Sep 01, 2024

# Xenograft snRNA-seq Integration and Normalization

DOI
**dx.doi.org/10.17504/protocols.io.81wgbzo4ngpk/v1**

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**DOI:** dx.doi.org/10.17504/protocols.io.81wgbzo4ngpk/v1

**Protocol Citation:** Zac Chatterton, Chiara Pavan, Clare Parish 2024. Xenograft snRNA-seq Integration and Normalization. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.81wgbzo4ngpk/v1**



**Protocol status:** Working
**We use this protocol and it's working**

**Created:** September 01, 2024

**Last Modified:** September 01, 2024

**Protocol Integer ID:** 106809

**Keywords:** ASAPCRN, snRNA-seq, Data integration, Normalization, Xenograft, Seurat

**Funders Acknowledgement:**
**Michael J Fox Foundation**
**Grant ID: ASAP-000497**

## Abstract

This protocol describes the integration and normalization of single-nucleus RNA sequencing (snRNA-seq) data obtained from xenograft samples. The process involves loading data from multiple Cell Ranger outputs, creating Seurat objects, performing data integration, and normalizing the datasets. The protocol includes the visualization of results and statistical analyses to assess the quality and characteristics of the integrated datasets. The protocol includes the assignment of cell-type and differential gene expression analysis.

## Materials

### *Equipment*

- High-performance computing system or workstation with R
- Seurat v5
- Cell Ranger output files (e.g., 10XGenomics)
- R packages: patchwork, Matrix, tidyverse, plyr, ggpubr, HGNChelper, EnhancedVolcano, ggbreak

## Before start

The user can access the code base at
**https://github.com/zchatt/ASAPSpatialTranscriptomics/tree/main/xenograft/snrna_seq**

## Setup and Data Loading:

1
- Set the working directory and specify the path for Cell Ranger outputs.
- Load the necessary R libraries and configure global options.

## Data Integration

2
- Import multiple Cell Ranger outputs.
- Create Seurat objects for each dataset.

## Quality Control (QC) and Data Normalization

3
- Filter cells based on unique feature counts (e.g., remove cells with < 2000 unique counts or > 0.1% mitochondrial reads).
- Integrate multiple datasets and normalize the data using Seurat's standard workflow.

## Selection of Human Cells

4
- Differentiate between human and rodent cells using per-cell read alignments to the respective genomes.

## Analysis and Visualization

5
- Perform dimensionality reduction (e.g., PCA, UMAP) and clustering.
- Visualize the integrated data using UMAP plots.

## Cell-Type Definition

6
- Classify human cell clusters using tools like CHETAH and scType.

## Statistical Analysis

7
- Conduct differential expression analysis between human cell types of interest.