10x GENOMICS®

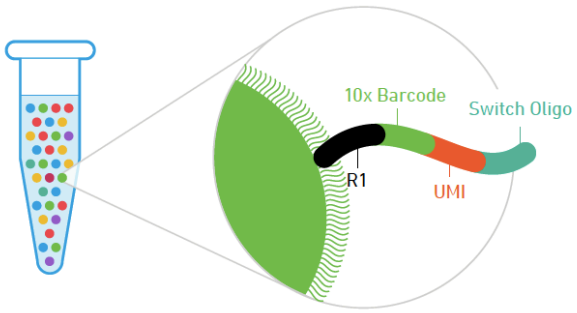
TECHNICAL NOTE

Assay Scheme and Configuration of Chromium™ Single Cell V(D)J Libraries

INTRODUCTION

The Chromium™ Single Cell V(D)J Solution produces Illumina® sequencer-ready libraries for profiling T-cell receptor (TCR) or B cell immunoglobulin (Ig) repertoires from single cells. In addition, users have the option of profiling 5' gene expression from the same cells if cDNA amplification is performed prior to target enrichment for TCR and/or Ig transcripts. During library preparation, sequence components essential for Illumina sequencing and downstream data analysis are incorporated into the final library construct. The sequence components are introduced via Gel Beads and during the library preparation steps of the workflow.

Each Gel Bead contains millions of oligo primers that are comprised of the following sequences (Figure 1):



i. Partial Illumina Read 1 Sequence (22 nucleotides (nt))
ii. 16 nt 10x™ Barcode
iii. 10 nt Unique Molecular Identifier (UMI)
iv. 13 nt Switch Oligo

Figure 1. Schematic of a Single Cell 5' Gel Bead oligo primer.

10x Genomics® GemCode™ Technology partitions thousands of cells into droplets, each called a Gel Bead-in-EMulsion (GEM). Once partitioned, the Gel Bead dissolves and its oligo primers are released into the aqueous environment of the GEM. The cell captured in the GEM is also lysed. The contents of the GEM (oligos, lysed cell components and Master Mix that contains the Poly-dT RT primer) are incubated in a reverse transcription (RT) reaction to generate full-length, barcoded cDNA from poly-adenylated mRNA. The reverse transcriptase incorporates the Gel Bead oligo via a template switching reaction at the 5' end of the transcript. All cDNA generated within an individual GEM share a common 10x Barcode.

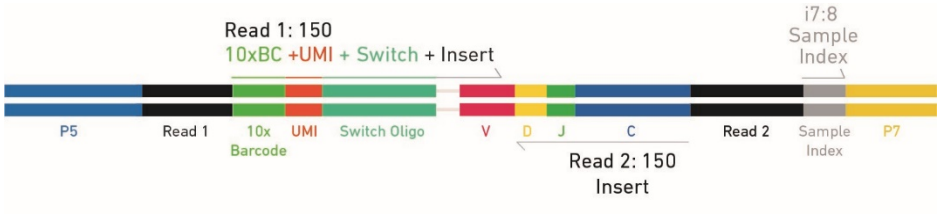
The Single Cell V(D)J Solution offers the option to generate:

i. Protocol Option 1: Direct Target Enrichment - enriched library from either T cells or B cells, directly from first-strand cDNA

ii. Protocol Option 2: cDNA Amplification followed by Target Enrichment - 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

The Single Cell V(D)J Protocols produce V(D)J enriched and 5' gene expression Illumina® sequencer-ready libraries. A library comprises standard Illumina paired-end constructs which begin and end with P5 and P7, respectively. For V(D)J enriched libraries, Read 1 encodes the 16 bp 10x™ Barcode, 10 bp UMI, and 13 bp Switch Oligo, as well as the 5' end of an enriched transcript. For 5' gene expression libraries, Read 1 encodes the 16 bp 10x Barcode and 10 bp UMI. Due to Enzymatic Fragmentation, for both libraries Read 2 encodes a random internal fragment of the corresponding insert. Sample index sequences are incorporated as the i7 index read. A schematic of the final library constructs is shown in Figure 2.

V(D)J Enriched Library Structure:



5' Gene Expression Library Structure:

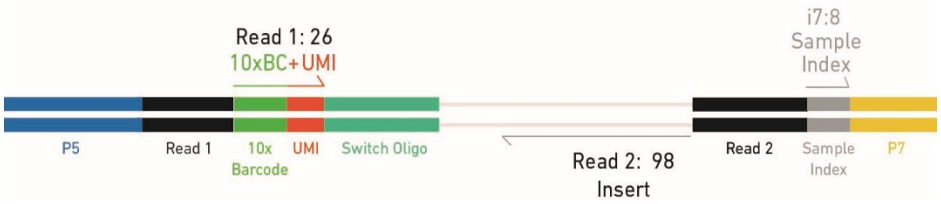


Figure 2. Schematic of final library constructs and recommended sequencing run parameters for the Single Cell V(D)J Protocol options.

An overview of the Single Cell V(D)J Protocol options and how individual sequence components are incorporated during library construction is presented in Figure 3 and Figure 4. Table 1 provides detailed reaction products and oligo sequences for Protocol Option 1: Direct Target Enrichment and Table 2 provides detailed reaction products and oligo sequences for Protocol Option 2: Target Enrichment from Amplified cDNA and 5' Gene Expression. Refer to the *Chromium™ Single Cell V(D)J Reagent Kits User Guide* (CG000086) for more details.

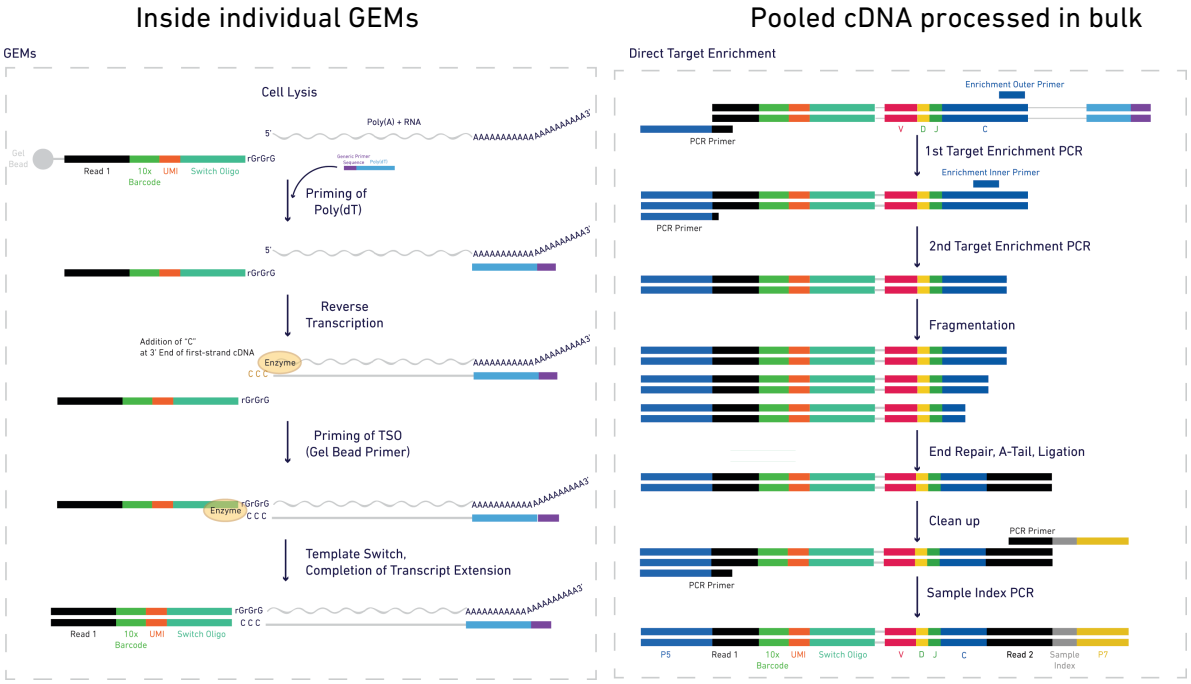


Figure 3. Assay schematic for Single Cell V(D)J Protocol Option 1: Direct Target Enrichment Library Construction. Fragmentation occurs through the length of the V(D)JC gene segments. Only fragmented products that contain the C region are shown for simplicity. TSO = Template Switch Oligo.

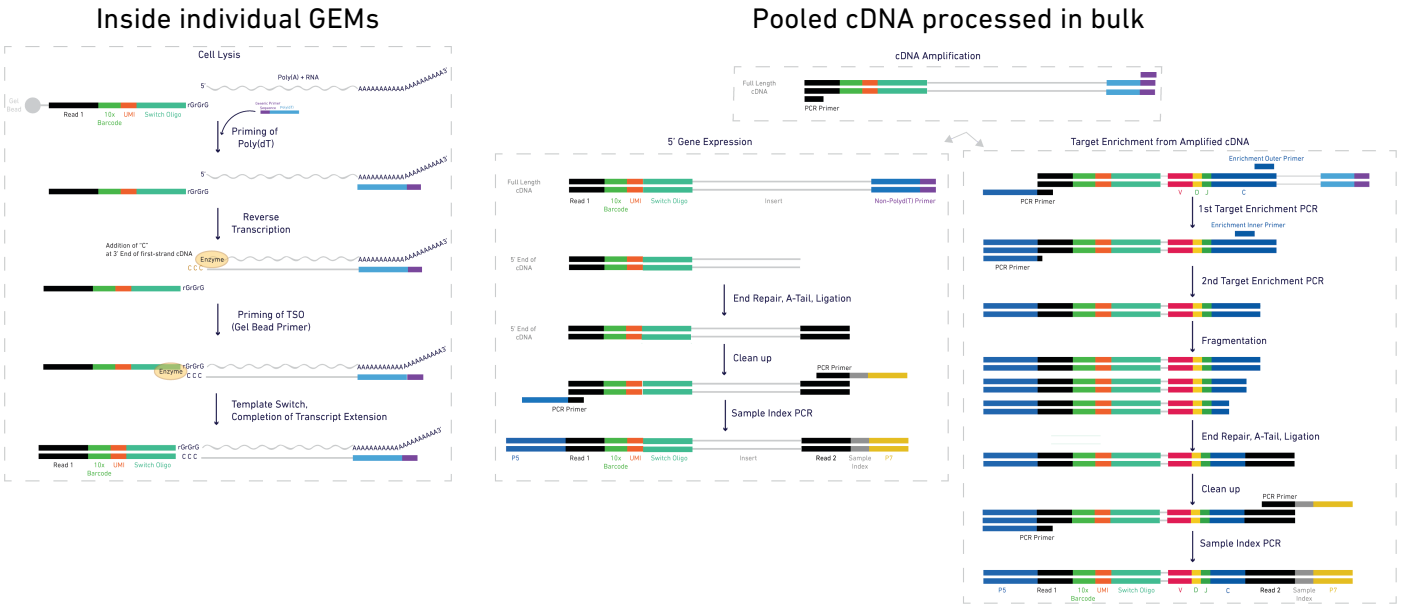


Figure 4. Assay schematic for Single Cell V(D)J Protocol Option 2: Target Enrichment from Amplified cDNA and 5' Gene Expression Library Construction. Fragmentation occurs through the length of the V(D)JC gene segments. Only fragmented products that contain the C region are shown for simplicity. TSO = Template Switch Oligo.

CONCLUSION

We have presented a detailed description of the assay configuration for the Single Cell V(D)J Solution, including enriched libraries and 5' gene expression libraries. Individual steps during library construction outlined here provide additional insight and may serve as a reference to customize the library preparation workflow for unsupported technical development and applications.

REFERENCES

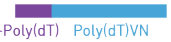
- *Chromium™ Single Cell V(D)J Reagent Kits User Guide* (CG000086)

Protocol Step 1.5 – GEM-RT Incubation

Gel Bead Oligo Primer (TSO)
(PN-220112)

5′–CTACACGACGCTCTTCCGATCT–NNNNNNNNNNNNNNNN–NNNNNNNNN–TTTCTTATATrGrGrG–3′

Poly-dT RT Primer
(PN-2000007)



5′AAGCAGTGGTATCAACGCAGAGTAC–TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN–3′

Reverse Transcript Product

3′–GATGTGCTGCGAGAAGGCTAGA–NNNNNNNNNNNNNNNN–NNNNNNNNN–AAAGAATATACCC–cDNA_Insert–NVTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT–CATGAGACGCAACTATGGTGACGAA–5′

Protocol Step 3.1 – Direct Target Enrichment 1

Human T Cell Mix 1
(PN-2000008)

Forward primer: (final concentration of 2 μM)

5′–AATGATACGGCGACCACCGA–GATCTACACTCTTTCCCTACACGACGCTC–3′

Reverse primers: (final concentration of 1 μM each)

5′–TGAAGGCGTTTGCACATGCA–3′
5′–TCAGGCAGTATCTGGAGTCATTGAG–3′

Human B Cell Mix 1
(PN-2000035)

Forward primer: (final concentration of 1 μM)

5′–AATGATACGGCGACCACCGA–GATCTACACTCTTTCCCTACACGACGCTC–3′

Reverse primers: (final concentration of 0.5 μM each)

5′–CAGGGCACAGTCACATCCT–3′
5′–TGCTGGACCACGCATTTGTA–3′
5′–GGTTTTGTTGTCGACCCAGTCT–3′
5′–TTGTCCACCTTGGTGTTGCT–3′
5′–CATGACGTCCTTGGAAGGCA–3′
5′–TGTGGGACTTCCACTG–3′
5′–TTCTCGTAGTCTGCTTTGCTCAG–3′

Protocol Step 3.3 – Direct Target Enrichment 2

Human T Cell Mix 2
(PN-2000009)

Forward primer: (final concentration of 2 μM)

5′–AATGATACGGCGACCACCGA–GATCT–3′

Reverse primers: (final concentration of 1 μM each)

5′–AGTCTCTCAGCTGGTACACG–3′
5′–TCTGATGGCTCAAACACAGC–3′

Human B Cell Mix 2
(PN-2000036)

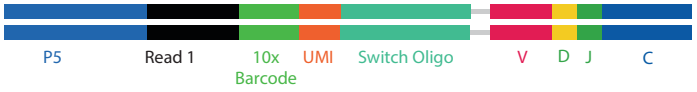
Forward primer: (final concentration of 1 μM)

5′–AATGATACGGCGACCACCGA–GATCT–3′

Reverse primers: (final concentration of 0.5 μM each)

5′–GGGAAGTTTCTGGCGGTCA–3′
5′–GGTGGTACCCAGTTATCAAGCAT–3′
5′–GTGTCCCAGGTCACCATCAC–3′
5′–TCCTGAGGACTGTAGGACAGC–3′
5′–CACGCTGCTCGTATCCGA–3′
5′–TAGCTGCTGGCCGC–3′
5′–GCGTTATCCACCTTCCACTGT–3′

Targeted Amplification Product



5′–AATGATACGGCGACCACCGA–GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT–NNNNNNNNNNNNNNNN–NNNNNNNNN–TTTCTTATATGGG–cDNA_Insert–Inner_Primer–3′
3′–TTACTATGCCGCTGGTGGCT–CTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA–NNNNNNNNNNNNNNNN–NNNNNNNNN–AAAGAATATACCC–cDNA_Insert–Inner_Primer–5′

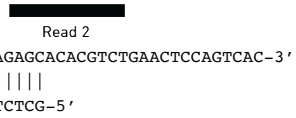
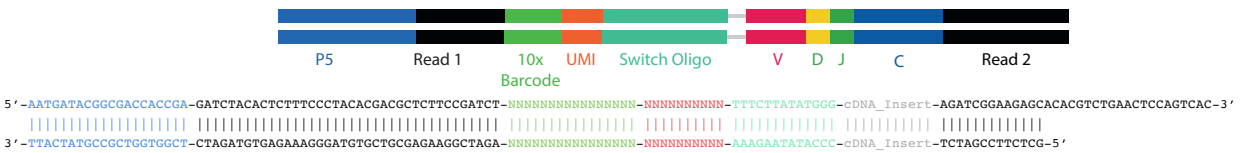
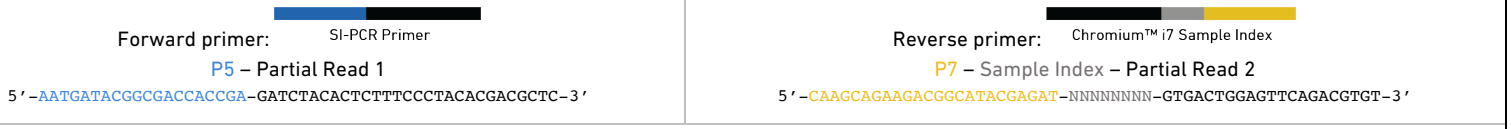
Protocol 7.2 – Adaptor Ligation	
Adaptor (Read 2) *(PN-220026)*	
Ligation Product	
Protocol 7.4 – Sample Index PCR	
Sample Index PCR Primer *(PN-220111 and PN-220103)*	
Sample Index PCR Product	

Table 1. Detailed reaction products and oligo sequences for the Single Cell V(D)J Protocol Option 1: Direct Target Enrichment Library Construction. Protocol steps correspond to the *Chromium™ Single Cell V(D)J Reagent Kits User Guide* (CG000086).

Protocol Step 1.5 – GEM-RT Incubation

Gel Bead Oligo Primer (TSO)
(PN-220112)

5′–CTACACGACGCTCTTCCGATCT–NNNNNNNNNNNNNNNN–NNNNNNNNN–TTTCTTATATrGrGrG–3′

Poly-dT RT Primer
(PN-2000007)

5′AAGCAGTGGTATCAACGCAGAGTAC–TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN–3′

Reverse Transcript Product

3′–GATGTGCTGCGAGAAGGCTAGA–NNNNNNNNNNNNNNNN–NNNNNNNNN–AAAGAATATACCC–cDNA_Insert–NVTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT–CATGAGACGCAACTATGGTGACGAA–5′

Protocol 4.1– cDNA Amplification

cDNA Primer Mix
(PN-220106)

Forward primer: Partial Read 1
5′–CTACACGACGCTCTTCCGATCT–3′

Reverse primer: Non-Poly(dT) Primer
5′– AAGCAGTGGTATCAACGCAGAG–3′

cDNA Amplified Product

5′–CTACACGACGCTCTTCCGATCT–NNNNNNNNNNNNNNNN–NNNNNNNNN–TTTCTTATATGGG–cDNA_Insert–GTACTCTGCGTTGATACCACTGCTT–3′

3′–GATGTGCTGCGAGAAGGCTAGA–NNNNNNNNNNNNNNNN–NNNNNNNNN–AAAGAATATACCC–cDNA_Insert–CATGAGACGCAACTATGGTGACGAA–5′

Protocol Step 5.1 – Target Enrichment 1 from Amplified cDNA

Human T Cell Mix 1
(PN-2000008)

Forward primer: (final concentration of 2 µM) PCR Primer
5′–AATGATACGGCGACCACCGA–GATCTACACTCTTTCCCTACACGACGCTC–3′

Reverse primers: (final concentration of 1 µM each) Enrichment Outer Primer
5′–TGAAGGCGTTTGCACATGCA–3′
5′–TCAGGCAGTATCTGGAGTCATTGAG–3′

Human B Cell Mix 1
(PN-2000035)

Forward primer: (final concentration of 1 µM) PCR Primer
5′–AATGATACGGCGACCACCGA–GATCTACACTCTTTCCCTACACGACGCTC–3′

Reverse primers: (final concentration of 0.5 µM each) Enrichment Outer Primer
5′–CAGGGCACAGTCACATCCT–3′
5′–TGCTGGACCACGCATTTGTA–3′
5′–GGTTTTGTTGTCGACCCAGTCT–3′
5′–TTGTCCACCTTGGTGTTGCT–3′
5′–CATGACGTCCTTGGAAGGCA–3′
5′–TGTGGGACTTCCACTG–3′
5′–TTCTCGTAGTCTGCTTTGCTCAG–3′

Protocol Step 5.3 – Target Enrichment 2 from Amplified cDNA

Human T Cell Mix 2
(PN-2000009)

Forward primer: (final concentration of 2 µM) PCR Primer
5′–AATGATACGGCGACCACCGA–GATCT–3′

Reverse primers: (final concentration of 1 µM each) Enrichment Inner Primer
5′–AGTCTCTCAGCTGGTACACG–3′
5′–TCTGATGGCTCAAACACAGC–3′

Human B Cell Mix 2
(PN-2000036)

Forward primer: (final concentration of 1 µM) PCR Primer
5′–AATGATACGGCGACCACCGA–GATCT–3′

Reverse primers: (final concentration of 0.5 µM each) Enrichment Inner Primer
5′–GGGAAGTTTCTGGCGGTCA–3′
5′–GGTGGTACCCAGTTATCAAGCAT–3′
5′–GTGTCCCAGGTCACCATCAC–3′
5′–TCCTGAGGACTGTAGGACAGC–3′
5′–CACGCTGCTCGTATCCGA–3′
5′–TAGCTGCTGGCCGC–3′
5′–GCGTTATCCACCTTCCACTGT–3′

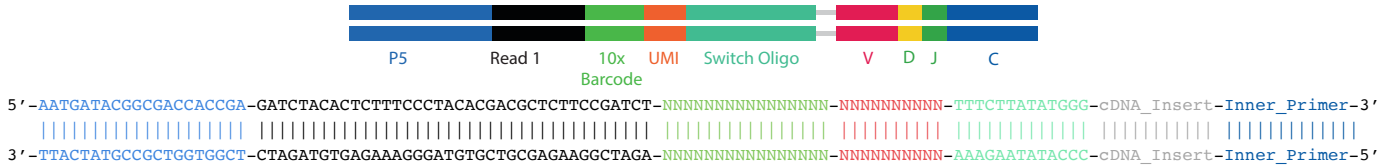
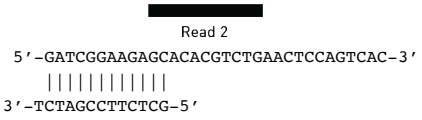
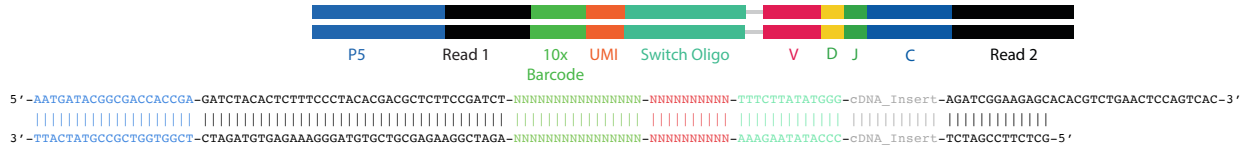
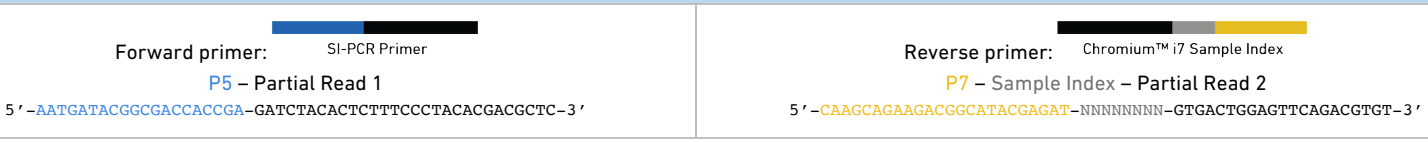
Targeted Amplification Product	
Protocol 7.2 – Adaptor Ligation	
Adaptor (Read 2) *(PN-220026)*	
Ligation Product	
Protocol 7.4 – Sample Index PCR	
Sample Index PCR Primer *(PN-220111 and PN-220103)*	
Sample Index PCR Product	

Table 2A. Detailed reaction products and oligo sequences for the Single Cell V(D)J Protocol Option 2: Target Enrichment from Amplified cDNA Library Construction. Protocol steps correspond to the *Chromium™ Single Cell V(D)J Reagent Kits User Guide* (CG000086).

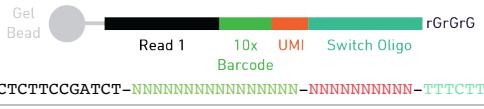
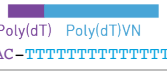
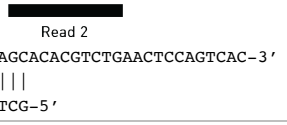
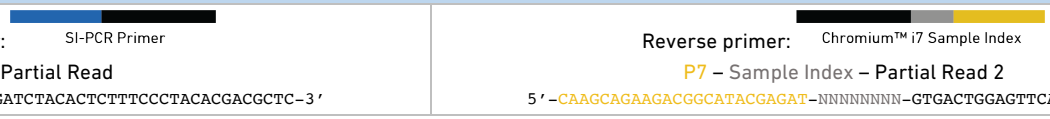
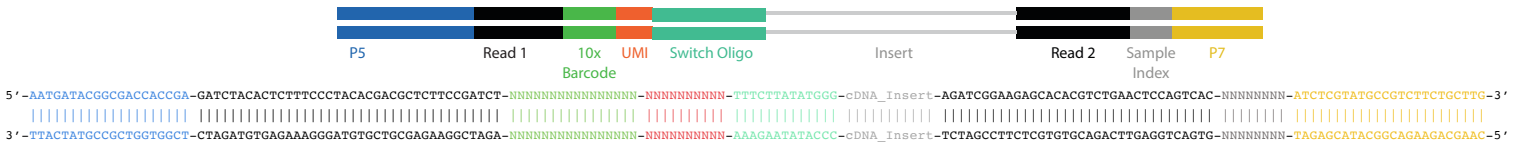
Protocol Step 1.5 – GEM-RT Incubation	
Gel Bead Oligo Primer (TSO) *(PN-220112)*	
Poly-dT RT Primer *(PN-2000007)*	
Reverse Transcript Product	
Protocol 4.1– cDNA Amplification	
cDNA Primer Mix *(PN-220106)*	
cDNA Amplified Product	
Protocol 6.3 – Adaptor Ligation	
Adaptor (Read 2) *(PN-220026)*	
Ligation Product	
Protocol 6.5 – Sample Index PCR	
Sample Index PCR Primer *(PN-220111 and PN-220103)*	
Sample Index PCR Product	

Table 2B. Detailed reaction products and oligo sequences for the Single Cell V(D)J Protocol Option 2: 5′ Gene Expression Library Construction. Protocol steps correspond to the *Chromium™ Single Cell V(D)J Reagent Kits User Guide* (CG000086).

Notices

Document Number

CG000109 Rev D *Technical Note*

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