Assignment 5

Gene Expression
Overview

- Due date: 2/23/2024
- **Start early, this assignment may take some time**

Look for TODO comments if you forget what things we want you to do or comment
Tips and Tricks: FUNCTIONS ARE YOUR FRIENDS

- Functions allow you to re-run the same code over and over again
  - Computer scientists are lazy and you should be too with your code!
  - Why copy paste if you can just call a function?
- You can provide functions with arguments to make them versatile
  - If only one small thing (like the data) is changing, use function arguments to run the same code on different objects
- When debugging functions help in determining which part of your code is breaking

As a general rule: Functions are really good at one thing, break up your steps into functions

TIP: You can write more functions than we specifically ask for if it is helpful to you

- Some simple tutorial for function in Python: https://www.w3schools.com/python/python_functions.asp
An example of a function used in this assignment

```python
# Library size
# Calculate library size of each sample (e.g. sum of RNA-seq counts)

def library_sizes(dictionary, list_of_samples):
    # Get the total number of samples
    num_samples = len(list_of_samples)
    # Initialize N, a list to hold the total counts from each sample
    N = []
    # For loop to iterate over each sample
    for i in range(num_samples):
        # Append a new float zero value for each sample (goes to index i)
        N.append(0.0)
        # For loop to iterate over each value in our dictionary
        for v in dictionary.values():
            # Get the count from the i index of this gene and add it to the total for sample i
            N[i] += v[i]
    # Return the list containing each sample's library size
    return(N)

# End of library sizes function #
```
Tips and Tricks:

1 is different than 1.0

Int and float are different and sometimes incompatible
Premise

- Have 20 subjects who are at a high risk for developing type 2 diabetes
  - You put them on a year-long exercise regimen
- You have muscle biopsies from each subject before beginning the exercise regimen and after one year (2 time points)
- You want to characterize their skeletal muscle transcriptomes and hope to better understand the genetic underpinnings of insulin signaling and insulin sensitivity after exercise in human skeletal muscle.
Important Files

- **raw_counts.txt**
  - RNA-Seq count data from the muscle biopsies before and after the exercise regime for the 20 individuals
  - Look at the data to get an rough idea (How many columns? Rows? What does those columns/rows mean?)
  - Do not copy this file to your folder to save space

- **gene_expression.py**
  - Python script that you will need to complete
  - Read through this script, see what is completed, what do you need to do

- **README.txt**
  - README to answer the questions in the assignment
RNA-Seq count data from the muscle biopsies before and after the exercise regime for the 20 individuals

Look at the data to get a rough idea (How many columns? Rows? What do those columns/rows mean?)
Part 0 - Setting up for success

- Make script exit if the number of input parameters is incorrect
- Fill in the code for the `translate_dictionary` function
  - Translate a dictionary (as input) from a dictionary of counts by sample for genes to a dictionary of counts of genes for each sample
  - `{gene:[list of counts by sample]}` to `{sample:[list of counts by gene]}`.
- Comment the `upper_quartile_norm` function to explain what each line does
- Create and comment a function `fishers_linear_discriminant`
  - Remember that functions should be able to work using different data sets, so make sure that your function would work with data from a different experiment with different numbers of before and after samples.
Part 1- Data filtering

1. Remove genes that have zero counts in all samples
   a. Create a dictionary with genes that pass your filter
   b. TIP: create a new dictionary for each filtering step
   c. **DO NOT ALTER THE ORIGINAL DATA FILE**

2. Calculate the counts per million (cpm) of each gene left in your data
   a. Use the `counts_per_million` function to your data that passed the filter

3. Create a dictionary of genes that pass your second filter
   a. Remove genes that have 20 or more samples have cpm < 1
   b. NOTE: this dictionary should be a dictionary of raw counts, not cpm. You are just doing the filtering based on cpm.
Part 2 - Data visualization

1. Plot the library sizes (total counts) for each sample using the genes that passed your filters in Part 1
2. Use the matplotlib library in python to plot things
3. Make sure your plot has labeled axes!
4. Save this plot as library_size.png
Graphing in Python

https://matplotlib.org/gallery/index.html

https://matplotlib.org/api/pyplot_summary.html

Ask Google about detailed questions : )

Or others if you are more comfortable with:
  Ggplot
  Pandas
  etc...
Part 3 - Data normalization

1. Use the `upper_quartile_norm` function to normalize the data you have from Part 1

2. Plot the normalized library sizes (total counts)
   a. TIP: Look at your code from Part 2 to make this easier!
   b. (optional) TIP: Make a function to plot your data
Part 4 - Data exploration

1. Use Fisher’s Linear Discriminant (FLD) to identify genes that are differentially expressed between the Before and After groups
   a. Use the FLD function you wrote in Part 0
   b. Output the genes with the ten highest FLD values (include gene name and FLD values)

NOTE: when calculating FLD for each gene, remember to split the expression values into group 1 (Before) and group 2 (After)
What to turn in

- Edited script: gene_expression.py
- Output files
  - library_size.png
  - library_size_normalized.png
  - mean_expression.png
- Your README.txt with the answers to the questions and the commands you used to answer the questions
- Extra credit only: dendrogram.png

REMEMBER TO COMMENT YOUR CODE

(for accurate grading and for your future self!)
Suggestions

1. Submit assignment on time
2. Discuss questions with classmates (but no copying)
3. Google is your best friend!
4. Post questions on Piazza