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Bulk RNA-seq and data analysis

In 1 collection

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We use this protocol and it's working

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Abstract

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1 Isolate total RNA from three biological replicates per sample of iPSC-derived neurons (treated for 24 hours), astrocytes, and microglia (treated for 6 hours) using the RNeasy Mini Kit (Cat. number 74106, QIAGEN, Germany) according to the manufacturer's instructions.

2 Enrich mRNA via poly(A) selection. (Done by GENEWIZ GmbH- Leipzig, Germany)

3 Prepare cDNA libraries using strand-specific RNA-seq with poly(A) selection (Illumina, CA, USA). (Done by GENEWIZ GmbH- Leipzig, Germany)

4 Perform sequencing on the Illumina NovaSeq platform (Illumina, CA, USA) to generate 150 bp paired-end reads.(Done by GENEWIZ GmbH- Leipzig, Germany)

5 Perform differential gene expression analysis with DESeq2 (version 1.44.0, RRID: SCR_015687) to normalize counts and identify differentially expressed genes (DEGs)

6 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).

7 Filter data to assess statistical significance (FDR < 0.05).

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

9 Data of the study were deposited into GEO under accession number GSE274283.