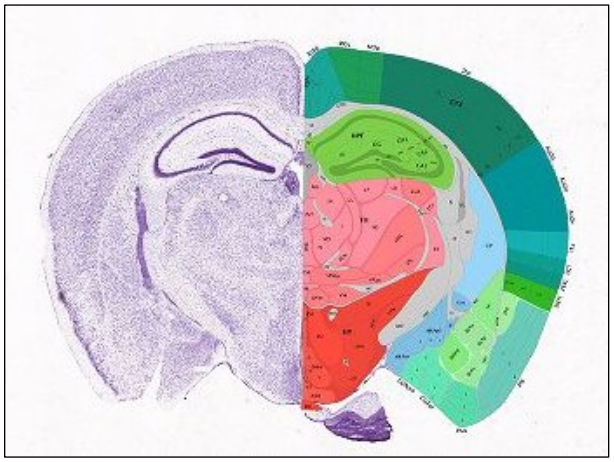
	SKILLS CENTER **STANDARD OPERATING PROCEDURE**	**A BIOFIZZ** **PRODUCTON**
Allen Brain Atlas: Comparing Animal and Human Models Using the Brain Explorer	**Effective Date: 03/01/2021**	**Revision # 1.0** **A. Siclair** **Checked: M. Guzie**

BACKGROUND

The Allen Institute, a bioscience nonprofit founded in 2003, focuses on furthering knowledge and innovation specifically in cell, immune and brain science. Each separate sector of the institute has made its own important contributions to research, including an integrated 3D cell explorer and the Allen Brain Atlas.



Allen Brain Atlas P56 coronal slice of a mouse brain

The Allen Brain Atlas project began with the original goal of mapping gene expression in the mouse brain (Gilbert, 2018). Since mice are such a common model organism, especially when studying the nervous system and its associated disorders, understanding the mouse genome and its homology to humans is extremely beneficial for research purposes. Since the dawn of its creation, the Allen Brain Atlas has expanded to include documentation of developing mice, the mouse spinal cord, adult and developing humans, and primate information (Gilbert, 2018).

The Allen Brain Atlas gives researchers many advantages when analyzing various aspects of the brain. The documentation of anatomy is important for biologists and neuroscientists who are just beginning to become familiar with nervous system structures. It provides detailed documentation of each structure on a brain slice and is a great resource to learn brain structures in a rostral to caudal order. *In situ* hybridization was utilized to create gene expression profiles for the most notable brain regions; the resulting values can be used for comparison of gene expression between animal models and humans, which will be the process explained in this procedure. It is also possible to utilize the gene expression values to determine the brain regions where the gene is most prevalent, which can assist in determining protein function. Understanding all of these components has implications in the study of the neurobiology and genetics of disease.

1. PURPOSE

The purpose of this procedure is to become comfortable with the techniques used to compare gene expression between mouse and human models using the Allen Brain Atlas.

2. SCOPE

This procedure applies to qualified skills center users.

3. RESPONSIBILITY

3.1. It is the responsibility of the user to understand and perform the procedure described in this document.
3.2. It is the responsibility of the user performing the procedure to fully document any deviations from the written procedure.
3.3. It is the responsibility of the user to become trained on the procedure.

4. DEFINITIONS

4.1. Allen Institute – A nonprofit, bioscience focused institute founded by Paul Allen in 2003 that emphasizes research in brain and cellular science.
4.2. Brain atlas – A series of various brain slices from either human or animal brains that provides anatomical information in addition to other details about specific brain sections.
4.3. Rostral – Towards the nose or beak, towards the front, anterior.
4.4. Caudal – Towards the tail, towards the back, posterior.
4.5. Sagittal – A longitudinal plane that divides the body into right and left sections.
4.6. Coronal – A vertical plane that divides the body into front and back sections.
4.7. Fold change value – The value that represents the average log2(intensity) of all samples in the target structure minus the average values in the contrast structure. It measures the degree of quantity change between final and original value.
4.8. *In situ* hybridization – An assay that utilizes nucleotide probes that quantitatively measures the presence of mRNA in a cell.
4.9. Z-score – The value that describes how many standard deviations the value falls from the mean. A positive z-score indicates a value higher than the mean, and a negative z-score indicates a value lower than the mean.

5. MATERIALS/EQUIPMENT

5.1. Allen Brain Atlas – https://portal.brain-map.org/

6. PROCEDURE

6.1. Installation of Brain Explorer

6.1.1. Go to the link to download the Brain Explorer 2 Software: https://mouse.brain-map.org/static/brainexplorer

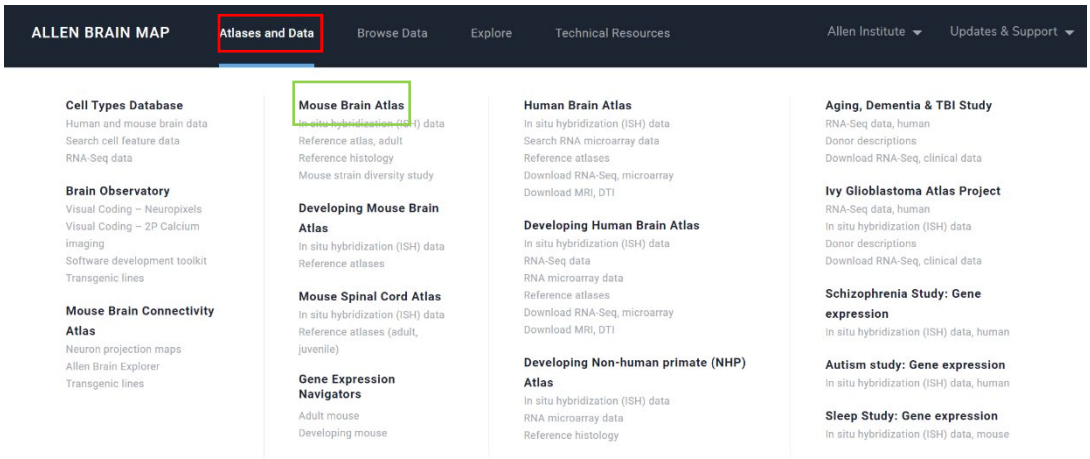
6.1.2. Download the proper software for the specific computer being used and follow all of the sequential steps until the installation is complete.

6.2. Becoming Comfortable with the Brain Explorer program

6.2.1. Go to the Allen Brain Atlas brain map website: https://portal.brain-map.org/

6.2.2. Hover over Atlases and Data and click on Mouse Brain Atlas. (Figure 1)

Figure 1

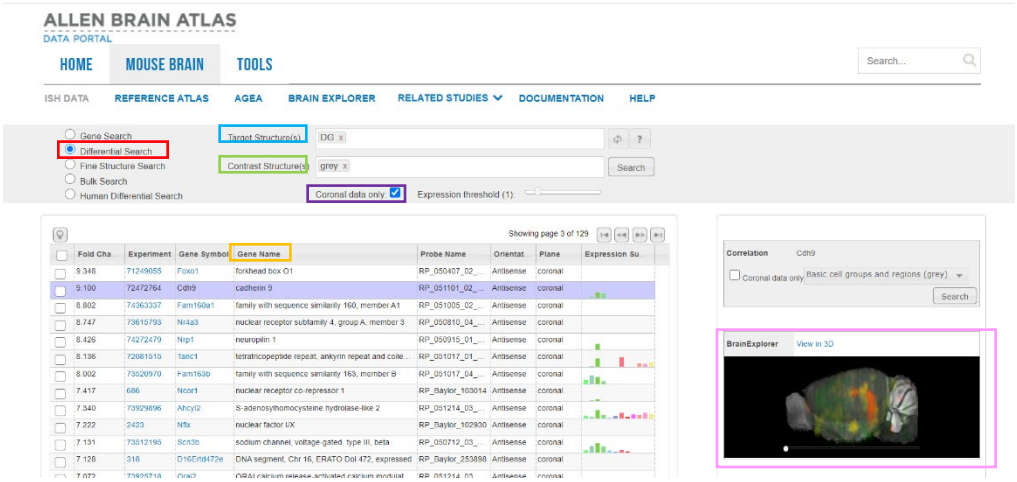


6.2.3. After selecting Differential Search, select the target brain structure of interest and search. (Figure 2)

- The dentate gyrus will be used in this example.
- Ensure that the Contrast Structure setting is marked as grey.
- Select the coronal data only box.

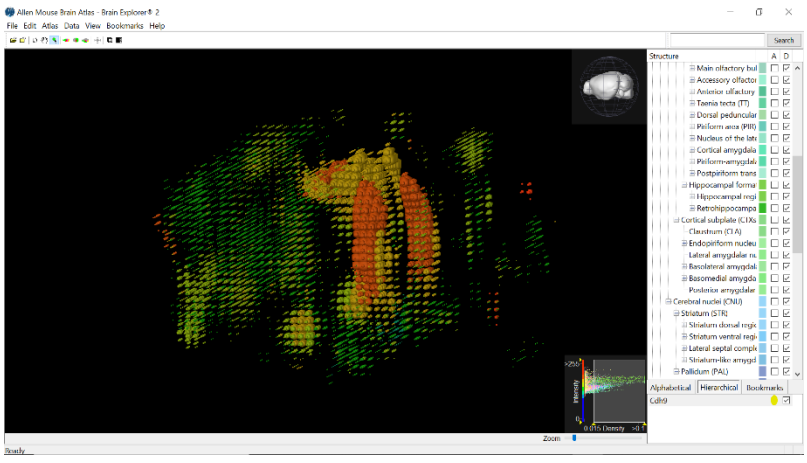
6.2.4. Scroll through the gene profiles and click on the gene name of the gene of interest. This will bring up a window to the right of the screen that showcases the Brain Explorer view. (Figure 2)
- For this example, the *Cdh9* gene will be used.

Figure 2



6.2.5. Click on the View in 3D button on the Brain Explorer view tab. (Figure 2)
6.2.6. Open the image in the Brain Explorer 2 application. (Figure 3)

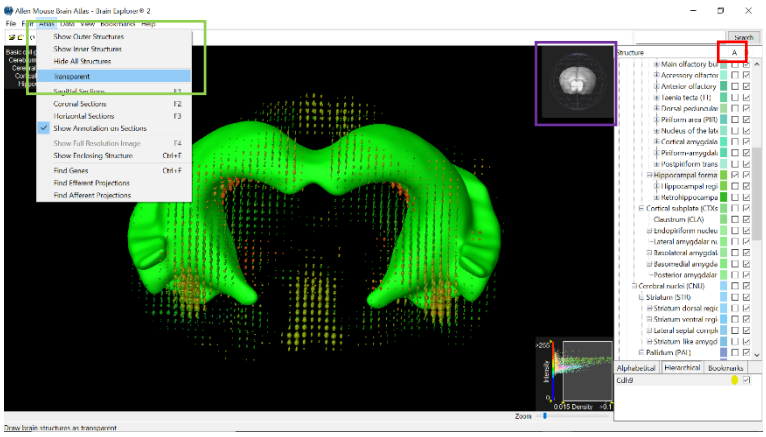
Figure 3



6.2.7. Once in the Brain Explorer application, click the desired brain structures or regions in the A column of the structure toggle to add the reference atlas structure. (Figure 4)

- The hippocampal formation was used in this example.
- The structure can be rotated by clicking and dragging the model brain in various directions.
- Make the reference atlas transparent via the Atlas and Transparent functions.

Figure 4

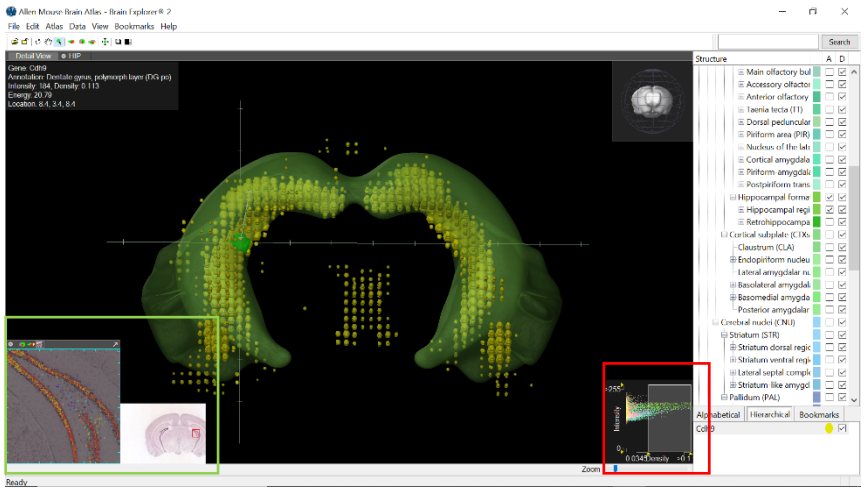


6.2.8. Moving the window of the density at the bottom makes the gene expression profile in the image more specific to dense regions if moved right, and broader to include less dense regions if moved left. (Figure 5)

6.2.9. Select a specific point on the gene expression map and make note of the gene expression annotation in the lower left-hand corner. It can be expanded into a new window to further evaluate gene expression if needed. (Figure 5)

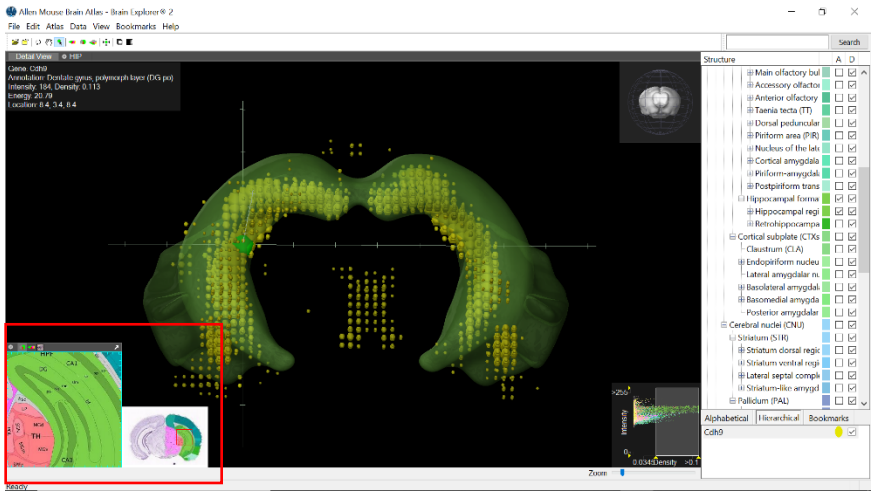
- Selecting the arrow in the top right corner of the gene expression annotation will open the experiment data for the gene. This page can be used according to the Allen Brain Atlas 1 SOP. (Figure 5)'

Figure 5



- Selecting either of the brain-shaped buttons in the lower left annotated map will bring up the reference atlas instead of a gene expression profile that can be used to evaluate the structures of interest anatomically. There are both coronal and sagittal visualizations. (Figure 6)

Figure 6

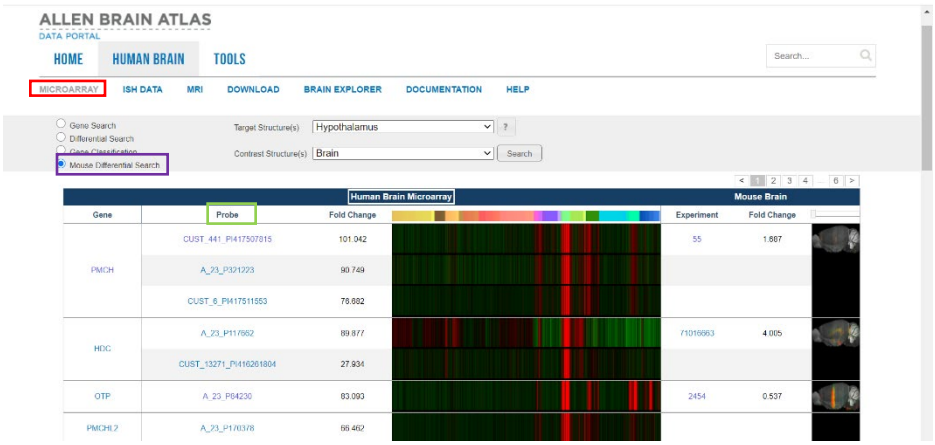


6.3. Human Brain Atlas – Mouse Differential Gene Search

6.3.1. Go to the Allen Brain Atlas brain map website: https://portal.brain-map.org/

6.3.2. Select the Human Brain Atlas this time, and make sure that the page is on the Microarray setting. (Figure 7)

Figure 7



6.3.3. Select the Mouse Differential Search function and type in the brain region of interest, with the whole brain as the contrast structure. Press search. (Figure 7)

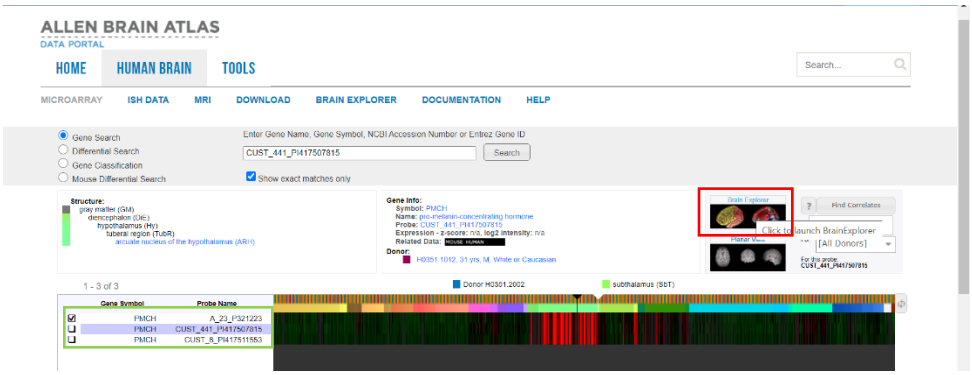
- This will open a comparison of the differential search for various genes in the mouse and human brain atlases.
- The human data will be viewed as a microarray with different probes, while the mouse data will pop up as a 3D visualization.
- The fold change values can be compared between the mouse and human data for the specific genes.

6.3.4. Clicking on the Experiment tab for a specific gene will bring up the gene expression map in the Brain Explorer application. The data can then be analyzed as described in part 6.2. of this document. (Figure 7)

6.3.5. Clicking on one of the probes for the human gene will open a new page with a heat map as seen in the Allen Brain Atlas 1 SOP. (Figure 7)

6.3.6. Select the probe of interest, click a corresponding point in the heat map and then click on the Brain Explorer tab to open the Brain Explorer application with the human data. (Figure 8)

Figure 8

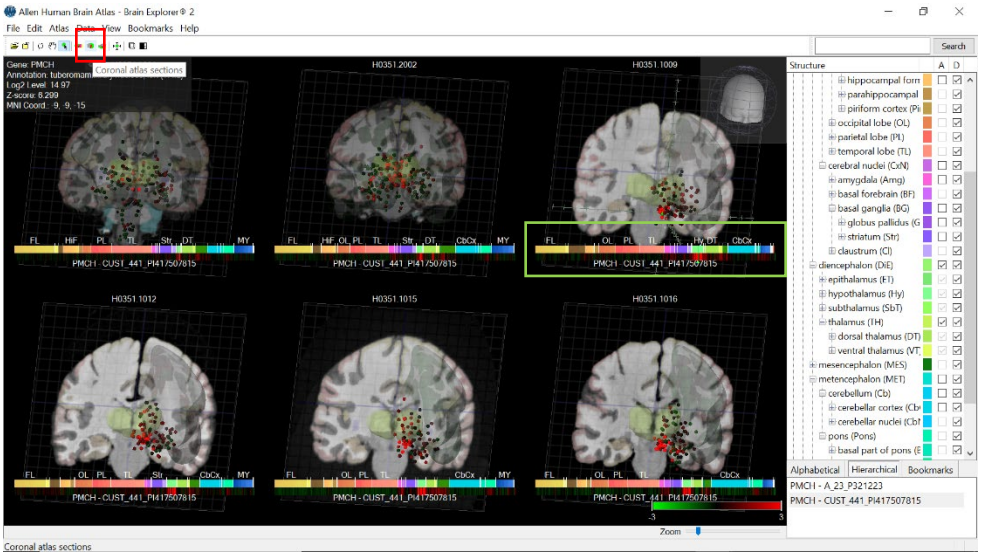


6.3.7. Add coronal slices to the brain images by clicking the coronal brain button. (Figure 9)

- Annotate the images with the same process as in section 6.2.

6.3.8. Click on a region in the region section of the heatmap on one of the images. This will bring up gene expression info in the top left corner of the screen. (Figure 9)

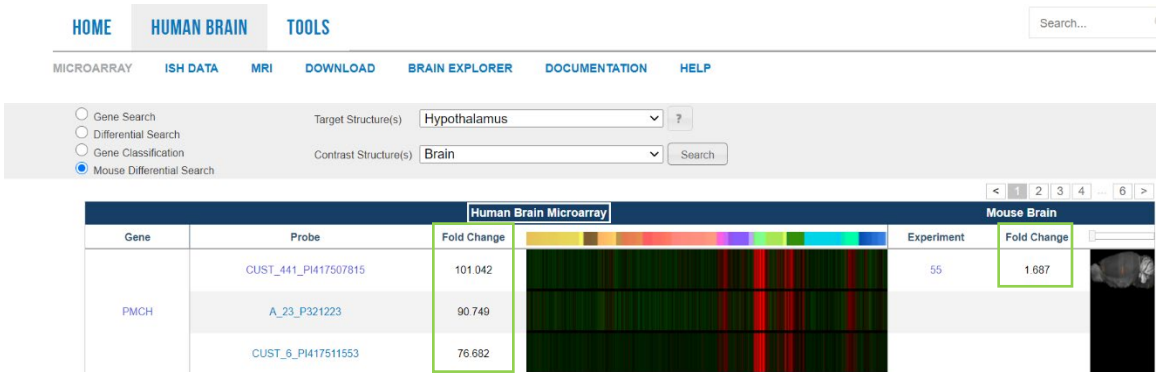
Figure 9



6.4. Comparing Human and Mouse Gene Expression

6.4.1. Return to the Mouse Differential Search in the Human Brain Atlas using the previously specified search parameters. Locate the gene of interest from part 6.3. (Figure 10)

Figure 10



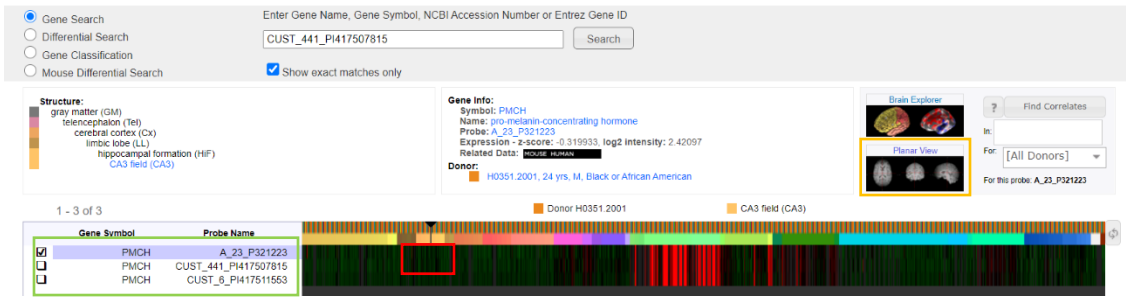
6.4.2. Comparing the Fold Change values can give some insight into variation in expression levels between the mouse and human brain. (Figure 10)

- For reference, a fold change value of 3 indicates that the expression level in the structure of interest is about 3x higher than in the reference structure.
- Note that human and mouse brain gene expression levels cannot be directly compared, as different probes and methodologies are used meaning that discrepancies exist. However, comparison can loosely be used as a tool to compare trends in the data.

6.4.3. Selecting a human probe from the differential search results will open a heat map. Click on a probe within the heat map and then Planar View to bring up the gene expression information. (Figure 11)

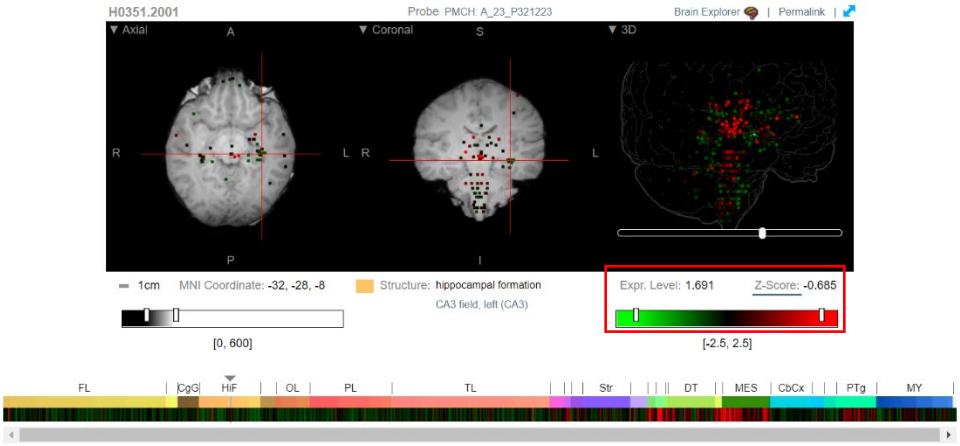
- Click within the heat map to choose the probe within a specific region.

Figure 11



6.4.4. The planar view will open a new window that specifies gene expression level data in varying brain regions. (Figure 12)

Figure 12

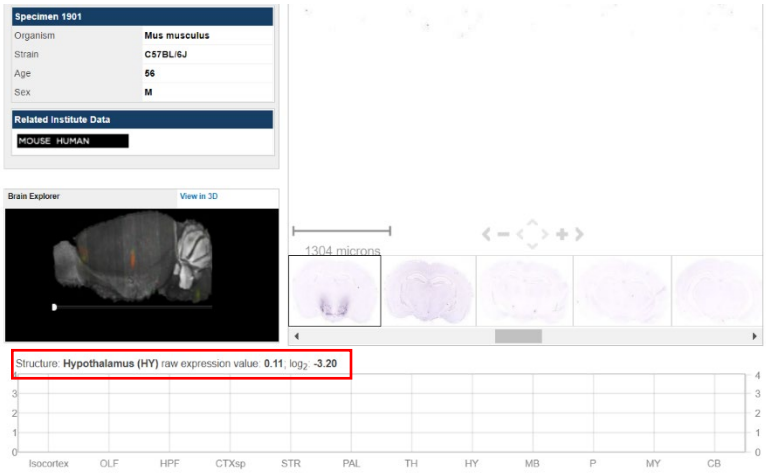


6.4.5. Selecting a mouse brain experiment from the differential search results will open a new window specific to that experiment. (Figure 10)

6.4.6. Farther down on the page is a bar chart which can be used to analyze gene expression in the mouse brain. (Figure 13)

- Note that in the example experiment, PMCH levels in the mouse brain are extremely low which is why there is a low expression value and no colored bar graphs are present.

Figure 13



7. TROUBLE SHOOTING

7.1. The Brain Explorer view is grayed out when assessing via the human brain atlas: make sure to click directly on a probe within the green and red heat map to select a probe of interest (See step 6.4.3. for figure)

8. REFERENCES

Allen Institute. (n.d.). *Allen Brain Map*. Allen Brain Institute. https://portal.brain-map.org/

[Allen Institute]. (2012, Mar.) *Tutorial: Using Differential Search* [Video]. YouTube. https://www.youtube.com/watch?v=sETee1fhT2k

Creative Proteomics. (n.d.). *Bioinformatic Fold Change Analysis Service*. Creative Proteomics. https://www.creative-proteomics.com/services/bioinformatic-fold change-analysis-service.

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Jenks, B.G. (2009). A Self Study Tutorial using the Allen Brain Explorer and Brain Atlas to Teach Concepts of Mammalian Neuroanatomy and Brain Function. *Journal of Undergraduate Neuroscience Education*, *8*(1), A21-A25. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3592706/

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9. MODULE MASTERY TASK

This quiz will test your knowledge of gene expression analysis techniques using the Allen Brain Atlas.

1. Run a differential search in the Mouse Brain Atlas and choose a gene of interest. Write down your choice for both the gene and target structure.
2. Open the gene in the Brain Explorer application. Add brain regions or structures to the map and make them transparent. Submit the resulting image.
3. Select a specific point on the gene expression map and change the gene expression annotation window so that it shows the reference atlas for that point. Name the function of one of the structures or regions present in the atlas at that point.
4. Run a Mouse Differential Search using the Human Brain Atlas. Select a gene and write it down along with the target structure.
5. What are the fold change values for the a mouse experiment and a human probe for that gene? What conclusion can be drawn from this?
6. Select a specific probe in the human data for the gene and open it in the Brain Explorer. Add coronal slices along with 2 transparent brain structures/regions of interest. Submit the resulting image.
7. Utilizing section 6.4, what is the gene expression value for your chosen region in the human brain?
8. Utilizing section 6.4, what is the gene expression value for your chosen region in the mouse brain?
9. Why do you think it is important to compare gene expression levels of a gene of interest in the mouse brain compared to the human brain?