

NEUROBIOLOGY 340

TUTORIAL 1: GENE EXPRESSION PATTERNS IN THE MOUSE BRAIN

Throughout this tutorial, you will be directed to answer questions about what you find. These questions can be found on the last page of the tutorial.

Part I: Exploring a Gene's Expression Pattern

Go to <http://portal.brain-map.org> and select Allen Mouse Brain Atlas from the options available. You can use the drop-down menu at the top of the page or search through the icons for this one:



MOUSE BRAIN ATLAS

A unique multimodal atlas of the adult mouse brain, featuring anatomic and genomic data.

[View Atlas →](#)

You will see a search page like the one below:

ALLEN BRAIN ATLAS
DATA PORTAL

HOME GET STARTED **MOUSE BRAIN** TOOLS

ISH DATA REFERENCE ATLAS AGEA BRAIN EXPLORER RELATED STUDIES DOCUMENTATION HELP

Gene Search Differential Search Fine Structure Search Bulk Search Human Differential Search

Enter Gene Name, Gene Symbol, NCBI Accession Number or Entrez Gene ID

Show exact matches only

Browse by Differential Expression

| | | |
|----------------------------|------------------------|-----------------------------------|
| Isocortex | Field CA1 | Reticular nucleus of the thalamus |
| Cerebral cortex, layer 2-3 | Field CA3 | Epithalamus |
| Cerebral cortex, layer 4 | Dentate gyrus | Hypothalamus |
| Cerebral cortex, layer 5 | Entorhinal area | Midbrain |
| Cerebral cortex, layer 6a | Striatum | Petaqueductal gray |
| Main olfactory bulb | Lateral septal complex | Pons |
| Accessory olfactory bulb | Pallidum | Medulla |
| Piriform area | Cerebellum | Inferior olivary complex |
| Hippocampal region | Thalamus | |

Allen Mouse Brain Atlas

Search the data

Use Gene Search to find ISH data for a specific gene of interest [\[more\]](#)

Use Correlation to find genes with similar spatial expression profiles to a gene selected from Gene Search returns [\[more\]](#)

About the Allen Mouse Brain Atlas

A genome-wide, high-resolution atlas of gene expression throughout the adult mouse brain

Key features:

- In situ hybridization image data with cellular-level resolution
- Anatomic reference atlas
- Advanced search and visualization tools
- Brain Explorer! 3-D viewer

[\[more\]](#)

See [publication](#) in Nature

To conduct a gene search, click the *Gene Search* radio button and enter the name of the gene in the search box. A new page will display like the one below:

ALLEN BRAIN ATLAS
DATA PORTAL

HOME GET STARTED MOUSE BRAIN TOOLS

ISH DATA REFERENCE ATLAS AGEA BRAIN EXPLORER RELATED STUDIES DOCUMENTATION HELP

Gene Search: Enter Gene Name, Gene Symbol, NCBI Accession Number or Entrez Gene ID

Differential Search
 Fine Structure Search
 Bulk Search
 Human Differential Search

Show exact matches only

Showing page 1 of 1

| Experiment | Gene Symbol | Gene Name | Probe Name | Orientati... | Plane | Expression Sum... |
|------------|-------------|---|------------------|--------------|----------|-------------------|
| 68797529 | Scn3b | sodium channel, voltage-gated, type III, beta | RP_050210_02_E10 | Antisense | sagittal | |
| 71064082 | Scn3b | sodium channel, voltage-gated, type III, beta | RP_050725_03_D01 | Antisense | coronal | |
| 73512195 | Scn3b | sodium channel, voltage-gated, type III, beta | RP_050712_03_D11 | Antisense | coronal | |
| 79762688 | Scn3b | sodium channel, voltage-gated, type III, beta | RP_071204_04_D08 | Antisense | sagittal | |

This shows the experiments available for that gene, the gene name, probe name, and the expression summary for each experiment. The top experiment in the list is a series of sagittal sections and the second one down is a series of coronal sections. The colored bars represent brain regions.

Click on the gene symbol for your gene and experiment of interest. This brings up all studies of that gene but at the top is some gene info. Write down the Entrez ID number.

| Gene Scn3b | | | |
|------------|--|------------|--------|
| Name | sodium channel, voltage-gated, type III, beta | | |
| Aliases | TC1517156, 4833414B02Rik, Scnb3, 1110001K16Rik | Entrez ID | 235281 |
| Organism | Mus musculus | Chromosome | 9 |

Scn3b - RP_050210_02_E10 - sagittal Metadata

Experiment
 Gene: Scn3b
 Probe Type: RNA
 Probe Orientation: Antisense
 Plane of Section: sagittal
 Treatments: ISH

Specimen b04-0420
 Organism: Mus musculus
 Strain: C57BL/6J
 Age: 56
 Sex: M

Related Institute Data
 MOUSE HUMAN NHP

Image Window

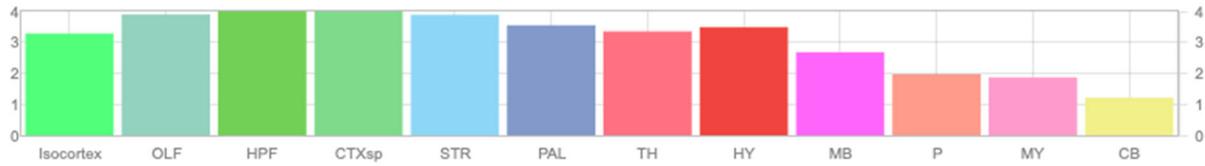
3D Viewer

Probe Info
 Type: RNA
 NCBI Accession: NM_153522.1
 GI: 23943798
 Orientation: Antisense
 Forward Primer: AAGGCCTGGAACAACCC
 Reverse primer: GCCATACAAAGCCCGATG

Gene Sequence
 Sequence: AAGGCCTGGAACAACCCGACACTTCCAAAGCCCAAGGAAGCATCCAGGACATTTTCAGTAGTTTTGAAAGGATATAACACTCCCGAGATTGGAAACAGCGATGATGG
 AA1TTTCTGGGTTGAATGCTCCTCACTTCTCAGTGTCTGTGATCAGTATTTCTCTGTTTACAGGAGCATCTGAGACTCACACAAGGCTGAACAGGATATGG
 AGGGGATTAAGCTGCCAGAGATGAGTTTCATGCTCCACTTCTACACTCCCTAAGCTTGAAGATGTTTTGAGGACTCTTAACATCAGCAGGGGAGAGCATGGGATTT
 GTCCAGCAGAGGCTACAGGTCAGATGAGACAGACTTAGGATTTACACAAGATTTGTAATTAAGCATCCTCCAAGACATGACCTGAGGGCAATAGCTATGG
 CAATAAATGTTTTAGTCTCAATGACCAATGAAACAAGAGACAAGATAGTGTGTAGCTCTAAGCTCCTCAGCTGATAGTCTATGTTGGGATTTGGTGTGTAAGATC
 CCGGAGCCCTAGCCCTGATGCCAGCCAGGAGAGCAGGGAAACAGTGAACAAGTCTTAGTCAATATCTCTTTTAAATGACTGTCTAAGTGCATATGTTTAGCCCTACA
 GATAGCAGGGCAGGAGCCCTATGATATATAAAGATGACAGTGAAGTCAAGTGAAGAGTGTGCTCACTCTGAGGAGCTATAAGAAAGCCCTCTCCACCCCAT
 CGGGCTTTGATGG

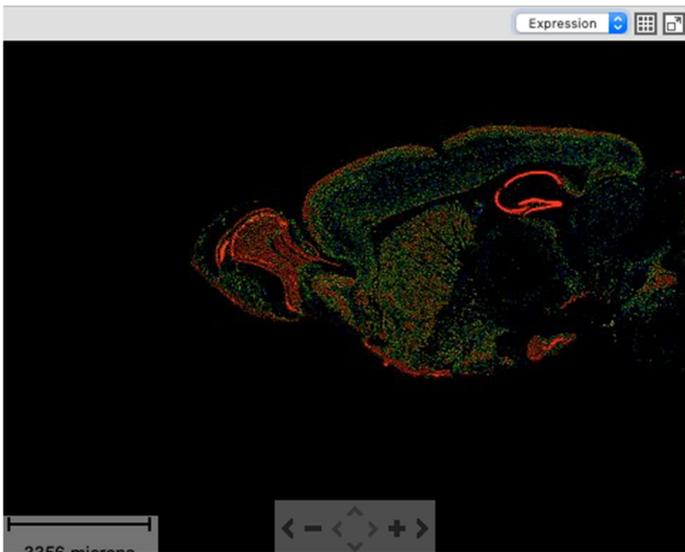
This experiment window has several sections. In the upper right is the experiment metadata (red arrow). The image window is to the right (orange arrow) with a tool bar in the upper right. Under the metadata is the 3D

viewer with an image of the brain showing regions of gene expression (blue arrow). At the bottom is the information about the probe used, the gene sequence, and the expression histogram (shown below).

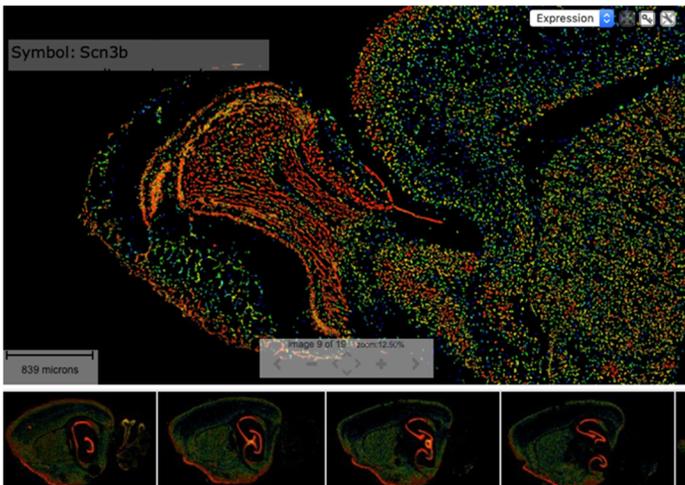


Mousing over each colored bar in the histogram changes the image window to that region of the brain. Note that these are fairly gross regions of the brain. This shows you the *In Situ* Hybridization patterns for each section of the brain. In the tools, click on ISH to bring up three view options and choose Expression. This shows the expression levels minus the background.

Notice that two areas show high expression levels for this gene (bright orange). If we want to know what brain regions those are, we can choose the tool on the far right (box with arrow). This brings you to a new high-resolution viewer window.

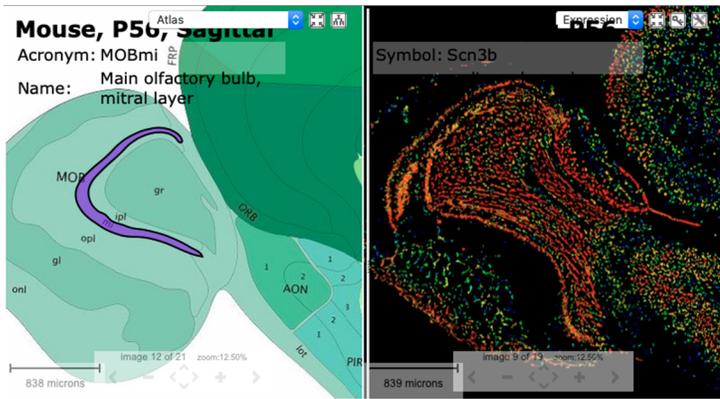


Now you can zoom into a region of high expression (as shown below).



To determine what brain region that is, we can access the brain reference atlas using the tool that looks like a key.

The new image is a split screen with the original expression view to the right and the reference atlas to the left.



The reference atlas is a color-coded map of the brain that has been verified for each region and sub-region of the mouse brain. Clicking on a colored brain region in the atlas displays the name of that region.

One region of high gene expression for SCN3b gene is the Main olfactory bulb, mitral layer (MOBmi). You should write down the acronyms (MOBmi) so you can remember it later. Repeat this for all regions of high gene expression.

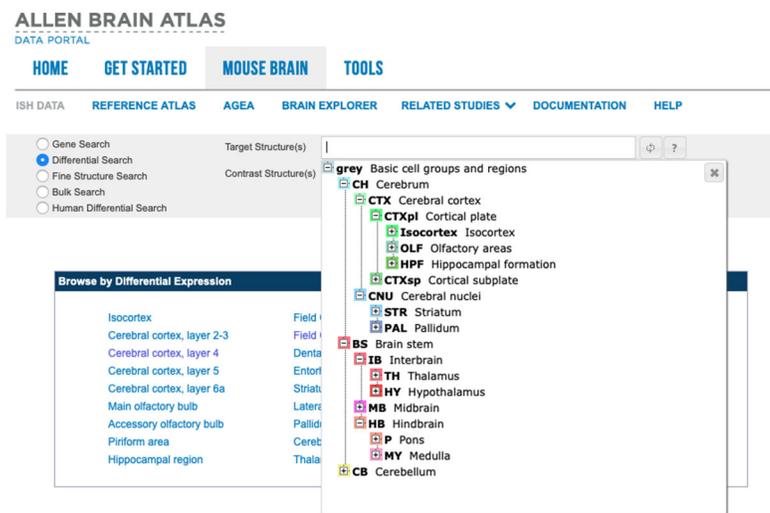
Answer questions 1-5 on the last page of this tutorial.

Part II: Exploring Gene Expression Patterns in a Brain Region

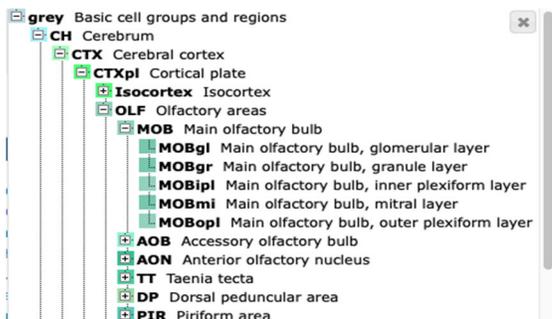
We will now explore the expression of all genes in a selected brain region, rather than searching for the pattern of one gene across the whole brain. For this use case, if you know the brain region you are interested in researching, but you don't know what genes may be expressed in that region, you can use the Differential Search option on the main Allen Mouse Brain Atlas page at <http://mouse.brain-map.org>. This search option will look for genes that are more highly expressed in your selected target brain region compared to your selected contrast region (the contrast region can be the whole brain).

Select the "Coronal data only" check box. You may want to use the "Expression threshold" slider to reduce artifacts. Slide it to the right to restrict expression to higher levels.

Check the button next to Differential Search and click in the Target Structure search box to bring up an expandable table of brain regions. Recall the abbreviation for your brain regions from part I (in this example, MOBmi and MOBgr).



The list can be expanded by clicking on the + symbols. In this example, click on the + symbol next OLF to see a dropdown list of other olfactory regions (figure below). Click on MOBmi and MOBgr to add them to the search box. You can also just type them in if you wrote them down in part I.



The contrast region will automatically be set to "grey:" the whole rest of the brain. Now click Search.

This brings up a long list of genes that are highly expressed in these two structures. They should be sorted in order of which genes are most highly expressed ("enriched") in those regions compared to all others.

Search through the list (there are many pages to the list) and find some genes that are enriched in these olfactory regions. Click on the check box for three of them and then on the View Selections button at the bottom left of the list. Be sure to write down their gene abbreviation code and names. Click on the gene name and record the Entrez ID code again for each of the three new genes.

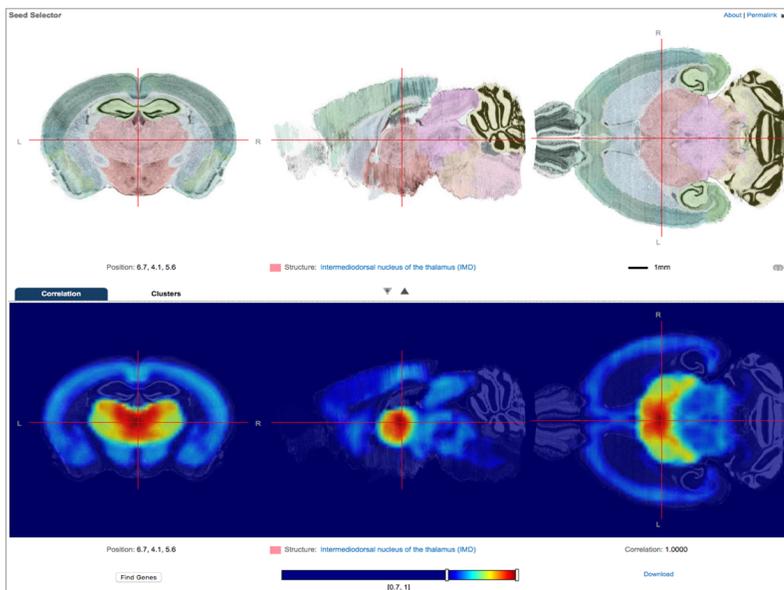
Go to <https://www.ncbi.nlm.nih.gov/gene/> and enter the gene ID number into the search field at the top of the NCBI page.

Answer question 6 on the last page of this tutorial.

Part III: Using the Anatomic Gene Expression Atlas (AGEA)

The Allen Institute has constructed a 3D viewer of the Allen Mouse Brain Atlas ISH gene expression images for the adult C57Bl/6J mouse brain. This viewer is called AGEA (or Anatomic Gene Expression Atlas). This atlas allows you to easily view and navigate the 3D spatial mouse brain maps (in Correlation mode) or search for specific genes (with Find Genes mode). Additionally, you can use Cluster mode to explore transcriptome derived brain organization.

To launch AGEA, click on the AGEA tab from the Allen Mouse Brain Atlas and you will see two rows of images like those in the following image. The AGEA shows the same data as you have been exploring up to this point in a different view.

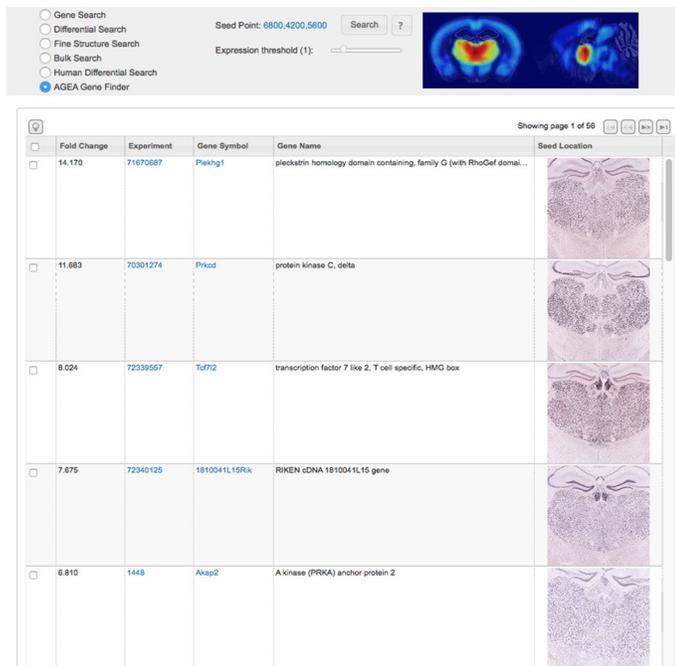


The upper row is the “seed selector” panel, a set of orthogonal views of the Nissel reference atlas. Positioning the red crosshairs over a spot selects a “seed” voxel (starting point) from which the gene expression profiles for all other brain regions are compared.

In the figure below the cross hairs are at position 6.7 (coronal), 4.1 (sagittal), and 5.6 (horizontal). The structure under the cross hairs is given as “Intermediate nucleus of the thalamus (IMD) and a scale bar is shown on the right.

The second row is the map panel. At the top are two tabs: Correlation and Clusters. The default view is Correlation. In Correlation mode, the color values are a measure of average co-expression between two voxels. Thus, red colors denote higher correlation values. This essentially means that genes expression from the seed voxel and these red voxels are more similarly co-expressed. If a high expressing region is distant from the “seed” voxel it likely means they share common cell types or functions.

Locate the Find Genes button in the lower left. Clicking this button allows you to find genes that are highly enriched in the voxel under the red cross hairs (the “seed”). It returns a list of genes enriched at the crosshairs in the upper panel.



Answer question 7 on the last page of this tutorial.

Complete these questions as you work through the tutorial.

- 1) Using other web resources (such as those used in Lab 1) describe the function of your brain regions.
- 2) Write a brief description of your gene by going to <https://www.ncbi.nlm.nih.gov/gene/> and enter the Entrez ID number into the search field at the top of the NCBI page.
- 3) Using the Allen Mouse Brain Atlas, describe two brain regions where your gene is expressed at high levels.
- 4) Briefly describe the function of these two brain regions.
- 5) Add images of your brain slices showing expression patterns for those areas you are describing above.
- 6) Write a brief description of these genes, their function, and the brain region.
- 7) For your brain region from part II, compare the genes located using AGEA with those you found using the differential search.