10x GENOMICS®

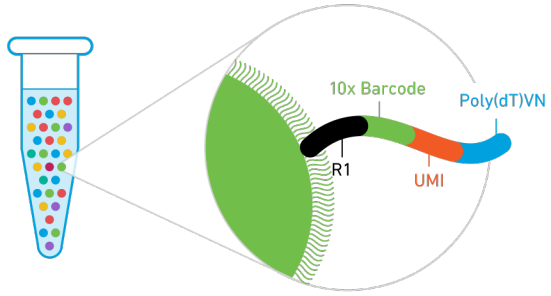
TECHNICAL NOTE

Assay Scheme and Configuration of Chromium™ Single Cell 3' v2 Libraries

INTRODUCTION

The Chromium™ Single Cell 3' v2 Protocol (CG00052) produces Single Cell 3' libraries ready for Illumina® sequencing. 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 the 10x™ Gel Beads and 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. 30 nt Poly(dT) primer sequence

Fig. 1. Schematic of a SC3' v2 Gel Bead oligo primer.

10x Genomics technology is based on the partitioning of samples and reagents 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) are incubated in an RT reaction to generate full-length, barcoded cDNA from the poly A-tailed mRNA transcripts. The reverse transcription reaction is primed by the barcoded Gel Bead oligo and the reverse transcriptase incorporates the template switch oligo via a template switching reaction at the 5' end of the transcript. The GEMs are then "broken", pooling single-stranded, barcoded cDNA molecules from every cell. A bulk PCR-amplification and Enzymatic Fragmentation follow. Size selection is then used to optimize the insert size of the double-stranded cDNA prior to library construction. During library construction Read 2 is added by Adapter ligation. Illumina P5 and P7 sequences and sample index sequences are added during the Sample Index PCR. The final library fragments contain the P5, P7, Read 1 and Read 2 sequences used in Illumina bridge amplification and sequencing. Additionally, each fragment contains the 10x Barcode, UMI and cDNA insert sequence used in data analysis (Figure 2). An overview of the Single Cell 3' v2 assay scheme and how individual sequence components are incorporated during library construction is presented in Figure 3.

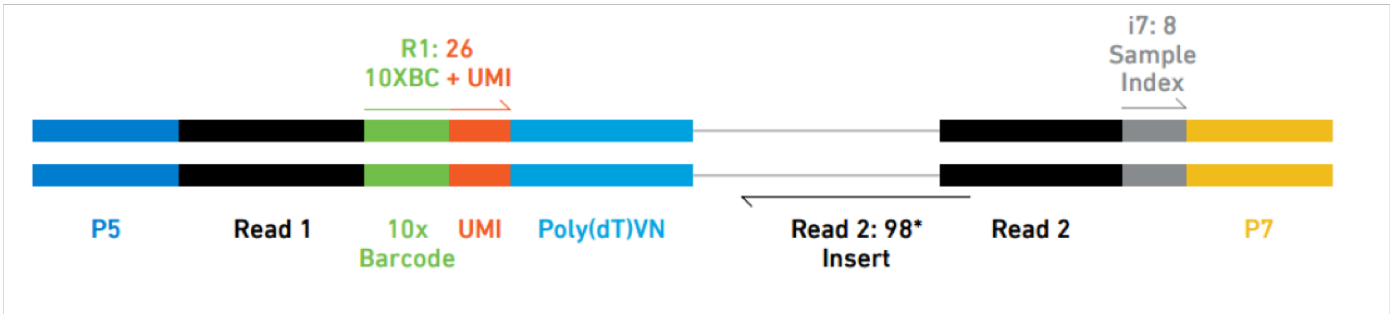


Fig. 2. Schematic of a fragment from a final Chromium™ Single Cell 3' v2 library. *Can be adjusted.

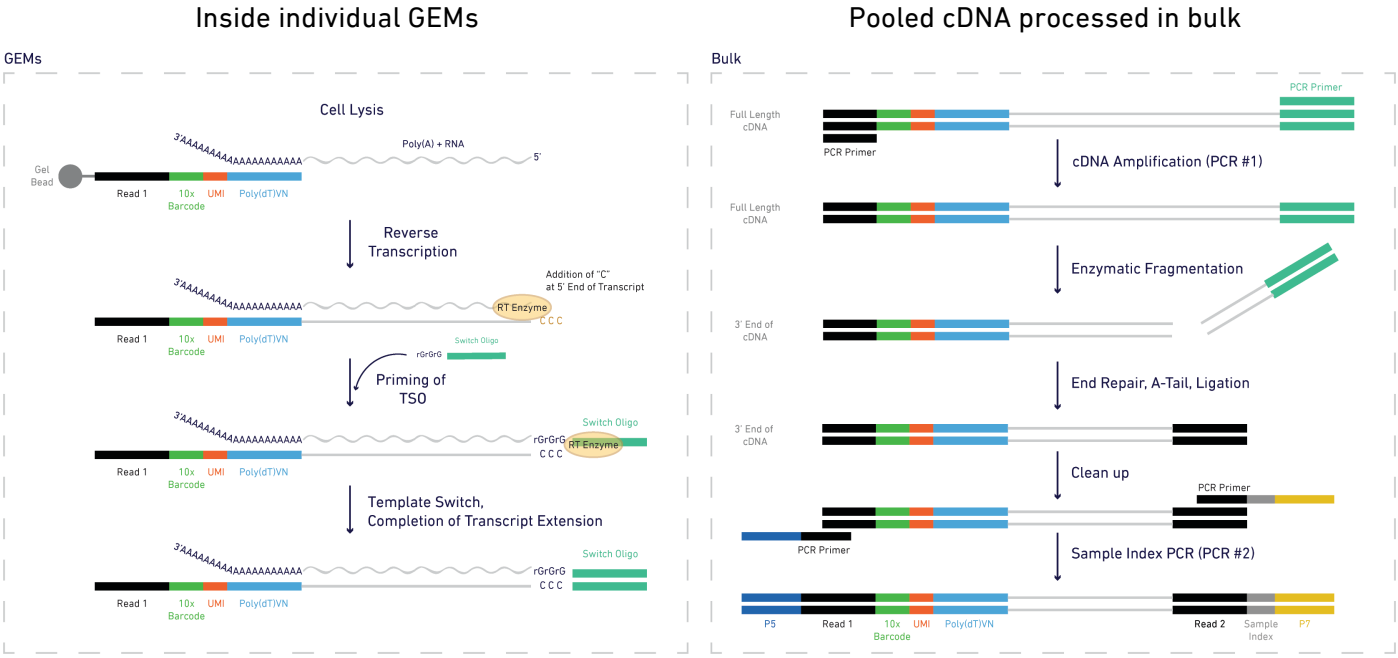


Fig. 3. Schematic of assay scheme for Chromium Single Cell 3' v2 library preparation.

Figure 4 provides a detailed description of the library preparation workflow. Individual protocol steps that are listed in Figure 4 refer to the *Chromium Single Cell 3' Reagent Kits v2 User Guide* (CG00052).

CONCLUSION

We have presented a detailed description of the assay configuration for Chromium Single Cell 3' v2 libraries. Individual steps during library construction outlined here provide additional insight and may serve as a reference to customize the library preparation workflow (e.g. targeted cDNA enrichment).

REFERENCES

- *Chromium™ Single Cell 3' Reagent Kits v2 User Guide* (CG00052)

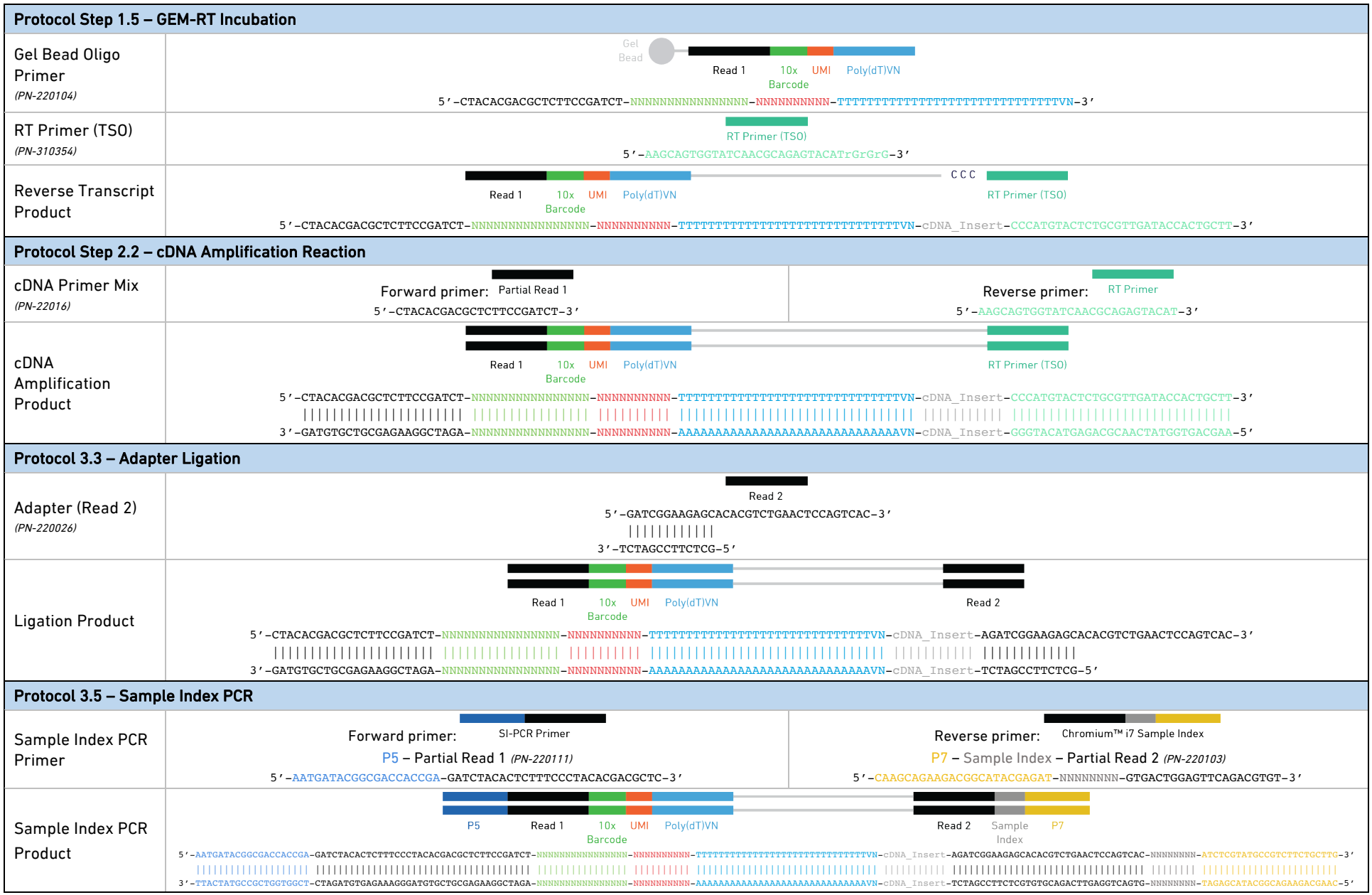


Fig. 4. Flow chart that outlines construction of Single Cell 3′ v2 libraries. Protocol steps and part numbers refer to *Chromium™ Single Cell 3′ Reagent Kits v2 User Guide* (CG00052).

Notices

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