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Single-cell sequencing data analysis

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We use this protocol and it's working

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Abstract

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1 Download the corresponding annotation file from: https://raw.githubusercontent.com/hbctraining/scRNA-seq/master/data/annotation.csv.

2 Demultiplex and filter sequencing data using the 10X Genomics Cell Ranger pipeline to generate filtered gene-barcode matrices.

3 Import filtered matrices into the R package Seurat (version 4.1.0, RRID: SCR_007322) for downstream analysis.

4 Filter out low-quality cells using the following criteria:
- Number of unique molecular identifiers (UMIs) < 2500,
- Number of detected genes < 500,
- Mitochondrial DNA ratio < 0.2

5 Remove genes with zero counts or those expressed in fewer than 10 cells.

6 Use DoubletFinder (version 3, RRID: SCR_018771) to eliminate doublets from the dataset.

7 Normalize the data using SCtransform (version 0.4.1, RRID: SCR_022146), regressing out cell cycle and mitochondrial reads.

8 Integrate datasets using the Harmony package (version 1.2.0, RRID: SCR_022206).

9 Identify principal components (PCs) and cluster cells using the first 40 PCs with a K-nearest neighbor graph.

10 Perform UMAP analysis and identify clusters using a resolution of 0.8, yielding 11 clusters across both conditions.

11 Visualize density estimation for cell type-specific markers in the UMAP plot using Nebulosa's kernel function (Nebulosa version 3.19).

12 Identify conserved markers for each cluster using the FindConservedMarkers function in Seurat with default settings (min.pct = 0.25, logfc.threshold = 0.25). Assign cell types to clusters based on these markers.

13 Use the UCell package (version 2.7.6) to evaluate UPRmt signatures in the single-cell datasets.

14 Merge neuronal, astrocytic, and microglial clusters after manual annotation to improve the statistical power for differential expression analysis.

15 Identify differentially expressed genes (DEGs) between conditions in each cluster using the FindMarkers function in Seurat with the MAST test (padj < 0.05, logFC > 2). Apply Bonferroni correction for p-value adjustment.

16 Generate Venn diagrams of shared DEGs between iPSC-derived neurons, microglia, and astrocytes in monoculture and triculture systems using Draw Venn Diagram (**https://bioinformatics.psb.ugent.be/webtools/Venn/**).

17 Conduct functional enrichment analysis of DEGs (fold change > 2; Benjamini-Hochberg FDR < 0.05) using ShinyGO (version 0.80, **http://bioinformatics.sdstate.edu/go/**, RRID: SCR_019213) with:
- KEGG PATHWAY Database (RRID: SCR_018145),
- Reactome Database (RRID: SCR_003485).

18 Use the CellChat package (version 2.1.0, RRID: SCR_021946) to analyze and visualize cell-cell communication events, following the developer's instructions.