



Oct 21, 2023

Version 1

LDW_BDC Muscle scRNAseq V.1

DOI

dx.doi.org/10.17504/protocols.io.ewov1q31ogr2/v1

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Protocol Citation: Lauren D Walter, Benjamin D Cosgrove 2023. LDW_BDC Muscle scRNAseq. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.ewov1q31ogr2/v1>

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Protocol status: Working

We use this protocol and it's working

Created: October 20, 2023



Last Modified: October 21, 2023

Protocol Integer ID: 89669

Keywords: single cell rna, cell rna sequencing, ldw-bdc muscle, cell rna, rna

Abstract

single-cell RNA sequencing of muscle

Troubleshooting

Skeletal muscle injury and collection

- 1 In a given mouse, both tibialis anterior (TA) muscles were injected with 10 μ l of notexin (10 μ g/ml; Latoxan, France).
- 2 At various days post injury, the mouse was sacrificed and the TAs were collected. Each TA was processed independently to generate single cell suspensions.

Generate single-cell suspensions

- 3 Muscles were enzymatically digested with 8 mg/ml Collagenase D (Roche, Basel, Switzerland) and 10 U/ml Dispase II (Roche, Basel, Switzerland) and then manually dissociated to generate cell suspensions.
- 4 Myofiber debris was removed by filtering the cell suspensions through a 100 μ m and then a 40 μ m filter (Corning Cellgro # 431752 and # 431750).
- 5 After filtration, erythrocytes were removed by incubating the cell suspension in erythrocyte lysis buffer (IBI Scientific # 89135-030).
- 6 After digestion, the single-cell suspensions were washed and resuspended in 0.04% BSA in PBS at a concentration of 10^6 cells/ml. A hemocytometer was used to manually count the cells to determine the concentration of the suspension.

Single-cell RNA library preparation and sequencing

- 7 Single-cell RNA-sequencing libraries were prepared using the Chromium Single Cell 3' reagent kit v3 (10x Genomics, Pleasanton, CA) following the manufacturer's protocol.
- 8 Cells were diluted into the Chromium Single Cell A Chip to yield a recovery of 6,000 single-cell transcriptomes with <5% doublet rate.
- 9 Libraries were sequenced on the NextSeq 500 (Illumina, San Diego, CA).