CeNGENApp Use Guide

1. I want to know expression levels for all or a subset of genes in a given neuron.

Use **Gene Expression by cell type.** For all genes, select the neuron of interest on the left drop-down menu and download the table. For a subset of genes, either manually filter the downloaded table, or enter the group of genes in the “Query multiple genes for download,” download the table, then filter for the neuron type or types of interest. A visual representation of a subset of genes across neuron types can be generated in **Heatmaps of gene expression**.

1. I want to know in which neurons a gene (or genes) of interest is expressed.

Use **Gene Expression by cell type**. Enter a single gene in the “Type gene name” box, select a threshold (we recommend 2 for most purposes), and click “Which cell types.” For multiple genes, enter the list of genes into “Query multiple genes for download.”

1. How can I interrogate non-neuronal data?

For searching by gene, use **Single cell plot**. In the “Choose dataset” dropdown menu, select “All cell types.” Enter the gene symbol and click “Plot data.” This returns a UMAP representation colored by relative expression of the gene and ridge plots of gene expression ranked by cell types with the highest expression (highest expression at the top of the plot).

For searching specific non-neuronal cell types, use **Enriched Genes by Cell Type**, and select “all cell types” in the “Choose dataset” dropdown menu. Select the specific cell type of interest to display a list of genes enriched in that particular cell type.

Differential expression tests can include non-neuronal cell types, and are performed in the **Find Differential Expression between Cell Types** tab.

1. I want to find genes that are differentially expressed between two cell types.

Use **Find Differential Expression by cell type.** Select two cell types on the left side drop-down menus, or enter groups of cells into the boxes on the right. In the Group 2 box, you can type “NEURONS” to compare Group 1 to all neurons or “ALL” to compare Group 1 to all cells.

1. I want to find a marker for genetic access to a particular neuron.

There are multiple possible ways to find possible candidates. We list strains we have used to isolate individual neuron types for bulk RNA-Seq profiling at <http://www.cengen.org/advancement-of-bulk-sequencing/>. Supplemental tables 8-11 of our preprint (<https://www.biorxiv.org/content/10.1101/2020.12.15.422897v2>) contain genes detected in single neuron classes in the single-cell data at the different thresholds. You can also use **Enriched Genes by Cell Type** and search for the neuron of interest. Genes with the lowest value in the ‘pct.2’ column will be the most selectively expressed. In this case, the ‘pct.2’ column refers to all other cell types or all other neurons, depending on the dataset selected. To search for markers specific within a subset of cells, you can use either **Find Differential Expression between Cell Types** or **Find markers based on percentage of expression**.