In Situ Hybridization (ISH) Data

**IN SITU** Hybridization (ISH) Data

- Searching
  - Gene Search
  - Search by Study
  - Gene Classification
- Viewing Images
  - Experiment Details Page
  - Zoom and Pan (ZAP) Image Viewer
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  - Specimen Detail Information

Gene expression data is available as colorimetric *in situ* hybridization (ISH) images for specific brain regions. Images are grouped into *image series* that comprise an experiment. An experiment consists of a sequence of slides from the same *specimen* and that receive the same treatment, whether it is Nissl staining or ISH with a probe for a particular *gene*.

ISH data is available for five distinct studies: The Cortex Study (1,000 Gene Survey in Cortex), Schizophrenia Study, Autism Study, Subcortex Study and Neurotransmitter Study. Please refer to the *in situ* hybridization white paper in the Allen Human Brain Atlas Documentation tab for details.

Choose among the following options for retrieving the data:

- Gene Search
- Search by Study
- Search by Gene Classification

Searching
You can search for enhanced gene expression in various categories by selecting a category from the tag cloud or by selecting multiple categories from the Gene Category drop down menu. The font size in the tag cloud is proportional to the number of genes associated with that category. The categories were created by online search engines such as PANTHER.

**Gene Search**

You can search for experiments associated with a gene using its gene symbol, name, or Entrez Gene ID. First, select the "Gene" radio button, type the search term in the input box and click the “Search” button. You will be offered suggestions to choose from while you type, but you can also search by typing the first three or more letters in a gene name or symbol and appending an asterisk (*) as a wildcard.

**Search by Study**

Data includes ISH experiments from five separate projects including: Neurotransmitter Study, 1,000 Gene Survey in Cortex (Cortex Study), Subcortex study, Schizophrenia Study and Autism Study. Please refer to the *in situ* hybridization white paper and the gene list under the Allen Human Brain Atlas Documentation page for details. A brief description of the study methods are included on the landing page for each study.
You can restrict your search to a single study by selecting the relevant radio button: Neurotransmitter Study, Cortex Study, Subcortex Study, Schizophrenia Study or Autism Study. You will then have a choice to limit your search based on additional criteria such as sex of the donor, hemisphere, etc., depending on the study. Once a radio button for a specific study is selected, you will have the choice to select experiments based on one or more gene categories from the drop-down menu. While in the Subcortex or Neurotransmitter Study (by selecting the appropriate radio button), you also have the option to filter your gene search by structure.

Type a search term in the input box and click the "Search" button. Clicking the "Search" button without an entry in the input box will return the complete list of genes and the accompanying data for that study.

The following search criteria can be specified in the text box to the right of the categories. If you copy and paste in a list of terms delimited by tabs or carriage returns they will automatically be converted into a list of search criteria separated by the OR operator ( | ).

- Gene symbol
- Gene name
- Entrez gene ID
- Homologene Group ID
- NCBI accession number
- Probe name

**Boolean Syntax**

The following special operators can be used to build queries:

<table>
<thead>
<tr>
<th>Operator</th>
<th>Example Search Type</th>
<th>Example Query</th>
</tr>
</thead>
<tbody>
<tr>
<td>AND operator: &amp;</td>
<td>Gene</td>
<td>dopamine &amp; receptor</td>
</tr>
<tr>
<td>OR operator:</td>
<td>Gene</td>
<td>ABAT</td>
</tr>
<tr>
<td>NOT operator: !</td>
<td>Gene</td>
<td>&quot;dopamine receptor&quot; !DRD3</td>
</tr>
</tbody>
</table>

- AND, OR and NOT may be used in place of their corresponding operators. They must be upper case.
- The AND operator (&) is implicit, so spaces between words that are not separated by an operator will be treated like an &.
- OR () has higher operator precedence than AND ( &).
- Parenthesis can be used to group criteria, but nested parenthesis are not supported at this time.
- The NOT operator (!) is not supported within parenthesis.

**Gene Classification**

At any time during your visit to this website, you can search on the categories in the tag cloud by selecting the "Gene Classification" radio button and then selecting a category from the drop down menu.
Gene Search Returns

Your search will return a list of specimen blocks which include experiments that fit your search criteria, and an interactive visual display to provide structural context of where the specimen was sampled from. Use the slider bar to rotate the top image, and the drop-down menu to view either coronal or sagittal planes in the lower image. You can filter the search results by clicking on one or more blocks from the visual display. To select more than one block, press Shift and click on the block. Click on the blocks a second time to deselect them or click on the "Clear Selections" button below the display.
The list of experimental returns includes:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Study</th>
<th>Donor</th>
<th>Tissue Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>78958040</td>
<td>Ctx</td>
<td>H07-0043, 31 yrs, Male, control</td>
<td>Visual cortex</td>
</tr>
<tr>
<td>78958955</td>
<td>Ctx</td>
<td>H07-0043, 31 yrs, Male, control</td>
<td>Temporal cortex</td>
</tr>
<tr>
<td>78489968</td>
<td>Ctx</td>
<td>H07-0072, 42 yrs, Male, control</td>
<td>Temporal cortex</td>
</tr>
<tr>
<td>80103298</td>
<td>Ctx</td>
<td>H08-0021, 30 yrs, Female, control</td>
<td>Visual cortex</td>
</tr>
<tr>
<td>80103338</td>
<td>Ctx</td>
<td>H08-0021, 30 yrs, Female, control</td>
<td>Temporal cortex</td>
</tr>
<tr>
<td>80431340</td>
<td>Ctx</td>
<td>H08-0032, 24 yrs, Male, control</td>
<td>Visual cortex</td>
</tr>
<tr>
<td>80431363</td>
<td>Ctx</td>
<td>H08-0032, 24 yrs, Male, control</td>
<td>Temporal cortex</td>
</tr>
</tbody>
</table>

Clicking on this link will take you to the Specimen Details page.

Column Description

- **Specimen**: Clicking on this link will take you to the Specimen Details page.
- **Study**: Indicates which ISH Study the sample is from.
- **Donor**: Donor Information including: Donor ID, Age, Gender and Disease State.
- **Tissue Location**: General location from where the experiment was sampled.

Clicking on the donor will open a panel on the right with more metadata regarding your specimen.

This panel includes demographic information of the donor including their race, gender, age, the tissue location, handedness and any relevant conditions. If your search was not gene specific, all genes that were assayed on this specimen block will be listed under “Related genes that match search criteria:”. You can open the Specimen Detail Information page by clicking the link labeled “Open specimen ISH and details page” or you can directly view the experimental detail by clicking on the gene abbreviation.

Viewing Images

**Experiment Details Page**

Clicking on the Experiment ID from the search results page will take you to an experimental details page which includes metadata on the experiment, the specimen and the probe as well as related institute data links and a ZAP viewer for all images in the series.
This data can be downloaded as an XML file by clicking the link in "This data is also available as XML".

Zoom and Pan (ZAP) Image Viewer

The ZAP image viewer allows you to navigate through an image series' thumbnails to select an image to view at higher resolution. Once selected, you can manipulate it with your mouse and use the keyboard or too bar to take additional actions. There are also navigation tools in the main viewer that will allow you to zoom and pan using your mouse.

The gene symbol or treatment type is displayed in the title bar along with the image series ID. Additional details are displayed across the top of the viewing area, including but not limited to the tissue index and tissue location.

 Thumbnails for the entire image series are displayed across the bottom of the viewer in section order. Click a thumbnail to select it for viewing, or use the k eyboard to navigate through the set. The current selection is outlined in black.

Scale Bar

Shows the current viewing resolution of the image, in microns. This value dynamically changes as you zoom in/out of the image. You can position the scale bar anywhere on the main image by dragging the scale bar by its ruler.
You can toggle the orientation of the scale bar from horizontal to vertical by clicking on the scale bar text.

**Toolbar**

<table>
<thead>
<tr>
<th>Icon</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjust contrast on the main image</td>
</tr>
<tr>
<td>ISH</td>
<td>Select ISH, Nissl, or Expression image type</td>
</tr>
<tr>
<td></td>
<td>View image series in contact sheet form</td>
</tr>
<tr>
<td></td>
<td>Show current image in a High Resolution Image Viewer</td>
</tr>
<tr>
<td></td>
<td>Open a new window to view the image series’ details</td>
</tr>
<tr>
<td></td>
<td>close the current image viewer</td>
</tr>
</tbody>
</table>

**Keyboard Commands**

<table>
<thead>
<tr>
<th>Key</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>Advance to the next image in the series</td>
</tr>
<tr>
<td>D</td>
<td>Go back to the previous image</td>
</tr>
<tr>
<td>R</td>
<td>Advance to the last image in the series</td>
</tr>
<tr>
<td>E</td>
<td>Go back to the first image in the series</td>
</tr>
</tbody>
</table>
**Expression Mask Colors**

The Expression mask image display highlights those cells that have the highest probability of gene expression using a heat map color scale (from low/blue to high/red).

**High Resolution Image Viewer**

**Side-by-Side Nissl Viewing**

Once you click on the full screen viewer button, you will be taken to a screen with side-by-side viewers. The left hand viewer shows the ISH image series and the right hand viewer displays the Nissl image series from the same specimen block. By default, the nearest Nissl section to the ISH image you are interested in will be shown and synched with the section you are viewing. Click the “Sync” checkbox to manually correct any synching between the images. Clicking on another ISH image will automatically display its nearest Nissl section. Clicking on an image thumbnail in the Nissl image series will automatically take you to the nearest ISH image. While the "Sync" box is checked the Pan and Zoom functions will affect both ISH and Nissl images.

Colored circles in the Nissl slides are hotspots - regions that when moused over list the brain regions manually labeled by our Annotation team. Clicking on one of the hotspots will take you to the Interactive Atlas Viewer, which will provide a spatial context for the structure you have indicated inside our Human Brain Atlas Guide.

To download an image, click on the button.

**Specimen Detail Information**
Clicking on the specimen link from the search results page or on the specimen link in the experiment details page will take you to detailed specimen information. Specimen detail information includes:

1. Specimen Information
2. Spatial Context
3. Section Information
4. Gene Information
5. Image Viewer
6. Nearest Nissl Image
7. Select experiments for the Multiple Experiment Viewer

**Donor/Specimen Metadata**
Specimen ID - internal ID
Age - years
Sex - male or female
Tissue Location - tissue origination in brain
Hemisphere - right or left hemisphere tissue origination
RNA Integrity Number - metric indicating RNA integrity from tissue. Ranges from 1 to 10 (degraded to intact RNA)
pH - tissue sample pH
Race - ethnicity
Handedness - right, left, or ambidextrous
Conditions - disease conditions, smoker

Spatial Context

Specimen blocks are drawn onto an MRI image of the donor brain in either the coronal or saggital plane. Click the drop-down menu in the top right-hand corner of the MRI to change orientation.

To view the Human Brain Atlas Guide, click on the link below the image to open an Interactive Atlas Viewer in a new window.

Section Information

<table>
<thead>
<tr>
<th>Section Information</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
<td>GAP43</td>
</tr>
<tr>
<td>Experiment</td>
<td>80499034</td>
</tr>
<tr>
<td>Section Number</td>
<td>1</td>
</tr>
<tr>
<td>Treatments</td>
<td>ISH</td>
</tr>
<tr>
<td>Study</td>
<td>Human Brain ISH Cortex Study</td>
</tr>
</tbody>
</table>

The section information box lists information from the current gene pictured in the image viewer including the gene name, the experiment ID, the section number, the treatment and which study the data came from.

Gene Information

The Gene information section lists the genes that were assayed in this specimen block.
All genes from this specimen block are listed, but only ones selected in your original search criteria are selected. You can select fewer more or less genes /histological stains to view in the image viewer by selecting the checkboxes next to the gene/stain(s) you would like to view.

**Image Viewer**

The viewer displays the image from the current gene highlighted in the gene information box. Below the image is an indicator of the position in the specimen block of that particular section, as well as a visualization of the depth of the section into the block. You can view the sections in order (default or by clicking the button) or grouped by gene ( button).
You can navigate these images similar to the ZAP Image Viewer; with the on-screen navigation tools or the Keyboard Commands.

Nearest Nissl Image

When the "Sync" box is checked in the Image Viewer, the closest reference section will be automatically loaded in this viewer. As you are browsing images in the Image Viewer, the appropriate Nissl image will also be loaded in the reference viewer. The colored spots on the Nissl image are "hotspots" that when hovered over will bring up the structure name and it's acronym above the image. Clicking on the hotspot will open an Interactive Atlas Viewer with the structure of interest displayed in the context of the brain.

Multiple Experiment Viewer

Multiple image series can be opened on the same Web page to enable side-by-side comparisons. If you are interested in seeing separate gene expression experiments in one window together, click the checkbox when you have a gene expression image displayed in the Image Viewer.
You can select as many experiments to view as you'd like and they will be saved until you click the "Clear Selections" button. Once you have selected the experiments you'd like to view, click the "View Selections" button. Once in the Multiple Image Viewer, you can choose a different table layout by clicking on the settings wheel above the viewers and then selecting a different number of columns.

You can swap viewer locations on the page by clicking on one viewer’s title bar and dragging it to another viewer's location.