

# Tutorial

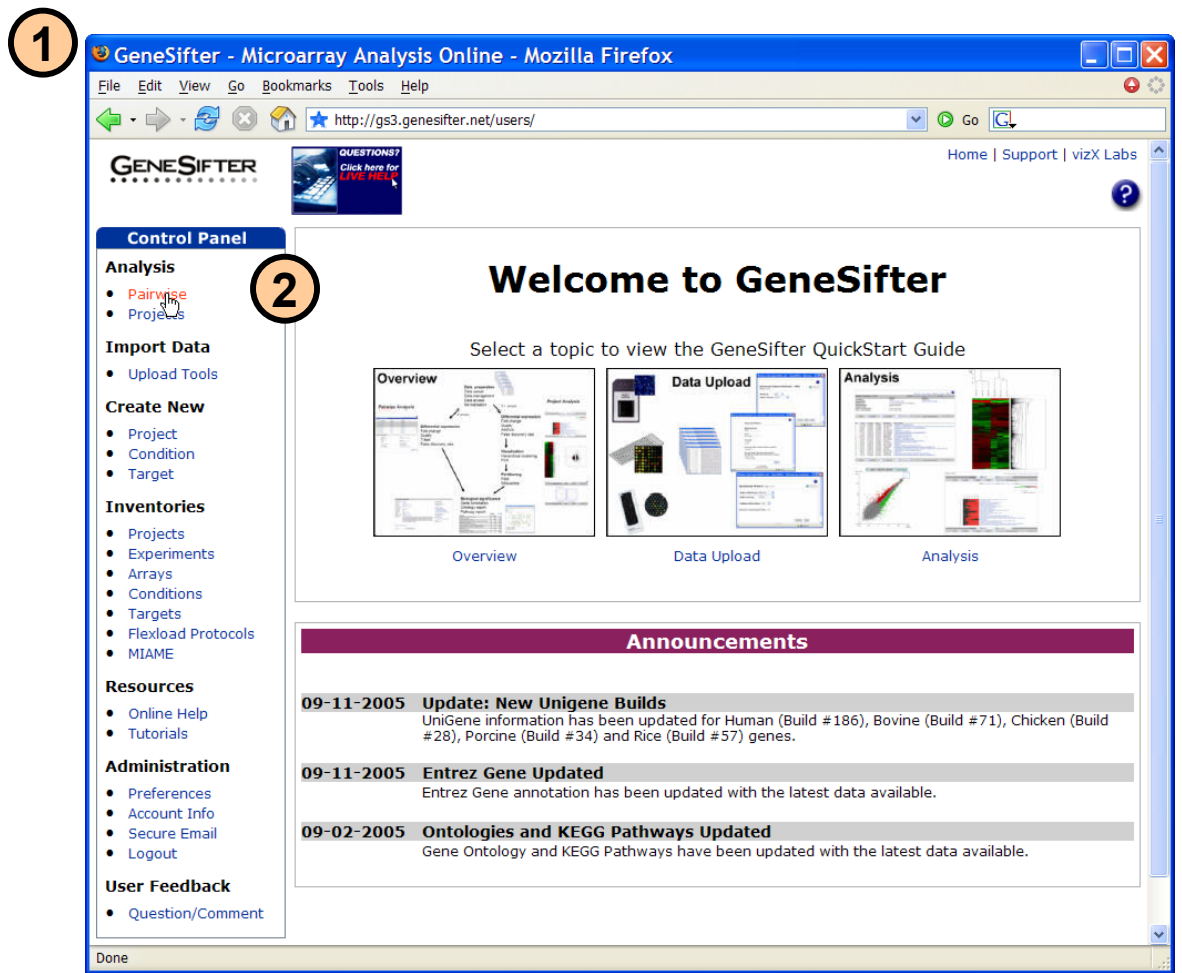
## Androgen Regulation of Gene Expression in the Mouse Meibomian Gland

The following tutorial walks through the analysis results presented in:

Schirra F, Suzuki T, Richards SM, Jensen RV, Liu M, Lombardi MJ, Rowley P, Treister NS, Sullivan DA. *Androgen control of gene expression in the mouse meibomian gland*. *Invest Ophthalmol Vis Sci*. 2005 Oct;46(10):3666-75.


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. After selecting the data set from the Data Center and entering the user name and password you will be logged into GeneSifter.
2. From the **Control Panel** select "Pairwise" within the **Analysis** section.
3. Click on the magnifying glass icon next to "Androgen studies" to begin the analysis. The data examined here was generated using the CodeLink Uniset I 10K Mouse array. There are approximately 10,000 transcripts represented on this array.



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Main (login: sullivan\_data) > Analysis > Pairwise

Array	Description
 Androgen studies	CodeLink Ms 10K
Lacrimal sex differences	CodeLink Ms 10K

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## Androgen Regulation of Gene Expression in the Mouse Meibomian Gland

- This example will compare gene expression in meibomian glands from placebo treated orchiectomized male mice to that in glands from testosterone treated orchiectomized mice. Select the three placebo treated gland samples (Male Placebo Mei) for **Group 1** and the three testosterone treated gland samples (Male Test Mei) for **Group 2**.
- Set the analysis settings as shown –
  - Normalization** – None (*data was normalized using CodeLink software and then loaded into GeneSifter*)
  - Statistics** – t-test (*Student's two-sample unpaired*)
  - Quality** – 0.50 (*this will filter out low intensity spots*)
  - Threshold** – None (*fold-change cutoff*)
  - Correction** – None
  - Data Transformation** – No Transformation

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Main (login: sullivan\_data) > Analysis > Arrays > Pairwise

**Pairwise Analysis: Androgen studies**

Group		Experiment	Target	Condition
1	2			
<input type="checkbox"/>	<input type="checkbox"/>	Male Placebo Lac A	Male Placebo Lac A	Male Placebo Lac
<input type="checkbox"/>	<input type="checkbox"/>	Male Placebo Lac B	Male Placebo Lac B	Male Placebo Lac
<input type="checkbox"/>	<input type="checkbox"/>	Male Placebo Lac C	Male Placebo Lac C	Male Placebo Lac
<input type="checkbox"/>	<input type="checkbox"/>	Male Test Lac A	Male Test Lac A	Male Test Lac
<input type="checkbox"/>	<input type="checkbox"/>	Male Test Lac B	Male Test Lac B	Male Test Lac
<input type="checkbox"/>	<input type="checkbox"/>	Male Test Lac C	Male Test Lac C	Male Test Lac
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Male Placebo Mei A	Male Placebo Mei A	Male Placebo Mei
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Male Placebo Mei B	Male Placebo Mei B	Male Placebo Mei
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Male Placebo Mei C	Male Placebo Mei C	Male Placebo Mei
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Male Test Mei A	Male Test Mei A	Male Test Mei
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Male Test Mei B	Male Test Mei B	Male Test Mei
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Male Test Mei C	Male Test Mei C	Male Test Mei
<input type="checkbox"/>	<input type="checkbox"/>	Male Placebo SMG A	Male Placebo SMG A	Male Placebo SMG
<input type="checkbox"/>	<input type="checkbox"/>	Male Placebo SMG B	Male Placebo SMG B	Male Placebo SMG
<input type="checkbox"/>	<input type="checkbox"/>	Male Placebo SMG C	Male Placebo SMG C	Male Placebo SMG
<input type="checkbox"/>	<input type="checkbox"/>	Male Test SMG A	Male Test SMG A	Male Test SMG
<input type="checkbox"/>	<input type="checkbox"/>	Male Test SMG B	Male Test SMG B	Male Test SMG
<input type="checkbox"/>	<input type="checkbox"/>	Male Test SMG C	Male Test SMG C	Male Test SMG

**Advanced Analysis Settings**

**5** **Normalization:** None

**Statistics:** t-test

**Quality:** 0.5

**Show genes that are:**

Up-regulated

Down-regulated

**Threshold:**

Lower: None Upper: None

**Correction:** None

**Data Transformation:**

No Transformation

Log Transform Data

Data Already Log Transformed

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- Select the **Analyze** button.

**Note:** This tutorial walks through the analysis used to generate figure 2-5 (Schirra et al., *Invest Ophthalmol Vis Sci.* 2005 Oct;46(10):3666-75.). Change the threshold to limit gene list based on fold change.

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## Androgen Regulation of Gene Expression in the Mouse Meibomian Gland

- The gene list shows the genes that passed all the analysis parameters. The genes are sorted by fold-change with the most changed genes shown first.
- To find out more about any gene in the list select the gene name.
- 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.
- Select the **Ontology** link to view a summary of the Gene Ontology terms associated with the genes in the list.

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Main (login: sullivan\_data) > Analysis > Pairwise > Results

**Pairwise Analysis: Androgen studies** [Reports: [Ontology](#) | [KEGG](#) | [Scatter Plot](#)] [Results: [Export](#) | [Save](#)]

	Group 1	Group 2
Conditions:	Male Placebo Mei	Male Test Mei
Experiments:	61106, 61107, 61108	61109, 61110, 61111
Significance:	1, t-test	
Normalization:	None	
Quality Cutoff:	0.5	
Data Transformation:	None	

Show: 20 Sort By: Ratio p Cutoff: 0.05 Search (1533 results found) [1 - 20] [21 - 40]

No.	Ratio	p-value	Identifier	Gene Name
1	3.96	0.04177	NM_011468	Small proline-rich protein 2A
2	3.31	0.00941	NM_011339	Chemokine (C-X-C motif) ligand 15
3	3.21	0.01047	NM_026523	Neuromedin B
4	3.01	0.00243	AF218416	Tocopherol (alpha) transfer protein
5	2.69	0.01485	NM_007894	Eosinophil-associated, ribonuclease A family, member 2
6	2.62	0.00129	NM_010898	Neurofibromatosis 2
7	2.52	0.00361	NM_008161	Glutathione peroxidase 3
8	2.43	0.00268	AK013634	Cyclin T2
9	2.42	0.00426	AK020020	AK020020_PROBE1
10	2.42	0.01357	NM_022984	Resistin
11	2.37	0.01213	AK002411	Splicing factor, arginine/serine-rich 11
12	2.34	0.00632	NM_007588	Calcitonin receptor
13	2.31	0.01184	AK005765	RIKEN cDNA 1700008G05 gene
14	2.30	0.00011	NM_009998	Cytochrome P450, family 2, subfamily b, polypeptide 10
15	2.28	0.04684	NM_011585	Cytotoxic granule-associated RNA binding protein 1
16	2.28	0.00322	NM_010220	FK506 binding protein 5
17	2.18	0.00657	AF250135	Splicing factor, arginine/serine-rich 2 (SC-35)
18	2.16	0.02742	NM_017370	Haptoglobin
19	2.16	0.00031	NM_019501	Trans-prenyltransferase
20	2.15	0.02630	NM_011478	Small proline-rich protein 3

Show: 20 Sort By: Ratio p Cutoff: 0.05 Search (1533 results found) [1 - 20] [21 - 40]

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» **One-Click Gene Summary™**

Accession No.: <a href="#">NM_011468</a>	<b>Gene Ontologies:</b> <b>Biological Process</b> <ul style="list-style-type: none"> <li>regulation of cell shape</li> <li>keratinization</li> </ul> <b>Molecular Function</b> <ul style="list-style-type: none"> <li>structural molecule activity</li> <li>structural constituent of cytoskeleton</li> </ul> <b>Cellular Component</b> <ul style="list-style-type: none"> <li>cornified envelope</li> </ul>
Cluster ID: <a href="#">Mm.6853</a>	
UG Title: Small proline-rich protein 2A	
Gene ID: <a href="#">Sprr2a</a>	
Homologene: <a href="#">Hs.11261, Rn.78577</a>	
Chromosome: 3	
Cytoband: -	
Seq Count: 478	
Entrez Gene: <a href="#">20755</a>	
Gene Name: small proline-rich protein 2A	
OMIM: -	
KEGG: <a href="#">20755</a>	
RefSeq mRNA: <a href="#">NM_011468</a> (FASTA)	
RefSeq Prot: <a href="#">NP_035598</a> (FASTA)	
Summary: -	

[ [Perform Sequence Analysis](#) ]

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## Androgen Regulation of Gene Expression in the Mouse Meibomian Gland

11. The Ontology Report lists the Gene Ontology terms associated with the 1533 genes in the pairwise results gene list. See the help documents for this page for more information about the Ontology Report.

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

12. Click on **z-score report**.

13. The z-score report lists the biological process ontologies that are significantly over or under-represented in the gene list. Ontologies with a z-score greater than 2 or less than -2 are over or under-represented, respectively (for the example shown, the ontology "cell proliferation" is significantly over-represented among the genes with lower expression in testosterone treated mice). Select the red arrow in the z-score column to sort the list by z-score for the up-regulated genes.

**Note:** The ontology terms are updated monthly so the exact numbers you see may be different than those presented here.

14. Select Molecular Function or Cellular Component to view the z-score report for these ontologies.

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GeneSifter - Microarray Analysis Online | Mozilla Firefox

http://gs3.genesifter.net/users/?&action=export&mod=arr&type=pairwise&file=97.txt&hkg=none&adjp

Group 1: Male Placebo Mei  
Group 2: Male Test Mei

[ Biological Process | Cellular Component | Molecular Function ]

[ Ontology Report | Z-score Report ]

Ontology	Genes	GO	List	Array	Totals	z-score
physiological process	869	574	295	5552	-0.25	2.17
cellular process	546	354	192	3675	-2.19	0.78
development	159	104	55	1146	-1.59	-0.40
regulation of biological process	35	18	17	187	-0.34	2.57
behavior	10	6	4	80	-0.85	-0.04
obsolete biological process	1	1	0	5	0.71	-0.51
viral life cycle	0	0	0	3	-0.59	-0.40

Done

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Group 1: Male Placebo Mei  
Group 2: Male Test Mei

[ Biological Process | Cellular Component | Molecular Function ]

[ Ontology Report | Z-score Report ]

Export Report

Ontology	Genes	GO	List	Array	Totals	z-score
physiological process	869	574	295	5552	-0.25	2.17
cellular process	546	354	192	3675	-2.19	0.78
protein modification	106	58	48	808	-3.18	1.26
morphogenesis	101	59	42	717	-2.00	1.07
catabolism	85	71	14	521	2.52	-2.55
cell organization and biogenesis	83	44	39	505	-1.29	2.86
response to stress	74	37	37	419	-1.08	3.67
cell proliferation	73	35	38	476	-2.25	3.05
response to biotic stimulus	70	38	32	441	-1.26	2.21
phosphate metabolism	67	34	33	481	-2.47	1.90
phosphorus metabolism	67	34	33	481	-2.47	1.90
lipid metabolism	65	50	15	300	3.65	-0.03
phosphorylation	62	32	30	399	-1.99	2.34
cytoplasm organization and biogenesis	61	31	30	333	-0.66	3.40
intracellular transport	61	46	15	313	2.56	-0.20
protein amino acid phosphorylation	59	29	30	369	-1.63	2.80
defense response	54	27	27	369	-1.99	2.06
ion transport	54	43	11	392	0.39	-2.08
macromolecule catabolism	54	45	9	377	1.02	-2.42
protein catabolism	53	45	8	371	1.13	-2.61
organelle organization and biogenesis	52	24	28	262	-0.66	4.27
proteolysis and peptidolysis	51	43	8	354	1.12	-2.45
cell cycle	49	22	27	329	-2.25	2.70
cell death	47	23	24	285	-1.31	2.67
death	47	23	24	289	-1.38	2.60
G-protein coupled receptor protein signaling pathway	47	43	4	336	1.49	-3.30
immune response	44	21	23	311	-2.15	1.95
carboxylic acid metabolism	42	33	9	213	2.48	-0.55
organic acid metabolism	42	33	9	213	2.48	-0.55
programmed cell death	42	21	21	261	-1.26	2.27
apoptosis	41	21	20	258	-1.20	2.04

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