

Huntington's disease and combination drug therapy

The following tutorial walks through the identification of biological themes in a microarray dataset examining Huntington's disease.

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" within the **Analysis** section.
2. Click on the magnifying glass icon next to "U74Av2 Mouse Genes" to begin the analysis.

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Welcome to GeneSifter

Select a topic to view the GeneSifter QuickStart Guide

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Announcements

02-06-2005 **LocusLink Updated**
LocusLink information has been updated with the most current data available.

01-23-2005 **LocusLink Updated**
LocusLink information has been updated with the most current data available.

01-21-2005 **Update: New Unigene Builds**
UniGene information has been updated for Human (Build #180), Mouse (Build #144), Rat (Build #139), and Zebrafish (Build #80) genes.

01-16-2005 **LocusLink Updated**

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Array Description

Array	Description
U74A	U74Av2 Mouse Genes

HD Tutorial (continued)

- 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 wild type and R6/2 strains, both untreated. Select the three **"WT untreated"** arrays to place them in Group 1.
- Select the four **"R6/2 untreated"** samples for Group 2.
- Set **Normalization** to **"All Median"** normalizing the data to the global median of gene expression, set **Statistics** to **"t-test"** and set **Quality** to **1**. This quality setting means that only those genes flagged as "P" by the Affymetrix MAS5 algorithm will be included.
- Leave **Lower Threshold** at **1.5** and **Upper Threshold** at **"None"**. Set **Correction** to **"Benjamini and Hochberg"** to perform multiple testing correction.
- Set **Data Transformation** to **"Log Transform Data"**.
- Click **"Analyze"** to process the data with these settings.

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Pairwise Analysis: U74A

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

Group	1	2	Experiment	Target	Condition
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		GSM13300	WT untreated 5241	WT untreated
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		GSM13355	WT untreated 5176	WT untreated
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		GSM13356	WT untreated 5296	WT untreated
<input type="checkbox"/>	<input type="checkbox"/>		GSM13357	WT treated 5217	WT treated
<input type="checkbox"/>	<input type="checkbox"/>		GSM13358	WT treated 5267	WT treated
<input type="checkbox"/>	<input type="checkbox"/>		GSM13359	WT treated 5289	WT treated
<input type="checkbox"/>	<input checked="" type="checkbox"/>		GSM13360	R6/2 untreated 5197	R6/2 untreated
<input type="checkbox"/>	<input checked="" type="checkbox"/>		GSM13361	R6/2 untreated 5213	R6/2 untreated
<input type="checkbox"/>	<input checked="" type="checkbox"/>		GSM13362	R6/2 untreated 5227	R6/2 untreated
<input type="checkbox"/>	<input checked="" type="checkbox"/>		GSM13363	R6/2 untreated 5270	R6/2 untreated
<input type="checkbox"/>	<input type="checkbox"/>		GSM13364	R6/2 treated 5185	R6/2 treated
<input type="checkbox"/>	<input type="checkbox"/>		GSM13365	R6/2 treated 5253	R6/2 treated
<input type="checkbox"/>	<input type="checkbox"/>		GSM13366	R6/2 treated 5211	R6/2 treated

Advanced Analysis Settings

Normalization: All Median

Statistics: t-test

Quality: 1

Threshold: Lower: 1.5 Upper: None

Correction:

GeneSifter - Microarray Analysis Online | Analysis - Microsoft Internet Explorer

Advanced Analysis Settings

Normalization: All Median

Statistics: t-test

Quality: 1

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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HD Tutorial

(continued)

9. At the top is a summary of the analysis just performed. The gene list below shows the genes that passed all our analysis parameters. By default, the most differentially expressed genes are shown first.

10. Change the **p Cutoff** from **raw p** to **adjusted p**, which reflects the p-value from the Benjamini and Hochberg false discovery rate (FDR) correction, and click **Search**. This will reduce the number of significantly affected genes to 439.

11. To find the most current information about a particular gene, click the gene name to bring up the **One Click Gene Summary**. It is a summary of the most current information, compiling data from over a dozen databases.

12. To get an overview of the gene families that have been differentially regulated, click on the **Ontology** link to bring up the Ontology Report.

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Pairwise Analysis: U74A
Main (login: huntingtons) > Analysis > Pairwise > Results
Results: Ontology | KEGG | Scatter Plot | Results: Export | Save
Conditions: WT untreated (Group 1), R6/2 untreated (Group 2)
Experiments: 28843, 28844, 28845
Significance: 1.5, t-test, Benjamini and Hochberg
Normalization: Median Intensity
Quality Cutoff: 1
Data Transformation: Log Transformed

Show: 20 | Sort By: Ratio | p Cutoff: adjusted p (selected) | Search (438 results found) [1 - 20] [21 - 40]

No.	Ratio	p-value	adj. p	Identifier	Gene Name
1	7.67	0.01716	0.04136	X87142	Calcium/calmodulin-dependent protein kinase II alpha
2	6.11	0.00139	0.01653	U20735	Mus musculus transcription factor junB (junB) gene, 5' region and complete cd
3	3.97	0.00055	0.01350	M24377	Early growth response 2
4	3.04	0.00211	0.01876	AB004315	Regulator of G-protein signaling 4
5	3.56	0.00453	0.02506	AA681998	CDC28 protein kinase regulatory subunit 2
6	3.56	0.00328	0.02271	AF002701	Glial cell line derived neurotrophic factor family receptor alpha 2
7	3.53	0.00068	0.01395	U61317	Myelin-associated oligodendrocytic basic protein
8	3.51	0.01447	0.03868	AF006466	Formin-like 1
9	3.32	0.00062	0.01395	AF045887	Angiotensinogen
10	3.32	0.00603	0.02791	D38215	Ryanodine receptor 1, skeletal muscle
11	3.27	0.00076	0.01395	X51468	Somatostatin
12	3.23	0.01612	0.04048	A1841709	Neurogranin
13	3.23	0.02339	0.04730	A1847120	UI-M-AP1-agn-g-11-0-UI.s1 Mus musculus cDNA, 3' end
14	3.20	0.00165	0.01719	AF053471	ATPase, Ca++ transporting, plasma membrane 2
15	3.14	0.00002	0.00444	V07836	Basic helix-loop-helix domain containing, class B2
16	3.03	0.01342	0.03705	U60150	Vesicle-associated membrane protein 2
17	3.01	0.00026	0.01131	AJ005983	CAMP-regulated phosphoprotein 19
18	2.99	0.00047	0.01321	AF022992	Period homolog 1 (Drosophila)
19	2.85	0.01262	0.03654	AA407367	Scn-like with four mbt domains 2

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Gene Summary: Calcium/calmodulin-dependent protein kinase II alpha
Main (login: huntingtons) > Analysis > Pairwise > Results > Gene Summary

Group	Condition	N	Mean	SEM	SEM/Mean	Quality
1	WT untreated	3	1.8658	+/- 0.2534	13.6%	1.0000
2	R6/2 untreated	4	-1.0733	+/- 0.6852	63.8%	0.0000

Intensity
3
2
1
0
-1
-2
WT untreated R6/2 untreated

R6/2 untreated down-regulated 7.67 fold compared to WT untreated

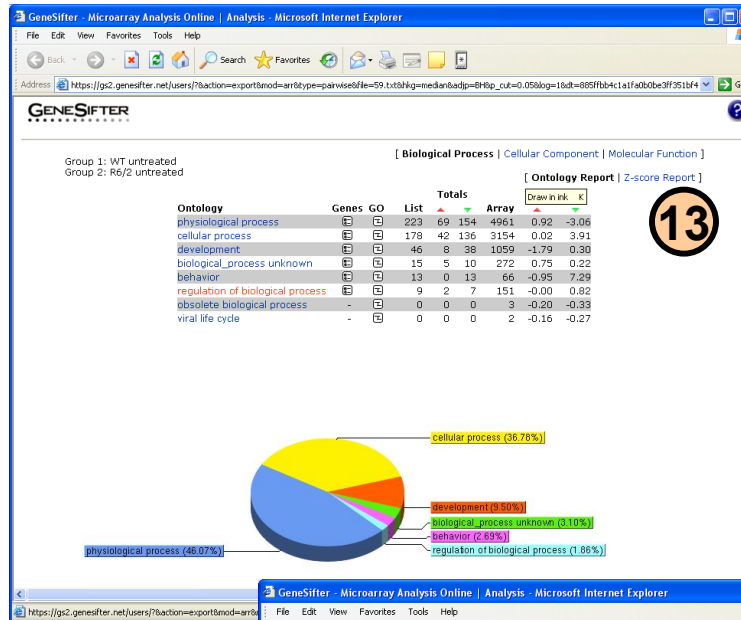
One-Click Gene Summary™
Probe Set ID: 93660_at
Accession No.: X87142
Cluster ID: Mm.131530
UG Title: Calcium/calmodulin-dependent protein kinase II alpha
Gene ID: Camk2a
Homologene: -
Chromosome: 18
Cytoband: -
Seq Count: 133

Gene Ontologies:
Biological Process
• G1/S transition of mitotic cell cycle
• regulation of neurotransmitter secretion
• autophosphorylation
• calcium ion transport
• protein amino acid phosphorylation
Molecular Function
• calcium- and calmodulin-dependent protein

HD Tutorial


(continued)

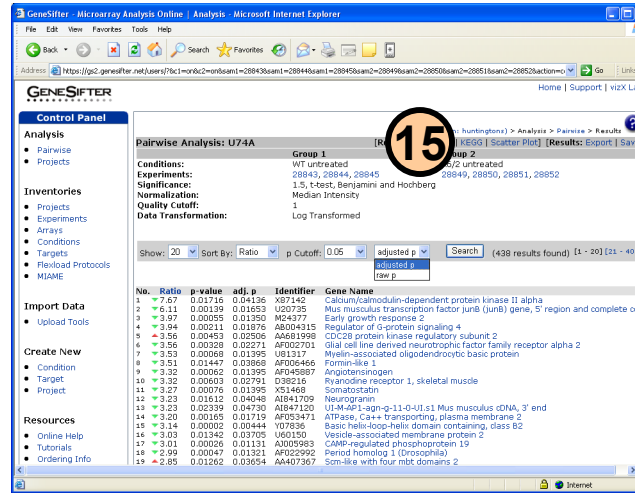
- The Ontology Report lists the GO terms of genes in the previous gene list. Currently, they are displayed hierarchically. Click on **Z-score Report** to show only the significant terms.
- This gives us the genes within Biological Process that are significantly affected. By default, the genes are ordered by number of genes affected (**List** column). Click on the green arrow to sort the downregulated genes by z-score. A positive z-score under downregulation, such as adult walking behavior, indicates that term is significantly overrepresented amongst the downregulated genes. Negative z-scores indicate that a term is showing up less often than expected by chance. For more information about the Ontology Report, please view the page-specific help in the upper right corner of the page ? .



Ontology	Genes	GO	List	Array	Totals	z-score
adult walking behavior	3	0	3	3	-0.20	9.18
calcium ion-dependent exocytosis	2	0	2	2	-0.16	7.50
locomotory behavior	7	0	7	22	-0.55	7.32
behavior	13	0	13	66	-0.95	7.29
synaptic transmission	13	0	13	80	-1.04	6.33
transmission of nerve impulse	13	0	13	82	-1.06	6.21
regulation of G-protein coupled receptor protein signaling pathway	3	0	3	7	-0.31	5.73
neurotransmitter secretion	4	0	4	12	-0.40	5.69
cell communication	88	10	78	1330	-2.08	5.51
circadian rhythm	4	0	4	13	-0.42	5.41
base conversion or substitution editing	1	0	1	1	-0.12	5.30
dopamine biosynthesis from tyrosine	1	0	1	1	-0.12	5.30
fatty acid desaturation	1	0	1	1	-0.12	5.30
long-chain fatty acid biosynthesis	1	0	1	1	-0.12	5.30
mystery cell fate differentiation (sensu Endopterygota)	1	0	1	1	-0.12	5.30
negative regulation of endocytosis	1	0	1	1	-0.12	5.30
positive regulation of small GTPase mediated signal transduction	1	0	1	1	-0.12	5.30
progesterone receptor signaling pathway	1	0	1	1	-0.12	5.30
regulation of cell size	1	0	1	1	-0.12	5.30
regulation of neurotransmitter secretion	1	0	1	1	-0.12	5.30
response to acid	1	0	1	1	-0.12	5.30
response to mechanical stimulus	1	0	1	1	-0.12	5.30
response to organic substance	1	0	1	1	-0.12	5.30
sensory transduction of mechanical stimulus	1	0	1	1	-0.12	5.30
T-helper 1 cell differentiation	1	0	1	1	-0.12	5.30
T-helper cell differentiation	1	0	1	1	-0.12	5.30
visual learning	1	0	1	1	-0.12	5.30

HD Tutorial (continued)

15. Return to the main analysis window and click **KEGG**.
16. This will bring up the list of KEGG pathways significantly affected. Click on the KEGG logo () for the **Calcium Signaling Pathway** to show the KEGG pathway diagram. Differentially regulated genes are highlighted in red.

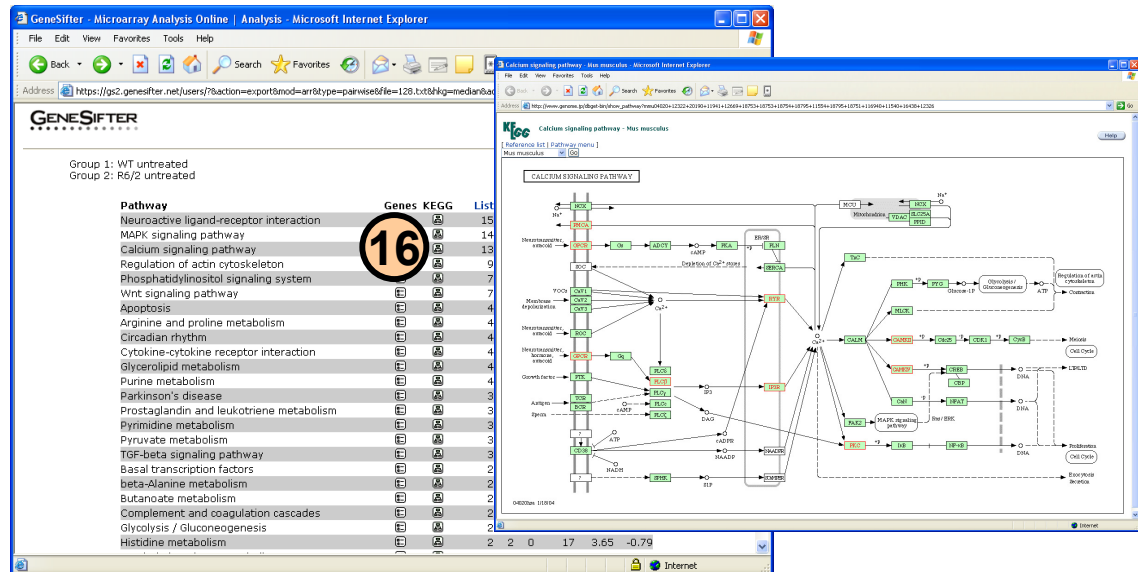


Pairwise Analysis: U74A

Conditions: WT untreated, R6/2 untreated
 Experiments: 28843, 28844, 28845
 Significance: 1.5, t-test, Benjamin and Hochberg
 Normalization: Median Intensity
 Quality Cutoff: 1
 Data Transformation: Log Transformed

Show: 20 | Sort By: Ratio | p Cutoff: 0.05 | adjusted p | Search (438 results found) 11 - 20 (11 - 40)

No.	Ratio	p-value	adj. p	Identifier	Gene Name
1	7.67	0.01745	0.04136	X87142	Calcium/calmodulin-dependent protein kinase II alpha
2	6.11	0.00139	0.01653	U20735	Mus musculus transcription factor JunB (JunB) gene, 5' region and complete co
3	5.97	0.00055	0.01250	M24371	Early growth response 2
4	5.94	0.00211	0.01076	A8084315	Regulator of G-protein signaling 4
5	3.56	0.00483	0.02556	A4481998	CD320 protein kinase regulatory subunit 2
6	3.56	0.00328	0.02271	AF002701	Gli3 cell line derived neurotrophic factor family receptor alpha 2
7	3.53	0.00048	0.01395	U91317	Myelin-associated oligodendrocytic basic protein
8	3.51	0.01447	0.03858	AF006466	Formin-like 1
9	3.32	0.00062	0.01395	AF049587	Angiogenin
10	3.32	0.00603	0.02791	D38216	Ryanodine receptor 1, skeletal muscle
11	3.27	0.00076	0.01395	X51468	Somatostatin
12	3.23	0.01312	0.04048	A1841709	Neurogranin
13	3.23	0.02339	0.04730	A1847120	U1-M-AP-1-gn-q-11-0-U1.s1 Mus musculus cDNA, 3' end
14	3.20	0.00105	0.01319	AF053479	ATPase, Ca++ transporting, plasma membrane 2
15	3.14	0.00002	0.00444	V07836	Basic helix-loop-helix domain containing, class B2
16	3.03	0.01942	0.03705	U60150	Vesicle-associated membrane protein 2
17	3.01	0.00026	0.01131	A3005983	CAMP-regulated phosphoprotein 19
18	2.99	0.00047	0.01321	AF025992	Period homolog 1 (Drosophila)
19	2.85	0.01362	0.03654	AA007367	Sch-1-like with four mitc domains 2



Group 1: WT untreated
 Group 2: R6/2 untreated

Pathway	Genes	KEGG	List
Neuroactive ligand-receptor interaction	15		15
MAPK signaling pathway	14		14
Calcium signaling pathway	13		13
Regulation of actin cytoskeleton	9		9
Phosphatidylinositol signaling system	7		7
Wnt signaling pathway	7		7
Apoptosis	4		4
Arginine and proline metabolism	4		4
Circadian rhythm	4		4
Cytokine-cytokine receptor interaction	4		4
Glycerolipid metabolism	4		4
Purine metabolism	4		4
Parkinson's disease	3		3
Prostaglandin and leukotriene metabolism	3		3
Pyrimidine metabolism	3		3
Pyruvate metabolism	3		3
TGF-beta signaling pathway	3		3
Basal transcription factors	2		2
beta-Alanine metabolism	2		2
Butanoate metabolism	2		2
Complement and coagulation cascades	2		2
Glycolysis / Gluconeogenesis	2		2
Histidine metabolism	2		2

Calcium signaling pathway - Mus musculus

Reference list: Pathway map 1
 Mus musculus

CALCIUM SIGNALING PATHWAY

The diagram illustrates the Calcium Signaling Pathway in Mus musculus. It shows the flow of information from extracellular signals (like Ca²⁺ and neurotransmitters) through various receptors (GPCRs, RTKs, etc.) to intracellular signaling molecules (G-proteins, PLC, PKC, etc.), ultimately leading to cellular responses such as gene expression, cell cycle regulation, and apoptosis. Key components include Ca²⁺, GPCR, PLC, PKC, and various transcription factors.

HD Tutorial

(continued)

- Return to the main analysis window and click **Scatter Plot**.
- This will bring up a scatter plot of the results. Upregulated genes are shown in red, and downregulated genes are green. The gray spots are those that did not pass our analysis parameters. Move the blue box around and click **Zoom** to see more detail of the scatter plot.
- Click on data points in the detail to bring up the gene summary for a specific gene.

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

