PathRings

Version 1.0

Manual

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This tutorial introduces PathRings, the user interface and the interaction. For better to learn, you will need to refer to the screenshots in this document.

You will learn:

- How pathways are visualized in PathRings

- How to overlay orthologous data on pathways

- How to overlay expression data on pathways

- How to interact with our tool

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What is PathRings?

PathRings is a web-based application of biological pathway visualization and analysis, allowing users to visualize, search pathways from Reactome, to upload their own gene orthologous data for ortholog analysis, and to upload their gene expression data for expression analysis. This is a web-version of PathBubbles project: https://sites.google.com/a/umbc.edu/pathbubbles/ .

The current version is "1.0". It could be run on any PCs (both Linus, Windows, Mac OS). Please use Chrome to use our tool.

It is freely available at http://raven.anr.udel.edu/~sunliang/PathRings/ (see *Figure 1*).

All the code is hosted on Github, you can fork it through the link: https://github.com/ivcl/PathRings .

We submit a paper to Bioinfomatics, if you would like to do analysis based on this tool, please quote the reference below.

> Yongnan Zhu, Liang Sun, Carl Schmidt, Keqin Wu, Zhigeng Pan, Jinglong Fang, J. C. *PathRings: a web-based tool for Exploration of Ortholog and Expression Data in Biological Pathways*[J] (submitted to BMC Bioinfomatics).
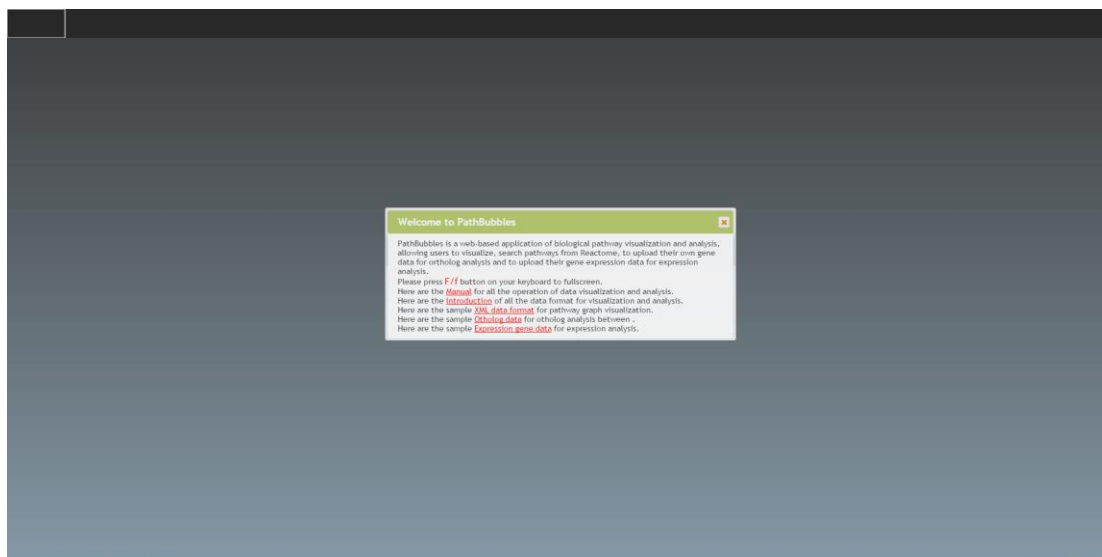

Figure 1. Homepage interface

What is this tutorial For?

This tutorial introduces features of PathRings using a combination of short explanations and examples. You will learn how to interact with PathRings, how to visualize pathways, how to do orthologous relationship analysis between two species, how to do expression data analysis and if necessary, you can contact us for help.
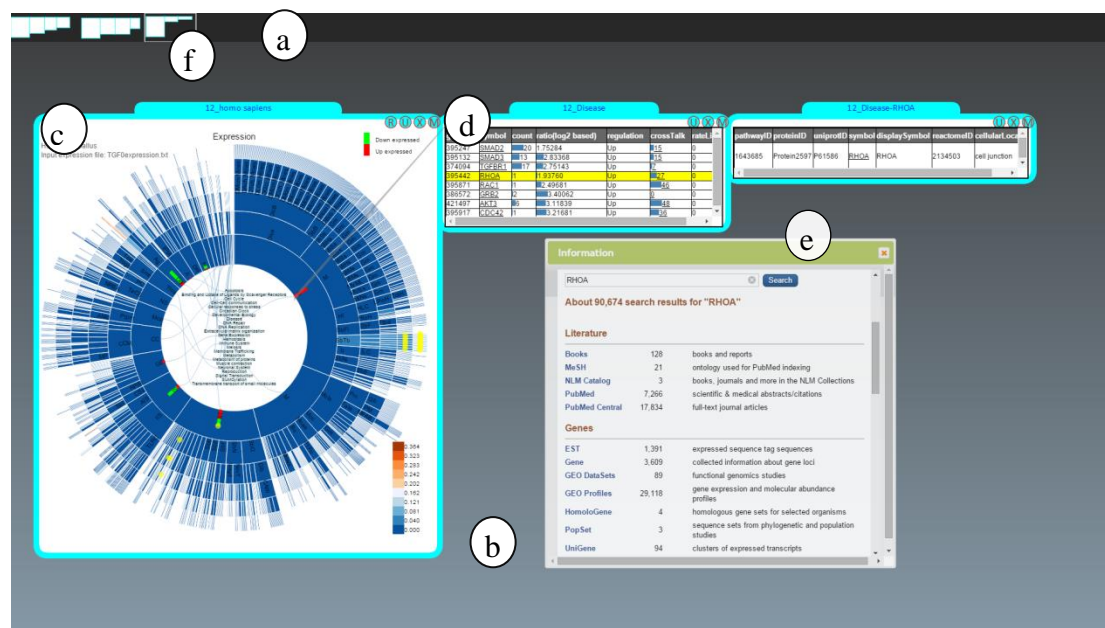
Overview

Figure 2. An overview of PathRings interface (a) Virtual working space: a panning bar to show an overview scene; (b) A large working space with lots of bubbles, including (c) Sunburst visualization bubble; (d) Sortable table bubble; (e) Query symbol from NCBI; (f) Current viewpoint to show the bubbles of current working space

PathRings supports data exploration of four types of pathway relationships, which includes hierarchical relationships, cross-talking relationships, orthologous relationships, gene expression relationships. In PathRings, we treat each functional view which is used to create and present visualization as a metaphor of Bubble. Bubble can be grouped and ungroup. *Figure 2*. provides an overview of the PathRings' interface. (a) is the virtual working space which is a panning bar to show the overview of the current bubbles in the scene with a shrunken view. (b) is a large working space (we will call it **scene** in this tutorial), which is used to present the current visualization bubbles. (c) is a Sunburst visualization of all the human pathways which overlays expression data. (d) is a table to show the detailed information of gene products. (e) is an information view to obtain the gene information for NCBI browser.

1 Operation Menu

1.1 Full Screen mode

You can press "F/f" on your keyboard to switch to "full screen" mode.If it is on the "Full screen" Mode, you can press "Esc" on your keyboard to exit the "Full screen" mode.

1.2 Menu of the scene

In PathRings, if you right click on the scene, it will pop out a menu. (see *Figure 3*). You can view individual pathway network graph by select "Open Pathway Graph"menu, it will add a Graph Bubble on the scene. You can view all the pathways in a tree-ring by select "Open Pathway TreeRing" menu. It will add a TreeRing Bubble on the scene. You can view the simple tutorial by select "Open Simple Tutorial"and you can delete all the bubbles on the scene by select "Delete All".
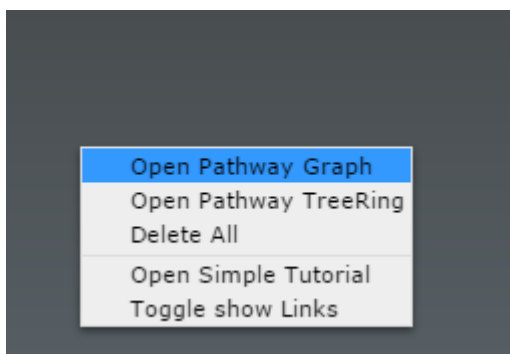

Figure 3 context menu on the scene

1.3 Menu of the Bubble

In PathRings, all the bubbles can be group and ungrouped, and also they can be deleted. Every Bubble (Figure 4 (a), (b), (c)) has a menu on the right corner with three buttons whose labels are (U/G, X, M). For the "U" button, this means the current bubble can be group (if it is overlap another bubble, they can be grouped), and if the "U" button become "G", it means this bubble can't be grouped (if it is overlap another bubble, they can't be grouped). "X" means close the current bubble, you can delete the bubble from the scene just by clicking "X" button. "M" means functional menu for this bubble. (Only Graph Bubble and Tree-Ring Bubble have this menu). In addition, there is an additional "R" button for Tree-Ring Bubble, if you zoom the tree-ring, you can click "R" button to reset the tree-ring to the default position.

There are three types of bubbles could be added on the scene: Pathway Graph Bubble (Figure 4 (a)), Pathway TreeRing Bubble (or Sunburst, we call tree ring here) (Figure 4 (b)), Table Bubble (Figure 4 (c)). You can open Pathway Graph Bubble (Figure 4 (a)) and Pathway TreeRing Bubble (Figure 4 (b)) by selecting the context menu on the scene. Table Bubble (Figure 4 (c)) is selected from Pathway TreeRing Bubble. (Detailed description will be introduced in sections below).

Figure 4 Description of three types of bubbles: (a) Pathway Graph Bubble, (b) Tree Ring Bubble (c) Sortable Table Bubble

(1) Pathway graph Bubble

You can view an individual pathway network graph by select "Open Pathway Graph" on the menu of **scene**. It will show you the default empty Bubble view (see Figure 5 (a)). And then you can click "M" button to open the functional menu (see Figure 5 (b)) on the right corner of the Bubble, you can choose the pathway network data to view the individual network graph (see Figure 5 (c)).

Figure 5 Viewing pathway graph: (a) Empty graph bubble (b) Menu of graph bubble (c) Load "ABCA transporters in lipid homeostadis.xml" pathway data

(2) Pathway Tree Ring Bubble

You can view all the pathways (1417 from Reactome) in a tree-ring by select "Open Pathway TreeRing" menu on the scene. It will show you the default view. (see Figure 6) On the left top corner of the bubble (Figure 6 (a)) is a label to show you the orthologous species or the name of your current input data file. On the right top corner of the Bubble (Figure 6 (b)) is a color mapping to show you the encoding in the ring. (Figure 6 (c)) is the menu of the tree-ring. Through this, you can change the orthologous species or input your orthologous table.

Figure 6 Tree Ring Bubble graph: (a) Label to show the input orthologous data (b) Legend to show the color mapping (c) Menu bar of Tree Ring Bubble

(3) Pathway Table Bubble

You can view the detailed protein information by "right click the bar (Figure 7 (a))" or "right click the arc of each pathway on the TreeRing Bubble View. It will add a bubble (Figure 7 (b)). From Table, if you are interested in a specify symbol, you can right click it, and it will add a Table Bubble with detailed protein information of this symbol (Figure 7 (c))".
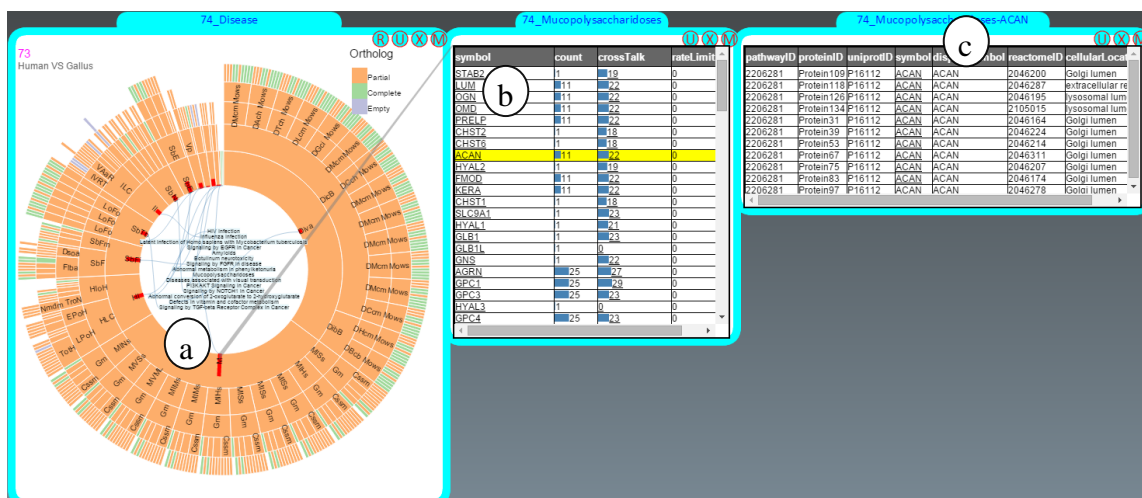

Figure 7 view detailed protein information through Table bubble.

2 Input Data Format

2.1 Supporting Pathway network format

The pathway graph in Pathbubbles supports pre-defined XML format.
We will first show you the sample example, and then tell you the detail format.

Table 1. XML file Format

```
<?xml version="1.0" encoding="utf-8"?>

<Pathway>

    <compartmentBlock Num="3">

        <compartment j="0">

            <Name>early endosome membrane</Name>

    <Position>(0.40767891088437735,0.2510594446429637,0.18464217823124535,0.22941258953095964)</Position>

    <Contain>(C,1,;S,1,;S,14,;S,4,;S,6,;S,8,;S,9,;S,15,;S,16,;S,19,;S,20,;S,24,;S,26,;S,29,;S,30,;S,34,;S,36,;R,1,;R,2,;R,3,;R,4,;

R,5,;R,6,;R,7,;)</Contain>

        </compartment>

    </compartmentBlock>

    <complexBlock Num="7">

        <complex j="1">

            <Name>PIKFYVEVAC14FIG4</Name>

    <Position>(0.6283649298613249,0.5706117201901926,0.042311567567274076,0.027386127875258303)</Position>

        </complex>

    </complexBlock>

    <physicalEntityBlock Num="1"/>

    <proteinBlock Num="15" />

    <DnaBlock   Num="1"/>

    <RnaBlock   Num="1"/>

    <smallMoleculeBlock Num="37"/>

    <reactionBlock   Num="9">

        <reaction j="1">

            <Name>PI(34)P2 is dephosphorylated to PI3P by INPP4AB at the early endosome membrane</Name>

            <Type>T</Type>

    <Position>(0.41048415733565263,0.2221266133316178,0.016924627026909632,0.027386127875258303)</Position>

        </reaction>

    </reactionBlock >

    <edgeBlock Num="41">

        <edge j="0">

            <Name>A</Name>

            <Ends>(P, 1, R, 1)</Ends>

        </edge>

    </edgeBlock>

</Pathway>
```

The first line is about the standard xml tag. The formal pathway is a hierarchical structure.

The first type is compartment. Compartment has attributes with "Name","Position","Contain". The position is a string with the combine of "x", "y", "w", "h" of this compartment. For the "Contain", it is consisted of all the biomolecules of the compartment. Which is splitted by ";". For each biomolecule, it begins with its type, and id. The id is the index of this type.

In PathBubble, it has 6 types.

"C" for complex;

"P" for protein;

"D" for Dna;

"Rna"for Rna;

"E" for"PhysicalEntity";

"S" for small molecule;

"R" for reaction

For each biomolecule, it has its id for "j" attribute.

Its "Name", its "Type"

At the end of the file is about the edges, which tells you the "Name" of the edge and Its two endpoints.

The endpoints is begins with the type of Biomolecule and the id of biomolecule, and ends with the type of Biomolecule and the id of biomolecule.

2.2 Supporting Pathway ortholog gene data format

You can upload you own gene data for ortholog analysis in PathBubbles.

Below we will you show the gene data format, which is **tab-delimited** format. The first column is about the symbol of gene, and the second column is about the gene orthologous relationship between this species and human, which is if this gene can be found in human pathway, it is the Reactome human database identification number or if this gene can't be found any human pathway, it is the "\N" tag.

Table 2 orthologous relationship of the input data between two species

symbol	dbId
ADA	\N
CDH2	414745
AKT3	421497
MED6	426282
NR2E3	395289

2.3 Supporting Pathway gene expression data format

You can upload you own gene expression data for expression analysis in PathBubbles.

Below we will show you the gene data format, which is a **tab-delimited** format. The first column is about the Entrez gene identification number, the second column is about

the gene symbol and the third column is about the ratio of the expression level of this gene between two biological states.

Table 2 gene expression of the experimental data between two biological states

gene_id	symbol	ratio
374096	SMAD6	0.6892992938620315
395132	SMAD3	7.128911138923654
395247	SMAD2	3.370212765957447
395543	SMAD9	Infinity
395679	SMAD5	3.013579576317219
395680	SMAD4	2.3619197482297403
429683	SMAD7	0.647887323943662
374094	TGFBR1	6.733865119651922
396399	TGFBR2	0.45263157894736844
395934	SHC1	1.4310163243812535
426482	SHC4	Infinity
431265	SHC3	NaN
770341	SHC2	3.3697916666666665
386572	GRB2	10.56062819576333
423572	SOS2	1.4514200298953663
425964	SOS1	1.5194805194805194

3 Introduction of PathRings Data Analysis

PathRings integrates all the existing pathways of Reactome (1417) in a single Sunburst visualization, and uses color to map interesting information, which is convenient to compare, query and validate hypothesis.

In PathRings, you can view each biological pathway network in a single Bubble. You can find cross-talking relationship between different pathways, you can compare the ortholog information between different species. You can upload your own experimental data (gene expression data) to compare the result between different pathways, and figure out the detailed connect protein information by the Table view.

3.1 Cross-talking Information

When you open a TreeRing, it will show you all the pathway names of the First Level of the TreeRing in the center. If you want to see the cross-talking relationship between pathways. You should click the "M" on the Menu Bar. And then, you should select "Show CrossTalk" option instead of the default "Show Pathway Name" option in the first dropdown menu.(see Figure 8 (c)) The crossTalking information showed in the graph represents the relationship between pathway in a same level. You can change the cross talking level by selecting the "crossTalkLevel" dropdown menu. You can right click the red bar to lookup the detailed cross-talking protein information. You can click the cross-talking cell of a gene(see Figure 8 (d)) in the table to see the cross-talking pathways, which will be highlight in the parent-treeRing using yellow dots.(see Figure 8 (b))
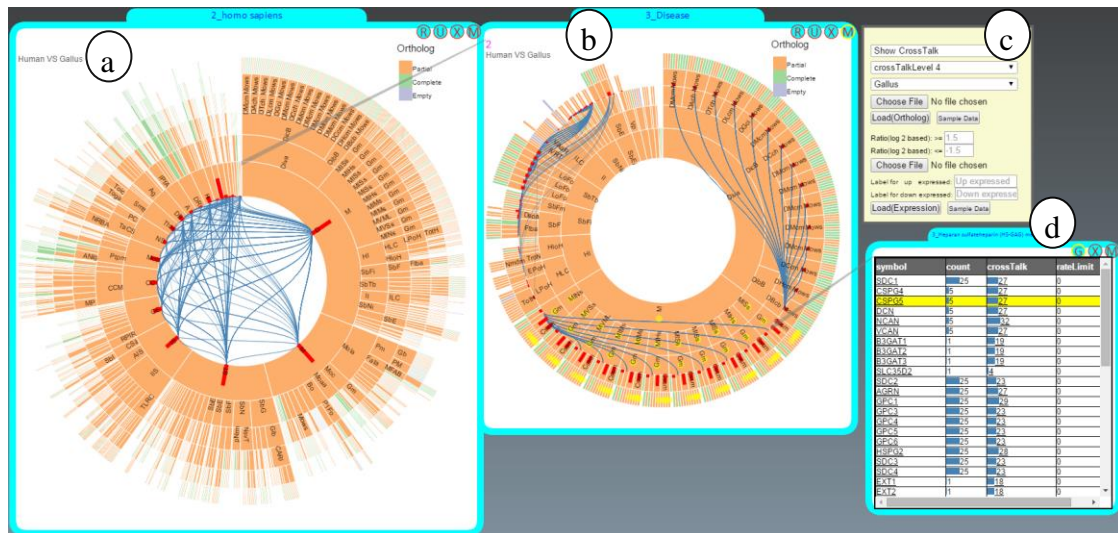
Figure 8. (a) Tree Ring visualization, (b) Sub-tree ring visualization, yellow dots attempt to represent the cross-talking pathways (c) Menu of tree ring visualization (d) Table contains cross-talking proteins

3.2 Ortholog Analysis

Our pathway is from Reactome, which is a database about human pathway. We can use the pathways of human to predict all the pathways of other species by providing their gene orthologous relationship between two species, so that we can know the pathway relationship between species. There are three types of relationships between a specie and human, Complete, Partial, Empty.

Complete: If all the gene of a species can be found in a pathway of human, we call this pathway is "Complete"to a species.

Partial: If just some gene of a species can be found in a pathway of human, we call this pathway is "Partial"to a species.

Partial: If all the gene of a species can not be found in a pathway of human, we call this pathway is "Empty"to a species.

In PathRings, all the three relationships of a pathway are encoded by color. ("Yellow" for "Partial","Green"for"Complete", and "Purple" for "Empty"). When you open a TreeRing, the default view will show you the ortholog relationship between Gallus and Human (see Figure 9 (g)). In the right corner of the Bubble, it will show you the color scale bar with 3 types of ortholog relationship (see Figure 9 (f)). And also you can view cross-talking information relationship by change "Show Pathway Name"(see Figure 9 (b)). You can change the default species by select "Choose Species" menu You can upload your own gene data of a species by select your own gene file and load it.
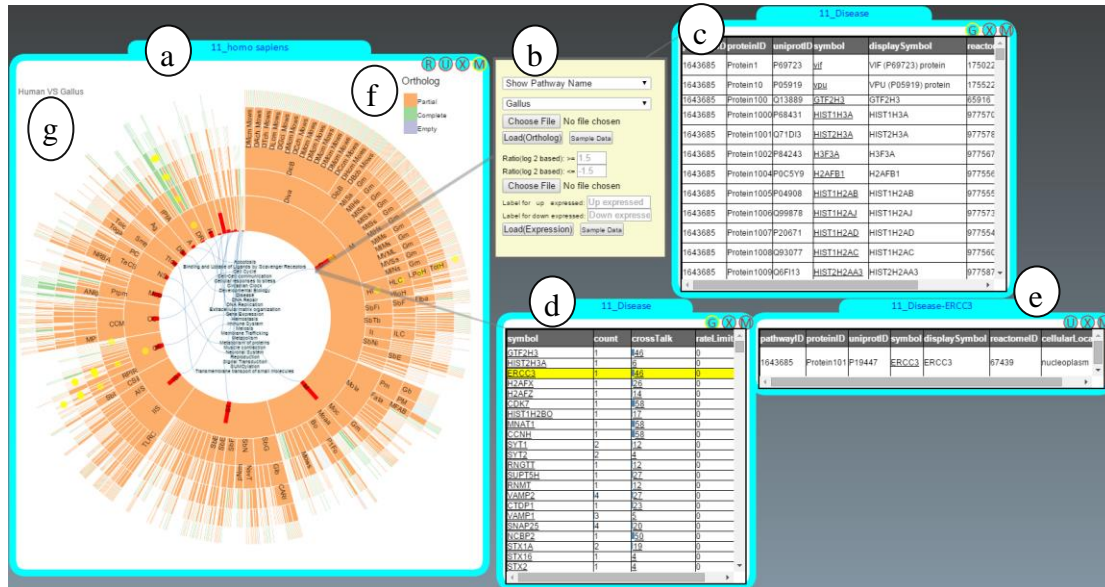
Figure 9 Orthologous analysis

If you want to see the detailed orthologous genes of a pathway between two species, you can right click a red bar on the ring. (see Figure 9) It will give a Table Bubble (Figure 9 (d)), which shows you the shared protein information of disease pathway between Human and Gallus. The Table is sortable, you can click the header of the table to sort it. And also If you care about the detailed symbol protein information, you can right click the interested symbol name.
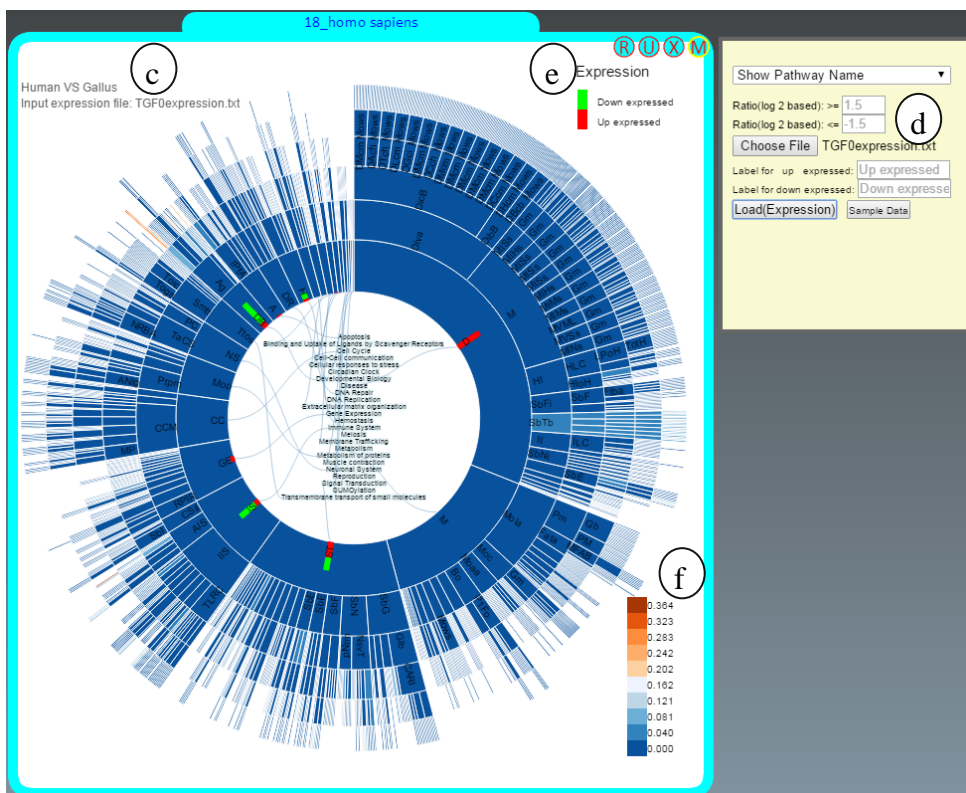
3.3 Gene Expression Analysis

PathRings provide gene expression analysis for a certain species. Gene Expression analysis is based on a certain species. So at first, you need to do the "Ortholog Analysis" (see the previous section).
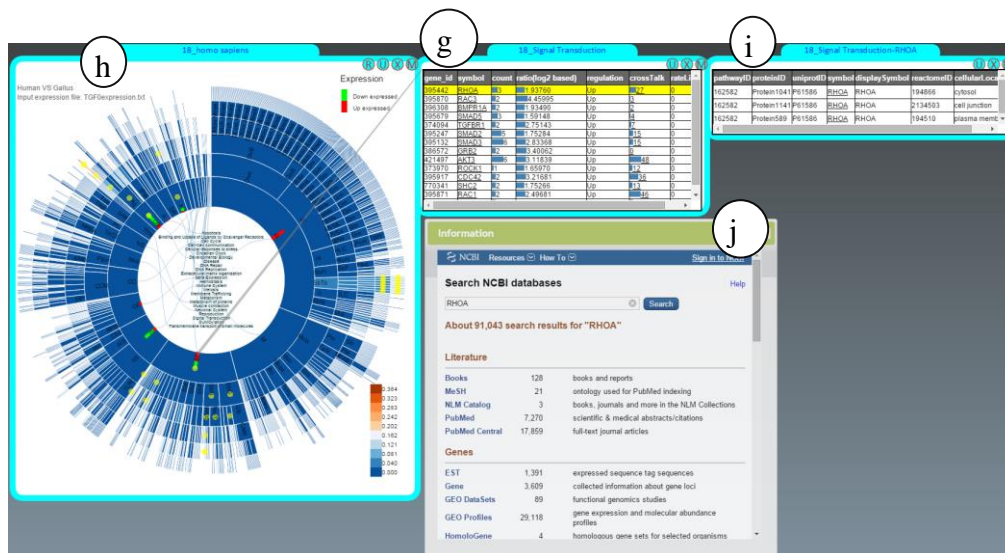
And then you can unpload your own gene expression data (see the expression data format section). You can input your cut-off of gene expression ratio, you can input your label for the up expressed and down expression bar. You can view the detailed expressed genes through table bubble. (Figure 10 (g)) You can obtain the gene information from the NCBI browser. (Figure 10(j)).
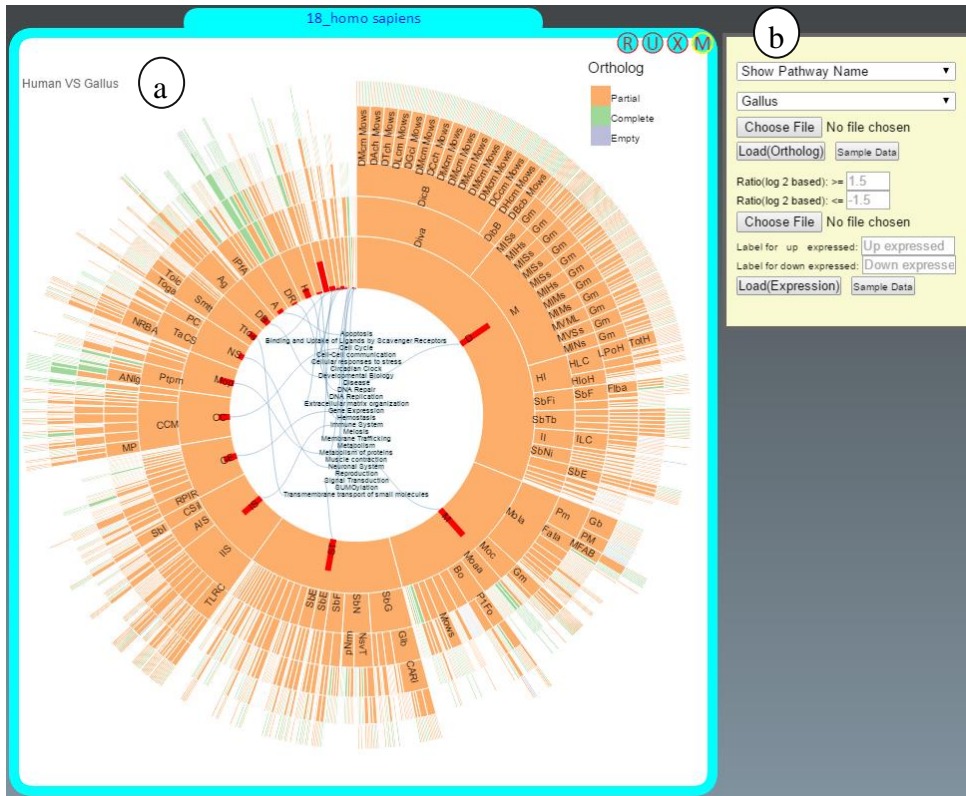
(1)

(2)

(3)

Figure 10 Gene expression analysis