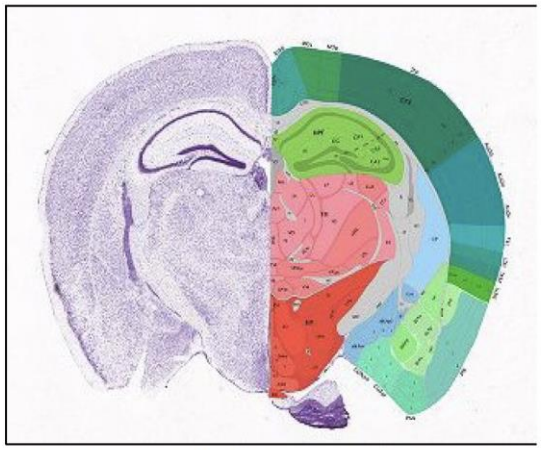
	SKILLS CENTER **STANDARD OPERATING PROCEDURE**	**A BIOFIZZ** **PRODUCTON**
Allen Brain Atlas 1: Gene Transcription Levels	**Effective Date: 02/12/2021** **Checked: M. Guzie**	**Revision # 1.1 A. Siclair edits: MK**

BACKGROUND

The Allen Institute, a bioscience nonprofit founded in 2003 initially focused on mapping gene activity in the mouse brain (see Gilbert, 2018), Since then its has expanded to include studies of "the human immune system; inner workings of our cells; and identifying transformative, paradigm-shifting science around the world". Each 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

Studying human brain development and function is ethically and experimentally challenging. Given their evolutionary relationship laboratory mice (a placental mammal like humans) have been invaluable in a range of biological studies as a powerful model organism. This has been particularly true for studies of the nervous system and its associated disorders, understanding where genes are expressed and how they influence the nervous system has lead to insights into the expression and function of homologous genes in humans. Since its inception, the Allen Brain Atlas has expanded to include gene expression during mouse and primate neural development, including the spinal cord and adult and developing humans (Gilbert, 2018).

a comparison of mouse & human brains

The Allen Brain Atlas gives researchers valuable tools for analyzing various aspects of brain development, gene expression, connections, and their functional roles in health and disease. The Atlas provides detailed documentation for the various brain structure, enabling the neuroscientists to gain familiarity with them. At the same time the *in situ* hybridization (ISH) technique can be used to visualize patterns of gene expression in different brain regions. Combining the two techniques (anatomical sections and ISH) makes possible comparisons of patterns of gene expression between homologous neural regions in animal models and humans. This module will introduce you to both while helping you learn how to navigate and use the Allen Brain Atlas with the goal of informing studies of human nervous system development, function, and dysfunction. Understanding these components has implications in the study of the neurobiology and genetics of disease.

	SKILLS CENTER Standard OPERATING Procedure **Allen Brain Atlas #1**	**A BIOFIZZ** **PRODUCTON**
Allen Brain Atlas: Gene Transcription Levels	Effective Date: 02/12/2021 Checked: M. Guzie	**Revision # 1.0 A. Siclair edits: MK (10/24)**

1. PURPOSE

The purpose of this module 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 sequential brain slices that provides anatomical information in addition to other details about specific brain regions.

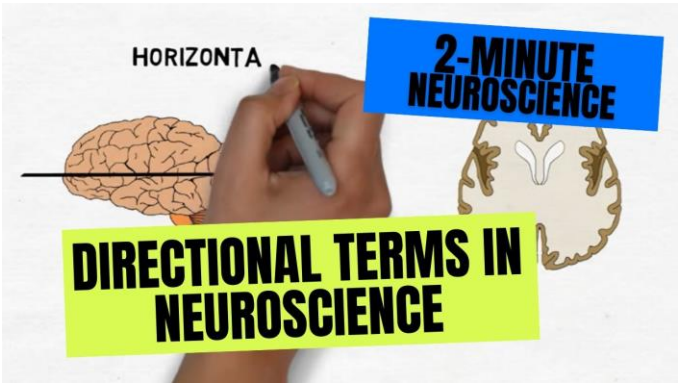
4.3. Directional terms in neuroanatomy: A good youtube introduction (→)

Rostral – Towards the nose or beak, towards the front, anterior.

Caudal – Towards the tail, towards the back, posterior.

Sagittal – A longitudinal plane that divides the body into right and left sections.

Coronal – A vertical plane that divides the body into front and back sections.



4.4. ***In situ* hybridization** (ISH) – An assay that utilizes the binding of short chemically modified "anti-sense" RNA probes to mRNAs to visualize the presence of specific mRNAs in specific brain regions (or in individual cells at higher magnifications).

4.5. **Fold change value** – The value that represents the average log2(intensity) of th ISH signal in a region minus the average values of a non-specific (control) anti sense RNA probe. It correlates with the expression (mRNA level) of a specific gene in a particularly region. .

4.6. **Z-score** – A 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. (a good introduction →)

5. MATERIALS/EQUIPMENT

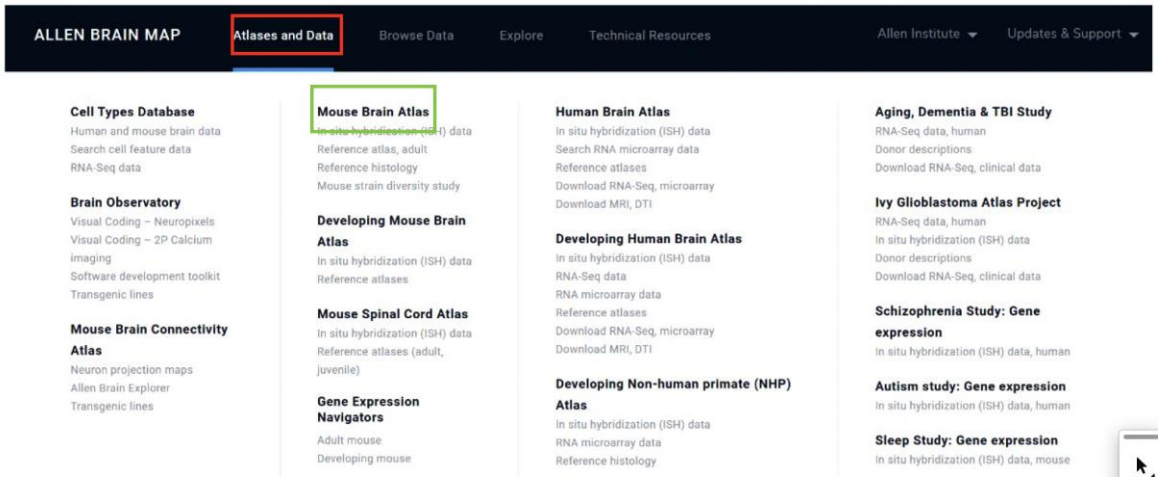
5.1. Allen Brain Atlas – https://portal.brain-map.org/

6. PROCEDURE

6.1. Mouse Brain Analysis

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

Figure 1



6.1.2. Hover over Atlases and Data and click on Mouse Brain Atlas. (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.
- Utilizing the Gene Search setting is applicable if you have a specific gene in mind. Today we will use the differential search since there is more versatility.

6.1.4. Scroll through the gene profiles and choose a gene that has both a relatively large

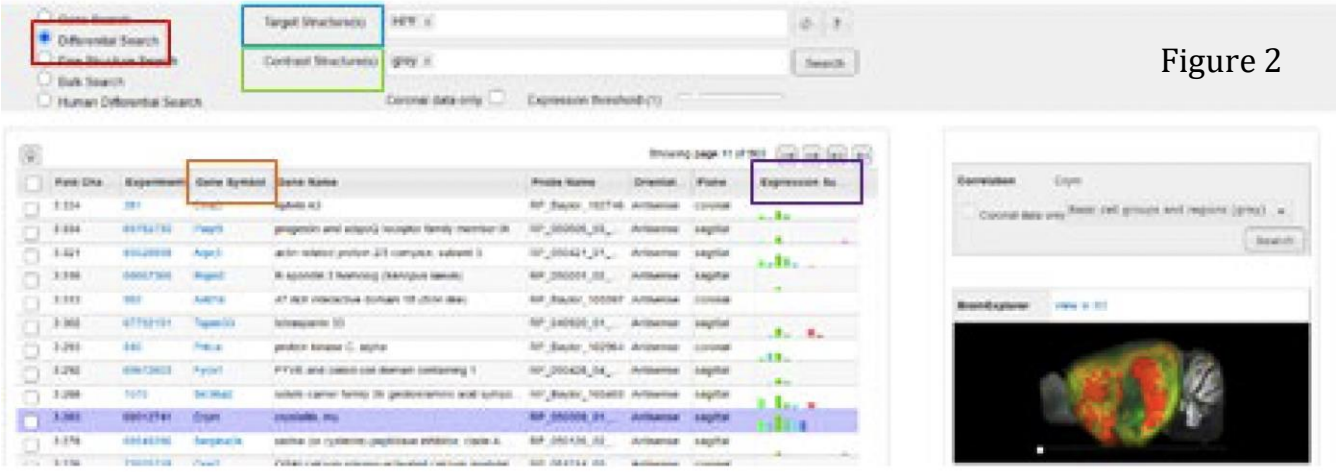


Figure 2

fold change value and an expression summary that includes multiple different brain regions. (Figure 2 ↑)

- The presence for a gene's expression 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↑)

6.1.6. Once viewing the experiment data for the specified gene, there are a few more key

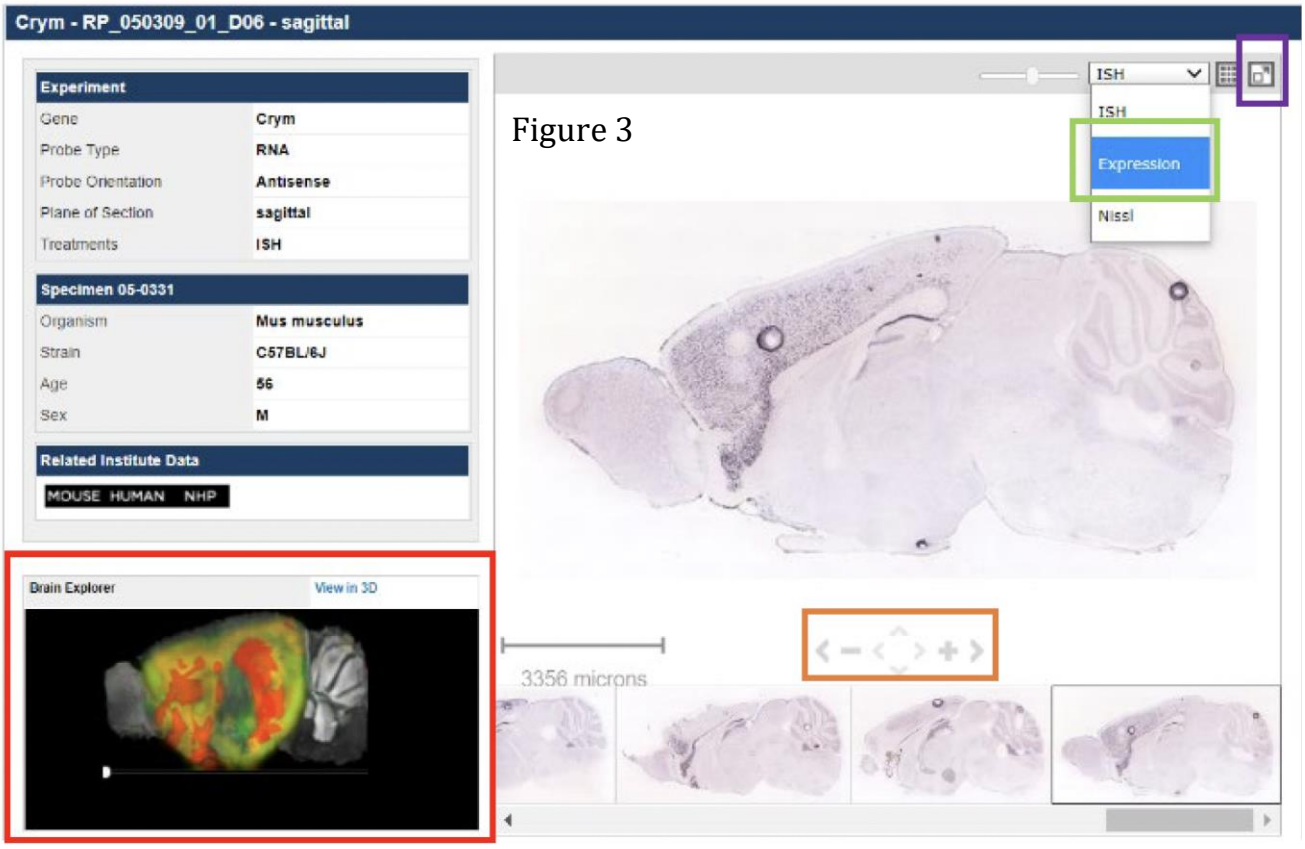
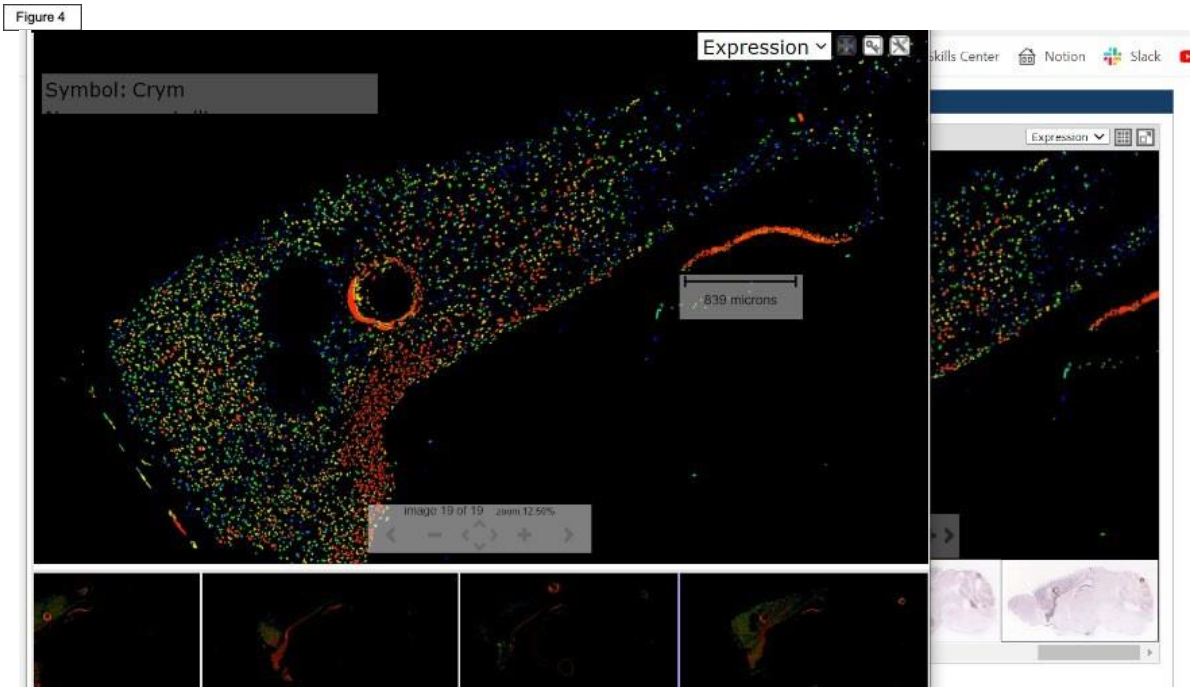


Figure 3

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.

Figure 4



6.1.7. Upon returning to the main screen and scrolling down, a bar graph will come into view. This graph denotes the gene expression in twelve of the major structures in the mouse brain. Bar height reflects relative gene expression (mRNA) levels.

(Figure 5 ↓)

6.2. Human Brain Analysis

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

6.2.2. Select the Human Brain Atlas this time, and make sure that the page

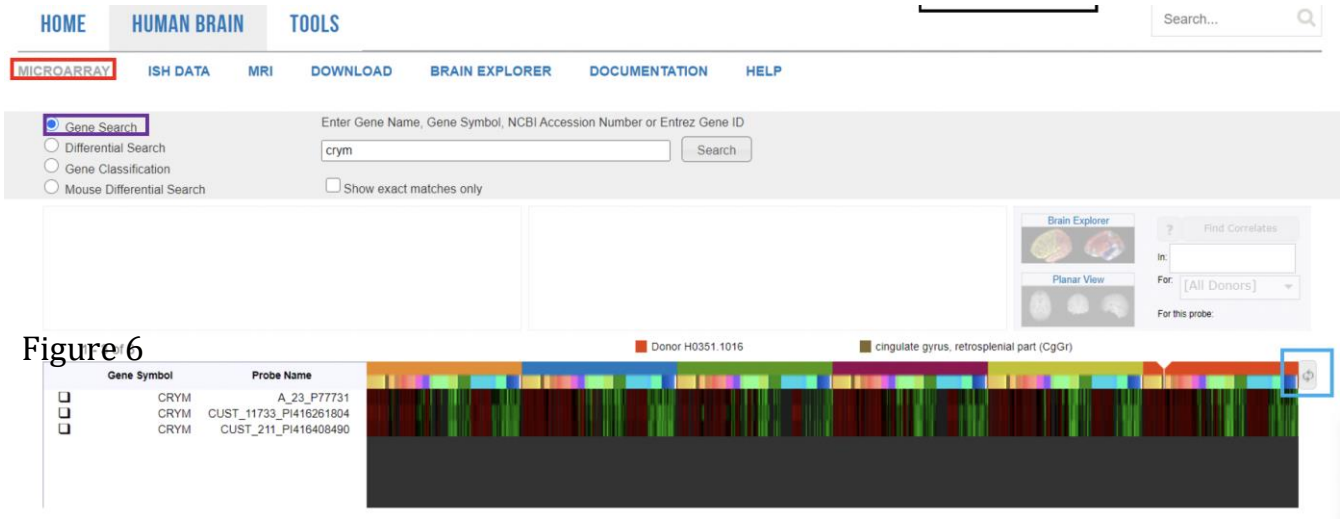


Figure 6

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.

Figure 7

This can either be read as a Z-score or as log2 intensity.

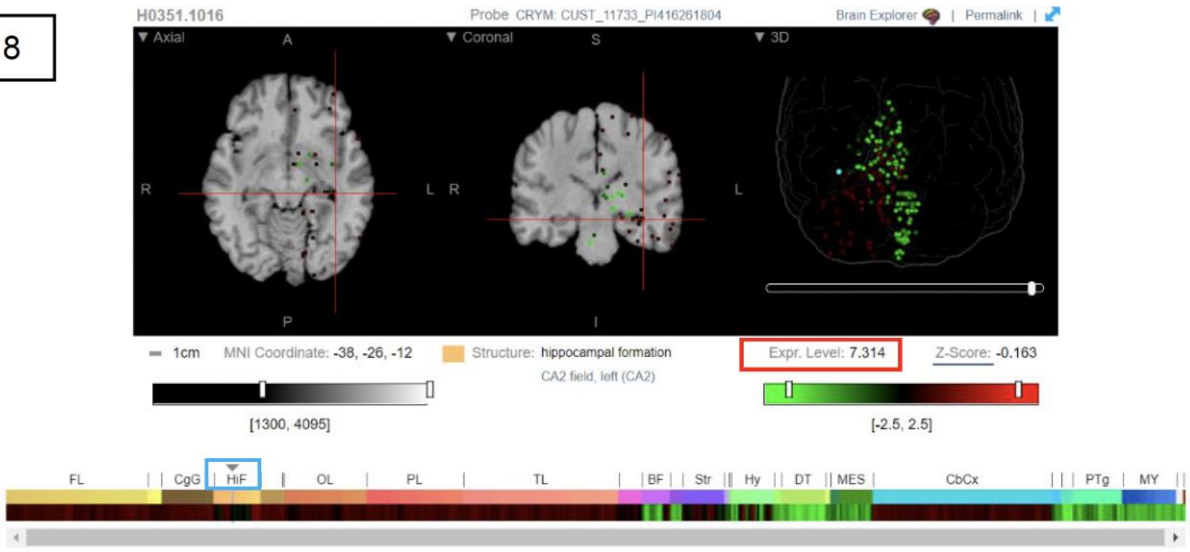
6.2.5. Clicking on the planar view for the probe will open a heat map, MRI and image viewers that are synced with each other. (Figure 7 ↑)

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.

Figure 8



- The expression level value can be compared to the mouse brain value.

7. TROUBLE SHOOTING

7.1. It is possible that the gene you chose from in the Mouse Brain Atlas is either not presence in humans, is not expressed in the brain, or has not been examined in the Human Brain:

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 (i.e. genes sharing a common evolutionary ancestor) to analyze. https://www.ncbi.nlm.nih.gov/homologene

Link to site for looking up the phenotypes of knock-out mutations in mouse (and mutations / alleles of genes in the human (gNomad)

8. REFERENCES

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9. MODULE METHODS TASK

This task 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. Explore 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.