Tutorial
Androgen Regulation of Gene Expression in the Mouse Meibomian Gland

The following tutorial walks through the analysis results presented in:


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 “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.
4. 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.

5. 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

6. 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.
7. 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.

8. To find out more about any gene in the list select the gene name.

9. 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.

10. Select the Ontology link to view a summary of the Gene Ontology terms associated with the genes in the list.
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 \( (\text{?}) \) 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.