

# Tutorial

## Orofacial development in mouse embryo

The following tutorial walks through the identification of biological themes associated with gene clusters identified in a murine orofacial development time series.

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 "Projects" within the **Analysis** section.
3. Select the magnifying glass icon (🔍) next to "Timecourse::Filter I::Filter II" from the list of projects.

**Note:** Timecourse::Filter I::Filter II is a sub-project of the Timecourse project, which contains data for all genes on GeneChip® U74Av2 Mouse Genome array. The filtered sub-project contains 411 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).

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GeneSifter - Microarray Analysis Online - Mozilla Firefox

http://gs2.genesifter.net/users/

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Control Panel

Analysis

- Pairwise
- **Projects**

Inventories

- Projects
- Experiments
- Arrays
- Conditions
- Targets
- Flexload Protocols
- MIAME

Import Data

- Upload Tools

Create New

- Condition
- Target
- Project

Resources

- Online Help
- Tutorials

Welcome to GeneSifter

Select a topic to view the GeneSifter QuickStart Guide

Overview Data Upload Analysis

Announcements

04-01-2005 Update: New UniGene Builds  
UniGene information has been updated for Human (Build #182), Rat (Build #141), and Drosophila (Build #36) genes.

03-11-2005 Update: New UniGene Builds  
UniGene information has been updated for Human (Build #181), Mouse (Build #145), Rat (Build #140), Arabidopsis (Build #48), and Zebrafish (Build #81) genes.

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Projects Main (login: orofacial) > Analysis > Projects

Project	Description	Date
🔍 All samples	-	03-23-2005
🔍 Timecourse	12-14 days gestation	03-23-2005
🔍 Timecourse :: Filter I	filter = P	03-23-2005
🔍 Timecourse :: Filter I :: Filter II	1.5 fold, 5% FDR	03-23-2005
🔍 Timecourse :: Filter I :: Filter II :: Transcription	Transcription factor activity	03-27-2005

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- The **Project Summary** section lists the time points examined in this project. The orofacial development time series examines gene expression in mouse orofacial tissue at 12, 13 and 14 days of gestation.
- Select the **Cluster** link from the **Project Analysis** section.
- Partition clustering will be used to separate the 411 genes into groups based on expression pattern. Set the **Clusters** pull-down menu to 2 in the **PAM** section and click **Search**.

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GeneSifter - Microarray Analysis Online | Analysis - Mozilla Firefox

File Edit View Go Bookmarks Tools Help

http://gs2.genesifter.net/users/?action=get\_project&mod=arr&study\_id=3587&array\_id=&rt=projects&view=cluster

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>> Project Summary: Filter II

Array:  
• U74A

Conditions:  
» Group 1  
• Day 12 [Control]  
• Day 13  
• Day 14

Show All Genes

(login: orofacial) > Analysis > Projects

>> Project Analysis: Filter II [Gene Navigation | Gene Function | Pattern Navigation | Cluster | Export]

Search by Name

Name:

Option: Match All Words  Statistics: No Statistics

Show: 20

Search by Accession, UniGene or Refseq ID

http://gs2.genesifter.net/users/?action=get\_project&mod=arr&study\_id=3587&array\_id=&rt=projects&view=cluster

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(login: orofacial) > Analysis > Projects

>> Project Analysis: Filter II [Gene Navigation | Gene Function | Pattern Navigation | Cluster | Export]

PAM - Partitioning around medoids

Clusters: 2  Distance: Euclidean   
Row Center: Row Mean

CLARA - Clustering Large Applications

Clusters: 4  Distance: Euclidean   
Row Center: Row Mean

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Project: Filter II

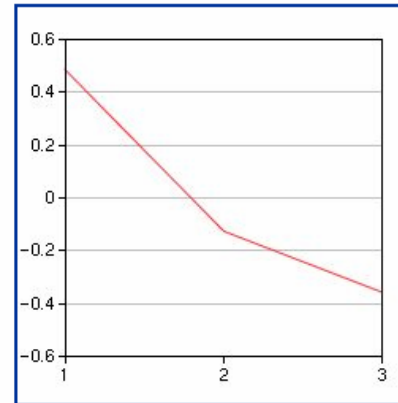
(login: orofacial) > Projects > Analysis > Cluster > PAM

K-medoids Clustering Clusters: 2 Distance: Euclidean Row Center: Row Mean

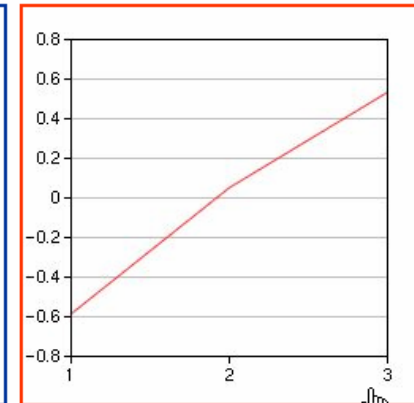
Search

Mean silhouette width: 0.636

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Cluster 1 - 142 Genes  
Mean sil. width: 0.760



Cluster 2 - 269 Genes  
Mean sil. width: 0.570

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K-medoids Clustering Clusters: 2 Distance: Euclidean Row Center: Row Mean

Search

7. Each line graph summarizes the gene expression pattern for that cluster (each gene in a cluster has an expression pattern more similar to the 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.

8. Silhouette widths measure how well 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.

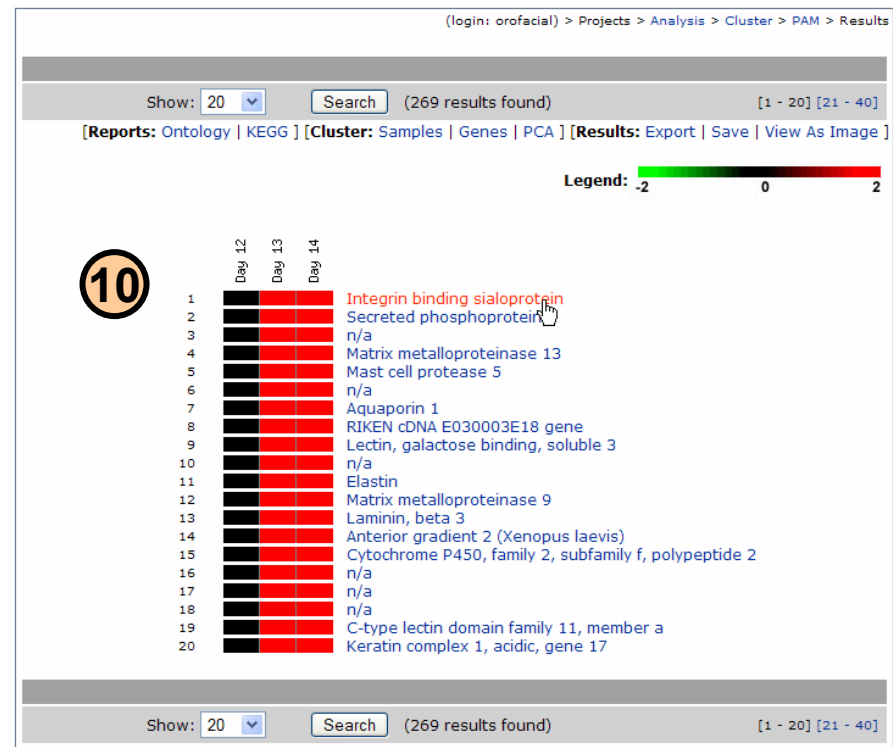
**Note:** To view page-specific help documents for this, or any page, select the question mark icon (?) located at the upper right page corner.

9. Select Cluster 2 (269 genes showing up-regulation during the time series).

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- The resulting heat map shows the expression pattern of the 269 genes in cluster 2. To find out more about any of the genes in the list, select the gene name to access the One-Click Gene Summary™ for that gene.
- The One-Click Gene Summary is a synopsis of current UniGene and LocusLink annotation for the gene.



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Project: Filter II

» One-Click Gene Summary™

Probe Set ID: 100476\_at  
Accession No.: L20232  
Cluster ID: Mm.4987  
UG Title: Integrin binding sialoprotein  
Gene ID: lbsp  
Homologene: Hs.121552, Rn.9721  
Chromosome: 5  
Cytoband: -  
Seq Count: 91  
Entrez Gene: 15891  
Gene Name: integrin binding sialoprotein  
OMIM: -  
KEGG: 15891  
RefSeq mRNA: NM\_008318 (FASTA)  
RefSeq Prot: NP\_032344 (FASTA)  
Summary: -

**Gene Ontologies:**

- Biological Process
  - cell adhesion
  - ossification
  - integrin-mediated signaling pathway
- Molecular Function
  - protein binding
- Cellular Component
  - extracellular space
  - extracellular region

[ Perform Sequence Analysis ]

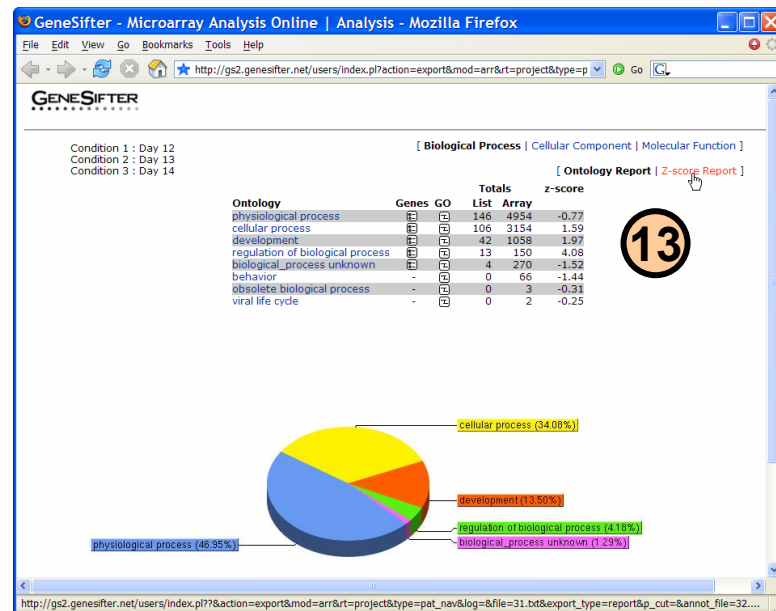
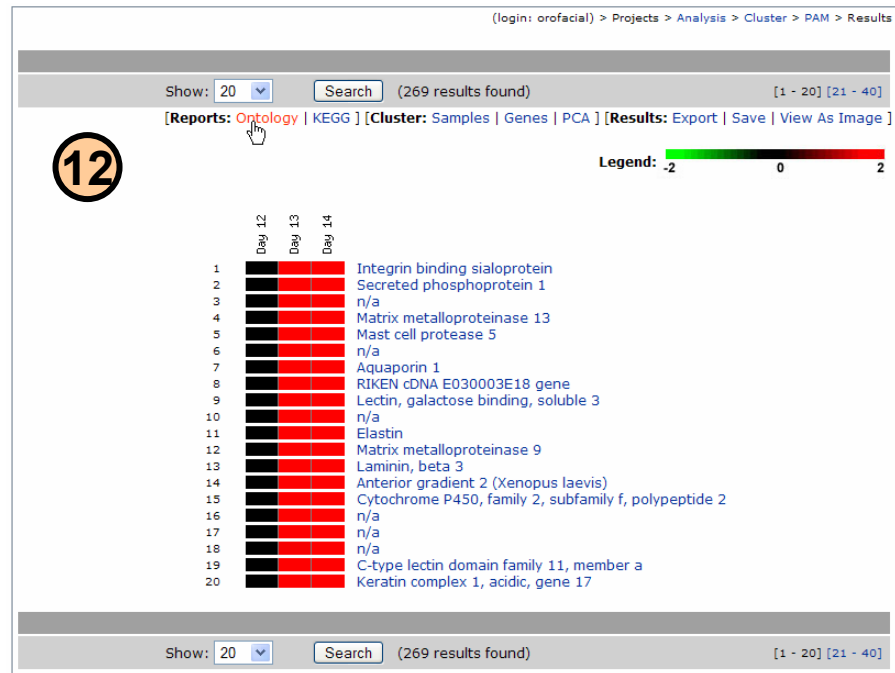
lbsp [Search PubMed]

**Search for Homologs:**  
Select a project [Search]

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- To view a summary of the Gene Ontologies associated with the genes in this list, click on the **Ontology** link to bring up the Ontology Report.
- The Ontology Report summarizes all of the Gene Ontology terms associated with the gene list. See the help documents for this page for more information about the Ontology Report.  
Click on **Z-score report**.



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14. The z-score report lists the biological process ontologies that are significantly over or under represented in the cluster. Select the **Molecular Function** link to view the z-score report for the molecular function ontologies.

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

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The screenshot shows the GeneSifter web application in a Mozilla Firefox browser. The page title is "GeneSifter - Microarray Analysis Online | Analysis - Mozilla Firefox". The browser address bar shows the URL: "http://gs2.genesifter.net/users/index.pl??action=export&moc". The GeneSifter logo is visible at the top left. The main content area has navigation links: "[ Biological Process | Cellular Component | Molecular Function ]" and "[ Ontology Report | Z-score Report | Export Report ]". Below these links, the conditions are listed: "Condition 1 : Day 12", "Condition 2 : Day 13", and "Condition 3 : Day 14". A table displays the Z-score report for Biological Process ontologies. The table has columns for "Ontology", "Genes", "GO", "List", "Array", and "Z-score". The data is as follows:

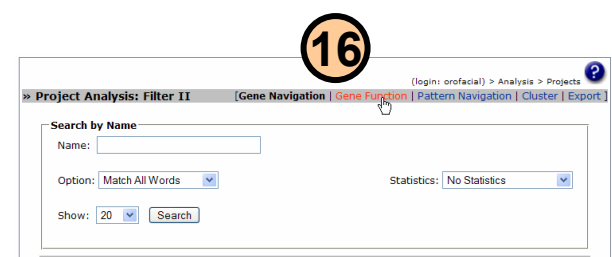
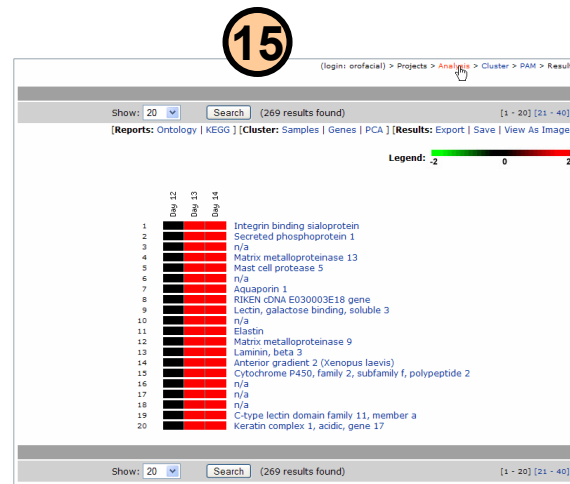
Ontology	Genes	GO	Totals		Z-score
			List	Array	
metabolism	75	3376		-4.12	
cell communication	52	1331		2.12	
morphogenesis	34	713		2.89	
organogenesis	32	651		2.98	
cell adhesion	26	265		6.59	
response to external stimulus	25	558		2.10	
nucleobase, nucleoside, nucleotide and nucleic acid metabolism	23	1416		-3.52	
response to biotic stimulus	23	448		2.71	
catabolism	21	446		2.16	
defense response	21	389		2.82	
transcription	19	956		-2.04	
immune response	18	314		2.88	
regulation of transcription	18	920		-2.06	
macromolecule catabolism	16	323		2.08	
protein catabolism	16	319		2.13	
proteolysis and peptidolysis	16	305		2.32	
organelle organization and biogenesis	15	256		2.70	
cytoskeleton organization and biogenesis	14	213		3.08	
regulation of biological process	13	150		4.08	
muscle development	12	106		5.03	
anion transport	11	94		4.95	
inorganic anion transport	11	70		6.23	
regulation of cellular process	11	123		3.87	
phosphate transport	10	36		8.69	
protein modification	10	680		-2.51	
response to pest/pathogen/parasite	10	172		2.17	
biosynthesis	9	581		-2.19	
skeletal development	9	70		4.83	
bone remodeling	7	42		5.18	
ossification	7	39		5.46	
regulation of cell proliferation	7	89		2.68	

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The last part of this tutorial walks through the use of the “Search by Gene Ontology” feature to isolate a sub set of genes based on their biological function. This feature will be used to identify a sub set of genes with the molecular function ontology “transcription factor activity”.

- Go back to the gene list and select the Analysis link in the upper right of the page.
- Select **Gene Navigation**.
- Select **Molecular Function**.
- Select “transcription factor activity” from the pull-down menu and then click on the Search button.
- There are 26 genes with the molecular function ontology “transcription factor activity” that are differentially expressed during the period of orofacial development examined here.



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