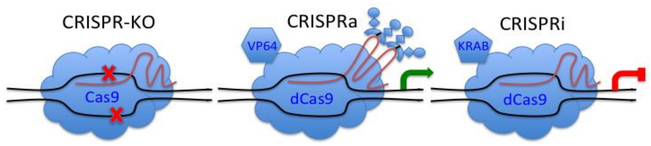
# New Strategies for Forward Genetic Screening and Single Cell Analysis with CRISPRi Transcriptional Repressors

Andrew Ravanelli[1], Tiffany Bernardo[1], Elliot Meer[2], Jessica Terry[2], Qingzhou Ji[1]
**MilliporeSigma,** [1]2909 Laclede Ave., St. Louis, MO 63103
**10x Genomics,** [2]6230 Stoneridge Mall Road, Pleasanton, CA 94566

## Introduction

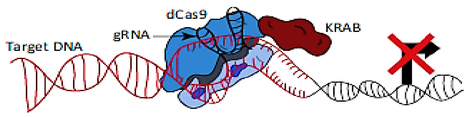
### Catalytically-Dead Cas9 Applications

- The full extent of CRISPR's utility extends beyond targeted DNA cutting.
- Nuclease-independent applications of CRISPR leverage the system's targeting specificity for **cargo delivery**.
- Transcription factor effector domains activate or inhibit **endogenous target gene expression**.




### Targeted Inhibition of Gene Function with CRISPRi

- CRISPRi is composed of a **KRAB-dCas9 + single guide RNA(sgRNA)** complex that functions by delivering a transcriptional repressor domain to the target sequence.
- sgRNAs are targeted within 200bp of the transcription start site, avoiding the nearest 50bp.
- KRAB-dCas9 sterically represses transcription by blocking either transcription initiation or elongation.
- This approach allows for **inhibition at the DNA level**, resulting in **reversible, highly specific gene repression** and the ability to **manipulate traditionally inaccessible regions of the genome** including long non-coding RNAs (lncRNAs) and microRNAs (miRNAs).




## Results

- **OBJECTIVE:** Determine which sgRNA design algorithm yields the highest knock down (KD) efficiency in traditionally **difficult to repress target genes**.

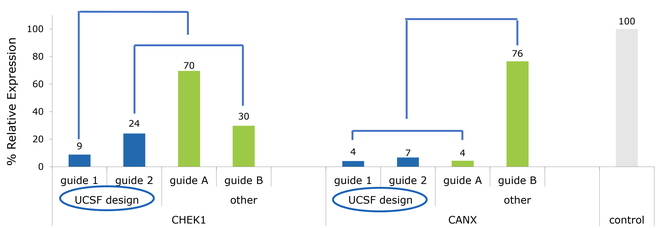
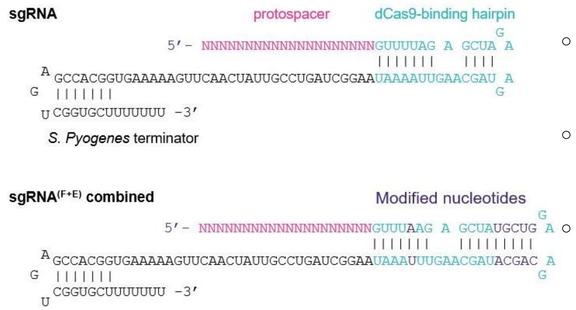



**Figure 1 – UCSF CRISPRi design algorithm improves KD efficiency across multiple sgRNAs.** Relative gene expression levels of *CHEK1* and *CANX* in A375 cells demonstrate increased KD efficiency by sgRNAs designed with UCSF v2.1 algorithm[1] as compared to those designed with Yeo et al[2] algorithm. Blue bars = UCSF design; Green bars = Yeo et al design. qPCR reactions were normalized to the *PPIA* gene.

## Improved sgRNA Scaffold Increases Knock Down Efficiency




- The Weissman lab made a series of **sgRNA modifications** to improve functionality.
- A-U **nucleotides** in the DNA-binding hairpin were **flipped** to eliminate a putative RNA pol-III termination signal.
- Stem loop extension **enhanced sgRNA stability**.

**Figure 2 – sgRNA scaffold modifications increase functionality in cells.** Diagram showing CRISPR sgRNA scaffold modifications[3].

## Enhanced sgRNA Functionality Confirmed in A375 Cells

- **Increased functionality** of enhanced sgRNA scaffolds were **confirmed** internally.
- *HEY1* and *HES1* represent easy-to-repress targets.
- *CANX* represents a difficult-to-repress target.
- *NES* previously showed a high degree of inconsistency across various sgRNAs.

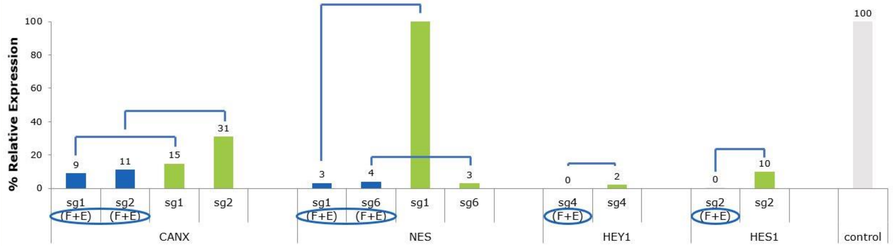



**Figure 3 – Modified sgRNAs demonstrate enhanced KD efficiency across various target genes.** Treatment with modified sgRNA scaffolds consistently resulted in decreased gene expression levels as compared to their unmodified counterparts in A375 cells. Blue bars = modified sgRNAs; Green bars = unmodified sgRNAs. (F+E) = A-U flip and extended stem loop. qPCR reactions were normalized to the *PPIA* gene.

## KRAB-dCas9 Outperforms dCas9-KRAB-MeCP2

- **OBJECTIVE:** Determine if additional effector domains could improve KRAB-dCas9 inhibitory function.
- The **MeCP2 construct did not enhance target gene repression** in internal evaluations.

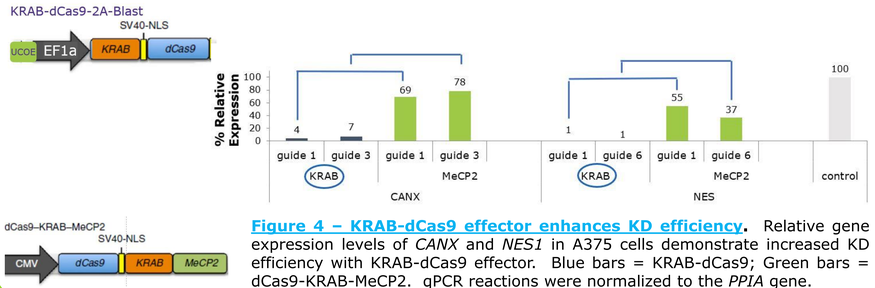



**Figure 4 – KRAB-dCas9 effector enhances KD efficiency.** Relative gene expression levels of *CANX* and *NES1* in A375 cells demonstrate increased KD efficiency with KRAB-dCas9 effector. Blue bars = KRAB-dCas9; Green bars = dCas9-KRAB-MeCP2. qPCR reactions were normalized to the *PPIA* gene.

## Reagents for Single-Cell Analysis with CRISPRi

- 10x Genomics-compatible CRISPRi reagents were developed **to facilitate screening at the single cell level.**
- Conduct **in-depth phenotypic analyses** and **follow-up on top hits** from a larger screen for a deeper understanding of functional genomics in a particular model system.
- **Capture sequences** are incorporated into the sgRNA scaffold and **do not interfere with KD efficiency.**

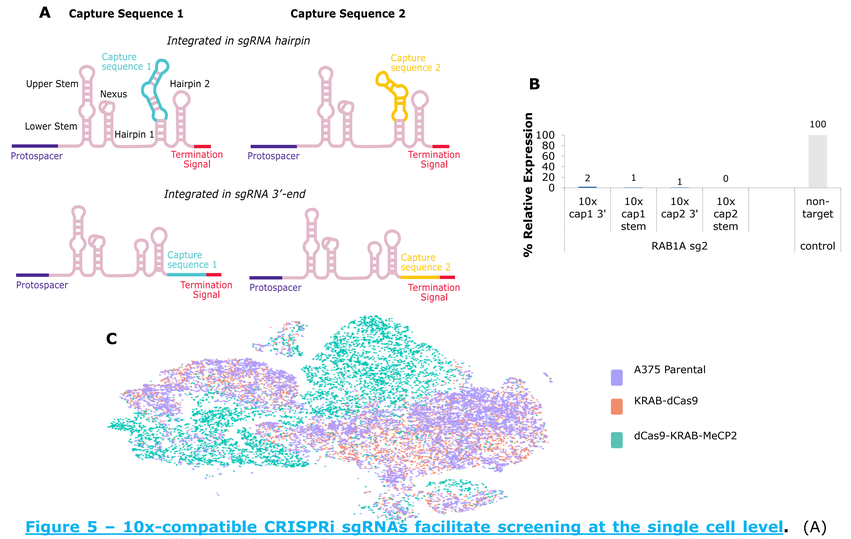


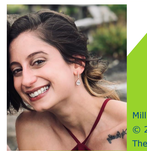

**Figure 5 – 10x-compatible CRISPRi sgRNAs facilitate screening at the single cell level.** (A) CRISPR sgRNA scaffold modifications for 10x Feature Barcoding. (B) Treatment with barcoded sgRNAs resulted in decreased gene expression as compared to non-targeting control in A375 cells. qPCR reactions were normalized to the *PPIA* gene. (C) KRAB-dCas9-expressing A375 cells more closely recapitulate the transcriptomic profile of the WT line as compared to dCas-KRAB-MeCP2-expressing cells.

## Conclusions

- A comprehensive evaluation of the CRISPRi technology revealed that **KRAB-dCas9 + sgRNA with improved scaffold** provides optimum KD efficiency in cells.
- **CRISPRi is well-suited for single-cell analysis** with 10x Genomics Feature Barcoding technology.

## References

1. Horlbeck MA, Gilbert LA et al. 2016. Compact and highly active next-generation libraries for CRISPR-mediated gene repression and activation. eLife. 5:e19760.
2. Yeo NA, Chavez A et al. 2018. An enhanced CRISPR repressor for targeted mammalian gene regulation. Nat Methods. 15:611-616.
3. Chen B, Gilbert LA et al. 2013. Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas9 system. Cell. 156(1-2):373.



**Primary Contact**
Tiffany Bernardo, PhD
Research Technology Specialist - Southeast
tiffany.bernardo@milliporesigma.com

MilliporeSigma and the M logo are trademarks of Merck KGaA, Darmstadt, Germany.
© 2016 EMD Millipore Corporation, Billerica, MA USA. All rights reserved.
The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada