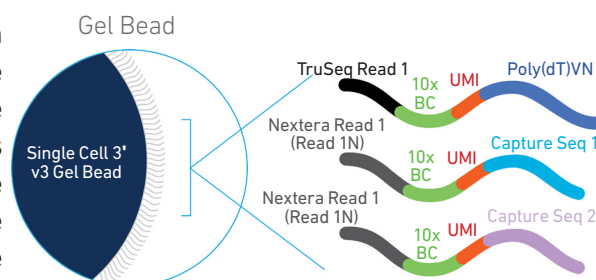## Stepwise Objectives

The Chromium Single Cell Gene Expression Solution with Feature Barcoding technology upgrades short read sequencers to deliver a scalable microfluidic platform for assessing cell surface protein expression combined with 3' digital gene expression of the same single cell by profiling 500-10,000 individual cells per sample. A pool of ~3,500,000 10x Barcodes are sampled separately to index each cell's transcriptome and cell surface protein. It is done by partitioning thousands of cells into nanoliter-scale Gel Beads-in-emulsion (GEMs), where all generated DNA molecules share a common 10x Barcode. Libraries are generated and sequenced from the DNA molecules and 10x Barcodes are used to associate individual reads back to the individual partitions.

This document outlines the protocol for generating Single Cell 3' Gene Expression and Cell Surface Protein libraries from the same cells.
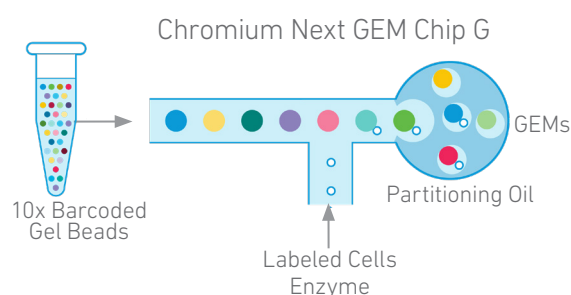
Single Cell 3' v3 Gel Beads

In addition to the poly(dT) primer that enables the production of barcoded, full-length cDNA from poly-adenylated mRNA, the Single Cell 3' v3.1 Gel Beads also include two additional primer sequences (Capture Sequence 1 and Capture Sequence 2), that enable capture and priming of Feature Barcode technology compatible targets or analytes of interest.

The poly(dT) primers along with one of the capture sequence primers are used in this protocol for generating Single Cell 3' Gene Expression and Cell Surface Protein libraries.

Step 1
GEM Generation & Barcoding

GEMs are generated by combining barcoded Single Cell 3' v3.1 Gel Beads, a Master Mix with cell surface protein labeled cells, and Partitioning Oil onto Chromium Chip B. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contain no cell, while the remainder largely contain a single cell.

Step 1
GEM Generation & Barcoding

Immediately following GEM generation, the Gel Bead is dissolved releasing the three types of primers and any co-partitioned cell is lysed. The poly(dT) and one of the capture sequence primers in the gel bead are engaged simultaneously in two different reactions inside individual GEMs (primer with Capture Sequence 2 is not shown in the illustrated example).

A. Primers containing:
- an Illumina TruSeq Read 1 (read 1 sequencing primer)
- 16 nt 10x Barcode
- 12 nt unique molecular identifier (UMI)
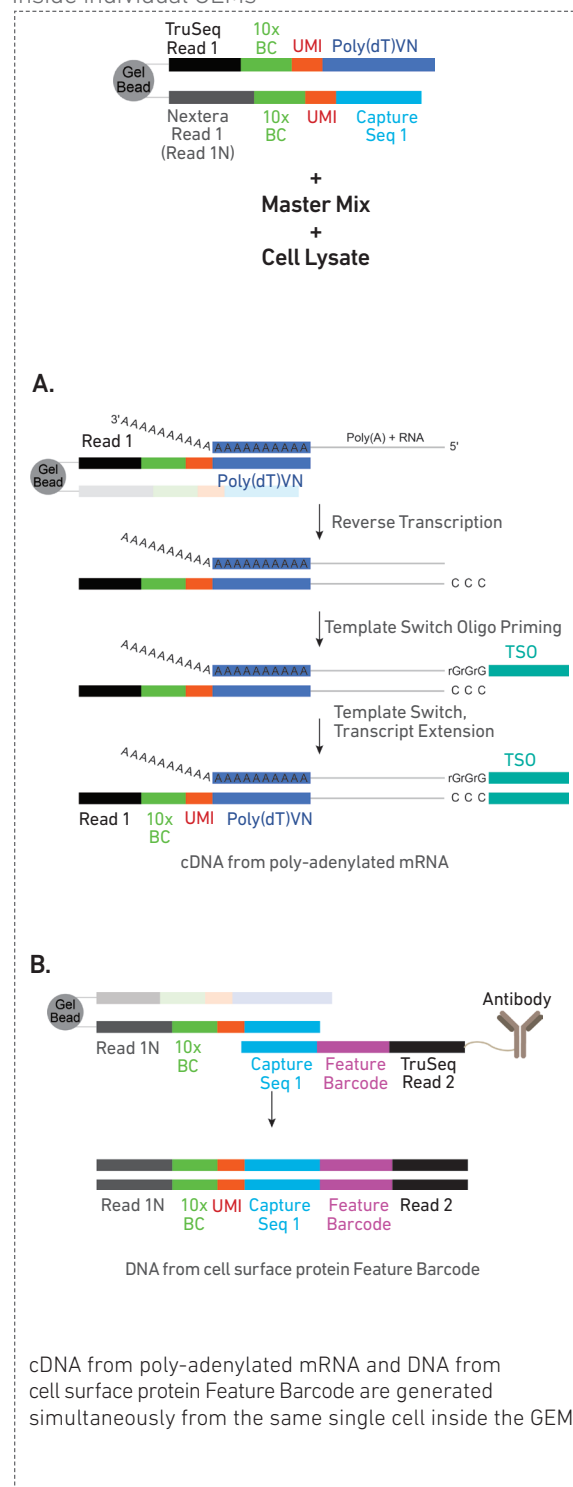- 30 nt poly(dT) sequence

are mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents. Incubation of the GEMs produces barcoded, full-length cDNA from poly-adenylated mRNA.

B. In the same partition, primers containing:
- an Illumina Nextera Read 1 (Read 1N; read 1 sequencing primer)
- 16 nt 10x Barcode
- 12 nt unique molecular identifier (UMI)
- Capture Sequence 1

are mixed with the cell lysate and the Master Mix containing the RT reagents. Incubation of the GEMs produces barcoded DNA from the cell surface protein Feature Barcode.
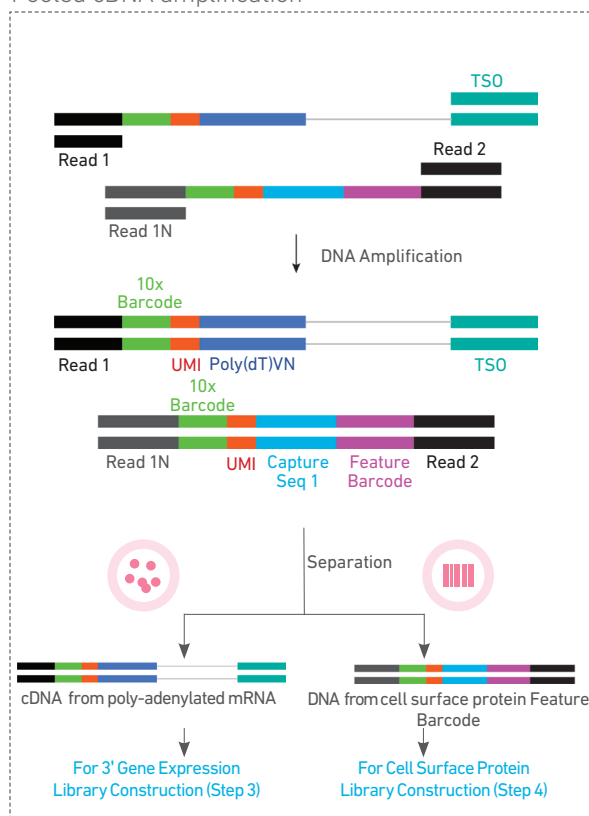
Inside individual GEMs

cDNA from poly-adenylated mRNA and DNA from cell surface protein Feature Barcode are generated simultaneously from the same single cell inside the GEM

Step 2
Post GEM-RT Cleanup & cDNA Amplification

After incubation, GEMs are broken and pooled fractions are recovered. Silane magnetic beads are used to purify the cell barcoded products from the post GEM-RT reaction mixture, which includes leftover biochemical reagents and primers. The cell barcoded cDNA molecules are amplified via PCR to generate sufficient mass for library constructions. Size selection is used to separate the amplified cDNA molecules for 3' Gene Expression and Cell Surface Protein library construction.
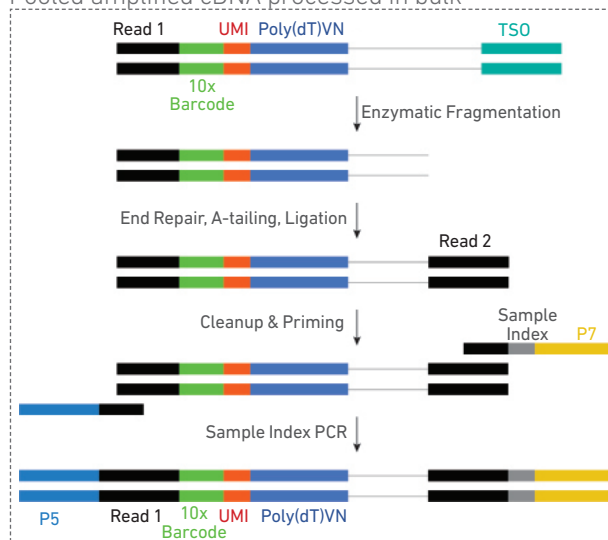

Pooled cDNA amplification

Step 3
3' Gene Expression Library Construction

Amplified full-length cDNA from poly-adenylated mRNA is used to generate 3' Gene Expression libraries. Enzymatic fragmentation and size selection are used to optimize the cDNA amplicon size. TruSeq Read 1 (read 1 primer sequence) is added to the molecules during GEM incubation. P5, P7, a sample index, and TruSeq Read 2 (read 2 primer sequence) are added via End Repair, A-tailing, Adaptor Ligation, and PCR. The final libraries contain the P5 and P7 primers used in Illumina bridge amplification.
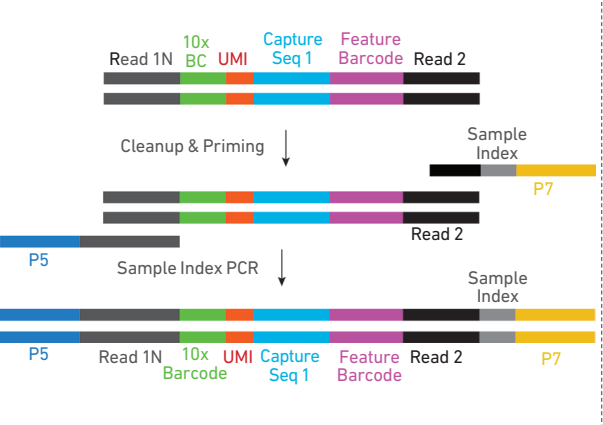

Pooled amplified cDNA processed in bulk

Step 4
Cell Surface Protein
Library Construction

Amplified DNA from cell surface protein Feature Barcodes is used for library construction. P5, P7, a sample index, and TruSeq Read 2 (read 2 primer sequence) are added via PCR. The final libraries contain the P5 and P7 primers used in Illumina bridge amplification.
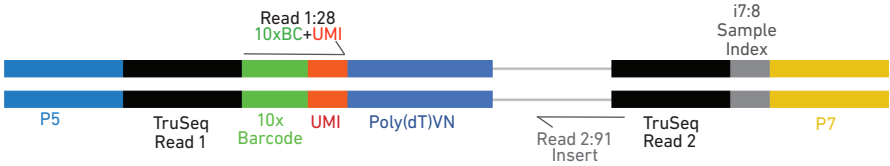

Pooled amplified DNA processed in bulk
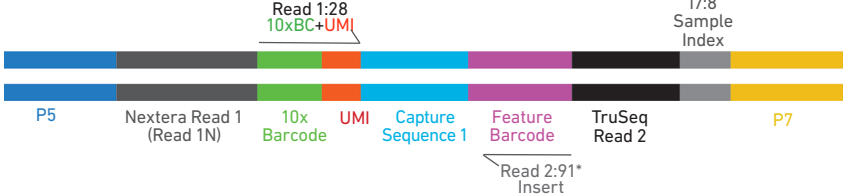
Step 5
Sequencing

The Single Cell 3' libraries comprise standard Illumina paired-end constructs which begin and end with P5 and P7. The 16 bp 10x Barcode and 12 bp UMI are encoded in Read 1, while Read 2 is used to sequence the cDNA fragment in 3' Gene Expression libraries and the Feature Barcode in the Cell Surface Protein libraries. Sample index sequences are incorporated as the i7 index read. Standard Illumina sequencing primer sites TruSeq Read 1 and TruSeq Read 2 in the 3' Gene Expression libraries and Nextera Read 1 and TruSeq Read 2 in the Cell Surface Protein libraries are used in paired-end sequencing.

Illumina sequencer compatibility, sample indices, library loading and pooling, recommended read depths and run parameters are summarized in step 5.

Chromium Single Cell 3' Gene Expression Library
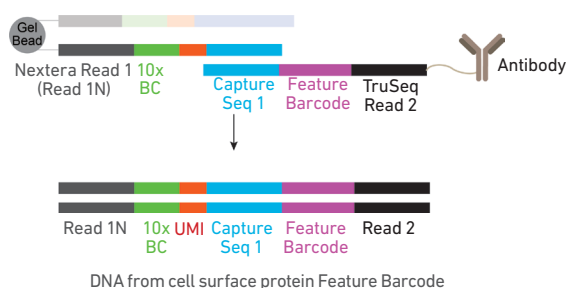

Chromium Single Cell 3' Cell Surface Protein Library

*Minimum required Read 2 length for Cell Surface Protein libraries is 25 bp

See Appendix for Oligonucleotide Sequences

Cell Surface Protein Labeling Guidelines

Overview

Cell surface proteins can be labeled using a Feature Barcode oligonucleotide conjugated to a specific protein binding molecule, such as an antibody. The Feature Barcode cojugated molecule bound to the cell surface protein can be directly captured by the Gel Bead inside a GEM during GEM generation and amplified (see Stepwise Objectives for assay scheme specifics). The amplified DNA generated form the Feature Barcode can be used for Cell Surface Protein Library Construction.

DNA from cell surface protein Feature Barcode

Demonstrated Protocols for cell surface protein labeling

For antibody-oligonucleotide conjugation guidance and cell surface protein labeling protocol, consult Demonstrated Protocol Cell Surface Protein Labeling for Single Cell RNA Sequencing Protocols with Feature Barcoding technology (Document CG000149).

Failure to label cell surface proteins with a Feature Barcode conjugated to a specific protein binding molecule prior to using the cells for GEM Generation & Barcoding will preclude generation of Cell Surface Protein library.