


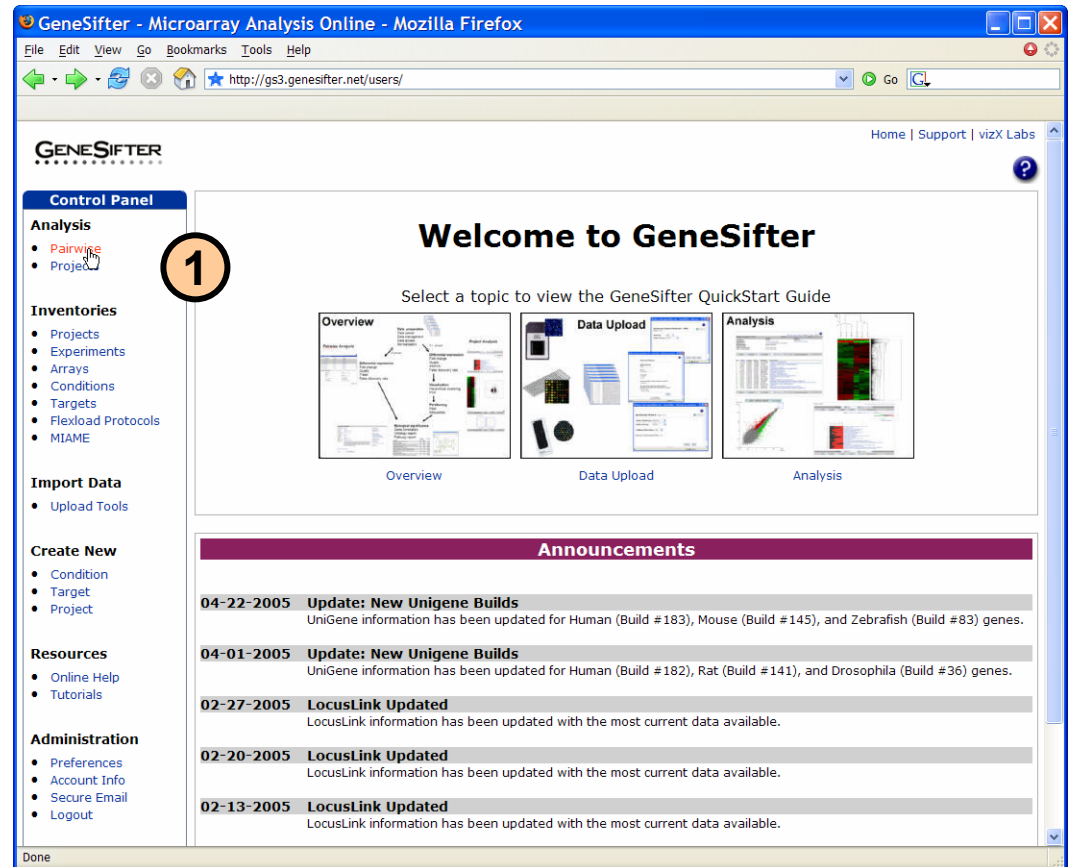
Tutorial

TNF-mediated gene expression in HUVEC

The following tutorial walks through the identification of genes regulated by TNF in HUVEC (Human Umbilical Vein Endothelial Cells). This analysis showed that genes involved in distinct biological processes, including immune response, cell cycle, apoptosis and cell adhesion, are regulated by TNF in HUVEC.

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

1. Select **Pairwise** from the **Analysis** menu.
2. Select the magnifying glass icon () next to "U133A Human Genome" in the list. The data presented here was generated using the Affymetrix® GeneChip® Human Genome U133A array. There are approximately 20,000 transcripts represented on this array. For this analysis, GC-RMA was used to derive expression measurements from the probe-level (CEL file) data.



GeneSifter - Microarray Analysis Online - Mozilla Firefox

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http://gs3.genesifter.net/users/

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GENESIFTER

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Welcome to GeneSifter

Select a topic to view the GeneSifter QuickStart Guide

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Announcements

04-22-2005 Update: New Unigene Builds
UniGene information has been updated for Human (Build #183), Mouse (Build #145), and Zebrafish (Build #83) genes.

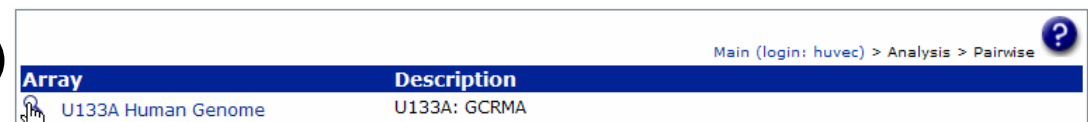
04-01-2005 Update: New Unigene Builds
UniGene information has been updated for Human (Build #182), Rat (Build #141), and Drosophila (Build #36) genes.

02-27-2005 LocusLink Updated
LocusLink information has been updated with the most current data available.

02-20-2005 LocusLink Updated
LocusLink information has been updated with the most current data available.

02-13-2005 LocusLink Updated
LocusLink information has been updated with the most current data available.

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Main (login: huvec) > Analysis > Pairwise

Array	Description
U133A Human Genome	U133A: GCRMA

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- Pairwise analysis is used to identify differentially expressed genes in two groups. There are four replicates for each of the two groups in this study. Select the four replicates for the untreated HUVEC samples (HUVEC) for group 1. Select the four replicates for the TNF treated HUVEC samples (HUVEC + TNF) for group 2.
- Pairwise analysis combines a fold-change cutoff and comparison statistics to generate a list of differentially expressed genes. Select the following settings:

Normalization: None

Data was already normalized during GC-RMA transformation.

Statistics: t-test

Performs a two-sample, unpaired t-test for each gene that passes the fold-change cutoff.

Quality: N/A

GC-RMA does not generate detection calls.

Threshold: 1.5

Filters out genes with less than a 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: Data Already Log Transformed

Data was log base2 transformed during GC-RMA transformation.

- Select the **Analyze** button.

Main (login: huvec) > Analysis > Arrays > Pairwise ?

Pairwise Analysis: U133A Human Genome

Group		Experiment	Target	Condition
1	2			
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	GSM50777	HUVEC con 1	HUVEC
<input checked="" type="checkbox"/>	<input type="checkbox"/>	GSM50778	HUVEC con 2	HUVEC
<input checked="" type="checkbox"/>	<input type="checkbox"/>	GSM50779	HUVEC con 3	HUVEC
<input checked="" type="checkbox"/>	<input type="checkbox"/>	GSM50780	HUVEC con 4	HUVEC
<input type="checkbox"/>	<input checked="" type="checkbox"/>	GSM50781	HUVEC TNF 1	HUVEC + TNF
<input type="checkbox"/>	<input checked="" type="checkbox"/>	GSM50783	HUVEC TNF 3	HUVEC + TNF
<input type="checkbox"/>	<input checked="" type="checkbox"/>	GSM50784	HUVEC TNF 4	HUVEC + TNF
<input type="checkbox"/>	<input checked="" type="checkbox"/>	GSM5082	HUVEC TNF 2	HUVEC + TNF

Advanced Analysis Settings (RMA)

Normalization: None

Statistics: t-test

Quality: N/A

Threshold: Lower: 1.5 Upper: None

Correction: Benjamini and Hochberg

Show genes that are:

- Up-regulated
- Down-regulated

Data Transformation:

- No Transformation
- Log Transform Data
- Data Already Log Transformed

Analyze Reset

Tutorial

TNF-mediated gene expression in HUVEC

6. 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 pairwise analysis settings selected. 764 genes passed the filtering criteria – a 1.5 fold or greater change in expression and a raw p-value of at least 0.05 from the t-test. The genes are sorted by fold change and the first 20 genes in the list are displayed.

7. To filter the list using the adjusted p-value (false discovery rate), select “adjusted p” from the pull-down menu and then select the **Search** button.

8. The list filtered on the adjusted p-value contains 624 genes with a false discovery rate less than 5%.

9. To view data and a gene summary for any gene in the list, select the **Gene Name**.

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Main (login: huvec) > Analysis > Pairwise > Results

Pairwise Analysis: U133A Human Genome [Reports: [Ontology](#) | [KEGG](#) | [Scatter Plot](#)] [Results: [Export](#) | [Save](#)]

Group 1		Group 2	
Conditions:	HUVEC	Conditions:	HUVEC + TNF
Experiments:	58528, 58529, 58530, 58531	Experiments:	58532, 58533, 58534, 58535
Significance:	1.5, t-test, Benjamini and Hochberg		
Normalization:	None		
Quality Cutoff:	None		
Data Transformation:	Log Transformed		

Show: 20 Sort By: Ratio p Cutoff: 0.05 raw p Search (764 results found) [1 - 20] [21 - 40]

No.	Ratio	p-value	adj. p	Identifier	Gene Name
1	▲ 125.37	0.00000	0.00006	L07555	CD69 antigen (p60, early T-cell activation antigen)
2	▲ 125.37	0.00000	0.00005	U84487	Chemokine (C-X3-C motif) ligand 1
3	▲ 124.50	0.00000	0.00005	NM_005658	TNF receptor-associated factor 1
4	▲ 102.54	0.00000	0.00008	NM_001078	Vascular cell adhesion molecule 1
5	▲ 94.35	0.00000	0.00017	NM_004591	Chemokine (C-C motif) ligand 20
6	▲ 74.54	0.00010	0.00173	NM_000201	Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor
7	▲ 72.00	0.00000	0.00006	NM_000450	Selectin E (endothelial adhesion molecule 1)
8	▲ 66.72	0.00000	0.00005	A1738896	Tumor necrosis factor, alpha-induced protein 3
9	▲ 64.89	0.00002	0.00060	NM_006290	Tumor necrosis factor, alpha-induced protein 3
10	▲ 64.45	0.00000	0.00017	NM_002341	Lymphotoxin beta (TNF superfamily, member 3)
11	▲ 61.39	0.00000	0.00008	NM_006398	Ubiquitin D
12	▲ 54.19	0.00004	0.00109	NM_002996	Chemokine (C-X3-C motif) ligand 1
13	▲ 42.52	0.00003	0.00087	AK026546	Chemokine (C-X-C motif) ligand 5
14	▲ 36.50	0.00007	0.00138	NM_002090	Chemokine (C-X-C motif) ligand 3
15	▲ 29.86	0.00000	0.00024	AF043337	Interleukin 8
16	▲ 27.28	0.00000	0.00007	NM_003855	Interleukin 18 receptor 1
17	▲ 26.72	0.00002	0.00060	U37546	Baculoviral IAP repeat-containing 3
18	▲ 23.92	0.00000	0.00008	U69563	Rho family GTPase 1
19	▲ 22.94	0.00000	0.00009	A1608725	Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor
20	▲ 22.01	0.01736	0.03863	NM_003046	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 2

Show: 20 Sort By: Ratio p Cutoff: 0.05 raw p Search (764 results found) [1 - 20] [21 - 40]

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Main (login: huvec) > Analysis > Pairwise > Results

Pairwise Analysis: U133A Human Genome [Reports: [Ontology](#) | [KEGG](#) | [Scatter Plot](#)] [Results: [Export](#) | [Save](#)]

Group 1		Group 2	
Conditions:	HUVEC	Conditions:	HUVEC + TNF
Experiments:	58528, 58529, 58530, 58531	Experiments:	58532, 58533, 58534, 58535
Significance:	1.5, t-test, Benjamini and Hochberg		
Normalization:	None		
Quality Cutoff:	None		
Data Transformation:	Log Transformed		

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Show: 20 Sort By: Ratio p Cutoff: 0.05 adjusted p Search (624 results found) [1 - 20] [21 - 40]

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TNF-mediated gene expression in HUVEC

10. Selecting a gene from the list will bring up a data summary and a One-Click Gene Summary™ for the gene. The One-Click Gene Summary provides a synopsis of current UniGene and LocusLink information for the gene.
11. Go back to the gene list by clicking the “Back” button in your browser.
12. 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 information about the other reports.

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

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» One-Click Gene Summary™

Probe Set ID: 202643_s_at
Accession No.: AI738896
Cluster ID: Hs.211600
UG Title: Tumor necrosis factor, alpha-induced protein 3
Gene ID: TNFAIP3
Homologene: Mm.56971
Chromosome: 6
Cytoband: 6q23
Seq Count: 250
Entrez Gene: 7128
Gene Name: tumor necrosis factor, alpha-induced protein 3
OMIM: 191163
RefSeq mRNA: NM_006290 (FASTA)
RefSeq Prot: NP_006281 (FASTA)
Summary: This gene was identified as a gene whose expression is rapidly induced by the tumor necrosis factor (TNF). The protein encoded by this gene is a zinc finger protein, and has been shown to inhibit NF-kappa B activation as well as TNF-mediated apoptosis. Knockout studies of a similar gene in mice suggested that this gene is critical for limiting inflammation by terminating TNF-induced NF-kappa B responses.

Gene Ontologies:

Biological Process

- ◆ negative regulation of transcription
- ◆ anti-apoptosis
- ◆ ubiquitin cycle
- ◆ apoptosis

Molecular Function

- ◆ DNA binding
- ◆ cysteine-type peptidase activity
- ◆ hydrolase activity
- ◆ protein binding
- ◆ zinc ion binding

Cellular Component

- ◆ nucleus

PubGene: TNFAIP3

[Perform Sequence Analysis]

TNFAIP3 Search PubMed

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Pairwise Analysis: U133A Human Genome [Reports: Ontology | KEGG | Scatter Plot] [Results: Export | Save]

Conditions: HUVEC HUVEC + TNF

Experiments: 58528, 58529, 58530, 58531 58532, 58533, 58534, 58535

Significance: 1,5, t-test, Benjamini and Hochberg

Normalization: None

Quality Cutoff: None

Data Transformation: Log Transformed

Show: 20 Sort By: Ratio p Cutoff: 0.05 adjusted p Search (624 results found) [1 - 20] [21 - 40]

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Show: 20 Sort By: Ratio p Cutoff: 0.05 adjusted p Search (624 results found) [1 - 20] [21 - 40]

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TNF-mediated gene expression in HUVEC

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13. The Ontology Report lists the Gene Ontology terms associated with the 624 genes in the pairwise results gene list. See the help documents for this page for more information about the Ontology Report.

14. Click on **Z-score report**.

15. The z-score report lists the biological process ontologies 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 by z-score for the up-regulated genes.

Z-score reports can be generated for the Molecular Function and Cellular Component ontologies as well.

GeneSifter - Microarray Analysis Online | Analysis - Mozilla Firefox

Group 1: HUVEC
Group 2: HUVEC + TNF

[Biological Process | Cellular Component | Molecular Function]

[Ontology Report | Z-score Report]

Ontology	Genes	GO	Totals			z-score	
			List	Array	z-score	z-score	
physiological process	328	180	148	7711	1.45	0.47	
cellular process	234	137	97	4903	3.86	0.67	
development	68	34	34	1398	0.54	1.62	
regulation of biological process	28	20	8	395	3.89	0.20	
biological_process unknown	11	5	6	279	-0.51	0.33	
viral life cycle	4	4	0	31	4.03	-0.77	
behavior	1	0	1	79	-1.35	-0.41	
obsolete biological process	-	0	0	7	-0.40	-0.37	

pie chart labels: physiological process (48.86%), cellular process (34.72%), development (10.05%), regulation of biological process (4.15%), biological_process unknown (1.63%), viral life cycle (0.53%), behavior (0.15%)

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[Biological Process | Cellular Component | Molecular Function]

Group 1: HUVEC
Group 2: HUVEC + TNF

[Ontology Report | Z-score Report]
Export Report

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Ontology	Genes	GO	Totals			z-score	
			List	Array	z-score	z-score	
cellular process	234	137	97	4903	3.86	0.67	
metabolism	210	102	108	5387	-2.62	0.96	
cell communication	133	92	41	2519	5.64	-1.13	
signal transduction	108	75	33	1990	5.23	-0.85	
nucleobase, nucleoside, nucleotide and nucleic acid metabolism	93	39	54	2258	-1.88	2.02	
response to external stimulus	84	70	14	1055	10.27	-1.42	
response to biotic stimulus	69	61	8	815	10.62	-1.99	
regulation of transcription, DNA-dependent	66	31	35	1338	0.22	2.11	
cell proliferation	65	30	35	924	2.19	4.47	
defense response	62	57	5	712	10.84	-2.42	
response to stress	61	44	17	755	6.97	0.76	
immune response	60	56	4	644	11.50	-2.45	
transport	42	25	17	1516	-1.69	-2.40	
cell cycle	41	13	28	609	-0.17	5.08	
organogenesis	41	26	15	762	2.30	0.17	
response to pest/pathogen/parasite	39	35	4	398	9.05	-1.32	
cell death	35	25	10	422	5.25	0.74	
death	35	25	10	426	5.19	0.71	
apoptosis	32	23	9	396	4.92	0.57	
programmed cell death	32	23	9	398	4.89	0.56	
response to wounding	29	27	2	253	9.21	-1.30	
regulation of biological process	28	20	8	395	3.89	0.20	
response to abiotic stimulus	27	20	7	311	5.09	0.48	
transcription from Pol II promoter	26	15	11	408	2.02	1.22	
cell adhesion	25	19	6	466	2.76	-0.98	
regulation of cell cycle	25	10	15	337	0.93	3.52	
DNA metabolism	23	3	20	432	-2.22	4.28	

Tutorial

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Group 1: HUVEC
Group 2: HUVEC + TNF

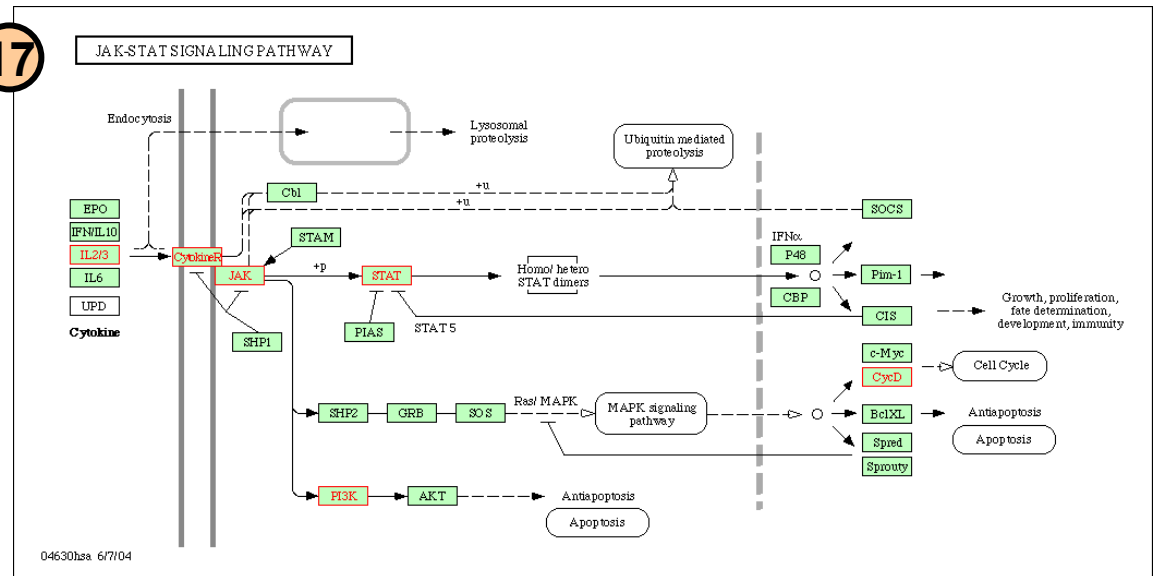
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Pathway	Genes	KEGG	Totals		Z-score	
			List	Array	▲	▼
Cytokine-cytokine receptor interaction	33	32	1	233	10.11	-1.68
MAPK signaling pathway	17	11	6	226	1.75	0.94
Apoptosis	16	13	3	91	6.46	1.04
Jak-STAT signaling pathway	15	13	2	142	4.45	-0.40
Cell cycle	12	1	11	93	-1.10	7.27
Toll-like receptor signaling pathway	11	11	0	94	5.07	-1.35
Calcium signaling pathway	9	7	2	177	0.80	-0.73
Wnt signaling pathway	8	2	6	126	-0.94	2.48
Regulation of actin cytoskeleton	7	7	0	185	0.67	-1.93
TGF-beta signaling pathway	7	5	2	76	1.88	0.51
Purine metabolism	6	1	5	133	-1.55	1.68
Neuroactive ligand-receptor interaction	5	2	3	263	-2.22	-0.90
Complement and coagulation cascades	4	3	1	67	0.73	-0.22
Focal adhesion	4	3	1	75	0.53	-0.34
Notch signaling pathway	4	3	1	34	2.02	0.48
Phosphatidylinositol signaling system	4	3	1	64	0.82	-0.17
Pyrimidine metabolism	4	0	4	74	-1.53	2.30
Glycerolipid metabolism	3	1	2	104	-1.23	0.06
Glycosphingolipid metabolism	3	3	0	39	1.75	-0.86
Inositol phosphate metabolism	3	2	1	70	-0.06	-0.26
Prostaglandin and leukotriene metabolism	3	2	1	37	0.88	0.39
Adherens junction	2	2	0	60	0.17	-1.07

16. Return to the **Pairwise Analysis Results Page** window and click on the **KEGG** link. This will bring up a z-score report for the KEGG pathway terms associated with the differentially expressed genes.

17. Click on the KEGG logo (📄) for the **Jak-Stat signaling pathway** to show the KEGG pathway diagram. Differentially regulated genes are highlighted in red.

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18. Return to the main analysis window and click **Scatter Plot**.

19. This will bring up a scatter plot of the results. Up-regulated genes are shown in red, and down-regulated genes are green. The gray spots are those that did not pass the analysis parameters. Move the blue box around and click **Zoom** to see more detail of the scatter plot.

20. 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.

Main (login: huvec) > Analysis > Pairwise > Results

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

Pairwise Analysis: U133A Human Genome	
Group 1	Group 2
Conditions:	HUVEC HUVEC + TNF
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