

Spinal cord injury in rats

The following tutorial walks through the identification of biological themes in a microarray dataset examining chronic spinal cord injury in rats.

Visit the GeneSifter Data Center (www.genesifter.net/web/dataCenter.html) to register for free access to the dataset.

1. From the **Control Panel** select “Pairwise” from the **Analysis** section.
2. Click on the magnifying glass icon next to “**U34A**” to begin the analysis. The data presented here was generated using the Affymetrix® GeneChip® Rat Genome U34A array. There are approximately 7000 genes represented on this array, and an additional 1000 ESTs.

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Welcome to GeneSifter

Select a topic to view the GeneSifter QuickStart Guide

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Announcements

05-20-2005 Update: New UniGene Builds
UniGene information has been updated for Human (Build #104), Rat (Build #143), Mouse (Build #147), Zebrafish (Build #33), Drosophila (Build #37), and Chicken (Build #26) genes.

05-13-2005 Update: New UniGene Builds
UniGene information has been updated for Rat (Build #142), Arabidopsis (Build #49), Bovine (Build #68) and Xenopus (Build #63) genes.

04-22-2005 Update: New UniGene Builds
UniGene information has been updated for Human (Build #103), Mouse (Build #145), and Zebrafish (Build #63) genes.

04-01-2005 Update: New UniGene Builds
UniGene information has been updated for Human (Build #182), Rat (Build #141), and Drosophila (Build #36) genes.

Array **2**

U34A

Description

U34A Rat Genes

Main (login: spinal_inj) > Analysis > Pairwise

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3. At the top of the page is a list of the different experiment groups contained in this analysis. We'll be comparing expression between the control and injured samples. Select the three "**Uninjured Control**" arrays to place them in Group 1. Select the three "**Contusion 35 days**" samples for Group 2.

4. Pairwise analysis combines a fold-change cutoff and comparison statistics to generate a list of differentially expressed genes. Select the following analysis parameters:

Normalization: None

Data was already normalized prior to loading.

Statistics: Welch's t-test

Performs a Welch's t-test for each gene that passes the fold change cutoff.

Quality: P

Only those genes marked as P (present) for each array within a group will be included.

Threshold: 1.5

Filters out genes with less than 1.5 fold change in expression.

Correction: Benjamini and Hochberg

Calculates a false discovery rate from the raw p-values using the method of Benjamini and Hochberg.

Data Transformation: No transformation

Data will not be logged.

5. Select the **Analyze** button.

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Main (login: spinal_inj) > Analysis > Arrays > Pairwise

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Pairwise Analysis: U34A

Group		Experiment	Target	Condition
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/> GSM49933	Contusion Rep 1	Contusion 35 days
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/> GSM49934	Contusion Rep 2	Contusion 35 days
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/> GSM49935	Contusion Rep 3	Contusion 35 days
<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> GSM49936	Uninjured Rep 1	Uninjured Control
<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> GSM49937	Uninjured Rep 2	Uninjured Control
<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> GSM49938	Uninjured Rep 3	Uninjured Control

Advanced Analysis Settings

Normalization: None

Statistics: Welch's t-test

Quality: P

Threshold: Lower: 1.5 Upper: None

Correction: Benjamini and Hochberg

Data Transformation:
 No Transformation
 Log Transform Data
 Data Already Log Transformed

Show genes that are:
 Up-regulated
 Down-regulated

Analyze Reset

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- After the analysis is performed, a gene list will be returned. The **Pairwise Analysis Results Page** lists the genes that are differentially expressed, based on the selected parameters. 707 genes passed the filtering criteria – 1.5 fold or greater change in expression, all present calls, and a raw p value less than 0.05. The genes are sorted by fold change, and the first 20 genes in the list are displayed.
- To filter the list using the adjusted p-value (false discovery rate), select “adjusted p” from the pull-down menu, and then click the **Search** button. Using the adjusted p-value reduces the gene list to 619 results with a false discovery rate of less than 5%.
- To view data and a gene summary for any gene in the list, select the **Gene Name**.

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Pairwise Analysis: U34A

Group 1: Uninjured Control
Experiments: 58541, 58542, 58543
Significance: 1.5, Welch's t-test, Benjamini and Hochberg
Normalization: None
Quality Cutoff: Present
Data Transformation: None

Group 2: Contusion 35 days
58538, 58539, 58540

Show: 20 Sort By: Ratio p Cutoff: 0.05 raw p Search (707 results found) [1 - 20] [21 - 40]

No.	Ratio	p-value	adj. p	Identifier	Gene Name
1	16.14	0.02185	0.04004	L09119	Neurogranin
2	10.14	0.00464	0.02096	L07114	Apolipoprotein B editing complex 1
3	8.49	0.01460	0.03244	J02722	J02722cds RATHOXA Rat heme oxygenase gene, complete cds
4	8.26	0.00238	0.01627	J02962	Lectin, galactose binding, soluble 3
5	8.17	0.00212	0.01565	A4817854	Ceruloplasmin
6	7.54	0.03108	0.04781	D00698	D00698 RATIGF1A Rat insulin-like growth factor 1 mRNA
7	6.50	0.00236	0.01627	AI169327	Tissue inhibitor of metalloproteinase 1
8	6.22	0.02340	0.04157	L32132	Lipopolysaccharide binding protein
9	6.18	0.00492	0.02122	X73371	Fc receptor, IgG, low affinity IIb
10	6.12	0.00023	0.00764	AA874803	Transcribed locus
11	6.01	0.00513	0.02169	J02585	Stearoyl-Coenzyme A desaturase 1
12	5.81	0.03779	0.05446	X17053	X17053mRNA RATIE Rat immediate-early serum-responsive IE gene
13	5.73	0.02079	0.03931	AI177004	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1
14	5.58	0.00104	0.01294	AA874803	Transcribed locus
15	5.33	0.01018	0.02822	M58364	GTP cyclohydrolase 1
16	5.24	0.01977	0.03834	AI176456	Transcribed locus, moderately similar to XP_529783.1 LOC454105 [Pan troglodytes]
17	5.23	0.00381	0.01886	A1639107	ADP-ribosylation factor-like 11 (predicted)
18	5.08	0.00002	0.00306	X71127	Complement component 1, d subcomponent, beta polypeptide
19	4.90	0.00029	0.00844	AI175764	Stearoyl-Coenzyme A desaturase 1
20	4.66	0.00182	0.01446	M32062	Fc receptor, IgG, low affinity III

Show: 20 Sort By: Ratio p Cutoff: 0.05 raw p Search (707 results found) [1 - 20] [21 - 40]

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9. Selecting a gene from the list will bring up the data summary and a One-Click Gene Summary for the gene. The One-Click Gene Summary provides a synopsis of current UniGene and Entrez Gene information for the gene.

Go back to the gene list by clicking the **Back** button in your browser.

10. Select the **Ontology** link to view a summary of the Gene Ontology terms associated with the genes in the list. See the online help system for more information about the other reports.

Note: To view page-specific help documents for any page, select the question mark icon (?) in the upper right corner of each page.

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» Gene Summary: Neurogranin

Group	Condition	N	Mean	SEM	SEM/Mean	Quality Mean
1	Uninjured Control	3	262.015	+/- 37.1811	14.2%	1.0000
2	Contusion 35 days	3	16.2324	+/- 2.0735	12.8%	0.0000

Contusion 35 days down-regulated 16.14 fold compared to Uninjured Control

» One-Click Gene Summary™

Probe Set ID: [L09119_g_at](#)
 Accession No.: [L09119](#)
 Cluster ID: [Rn.11236](#)
 UG Title: Neurogranin
 Gene ID: [Nrgn](#)
 Homologene: [Mm.201107](#), [Hs.26944](#), [Mm.199652](#), [Mm.191628](#), [Mm.29857](#)
 Chromosome: 8
 Cytoband: 8q21
 Seq Count: 35
 Entrez Gene: [64356](#)
 Gene Name: neurogranin
 OMIM: -
 KEGG: [64356](#)
 RefSeq mRNA: [NM_024140](#) (FASTA)
 RefSeq Prot: [NP_058103](#) (FASTA)
 Summary: -

Gene Ontologies:
 Biological Process
 • protein kinase cascade
 Molecular Function
 • calmodulin binding

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Pairwise Analysis: U34A

[Reports: [Ontology](#) | [KEGG](#) | [Scatter Plot](#)] [Results: [Export](#) | [Save](#)]

Conditions: Uninjured Control (58541, 58542, 58543) | Contusion 35 days (58538, 58539, 58540)
 Experiments: 1,5, Welch's t-test, Benjamini and Hochberg
 Significance: Present
 Normalization: None
 Data Transformation: None

Show: 20 | Sort By: Ratio | p Cutoff: 0.05 | raw p | Search (707 results found) [1 - 20] [21 - 40]

No.	Ratio	p-value	adj. p	Identifier	Gene Name
1	16.14	0.02185	0.04004	L09119	Neurogranin
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9	6.18	0.00492	0.02122	X73371	Fc receptor, IgG, low affinity IIb
10	6.12	0.00253	0.00764	A0874803	Transcribed locus
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12	5.81	0.03779	0.05446	X17053	X17053mRNA RAT3E Rat immediate-early serum-responsive 3E gene
13	5.73	0.02079	0.03931	AI177004	3-hydroxy-5-methylglutaryl-Coenzyme A synthase 1
14	5.58	0.00104	0.01234	AA074803	Transcribed locus
15	5.33	0.01018	0.02822	M08964	GTP cyclohydrolase 1
16	5.24	0.01577	0.03834	AI174656	Transcribed locus, moderately similar to XP_529783.1 LOC454105 [Pan troglodytes]
17	5.23	0.00381	0.01886	A1639107	ADP-ribosylation factor-like 11 (predicted)
18	5.08	0.00002	0.00306	X71127	Complement component 1, alpha subcomponent, beta polypeptide
19	4.90	0.00028	0.00844	AI175764	Stearoyl-Coenzyme A desaturase 1
20	4.66	0.00182	0.01446	M32062	Fc receptor, IgG, low affinity III

Show: 20 | Sort By: Ratio | p Cutoff: 0.05 | raw p | Search (707 results found) [1 - 20] [21 - 40]

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11. The Ontology Report lists the Gene Ontology terms associated with the 619 genes from the pairwise results list. See the page-specific help for a complete description of the Ontology Report.
12. Click on **Z-score Report**.
13. The z-score report lists the biological process ontology terms that are significantly over or under-represented in the gene list (z-score greater than 2, or less than -2, respectively). Select the red arrow in the z-score column to sort the list so that the most over-represented genes are at the top.

A z-score report can also be generated for the molecular function and cellular component ontology terms.

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Group 1: Uninjured Control
Group 2: Contusion 35 days

[Biological Process | Cellular Component | Molecular Function]

[Ontology Report | Z-score Report]

Ontology	Genes	GO	List	Totals	Array	z-score	
physiological process	313	150	163	2576	2.01	-0.45	
cellular process	217	97	120	1871	-0.91	0.01	
development	71	36	35	639	0.19	-1.08	
regulation of biological process	21	11	10	147	1.09	0.20	
behavior	4	1	3	72	-1.54	-0.79	
viral life cycle	-	-	0	0	2	-0.34	-0.37

cellular process (34.66%)
development (11.34%)
regulation of biological process (3.35%)
behavior (0.64%)
physiological process (50.00%)

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Group 1: Uninjured Control
Group 2: Contusion 35 days

[Biological Process | Cellular Component | Molecular Function]


[Ontology Report | Z-score Report]
Export Report

Ontology	Genes	GO	List	Totals	Array	z-score	
physiological process	313	150	163	2576	2.01	-0.45	
transport	80	25	55	708	-2.60	1.69	
response to external stimulus	58	45	13	390	5.63	-2.66	
catabolism	48	25	23	283	2.60	1.24	
response to biotic stimulus	46	40	6	271	7.04	-2.96	
response to stress	45	36	9	272	5.89	-2.19	
lipid metabolism	42	18	24	208	2.08	3.13	
defense response	36	33	3	227	6.23	-3.25	
cell-cell signaling	35	5	30	188	-1.75	5.52	
immune response	33	30	3	195	6.28	-2.87	
ion transport	30	7	23	299	-2.51	0.95	
alcohol metabolism	29	8	21	104	1.01	5.84	
nucleobase, nucleoside, nucleotide and nucleic acid metabolism	29	15	14	481	-2.48	-3.42	
cell death	28	15	13	145	2.64	1.29	
death	28	15	13	147	2.58	1.24	
neurogenesis	28	8	20	202	-0.98	2.10	
transmission of nerve impulse	28	1	27	154	-2.70	5.78	
synaptic transmission	26	1	25	149	-2.65	5.30	
vesicle-mediated transport	25	8	17	137	0.19	2.93	
G-protein coupled receptor protein signaling pathway	24	5	19	274	-2.79	0.37	
cell adhesion	23	18	5	163	3.21	-1.79	

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14. Return to the **Pairwise Analysis Results Page** and click on the **KEGG** link. This will bring up a z-score report for the KEGG pathway terms associated with the gene list.

15. Click on the KEGG logo () for **Wnt signaling pathway** to show the KEGG pathway diagram. Differentially regulated genes are shown in red.

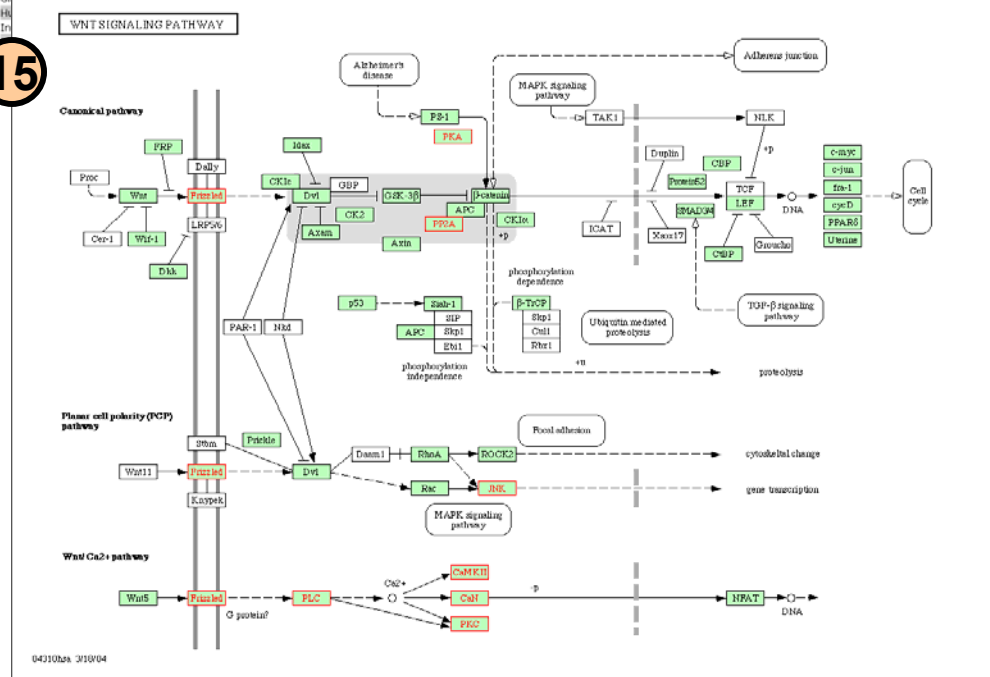
GeneSifter - Microarray Analysis Online | Analysis - Microsoft Internet Explorer

Group 1: Uninjured Control
Group 2: Contusion 35 days

Pathway	Genes	KEGG	List	Array	Totals	z-score
MAPK signaling pathway			21	10	11	132 1.39 0.42
Calcium signaling pathway			17	3	14	143 -1.72 1.14
Neuroactive ligand-receptor interaction			17	3	14	204 -2.56 -0.34
Regulation of actin cytoskeleton			12	6	6	92 0.66 -0.35
Wnt signaling pathway			11	3	8	57 0.07 1.94
Biosynthesis of steroids			9	0	9	11 -0.77 0.29
Glycerolipid metabolism			8	3	5	51 0.27 0.66
Oxidative phosphorylation			8	0	8	52 -1.70 2.23
Cytokine-cytokine receptor interaction			7	7	0	82 1.48 -2.66
Glycolysis / Gluconeogenesis			6	2	4	32 0.31 1.11
Phosphatidylinositol signaling system			6	1	5	39 -0.72 1.30
Tight junction			6	4	2	46 1.14 -0.81
Toll-like receptor signaling pathway			6	5	1	36 2.45 -1.08
Adherens junction			5	4	1	29 2.17 -0.93
Alzheimer's disease			5	3	2	16 2.51 0.78
Amotrophic lateral sclerosis (ALS)			5	0	5	15 -0.90 3.85
Complement and coagulation cascades			5	5	0	26 2.45 -1.73
TOF-beta signaling pathway			5	3	2	50 0.31 -0.94
Apoptosis			4	0	4	51 -1.68 0.11
Carbon fixation			4	0	4	15 -0.90 2.86
Purine metabolism			4	2	2	46 -0.23 -0.81
Terpenoid biosynthesis			4	0	4	4 -0.46 7.07
Alanine and aspartate metabolism			3	0	3	11 -0.77 2.52
butanoate metabolism			3	1	2	22 -0.11 0.30
Fatty acid metabolism			3	1	2	41 -0.78 -0.63
Fructose and mannose metabolism			3	1	2	20 -0.01 0.44
Glutamate metabolism			3	0	3	16 -0.93 1.74

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16. Return to the **Pairwise Analysis Results Page** and click **Scatter Plot**.

17. This will bring up a scatter plot of the results. Upregulated genes are shown in red, while downregulated genes are green. The gray spots are those genes that did not pass the analysis criteria (statistics, fold change, etc.). Drag the blue box to an area, and click **Zoom** to see more detail.

18. Click on data points in the detail to bring up the One-Click Gene Summary for a specific gene.

Only a few specific aspects of the data set have been demonstrated here. Feel free to examine the data further on your own.

The screenshot displays the GeneSifter web application interface. At the top, the title is "Pairwise Analysis: U3:4A". The interface is divided into several sections:

- Control Panel:** Contains navigation options like "Analysis", "Inventories", "Import Data", "Create New", "Resources", "Administration", and "User Feedback".
- Main Results Table:** A table with columns: No., Ratio, p-value, adj. p, Identifier, and Gene Name. It lists 20 genes, with the top one being Neurogranin (L09119) with a ratio of 16.14 and a p-value of 0.02185.
- Scatter Plot:** A log-log plot comparing "Contusion 35 days" (y-axis) to "Uninjured Control" (x-axis). Red dots represent upregulated genes, green dots represent downregulated genes, and gray dots represent non-significant genes. A blue box highlights a specific region of upregulated genes.
- Gene Info Detail:** A detailed view for the Neurogranin gene, showing a table of mean and SEM values for the two groups and a bar chart comparing their intensities. The bar chart shows a significant increase in intensity for the contusion group compared to the control.

Annotations 16, 17, and 18 are placed on the interface to guide the user through the workflow: 16 points to the "Scatter Plot" button, 17 points to the "Zoom" button, and 18 points to a data point in the detailed view.