


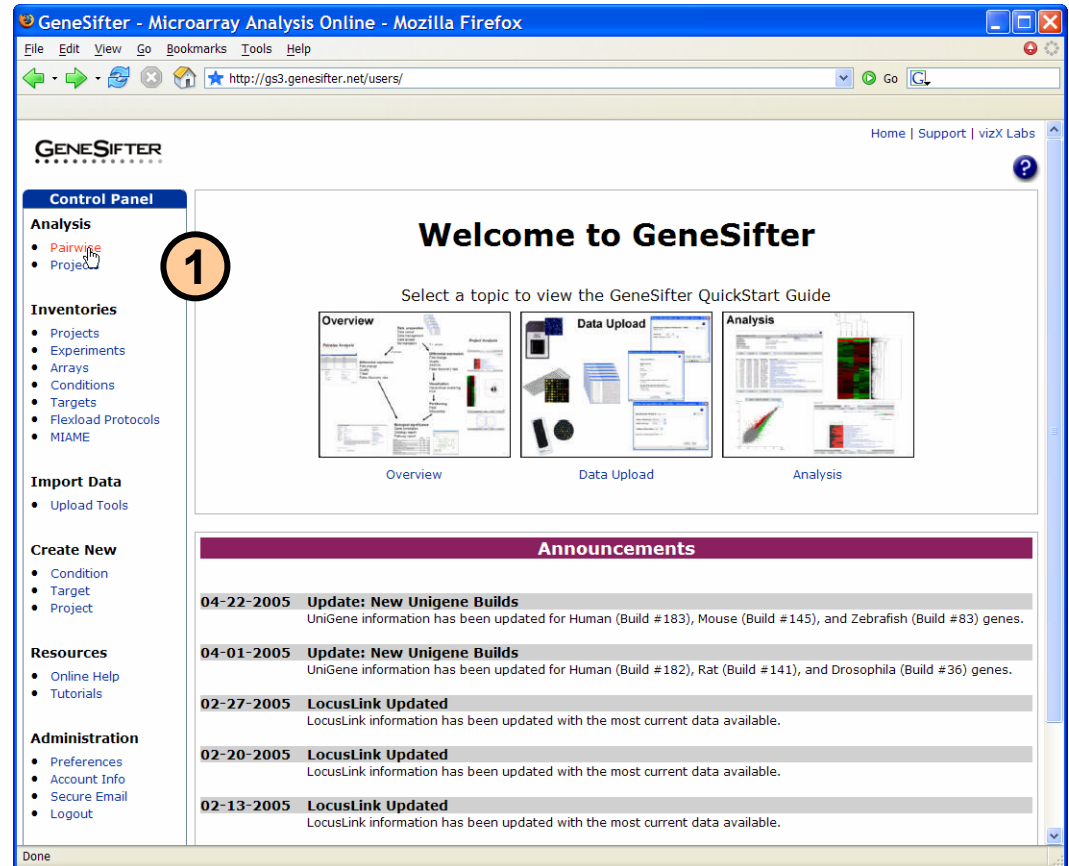
# Tutorial

## Transcriptome analysis of apoE $-/-$ mouse aortas

The following tutorial walks through a comparison of gene expression in aortas from wild type and apoE  $-/-$  mice. Genes involved in distinct biological processes, including immune response and cell adhesion, are differentially regulated in apoE  $-/-$  mice.

Visit the GeneSifter Data Center ([www.genesifter.net/web/dataCenter.html](http://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 "430 2.0 Mouse" in the list. The data presented here was generated using the Affymetrix® GeneChip® Mouse Genome 430 2.0 array. There are approximately 39,000 transcripts represented on this array.



GeneSifter - Microarray Analysis Online - Mozilla Firefox

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

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### Control Panel

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## Welcome to GeneSifter

Select a topic to view the GeneSifter QuickStart Guide

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### Announcements

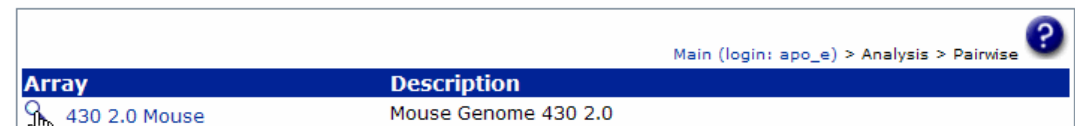
**04-22-2005 Update: New Unigene Builds**  
UniGene information has been updated for Human (Build #183), Mouse (Build #145), and Zebrafish (Build #83) genes.

**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.



Array	Description
430 2.0 Mouse	Mouse Genome 430 2.0

Main (login: apo\_e) > Analysis > Pairwise

# Tutorial

## Transcriptome analysis of apoE mouse aortas

- Pairwise analysis is used to identify differentially expressed genes in two groups. There are three replicates for each of the two groups in this study. Select the three replicates for the wild type mice (WT Aorta) for group 1. Select the three replicates for the apoE  $-/-$  mice (apoE Aorta) for group 2.
- Pairwise analysis combines a fold-change cutoff with a quality filter and comparison statistics to generate a list of differentially expressed genes. Select the following settings:

**Normalization:** None

Data was already normalized using MAS 5.

**Statistics:** t-test

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

**Quality:** P

Filters out genes that received absent or marginal detection 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 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.

Main (login: apo\_e) > Analysis > Arrays > Pairwise

### Pairwise Analysis: 430 2.0 Mouse

Group		Experiment	Target	Condition
1	2			
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	GSM44658	WT Aorta 1	WT Aorta
<input checked="" type="checkbox"/>	<input type="checkbox"/>	GSM44659	WT Aorta 3	WT Aorta
<input checked="" type="checkbox"/>	<input type="checkbox"/>	GSM44663	WT Aorta 2	WT Aorta
<input type="checkbox"/>	<input checked="" type="checkbox"/>	GSM44660	apoE Aorta 1	apoE Aorta
<input type="checkbox"/>	<input checked="" type="checkbox"/>	GSM44661	apoE Aorta 2	apoE Aorta
<input type="checkbox"/>	<input checked="" type="checkbox"/>	GSM44662	apoE Aorta 3	apoE Aorta

#### Advanced Analysis Settings

**Normalization:** None

**Statistics:** 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**

# Tutorial

## Transcriptome analysis of apoE mouse aortas

- After the analysis is performed a gene list will be returned. This list contains the genes that are differentially expressed based on the pairwise analysis settings selected. 1025 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 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.
- 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.
- The list filtered on the adjusted p-value contains 861 genes with a false discovery rate less than 5%.
- To view data and a gene summary for any gene in the list, select the **Gene Name**.

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Main (login: apo\_e) > Analysis > Pairwise > Results

Pairwise Analysis: 430 2.0 Mouse [Reports: Ontology | KEGG | Scatter Plot] [Results: Export | Save]

	Group 1	Group 2
Conditions:	WT Aorta	apoE Aorta
Experiments:	58266, 58267, 58271	58268, 58269, 58270
Significance:	1.5, t-test, Benjamini and Hochberg	
Normalization:	None	
Quality Cutoff:	Present	
Data Transformation:	Log Transformed	

Show: 20 Sort By: Ratio p Cutoff: 0.05 raw p Search (1025 results found) [1 - 20] [21 - 40]

No.	Ratio	p-value	adj. p	Identifier	Gene Name
1	▲ 192.67	0.00048	0.01921	BC019135	Matrix metalloproteinase 12
2	▲ 119.43	0.00000	0.00380	NM_009263	Secreted phosphoprotein 1
3	▲ 44.32	0.00178	0.01965	A1573431	Interleukin 7 receptor
4	▲ 43.41	0.00629	0.02609	AV204216	ATPase, H+ transporting, V0 subunit D, isoform 2
5	▲ 41.93	0.00002	0.00752	NM_009690	CD5 antigen-like
6	▲ 38.32	0.01629	0.03579	NM_008607	Matrix metalloproteinase 13
7	▲ 34.54	0.00644	0.02629	NM_009052	Reduced expression 3
8	▲ 33.82	0.00056	0.01921	X14607	Lipocalin 2
9	▲ 29.24	0.00038	0.01921	AV313762	Mucopolin 3
10	▲ 28.64	0.00367	0.02301	NM_031168	Interleukin 6
11	▼ 28.05	0.00606	0.02603	AK019319	Apolipoprotein E
12	▲ 26.35	0.00009	0.01496	BB078052	G-protein coupled receptor 88
13	▲ 23.92	0.00149	0.01941	NM_023044	Solute carrier family 15, member 3
14	▲ 22.63	0.00472	0.02456	BB134696	Membrane-associated ring finger (C3HC4) 1
15	▲ 22.47	0.01324	0.03278	BB770873	CD200 receptor 1
16	▲ 19.03	0.00067	0.01921	BB769890	ATPase, H+ transporting, V0 subunit D, isoform 2
17	▲ 18.64	0.00489	0.02498	AF108501	Chloride channel calcium activated 2
18	▲ 17.63	0.03118	0.05096	BB770972	Growth arrest-specific 2 like 3
19	▲ 16.34	0.00217	0.02075	NM_023270	Ring finger protein 128
20	▲ 15.67	0.00670	0.02630	NM_011087	Paired-Ig-like receptor A1

Show: 20 Sort By: Ratio p Cutoff: 0.05 raw p Search (1025 results found) [1 - 20] [21 - 40]

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Main (login: apo\_e) > Analysis > Pairwise > Results

Pairwise Analysis: 430 2.0 Mouse [Reports: Ontology | KEGG | Scatter Plot] [Results: Export | Save]

	Group 1	Group 2
Conditions:	WT Aorta	apoE Aorta
Experiments:	58266, 58267, 58271	58268, 58269, 58270
Significance:	1.5, t-test, Benjamini and Hochberg	
Normalization:	None	
Quality Cutoff:	Present	
Data Transformation:	Log Transformed	

Show: 20 Sort By: Ratio p Cutoff: 0.05 adjusted p Search (861 results found) [1 - 20] [21 - 40]

No.	Ratio	p-value	adj. p	Identifier	Gene Name
1	▲ 192.67	0.00048	0.01921	BC019135	Matrix metalloproteinase 12
2	▲ 119.43	0.00000	0.00380	NM_009263	Secreted phosphoprotein 1
3	▲ 44.32	0.00178	0.01965	A1573431	Interleukin 7 receptor
4	▲ 43.41	0.00629	0.02609	AV204216	ATPase, H+ transporting, V0 subunit D, isoform 2
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20	▲ 15.56	0.00356	0.02282	AK004847	Ring finger protein 128

Show: 20 Sort By: Ratio p Cutoff: 0.05 adjusted p Search (861 results found) [1 - 20] [21 - 40]

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# Tutorial

## Transcriptome analysis of apoE mouse aortas

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: 1448576\_at  
Accession No.: A1573431  
Cluster ID: Mm.389  
UG Title: Interleukin 7 receptor  
Gene ID: IL7r  
Homologene: Rn.6633, Rn.17789, Hs.237868  
Chromosome: 15  
Cytoband: -  
Seq Count: 246  
Entrez Gene: 16197  
Gene Name: interleukin 7 receptor  
OMIM: -  
KEGG: 16197  
RefSeq mRNA: NM\_008372 (FASTA)  
RefSeq Prot: NP\_032398 (FASTA)  
Summary: Interleukin-7 is a glycoprotein involved in the regulation of lymphopoiesis. Response of cells to IL7 is dependent on the presence of the interleukin 7 receptor (IL7R); the active receptor is a alpha/gamma chain heterodimer. The gamma(c) chain, which also associates with the interleukin-2 receptor, serves primarily to activate signal transduction by the IL7R complex, while the alpha chain of IL7R determines specific signaling events through its association with cytoplasmic signaling molecules.

**Gene Ontologies:**

**Biological Process**

- ♦ lymph gland development
- ♦ cell surface receptor linked signal transduction

**Molecular Function**

- ♦ interleukin receptor activity
- ♦ hematopoietin/interferon-class (D200-domain) cytokine receptor activity
- ♦ receptor activity

**Cellular Component**

- ♦ integral to membrane
- ♦ external side of plasma membrane
- ♦ membrane

**KEGG Pathways:**

- ♦ Jak-STAT signaling pathway
- ♦ Cytokine-cytokine receptor interaction

[ Perform Sequence Analysis ]

IL7r

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Main (login: apo\_e) > Analysis > Pairwise > Results ?

**Pairwise Analysis: 430 2.0 Mouse** [Reports: Ontology | KEGG | Scatter Plot] [Results: Export | Save]

	Group 1	Group 2
Conditions:	WT Aorta	apoE Aorta
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Significance:	1.5, t-test, Benjamini and Hochberg	
Normalization:	None	
Quality Cutoff:	Present	
Data Transformation:	Log Transformed	

Show: 20 Sort By: Ratio p Cutoff: 0.05 adjusted p Search (861 results found) [1 - 20] [21 - 40]

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17	▲ 18.64	0.00489	0.02498	AF108501	Chloride channel calcium activated 2
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Show: 20 Sort By: Ratio p Cutoff: 0.05 adjusted p Search (861 results found) [1 - 20] [21 - 40]

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## Transcriptome analysis of apoE mouse aortas

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13. The Ontology Report lists the Gene Ontology terms associated with the 861 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.

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[ **Biological Process** | Cellular Component | Molecular Function ]

Group 1: WT Aorta  
Group 2: apoE Aorta

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

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Ontology	Genes	GO	Totals		z-score	
			List	Array		
physiological process	337	300	37	7777	0.38	-2.29
cellular process	264	226	38	4956	3.85	2.67
metabolism	190	166	24	5321	-3.92	-1.64
cell communication	136	119	17	2118	4.87	1.64
signal transduction	111	98	13	1623	5.10	1.38
response to external stimulus	98	95	3	750	13.15	-0.63
response to biotic stimulus	85	83	2	574	13.70	-0.72
transport	83	67	16	1724	0.16	2.21
defense response	82	82	0	498	15.11	-1.73
immune response	74	74	0	398	15.70	-1.54
cell surface receptor linked signal transduction	64	55	9	897	3.79	1.83
morphogenesis	60	51	9	954	2.59	1.64
organogenesis	53	45	8	868	2.20	1.46
response to stress	52	50	2	494	7.50	-0.49
nucleobase, nucleoside, nucleotide and nucleic acid metabolism	47	42	5	2247	-5.49	-2.47
catabolism	42	38	4	680	2.49	0.08
response to pest/pathogen/parasite	40	40	0	218	11.32	-1.13
cell proliferation	37	36	1	686	2.02	-1.52
cell adhesion	36	31	5	431	3.74	1.68
macromolecule catabolism	35	32	3	494	3.16	0.13
protein catabolism	35	32	3	485	3.27	0.16
proteolysis and peptidolysis	35	32	3	465	3.53	0.23
regulation of transcription	34	31	3	1468	-3.70	-2.00
regulation of transcription, DNA-dependent	34	31	3	1443	-3.58	-1.96
transcription	34	31	3	1522	-3.94	-2.08

# Tutorial

## Transcriptome analysis of apoE mouse aortas

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16. Return to the main analysis window and click **KEGG**. 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.

GeneSifter - Microarray Analysis Online | Analysis - Mozilla Firefox

File Edit View Go Bookmarks Tools Help

http://gs3.genesifter.net/users/?&action=expo

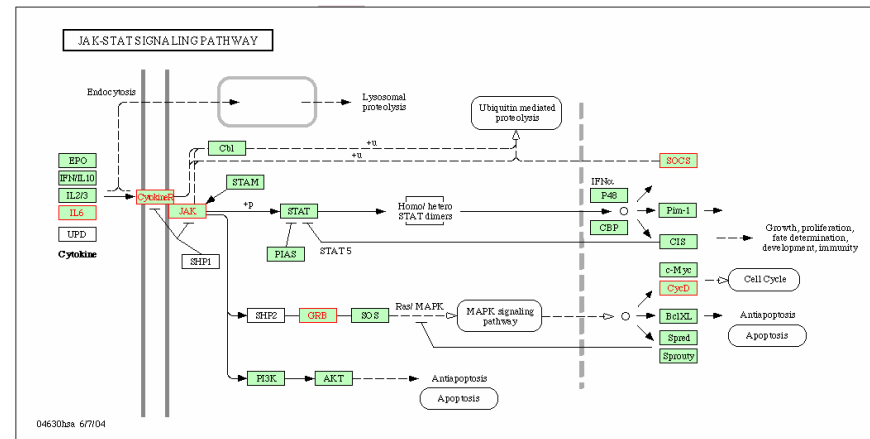
GENESIFTER

Group 1: WT Aorta  
Group 2: apoE Aorta

Pathway	Genes	KEGG	Totals		z-score	
			List	Array		
Cytokine-cytokine receptor interaction	39	36	3	218	7.78	1.55
Jak-STAT signaling pathway	18	17	1	133	3.97	0.23
Calcium signaling pathway	15	12	3	179	0.88	1.93
Regulation of actin cytoskeleton	15	12	3	191	0.64	1.81
MAPK signaling pathway	14	11	3	227	-0.31	1.47
Neuroactive ligand-receptor interaction	9	8	1	248	-1.54	-0.43
Toll-like receptor signaling pathway	8	8	0	88	1.62	-0.74
Apoptosis	7	7	0	91	1.04	-0.76
Complement and coagulation cascades	6	6	0	68	1.32	-0.65
Glycosphingolipid metabolism	5	5	0	32	2.63	-0.44
Purine metabolism	5	5	0	104	-0.22	-0.81
Globoside metabolism	4	4	0	21	2.83	-0.36
Glycerolipid metabolism	4	3	1	82	-0.67	0.74
N-Glycan degradation	4	4	0	12	4.35	-0.27
Wnt signaling pathway	4	4	0	140	-1.33	-0.95

Done

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# Tutorial

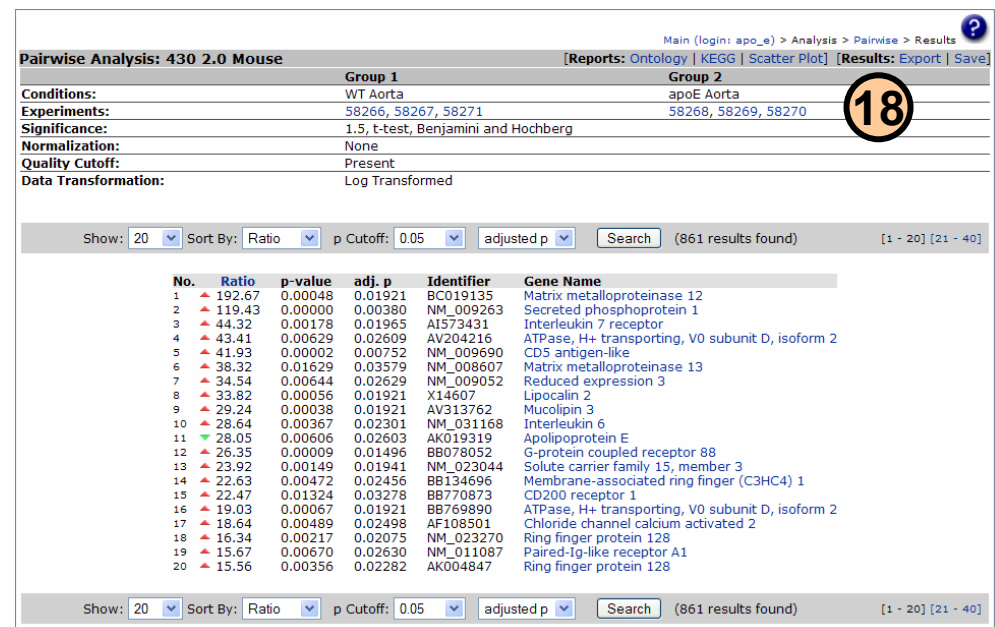
## Transcriptome analysis of apoE mouse aortas

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

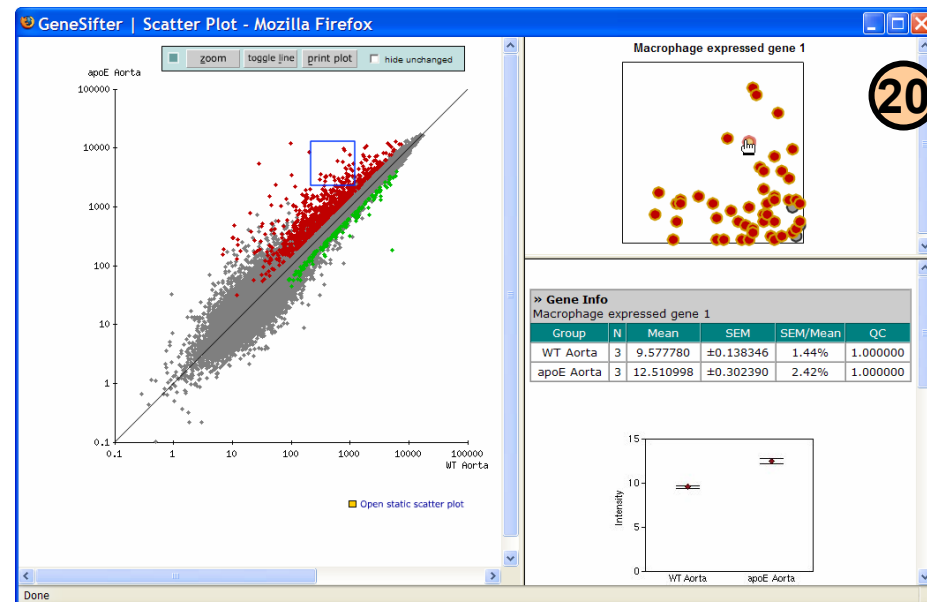
19. This will bring up a scatter plot of the results. Up-regulated genes are shown in red, and down-regulated genes are green. The gray spots are those that did not pass the analysis parameters. Move the blue box around and click **Zoom** to see more detail of the scatter plot.

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.



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