

Tutorial

Sex-related Differences in Gene Expression in Salivary Glands of BALB/c Mice

The following tutorial walks through the analysis results presented in -

Treister N.S., Richards S.M., Lombardi M.J., Rowley P., Jensen R.V., Sullivan D.A. *Sex-related differences in gene expression in salivary glands of BALB/c mice* **J Dent Res.** 2005 Feb;84(2):160-5.

Visit the GeneSifter Data Center (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 "Mouse 10K" 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.

1

The screenshot shows the GeneSifter website in a Mozilla Firefox browser window. The address bar shows the URL <https://gs3.genesifter.net/users/>. The page features a "Control Panel" on the left with sections for Analysis, Inventories, Import Data, Create New, and Resources. The main content area displays a "Welcome to GeneSifter" message with three navigation options: Overview, Data Upload, and Analysis. Below this is an "Announcements" section with several updates from 2005. A magnifying glass icon is visible next to the "Analysis" link in the navigation area.

3

Main (login: treister1) > Analysis > Pairwise

Array	Description
 Mouse 10K	CodeLink

Tutorial

Sex Differences in Mouse Salivary Glands

- This example will compare gene expression in submandibular glands from male mice with glands from female mice. Select the three male SMG samples for **Group 1** and the three female SMG samples for **Group 2**.
- Select 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 – 0.75 (*this will filter out low intensity spots*)
Threshold – 1.5 (*fold-change cutoff*)
Correction – None
Data Transformation – No Transformation
- Select the **Analyze** button.

Note: This tutorial walks through the analysis used to generate figure 2 (Treister et al. **J Dent Res.** 2005 Feb;84(2):160-5). Changing the threshold and selecting other samples will generate the data used for figure 1.

4

Main (login: treister1) > Analysis > Arrays > Pairwise

Pairwise Analysis: Mouse 10K

Group		Experiment	Target	Condition
1	2			
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	SMG B M	SMG B M	SMG M
<input checked="" type="checkbox"/>	<input type="checkbox"/>	SMG C M	SMG C M	SMG M
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Submandibular A M	Submandibular A M	SMG M
<input type="checkbox"/>	<input type="checkbox"/>	SUBLINGUAL A M	SUBLINGUAL A M	SUBLINGUAL M
<input type="checkbox"/>	<input type="checkbox"/>	SUBLINGUAL B M	SUBLINGUAL B M	SUBLINGUAL M
<input type="checkbox"/>	<input checked="" type="checkbox"/>	SMG A F	SMG A F	SMG F
<input type="checkbox"/>	<input checked="" type="checkbox"/>	SMG B F	SMG B F	SMG F
<input type="checkbox"/>	<input checked="" type="checkbox"/>	SMG C F	SMG C F	SMG F
<input type="checkbox"/>	<input type="checkbox"/>	PAROTID A M	PAROTID A M	PAROTID M
<input type="checkbox"/>	<input type="checkbox"/>	PAROTID B M	PAROTID B M	PAROTID M
<input type="checkbox"/>	<input type="checkbox"/>	PAROTID A F	PAROTID A F	PAROTID F
<input type="checkbox"/>	<input type="checkbox"/>	PAROTID B F	PAROTID B F	PAROTID F
<input type="checkbox"/>	<input type="checkbox"/>	SUBLINGUAL A F	SUBLINGUAL A F	SUBLINGUAL F
<input type="checkbox"/>	<input type="checkbox"/>	SUBLINGUAL B F	SUBLINGUAL B F	SUBLINGUAL F

Advanced Analysis Settings

5

Normalization: None

Statistics: t-test

Quality: 0.75

Threshold: Lower: 1.5 Upper: None

Correction: None

Show genes that are:

- Up-regulated
- Down-regulated

Data Transformation:

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

Analyze Reset

6

Tutorial

Sex Differences in Mouse Salivary Glands

- 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.
- Select the **Ontology** link to view a summary of the gene Ontology terms associated with the genes in the list.

7

10

Main (login: treister1) > Analysis > Pairwise > Results

Pairwise Analysis: Mouse 10K [Reports: Ontology | KEGG | Scatter Plot] [Results: Export | Save]

	Group 1	Group 2
Conditions:	SMG M	SMG F
Experiments:	57526, 57537, 57539	57528, 57529, 57538
Significance:	1.5, t-test	
Normalization:	None	
Quality Cutoff:	0.75	
Data Transformation:	None	

Show: 20 Sort By: Ratio p Cutoff: 0.05 Search (464 results found) [1 - 20] [21 - 40]

No.	Ratio	p-value	Identifier	Gene Name
1	276.92	0.00893	AK015750	Sulfotransferase family 1E, member 1
2	59.69	0.00136	NM_013609	Nerve growth factor, beta
3	45.44	0.00766	NM_012011	Eukaryotic translation initiation factor 2, subunit 3, structural gene Y-linked
4	38.56	0.00042	NM_010643	Kallikrein 1
5	27.42	0.03283	AF141934	Solute carrier family 4 (anion exchanger), member 4
6	27.01	0.00914	AK007264	Uridine phosphorylase 2
7	24.59	0.00320	NM_009998	Cytochrome P450, family 2, subfamily b, polypeptide 20
8	20.74	0.00013	NM_010113	Epidermal growth factor
9	17.92	0.03491	AJ007376	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked
10	16.15	0.00471	NM_008291	Hydroxysteroid (17-beta) dehydrogenase 3
11	15.14	0.00260	AK009417	RIKEN cDNA 2310020F24 gene
12	13.00	0.00120	NM_008340	Insulin-like growth factor binding protein, acid labile subunit
13	11.71	0.00524	NM_009177	ST3 beta-galactoside alpha-2,3-sialyltransferase 1
14	11.25	0.00346	NM_010609	Potassium channel, subfamily K, member 7
15	10.89	0.04217	NM_009180	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide
16	9.94	0.00219	NM_010784	Midkine
17	9.78	0.00990	NM_028104	Protein phosphatase 1, regulatory (inhibitor) subunit 14D
18	9.26	0.02962	NM_031193	Renin 1 structural
19	9.23	0.00603	NM_009161	Sarcoglycan, alpha (dystrophin-associated glycoprotein)
20	9.19	0.00025	NM_009263	Secreted phosphoprotein 1

Show: 20 Sort By: Ratio p Cutoff: 0.05 Search (464 results found) [1 - 20] [21 - 40]

8

9

Main (login: treister1) > Analysis > Pairwise > Results > Gene Summary

» Gene Summary: Nerve growth factor, beta

Group	Condition	N	Mean	SEM	SEM/Mean	Quality Mean
1	SMG M	3	124.943	+/- 15.4560	12.4%	124.943
2	SMG F	3	2.0931	+/- 0.5484	26.2%	2.0931

SMG F down-regulated 59.69 fold compared to SMG M

Group	Sample	N	Mean	SEM	SEM/Mean	Quality Ave
1	Submandibular A M	1	114.840	-	-	114.84
1	SMG C M	1	104.694	-	-	104.694
1	SMG B M	1	155.295	-	-	155.295
2	SMG B F	1	1.6793	-	-	1.67931
2	SMG A F	1	3.1797	-	-	3.17965
2	SMG C F	1	1.4205	-	-	1.42046

» One-Click Gene Summary™

Accession No.: NM_013609
 Cluster ID: Mm.1259
 UG Title: Nerve growth factor, beta
 Gene ID: Ngfb
 Homologene: Hs.2561, Rn.22168
 Chromosome: 3
 Cytoband: -
 Seq Count: 38
 Entrez Gene: 18049
 Gene Name: nerve growth factor, beta
 OMIM: -
 KEGG: 18049
 RefSeq mRNA: NM_013609 (FASTA)
 RefSeq Prot: NP_038637 (FASTA)
 Summary: -

[Perform Sequence Analysis]

Ngfb Search PubMed

Gene Ontologies:
 Biological Process
 • peripheral nervous system development
 • perception of pain
 Molecular Function
 • growth factor activity
 • receptor signaling protein activity
 Cellular Component
 • extracellular space
KEGG Pathways:
 • Apoptosis
 • MAPK signaling pathway

Tutorial

Sex Differences in Mouse Salivary Glands

11. The Ontology Report lists the Gene Ontology terms associated with the 464 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. Select the red arrow in the z-score column to sort the list by z-score for the up-regulated genes.

Note: Ontology reports can be generated for each gland by performing pairwise analysis for each set of glands and viewing the Ontology Report for each gene list.

Group 1: SMG M
Group 2: SMG F

Ontology	Genes	GO	List	Array	z-score
physiological process	264	93	191	5373	0.84 -0.08
cellular process	178	55	123	3554	-0.41 0.12
development	61	14	47	1133	-1.09 1.44
biological process unknown	18	5	13	336	-0.18 0.45
regulation of biological process	12	2	10	166	-0.42 1.85
behavior	6	3	3	73	1.71 0.32
obsolete biological process	0	0	0	5	-0.29 -0.42
viral life cycle	0	0	0	3	-0.22 -0.33

pie chart labels:
 physiological process (20.81%)
 cellular process (31.84%)
 development (10.51%)
 biological process unknown (0.22%)
 regulation of biological process (2.15%)
 behavior (1.07%)

Group 1: SMG M
Group 2: SMG F

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

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

Export Report

Ontology	Genes	GO	Totals		z-score	
			List	Array	▲	▼
metabolism	179	66	113	3863	0.75	-2.64
nucleobase, nucleoside, nucleotide and nucleic acid metabolism	57	25	32	1603	-0.18	-3.62
signal transduction	44	15	29	1175	-0.99	-2.00
biosynthesis	33	16	17	601	2.15	-0.86
transcription	31	13	18	1080	-1.15	-3.48
transcription, DNA-dependent	30	13	17	1044	-1.01	-3.48
regulation of transcription	29	13	16	1049	-1.03	-3.69
regulation of transcription, DNA-dependent	29	13	16	1029	-0.95	-3.59
macromolecule biosynthesis	28	15	13	502	2.55	-1.08
carbohydrate metabolism	17	9	8	211	3.12	0.29
cell death	17	1	16	276	-1.68	2.20
death	17	1	16	280	-1.70	2.14
apoptosis	16	1	15	246	-1.53	2.33
programmed cell death	16	1	15	249	-1.54	2.28
cytoplasm organization and biogenesis	14	0	14	325	-2.36	0.88
organelle organization and biogenesis	13	0	13	258	-2.09	1.44
cell motility	12	2	10	155	-0.32	2.09
nucleotide metabolism	11	6	5	90	3.84	1.11
amine metabolism	10	6	4	140	2.54	-0.38
coenzyme and prosthetic group metabolism	10	5	5	85	3.15	1.25
G-protein coupled receptor protein signaling pathway	10	5	5	343	-0.23	-2.06
coenzyme metabolism	9	4	5	73	2.64	1.61
blood vessel development	8	1	7	76	-0.20	2.78
cell migration	8	1	7	98	-0.47	2.03
glycoprotein biosynthesis	8	3	5	46	2.66	2.78
glycoprotein metabolism	8	3	5	51	2.44	2.51