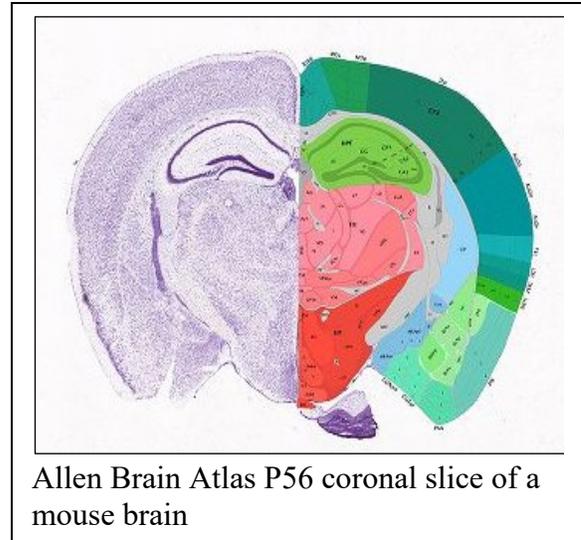


	SKILLS CENTER STANDARD OPERATING PROCEDURE	A BIOFIZZ  PRODUCTON
Allen Brain Atlas: Gene Transcription Levels	Effective Date: 02/12/2021 Checked: M. Guzie	Revision # 1.0 A. Siclair

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.

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.

	SKILLS CENTER STANDARD OPERATING PROCEDURE	A BIOFIZZ  PRODUCTON
Allen Brain Atlas: Gene Transcription Levels	Effective Date: 02/12/2021 Checked: M. Guzie	Revision # 1.0 A. Siclair

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 $\log_2(\text{intensity})$ 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/>

	SKILLS CENTER STANDARD OPERATING PROCEDURE	A BIOFIZZ PRODUCTON 
Allen Brain Atlas: Gene Transcription Levels	Effective Date: 02/12/2021 Checked: M. Guzie	Revision # 1.0 A. Siclair

6. PROCEDURE

6.1. Mouse Brain Analysis

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

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

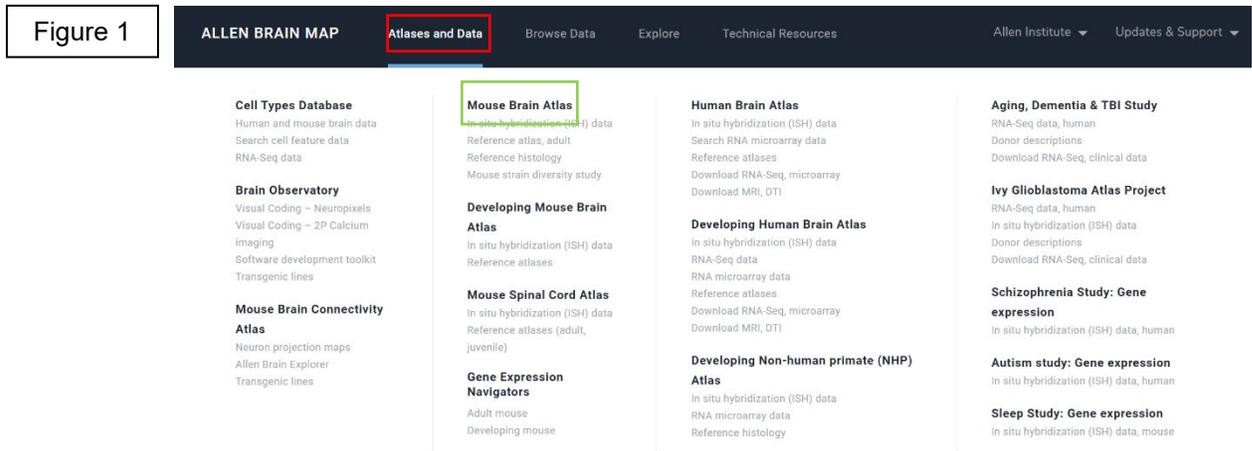


Figure 1

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

- Ensure that the **Contrast Structure** setting is marked as grey.

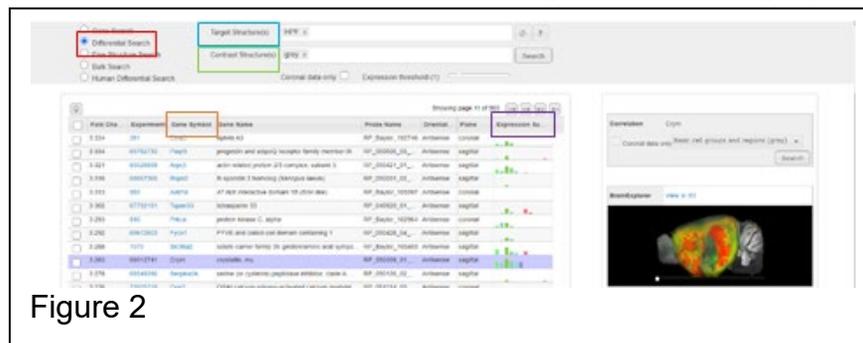


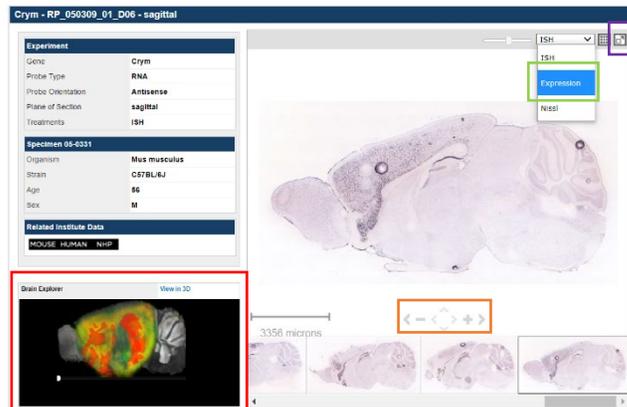
Figure 2

- Utilizing the Gene Search setting is more applicable if you already have a gene in mind. Today we will use the differential search since there is more versatility.

	SKILLS CENTER STANDARD OPERATING PROCEDURE	A BIOFIZZ  PRODUCTON
Allen Brain Atlas: Gene Transcription Levels	Effective Date: 02/12/2021 Checked: M. Guzie	Revision # 1.0 A. Siclair

- 6.1.4. Scroll through the gene profiles and choose a gene that has both a relatively large fold change value and an **expression summary** that includes multiple different brain regions. (Figure 2)
- The presence in multiple brain regions is denoted by multiple bars in the expression summary graph.
 - For this example, the *Crym* gene will be used.
- 6.1.5. Clicking the **Gene Symbol** link will provide access to the data from all of the Allen Institute projects involving the gene. The brain slices will either be displayed in a sagittal or coronal manner. (Figure 2)

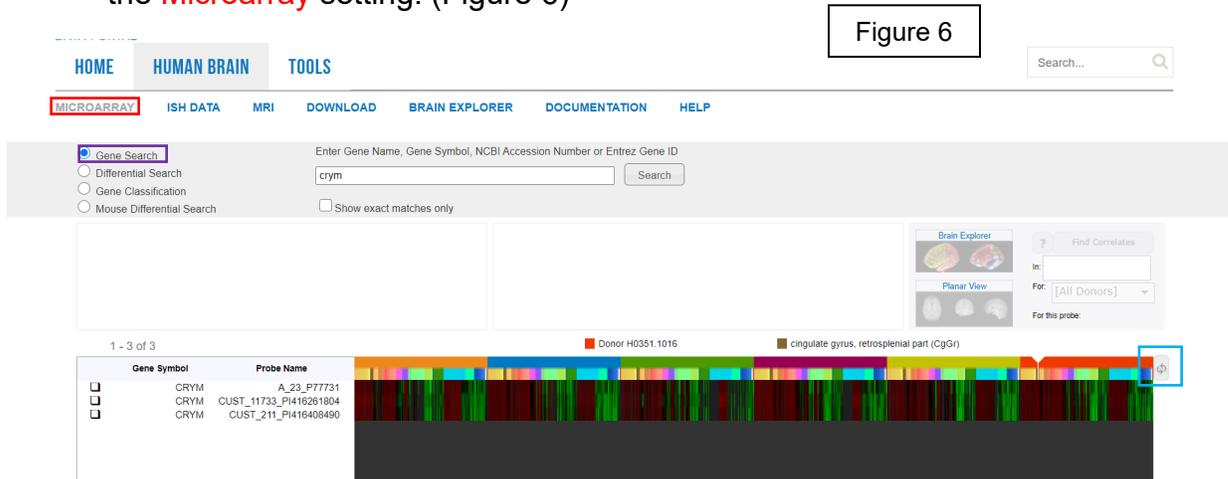
Figure 3



- 6.1.6. Once viewing the experiment data for the specified gene, there are a few more key features that allow for analysis of gene expression. (Figure 3)
- The **Brain Explorer** feature allows for a rotational view of the gene expression pattern using the white rotator knob.
 - The **+ and - symbols** allow for zooming in and out to closely examine the brain slice
 - Clicking the dropdown bar and selecting **Expression** allows for removal of any background signal which helps with analysis. Warmer colors mean a higher signal is expressed.
 - Clicking the **high-resolution viewer button** will allow for the image to open in a separate, enlarged window that can be used for further detailed analysis. Here, measurements can be documented using the scale in the bottom left corner of the image.

	SKILLS CENTER STANDARD OPERATING PROCEDURE	A BIOFIZZ PRODUCTON 
Allen Brain Atlas: Gene Transcription Levels	Effective Date: 02/12/2021 Checked: M. Guzie	Revision # 1.0 A. Siclair

6.2.2. Select the Human Brain Atlas this time, and make sure that the page is on the **Microarray** setting. (Figure 6)

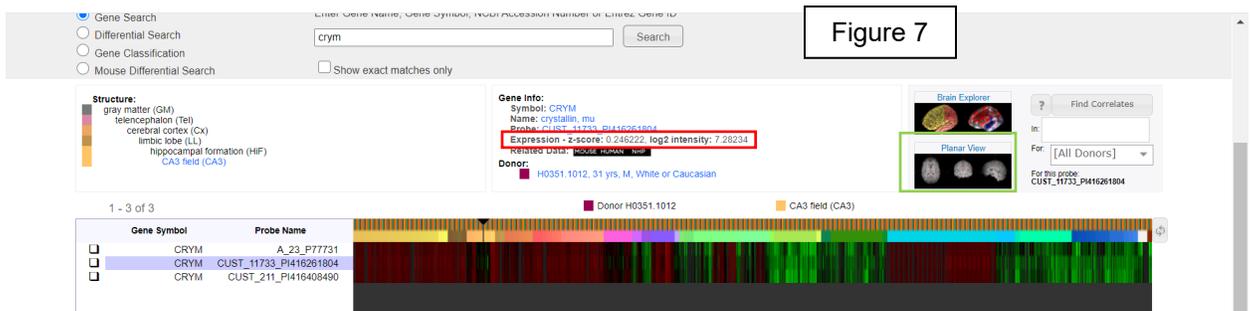


6.2.3. Select the **Gene Search** function and type in the gene previously used in the Mouse Brain Atlas. (Figure 6)

- A heat map will pop up as seen in the figure.
- The top row of colors = donor
- The second row of colors = structure in the brain
- The rest of the rows = gene data
- The **toggle button** in the top right corner of the heat map allows for organization by structure instead of by donor.

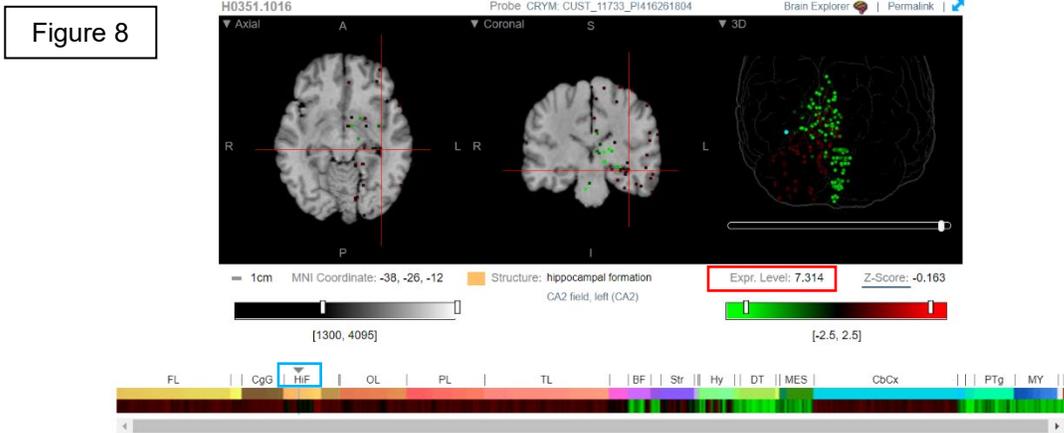
6.2.4. Clicking on one of the data points will bring up information about that single probe. (Figure 7)

- Some information regarding **gene expression** for the probe can be found here. This can either be read as a Z-score or as log2 intensity.



	SKILLS CENTER STANDARD OPERATING PROCEDURE	A BIOFIZZ  PRODUCTON
Allen Brain Atlas: Gene Transcription Levels	Effective Date: 02/12/2021 Checked: M. Guzie	Revision # 1.0 A. Siclair

6.2.5. Clicking on the **planar view** for the probe will open a heat map, MRI and image viewers that are synced with each other. (Figure 7)



6.2.6. The top row of the heat map designates structures, while the bottom portion of the heat map describes the gene expression levels within the structures. Shifting the **small arrow** within the structure row will shift the location that is being analyzed for gene expression levels. (Figure 8)

- Change in analysis of gene expression can also be accomplished by moving the red lines in the MRI image.
- The **expression level** value can be compared to the mouse brain value.

7. TROUBLE SHOOTING

7.1. The gene chosen in the Mouse Brain Atlas is not expressed in the Human Brain Atlas: This could mean that there is no homology between the chosen mouse gene and the human genome. The NCBI HomoloGene site can be useful to find homologous genes to analyze. <https://www.ncbi.nlm.nih.gov/homologene>

8. REFERENCES

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	SKILLS CENTER STANDARD OPERATING PROCEDURE	A BIOFIZZ  PRODUCTON
Allen Brain Atlas: Gene Transcription Levels	Effective Date: 02/12/2021 Checked: M. Guzie	Revision # 1.0 A. Siclair

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	SKILLS CENTER STANDARD OPERATING PROCEDURE	A BIOFIZZ  PRODUCTON
Allen Brain Atlas: Gene Transcription Levels	Effective Date: 02/12/2021 Checked: M. Guzie	Revision # 1.0 A. Siclair

9. MODULE MASTERY TASK

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

1. Choose a gene of interest and write down your choice. Click on the gene symbol in the Mouse Brain Atlas.
2. Use the high-resolution feature to open the mouse brain slice in a new window and measure part of the image of your chosen gene and brain region. Save and submit the image in both ISH and Expression view.
3. Document the gene expression values for 2 of the mouse brain regions in one of the experiments shown for your gene.
4. Document the expression level value from the Human Brain Atlas for the same gene and brain regions that you chose for the Mouse Brain Atlas.
5. Play around with the features of the brain slice depictions at the bottom of the Human Brain Atlas planar view. Submit a picture of your chosen image.
6. Was the gene expression level similar in the mouse compared to the human brain? If not, explain the differences.