Jan 28, 2026

Bulk RNA-seq

DOI
https://dx.doi.org/10.17504/protocols.io.81wgbon1olpk/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.81wgbon1olpk/v1

Protocol Citation: Boxun Li, Charles Gersbach 2026. Bulk RNA-seq. **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgbon1olpk/v1

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

Created: January 23, 2026

Last Modified: January 28, 2026

Protocol Integer ID: 240233

Keywords: RNA-seq, neurons, astrocytes, performing bulk rna, bulk rna, mouse primary astrocyte, primary astrocyte, seq on ipsc, seq, seq this protocol, derived neuron

Funders Acknowledgements:
NIH
Grant ID: HG012053

Disclaimer

This protocol was adapted from the work of Boxun Li in the Gersbach lab at Duke University.

Abstract

This protocol describes methods for performing bulk RNA-seq on iPSC-derived neurons and mouse primary astrocytes.

Materials

Mr. Frosty Freezing Container (Thermo Scientific, 5100-0001)
dimethyl sulfoxide (Sigma-Aldrich, D8418)
KnockOut Serum Replacement (Gibco, 10828010)
RNeasy Mini Kit (Qiagen 74104)
Qubit RNA Broad Range Assay Kit (Invitrogen Q10211)
RNase-free DNase Set (Qiagen 79254)

Bulk RNA-seq

1 Collect neuron and mouse primary astrocyte monocultures, as well as neuron-astrocyte co-cultures after 3, 7, 14, and 28 days into neuronal maturation (cell re-seeding on PLO-coated plate and switching to Maturation Medium (as detailed in protocol [Ref 1.] Ngn2 neuron differentiation) (i.e., 2, 6, 13, and 27 days after co-culturing, respectively).

- Note: Astrocyte monoculture is incubated in the identical medium as neuron mono- and co-cultures.

2 On the collection days mentioned above, collect the neuron/astrocyte mono- and co-cultures for RNA extraction. Either viably freeze scraped-off cell sheets, dissociated single cells (as detailed in protocol [Ref 2.] neuron dissociation), or directly lyse cells on plate.

1. Option 1: To scrape the cells, use a cell scraper to scrape off >90% of the cells. Collect them into a 15mL conical with Neuronal Maturation Medium. Spin at 300xg for 5 mins. Resuspend the pellet in freeze medium (90% KnockOut Serum Replacement (Gibco, 10828010) + 10% dimethyl sulfoxide [Sigma-Aldrich, D8418]). Freeze cells at -80C in Mr. Frosty Freezing Container (Thermo Scientific, 5100-0001). Transfer frozen vials to liquid nitrogen for storage until ready for RNA extraction.
2. Option 2: To dissociate the cultures into single cells (refer to protocol [Ref 2.] Neuron dissociation). Store frozen vials in liquid nitrogen until ready for RNA extraction.
3. Option 3: Lyse cells on tissue culture plate. Remove medium and add 350µL/well (for 6-well plates) RLT buffer (RNeasy Mini Kit, Qiagen 74104) to lyse the cells. Collect and freeze the lysates at -80C until ready for RNA extraction.

3 When all time points are collected, perform RNA extraction following the manufacturer's recommended protocol for silica-column purification (RNeasy Mini Kit, Qiagen 74104), with optional incorporation of an on-column DNase digestion step to remove residual genomic DNA (RNase-free DNase Set, Qiagen 79254). Elute RNA in small volumes (typically 30µL), optionally as two sequential elutions, in RNase-free water

1. For frozen cell sheets or dissociated cells, thaw cells at 37C. Resuspend cells in 5mL Maturation Medium by adding medium dropwise to cells in freeze medium in a conical tube. Spin for 5 mins at 300 xg. Lyse each pellet in 350µL Buffer RLT.

4 Measure RNA concentrations using a fluorometric assay (Qubit RNA Broad Range Assay Kit, Invitrogen Q10211) on Invitrogen Qubit 4 Fluorometer.

5 Submit RNA samples to Genewiz for RNA-seq library prep (Library preparation, Illumina, RNA with rRNA depletion) and sequencing (Illumina, 2×150bp, ~350M PE reads) services, with targeted sequencing depth for the mono-cultured neurons and astrocytes samples at 30 million paired-end reads per sample, and for the co-cultured neurons and astrocytes samples at 60 million per sample.

Protocol references

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2. Li B, Gersbach CA. Neuron dissociation protocol for single-cell sequencing applications. https://www.protocols.io/view/neuron-dissociation-protocol-for-single-cell-seque-j8nlk1or5g5r/v1