## Stepwise Objectives

The Single Cell V(D)J protocols offer comprehensive, scalable solutions for measuring immune repertoire information and gene expression from the same cell. Profile full-length (5' UTR to constant region), paired T-cell receptor (TCR), or B-cell immunoglobulin (Ig) transcripts from 100-10,000 individual cells per sample. A pool of ~750,000 barcodes are sampled separately to index each cell's transcriptome. It is done by partitioning thousands of cells into nanoliter-scale Gel Beads-in-emulsion (GEMs), where all generated cDNA share a common 10x Barcode. Libraries are generated and sequenced and 10x Barcodes are used to associate individual reads back to the individual partitions. This document outlines the protocol to generate an enriched T-cell library and/or an enriched B-cell library, and/or a 5' Gene Expression library from amplified cDNA from the same cells.

Step 1
GEM Generation & Barcoding

GEMs are generated by combining barcoded Single Cell VDJ 5' Gel Beads v1.1, a Master Mix with cells, and Partitioning Oil on Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90 – 99%) of generated GEMs contains no cell, while the remainder largely contain a single cell.

Immediately following GEM generation, the Gel Bead is dissolved and any co-partitioned cell is lysed. Oligonucleotides containing (i) an Illumina R1 sequence (read 1 sequencing primer), (ii) a 16 nt 10x Barcode, (iii) a 10 nt unique molecular identifier (UMI), and (iv) 13 nt template switch oligo (TSO) are released and mixed with the cell lysate and a Master Mix containing reverse transcription (RT) reagents and poly(dT) RT primers. Incubation of the GEMs produces 10x Barcoded, full-length cDNA from poly-adenylated mRNA.

Chromium Next GEM Chip G
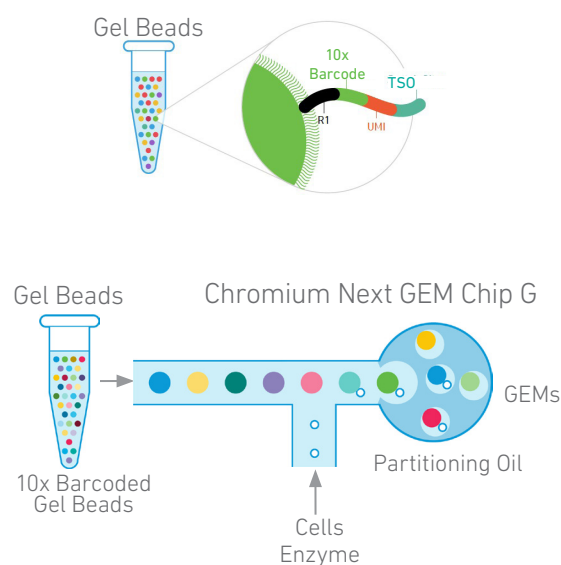
Inside individual GEMs
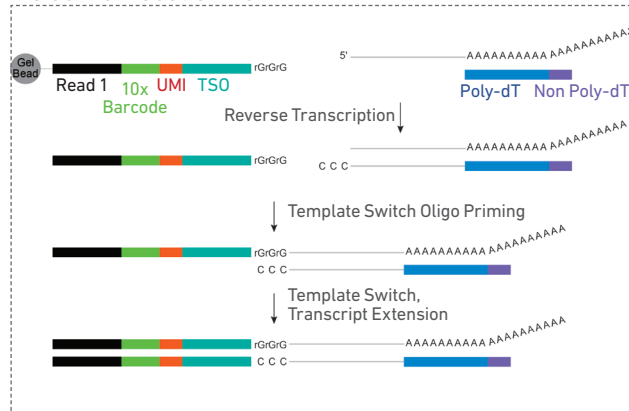
Step 2
Post GEM-RT Cleanup & QC

GEMs are broken and pooled after GEM-RT reaction mixtures are recovered. Silane magnetic beads are used to purify the 10x Barcoded first-strand cDNA from the post GEM-RT reaction mixture, which includes leftover biochemical reagents and primers. After cleanup a user may decide to pursue target enrichment directly from first-strand cDNA, in which case, consult Demonstrated Protocol - Chromium Single Cell V(D)J Reagent Kits-Direct Target Enrichment (Document CG000166). Otherwise, users should proceed to cDNA amplification in this protocol.

Step 3
cDNA Amplification & QC

10x Barcoded, full-length cDNA is amplified via PCR with primers against common 5' and 3' ends added during GEM-RT. Amplification generates sufficient material to construct multiple libraries from the same cells, e.g. both T cell and/or B cell enriched libraries (steps 4 and 5) and 5' Gene Expression libraries (step 6).
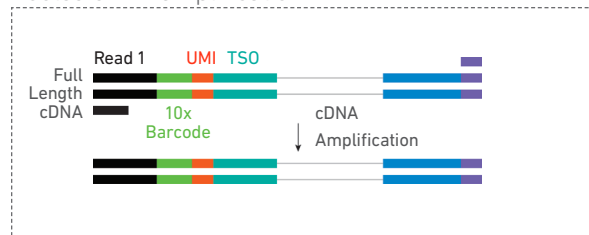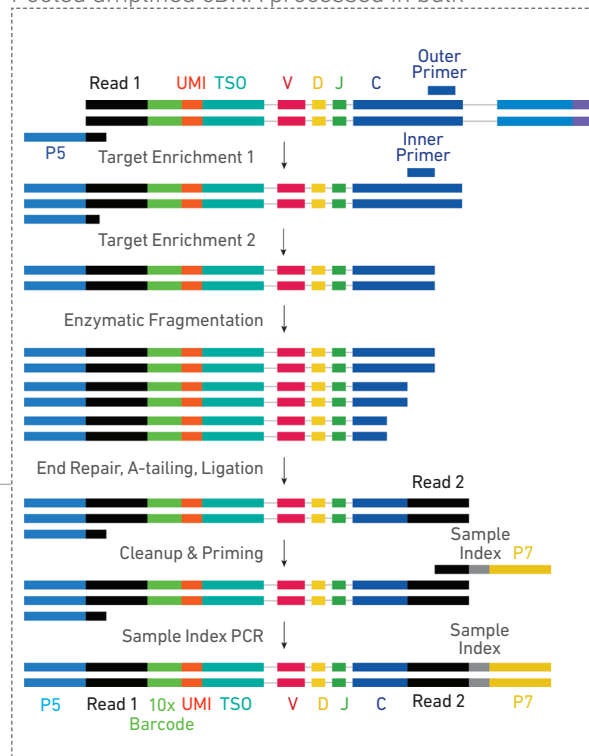

Step 4
Target Enrichment from cDNA

Full-length V(D)J segments (10x Barcoded) are enriched from amplified cDNA via PCR amplification with primers specific to either the TCR or Ig constant regions. If both T and B cells are expected to be present in the partitioned cell population, TCR and Ig transcripts can be enriched in separate reactions from the same amplified cDNA material. P5 is added during enrichment.

## Step 5
Enriched Library Construction

Enzymatic fragmentation and size selection are used to generate variable length fragments that collectively span the V(D)J segments of the enriched TCR or Ig transcripts prior to library construction.

P7, a sample index, and an Illumina R2 sequence (read 2 primer sequence) are added via End Repair, A-tailing, Adaptor Ligation, and Sample Index PCR. The final libraries contain the P5 and P7 priming sites used in Illumina sequencing.

Step 6
5' Gene Expression (GEX)
Library Construction

Enzymatic fragmentation and size selection are used to optimize the cDNA amplicon size prior to 5' Gene Expression library construction. P5, P7, a sample index, and Illumina R2 sequence (read 2 primer sequence) are added via End Repair, A-tailing, Adaptor Ligation, and Sample Index PCR. The final libraries contain the P5 and P7 priming sites used in Illumina sequencers.

Pooled amplified cDNA processed in bulk
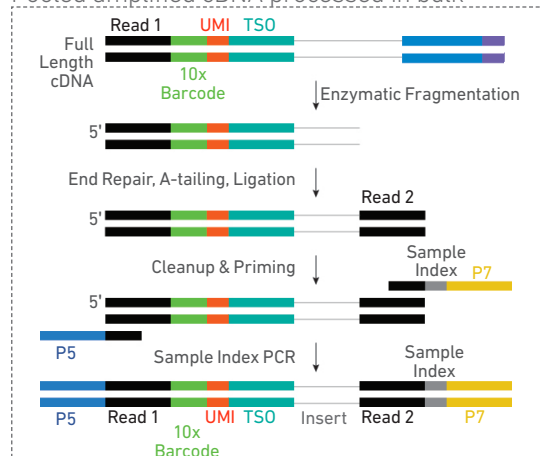
Step 7
Sequencing

Illumina-ready sequencing libraries can be sequenced at the recommended depth & run parameters. Illumina sequencer compatibility, sample indices, library loading and pooling for sequencing are summarized in step 7.

Chromium Single Cell V(D)J Enriched Library

Chromium Single Cell 5' Gene Expression Library
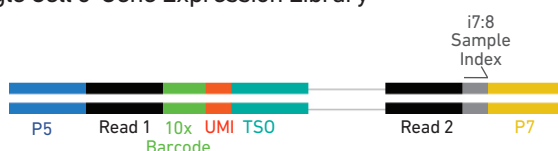
See Appendix for Oligonucleotide Sequences