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.

There are two options for this module:

1. a companion and expansion to the C-MOOR RNA-seq miniCURE. This module adds an additional RNA-seq dataset that is suitable for an independent research project.
2. a standalone activity that fits into a single laboratory session.

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:** miniCURE: Three lab periods; standalone activity: 1-2 hours

## 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, ggrepel (miniCURE only)
* Data hosting:
  + [Sample metadata](mouse_gutbrain_metadata.csv)
  + [Gene expression count data for all control-type mice](https://genomicseducation.org/data/mouse_gutbrain_de_counts_controls.csv)
  + [Gene expression count data for all ASD-type mice](https://genomicseducation.org/data/mouse_gutbrain_de_counts_asd.csv)
  + [Gene expression count data for all prefrontal cortex samples](https://genomicseducation.org/data/mouse_gutbrain_de_counts_prefrontalcortex.csv)
  + [Gene expression count data for all striatum samples](https://genomicseducation.org/data/mouse_gutbrain_de_counts_striatum.csv)
  + [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)
* Download the miniCURE R student activity as:
  + [Web page](https://genomicseducation.org/module/mouse_gutbrain_de_miniCURE_guide.html)
  + [Quarto (qmd)](https://github.com/fhdsl/GEMs/blob/main/module/mouse_gutbrain_de_miniCURE_guide.qmd)
  + [Word (docx)](https://github.com/fhdsl/GEMs/raw/main/docs/docx/module/mouse_gutbrain_de_miniCURE_guide.docx)
  + Google Doc - coming soon!
* Download the standalone R student activity as:
  + [Web page](https://genomicseducation.org/module/mouse_gutbrain_de_student_guide.html)
  + [Quarto (qmd)](https://github.com/fhdsl/GEMs/blob/main/module/mouse_gutbrain_de_student_guide.qmd)
  + [Word (docx)](https://github.com/fhdsl/GEMs/raw/main/docs/docx/module/mouse_gutbrain_de_student_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).

## 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” [1]. 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
* Neurodevelopmental disorders: disorders that are the result of altered brain development (miniCURE only)
* Neurodegenerative disorders: disorders that that occur when brain structures and systems experience degradation (miniCURE only)
* 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 RNA-seq miniCURE Extension

* Background and Setup
* Developing a Hypothesis; Identifying Genes of Interest
* Exploring Gene Expression Data
* Analyzing Differential Expression Data

## Outline for RNA-seq standalone activity

* Background and Setup
* Exploring Gene Expression Data
* Analyzing Differential Expression Data

## Evaluation

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| Help us improve! |
| GEMs is an NIH-funded program. Part of our mission is understanding the impact of our materials. Please take the time to review our program as an instructor. We also appreciate you distributing our survey to students after they participate in GEMs content.  [Instructor survey](https://forms.gle/Qd9KLiDsUFY4s1LV8)  Student survey |

[1] G. Sharon *et al.*, “Human gut microbiota from autism spectrum disorder promote behavioral symptoms in mice,” *Cell*, vol. 177, no. 6, pp. 1600–1618, 2019, Available: <https://pubmed.ncbi.nlm.nih.gov/31150625/>