CHRD 2024: Abstract Submission Form

Presenter Name
John Pham

Presenter Status
Non-Trainee

Role in the project
Design
Analyze Data
Write Abstract

Research Category
Basic Science

Title
Development of a single cell analysis pipeline: Pilot application to Rett syndrome forebrain organoids

Background
Single-cell RNA-sequencing (scRNA-seq) technology is a main focus of several genomic and bioinformatic studies due to its ability to analyze a distribution of gene expression for individual cell types. However, many scRNA-seq analyses purely focus on differential gene expression, where these rich data could provide additional biological context and information.

Objective
To address this often vacant gap of analyses, we analyzed human stem cell-derived brain organoids from the neurodevelopmental disorder, Rett syndrome (RTT), using a standardized pipeline that performs several scRNA-seq analyses.

Methods
Organoid data (n = 16) from Samarasinghe et al., which is aligned using 10X Cellranger to a human reference (GRCh38), is passed into the pipeline that performs quality control, dimensionality reduction, and clustering (Seurat, soupX, and sclDoubletFinder). Afterwards, complex analyses are carried out, namely, trajectory inference (slingshot), differential abundance (miloR), single-cell gene-set enrichment (escape), and ligand-receptor interactions (CellChat).

Results
Differential abundance analysis displayed similar proportional differences in the cell types identified in Samarasinghe et al.'s findings, while also showing that pathways related to the brain, axons, and neurons are upregulated in the RTT brain organoids compared to the wildtype. This is further supported when performing a ligand-receptor analysis through CellChat, where cadherin and pleiotrophin pathways were significantly dysregulated ($P < 0.05$) in RTT organoids. These pathways are related to development, cell growth, and proliferation in neurons.

Conclusion
We were able to confirm previous reports of earlier differentiation to neuronal cell types in RTT by ensuring reproducibility, avoiding arbitrary cutoffs, and having a wide coverall of scRNA-seq analyses in the pipeline. In particular, the pathways found from our enhanced pipelines which incorporated miloR and CellChat analyses are in alignment with results from RTT animal models. Future directions aim to use this pipeline to assess six-month-old dorsal forebrain RTT organoids that have been cultured locally to confirm our results

Do you have a table/figure to upload?
Yes

Authors

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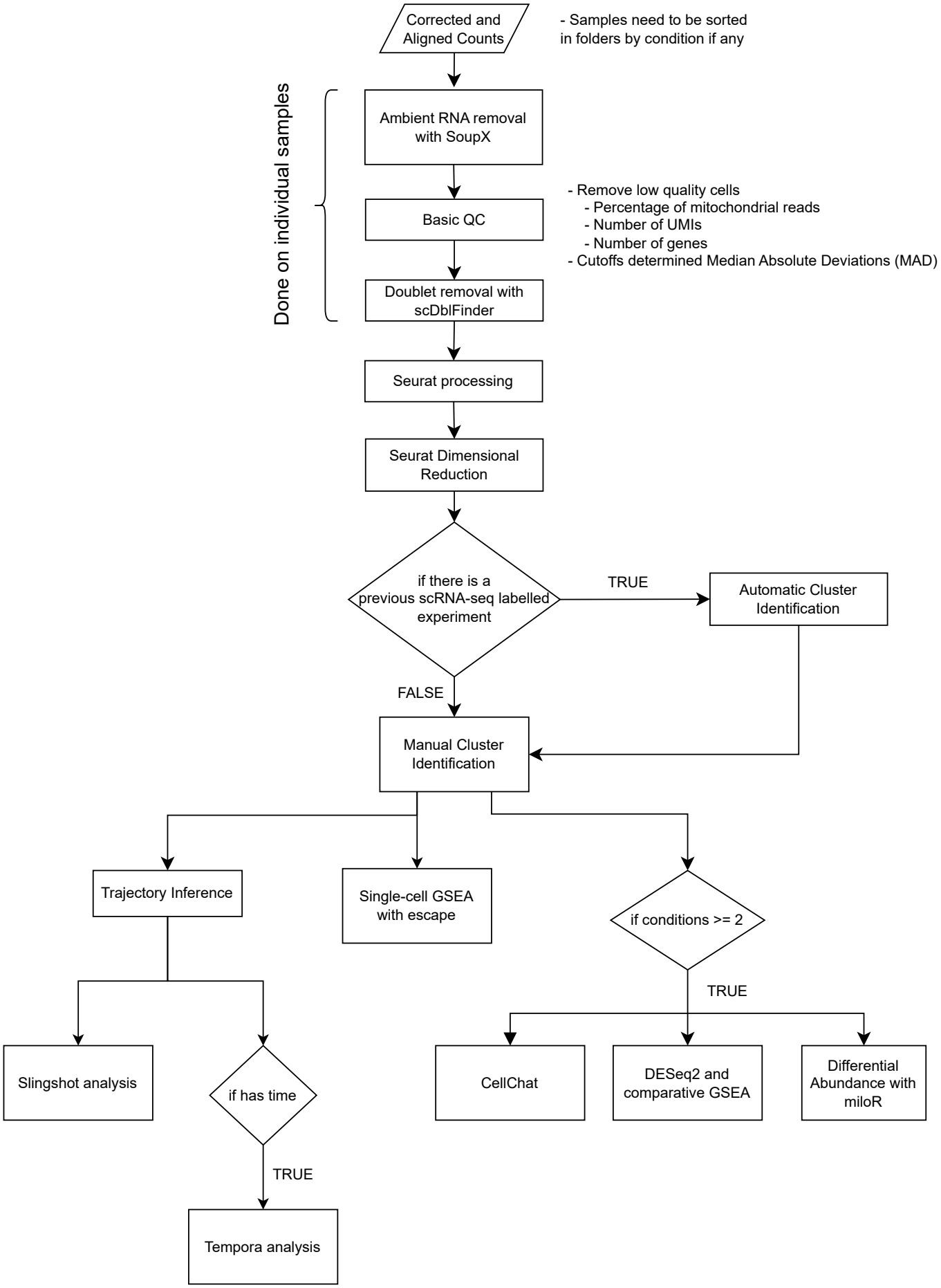
Corrected and Aligned Counts
- Samples need to be sorted in folders by condition if any
Done on individual samples
Ambient RNA removal with SoupX
Basic QC
- Remove low quality cells
- Percentage of mitochondrial reads
- Number of UMIs
- Number of genes
- Cutoffs determined Median Absolute Deviations (MAD)
Doublet removal with scDblFinder
Seurat processing
Seurat Dimensional Reduction
if there is a previous scRNA-seq labelled experiment
TRUE
Automatic Cluster Identification
FALSE
Manual Cluster Identification
Trajectory Inference
Single-cell GSEA with escape
if conditions >= 2
TRUE
Slingshot analysis
if has time
TRUE
Tempora analysis
CellChat
DESeq2 and comparative GSEA
Differential Abundance with miloR