Deconvoluting cell types through scRNA-Seq

Erica Chio

Data

SCG Rattus norvegicus Cell Cultures

- 2 untreated cultured (mock-treated with DMSO)
- 1 treated with LY294002 (LY) (dissolved in DMSO)
- 1 treated with MIRIN (dissolved in DMSO)

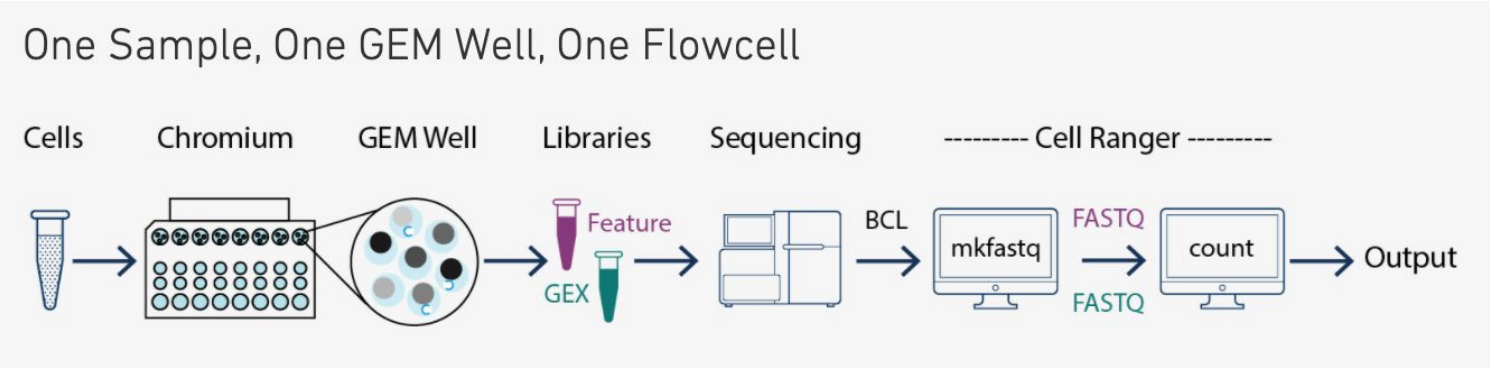
Background

- Superior Cervical Ganglion (SCG)
  - Part of the nervous system
- LY294002 (LY)
  - Affects to PI3K pathway
    - PI3K pathway affects cell metabolism, growth, proliferation, survival
- Mirin
  - Affects MRN-ATM pathway
    - MRN-ATM pathway affects response to DNA repair breaks and homology directed repairs

Aligning and Generating Counts (Cell Ranger)

Cell Ranger: analysis pipeline that processes single cell RNA-seq output to align reads and generate feature counts

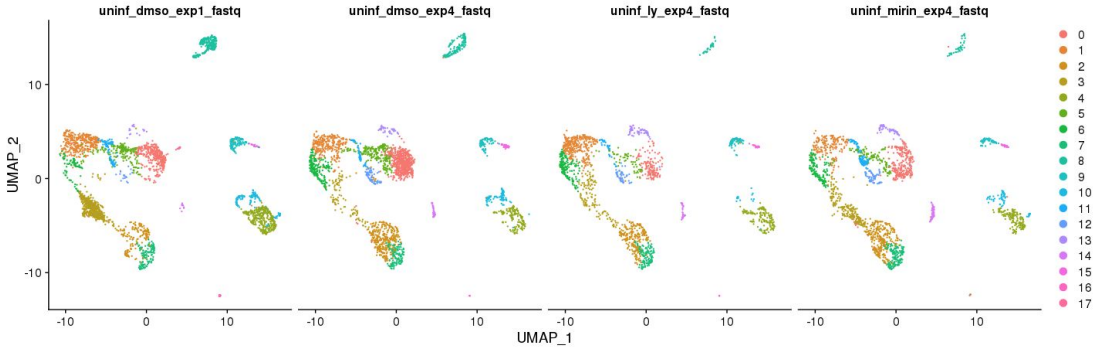
- Fastq files already provided, so only Cell Ranger count was utilized

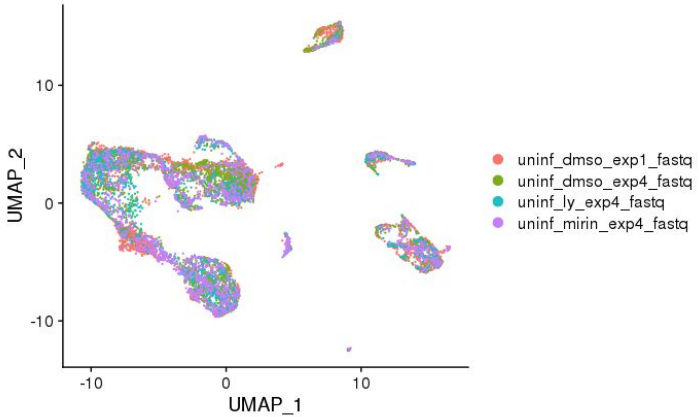


Integration of Datasets (Seurat)

Seurat: Datasets are integrated to promote the identification of common cell types and allow comparative analysis

- Achieved through defining “anchors” (pairwise correspondence between cells in different datasets

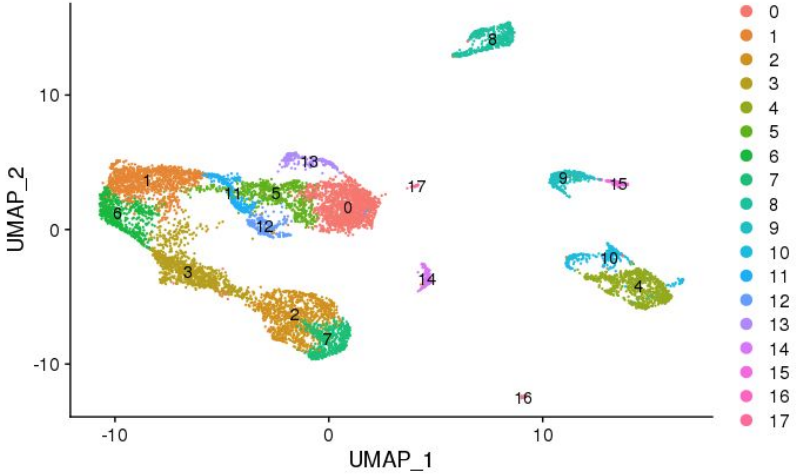


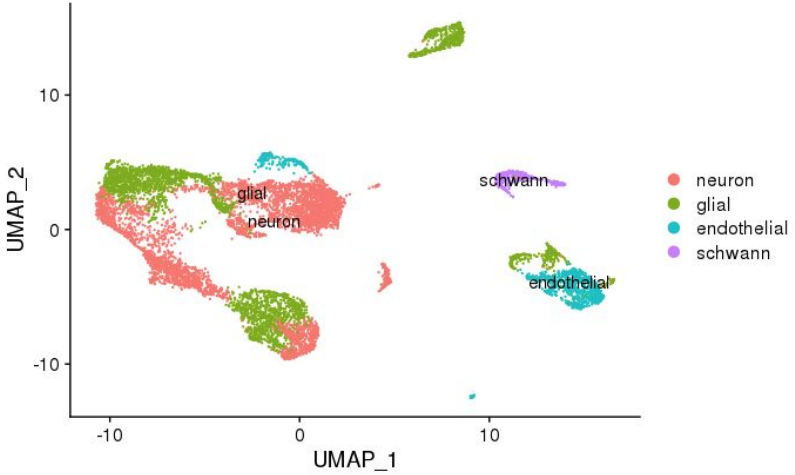


Cell Clusters (Seurat)

Seurat: Utilized graph based clustering approach to cluster the cells

- FindConservedMarkers to determine the conserved cell type marker

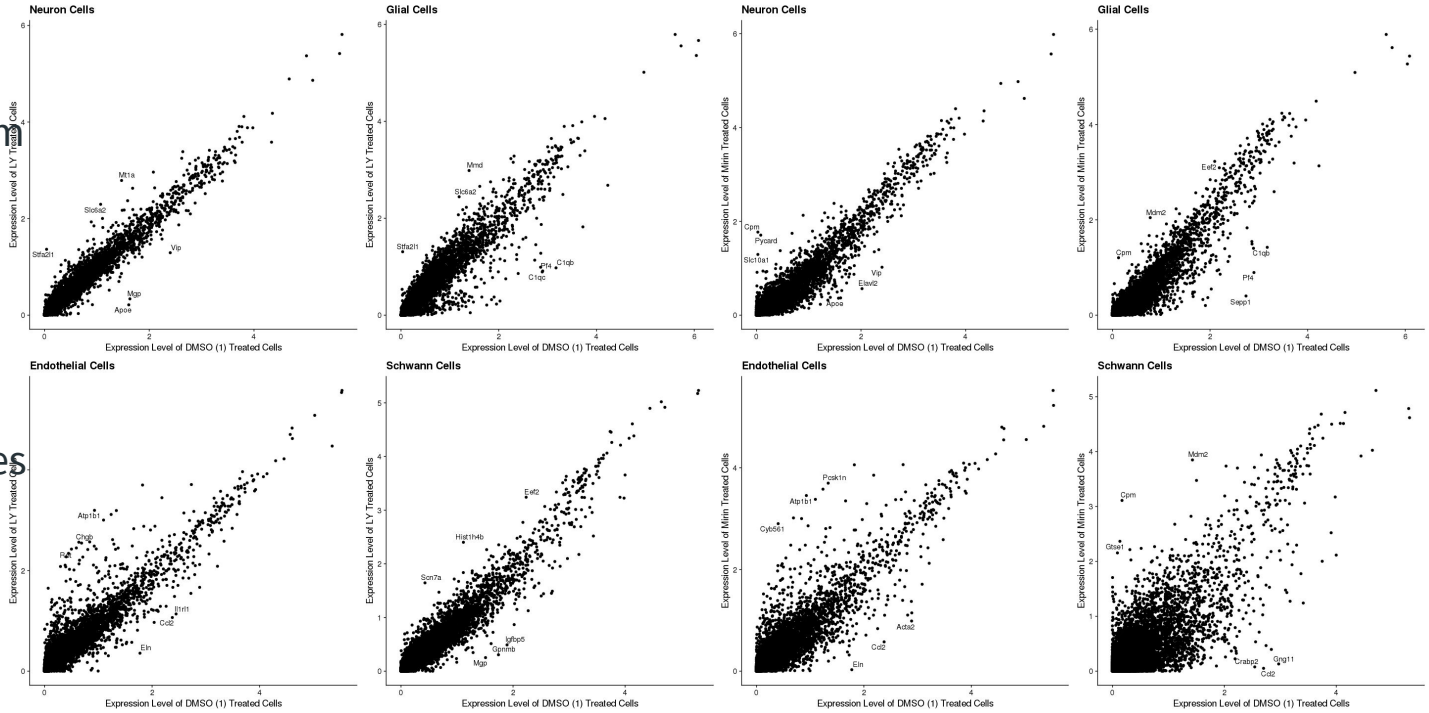




Average Cell Expression (Seurat)

After aligning, the differences induced from stimulation / treatment can be visualized by plotting average cell expression

- Outliers have dramatic responses to treatment



Up Regulated Genes in Different Conditions (Seurat)

FindMarkers is utilized to identify the changes in gene across different conditions

Experiment	Cell Type	Gene
Control (DMSO)	Neuron	Tuba1a
		Tuba1b
	Glial	Apoe
		Prdx1
	Endothelial	Gapdh
		Ifitm3
	Schwann	Npc2
		Prdx1
LY treated	Neuron	Stfa2l1
		Hist1h4b
	Glial	Th
		Rsrp1
	Endothelial	Ret
		Atp1b1
	Schwann	Eef2
		Eef1a1

Experiment	Cell Type	Gene
Control (DMSO)	Neuron	Elavl2
		Bri3
	Glial	Rps27l
		Bri3
	Endothelial	Fam111a
		Sod2
	Schwann	Gng11
		Nudt4
mirin treated	Neuron	Slc10a1
		Pycard
	Glial	Rpl9
		Eef2
	Endothelial	Atp1b1
		Ret
	Schwann	Cpm
		Gtse1

Pathways Impacted (DAVID)

Database for Annotation, Visualization and Integrated Discovery (DAVID)

- List of genes is obtained from Seurat's FindMarkers
- Top 2 pathways statistically significant) (determined through p-value)

Experiment	Cell Type	Pathway
LY treated	Neuron	negative regulation of neuron apoptotic process cellular response to manganese ion
	Glial	aging positive regulation of neuron projection development
	Endothelial	response to oxidative stress aging
	Schwann	translation protein folding
mirin Treated	Neuron	translation negative regulation of neuron apoptotic process
	Glial	translation aging
	Endothelial	aging response to drug
	Schwann	translation cell-cell adhesion

Experiment	Neuron	Glial	Endothelial	Schwann
DMSO Biological Replicate 1	1444	1075	437	147
DMSO Biological Replicate 2	1487	927	203	128
Treated with LY	717	638	207	87
Treated with mirin	1073	696	227	81

Thank You

Thank You for a Great Class