**Differential Expression and the Mouse Gut-Brain Axis**

## Overview

This module introduces students to differential expression analyses using the R programming language. Previous programming experience is helpful but not necessary. Students will work with real data from a mouse RNA-seq study to explore how the gut-brain axis might impact symptoms in Autism Spectrum Disorder.

This module is a companion and expansion to the C-MOOR RNA-seq miniCURE. **It can be used as a stand-alone activity or as part of a broader miniCURE experience.** Students do not have to have completed the miniCURE in order to use the stand-alone guide. If you are using the RNA-seq miniCURE in your class, this module adds an additional RNA-seq dataset that is suitable for an independent research project.

You can find out more about the RNA-seq miniCURE and other C-MOOR activities at the [C-MOOR website](https://www.c-moor.org/). An online guide to the miniCURE is [here](https://science.c-moor.org/miniCURE-RNA-seq/index.html).

**Duration:**

## Learning Objectives

1. Explore differential expression data in mice.
2. Identify genes that are differentially expressed in an RNA-seq dataset.
3. (Optional) Formulate and test a hypothesis about differential expression of genes as a result of the gut-brain axis.

## Materials and Setup

* Students will need either:
  + An internet connection for this activity as written (using Posit Cloud)
  + A local installation of R or RStudio (with data downloaded and tidyverse package installed ahead of time)
* Required R package: tidyverse
* Data hosting:
  + [Comparing gene expression between ASD and control mice, both brain regions](https://genomicseducation.org/data/mouse_gutbrain_de_autismVcontrol.csv)
  + [Comparing gene expression between ASD and control mice, prefrontal cortex only](https://genomicseducation.org/data/mouse_gutbrain_de_autismVcontrol_in_prefrontalcortex.csv)
  + [Comparing gene expression between ASD and control mice, striatum only](https://genomicseducation.org/data/mouse_gutbrain_de_autismVcontrol_in_striatum.csv)
  + [Comparing gene expression between prefrontal cortex and striatum, all mice](https://genomicseducation.org/data/mouse_gutbrain_de_tissuetype.csv)
  + [Comparing gene expression between prefrontal cortex and striatum, only ASD mice](https://genomicseducation.org/data/mouse_gutbrain_de_tissuetype_in_ASDmice.csv)
  + [Comparing gene expression between prefrontal cortex and striatum, only control mice](https://genomicseducation.org/data/mouse_gutbrain_de_tissuetype_in_controlmice.csv)
* **If you are using this as a stand-alone activity**: download the R student activity as:
  + [Web page](https://genomicseducation.org/module/mouse_gutbrain_de_student-guide.html)
  + [Quarto (qmd)](https://genomicseducation.org/module/mouse_gutbrain_de_student-guide.qmd)
  + [Word (docx)](https://genomicseducation.org/module/mouse_gutbrain_de_student-guide.docx)
  + Google Doc - coming soon!
* **If you are using this as an extension of the C-MOOR miniCURE**: download the R student activity as:
  + [Web page](https://genomicseducation.org/module/mouse_gutbrain_de_independent_guide.html)
  + [Quarto (qmd)](https://genomicseducation.org/module/mouse_gutbrain_de_independent_guide.qmd)
  + [Word (docx)](https://genomicseducation.org/module/mouse_gutbrain_de_independent_guide.docx)
  + Google Doc - coming soon!
* Google Slides presentation available for borrowing images [here](https://docs.google.com/presentation/d/1vikcyml6uw-_DaC1yFpS-UWoWNEGxFukMB_eCi42BqY/edit?usp=sharing).
* Answer key available here.

## Scientific Topics

The activity uses data from a [published research study](https://www.cell.com/cell/fulltext/S0092-8674(19)30502-1) “Human Gut Microbiota from Autism Spectrum Disorder Induces Behavioral Deficits in Mice.” Students will gain exposure to the following:

* Differential expression: the process where different cells within an organism, even those with the same DNA, express different genes, with some genes being expressed or “turned on”, while others are “turned off”
* Gut-brain axis: the proposed connection between the gut microbiome and gene expression in the brain
* Autism Spectrum Disorder: a neurological disorder that affects behavioral and social interactions
* Prefrontal cortex: the part of the brain that is primarily in charge of decision making, reasoning, personality, maintaining social appropriateness, and other complex behaviors that fall under the umbrella of executive functions
* Striatum: the part of the brain involved in motor control and cognitive tasks like reward processing, decision-making, and social interactions

## Outline for Stand-Alone Activity

* Part 1: Background and Setup
* Part 2: Exploring Differential Expression Data
* Part 3: Exploring Differential Expression of Individual Genes

## Outline for RNA-seq miniCURE Extension

* Background and Setup
* Exploring Differential Expression Data
* Creating Lists of Differentially Expressed Genes
* Cluster Analysis of Gene Lists