


# Drosophila Immune Response Tutorial

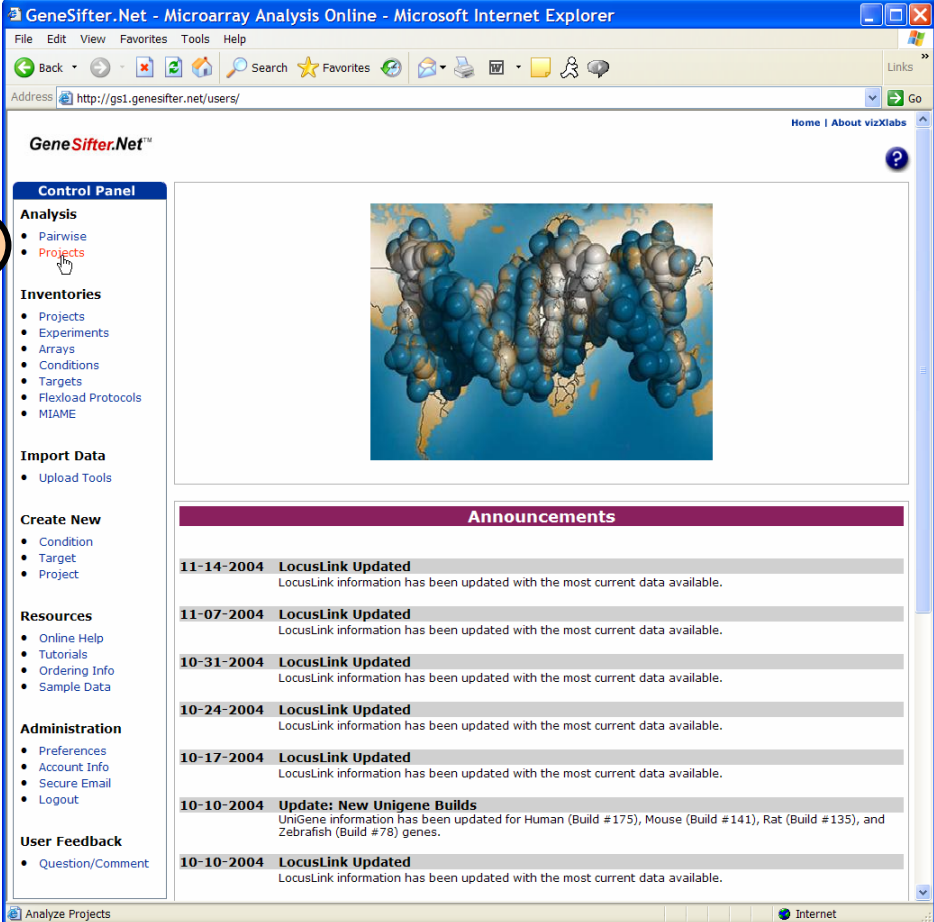
The following tutorial walks through the identification of biological themes associated with gene clusters identified in an immune response time series.

Visit the GeneSifter Data Center to obtain login information to access the dataset. You should receive a confirmation email shortly. Login to GeneSifter using the provided username and password.

1. Select **Projects** from the **Analysis** menu.
2. Select the magnifying glass icon (  ) next to "Immune Response::Filtered" from the list of projects.

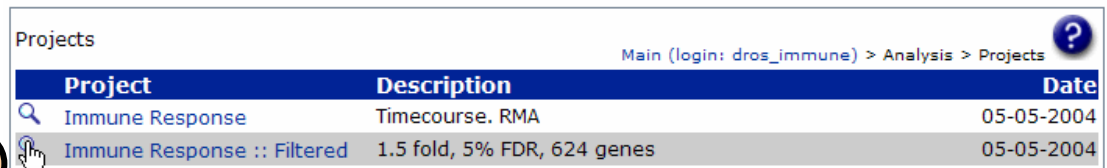
**Note:** Immune Response::Filtered is a sub-project of Immune Response, which contains all genes on the Drosophila Genome I array. The filtered sub-project contains 624 genes that were differentially regulated with at least a 1.5 fold change across the time series and statistically significant (5% false discovery rate based on ANOVA followed by correction for multiple testing using the method of Benjamini and Hochberg).

1





The screenshot shows the GeneSifter.Net website in a Microsoft Internet Explorer browser window. The address bar shows the URL http://gst.genesifter.net/users/. The page title is "GeneSifter.Net™". The main content area features a "Control Panel" with several sections: "Analysis" (with "Projects" selected), "Inventories", "Import Data", "Create New", "Resources", "Administration", and "User Feedback". To the right of the Control Panel is a large 3D molecular model of a protein structure. Below the model is an "Announcements" section with several entries dated from 10-10-2004 to 11-14-2004, all titled "LocusLink Updated".

2



The screenshot shows a table of projects on the GeneSifter.Net website. The table has columns for "Project", "Description", and "Date". The "Immune Response :: Filtered" project is highlighted, and a magnifying glass icon is visible next to it. The breadcrumb navigation at the top right reads "Main (login: dros\_immune) > Analysis > Projects".

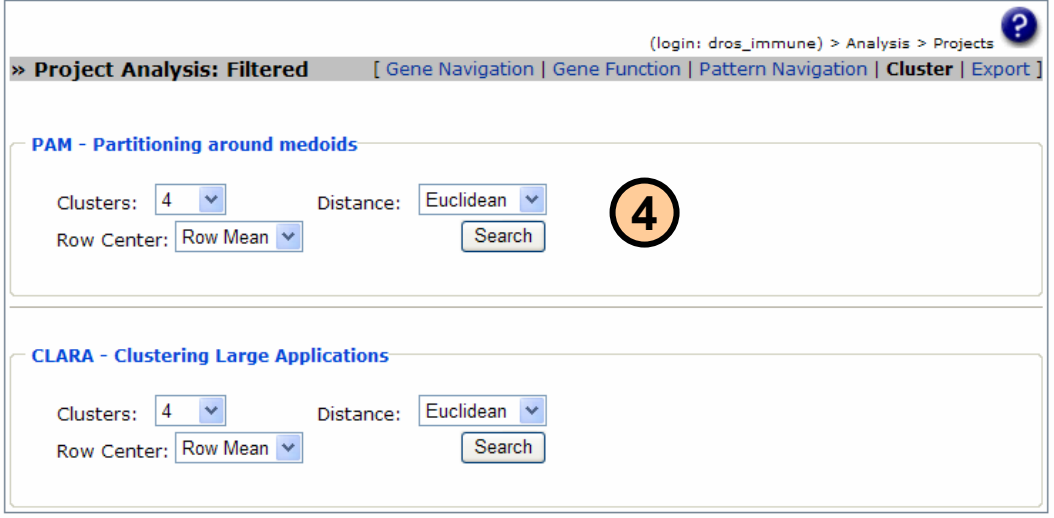
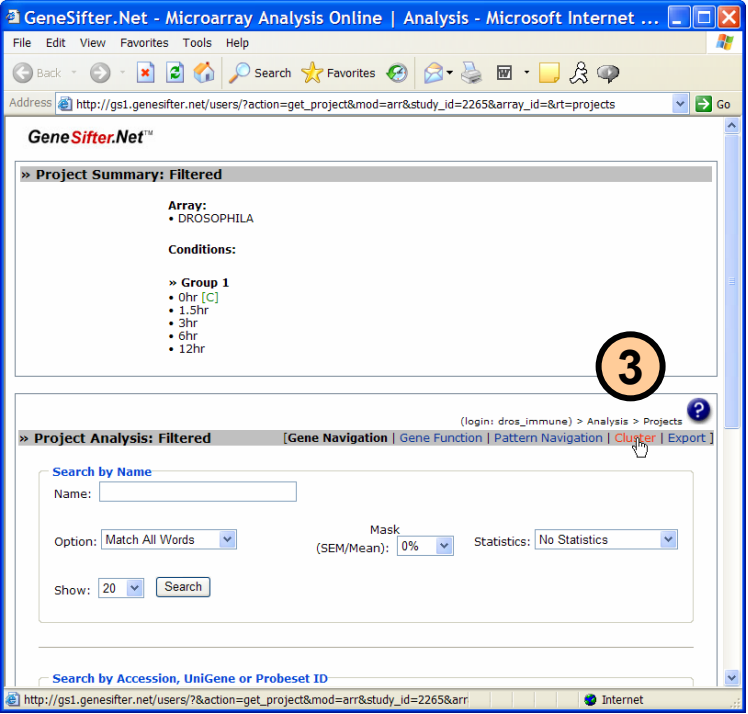
Project	Description	Date
 Immune Response	Timecourse. RMA	05-05-2004
 Immune Response :: Filtered	1.5 fold, 5% FDR, 624 genes	05-05-2004

# Immune Response Tutorial (continued)

3. The **Project Summary** section lists the time points examined in this project. The immune response time series examines gene expression in uninfected flies (0hr) and at 1.5, 3, 6, and 12 hours after infection.

Select the **Cluster** link from the **Project Analysis** section.

4. Partition clustering will be used to separate the 624 genes into groups based on expression pattern. Set the **Clusters** pull-down menu to 4 in the PAM section and click **Search**.



# Immune Response Tutorial

(continued)

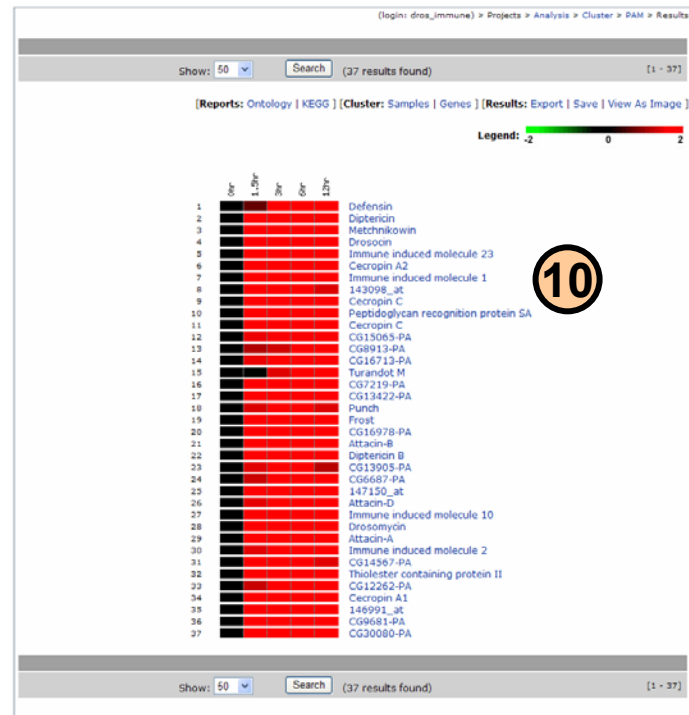
- Each line graph summarizes the gene expression pattern for that cluster (each gene in a cluster has an expression pattern more similar to pattern shown in the graph than to the pattern associated with each of the other clusters). The number of genes in each cluster is listed below the graphs. Silhouette widths measure how the genes in each group are clustered and can be used to select the best number of clusters for a set of genes. See the help documents for this page for more information about silhouettes.
- To view page-specific help documents for this, or any page, select the question mark icon (?) located at the upper right page corner.
- Select Cluster 4 (37 genes showing dramatic up-regulation during the time series).



# Immune Response Tutorial (continued)

- The resulting heat map shows the expression pattern of the 37 genes in cluster 4.
- To view an summary of the Gene Ontologies associated with the genes in this list, click on the **Ontology** link to bring up the Ontology Report (go to step 12 in this tutorial).
- To view a data and gene summary for any of the genes in the list select the gene title.
- The One Click Gene Summary is a synopsis of current UniGene and LocusLink for the gene.

8



9

10

11

Project: Filtered

### » One-Click Gene Summary™

Probe Set ID: 143100\_f\_at  
Accession No.: NM\_079852  
Cluster ID: Dm.20562  
UG Title: Cecropin C  
Gene ID: CecC  
Homologene: -  
Chromosome: 3R  
Cytoband: -  
Seq Count: 19  
LocusLink: 43599  
Gene Name: Cecropin C  
OMIM: -  
KEGG: 43599  
RefSeq mRNA: NM\_079852 (FASTA)  
RefSeq Prot: NP\_524591 (FASTA)  
Summary: -

**Gene Ontologies:**

**Biological Process**

- defense response to bacteria
- defense response to Gram-negative bacteria
- defense response to Gram-positive bacteria
- antibacterial humoral response (sensu Protostomia)
- defense response to fungi

**Cellular Component**

- extracellular region

[Perform Sequence Analysis]

CecC

**Search for Homologs:**

# Immune Response Tutorial

(continued)

13. The Ontology Report lists the Gene Ontology terms associated with the 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. Genes involved in the defense response are greatly enriched in this cluster.

Z-score reports can be generated for each of the clusters. The biological themes associated with cluster are very different.

GeneSifter.Net - Microarray Analysis Online | Analysis - Microsoft Internet Explorer

Address: http://gs1.genesifter.net/users/index.pl?action=export&mod=arr&rt=project&type=pat\_nav&file=78.tdf&state=&p\_out=&dt=a38820b52eabc2851

Condition 1 : 0hr  
Condition 2 : 1.5hr  
Condition 3 : 3hr  
Condition 4 : 6hr  
Condition 5 : 12hr

[ Biological Process | Cellular Component | Molecular Function ]

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

Ontology	Genes	GO	Totals List	Array	z-score
physiological process	19	4795	-1.05		
development	2	1261	-1.69		
biological_process unknown	1	96	0.92		
cellular process	1	2838	-4.62		
behavior	0	123	-0.74		
obsolete biological process	0	2	-0.09		
regulation of biological process	0	42	-0.43		
viral life cycle	0	1	-0.07		

physiological process (12.61%)  
development (0.70%)  
biological\_process unknown (4.55%)  
cellular process (4.35%)

Condition 1 : 0hr  
Condition 2 : 1.5hr  
Condition 3 : 3hr  
Condition 4 : 6hr  
Condition 5 : 12hr

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

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

Export Report

Ontology	Genes	GO	Totals		z-score
			List	Array	
response to external stimulus	17	531	10.22		
defense response	16	407	11.17		
response to biotic stimulus	16	418	10.99		
antibacterial humoral response (sensu Protostomia)	11	18	39.27		
defense response to bacteria	11	43	25.21		
response to bacteria	11	45	24.63		
defense response to Gram-negative bacteria	7	12	30.58		
defense response to Gram-positive bacteria	6	10	28.71		
defense response to fungi	5	6	30.94		
response to fungi	5	17	18.23		
metabolism	4	3624	-5.02		
immune response	3	58	5.53		