Project 2

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In [ ]:
```
import pandas as pd
import matplotlib.pyplot as plt
from pydeseq2.dds import DeseqDataSet
from pydeseq2.ds import DeseqStats
import numpy as np
from sklearn.decomposition import PCA
import seaborn as sns
from scipy.stats import zscore
```

Preprocessing

Only raw counts were supplied with this paper, so I processed the data using pydeseq2 for differential expression analysis.

In [17]:
```
#read in counts data and drop gene_name column as it is unnecessary for analys
counts = pd.read_csv("GSE221115_R-G-spMN.txt", sep='\t').drop("gene_name",axis
counts
```

Out[17]:

	gene_id	G1	G2	G3	R1	R2	R3
0	ENSMUSG00000000001	72	80	141	71	43	127
1	ENSMUSG00000000003	0	0	0	0	0	0
2	ENSMUSG00000000028	13	6	18	0	1	0
3	ENSMUSG00000000031	0	0	0	0	2	0
4	ENSMUSG00000000037	4	0	0	0	1	0
...	...	...	...	...	...	...	...
53853	ENSMUSG00000116995	14	0	13	61	0	11
53854	ENSMUSG00000116996	0	0	0	0	0	0
53855	ENSMUSG00000116997	0	0	0	0	0	0
53856	ENSMUSG00000116998	0	0	0	0	0	0
53857	ENSMUSG00000116999	0	0	0	0	0	0

53858 rows × 7 columns

In [18]:
```
#set the index to be gene_id (needed for DESeq2)
counts = counts.set_index("gene_id")
counts
```

Out[18]:

gene_id	G1	G2	G3	R1	R2	R3
ENSMUSG00000000001	72	80	141	71	43	127
ENSMUSG00000000003	0	0	0	0	0	0
ENSMUSG00000000028	13	6	18	0	1	0
ENSMUSG00000000031	0	0	0	0	2	0
ENSMUSG00000000037	4	0	0	0	1	0
...	...	...	...	...	...	...
ENSMUSG00000116995	14	0	13	61	0	11
ENSMUSG00000116996	0	0	0	0	0	0
ENSMUSG00000116997	0	0	0	0	0	0
ENSMUSG00000116998	0	0	0	0	0	0
ENSMUSG00000116999	0	0	0	0	0	0

53858 rows × 6 columns

In [19]:
```
#filter out rows that only contain 0s
counts = counts[counts.sum(axis = 1) > 0]
counts
```

Out[19]:

gene_id	G1	G2	G3	R1	R2	R3
ENSMUSG00000000001	72	80	141	71	43	127
ENSMUSG00000000028	13	6	18	0	1	0
ENSMUSG00000000031	0	0	0	0	2	0
ENSMUSG00000000037	4	0	0	0	1	0
ENSMUSG00000000049	24	5	16	27	23	0
...	...	...	...	...	...	...
ENSMUSG00000116976	3	0	0	0	0	0
ENSMUSG00000116980	0	0	2	0	0	0
ENSMUSG00000116984	6	0	0	0	0	0
ENSMUSG00000116989	11	0	0	0	0	0
ENSMUSG00000116995	14	0	13	61	0	11

28513 rows × 6 columns

In [20]:
```
#get sample list from counts dataframe
samples = list(counts.columns)
samples
```

```
Out[20]: ['G1', 'G2', 'G3', 'R1', 'R2', 'R3']
```

```
In [ ]: #create a metadata dataframe (needed for DESeq)
        #for each sample, get the type of neuron -- regenerative (R) and non-regenerati
        sample_info = [[i, i[0]] for i in samples]

        #put sample_info into metadata dataframe
        metadata = pd.DataFrame(sample_info, columns = ['sample', 'condition'])

        #set sample column to be the index (needed for DESeq)
        metadata = metadata.set_index("sample")
        metadata
```

Out[ ]:

sample	condition
G1	G
G2	G
G3	G
R1	R
R2	R
R3	R

```
In [22]: #transpose the counts dataframe to input into DESeq
         counts = counts.T
         counts
```

Out[22]:

gene_id	ENSMUSG00000000001	ENSMUSG00000000028	ENSMUSG00000000031	ENSMUSG(
G1	72	13	0	
G2	80	6	0	
G3	141	18	0	
R1	71	0	0	
R2	43	1	2	
R3	127	0	0	

6 rows × 28513 columns

```
In [23]: #make Deseq dataset
         dds = DeseqDataSet(counts=counts,
                     metadata=metadata,
                     design_factors="condition")
         #run deseq
         dds.deseq2()
```

```
Fitting size factors...
... done in 0.01 seconds.

Fitting dispersions...
... done in 1.74 seconds.

Fitting dispersion trend curve...
... done in 0.45 seconds.

Fitting MAP dispersions...
... done in 1.81 seconds.

Fitting LFCs...
... done in 1.62 seconds.

Calculating cook's distance...
... done in 0.01 seconds.

Replacing 0 outlier genes.
```

In [24]:
```
#get statistics of R vs. G for differential expression analysis
stat_res = DeseqStats(dds, contrast = ('condition',"R", "G"))
stat_res.summary()
```

```
Running Wald tests...
Log2 fold change & Wald test p-value: condition R vs G
                     baseMean  log2FoldChange     lfcSE      stat    pvalue  \
gene_id
ENSMUSG00000000001  84.515515       -0.356183  0.568892 -0.626099  0.531250
ENSMUSG00000000028   5.680290       -4.850814  2.177320 -2.227883  0.025888
ENSMUSG00000000031   0.577809        2.663581  4.289841  0.620904  0.534663
ENSMUSG00000000037   0.737856       -1.267320  4.066582 -0.311643  0.755312
ENSMUSG00000000049  17.074460        0.819661  1.317660  0.622058  0.533904
...                       ...             ...       ...       ...       ...
ENSMUSG00000116976   0.336714       -1.948040  4.324183 -0.450499  0.652351
ENSMUSG00000116980   0.261784       -1.653760  4.348713 -0.380287  0.703732
ENSMUSG00000116984   0.673427       -2.887404  4.271295 -0.676002  0.499039
ENSMUSG00000116989   1.234617       -3.748069  4.244845 -0.882970  0.377253
ENSMUSG00000116995  14.190982        1.712189  1.549895  1.104713  0.269284

                        padj
gene_id
ENSMUSG00000000001  0.981100
ENSMUSG00000000028       NaN
ENSMUSG00000000031       NaN
ENSMUSG00000000037       NaN
ENSMUSG00000000049  0.981100
...                      ...
ENSMUSG00000116976       NaN
ENSMUSG00000116980       NaN
ENSMUSG00000116984       NaN
ENSMUSG00000116989       NaN
ENSMUSG00000116995  0.932387

[28513 rows x 6 columns]
... done in 0.98 seconds.
```

In [31]:
```
#create a results dataframe
results = stat_res.results_df
results
```

Out[31]:

	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
gene_id						
ENSMUSG00000000001	84.515515	-0.356183	0.568892	-0.626099	0.531250	0.981100
ENSMUSG00000000028	5.680290	-4.850814	2.177320	-2.227883	0.025888	NaN
ENSMUSG00000000031	0.577809	2.663581	4.289841	0.620904	0.534663	NaN
ENSMUSG00000000037	0.737856	-1.267320	4.066582	-0.311643	0.755312	NaN
ENSMUSG00000000049	17.074460	0.819661	1.317660	0.622058	0.533904	0.981100
...	...	...	...	...	...	...
ENSMUSG00000116976	0.336714	-1.948040	4.324183	-0.450499	0.652351	NaN
ENSMUSG00000116980	0.261784	-1.653760	4.348713	-0.380287	0.703732	NaN
ENSMUSG00000116984	0.673427	-2.887404	4.271295	-0.676002	0.499039	NaN
ENSMUSG00000116989	1.234617	-3.748069	4.244845	-0.882970	0.377253	NaN
ENSMUSG00000116995	14.190982	1.712189	1.549895	1.104713	0.269284	0.932387

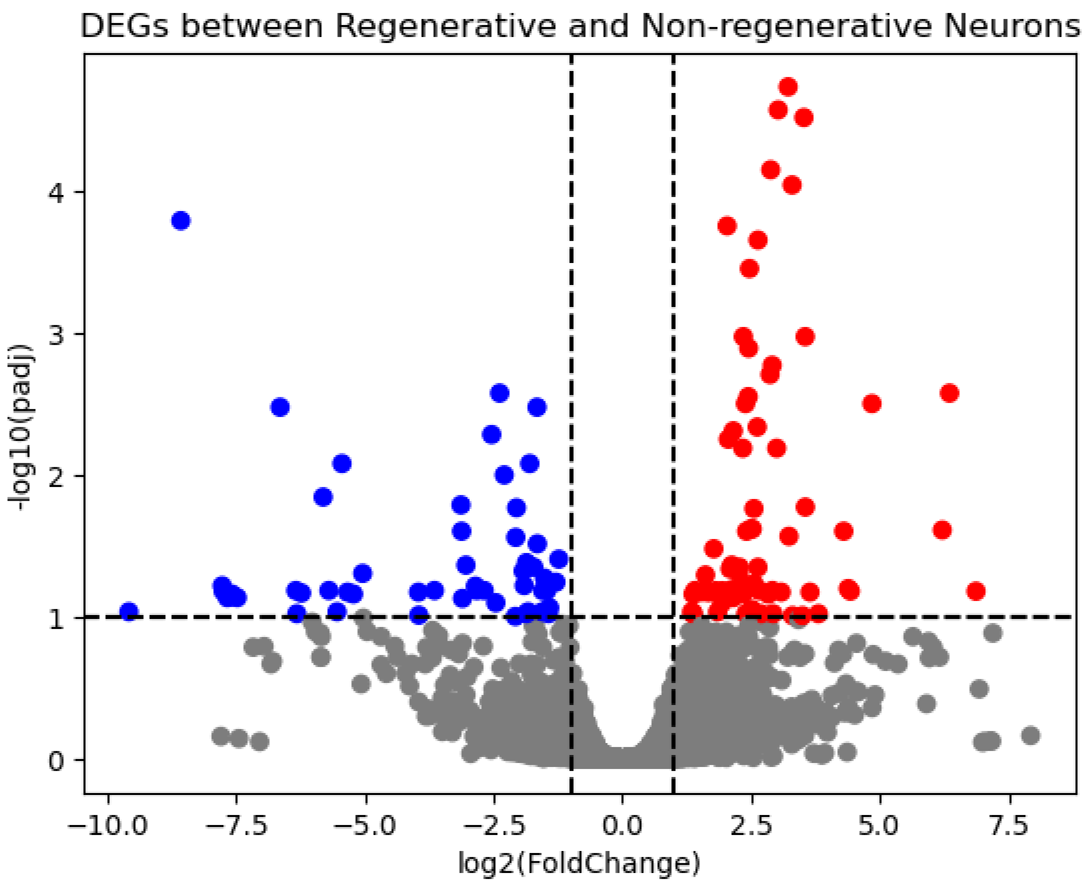
28513 rows × 6 columns

Plot 1

Volcano plot of differentially expressed genes between regenerative and non-regenerative neurons. Significantly upregulated genes (log2FC > 1 and padj < 0.1) are indicated in red and significantly downregulated genes (log2FC < -1 and padj < 0.1) are indicated in blue. Recreation of figure 1E.

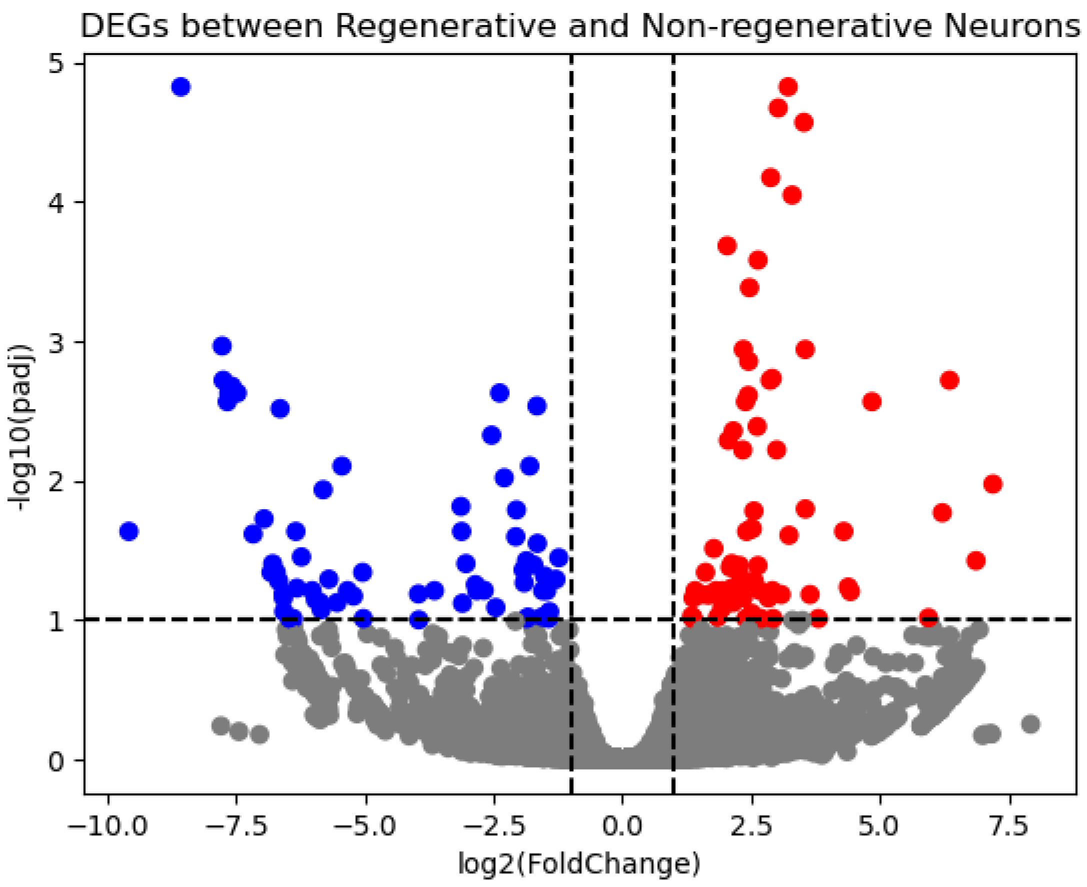
In [26]:
```
#create list of colors for volcano plot that indicate significance
#for the volcano plot in this paper, the significance thresholds were set at pa
colors = np.where((results['log2FoldChange'] > 1) & (results['padj'] < 0.1), '
        (results['log2FoldChange'] < -1) & (results['padj'] < 0.1), 'blue',  #
        'gray'  # Non-significant
    )
)
```

In [ ]:
```
#scatter log2FoldChange and -log10 scaled padj with indicated colors
plt.scatter(results['log2FoldChange'], -np.log10(results['padj']), c=colors)

#set labels
plt.xlabel("log2(FoldChange)")
plt.ylabel("-log10(padj)")
plt.title("DEGs between Regenerative and Non-regenerative Neurons")

#lines that indicate significance thresholds
plt.axvline(1, color = "black", linestyle="--")
plt.axvline(-1, color="black",linestyle="--")
plt.axhline(-np.log10(0.1), color = "black", linestyle="--")
plt.axhline(-np.log10(0.1), color="black",linestyle="--")
plt.savefig("volcano_plot.png")
plt.show()
```



The original paper used the R DESeq2 library to make their calculations. The python pydeseq2 package makes slightly different calculations when analyzing counts data. Because of this, the volcano plot I have produced looks slightly different than the plot included in the paper, however the overall shape of the plot aligns to the paper. I ran DESeq2 in R on the counts data and saved the calculations to the file r_deseq2_output.csv. The code used for running this analysis is included in deseq.Rmd. The following plot is a volcano plot created from the R calculations, and matches up exactly to the plot in the paper.

In [ ]:
```
#read in the csv output by the R script
r_deseq_output = pd.read_csv("r_deseq2_output.csv")

#set the colors
r_colors  = np.where((r_deseq_output['log2FoldChange'] > 1) & (r_deseq_output[
          (r_deseq_output['log2FoldChange'] < -1) & (r_deseq_output['padj'] < 0.
          'gray'  # Non-significant
     )
)

#plot the datapoints
plt.scatter(r_deseq_output['log2FoldChange'], -np.log10(r_deseq_output['padj']

#set the labels
plt.xlabel("log2(FoldChange)")
plt.ylabel("-log10(padj)")
plt.title("DEGs between Regenerative and Non-regenerative Neurons")

#lines that indicate significance thresholds
plt.axvline(1, color = "black", linestyle="--")
plt.axvline(-1, color="black",linestyle="--")
plt.axhline(-np.log10(0.1), color = "black", linestyle="--")
plt.axhline(-np.log10(0.1), color="black",linestyle="--")
plt.savefig("r_volcano_plot.png")
plt.show()
```



Plot 2

Heatmap of top 1000 differentially expressed genes between regenerative and non-regenerative neurons, ranked by FDR/padj (not indicated in paper, assumption). Recreation of supplementary figure 2C.

```
In [ ]: #sort results by padj
        sorted_results = results.sort_values('padj')

        #get the top 1000 differentially expressed genes post ranking
        top1000 = sorted_results.head(1000)
        top1000
```

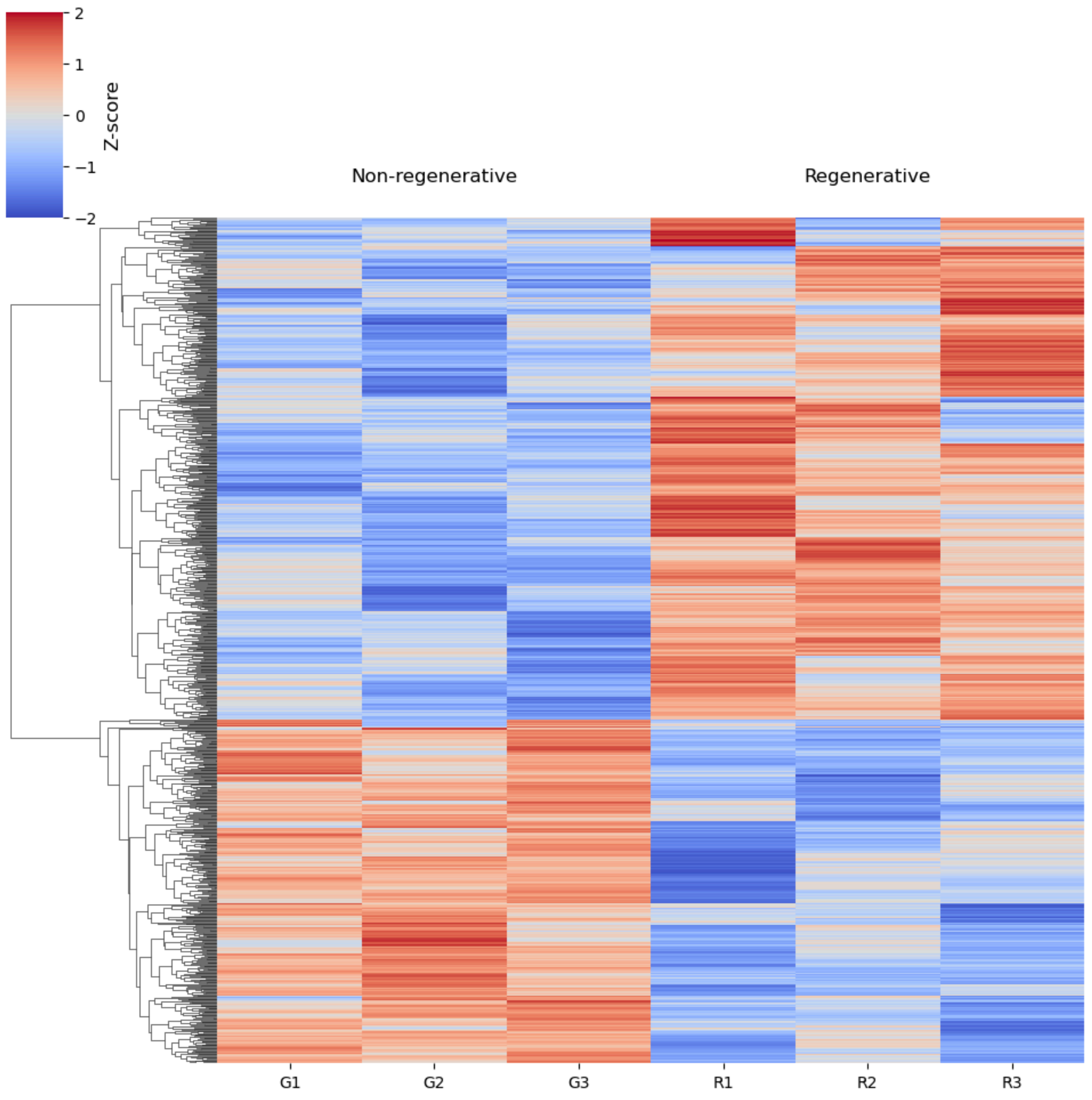
Out[ ]:

gene_id	baseMean	log2FoldChange	lfcSE	stat	pvalue	
ENSMUSG00000002985	1131.925246	3.218768	0.531728	6.053411	1.418102e-09	0.00
ENSMUSG00000041607	6157.616201	3.025973	0.514693	5.879185	4.122909e-09	0.00
ENSMUSG00000041329	144.403867	3.524700	0.608668	5.790840	7.003510e-09	0.00
ENSMUSG00000032517	672.757626	2.879878	0.514525	5.597160	2.178914e-08	0.00
ENSMUSG00000000296	246.502424	3.296093	0.597652	5.515071	3.486390e-08	0.00
...	...	...	...	...	...	
ENSMUSG00000028207	816.924644	0.747575	0.381484	1.959646	5.003718e-02	0.646
ENSMUSG00000021843	1383.492688	-0.747444	0.380918	-1.962216	4.973731e-02	0.646
ENSMUSG00000069355	76.181162	-1.225133	0.624991	-1.960242	4.996755e-02	0.646
ENSMUSG00000023262	25.359535	2.037170	1.038649	1.961365	4.983649e-02	0.646
ENSMUSG00000028063	822.384149	-0.681389	0.347668	-1.959885	5.000929e-02	0.646

1000 rows × 6 columns

In [48]:
```
#log1p normalize the normed counts (ln(1+normed_count)) for proper calculation
dds.layers['log1p'] = np.log1p(dds.layers['normed_counts'])

#get the dds statistics for the top 1000 genes
dds_sigs = dds[:, top1000.index]

#get the normalized counts for the top 1000 genes
top1000_normalized = pd.DataFrame(dds_sigs.layers['log1p'].T,index=dds_sigs.va

#plot the heatmap using a zscore axis of 0 (z_score across row) and blue/red co
#col_cluster set to false to keep in order of the dataframe
clustergrid = sns.clustermap(top1000_normalized, z_score=0, vmin =-2, vmax = 2

#style axes
ax = clustergrid.ax_heatmap

#Label first 3 columns with non-regenerative and last 3 columns with regenerat
ax.text(0.25, 1.05, 'Non-regenerative', fontsize=12, ha='center', va='center',
ax.text(0.75, 1.05, 'Regenerative', fontsize=12, ha='center', va='center', tra

#label the color bar with Z-score
colorbar = ax.collections[0].colorbar
colorbar.set_label('Z-score', fontsize=12)
colorbar.ax.tick_params(labelsize=10)
```

```
#remove x and y labels
ax.set_xlabel('')
ax.set_ylabel('')

plt.savefig("heatmap.png")
plt.show()
```


Z-score
Non-regenerative
Regenerative
G1
G2
G3
R1
R2
R3