LDW_BDC Muscle scRNAseq

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ABSTRACT

single-cell RNA sequencing of muscle

OCT 20, 2023

OPEN ACCESS

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

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

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

Created: Oct 20, 2023

Last Modified: Oct 20, 2023

PROTOCOL integer ID:
89669

Skeletal muscle injury and collection

1 In a given mouse, both tibialis anterior (TA) muscles were injected with 10 µl of notexin (10 µg/ml; Latoxan, France).

2 At various days post injury, the mouse was sacrificed and the TAs were collected. Each TA was proccessed 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 µm and then a 40 µm 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).