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Lab & Production Materials

Product Guide

Genome Modulation with CRISPRi & CRISPRa: Complementary Tools for Gene Knockdown and Overexpression

Introduction

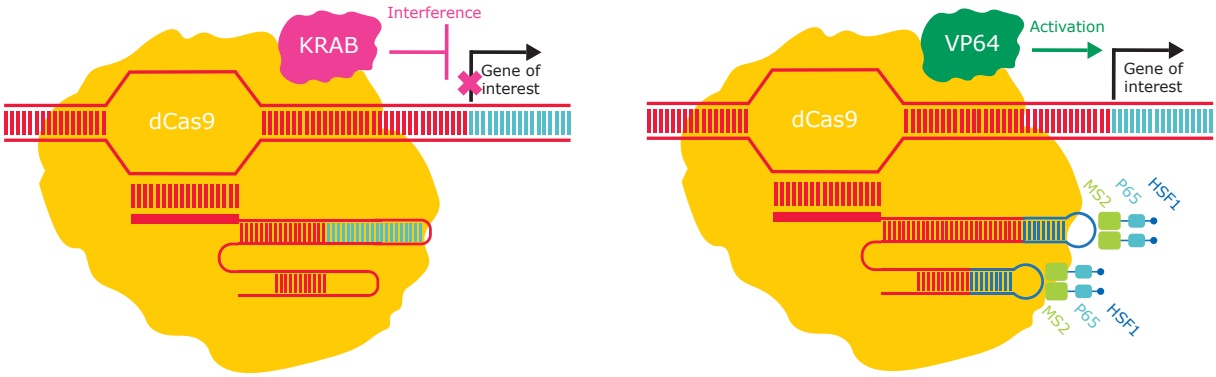
Functional genomics is a critical component of the drug discovery process. Screening tools have made it possible to capture large and highly informative datasets enabling rapid exploration of gene pathways involved in cell function and disease. One tool that in recent times has commanded significant attention, even from non-scientists, is the CRISPR Cas9 system, which supports controlled manipulation of genomes with high precision and efficiency including genetic modifications that knock-in, knockout, knockdown or activate genes. CRISPR inhibition and activation modulate transcription by targeting the transcriptional start site of a gene using effector domains that inhibit or activate transcription.
This product guide is a reference for scientists looking to incorporate gene editing technology in their research toolbox. It covers two common types of gene editing systems - CRISPRa and CRISPRi.

Types of CRISPR Screens

There are two primary formats of CRISPR screens that have similar workflows:

Pooled screens – These high-throughput screens are the most widely used but require more vested time in data deconvolution and validation downstream. These screens combine thousands of guide RNAs in a single tube and support rapid identification of lists of target genes. They can be used to screen whole genomes efficiently, and next-generation sequencing is used to analyze the data gleaned from pooled screens.

Arrayed screens – These screens need a larger investment of labor upfront but can yield a broad variety of data points and require less effort on the backend compared to pooled screens. Screens consist of several thousand unique wells that contain individual guide RNAs. Arrayed screens are well suited for experiments that have high content imaging and cellular phenotyping. Lastly, unlike pooled screens, next-generation sequencing isn't required for downstream analysis.



CRISPRi **CRISPRa**

Figure 1. Comparison of CRISPRi and CRISPRa. Both systems use catalytically dead Cas9 whereas CRISPRi employs a dCas9 fused to the primary transcriptional repressor, the Krüppel associated box (KRAB) domain, which silences the targeted gene. In the CRISPRa system, dCas9 is fused to the transcriptional activator VP64 which complexes with the CRISPR gRNA and activators MS2-p65-HSF1 which increases gene expression.

Nuclease-independent CRISPR Screening

Traditional CRISPR approaches rely on nuclease-based creation of double-stranded breaks. These methods cleave DNA at specific sites and then rely on the endogenous DNA repair mechanism of cells to fix the damage using either non-homologous end joining (NHEJ) or homology-directed repair (HDR). The predominant cellular repair mechanism for mammalian cells, NHEJ, is more error-prone and can introduce changes such as frameshifts or loss-of-function mutations that knockout gene function. A different category of CRISPR systems are nuclease independent, which offer similar target specificity to nuclease dependent CRISPR systems and are often used for cargo delivery in the context of gene regulation. These applications use a version of the Cas9 protein called dCas9, or dead Cas9, which has specific point mutations that deactivate the DNA cleavage functionality. As with standard CRISPR, they utilize guide RNAs for gene targeting. Two nuclease-independent CRISPR systems currently available on the market are **CRISPRa, used for gene activation, and CRISPRi, used for gene inhibition**. The differences and similarities between the two systems are shown in **Figure 1**.

CRISPRa for Gene Activation

Studies carried out by Chavez et al. (2016) and Yang et al. (2019) that compared various CRISPRa systems showed that the CRISPR SAM system (Synergistic Activation Mediator) offers the strongest transcriptional activation.[1, 2] Results from one such comparison are shown in **Figure 2**. The CRISPR SAM system features a three-part transcriptional activator complex with a dCas9-VP64 fusion protein at its core that forms the basic synthetic transcription factor. It complexes with a guide RNA to form the ribonucleoprotein (RNP) targeting complex, but includes modified stem and guide scaffold tetraloops containing RNA aptamers that can bind to an MS2-p65-HSF1 viral protein.

For CRISPR screening experiments, the use of lentiviral transduction provides more consistent and robust results across a variety of cell lines. Sigma-Aldrich® CRISPR SAM lentiviral plasmids have been improved to yield higher levels of functional virus compared to other methods as demonstrated in **Figure 3**.

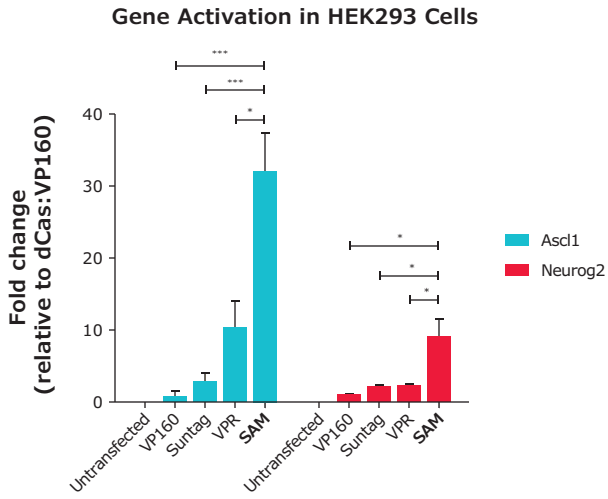


Figure 2. SAM CRISPRa Outperforms Other CRISPRa Systems. Activation of Ascl1 and Neurog2 in HEK293 cells. Cells were transfected with one sgRNA per target and four different dCas9 versions. qRT-PCR normalized to Gapdh, fold change relative to dCas9:VP160. In both targets the SAM system increases gene activation significantly more than other systems. Adapted from Yang et al.[2]

Why use CRISPRa?

- CRISPRa complements existing loss-of-function screening technologies by enriching different sets of genes responsible for observed phenotypes.
- CRISPRa offers unique functionality for gain-of-function screening.
- There is little risk of genome instability from double-stranded breaks since the dCas9 protein does not cleave DNA.
- The effect of high copy number on gene knockout is mitigated since the targeting complex needs to bind to a single copy and then recruits co-activators.
- CRISPRa activates transcription at the endogenous locus rather than via transgenic overexpression of a defined isoform. This leads to more natural activation that allows cells to choose the isoform expression and splice variation that is used.

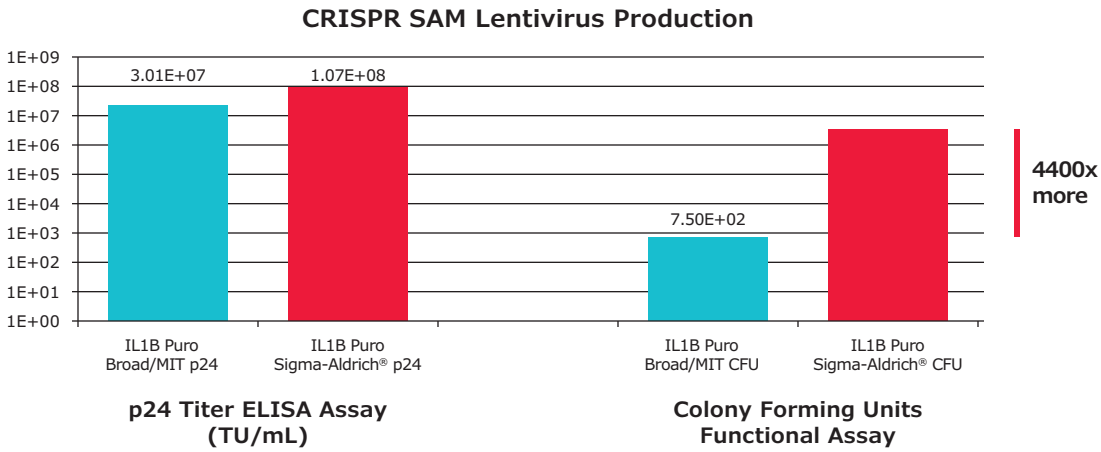


Figure 3. The Sigma-Aldrich® modified CRISPR SAM vector showed an increase in lentiviral titer and substantially higher functional virus levels.

CRISPRi for Gene Inhibition

CRISPRi offers an alternative to RNAi technologies for gene knockdown. The technology has improved in recent years as a result of the development and application of efficient guide design algorithms as well as genetic modifications that improve CRISPRi transcription and guide RNA stability in cells. Gilbert et al. showed that CRISPRi and CRISPRa technologies yield complementary phenotypes for the same target genes, which illustrates the strength of pairing the two technologies.[3] Furthermore, using the SigmaAldrich® improved guide RNA scaffold developed by researchers at the University of California, San Francisco, enhances CRISPR knockdown efficiency.[4] Our CRISPRi offerings provide these optimized tools for gene knockdown in various cell lines.

Why use CRISPRi?

- CRISPRi compliments RNAi and other loss-of-function technologies by providing additional gene pathway insights.
- It yields more significant phenotypes.
- CRISPRi complements gain-of-function screening technologies.
- It does not compete for endogenous cellular machinery.
- Inhibition of non-coding portions of the genome can reveal novel gene functions.
- Knockdown using CRISPRi can reveal essential genes that might otherwise have been overlooked if a standard knockout screen were used.

Considerations for CRISPR Screening

It is important to note that although both systems offer different functionality, CRISPRa and CRISPRi are complementary systems and are designed to be used as such. For some experiments, it may be beneficial and even necessary to have both systems on hand. Some considerations which can influence the type of technology and format to use in a CRISPR screen for a given study include:

- Available cell lines and their features including karyotype, proliferation rates, and drug resistance
- Effect of the drug being tested on the cell lines
- The expected phenotypic outcome
- Drug validation methods
- Sequencing capabilities and bioinformatics analysis methods available

Complementary Technologies

CRISPRi and CRISPRa enable rapid screening of loss-of-function and gain-of-function variation in cells but are not mutually exclusive. Both work by modulating gene expression at the transcriptional level. The transcript repression functionality that CRISPRi offers, coupled with the gene activation capabilities of CRISPRa, provide complementary data that can be used to map complex genomic pathways, and to control transcript expression levels in the genome over a 1000-fold range. Together, these tools can help define the relationship between a phenotype and gene expression levels in an isogenic background.

An example of the complementary nature of CRISPRi and CRISPRa screening technologies is shown in Gilbert et al. where researchers used insights from a genome-scale CRISPRa screen of cholera diphtheria toxin to build on results from a CRISPRi screen.[3] In this case, CRISPRa was used to complement CRISPRi by querying the effects of upregulating otherwise inactive gene pathways. Each of these technologies can also provide additional gene pathway insights to knockout screening results.

Single-Cell Analysis

CRISPR screening is a powerful approach to interrogate mechanisms underlying development, disease, and therapeutic responses. Single cell CRISPR screens extend this approach by directly linking CRISPR perturbations and single cell gene expression readouts, cell by cell. After you have completed your whole genome screen, a follow up screen using **Sigma-Aldrich® custom CRISPR pools with 10x Genomics compatibility are a powerful method to validate your candidate list at single cell resolution**. This allows profiling of hundreds of different CRISPR perturbations and the ability to detect individual sgRNAs with directly linked gene expression phenotypes in hundreds to tens of thousands of cells, without prior knowledge of cell types or markers. This technology greatly expands the accessibility, scalability, and resolution of high-throughput functional screens, for mechanistic insights into biology.

Conclusion

The power of CRISPR technology is its ability to enable virtually any genomic manipulation. The ease with which the technology can target multiple genes at the same time opens the possibility of doing unbiased whole-genome screening. It is also simpler to devise targeting strategies and design guides for both CRISPRa and CRISPRi compared to nuclease dependent CRISPR systems. Both systems share some functionality, for example, both CRISPRa and CRISPRi operate within a specific targeted window near a transcriptional start site. But they also have capabilities that make them optimal for different tasks. One promising new application is the possibility of combining CRISPR screening with single-cell analysis. The Sigma-Aldrich® CRISPRi system is a 10x Genomics compatible product that has their capture sequences incorporated directly into the vector, for discovery at single-cell resolution without impairing knockdown efficiency.

Product links

SigmaAldrich.com/CRISPRa

SigmaAldrich.com/CRISPRi

SigmaAldrich.com/10xCRISPRpools

References

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2. Yang, J. et al. Genome-Scale CRISPRa Screen Identifies Novel Factors for Cellular Reprogramming. Stem Cell Reports (2019) 12: 757-771. doi:10.1016/j.stemcr.2019.02.010
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