Motor Neuron iPSC time-course scRNA-seq

Statement of Work

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# Background

Induced pluripotent stem cell technology is used to generate patient-specific stem cells that can undergo directed-differentiation to multiple lineages in culture. Diseases being explored include Duchenne muscular dystrophy, X-linked myotubular myopathy and autistic syndrome disorder. The iPSC model is employed in the current investigation into the physiologic effect and mechanism of astrocyte-mediated neuromodulation in normal and ALS mutant condition. This would identify molecular drivers of the observed phenotypic change using human iPSC-derived spinal astrocytes and motor neurons.

We ran a longitudinal single-cell RNAseq with the differentiation motor neurons from iPSCs and got the gene expression table using Cell Ranger (10x Genomics) of each timepoint. We collected cells from undifferentiated iPSC, day3 of differentiation, day5, day8… until day 45. We aim to identify the regional identity and subtypes of iPSC motor neurons and transcriptomic (dis)similarity with native MNs in the fetal spinal cord.

# Data provided

* Counts matrices for each time-point scRNA-seq
  + Genetically normal hIPSC cells
  + Cellranger pipeline
  + Single replicate 10x sample per time-point
  + Later time-points have scRNA-seq and microscopy to validate state of cellular differentiation
* Sample metadata sheet

# Goals/Deliverables

* Merging individual scRNA-seq samples (time-points) into a single object. Check for batch effects, doublets, ambient DNA fraction, and other QC metrics.
  + Use cellranger raw counts –> doublet detection (scDblFinder, simulation) –> soupX ambient dna detection –> Seurat object
  + Seurat object will be shared to UW to allow for con-current transcriptome analyses
* Gene quantification normalization and data dimension reduction (PCA, UMAP)
* Cell-type annotation using public references
  + Seurat cell-type annotation
  + Integrate > 1 public scRNA-seq reference dataset as reference
* Track the gene expression changes of the same type of cells along the differentiation. Are there cell pops that disappear and appear over time?
  + Utilize [Monocle](http://cole-trapnell-lab.github.io/monocle-release/) for pseudotime trajectory analysis and assist with troubleshooting
  + Utilize [psupertime](https://academic.oup.com/bioinformatics/article/38/Supplement_1/i290/6617492) for time-series analysis of gene-expression
* Assist with figure generation
  + Examples are provided in this [publication](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8353162/).

# Scope

We will assist with computational analysis including QC, normalization, and cell-type annotation. Down-stream analyses for trajectory inference and time-series analyses will be completed.

This work will be supported by hourly charging of $170/hr. All scripts, figures, and data will be shared using Miscrosoft Azure container. Estimate about 6-8 months to completion, where we consider completion the agreed-upon final publication ready figures and methods section paragraph(s).

To help facilitate communication and collaboration on research findings, we will set-up a re-curring meeting every 3 weeks to review the data analyses and results. Frequent email communication is always encouraged and additional meetings can be scheduled as needed for questions and clarifications regarding the results/analysis. We will also create a google document to curate a set of running notes for continued tracking of progress and next steps.

# Caveats and Unknowns

* Need to find a better in-vivo reference for spinal cord tissue, since the Allen Institute motor cortex dataset is not fully sufficient for the cell types expected to be present in the iPSC model.
* A technical issue with this (in singlicets) is that the gain/loss of the cell pops overtime difficult to track due to biological variability between wells
* What is our expected number of cell-pops per well? What is the general variability in induced pluripotent cells?

# Collaborators

* David Mack, UW ISCRM Principal Investigator
* Changho Chun, UW ISCRM phD candidate
* Lisa Maves, SCRI Principal Investigator