Direct capture of guide RNAs enables scalable and combinatorial single cell CRISPR screens

Researchers at the University of California, San Francisco (San Francisco, CA, USA), with scientists from 10x Genomics, described a method to streamline CRISPR screens by directly capturing and sequencing guide RNAs in single cells, without the need for a separate indexing transcript. Direct capture of guide RNAs enables accurate identification at the single cell level, and opens the door for researchers to test combinatorial CRISPR perturbations and detect the resulting cell phenotypes. This scalable method to simultaneously capture and analyze guide RNAs, together with their effect on the transcriptome, is enabled on the 10x Genomics Single Cell Gene Expression Solution with Feature Barcode technology. JM Replogle et al., *Nat. Biotechnol.* (2020).

Snapshot	10x Genomics product
Research questions: How does direct capture of guide RNAs enable an increase in scale for single cell CRISPR screens? How well does direct capture of guide RNAs perform relative to previous methods? How do CRISPR screens at single cell resolution enhance my understanding of complex biology? **Research area:** CRISPR screening **Organism:** Human **Sample type:** K562 human cancer cell line	**Chromium Single Cell Gene Expression v3 with Feature Barcode technology*** • Chromium Single Cell 3′ GEM, Library & Gel Bead Kit v3 • Chromium Single Cell 3′ Feature Barcode Library Kit • Chromium Chip B Single Cell Kit • Chromium i7 Multiplex Kit • Cell Ranger Analysis Pipelines * This assay is also supported on v3.1 Next GEM technology

Experiment overview

Enable accurate and direct identification of guide RNAs in large-scale CRISPR screens

- Design unique capture sequences within guide RNAs that do not interfere with guide activity
- Evaluate guide RNA capture from Feature Barcode primers present on Single Cell Gene Expression v3 Gel Beads
- Computationally assign guide identity to individual cells

Compare direct capture of guide RNAs with indirect detection of guide RNAs via paired guide barcodes (GBC Perturb-seq)

- Screen five CRISPRi libraries in K562 cells
- Sequence to matched read depth of 25 million index reads per experiment
- Compare capture rate, guide identity assignment, and transcriptional responses between methods
- Annotate t-SNE projection of single cells by guide RNA identity

Why single cell?

CRISPR screens provide the opportunity to efficiently test disruption of thousands of genes at once, with throughput limited only by the chosen method of phenotypic screening. Using single cell sequencing, screening for transcriptional changes can occur hand-in-hand with guide identification. Direct capture of guide RNAs paired with single cell gene expression analysis is now commercially available as the Chromium Single Cell Gene Expression Solution with Feature Barcode technology.

Results

Previous methods for single cell CRISPR screening, including Perturb-seq with unique guide barcodes (GBC Perturb-seq) or CROP-seq, required vectors that expressed polyadenylated index transcripts in conjuction with non-polyadenylated guide RNAs. This limited the scale possible for single cell CRISPR screens, making combinatorial perturbations difficult or impossible to pursue. These limitations are overcome by using specialized capture sequences and unique RT barcodes within partitioning droplets to enable direct capture of guide RNAs (Figure 1).

Comparison of capture rate, guide identity assignment, and transcriptional response show that direct capture of guide RNAs performs as well or better than GBC Perturb-seq. Direct capture can have higher capture rates, dependent on the guide RNA used (4.1-fold higher for sgRNA^{cs1} and 0.56-fold for sgRNA^{cs2}, Figure 2) and equivalent guide identity assignment (89% for GBC Perturb-seq versus 84–94% for direct capture methods). Target knockdown is also comparable (90% for GBC Perturb-seq versus 93–94% for direct capture).

Importantly, transcriptional responses to CRISPR perturbation are consistent across these methods, suggesting the addition of capture sequences to guide RNAs does not impact cellular responses. In addition to tight correlation of differentially expressed genes, cluster analysis for both methods segregated cells appropriately (Figure 3).

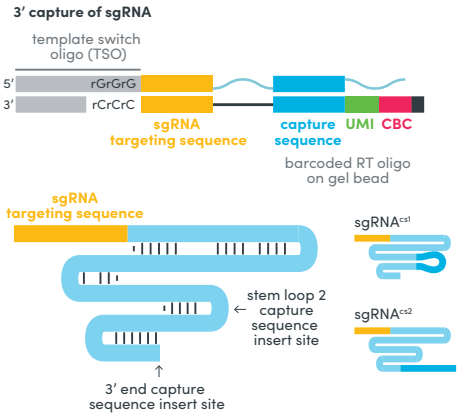

Figure 1. Schematic of designs for direct capture of guide RNAs. RT oligo on Gel Bead includes capture sequence 1 or 2, unique molecular identifier (UMI), and cell barcode (CBC). Also shown are sites for capture sequence 1 (sgRNAcs1), located within the stem loop, and capture sequence 2 (sgRNAcs2), located at the 3′ end of the guide RNA.

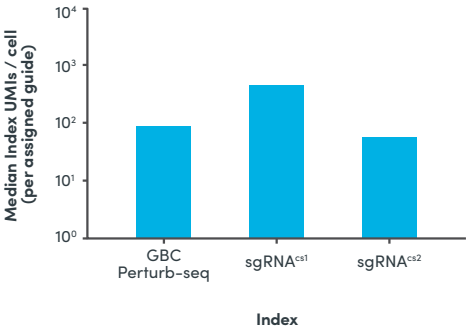

Figure 2. Comparison of index capture rate. Compared to GBC Perturb-seq, direct capture of guide RNAs can detect more index UMIs/cell.

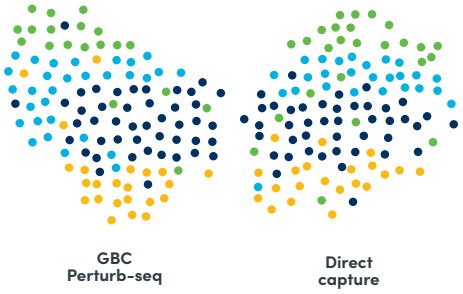

Figure 3. Single cell t-SNE projection of CRISPRi screens for GBC Perturb-seq versus the direct capture method. Cells are color-coded according to guide identity (dark blue = negative control).

References

1. JM Replogle et al., Combinatorial single-cell CRISPR screens by direct guide RNA capture and targeted sequencing. *Nat. Biotechnol.* (2020). doi.org/10.1038/s41587-020-0470-y

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