Prenatal LMD Microarray

SEARCHING THE PRENATAL LASER MICRODISSECTION (LMD) MICROARRAY DATA

- Gene Search
- Differential Search
- Correlative Search
- Gene Classification
- Data Visualization
  - Heatmaps
  - Detailed Information Section

Three search types are available: (1) Gene Search, to obtain gene expression data for specific genes of interest, (2) Differential Search, to compare expression between two sets of brains structures, and (3) Correlative Search, to find genes that have an expression pattern similar to a "seed gene" selected from the results of a Gene or a Differential search.

Clicking on the "?” button from any search type will take you to the appropriate help section.

Gene Search

To search for probes associated with a specific gene, select the Gene Search radio button, type the gene name, gene symbol, Entrez Gene ID or probe name in the search box and click the "Search" button. Alternatively, select a category from the tag cloud. The font size of the words in the cloud correspond to the number of genes linked to each category. Genes were linked to each category using online tools such as the PANTHER.

To restrict the set of probes displayed, check the boxes next to the Gene Symbols and click the View Selection Heatmap button at the bottom of the page (see heat map figure below).

Differential Search

Another common usage of gene expression databases is to find genes that show enrichment of expression in one region compared to another region. This type of query is supported by the Differential Search mode. Select the "Differential Search" radio button. To find genes or probes with an enhanced gene expression profile in one or more structures when compared to one or more other structures, enter the target brain structure in the top search box and your contrast regions in the bottom search box. You also have the option to filter your search by donor. Once you have made your selections, click on the Search button.

To enter the structures, you can simply type the acronyms (separated by semi-colons) in the search boxes, or select them from the drop down ontology viewer.
Your search will return genes exhibiting higher expression in the target domain compared to the contrast domain. You can filter the displayed data on the heatmap by clicking the “Filter Heatmap” function below the heatmap. A menu will open allowing you to select both the structures and the developmental stages. Search results are sorted either by p-value or fold change, indicated by the arrow on the buttons over the column of genes. To alter the sort parameter, click on either the “p value” or the “fold-change” buttons.

To perform the previous search in reverse, click the toggle button to the left of the Search button.

**Correlative Search**

In using gene expression databases, a “search by example” feature is also highly desirable as genes with similar expression patterns may be related in function. The Find Correlates search utility will accomplish this function. This search by example facility is also available in the Allen Human Brain Atlas, and in the Allen Mouse Brain Atlas and the Allen Developing Mouse Brain Atlas as the NeuroBlast function.

Once you have identified a gene of interest, to find other genes with spatial expression profiles similar to your gene of interest, first select your probe by clicking on any cell in the heat map related to that probe. You will see that probe listed in the box above the right hand side of the heat map. Then select the brain structure(s) and donor(s) in which you would like to see a similar expression pattern, and click “Find Correlates”. This action will return probes with a similar expression profile in the brain region(s) and donor(s) in which you are interested.

Only regions selected for the search will be displayed. To see the search results across the entire brain, turn off the “Restrict Domains” function at the bottom of the heat map. You can see “anti-correlated” genes by toggling the sort order on column “r” or scrolling to the bottom of the heat map.

**Gene Classification**

When you are viewing heatmap data, there will be a “Gene Classification” radio button available. When the gene classification radio button is selected, you can search for enhanced gene expression from one of the categories in the drop down menu.
Data Visualization

The visualization page for the prenatal LMD microarray data is divided into two sections:

The bottom section contains the heatmap representation of the data returned by a search operation.
The top section contains probe, sample and expression information for a data point selected by clicking a location in the heat map.

Heatmaps

Based on your search, the resulting microarray data sets are presented as a matrix with brain structure and donor on the horizontal x-axis and gene probes on the vertical y-axis. On the horizontal axis, donors are represented in the first row and by the three distinct blue colors. The next row represents brain structures and are organized in ontological order. To toggle between sorting initially by structure or by donor, click the toggle button. On the vertical axis, each row represents a probe rather than a gene, since multiple probes were used to measure gene expression for a gene.

The microarray data is presented in a heat map format where the colors of the heat map correspond either to raw data or to a normalized (z-score) expression level of a probe. Default heat map colors are in the green - red scale, where green represents low expression values and red, high expression values. The window and level for the colors may be adjusted, and other color schemes may be selected by using the Color Map control under the heat map.

You can select a set of probes for later use by selecting the check-boxes beside the list of gene symbols. Your choices are stored in a browser cookie in your computer and will remain in effect until you click the “Clear Selections” button or clear your Web browser’s cookie cache. Click the “View Selections” button to see your selections.

Detailed Information Section

Clicking on a cell of the heat map populates the top section of the page with detailed information about the selected cell. This information includes our structure ontology which when you click the blue link at the bottom will take you to the Reference Atlas. It also includes Gene Information: gene symbol, gene name, probe name (when the gene or probe links are clicked you will be taken to a Gene Details page), expression values (log2 intensity and z-score), and the donor details.

The section also contains buttons to navigate to related data in other Allen Brain Atlas resources, as well as links to external pages with additional data on the specific gene and probe.
Gene Details

Clicking on the Gene Symbol or Gene Name takes you to the gene details page.

The gene details page displays information about the gene in three parts. The first lists metadata specifically about the gene, including Gene Name, Aliases, Organism, related data from other Allen Brain Atlas resources, Entrez gene ID and Chromosome.

The second part outlines information specific for the probe originally selected from the heatmap, including Probe Name, NCBI Accession Number, Probe length, Sequence Data, probe type, transcript GI identifier and GC percentage.

The third part of the gene detail page is a visual representation of gene expression throughout the brain. The first row of images includes seven representative sections from the Reference Atlas with each structure color coded as in the original heatmap. The next row of four images illustrates gene expression in a select section from each of the donors.

Navigating this structural representation of gene expression:

1. **Probe**: The dropdown menu allows you to navigate to other probes for this particular gene.
2. **Donor**: The dropdown menu allows you to navigate to other donors.
3. **Section**: Indicates which of the seven sections is portrayed in the second row of images. The dropdown menu allows you to choose which representative section is portrayed over the four different donors.
4. **Color Map Bar**: Displays different normalized (z-score) color representations of the heat map data (i.e. blue-red vs. green-red) or log2 normalized expression where the color scale ranges from dark blue, representing low expression, and passes through cyan, yellow, orange and finally to dark red, representing high expression. Clicking on the arrow will allow you to change the color map.
5. **View Heatmap**: Clicking on "View Heatmap" will take you back to the microarray heatmap data.
6. **Atlas Sections**: Seven sections from the reference atlas representing gene expression in each structure. Clicking on the link above one of the sections will select that section to be displayed in the second row if images. Hovering over a section with your mouse will bring up the ontology below the sections and indicate the name of the region/structure. Clicking on an image will highlight that structure in all of the sections that include that structure. When a structure is selected in this fashion, hovering your mouse over another structure will bring up the name and gene expression of that structure for comparison.
7. **Ontology**: The name of the structure and gene expression values of that structure for the probe and donor selected. Clicking on the link will take you to the Reference Atlas.

The second row of images shows a representative section from each of the donors to allow for comparison of gene expression values between donors.