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# Dose-response pathway analysis for gene expression: Graphical User Interface

User Manual



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# 1. About DR Pathway Analysis

Dose-response (DR) modeling is a key component of quantitative risk assessments. When performing microarray studies of transcriptional dose-response, it is of interest to test the coordinated involvement of transcripts (genes) from known biological pathways or functional categories. However, existing gene-set testing methods are not well-suited for DR pathway analysis, which requires careful control of false positive rates and fast DR curve fitting.

DR Pathway Analysis GUI was made for DR analysis, with fast curve fitting procedures and the introduction of a *pathway* dose-response profile. A bootstrap procedure is used to obtain confidence envelopes for the pathway DR profile.

\* DR GUI is written in Java, and for the testing phase uses ideas from the Significance Analysis of Function and Expression (SAFE) package (Barry et al., 2005) written in R (R Development Core Team, 2006).

## 2. Installation

## 2.1 Requirements

In order to use DR GUI user need to have R and the latest Java installed on your PC. **NOTE: R software should not be older than version 2.15.1.** R software could be downloaded from many mirrors around the world. You could find these mirrors here <u>http://www.r-project.org/</u>. Please select Windows operation system and then "base" as a subdirectory. One can find Java software here: <u>http://www.java.com/en/</u>.

After you have successfully installed R and Java you need to download DR Pathway Analysis installation package. The most recent version of DR Pathway Analysis is located here <a href="http://comptox.unc.edu/resources.html">http://comptox.unc.edu/resources.html</a>.

Before installation make sure that you have Full Control over your R installation directory.

- 1. To check that right click on the R folder (usually it's here: C://Program Files/R).
- 2. Select "Properties" from menu that will appear.
- 3. Navigate to "Security" tab.
- 4. Click "Edit" button.
- 5. Select Users in the list of Group or user names.
- 6. Check "Full control" box under Allow.



#### 7. Click OK.

#### 2.2 Installation process

Please unzip DR GUI archive to any place on your PC after you have downloaded **DRGUI.zip** file. Then open DRGUI folder and double click on **DRGUIInstaller.jar** file. The Installation window should appear.

DR Pathway installer will look for installed versions of R inside default locations and if you have administrative rights on your PC, Java and R properly installed into default directories you'll be able to use the "Automatic installation" function.

If you don't have administrative rights on your PC you will need to install R into any folder under your user account where you have rights to read/write/modify/execute files. In order to install DR GUI you will need to use the "Manual installation" option. One will need to click "Select file" button and select the folder where R was installed. The next step is click "Manual installation" button.

📓 Instalation of DR GUI
Automatic instalation
You can use this option if you have read/wright/execute user rights over the default R installation folder:
C:\Program Files\R\R-2.15.1
Please click the button to proceed. Automatic installation
Please use manual instalation if you don't have Administrator rights to read/write/execute on system folders or you have R installed into specific folder and you have rights to read/write/execute on the files in that folder. Plese select the folder with your R version, and then dick "Manual Installation" button.
Select file
Manual instalation

#### Figure 1. Installer main screen

DR Pathway GUI will try to download and install all necessary R libraries from the Internet, so Internet connection is required at this stage. During the installation you will see the progress window and occasionally the R progress window.



Installing DR Pathway Analysis GUI	
Loading initial packages	
<u></u>	

Figure 2. Progress window

If installation was successful you will see a successful install message displayed. Installer will place startup and uninstall link inside **R\DRGUI** directory in case it's unable to create startup menu items and Desktop shortcuts for DR Pathway GUI.



Figure 2. Installer success message



Figure 4. DR GUI folder in Windows Start menu

If installation fails with error message below, please follow installation instructions for User Access Control below.

#### 2.2.1 Installation under Windows Vista

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As Windows Vista has UAC (User Access Control) which doesn't allow copy files in Windows you should undertake some additional steps.

1) Before installation make sure that you have Full Control over your R installation directory.

8. To check that right click on the R folder (usually it's here: C://Program Files/R).



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- 9. Select "Properties" from menu that will appear.
- 10. Navigate to "Security" tab.
- 11. Click "Edit" button.
- 12. Select Users in the list of Group or user names.
- 13. Check "Full control" box under Allow.
- 14. Click OK.

2) Double click on DRGUIInstaller.jar file.

3) Click "Install" button to proceed

4) If you will see message "Cannot create Start menu! Shortcuts will be created in R directory" the program will not create Desktop shortcut and Start menu items.

But you'll find the shortcuts inside your R directory (usually it's here: C://Program Files/R/SAFEGUI).

\*You can avoid changing user rights by switching off UAC before installation. <u>But we do not</u> recommend you to do this.



## 3. Main window

#### 3.1. DR Pathway Analysis Settings

📉 DR Pathway Analysis GUI		
File Settings Help		
		AUSAY
	Select file	Results
		Show input data
Microarray Platform	hu6800.db	Show input data
Testing		
Method	Express	
Global statistic	D	THE
Local statistic	Score 👻	UNIVERSITY of NORTH
Pathway Database	60.CC •	CAROLINA
Correction method	Benjamini & Hochberg FDR 🔹	Gillings School of Global Public Health
Confidence an	alysis	Department of Biostatistics
P-value threshold for	inclusion: 0.1	
Number of data re-sa	mples: 100	
Pathway FDR thresho	old: 0.05	Run
-	Savanyanyayaya	2 hove h

Figure 3. DR Pathway Analysis main screen

On Figure 3 you can see the DR Pathway Analysis main screen which allows you to set input parameters for your data analysis.

First of all you need to set a path to the input data file. To do that, click on the "Select file" button. File browsing dialog should appear. Browse to your data file. Click "Open".

Select following parameters when you have selected input data file:

• **Microarray platform** - name of a Bioconductor annotation package to build gene pathways that corresponds to the array platform that was used to collect the data.

## **TESTING OPTIONS**

"Testing" refers to testing pathways for a significant dose-response relationship among numerous genes in the pathway



- **Method** Currently "express" is the only option. This is a mathematical approximation to permutation, described in Zhou et al. (2013). Other options may be added in future releases.
- **Global statistic** Specifies the global statistic for a pathway (gene set or category). The statistic D is currently the only option. It is a competitive statistic that compares the mean squared score statistic within the pathway to the mean squared score statistic among the remaining genes.
- Local statistic Specifies the gene-specific statistic "Score" is the current default.
- Pathway Database "GO.MF", "GO.BP", "GO.CC" specify the three Gene Ontologies. "GO.CC" will form categories from Cellular Compartment ontology, while GO.BP and GO.MF will work with Biological Processes and Molecular Function, respectively. It is important to note that in the hierarchical structure of the GO vocabularies, a gene category is generally thought of as containing the set of genes directly annotated to a term, and also to any terms beneath it in the ontology.
- **Correction method** Specifies the method for computing false positive error rates, accounting for multiple comparisons across numerous pathways. A Bonferroni, ("Bonferroni FWER"), Holm's step-up ("Holm FWER"), and Benjamini- Hochberg step down ("Benjamini & Hochberg FDR") adjustment can be specified.



## **CONFIDENCE ANALYSIS OPTIONS**

"Confidence analysis" refers to quantifying uncertainty in the *pathway* doseresponse curve. This is performed only for pathways meeting the "Pathway FDR threshold." The pathway dose response curve is created by

(i) selecting genes meeting "threshold for inclusion." For each of these genes, a four-parameter logistic curve is fit to represent the gene. For the included genes, bootstrap resampling of the gene expression and dose values is performed. In contrast to the testing phase, the confidence phase treats the entire expression profile and dose for each sample as a combined unit for bootstrap resampling.

(ii) For the observed data and each resample, a pathway dose response curve is created either by a) summing the individual dose-response curves (rescaled to the unit interval) or b) fitting a single logistic curve to all of the genes, which have been rescaled to the unit interval.

- **P-value threshold for inclusion** gene-specific p-value threshold to select genes that are to be included in the confidence analysis. Default is 0.1.
- Number of data re-samples An integer value to specify the number of bootstrap resamples performed. The entire curve fitting procedure to create the pathway dose-response curve is performed for each bootstrap.
- Pathway FDR (or Bonferroni/Holm) threshold The criterion for deciding which pathways should be included in the confidence analysis. Setting this value too high will result in performing the confidence analysis step for a large number of pathways, and thus will take a long time for computations to complete. By default, value is 0.05.

## BUTTONS

- **Results** (*button*) will show the results window on click. Note that this button became enabled after you run DR Pathway Analysis.
- Show input data (button) will show your input data file in a table format.
- **RUN** (button) runs DR Pathway Analysis method with selected parameters



#### 3.2. Main menu



Figure 6. DR Pathway Analysis menu

On the main DR Pathway GUI window you will see a top positioned menu with three main items – "File", "Settings" and "Help".

#### 3.2.1. File Menu

e Settings Help			
Print input data tab	ole		<u>auza</u>
Save Project	Ctrl+S	Select file	Results
Open Project	Ctrl+O		Show input data
Open Recent Project	ct 🕨		
Exit			

Figure 7. DR Pathway Analysis File menu

- Print input data table prints the data in table format from selected data file.
- Save Project saves current project.
- **Open Project** allow user to load previously saved project.
- **Open Recent Project** gives user a list of recent projects to load.
- Exit closes DR Pathway Analysis.

#### 3.2.2. Settings Menu

ſ	💟 D	R Pathway Analysis GUI		
	File (	Settings Help		
L		Advanced settings		AUZAY
L	$\langle \mathbf{I}$	Color settings	Select file	Results

#### Figure 8. DR Pathway Analysis Settings menu

- Advanced settings will open Advanced Settings window
- Color settings will open Color Settings window

#### 3.2.2.1. Advanced settings

Advanced Settings	
Category size options	Randomization options
Min Category Size: 10 Max Category Size: 100	<ul> <li>Use random begining seed</li> <li>Use constant begining seed</li> <li>Begining seed: 12345</li> </ul>
Permutation options (Not Applicable) Number of resamplings:	MLE Optimization options
Genelist cutoff value for Trend Fisher statistic:	Stopping criteria: 1E-3  Method: Nelder-Mead  Gradient
Delimiter options Delimiter for data output	Max iter: 1000
<ul> <li>○ Comma [,]</li> <li>○ TAB [\t]</li> <li>○ Space []</li> <li>○ Pipe []</li> </ul>	Safe Express options Grid length: 1000
	Cancel Save

Figure 9. DR Pathway Analysis Advanced Settings window

- **Category size options** allows user to set minimum and maximum category (pathway) size for the pathways to be included in the results.
- **Randomization options** allows user to set random seed or constant seed for the bootstrap resmapling. With constant seed option selected you'll need to input sthe eed value, and expect that you will obtain reproducible results.
- **Permutation options** these options are intended for future versions of DR Pathway.
- **MLE Optimization options** allows user to set options for MLE optimization for logistic curve fitting
- **Delimiter options** allows users to set delimiter for the tables which are produced when user tries to save the result data table. Usually delimiter is tab ("""), space (""), comma(",") or pipe ("|").
- Safe express options allows user to set "grid length" to be used for approximating permutation p-values. Higher grid values produce more accurate p-values. The default of 1000 should suffice unless the sample size is large (>200).



#### 3.2.2.2. Color settings

🔪 Color scheme 📃 📃 💌 🔀						
SAFE trees:						
p< 0.1	Color					
0.1 <= p < <u>0.5</u>	Color					
0.15 <= p < <u>0.7</u>	Color					
p>= 0.7	Color					
SAFE plots:						
Step line color:	Color					
Plot background color:	Color					
Plot shaded area color left:	Color					
Plot shaded area color right:	Color					
Plot shaded area one sided:	Color					
Cancel	Save					

Figure 10. DR Pathway Analysis Color Settings window

#### 3.2.3. Help menu

DR Pathway Analysis GUI								
File Settings Help								
About	ADEADEADE	AUZAY						
Input data format	Select file	Results						
Load example dataset		Show input data						

Figure 11. DR Pathway Analysis Color Settings window

- About contains information about the DR GUI software
- Input data format shows examples of the input data file structure.



THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL • Load example dataset – loads example dataset of Rat expression data, on the RAE230 platform, for demonstration purposes.

## 4. Input Data Format

Data in the input data file should be separated using "," (comma) delimiter. File extension usually should be .csv. Input data file should be in the following format:

First row – sample names,

Second row through n-row – expression data.

Dose values should be on an original meaningful scale, but will be analyzed on the log scale. To avoid difficulties with log(0), zero values will be transformed on the log scale to be an increment x lower than log(lowest positive dose), where x is the average increment between successive log(positive dose) values.

The expression data should be in the form of an mXn matrix, where appropriate normalization and other pre-processing steps have been taken. It should be noted that in the current version of DR Pathway Analysis, missing values are not allowed in the expression data, and must be imputed prior to analysis.



# 5. Running DR Pathway Analysis

After you click "RUN" button main window will disappear and Status window will appear.



N	Performed 200 genes out of 514.	
	PLATFORM INFO	
	Platform rae230a.db version is 2.8.1 built with R version 2.15.1	
	Building GO.BP categories from rae230a.dbGO2ALLPROBES	
	have C.list	
	finished safe.express	
	Categories completed:	
	40%	
	60%	-
	Clear	Stop

Figure 12. DR Pathway Analysis Progress window

You can see how Status window looks like on Figure 6. In this window DR Pathway Analysis displays status of the current process. You can halt execution by clicking **"Stop"** button. You can clear status window by clicking **"Clear"**.



## 6. Results

Results File Settings								
Category name	Size	P-value	FDR	Description				
GO:0000028	12	0.0033	0.041	ribosomal small subunit assem				
O:000038	17	0.0007	0.015	very long-chain fatty acid me				
O:0000303	17	0.0003	0.0078	response to superoxide				
O:0000305	18	0.0006	0.013	response to oxygen radical				
O:0001523	35	0.0011	0.019	retinoid metabolic process				
O:0001541	59	0.0019	0.029	ovarian follicle development				
O:0001556	12	0.0008	0.016	oocyte maturation				
O:0001569	45	0.0021	0.03	patterning of blood vessels				
O:0001570	64	0.0025	0.034	vasculogenesis				
D:0001658	67	0.0001	0.005	branching involved in ureteric				
D:0001676	46	0.0008	0.016	long-chain fatty acid metaboli				
D:0001678	67	0.0002	0.0069	cellular glucose homeostasis				
D:0001935	88	0.002	0.029	endothelial cell proliferation				
D:0001938	55	0.0005	0.013	positive regulation of endoth				
D:0001954	23	0.00007	0.0028	positive regulation of cell-mat				
D:0001956	11	0.0002	0.0059	positive regulation of neurotr				
O:0002053	42	0.0008	0.016	positive regulation of mesenc				
D:0002092	10	0.0036	0.043	positive regulation of recepto				
Local statistic: Score		Erro	or rate: FDR.BH					
Global statistic: D		Me	thod: express					
Threshold results by FI	DR: 0.05							
_ <u>_</u>								
0.1	0.2 0.3 0	0.4 0.5	0.6 0.7	0.8 0.9 1				

Results window will appear after DR Pathway GUI finishes with all permutations.

Figure 13. DR Pathway Analysis Results window

Result window is divided by two logical parts – Categories part and Specific category part. Categories part consists of the categories table, which we will describe more precisely below, and Analysis parameters, such as:

- Local statistic used for analysis
- Global statistic used for analysis
- Error rate
- **Method** (Express is the only option)
- Threshold results by FDR. User could change this value by dragging the slider



#### 6.1. Categories (pathways) table

The "Categories" table consists of categories that attain a certain level of significance. Figure 14 shows significant results for categories that have empirical p-values <= 0.25. For each category, the category name, and number of annotated genes in the dataset is displayed along with the p-value, FDR value and Category description.

Specific category information including genes contained in selected category will appear when user clicks on any category. Processing times for specific results depend on category size. Results for smaller categories will appear immediately, while results for bigger categories will take some time but only the first time a user click on the category.

Results											<u> </u>
File Seturigs											
Categor	Size	P-value	FDR	Descript	Table	Graph	Tree DR Pathway P	lot Change DR P	athway Plot Up	DR Pathway Plot [	Down
GO:0000	12	0.0033	0.041	ribosoma 🔺		oropri				2	
GO:0000	17	0.0007	0.015	very lon	Up	regulate	ed				
GO:0000	17	0.0003	0.0078	respons		Name	Local statistics	Empirical p. unlug	Cumbal	Description	
GO:0000	18	0.0006	0.013	respons		iname	LOCAI STAUSUCS	Empirical p-value	Symbol 1200100 a. ab	Description	
GO:0001	35	0.0011	0.019	retinoid	Syl	- 2	0.012	0.99	1368186_a_at	spieen tyrosin	
GO:0001	59	0.0019	0.029	ovarian f		q3 (- 1	0.052	0.96	136/818_at	coenzyme Q3	
GO:0001	12	0.0008	0.016	oocyte	SIC	581	0.082	0.93	13681/0_at	solute carrier t	
GO:0001	45	0.0021	0.03	patterni	SIC	2/a2	0.204	0.84	1368150_at	solute carrier t	
GO:0001	64	0.0025	0.034	vasculog	Psr	nd9	0.243	0.81	1368184_at	proteasome (p	
GO:0001	67	0.0001	0.005	branchin	Ph	pp 1	0.34	0.73	1368262_at	PH domain an	
GO:0001	46	0.0008	0.016	long-chai	Ptg	es	0.366	0./1	1368014_at	prostaglandin	
GO:0001	67	0.0002	0.0069	cellular g	Tb:	(asl	0.394	0.69	1368027_at	thromboxane	
GO:0001	88	0.002	0.029	endothel	Gu	cy1a3	0.49	0.62	1368154_at	guanylate cycl	
GO:0001	55	0.0005	0.013	positive	Jai	3	0.59	0.56	1368251_at	Janus kinase 3	
GO:0001	23	0.00007	0.0028	positive							
GO:0001	11	0.0002	0.0059	positive	Do	wn reaul	ated				
GO:0002	42	0.0008	0.016	positive							— I
GO:0002	10	0.0036	0.043	positive 🖵		Name	Local statistics	Empirical p-value	Symbol	Description	
····			1		Sp	nk1	-3.754	0.00020	1368254_a_at	sphingosine ki	<b>_</b>
				500 011	Dh	cr7	-3.228	0.0012	1368189_at	7-dehydrochol	
Local sta	tistic: So	tore	Error rate	E FDR.BH	Mt	nfd1	-2.603	0.0092	1368181_at	methylenetetr	
Global st	atistic: D		Method:	express	Ga	nt	-2.248	0.025	1368253_at	guanidinoacet	-
Threshol	d results	by FDR:	0.05				1	1	1		_
0.1	0.2 0.3 (	0.4 0.5	0.6 0.7 (	0.8 0.9 1		Close view	/				
0.1	0.2 0.0 (		0.0 0.7 (								

Figure 14. DR Pathway Analysis Results window



#### 6.2. Genes table

able Gr	aph Tree	DR Pathway Plot Change	DR Pathway Plot Up	DR Pathway Plot Do	wn	
Up reg	ulated					
Name		Local statistics	Empir	ical p-value	Symbol	Description
Katnb 1		0.012	0.99		1375188_at	katanin p80 (WD repeat con
Prtfdc1		0.026	0.98		1374784_at	phosphoribosyl transferase
Plxna3		0.106	0.92		1376139_at	plexin A3
Lamb2		0.165	0.87		1367880_at	laminin, beta 2
Rarg		0.621	0.53		1376023_at	retinoic acid receptor, gamma
Vefl		0.903	0.37		1370058_at	neurofilament, light polypep
Vapsa		0.927	0.35		1368521_at	napsin A aspartic peptidase
łrk		1.689	0.091		1368535_at	harakiri, BCL2 interacting pr
Guca 1b		2.135	0.033		1376048_at	guanylate cyclase activator 18
Vegfa		3.257	0.001	1	1370081 a at	vascular endothelial growth
Name		Local statistics	Empir	ical p-value	Symbol	Description
Name		Local statistics	Empir	ical p-value	Symbol	Description
SIC11a2		-3.8/1	0.000	10	136/8//_at	solute carrier family 11 (prot
-asn		-2,313	0.021		1367/08_a_at	fatty acid synthase
sanı		-2,212	0.027		1368387_at	3-nydroxybutyrate denydro
Vnto		-2,134	0.033		13/6063_at	wingless-type MMI V integra
boat1		-2.05	0.040		13/49/6_a_at	sterol O-acyltransferase 1
Ltsa		-1.509	0.13		130/051_at	Cathepsin D
Loasy		-1.028	0.30		13/3304_at	COA synthase
		-0.676	0.50		13/0332_at	unc-13 nomolog D (C. elegans)
JITIS Dece 1		-0.0+3	0.52		1375971_dt	ceroid-lipotuscinosis, neuron
vbcd1		-0.323	0.00		1376075_at	ATP binding cassotte, subfa
ADCU1		-0.205	0.70		1276002 at	molybdonum cofactor synth
Nocs1		-0.135	0.80		1370055_at	phospholipase A2, group V
		-0.117	0.05		1375250 at	LIDB-Gal-betaGlcNAc beta 1
R4nal+1		-0.117	0.91		1376145 at	eukarvotic translation initiati
34galt1		-0.075	0.94		1570145_at	Eukaryouc u ansiauon muau
84galt1 Eif2b5						
B4galt1 Eif2b5						
B4galt1 Eif2b5						

#### Figure 15. DR Pathway Analysis Results window (Genes table)

The "Genes" table is located under the Table tab and could consist of one or two tables. On Figure 15 you can see *Up regulated* genes table versus *Down regulated* genes table. Up regulated are genes with local statistic values more than 0. Down regulated are genes with local statistic value under 0.



Each genes table contains the following information:

- Gene name
- Gene local statistic
- Empirical p-value
- Gene symbol
- Gene description

#### 6.3. Graph



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The graph tab contains a plot for a single pathway. We have proposed that the differential expression of genes be plotted as a SAFE-plot (Barry et al., 2005). SAFE-plots show the cumulative distribution function (CDF) for the ranked local statistics from a given category (solid line). A significant category will have more extreme associations to the response of interest than its complement, resulting in a rightward, leftward, or bidirectional shift in the CDF away from the unit line (dashed line). The shaded regions of the plot correspond to the genes that pass a nominal level of significance (empirical p-values <= 0.05 by default). A user can select SAFE-plot representation by switching from ranked local statistic to simple local statistic.

To zoom in/out the plot a user can use "+"/"-" buttons or by rotating mouse scroll. To move zoomed plot, push and hold left mouse button and drag the plot right or left.

If you click on the gene symbol, DR Pathway Analysis will open extended gene information in your default browser.



Figure 17. DR Pathway Analysis Results window (Genes Plot)

#### 6.4. Tree

The Tree tab utilizes the structured vocabulary whereby genes are annotated in GO from broad to narrow levels of classification in a directed acyclic graph (DAG). As such, many categories are highly related in their gene membership, and visualizing results across the ontology can be useful in ascertaining the relationship among multiply significant categories.



THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL By default, nodes with unadjusted p-values less than 0.1 are drawn in brown; less than 0.5 are drawn in green; and less than 0.7 are drawn in purple, more than 0.7 in grey, all other categories are colored in cyan. User-defined cutoffs for these colors can be specified using the menu **Options->Color scheme**.

By clicking on any tree, the node graph will be refreshed and the selected node will become the top of the tree showing child categories. You can easily undo that operation by pushing **"Undo" button.** 

By pushing the **"Big tree"** button, a new window will open showing the tree containing all the categories in selected package.

By checking **"Show all children"** DR Pathway Analysis will show all the categories which are not in threshold results.



Figure 18. DR Pathway Analysis Results window (Tree tab)



#### 6.5. DR Path Detector Plot tabs



Figure 19. DR Pathway Analysis Results window (DR Path Change tab)

DR Path Detector (Change, Up, Down) Plot tab contains information and graphical representation of the pathway dose response profiles. Users can see the EC5, EC10 and EC50 values on the table and on the plot by selecting one of the radio buttons. The EC values and 95% confidence intervals are in red. Clicking on the EC buttons will also cause them to appear in the table of all significant pathways.

The shaded areas indicate extrapolation of results beyond the actual range tested. As with any extrapolation, these should be interpreted with caution.

Zooming ability is available and behaves in the same manner as Gene plots.





Figure 20. DR Pathway Analysis Results window (DR Path Up tab)







Figure 20. DR Pathway Analysis Results window (DR Path Down tab)

Results can be printed or saved from the Results window.

