TECHNICAL NOTE

Guide RNA Specifications Compatible with Feature Barcoding technology for CRISPR Screening

Introduction

The Single Cell Gene Expression Solution coupled with Feature Barcoding technology enables profiling of gene expression in conjunction with additional cellular features from the same single cell. Specifically, the Single Cell Gene Expression and CRISPR Screening Solution provides a high-throughput and scalable approach to obtain gene expression profiles along with CRISPR-mediated perturbation phenotypes in transduced cells via direct capture of poly-adenylated mRNAs and single-guide RNAs (sgRNAs) (Figure 1). This document provides specifications for engineering Feature Barcoding technology compatible sgRNAs for use with the Chromium Single Cell 3' workflow (Figure 2) for CRISPR screening.

Chromium Single Cell 3' v3 Gel Beads

In addition to a poly(dT) primer sequence that enables the production of barcoded, full-length cDNA from poly-adenylated mRNA, the Single Cell 3' v3 Gel Beads (Figure 1) also include two additional primer sequences (Capture Sequence 1 and Capture Sequence 2) for direct capture and priming of Feature Barcoding technology compatible sgRNAs present in a cell inside a Gel-bead-in-emulsion (GEM).

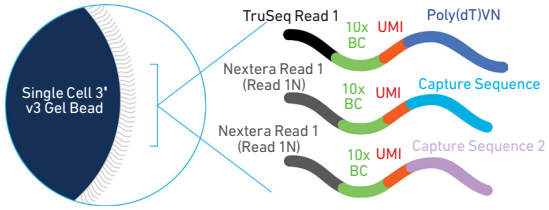


Figure1. Schematic of a Chromium Single Cell 3' v3 Gel Bead, including the Capture Sequence 1 and the Capture Sequence 2 for direct capture of sgRNA molecules from a cell inside a GEM.

Capture Sequence Integration in sgRNA

To enable direct capture, each sgRNA should be engineered to contain either Capture Sequence 1 or Capture Sequence 2, along with a protospacer (Feature Barcode), designed to target gene/s of interest. Two possible locations for integrating the capture sequence in the sgRNA include (1) within the sgRNA hairpin structure, or (2) immediately before the sgRNA termination signal, elongating the 3' end of the sgRNA (Table 1). However, alternate sgRNA integration locations for either of the two capture sequences may be possible depending on the specific application, type of construct used etc.

Performing sgRNA QC by qPCR, NGS or other methods is recommended before proceeding with 10x Genomics Single Cell Solutions. The Chromium Single Cell Gene Expression and CRIPSR Screening Solution has been shown to be compatible with sgRNA constructs in several CRISPR applications, including interference (CRISPRi), activation (CRISPRa), and cutting.

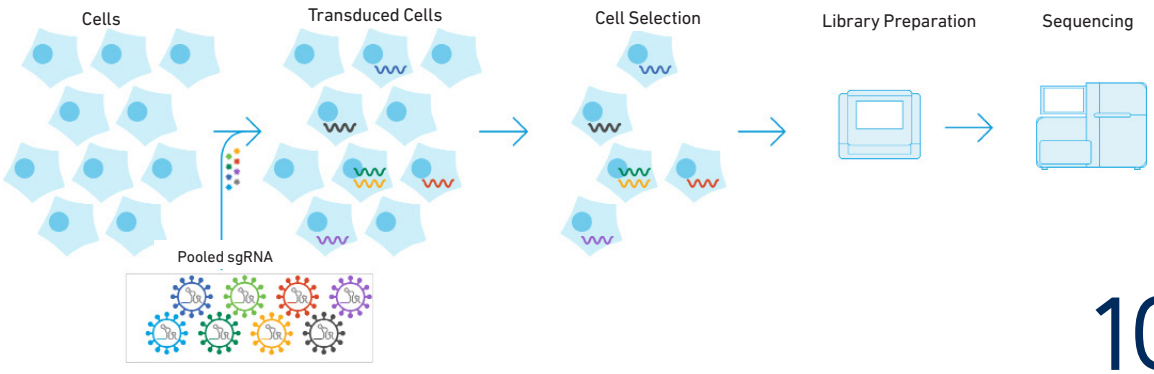
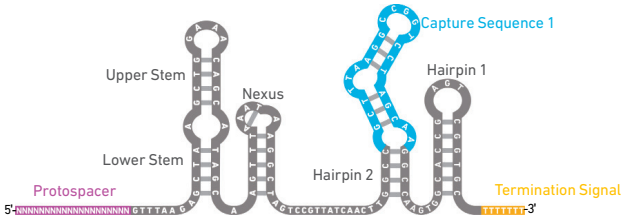


Figure 2. Transduced cells with integrated sgRNA for the Chromium Single Cell 3' workflow for CRISPR screening.

Capture Sequence 1

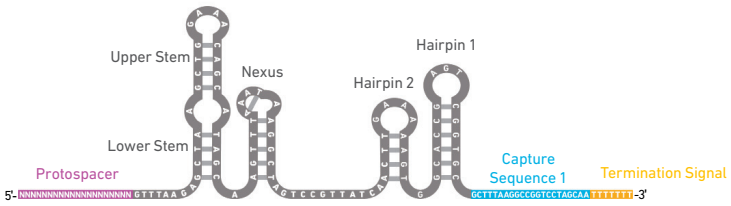
Capture Sequence 1 on Gel Bead: 5'-TTGCTAGGACCGGCCTTAAAGC-3'

Capture Sequence 1 integrated in sgRNA hairpin



5'-NNNNNNNNNNNNNNNNNNNNGTTTAAGAGCTAAGCTGGAAACAGCATAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTggccGCTTTAAGGCCGGTCCTAGCAAggccAAGTGGCACCGAGTCGGTGCTTTTTTT-3'

Capture Sequence 1 integrated in sgRNA 3'-end

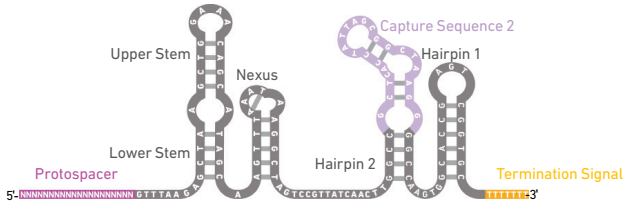


5'-NNNNNNNNNNNNNNNNNNNNGTTTAAGAGCTAAGCTGGAAACAGCATAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTgaaaAAGTGGCACCGAGTCGGTGCGCTTTAAGGCCGGTCCTAGCAATTTTTTT-3'

Capture Sequence 2

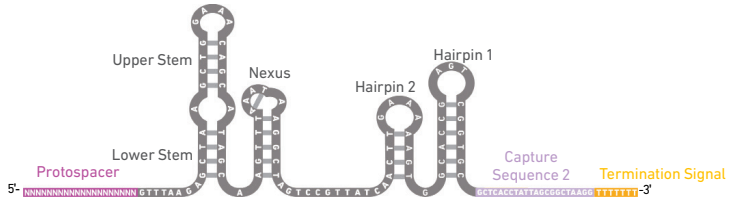
Capture Sequence 2 on Gel Bead: 5'-CCTTAGCCGCTAATAGGTGAGC-3'

Capture Sequence 2 integrated in sgRNA hairpin



5'- NNNNNNNNNNNNNNNNNNNNGTTTAAGAGCTAAGCTGGAAACAGCATAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTggccGCTCACCTATTAGCGGCTAAGGggccAAGTGGCACCGAGTCGGTGCTTTTTTT-3'

Capture Sequence 2 integrated in sgRNA 3'-end



5'- NNNNNNNNNNNNNNNNNNNNGTTTAAGAGCTAAGCTGGAAACAGCATAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTgaaaAAGTGGCACCGAGTCGGTGCGCTCACCTATTAGCGGCTAAGGTTTTTTT-3'

Table 1. Integration of Capture Sequence 1 and Capture Sequence 2 in sgRNA.

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