

Feb 03, 2025

Bulk RNA-seq and data analysis

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<https://dx.doi.org/10.17504/protocols.io.ewov1d46pvr2/v1>

Maria Jose Perez J.¹

¹Institut Imagine

Team Deleidi



Federico Bertoli

ASAP

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Protocol Citation: Maria Jose Perez J. 2025. Bulk RNA-seq and data analysis. **protocols.io**

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Protocol status: Working

We use this protocol and it's working

Created: November 26, 2024

Last Modified: February 03, 2025

Protocol Integer ID: 112821

Keywords: data analysis bulk rna, seq, data analysis, data

Funders Acknowledgements:

ASAP

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.