TECHNICAL NOTE

Assay Scheme & Configuration of Chromium Single Cell DNA Libraries

Introduction

The Chromium Single Cell DNA Reagent Kits protocol (Document CG000153) produces Single Cell DNA libraries ready for Illumina sequencing. Using the "Cell Bead-Gel Bead" (CBGB) technology, which involves a two-step microfluidics process to partition individual cells into Cell Beads, followed by partitioning of each Cell Bead with a 10x Gel Bead in a Gel Bead-in-Emulsion (GEM), single cell DNA barcoding is achieved. During library preparation, sequence components essential for Illumina sequencing and downstream data analysis are incorporated into the final library construct.

An overview of the Single Cell DNA workflow is presented in Figures 1-3. A single cell or nuclei suspension is combined with Cell Bead reagents in Chromium Chip C and run on the Chromium Controller or Chromium Single Cell Controller to generate Cell Beads. The Cell Beads are collected and processed to lyse the cells and denature the genomic DNA (gDNA), while retaining the gDNA within the Cell Bead. The gDNA inside the Cell Bead is now accessible for downstream amplification and barcoding (Figure 1).

Gel Beads that each deliver oligonucleotides consisting of a 10x Barcode sequence (Figure 2) are then combined with the Cell Beads and a reaction mix containing enzyme in Chromium Chip D (Figure 3). The chip is run on the Chromium Controller or Chromium Single Cell Controller to generate Cell Bead-Gel Bead (CBGB) GEMs, which are droplets containing one Cell Bead and one Gel Bead.

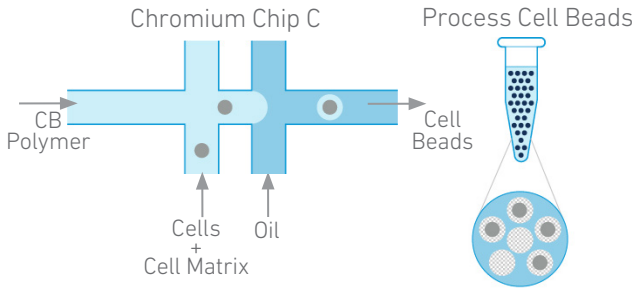

Figure 1. Single cell suspensions are partitioned into Cell Beads. Cell Beads are collected and processed to lyse the cells and denature the gDNA, while retaining the gDNA within the Cell Bead.

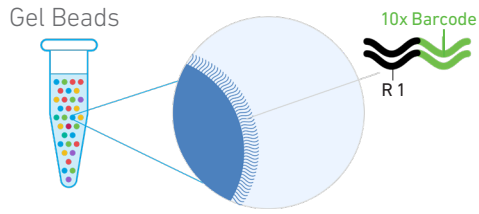

Figure 2. Schematic of Gel Bead oligo sequence. Each Gel Bead comprises of many oligos containing (i) a partial Illumina Read 1(R1) sequence (22 bp) and (ii) a 16 bp 10x Barcode. The 10x Barcode sequence is unique to each Gel Bead.

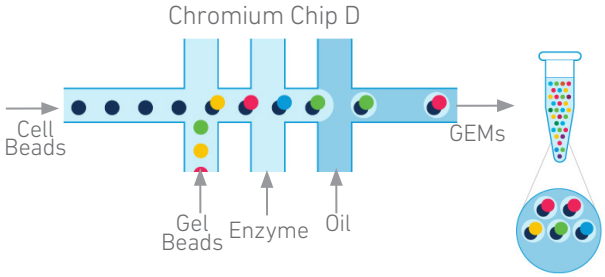

Figure 3. Cell Beads are partitioned into GEMs containing the components necessary for barcoding and amplifying DNA from single cells.

Once partitioned, the Gel Bead dissolves, releasing Gel Bead oligonucleotides into the aqueous environment of the GEM. The GEMs are isothermally incubated to amplify gDNA using random hexamers supplied in the Reaction Mix (Figure 4). During the isothermal incubation, the Gel Bead oligonucleotides are ligated to the amplified DNA to produce barcoded fragments ranging from a several hundred basepairs to a few kilobases.

The GEMs are then "broken", pooling the barcoded DNA molecules from the single cell partitions (Figure 4). During library construction, Read 2 is added during Adaptor Ligation. Illumina P5 and P7 sequences and i7 sample index are added during Sample Index PCR. The final library fragments contain a 10x Barcode and the genomic insert, flanked by sequences used in Illumina amplification (P5, P7) and indexing (i7). Detailed information regarding the sequences added during library preparation is provided in Figure 5.

Conclusion

This Technical Note presents an overview of the Single Cell DNA assay scheme and configuration of the sequencing library after completing the protocol outlined in the Chromium Single Cell DNA Reagent Kits User Guide (Document CG000153). The workflow and sequences presented here provide additional insight into Chromium Single Cell DNA libraries and may serve as a reference to customize library preparation.

References

- Chromium Single Cell DNA Reagent Kits User Guide (Document CG000153)

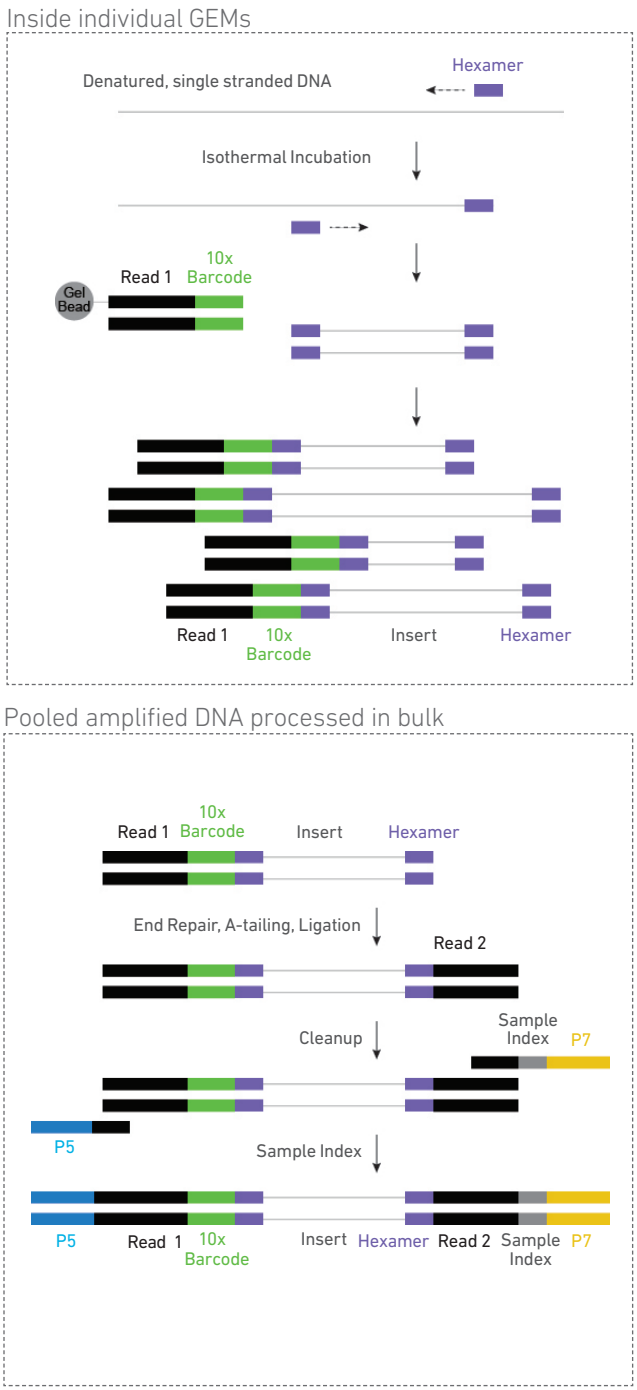

Figure 4. Assay scheme for Single Cell DNA Library Preparation.

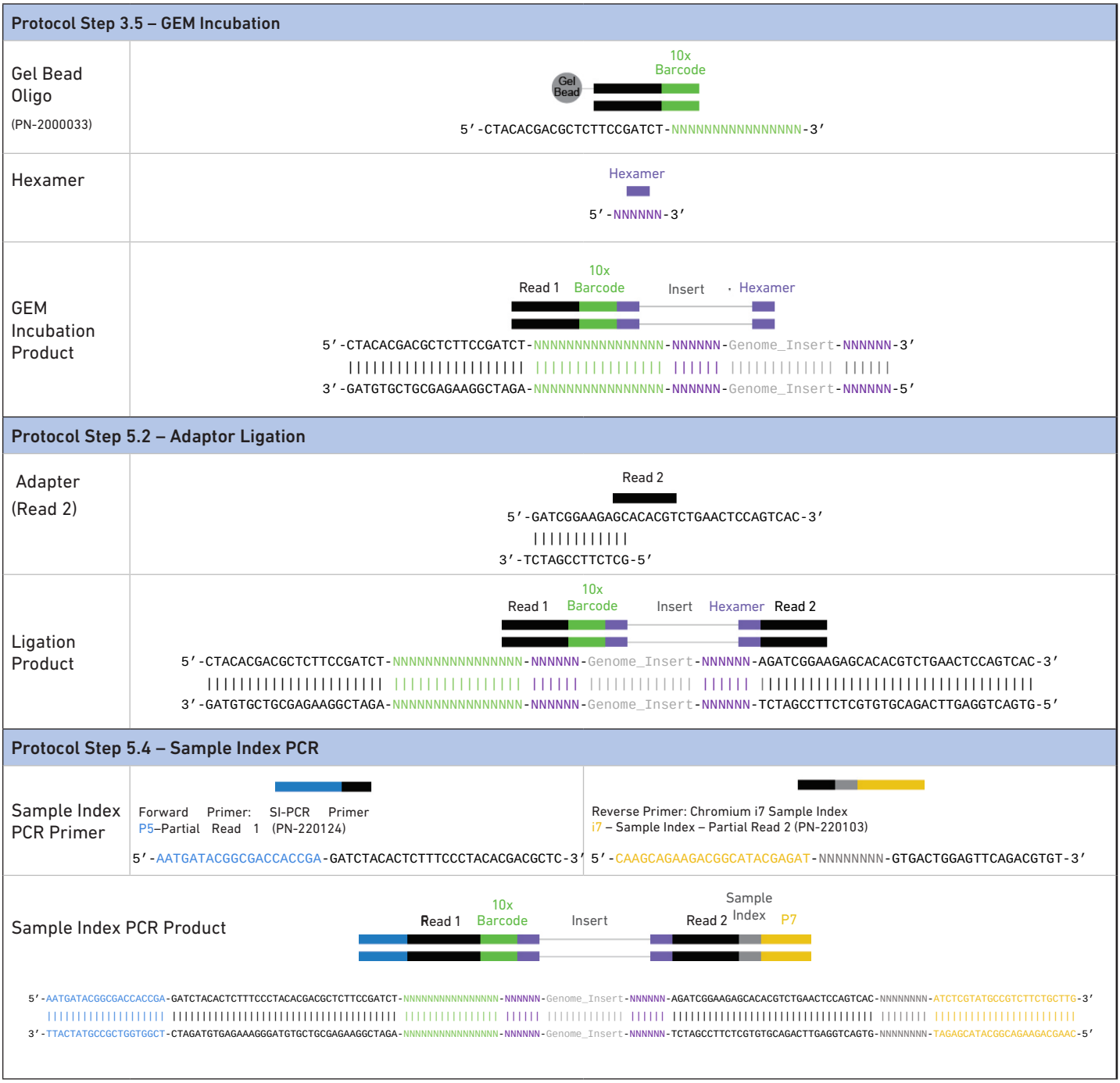

Figure 5. Sequences added during Single Cell DNA library construction. Protocol steps and part numbers refer to Chromium Single Cell DNA Reagent Kits User Guide (Document CG000153).

Contact:
support@10xgenomics.com
10x Genomics
7068 Koll Center Parkway
Suite 401
Pleasanton, CA 94566 USA