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Data Sheet

CRISPRi Pools with 10x Genomics Compatibility

10x GENOMICS Compatible Products

Sigma-Aldrich® custom CRISPR pools are powerful research tools for the discovery of novel genes and cellular pathways essential to understanding human health and disease. As a world leader in the production of lentiviral libraries, we design and build custom pools to any specifications, and our proven KRAB-dCas9 system for CRISPR interference drives superior knockdown efficiency (**Figure 1**). With this capability, we have partnered with 10x Genomics to bring innovative pooled screening products to enable discovery at single-cell resolution.

CRISPR-based functional genomics screening provides a broad overview of the genetic contributions to a measurable phenotype. Bulk screening, however, only provides limited functional information beyond the selectable phenotype. At the other end of the spectrum, single-cell analysis provides high content information centered around genome-wide transcriptome profiling for candidate genes. We offer optimized, custom lentiviral vectors compatible with Chromium Single Cell Gene Expression utilizing Feature Barcode technology, perfect for your candidate list validation.

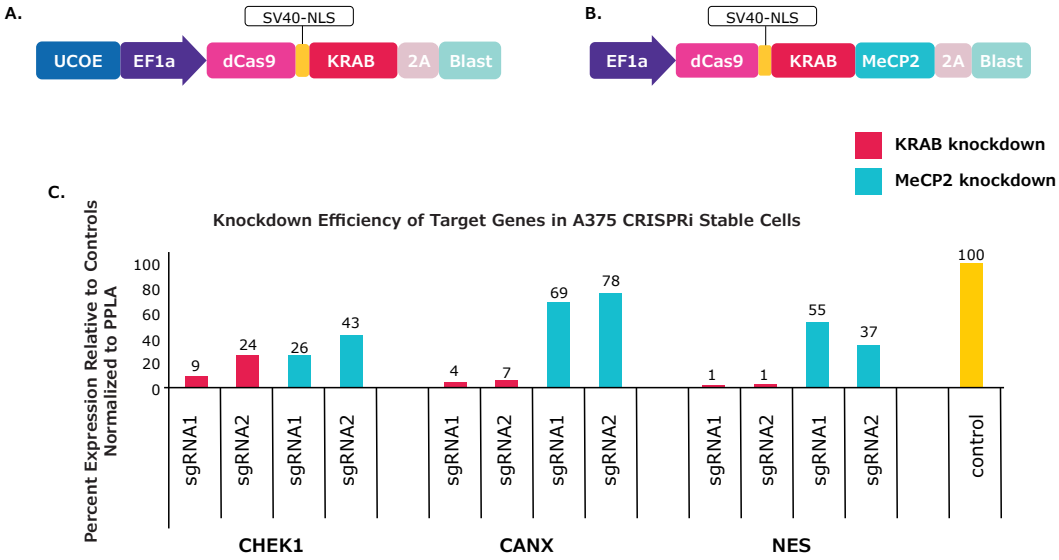
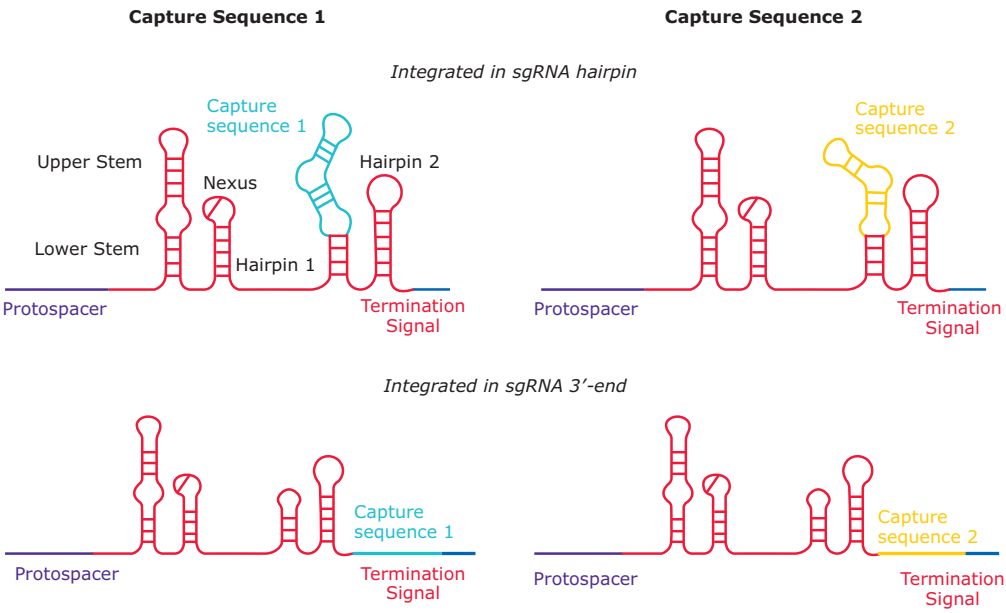


Figure 1. Sigma-Aldrich® KRAB-dCas9 drives superior targeted knockdown efficiency. (A) The Sigma-Aldrich® developed CRISPRi construct **(B)** Constructs adapted from Yeo et al.[1] with an additional MeCP2 domain added to the KRAB domain. **(C)** Relative expression levels of target genes in stable CRISPRi KRAB-dCas9 cells as determined using qRT-PCR. The Sigma-Aldrich® system shows superior knockdown efficiency when compared to the MeCP2 system in all targets tested. Demonstrated in CHEK1 and CANX, known difficult-to-repress targets as well as a target that knocks down more efficiently in this cell line, NES.

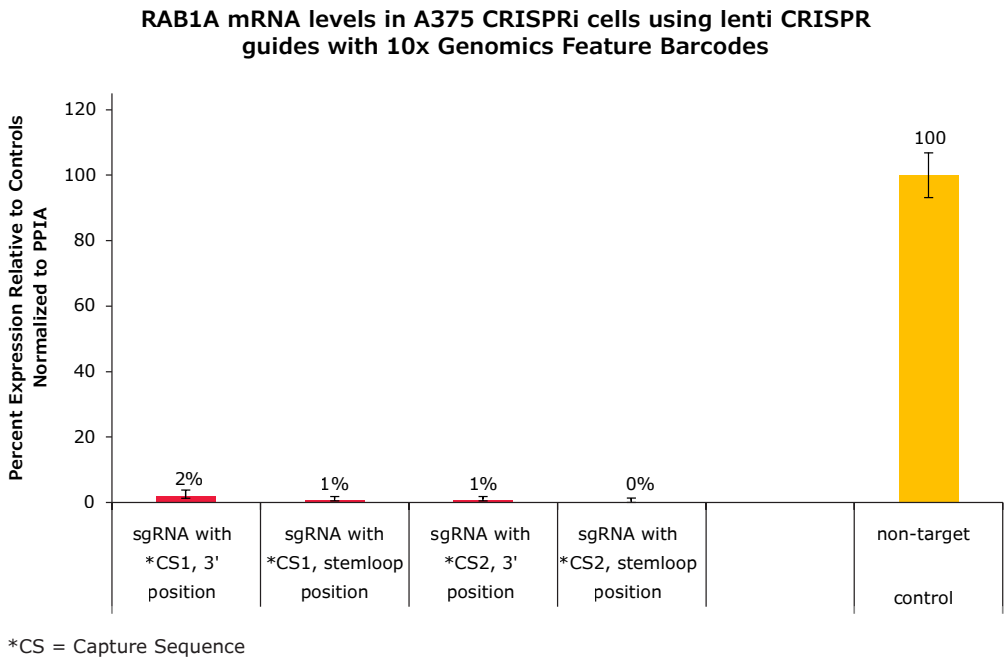
Single-Cell Analysis: 10x Genomics-compatible vectors and controls

We have developed and tested CRISPR guide RNA vectors that incorporate a capture sequence directly into the guide scaffold to allow direct capture of sgRNAs during single-cell RNAseq, **Figure 2A**. These sequences do not impair CRISPRi function, **Figure 2B**.

A



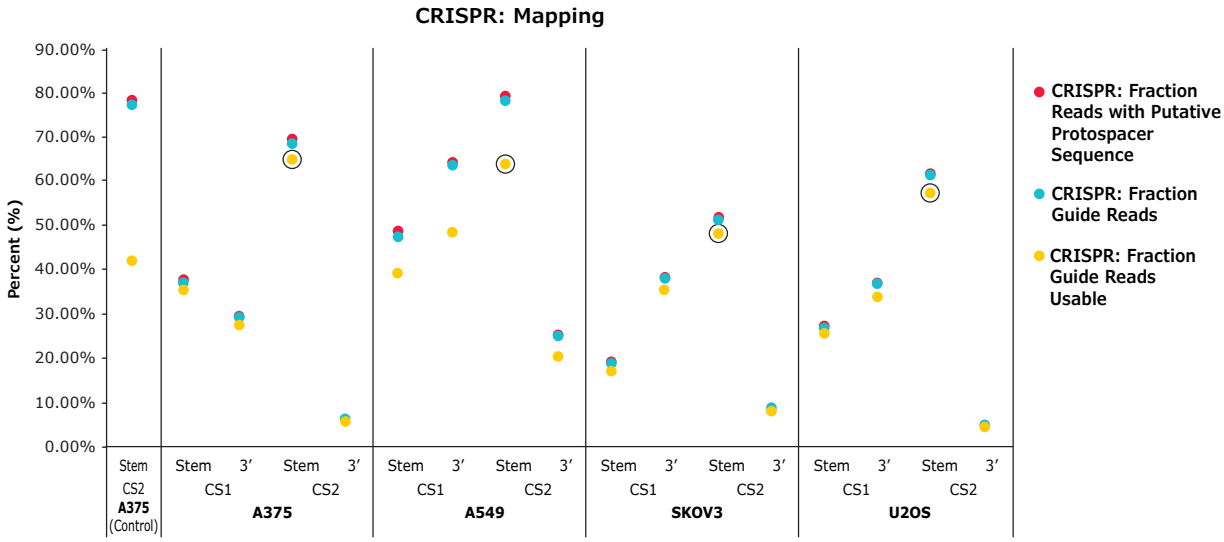
B



*CS = Capture Sequence

Figure 2. Uniform and Efficient Knockdown using sgRNAs containing 10x Genomics Feature Barcoding (A) Modifications to the CRISPR sgRNA scaffold incorporate two capture sequences in the 3′ and stem loop positions. **(B)** Relative RNA knockdown expression levels of our provided positive control, RAB1A, in A375 CRISPRi KRAB-dCas9 stable cells as determined using qRT-PCR. With each capture sequence a minimum of 98% knockdown was observed.

Preliminary data suggested that guide capture efficiency varies depending on capture motif and cell line. Further analysis suggests that the stem-loop position drives more efficient capture for most cell lines. However, we developed an optimization kit (CRISPRI10X) that allows researchers to easily determine which capture motif is ideal for their cell line of interest and their CRISPR assay.



*CS = Capture Sequence, Stem = Stem loop position, 3′ = 3′ position

Figure 3. Capture sequence optimization kit reveals guide capture efficiency differences across cell lines. The 10x Feature Barcode Optimization Kit is used to calculate key 10x Genomics CRISPR application metrics after undergoing several levels of filtering. First filter, represented in red: Only reads in which a predefined constant region of the gRNA can be found (supplied by the user as part of a “Feature Reference File”) are retained. These reads are termed as "Reads with Putative Protospacer Sequence". Second filter, represented in blue: After removing reads without a constant sequence, reads that contain a protospacer sequence mentioned in the Feature Reference File are retained. These reads are termed as “Fraction Guide Reads Usable” (circled above) and are used to perform guide calling per cell. Third filter, represented in yellow are the most relevant: Retains Guide Reads that contain a valid cell barcode, valid UMI (Unique Molecular Identifier), and the cell barcode is associated with a cell containing partition, as defined by the gene expression based cell-calling algorithm. The reads that pass through these three filters are defined as "Fraction Guide Reads Usable" (circled above), and are used to perform guide calling per cell.

Capture sequence 2 in the stem-loop position yielded the highest capture efficiency in each of these example cell lines. Based on the observed variability in capture efficiency between the capture motifs, we recommend using the optimization kit to determine the best capture motif for your cell line of interest. Additionally, the reagents included have utility in optimizing knockdown conditions in your specific cell lines.

Small Pools and Screening:

To demonstrate the power of CRISPR screens at single cell resolution we designed a small pool of ~100 CRISPRi guides and subjected cells to different drug treatments to alter gene expression. 40% of guides showed greater than 2-fold knockdown. Drug treatments drove differential gene expression, results can be seen in **Figure 4**.

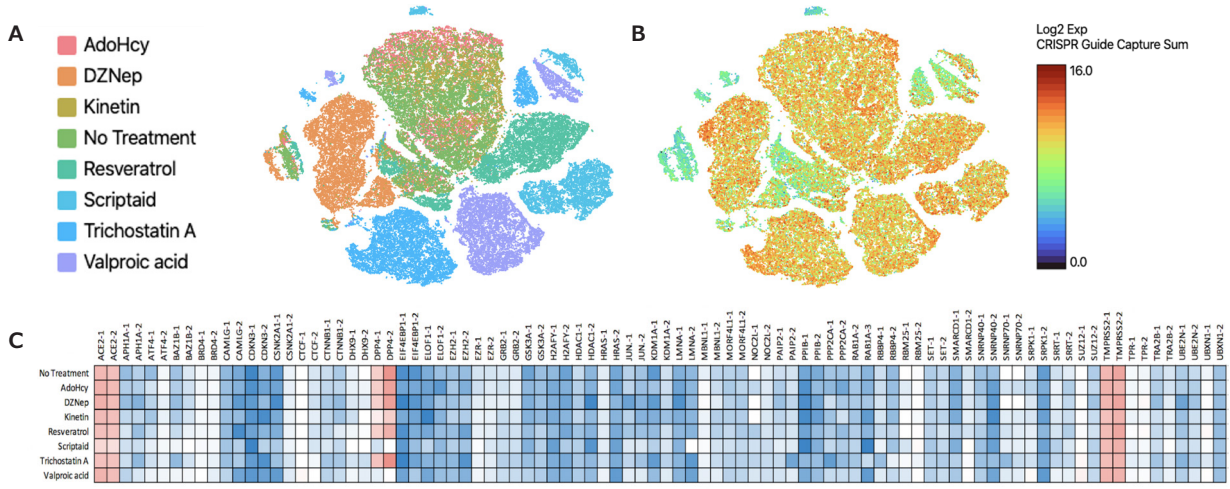


Figure 4. Exploring the combinatorial effects of CRISPR perturbations and drug treatments. (A) Aggregated 10x Single Cell gene expression data from each of 8 conditions tested, presented using 10x Loupe Cell Browser™. Clustering of data points are based on tSNE; cells which cluster together have similar gene expression profiles. The effects of select drug conditions, vs control, show large changes in overall gene expression profiles. **(B)** 10x Genomics data provides a digital readout of the genes expressed and the number of transcripts present, allowing for the ability to quantify changes in gene expression due to CRISPR perturbations at the individual cell level. **(C)** Knockdown of target genes (listed across the top) in each drug treatment context (listed on the left). When combined with the 10x Genomics, 3' Single Cell assay, large pooled CRISPR screens provide a massively parallelized system to quickly screen perturbation efficiencies across a large number of gRNAs/targets while simultaneously being able to understand the effects of these perturbations in a range of contexts (in this case drug conditions).

CONCLUSIONS

We performed side-by-side comparisons of competing CRISPRi technologies and have further improved the libraries and pools created by the University of California San Francisco (UCSF), making them single cell RNAseq compatible and available for distribution to advance your scientific discovery. Performance differentiators include:

- Targeted sub pools including druggable genome, cancer & apoptosis, non-coding novel targets, and more
- A KRAB-dCas9 helper construct containing a proprietary expression-stabilizing UCOE element
- Our 10x Genomics-compatibility allows functional analysis of CRISPRi perturbations and are the perfect follow up to your functional genomic screens
- A sortable BFP marker is included in our vectors
- An optimization kit to identify the most appropriate capture sequence in your cell lines and your CRISPR assay

To learn more or request a quote please visit:

SigmaAldrich.com/CRISPRi

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Further information and references:

1. Yeo NC, et al. An enhanced CRISPR repressor for targeted mammalian gene regulation. Nat Methods. 2018 Aug;15(8):611-616. doi: 10.1038/s41592-018-0048-5. Epub 2018 Jul 16. PMID: 30013045; PMCID: PMC6129399.
2. Dixit A, et al. Perturb-Seq: Dissecting Molecular Circuits with Scalable Single-Cell RNA Profiling of Pooled Genetic Screens. Cell. 2016 Dec 15;167(7):1853-1866.e17. doi: 10.1016/j.cell.2016.11.038. PMID: 27984732; PMCID: PMC5181115.
3. Horlbeck MA, et al. Compact and highly active next-generation libraries for CRISPR-mediated gene repression and activation. Elife. 2016 Sep 23;5:e19760. doi: 10.7554/eLife.19760. PMID: 27661255; PMCID: PMC5094855.
4. Luke A. Gilbert, et al. Genome-Scale CRISPR-Mediated Control of Gene Repression and Activation, Cell, Volume 159, Issue 3, 2014, 647-661.

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