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

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dx.doi.org/10.17504/protocols.io.bp2l6175rvqe/v1

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molmer

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.

CG000315_ChromiumNextGEMSingleCell3-_GeneExpression_v3.1_DualIndex__RevC.pdf

DOI

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protocol ,

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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 femoral articular cartilage using doi dx.doi.org/10.17504/protocols.io.14egn765qv5d/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.

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.

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