


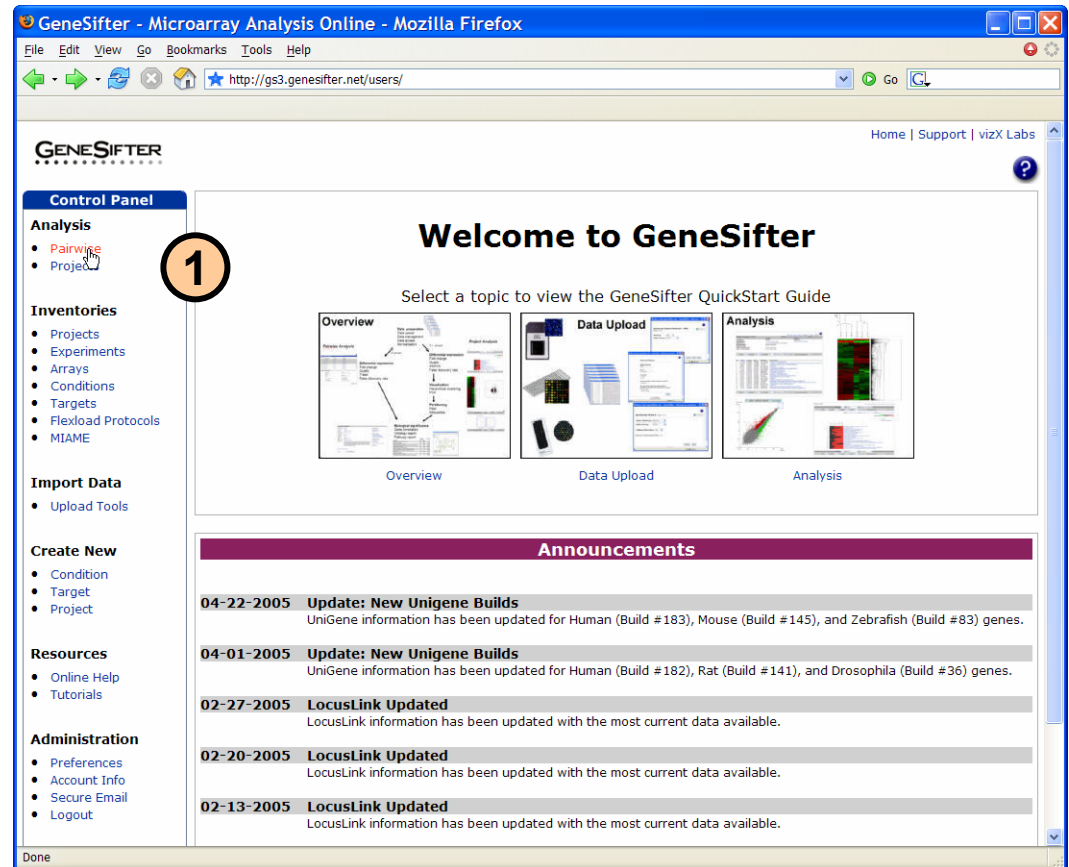
Tutorial

Microarray analysis of CD40-mediated gene expression in Ramos cells

The following tutorial walks through the identification of genes regulated by CD40 stimulation in the Ramos Burkitt lymphoma cell line. This analysis showed that genes involved in distinct biological processes, including immune response, cell cycle, apoptosis and cell-matrix adhesion, are regulated by CD40 in Ramos cells.

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 "U95A" in the list. The data presented here was generated using the Affymetrix® GeneChip® Human Genome U95A array. There are approximately 12,000 transcripts represented on this array.



GeneSifter - Microarray Analysis Online - Mozilla Firefox

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

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GENESIFTER

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Select a topic to view the GeneSifter QuickStart Guide

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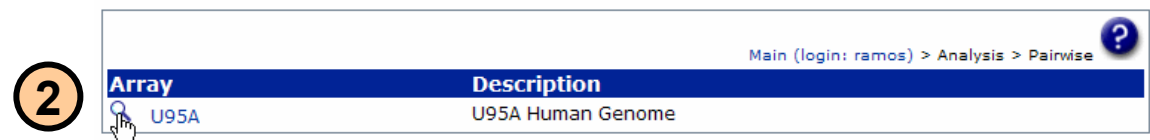
04-22-2005 Update: New Unigene Builds
UniGene information has been updated for Human (Build #183), Mouse (Build #145), and Zebrafish (Build #83) genes.

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.



Main (login: ramos) > Analysis > Pairwise

Array	Description
 U95A	U95A Human Genome

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Microarray analysis of CD40-mediated gene expression in Ramos cells

- Pairwise analysis is used to identify differentially expressed genes in two groups. There are six replicates for each of the two groups in this study. Select the six replicates for the untreated Ramos samples (Ramos) for group 1. Select the six replicates for the anti-CD40 treated Ramos samples (Ramos + anti-CD40) 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: All median

Normalizes each array to its median intensity.

Statistics: t-test

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

Quality: 1

Filters out genes that received absent or marginal calls in both groups.

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 (FDR) from the raw p-values using the method of Benjamini and Hochberg.

Data transformation: Log Transform Data

Log base2 transforms the signal values.

- Select the **Analyze** button.

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

Pairwise Analysis: U95A

Group		Experiment	Target	Condition	
1	2				
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	GSM43967	1UT24h	Ramos	
<input checked="" type="checkbox"/>	<input type="checkbox"/>	GSM43968	2UT24h	Ramos	
<input checked="" type="checkbox"/>	<input type="checkbox"/>	GSM43969	3UT24h	Ramos	
<input checked="" type="checkbox"/>	<input type="checkbox"/>	GSM43970	4UT24h	Ramos	
<input checked="" type="checkbox"/>	<input type="checkbox"/>	GSM43971	5UT24h	Ramos	
<input checked="" type="checkbox"/>	<input type="checkbox"/>	GSM43972	6UT24h	Ramos	
<input type="checkbox"/>	<input checked="" type="checkbox"/>	GSM43973	7aCD4O24h	Ramos + anti-CD40	
<input type="checkbox"/>	<input checked="" type="checkbox"/>	GSM43974	8aCD4O24h	Ramos + anti-CD40	
<input type="checkbox"/>	<input checked="" type="checkbox"/>	GSM43975	9aCD4O24h	Ramos + anti-CD40	
<input type="checkbox"/>	<input checked="" type="checkbox"/>	GSM43976	10aCD4O24h	Ramos + anti-CD40	
<input type="checkbox"/>	<input checked="" type="checkbox"/>	GSM43977	11aCD4O24h	Ramos + anti-CD40	
<input type="checkbox"/>	<input checked="" type="checkbox"/>	GSM43978	12aCD4O24h	Ramos + anti-CD40	

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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Microarray analysis of CD40-mediated gene expression in Ramos cells

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. 598 genes passed the filtering criteria – a 1.5 fold or greater change in expression, present calls in at least one of the groups and a raw p-value of less than 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 598 genes with a false discovery rate less than 5% (all of the genes pass the more stringent cutoff of a false discovery rate less than 0.05).

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

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

Pairwise Analysis: U95A [Reports: Ontology | KEGG | Scatter Plot] [Results: Export | Save]

Group 1		Group 2	
Conditions:	Ramos	Conditions:	Ramos + anti-CD40
Experiments:	58458, 58459, 58460, 58461, 58462, 58463	Experiments:	58464, 58465, 58466, 58467, 58468, 58469
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: 0.05 raw p Search (598 results found) [1 - 20] [21 - 40]

No.	Ratio	p-value	adj. p	Identifier	Gene Name
1	▲ 8.63	0.00010	0.00030	U20816	HSU20816 Human nuclear factor kappa-B2 (NF-KB2) gene, partial cds
2	▲ 8.22	0.00000	0.00000	X83490	Fas (TNF receptor superfamily, member 6)
3	▲ 7.11	0.00076	0.00132	X83492	Fas (TNF receptor superfamily, member 6)
4	▲ 6.23	0.00000	0.00002	U27467	BCL2-related protein A1
5	▼ 5.86	0.00034	0.00071	M34276	Human plasminogen gene
6	▲ 5.62	0.00000	0.00001	Z70519	Fas (TNF receptor superfamily, member 6)
7	▲ 5.46	0.00000	0.00001	M16441	HUMTNFAB Human tumor necrosis factor and lymphotoxin genes, complete cds
8	▲ 4.96	0.00000	0.00001	X63717	Fas (TNF receptor superfamily, member 6)
9	▲ 4.23	0.00000	0.00000	D13891	Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
10	▲ 3.97	0.00000	0.00002	D12614	Lymphotoxin alpha (TNF superfamily, member 1)
11	▲ 3.97	0.00000	0.00000	M69043	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, al
12	▲ 3.63	0.00000	0.00000	U19261	TNF receptor-associated factor 1
13	▲ 3.58	0.00000	0.00000	AA868382	ak41e04.s1 Homo sapiens cDNA, 3 end
14	▲ 3.46	0.00008	0.00026	AL050141	AG1 protein
15	▲ 3.27	0.00000	0.00000	M24283	Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor
16	▼ 3.12	0.00001	0.00006	AF035296	DC12 protein
17	▲ 3.03	0.00000	0.00002	S76638	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)
18	▲ 3.03	0.00043	0.00086	X80200	TNF receptor-associated factor 4
19	▲ 3.01	0.00000	0.00001	S76638	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)
20	▲ 2.99	0.00000	0.00000	Y14768	Homo sapiens DNA, cosmid clones TN62 and TN82

Show: 20 Sort By: Ratio p Cutoff: 0.05 raw p Search (598 results found) [1 - 20] [21 - 40]

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

Pairwise Analysis: U95A [Reports: Ontology | KEGG | Scatter Plot] [Results: Export | Save]

Group 1		Group 2	
Conditions:	Ramos	Conditions:	Ramos + anti-CD40
Experiments:	58458, 58459, 58460, 58461, 58462, 58463	Experiments:	58464, 58465, 58466, 58467, 58468, 58469
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: 0.05 adjusted p Search (598 results found) [1 - 20] [21 - 40]

No.	Ratio	p-value	adj. p	Identifier	Gene Name
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Show: 20 Sort By: Ratio p Cutoff: 0.05 adjusted p Search (598 results found) [1 - 20] [21 - 40]

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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.

» One-Click Gene Summary™

Probe Set ID: 1440_s_at
Accession No.: X83490
Cluster ID: Hs_244139
UG Title: Fas (TNF receptor superfamily, member 6)
Gene ID: FAS
Homologene: -
Chromosome: 10
Cytoband: 10q24.1
Seq Count: 156
Entrez Gene: 355
Gene Name: Fas (TNF receptor superfamily, member 6)
OMIM: 134637
RefSeq mRNA: NM_152877 (FASTA)
RefSeq Prot: NP_690616 (FASTA)
Summary: The protein encoded by this gene is a member of the TNF-receptor superfamily. This receptor contains a death domain. It has been shown to play a central role in the physiological regulation of programmed cell death, and has been implicated in the pathogenesis of various malignancies and diseases of the immune system. The interaction of this receptor with its ligand allows the formation of a death-inducing signaling complex that includes Fas-associated death domain protein (FADD), caspase 8, and caspase 10. The autoproteolytic processing of the caspases in the complex triggers a downstream caspase cascade, and leads to apoptosis. This receptor has been also shown to activate NF-kappaB, MAPK3/ERK1, and MAPK8/JNK, and is found to be involved in transducing the proliferating signals in normal diploid fibroblast and T cells. At least eight alternatively spliced transcript variants encoding seven distinct isoforms have been described. The isoforms lacking the transmembrane domain may negatively regulate the apoptosis mediated by the full length isoform.

Gene Ontologies:

Biological Process

- signal transduction
- anti-apoptosis
- immune response
- induction of apoptosis
- protein complex assembly
- regulation of apoptosis
- apoptosis

Molecular Function

- protein binding
- transmembrane receptor activity

Cellular Component

- membrane
- cytosol
- integral to membrane
- soluble fraction

KEGG Pathways:

- MAPK signaling pathway
- Cytokine-cytokine receptor interaction
- Apoptosis

[Perform Sequence Analysis]

FAS

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Pairwise Analysis: U95A [Reports: Ontology | KEGG | Scatter Plot] [Results: Export | Save]

Conditions: Ramos Group 1 Ramos + anti-CD40 Group 2

Experiments: 58458, 58459, 58460, 58461, 58462, 58463, 58464, 58465, 58466, 58467, 58468, 58469

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: 0.05 adjusted p Search (598 results found) [1 - 20] [21 - 40]

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Show: 20 Sort By: Ratio p Cutoff: 0.05 adjusted p Search (598 results found) [1 - 20] [21 - 40]

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Microarray analysis of CD40-mediated gene expression in Ramos cells

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13. The Ontology Report lists the Gene Ontology terms associated with the 598 genes in the pairwise results gene list. See the help documents for this page for more information about the Ontology Report.

14

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

15

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.

[Biological Process | Cellular Component | Molecular Function]

Group 1: Ramos
Group 2: Ramos + anti-CD40

[Ontology Report | **Z-score Report**]
Export Report

Ontology	Genes	GO	Totals		Z-score
			List	Array	
metabolism	256	42	214	4031	0.71 2.77
cellular process	203	44	159	3826	1.69 -2.43
nucleobase, nucleoside, nucleotide and nucleic acid metabolism	140	18	122	1686	0.46 5.63
cell communication	101	25	76	2017	1.46 -2.38
transcription	94	15	79	1096	1.46 4.25
regulation of transcription	93	15	78	1042	1.67 4.59
transcription, DNA-dependent	89	14	75	1041	1.33 4.12
regulation of transcription, DNA-dependent	88	14	74	1004	1.48 4.30
cell proliferation	55	6	49	797	-0.67 2.03
response to external stimulus	48	18	30	861	3.58 -1.82
development	45	9	36	1099	-0.56 -2.45
transport	45	8	37	1122	-0.96 -2.44
cell cycle	42	2	40	522	-1.43 3.31
response to biotic stimulus	40	17	23	672	4.33 -1.66
defense response	33	15	18	591	4.06 -2.00
immune response	33	15	18	536	4.49 -1.54
DNA metabolism	32	3	29	327	-0.10 3.63
apoptosis	27	15	12	314	7.04 -0.76
cell death	27	15	12	336	6.69 -1.01
death	27	15	12	338	6.66 -1.03
programmed cell death	27	15	12	314	7.04 -0.76
mitotic cell cycle	24	0	24	221	-1.50 4.38
regulation of cell cycle	24	1	23	298	-1.14 2.50
ubiquitin cycle	23	4	19	236	1.15 2.46
morphogenesis	22	4	18	716	-1.19 -2.93
response to pest/pathogen/parasite	22	7	15	328	2.20 -0.12
organogenesis	19	4	15	635	-0.92 -2.93

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Microarray analysis of CD40-mediated gene expression in Ramos cells

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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 **Cell cycle** to show the KEGG pathway diagram. Differentially regulated genes are highlighted in red.

GeneSifter - Microarray Analysis Online | Analysis - Mozilla Firefox

File Edit View Go Bookmarks Tools Help

http://gs6.genesifter.net/users/?&action=export&mo

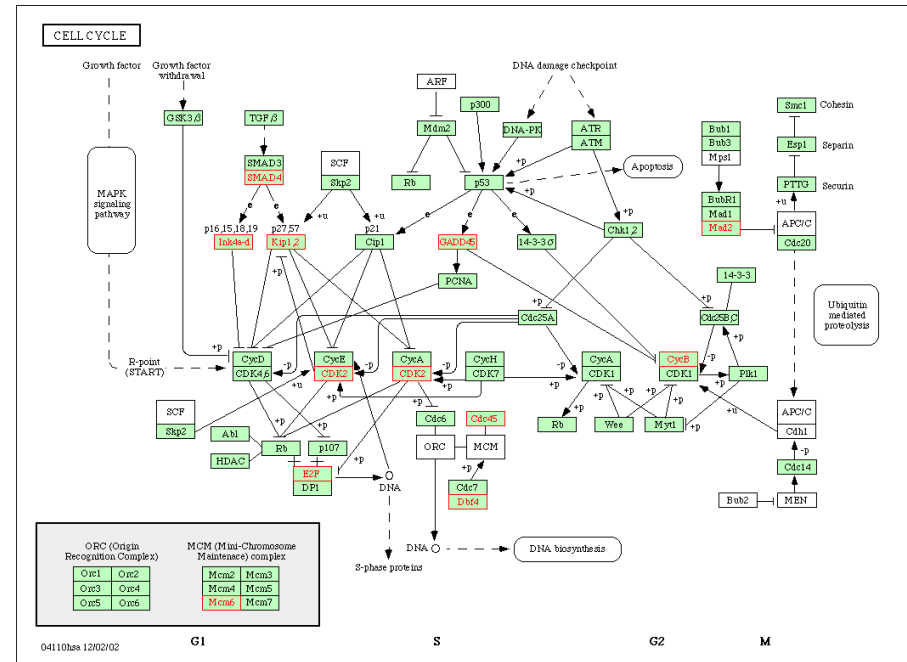
GENESIFTER

Group 1: Ramos
Group 2: Ramos + anti-CD40

Pathway	Genes	KEGG	Totals		z-score	
			List	Array	▲	▼
MAPK signaling pathway	18	8	10	197	3.46	0.63
Regulation of actin cytoskeleton	14	4	10	161	1.30	1.31
Cytokine-cytokine receptor interaction	13	7	6	199	2.78	-0.88
Cell cycle	12	0	12	90	-1.13	4.39
Apoptosis	10	7	3	83	5.71	-0.28
Calcium signaling pathway	10	3	7	159	0.61	0.12
Jak-STAT signaling pathway	9	6	3	127	3.40	-1.07
Purine metabolism	9	1	8	115	-0.45	1.50
Pyrimidine metabolism	8	1	7	62	0.18	2.81
Wnt signaling pathway	8	0	8	100	-1.19	1.93
TGF-beta signaling pathway	7	2	5	67	1.18	1.34
Toll-like receptor signaling pathway	7	5	2	85	3.70	-0.87
Glycolysis / Gluconeogenesis	5	0	5	52	-0.85	1.96
Tight junction	5	1	4	85	-0.14	0.23
Arginine and proline metabolism	4	1	3	46	0.49	0.79
Basal transcription factors	4	0	4	24	-0.58	3.05
Focal adhesion	4	0	4	72	-1.01	0.58

Done

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Microarray analysis of CD40-mediated gene expression in Ramos cells

- Return to the main analysis window and click **Scatter Plot**.
- 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.
- 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: ramos) > Analysis > Pairwise > Results

Pairwise Analysis: U95A

[Reports: Ontology] [KEGG] [Scatter Plot] [Results: Export] [Save]

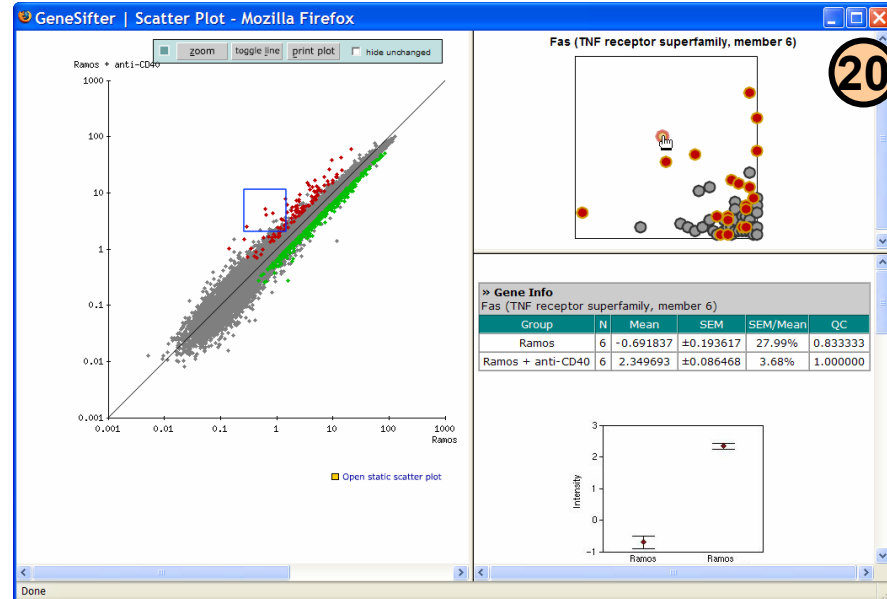
	Group 1	Group 2
Conditions:	Ramos	Ramos + anti-CD40
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Significance:	1.5, t-test, Benjamini and Hochberg	
Normalization:	Median Intensity	
Quality Cutoff:	1	
Data Transformation:	Log Transformed	

Show: 20 Sort By: Ratio p Cutoff: 0.05 adjusted p Search (598 results found) [1 - 20] [21 - 40]

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