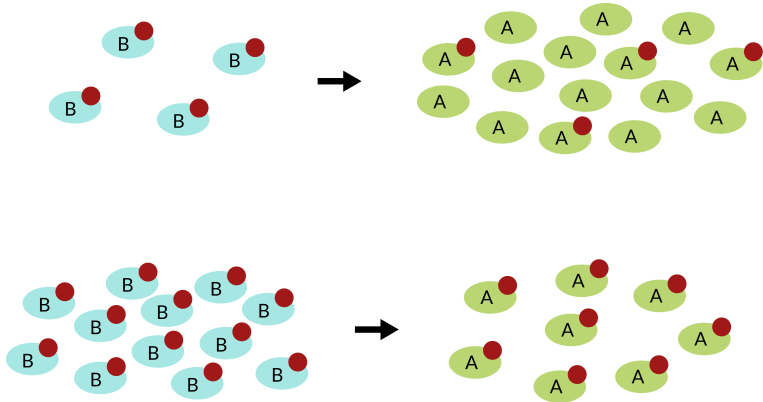
# Intuitive idea

---



# Intuitive idea

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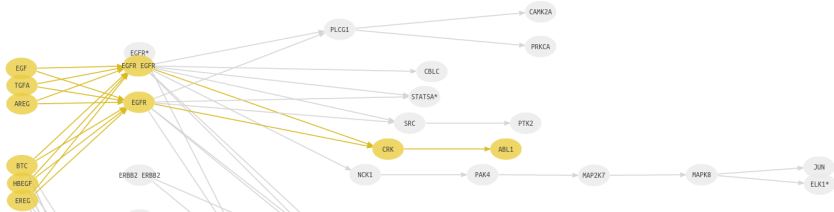




# Meaningful subpathways

## Effector subpathway

Subpathway including any node from any receptor to one effector protein



# Computing the signal

- 1 Compute a node score based on the expression
- 2 Compute signal passing through each node  $n$

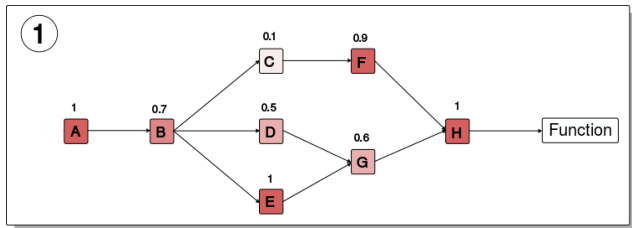
$$S_n = v_n \cdot \left(1 - \prod_{s_j \in A} (1 - s_j)\right) \cdot \prod_{s_j \in I} (1 - s_j)$$

$S_n$ : Signal value through  $n$

$v_n$ : Node value

$A$ : Activation edges

$I$ : Inhibition edges



# Computing the signal

- 1 Compute a node score based on the expression
- 2 Compute signal passing through each node  $n$

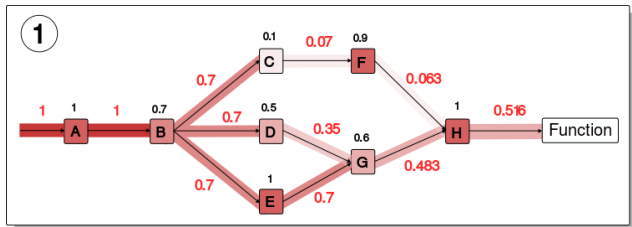
$$S_n = v_n \cdot \left(1 - \prod_{s_i \in A} (1 - s_i)\right) \cdot \prod_{s_j \in I} (1 - s_j)$$

$S_n$ : Signal value through  $n$

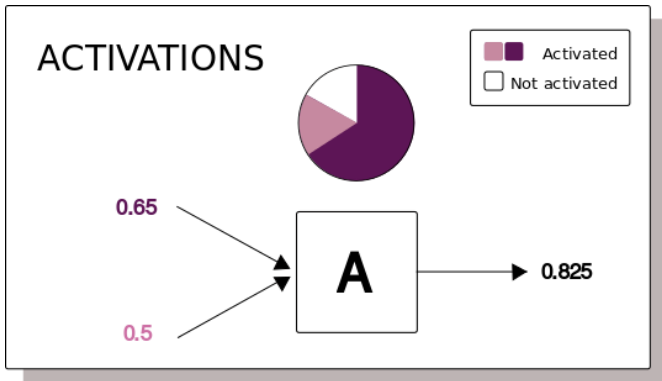
$v_n$ : Node value

A: Activation edges

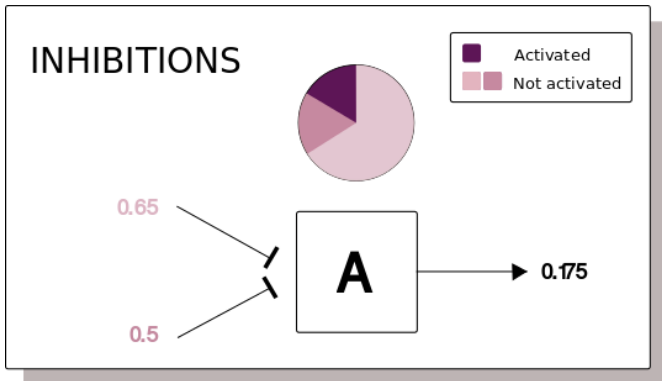
I: Inhibition edges



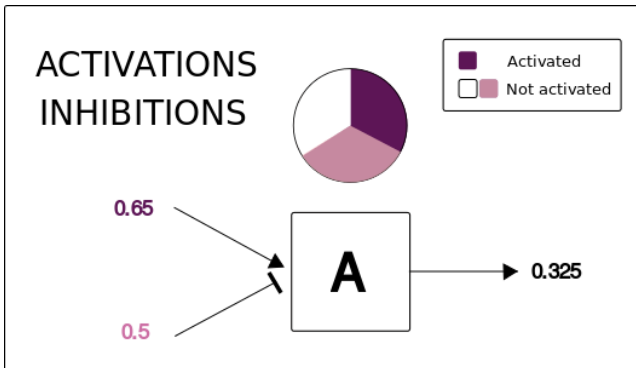
# Computing the signal



# Computing the signal

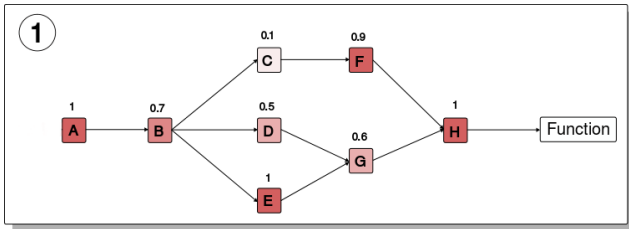


# Computing the signal



# Signal values

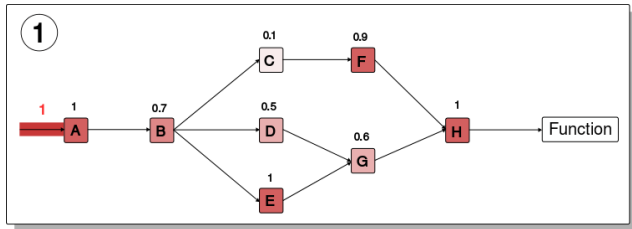
- Input signal 1 in any input node
- Compute the signal through each node iteratively
- Loops can be processed
- Subpathway signal: last node signal





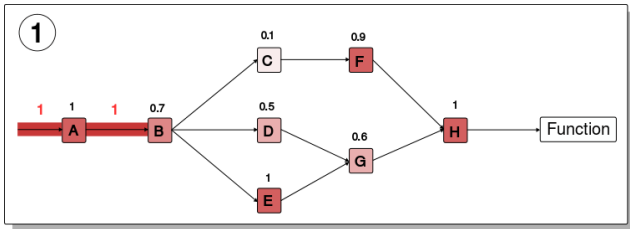
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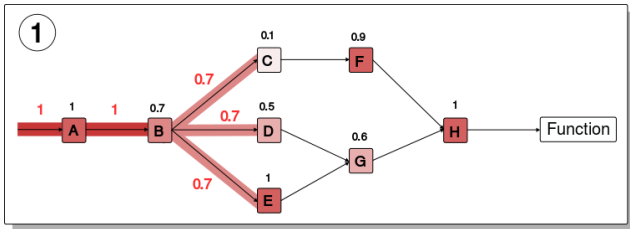
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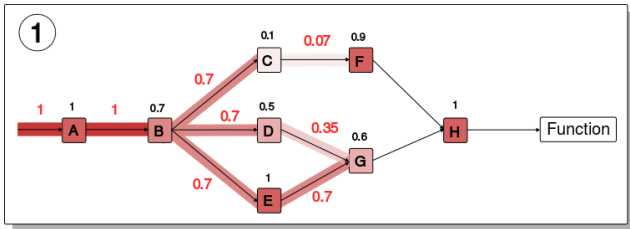
# Signal values

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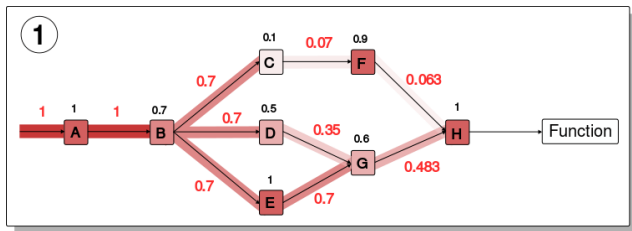
# Signal values

- Input signal 1 in any input node
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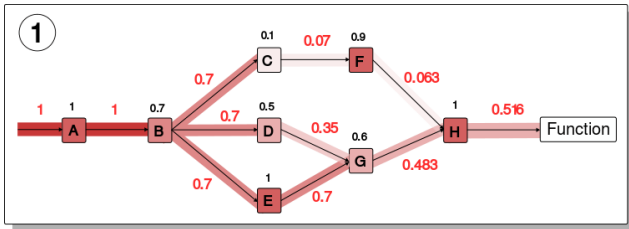
# Signal values

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# Signal values

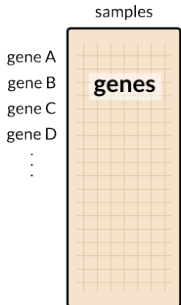
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- Loops can be processed
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# Functional annotation

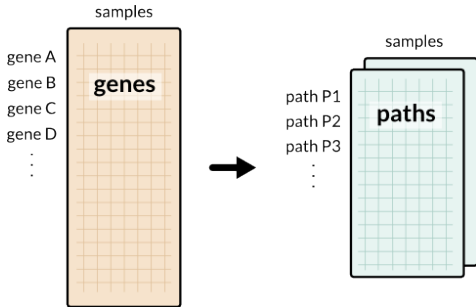
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- 1 Estimate effector proteins activation
- 2 Annotate effector proteins functions
  - Uniprot keywords
  - GO annotation



# Functional annotation

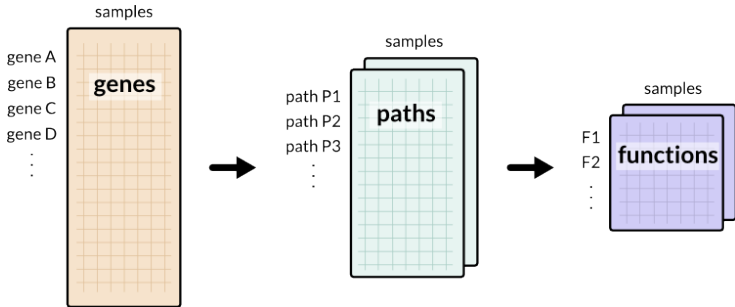
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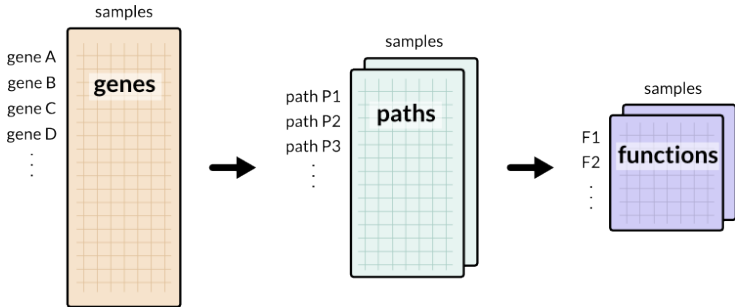
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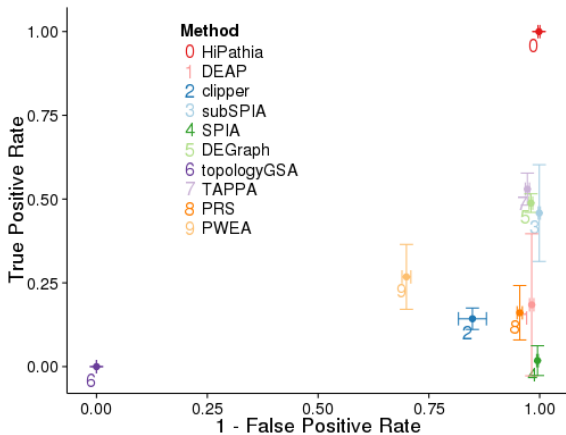


# Functional annotation

- 1 Estimate effector proteins activation
- 2 Annotate effector proteins functions
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  - GO annotation



# Method comparison



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# HiPathia

Web tool

# Logging in

hipathia.babelomics.org

hiPathia Pathways analysis suite Differential signaling Prediction Login Sign up

## hiPathia

HIGH THROUGHPUT PATHWAY  
INFERENCE ANALYSIS

1 Start

2 3

hiPathia is a web tool for the interpretation of the consequences of the combined changes of gene expression levels and/or genomic mutations in the context of signalling pathways. hiPathia transforms uninformative gene expression and/or genomic variation data into signalling circuit activities, which carry information on the different cell functionalities triggered by them. Such signalling activities not only account for the underlying molecular mechanisms of diseases or the mode of action of drugs but they can also be used as mechanistic features for the prediction of complex phenotypes.

Note:

hiPathia web application makes an intensive use of the HTML 5 standard and other cutting-edge web technologies such as Web Components, so only modern web browsers are fully supported.

hiPathia v1.1.0  
Created by Computational Genomics Department  
Príncipe Felipe Research Center, Valencia, Spain  
2016

# Upload data

The screenshot displays the hiPathia web application interface. At the top, the navigation bar includes the hiPathia logo, the text "Pathways analysis suite", and links for "Differential signaling" and "Prediction". On the right side of the navigation bar, there is a "My data" button (circled with a 1), a search bar containing "gta2016ciberer", and links for "My jobs", "Profile", and "Logout".

The main content area is divided into two sections. On the left, a "Browse My Data" window is open, showing a file named "Exercise\_1" with a size of "-" and a date of "Sep 26, 2016". An "Upload" button is highlighted with a circled 3. Below the file list, it indicates "0B of 1.0 GB" and a search bar with the text "Search by name...".

On the right, the main header area features the text "hiPathia" in a large font, followed by "HIGH THROUGHPUT PATHWAY INFERENCE ANALYSIS" in a smaller font. A circled 1 is placed above this text.

Below the header, there is a paragraph of text: "interpretation of the consequences of the combined changes of gene expression levels and/or genomic mutations in the context of signalling pathways. hiPathia transforms uninformative gene expression and/or genomic variation data into signalling circuit activities, which carry information on the different cell functionalities triggered by them. Such signalling activities not only account for the underlying molecular mechanisms of diseases or the mode of action of drugs but they can also be used as mechanistic features for the prediction of complex phenotypes."

Below this paragraph, there is a "Note:" section with the text: "hiPathia web application makes an intensive use of the HTML 5 standard and other cutting-edge web technologies such as Web Components, so only modern web browsers are fully supported."

At the bottom of the page, the footer contains the text: "hiPathia v1.1.0  
Created by Computational Genomics Department  
Príncipe Felipe Research Center, Valencia, Spain  
2016"

# Upload data

The screenshot displays the hiPathia Pathways analysis suite interface. A modal dialog titled "Upload File" is open, showing the following components:

- Step 5:** "File upload:" section with a "Choose file..." button.
- Step 4:** "Bioformat:" section with three radio button options: "Data matrix expression", "Variant (VCF)", and "Experimental design".
- Step 6:** "Upload" button.
- Selected file:** A text field containing "None" and a "Revalidate" button.
- File validation log:** A table with columns "Line", "Type", and "Message".
- Progress:** A progress bar showing "0%" and a "Stop" button.
- Summary:** "Errors: 0", "Warning: 0", "Info: 0", and "Lines: 0".

The background interface shows a file browser with a folder named "Exercise\_1" selected. The top navigation bar includes "My data", "My jobs", "gda2016cberer", "Profile", and "Logout".

Note:  
hiPathia web application uses an extensive use of the HTML5 standard and other cutting-edge web technologies, such as Web Components, so only modern web browsers are fully supported.

hiPathia v1.0  
Created by Computational Genomics Department  
Príncipe Felipe Research Center, Valencia, Spain  
2016

# Workflow

The screenshot displays the hiPathia web application interface. At the top, the navigation bar includes the hiPathia logo, the text "Pathways analysis suite", and menu items for "Differential signaling" and "Prediction". On the right side of the navigation bar, there are links for "My data", "My jobs", "gda2016ciberer", "Profile", and "Logout".

On the left side, a "Browse My Data" window is open, showing a file browser for the user "gda2016ciberer". The browser contains a table with the following data:

Name	Size	Date
Exercise_1	-	Sep 26, 2016

Below the table, it indicates "0B of 1.0 GB" and a search bar with the placeholder "Search by name...".

The main content area on the right features the hiPathia logo and the text "HIGH THROUGHPUT PATHWAY INFERENCE ANALYSIS". Three orange circles with numbers 1, 2, and 3 are overlaid on the interface: circle 1 is near the "My data" link, circle 2 is near the hiPathia logo, and circle 3 is near the "Differential signaling" and "Prediction" menu items.

Interpretation of the consequences of the combined changes of gene expression levels and/or genomic mutations in the context of signalling pathways. hiPathia transforms uninformative gene expression and/or genomic variation data into signalling circuit activities, which carry information on the different cell functionalities triggered by them. Such signalling activities not only account for the underlying molecular mechanisms of diseases or the mode of action of drugs but they can also be used as mechanistic features for the prediction of complex phenotypes.

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hiPathia v1.1.0  
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2016



# Workflow

The screenshot displays the hiPathia Pathways analysis suite interface. The main navigation bar includes the logo, the text "hiPathia Pathways analysis suite", and tabs for "Differential signaling" and "Prediction". On the right side of the navigation bar, there are links for "My data", "My jobs", "Libraries", "Profile", and "Logout".

The main content area is divided into several sections:

- Function level analysis:** Contains checkboxes for "Gene ontology" and "UniProt keywords".
- Pathways:** A list of biological pathways with checkboxes, including "Focal adhesion", "Adherens junction", "Tight Junction", "Gap Junction", "Platelet activation", "Toll-like receptor signaling pathway", "NOD-like receptor signaling pathway", "RIG-I like receptor signaling pathway", "Natural killer cell mediated cytotoxicity", "T cell receptor signaling pathway", "B cell receptor signaling pathway", "Fc epsilon RI signaling pathway", "Fc gamma R-mediated phagocytosis", and "Leukocyte transendothelial migration".
- Job information:** Includes a "Output folder:" section with a "File browser" button and a note: "You can create folders using the button  inside file browser:". Below this are input fields for "Job name:" (containing "Differential signaling.job") and "Description:".

On the right side, a "Browse My Jobs" panel is visible, showing a list of jobs:

- ✓ Prediction train example  
Prediction Train Done 10/27/2016, 12:03:49 PM
- ✓ Differential signaling example  
Differential Signaling Done 10/27/2016, 12:03:49 PM

At the bottom of the job list, it shows "Total: 2" and an "Enable job notifications" checkbox.

Numbered callouts (4, 5, 6, 7, 8) highlight specific elements: 4 points to the "Function level analysis" section; 5 points to the "Launch Job" button; 6 points to the "My jobs" link in the navigation bar; 7 points to the "My data" link; and 8 points to the job list in the "Browse My Jobs" panel.

# Tools

---

## ① Differential signaling

- Compare signal activity between two conditions
- Correlate path value with a continuous variable

## ② Perturbation effect

- Predict effects of gene overexpressions and KOs
- Predict drug effects

# Tools

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## ① Differential signaling

- Compare signal activity between two conditions
- Correlate path value with a continuous variable

## ② Perturbation effect

- Predict effects of gene overexpressions and KOs
- Predict drug effects

---

# HiPathia

Differential signaling tool

# Formular details

---

① **Species:** *hsa, mmu, rno*

② **Parameters**

- Paired
- Unadjusted

③ **Function level analysis**

Perform analysis with the following functional annotations:

- Gene ontology
- Uniprot keywords

# Formular details

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① **Species:** *hsa, mmu, rno*

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② **Parameters**

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③ **Function level analysis**

Perform analysis with the following functional annotations:

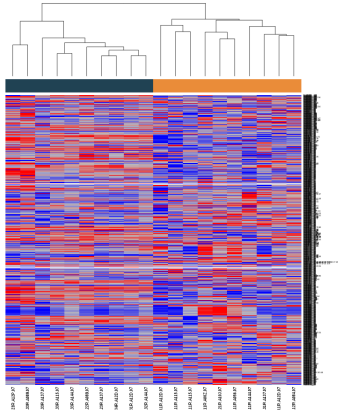
- Gene ontology
- Uniprot keywords

# Heatmap

Graphical representation of data where values in a matrix are represented as colors

Path values

- 📄 Path values
- 📄 Heatmap

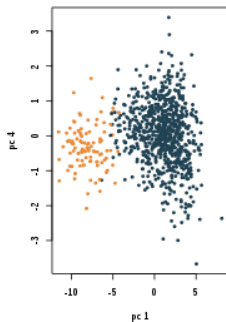
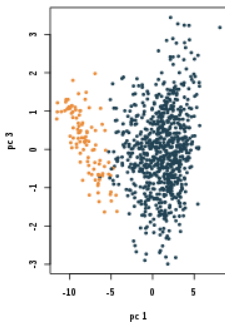
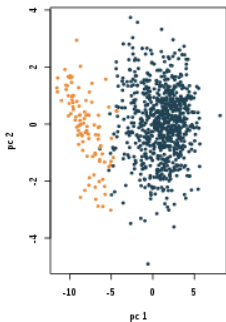




# Principal Components Analysis (PCA)

Statistical procedure to convert a set of observations into a set of values of linearly uncorrelated variables

 PCA 



# Results table

- Table of results for the comparison.
- Ordered by the FDR p-value.

Path significance 

circuit/term	UP/DOWN	statistic	p.value	FDRp.value
ErbB signaling pathway: STAT5A	DOWN	-16.076	0.000	0.000
Adrenergic signaling in cardiomyocytes: SCN1B	DOWN	-15.987	0.000	0.000
Thyroid hormone signaling pathway: RCAN1	DOWN	-15.966	0.000	0.000
cGMP-PKG signaling pathway: PDE2A	DOWN	-15.909	0.000	0.000
cGMP-PKG signaling pathway: C00144	DOWN	-15.786	0.000	0.000
AMPK signaling pathway: LEPR	DOWN	-15.761	0.000	0.000
Hippo signaling pathway: FGF1	DOWN	-15.728	0.000	0.000
Adherens junction: SMAD4 SMAD2	DOWN	-15.727	0.000	0.000
Adipocytokine signaling pathway: PTPN11	DOWN	-15.727	0.000	0.000
p53 signaling pathway: CDK1 CCNB3	UP	15.693	0.000	0.000

# Viewer

hiPathia Pathways analysis suite

Differential signaling Prediction

My data My genes g:K01170:cbcr1:1:Protein Login

## Pathways

**Shape**

**Nodes**

- Gene
- Metabolite

**Edges**

- Inhibition
- Activation

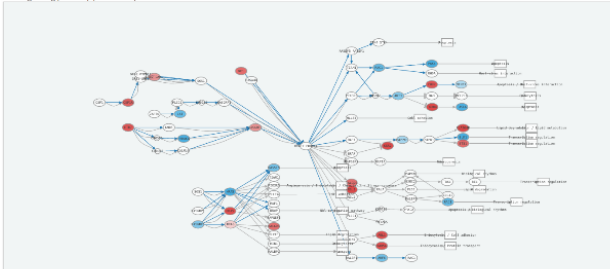
**Color**

- Down-regulated gene (DE)
- Up-regulated gene (UE)
- Not differentially regulated path
- Down-regulated path
- Up-regulated path

**Stroke color**

- Gene expression
- Expression

Ras signaling pathway (hsa04014)



**1**

**2**

**3**

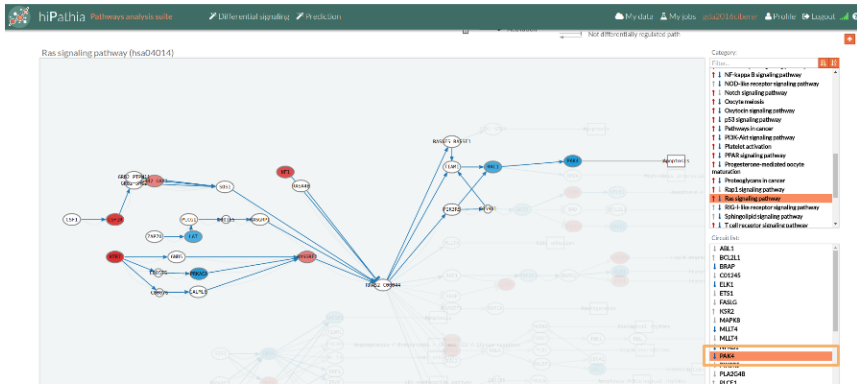
**Categories**

- 1 | Raf-Mek1/2 signaling pathway
- 1 | NG2-like receptor signaling pathway
- 1 | Notch signaling pathway
- 1 | Oncostatin receptor
- 1 | Osteonin signaling pathway
- 1 | p53 signaling pathway
- 1 | Pathways overview
- 1 | PI3K-Akt signaling pathway
- 1 | Platelet activation
- 1 | PPAR signaling pathway
- 1 | Prostaglandin-mediated cyclooxygenase inhibition
- 1 | Proteoglycans in cancer
- 1 | Rgs1 signaling pathway
- 1 | Ras signaling pathway
- 1 | RIG-I-like receptor signaling pathway
- 1 | Sphingosin1-phosphate signaling pathway
- 1 | Tyrosine receptor signaling pathway

**Genes**

- 1 | ABL1
- 1 | BCL2L1
- 1 | BDNF
- 1 | CD44
- 1 | ELK1
- 1 | ERK1
- 1 | FAK3
- 1 | HSP2
- 1 | MAPK3

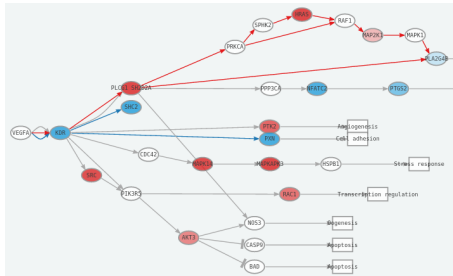
# Viewer



# Viewer



# Viewer



---

# HiPathia

Perturbation effect tool

# Formular details

---

① **Species:** *hsa, mmu, rno*

② **Data**

- Matrix with one column
- Matrix with many columns, selection of one of them



# Formular details

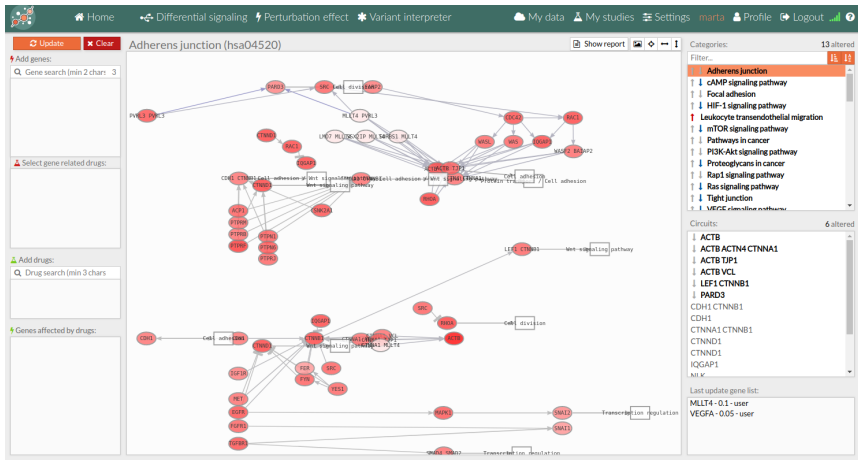
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① **Species:** *hsa, mmu, rno*

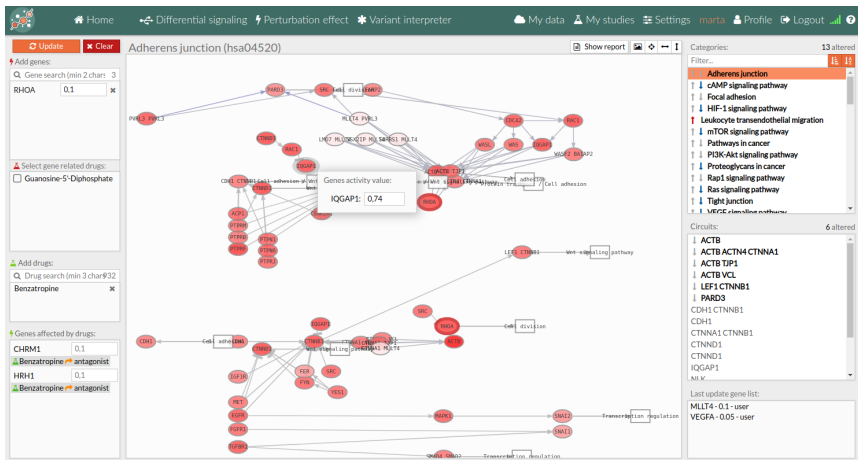
② **Data**

- Matrix with one column
- Matrix with many columns, selection of one of them

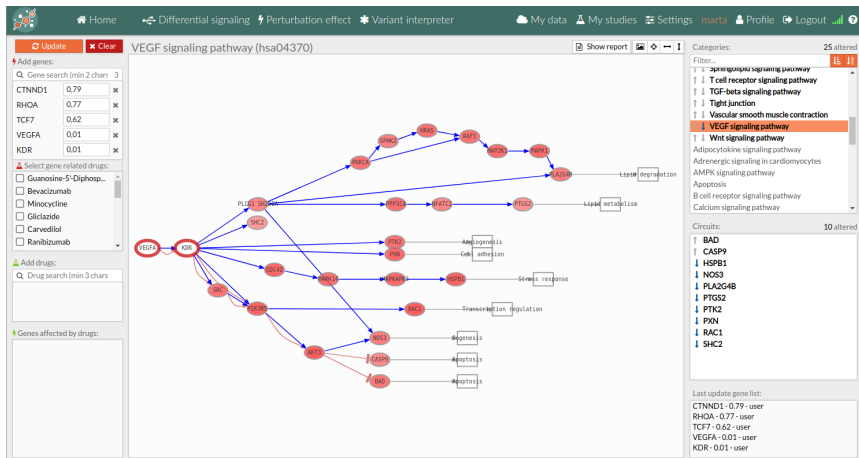
# Perturbation effect



# Perturbation effect



# Perturbation effect



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# HiPathia

Bioconductor package

# Bioconductor package

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## hipathia

platforms **all** rank **690 / 1823** posts **0** in Bioc **1.5 years**  
build **ok** updated **before release** dependencies **106**

DOI: [10.18129/B9.bioc.hipathia](https://doi.org/10.18129/B9.bioc.hipathia) [f](#) [t](#)

## HiPathia: High-throughput Pathway Analysis

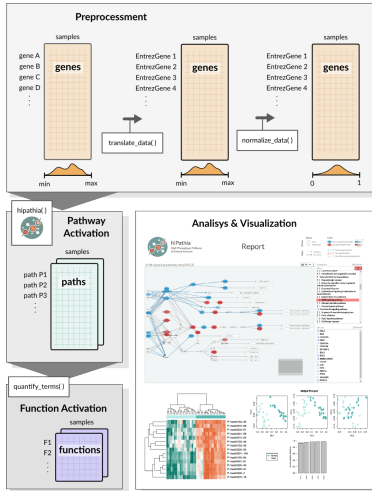
Bioconductor version: Release (3.10)

Hipathia is a method for the computation of signal transduction along signaling pathways from transcriptomic data. The method is based on an iterative algorithm which is able to compute the signal intensity passing through the nodes of a network by taking into account the level of expression of each gene and the intensity of the signal arriving to it. It also provides a new approach to functional analysis allowing to compute the signal arriving to the functions annotated to each pathway.

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# Bioconductor package



# Bioconductor functions

---

```
# data: matriz normalizada de expresión de los datos
# clases: vector con las clases a las que pertenece cada sample ("Clase 1", "Clase 2", y otras posibles)

# Hipathia
pathways <- load_pathways("hsa")
results <- hipathia(data, pathways)
paths <- get_paths_data(results)

# Comparison
comp <- do_wilcoxon(paths, clases, "Clase 1", "Clase 2")
summ <- get_pathways_summary(comp, pathways)

# Save and serve all results to browser
node_colors <- node_color_per_de(results, pathways, clases, "Clase 1", "Clase 2")
folder <- create_report(comp, pathways, node_colors = node_colors)
visualize_report(folder, port = 4001)

# Functional analysis
uniprot <- quantify_terms(results, pathways, "uniprot")
comp_uniprot <- do_wilcoxon(uniprot, clases, "Clase 1", "Clase 2", order = T)
```



# Bioconductor functions

```
# data: matriz normalizada de expresión de los datos
# clases: vector con las clases a las que pertenece cada sample ("Clase 1", "Clase 2", y otras posibles)

# Hipathia
pathways <- load_pathways("hsa") # Carga objeto de pathways
results <- hipathia(data, pathways) # Aplica Hipathia
paths <- get_paths_data(results) # Extrae matriz de pathways

# Comparison
comp <- do_wilcoxon(paths, clases, "Clase 1", "Clase 2") # Hace comparación por pathways
summ <- get_pathways_summary(comp, pathways) # Resumen de resultados

# Save and serve all results to browser
node_colors <- node_color_per_de(results, pathways, clases, "Clase 1", "Clase 2") # ED de nodos
folder <- create_report(comp, pathways, node_colors = node_colors) # Guarda resultados en una carpeta
visualize_report(folder, port = 4001) # Sirve los resultados a un navegador

# Functional analysis
uniprot <- quantify_terms(results, pathways, "uniprot") # Calcula matriz de funciones Uniprot
comp_uniprot <- do_wilcoxon(uniprot, clases, "Clase 1", "Clase 2", order = T) # Comparación
```

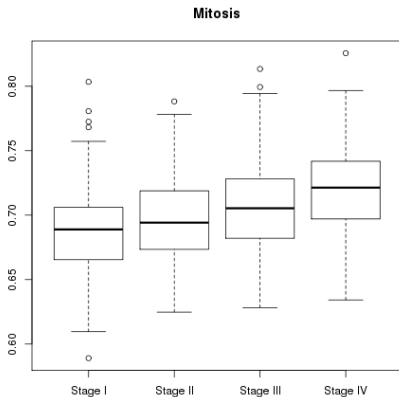
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# HiPathia

Further analysis

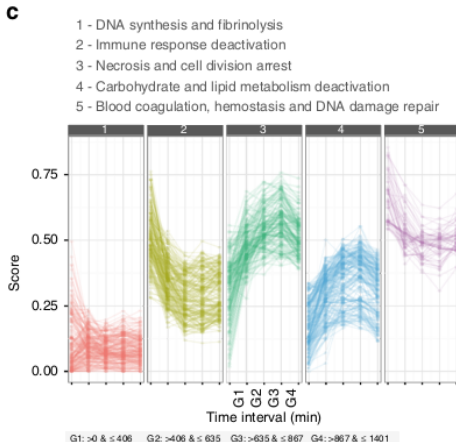
# Disease progression analysis

Search for features which increase with the progression of the disease



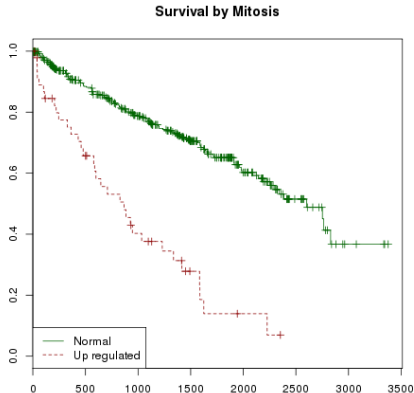
# Disease progression analysis

Search for features which increase with the progression of the disease



# Survival analysis

Analysis of time duration until one or more events happen



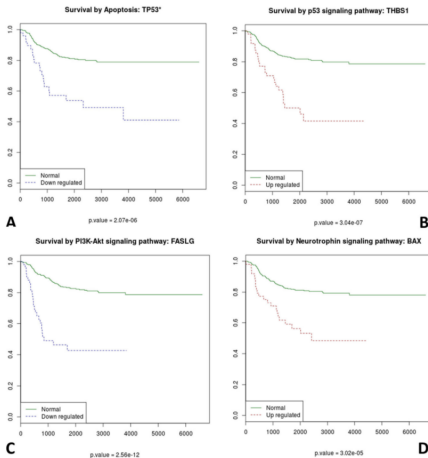
# Supervivencia

Inhibición  
apoptosis

Metástasis

Angiogénesis

Evasión  
sistema  
inmune



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# Exercises

# HiPathia exercises

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## Exercise 1

Do the [Differential signaling worked example](#)

## Exercise 2

Do the [Perturbation effect worked example](#)

## Further exercises

Do the [Differential signaling exercises](#)