Direct capture of CRISPR guides enables scalable, multiplexed, and multi-omic genetic screens

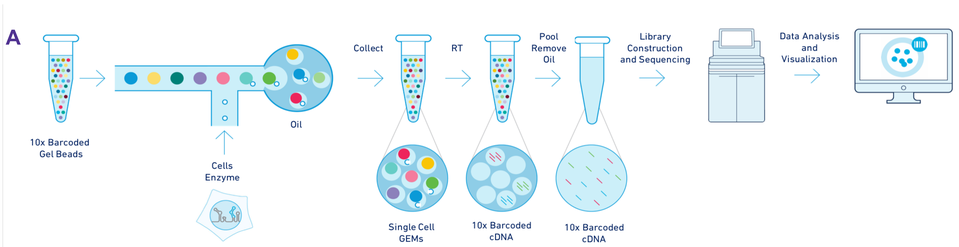
Joseph M. Replogle[1], Albert Xu[1], Thomas M. Norman[1], Elliott J. Meer[3], Jessica M. Terry[3], Daniel Riordan[3], Niranjan Srinivas[3], Andrew Ravanelli[4], Tarjei S. Mikkelsen[3], Jonathan S. Weissman[1] & Britt Adamson[2]

[1]University of California, San Francisco (UCSF), [2]Princeton University, [3]10x Genomics, [4]Merck KGaA, Darmstadt, Germany

Introduction

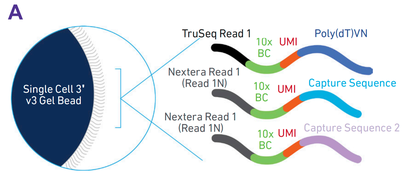
Single cell analysis can provide novel insights into the molecular mechanisms underlying both normal cellular function and disease states, and is crucial in understanding the complex interplay between cell lineages, transcription factors, and signaling pathways. Pairing CRISPR-based genetic screens with single-cell transcriptional phenotypes (Perturb-seq) has advanced efforts to explore the function of mammalian genes and genetic networks. Single cell genomics technologies from 10x Genomics provide comprehensive, scalable solutions for cell characterization and gene expression profiling of hundreds to tens of thousands of cells, without a need for prior knowledge of cell types or markers. Here, we introduce the Single Cell Gene Expression Solution combined with the Feature Barcoding technology that enables the simultaneous measurement of CRISPR-mediated perturbations alongside cellular transcripts. We present strategies for Perturb-seq that enable direct capture of CRISPR sgRNAs within 3’ or 5’ single-cell RNA-sequencing libraries using the 10x Genomics platform. This technology greatly expands the accessibility, scalability, and flexibility of high-throughput and scalable functional screens, specifically enabling use with programmed combinatorial perturbations and multiplexing with multi-omic measurements.

Overview of 10X Single Cell Gene Expression Workflow

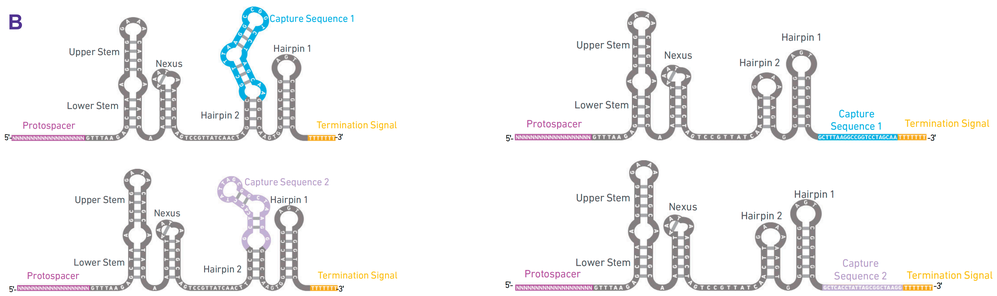


The Chromium Single Cell Gene Expression Solution combined with the Feature Barcoding Technology enables functional genetic screens at scale and high resolution.
(A) Workflow of the Chromium Single Cell Gene Expression Solution. (B) Schematic of the library constructs produced during the workflow. (C) Transduced cells with integrated sgRNA for the Chromium Single Cell 3' workflow for CRISPR screening.

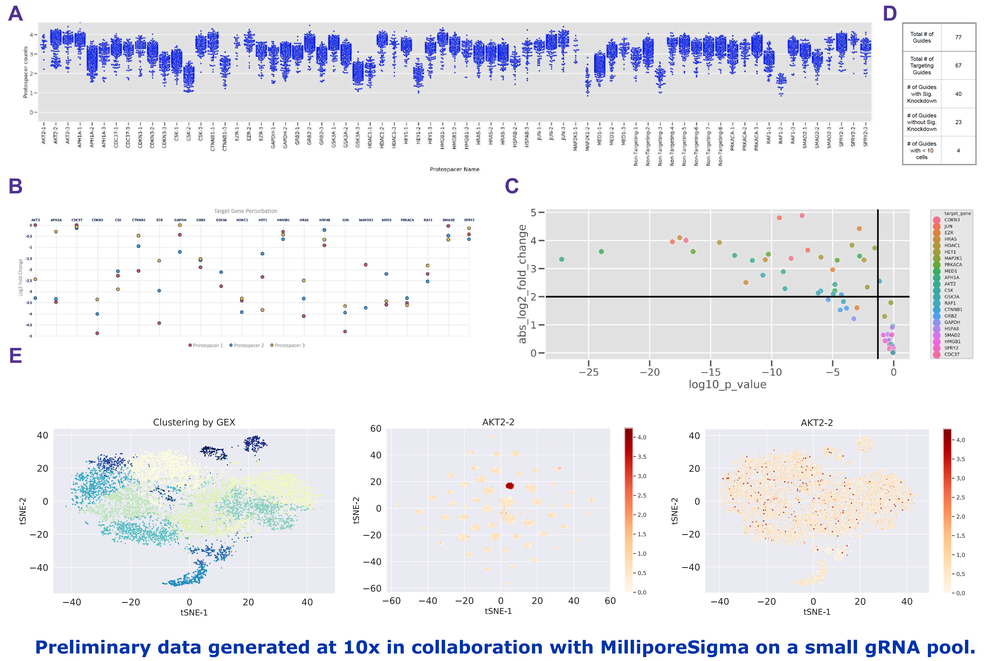
Direct Capture of gRNAs via 10x Gel Beads



Simultaneous capture of polyadenylated mRNA transcripts and polyA-independent gRNAs in the same cell. (A) Schematic of the Single Cell 3’v3 gel beads. Three different oligonucleotide types are attached to the gel bead. Oligo type one captures polyadenylated mRNA transcripts via the poly(dT)VN tail. Oligo types two and three contain a capture sequence 1 and 2, respectively that can capture the reverse complement sequence that is incorporated to the gRNA. (B) Integration of Capture Sequence 1 and Capture Sequence 2 in sgRNA at either the 3’ end or the hairpin.

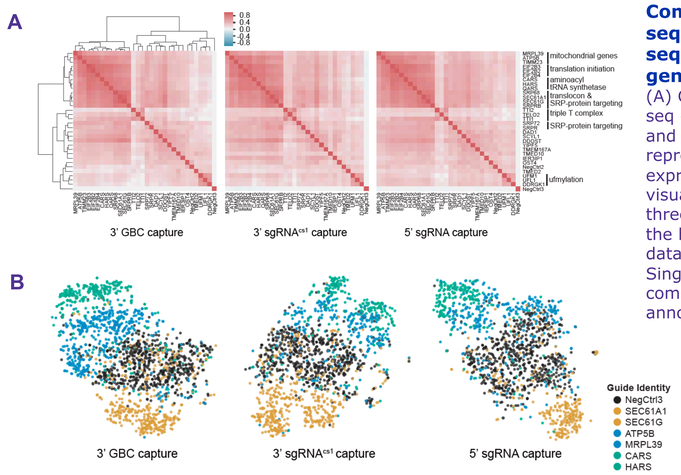


Small gRNA library pool data



Preliminary data generated at 10x in collaboration with MilliporeSigma on a small gRNA pool.
(A) Guide UMI counts for cells called with a single guide. (B) Knockdown efficiencies: comparing protospacers for the same target gene. (C) Knockdown efficiencies: log2 fold change vs p-value (D) Summary of knockdown efficiencies in small gRNA pool. (E) Left: Clustering based on gene expression. Sub-clusters colored based on graph-based clustering. Middle: Clustering based on CRISPR gRNAs. Highlighted expression of one gRNA. Right: One example gRNA expression shown in clustering based on gene expression.

Scalable, Multiplexed, and Multi-Omic Genetic Screens (Replogle et al)



Comparison of direct capture Perturb-seq and GBC (guide barcodes) Perturb-seq for interrogating gene function and genetic regulation using 10x reagents.
(A) Clustering of perturbations from UPR Perturb-seq experiments conducted with GBC Perturb-seq and direct capture Perturb-seq. Heatmap represents correlations between pseudo-bulk expression profiles for each perturbation. For visual comparison, the rows and columns of all three heatmaps are ordered identically based on the hierarchical clustering of GBC Perturb-seq data. Functional annotations are indicated. (B) Single-cell projections based on 10 independent components followed by t-sne. Colors indicate annotation of guide identity.

10x GENOMICS

www.sigmaaldrich.com