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cDNA Library Preparation for scRNA-seq of Human Meniscus (10x Genomics)

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

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Keywords: 10x genomic, chemistry kits of 10x genomic, cdna library preparation, scrna, barcoded antibody, cells from individual donor, cell hashing, cdna, barcoding chemistry kit, cell

Abstract

Samples are processed using V2 barcoding chemistry kits of 10x Genomics. For each run, 10,000 cells from individual donors are labeled with distinct oligo-barcoded antibodies (cell hashing), enabling us to pool samples from both conditions on each 10X Genomics run and reduce batch effects. All samples from a given experiment are processed in parallel in the same thermal cycler. The 10x user guide can be found https://assets.ctfassets.net/an68im79xiti/1C16trEdzy1Folq5xbOijE/7e6fb1f504e130bd561d898384da99d9/CG000315_ChromiumNextGEMSingleCell3-_GeneExpression_v3.1_DualIndex__RevB.pdf and is also an attached document.

Attachments

PDF

CG000315_ChromiumNex...

4.5MB

Materials

Chromium Next GEM Single Cell 3′ GEM Kit v3.1, 16 rxns PN-1000123
Library Construction Kit, 16 rxns PN-1000190
Chromium Next GEM Single Cell 3′ Gel Bead Kit v3.1, 16 rxns PN-1000122
Dynabeads™ MyOne™ SILANE PN-2000048
Chromium Next GEM Chip G Single Cell Kit, 16 rxns PN-1000127
Dual Index Kit TT Set A, 96 rxns PN-1000215
BD Hu Single Cell Sample Multiplexing Kit cat# 633781

1 Single cells are isolated from human knee menicus using doi **dx.doi.org/10.17504/protocols.io.n2bvj6k7blk5/v1**.

2 Single cell suspensions are multiplexed and converted to barcoded scRNAseq libraries using the Chromium Single Cell 3' Library, Gel Bead and Chip Kit (10x Genomics), and the **BD™ Hu Single Cell Sample Multiplexing Kit**.

Note

Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (Dual Index) User Guide https://assets.ctfassets.net/an68im79xiti/1C16trEdzy1Folq5xbOijE/7e6fb1f504e130bd561d898384da99d9/CG000315_ChromiumNextGEMSingleCell3-_GeneExpression_v3.1_DualIndex__RevB.pdf

3 Libraries are sequenced on an Illumina NextSeq2000 sequencer targeting 20,000-25,000 reads per cell.