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A flexible and efficient approach for single-cell CRISPR perturbation screens combined with targeted RNA-seq expression analysis

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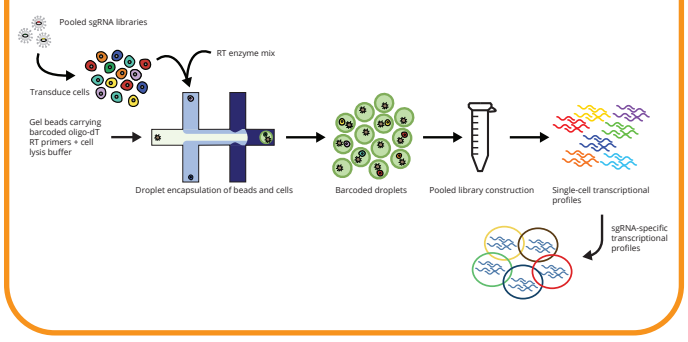
Abstract

This study aims to compare the performance of two distinct single-cell CRISPR methodologies: the standard 10X Genomics protocol (CS1) and the CROP-Seq (U6d1) approach. In the standard 10X protocol, the CS1 capture sequence inserted in the sgRNA tracr is used to detect the expressed sgRNA in each single cell, while in the CROP-Seq protocol the sequence of the expressed sgRNA is detected within the poly-dT cDNA pool.
We designed a custom sgRNA library focused on a small subset of genes associated with the TNFα response, and cloned it in the 10X CS1 and CROP-Seq lentivectors. HEK293-Cas9 cells were transduced in parallel with the two lentiviral libraries at MOI ~0.05, Puromycin-selected to enrich for transduced cells, treated for 72h with TNFα, and analyzed at single cell level for the activation of the TNFα response pathways.
The findings of this study indicate that the 10X-CS1 and the CROP-Seq approach yield comparable results in single-cell CRISPR-seq, leaving the choice of which approach to use to the investigator's preference and study-specific factors.

Introduction

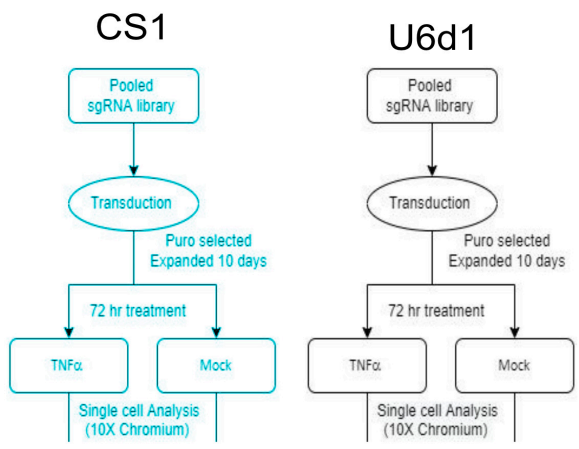
- Single-cell CRISPR (sc-CRISPR) is a functional genomics platform that combines **pooled CRISPR (sgRNA)** genetic screening with single-cell expression analysis.
- Cells transduced with pooled sgRNA libraries are loaded in the 10X reaction which co-encapsulates barcoded beads (each bead a unique barcode) and cells in discrete droplets, most droplets containing one cell and one bead.
- The sgRNA and mRNAs from each single cell are then labelled with bead barcodes, and used for library construction and the generation of single-cell transcriptional profiles.
- Changes in gene expression associated with specific sgRNAs can be directly linked to specific gene knockouts.
- Cellecta offers all services required to run sc-CRISPR screens including construction and packaging of custom pooled sgRNA libraries, cell culture work, single-cell partitioning on the 10X Chromium, RNA-Seq NGS and data analysis.



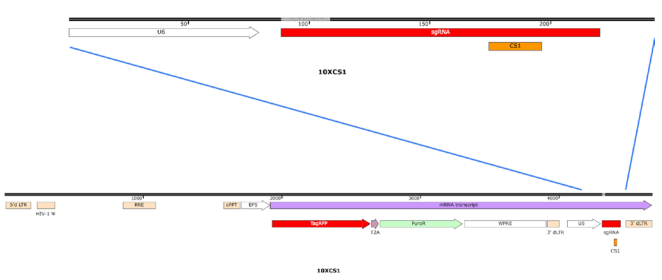
Method

- Two sc-CRISPR lentiviral libraries (CS1 and U6d1) with identical set of sgRNAs targeting mediators of the TNFα response were constructed.
- Cells transduced with **CS1** sc-CRISPR library are processed with the standard 10X 3' sc-CRISPR protocol, which utilizes the CS1 capture sequence to detect the expressed sgRNAs.
- Cells transduced with the CROP-Seq library are processed with an optimized CROP-Seq approach which utilizes the U6d1 primer to detect the expressed sgRNAs.
- Two parallel sc-CRISPR screens utilizing the 10X-CS1 and the CROPseq library were run to compare the performance of the two approaches.

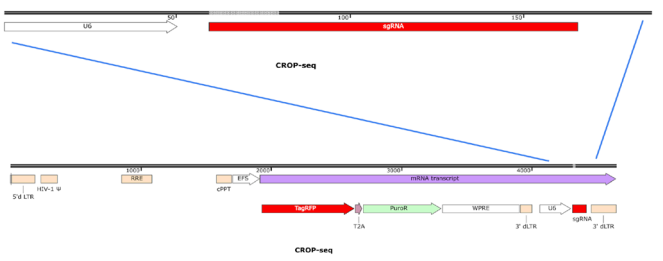
Method (cont.)



Library Vector Design



Cellecta's scCRISPR vector for 10X CS1 (can also be used for CROP-seq)



Cellecta's scCRISPR vector for CROP-seq only

Results

Sample Name	Cell numbers	Detected guides	Cell Rate assigned with guide	Unique guide rate
NoTNFa_U6d1	19974	192	61.8%	56.8%
TNFa_U6d1	25908	192	70.9%	61.5%
NoTNTa_CS1	21472	191	66.2%	59.0%
TNTa_CS1	23882	192	71.7%	62.8%

Table 1: sgRNA assignment across CS1 library (+TNFα/-TNFα) and U6d1 library (+TNFα/-TNFα) using 10X Cell-Ranger Software (Raw data without filtering). For each sample, ~60% of detected cells could be assigned to a single sgRNA, while additional 5%-10% cells were assigned to 2 or more sgRNAs. ~30%-40% cells could not be assigned to any sgRNA.

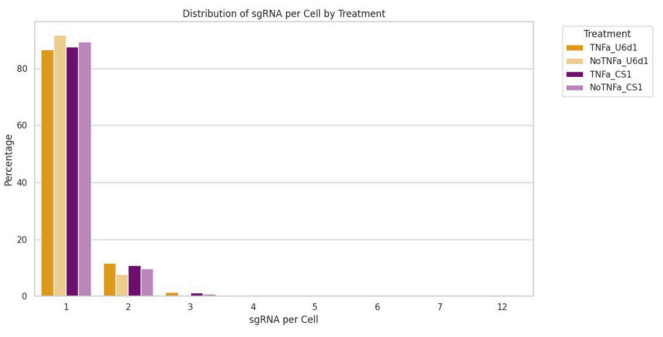


Fig 1: sgRNA distribution in the cell population where guide is assigned. Among this population >90% of cells are with single sgRNA.

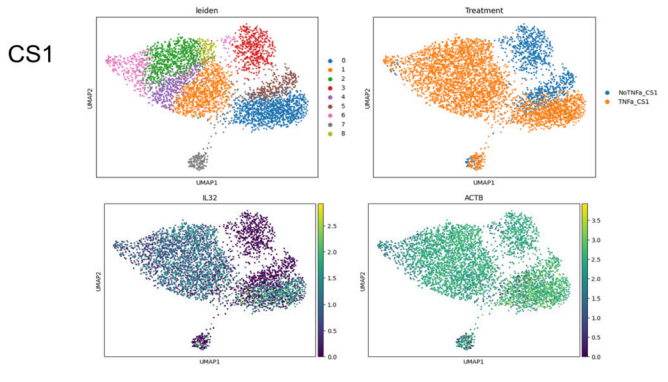


Fig 2: General gene expression UMAP for +TNFα/-TNFα cells for CS1 Library. Low expression of IL32 in untreated versus treated cells as compared to housekeeping gene (ACTB).

Results (cont.)

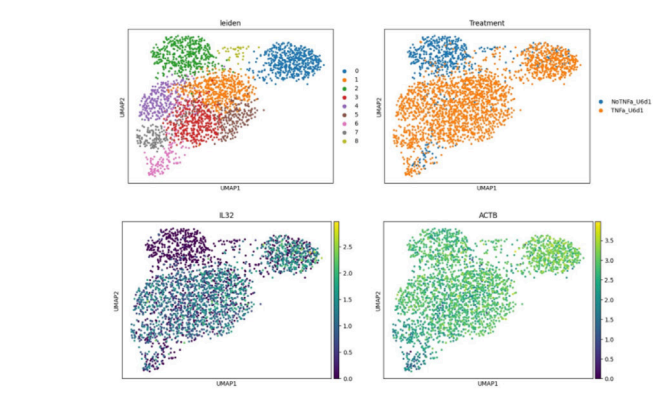


Fig 3. General gene expression UMAP for +TNFα/-TNFα cells cells for U6d1 Library. Low expression of IL32 in untreated versus treated cells as compared to housekeeping gene (ACTB).

Fig 4. Comparable results for CS1 and U6d1 design approach for different sgRNA versus NT control. CRISPR-KO of TNFα pathway key genes (X-axis: CHUK, TNFRSF1A, TRADD, RIPK1) downregulated the expression of the TNFα responsive IL32 gene (Y-axis).

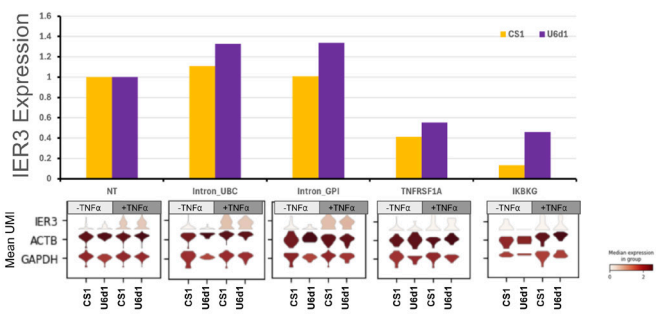


Fig 5. Comparable results for CS1 and U6d1 design approach for different sgRNA versus NT control. CRISPR-KO of TNFα pathway key genes (X-axis: TNFRSF1A, IKBKG) downregulated the expression of the TNFα responsive IER3 gene (Y-axis).

Discussion

- Our results showed the comparable performance of 10X Genomics protocol (CS1 library) and CROP-Seq (U6d1) approach in single-cell CRISPR screens.
- Based on the positive outcome of the CROP-Seq approach, we have been working on the development of a CROP-Seq/Targeted-RNAseq protocol which presents the potential benefits of significantly reducing NGS costs and increasing the sequencing depth for better expression level quality data. Results of these new studies will be available soon.
- Additional studies were also performed to assess the performance of the 10X 5' protocol, which does not rely on CS1 capture sequence, therefore does not need any sgRNA modifications and can be used with any sgRNA library. Results of these studies will also be available soon.