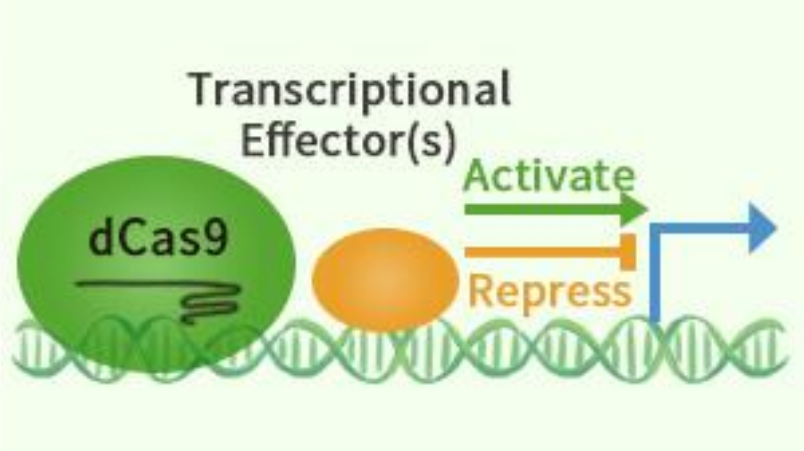
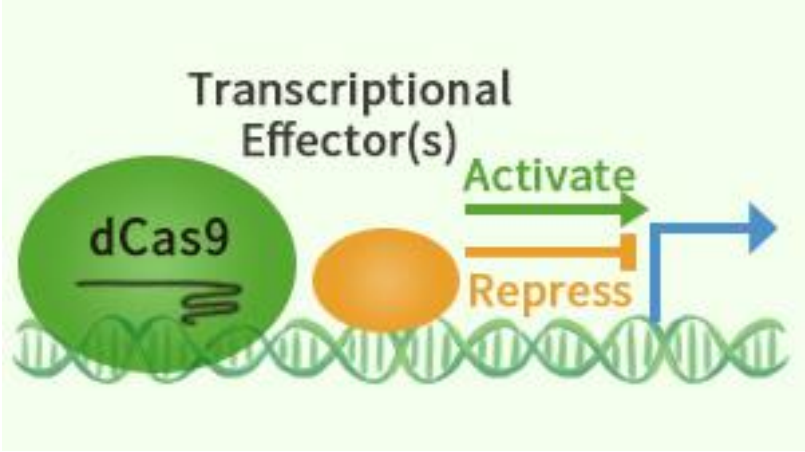
CRISPR Activation and Interference



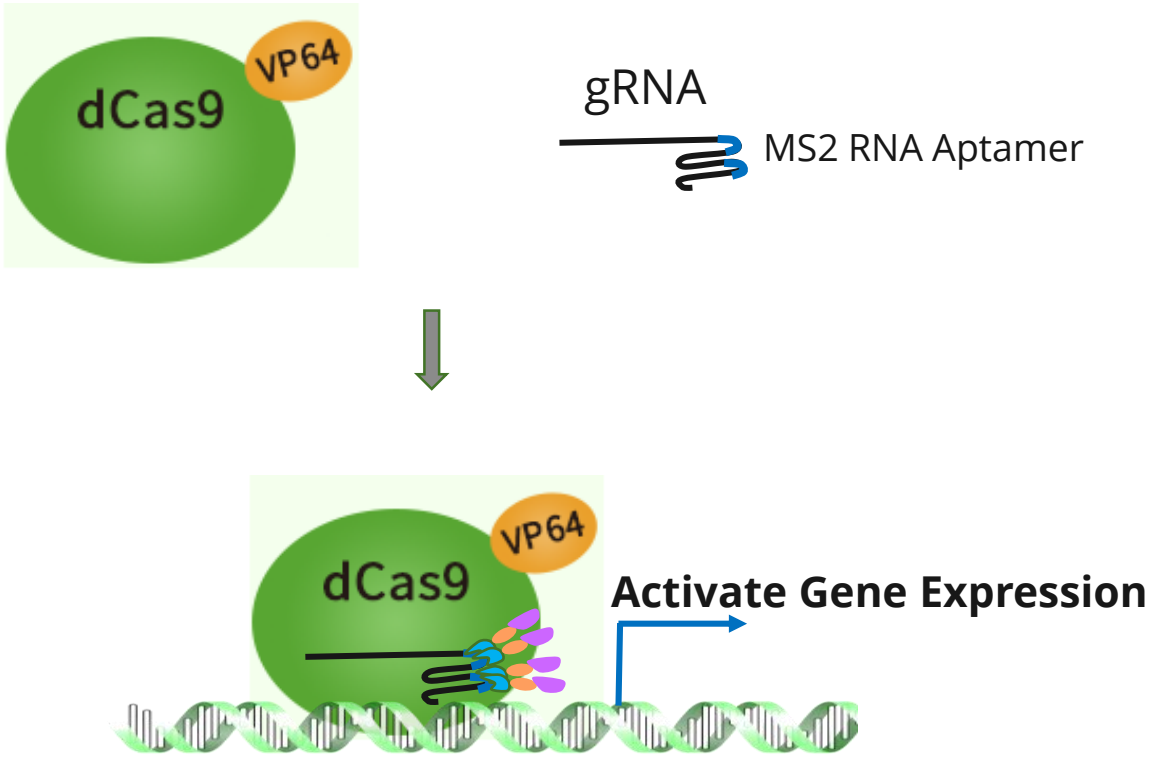
CRISPRa and CRISPRi in Gene Regulation

- dCas9 – Enzymatically deficient Cas9
- dCas9 -Binds DNA, but not cutting
- dCas9 + Activator/repressor - Regulate Gene Expression

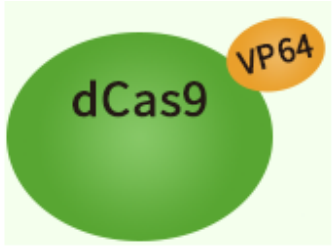


CRISPRa SAM System

CRISPRa SAM – CRISPR activation, Synergistic Activation Mediator

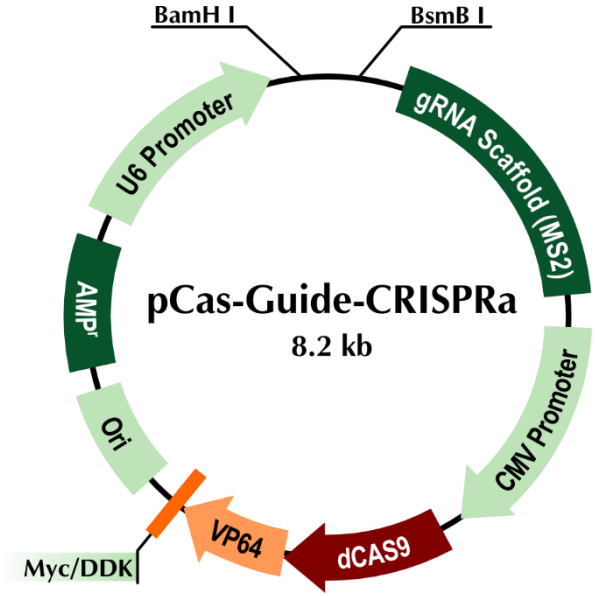


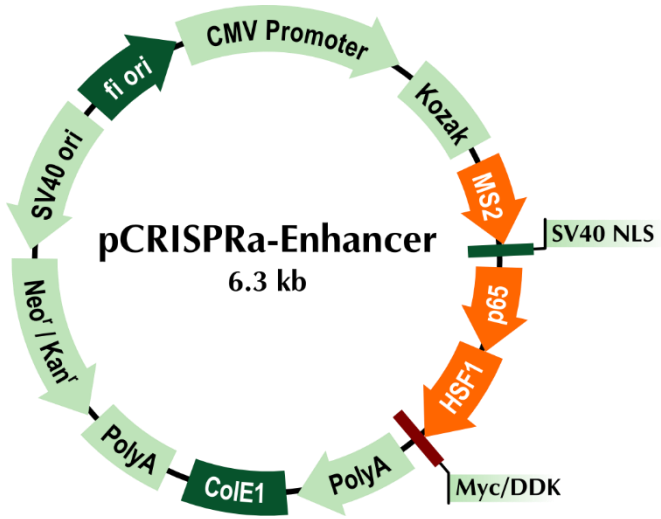
CRISPRa SAM Vectors



gRNA

MS2 RNA Aptamer





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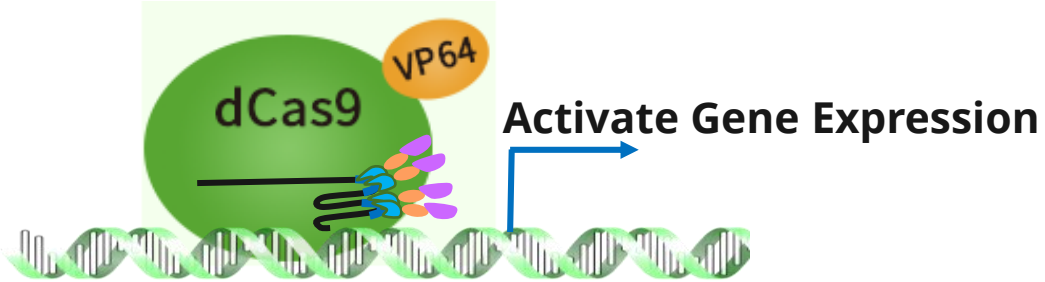
Summary of CRISPRa SAM

- ✓ **Robust CRISPR gene activation system**
- ✓ **Synergistic activation by three activation domains, VP64, p65 and HSF1**
- ✓ **Two vector system, dCas9-VP64-gRNA(MS2) (SKU GE100055) and MS2-p65-HSF1 (SKU GE100056)**



Genome-wide CRISPRa Kits

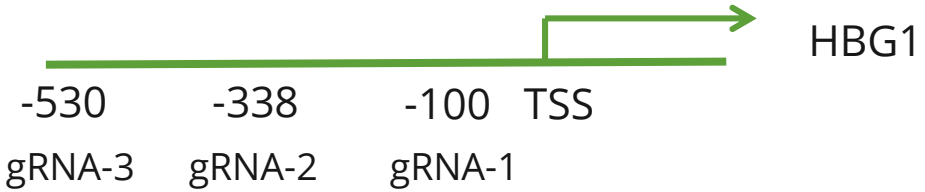
- **Turn-key solution for endogenous gene activation**
- **Locus specific**
- **3 gene specific gRNA constructs**
- **1 Enhancer**
- **1 gRNA scramble**

Human HBG1 Activation using CRISPRa SAM Kit

gRNA 1: CTTGACCAATAGCCTTGACA

gRNA 2: GCTAAACTCCACCCATGGGT

gRNA 3: TATCTGTCTGAAACGGTCCC



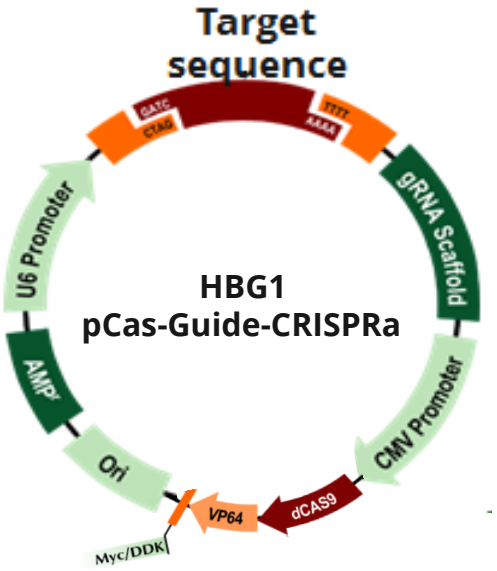
Gene Activation Protocol Using CRISPRa Kit

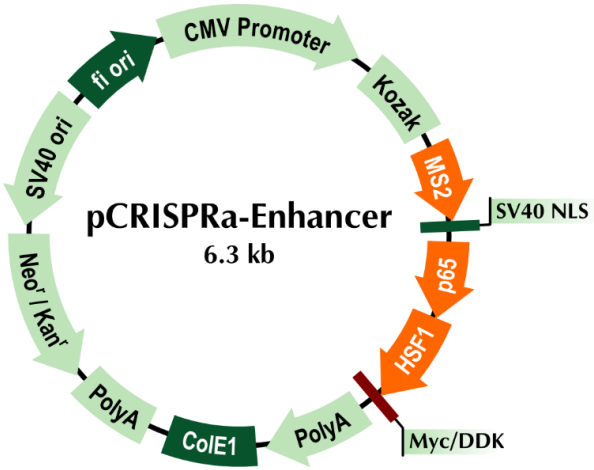
1. **Cotransfection into cells:**
   gRNA vector + Enhancer vector

Four separate transfections (3 gRNAs + Scramble)

gRNA Enhancer

scramble Enhancer

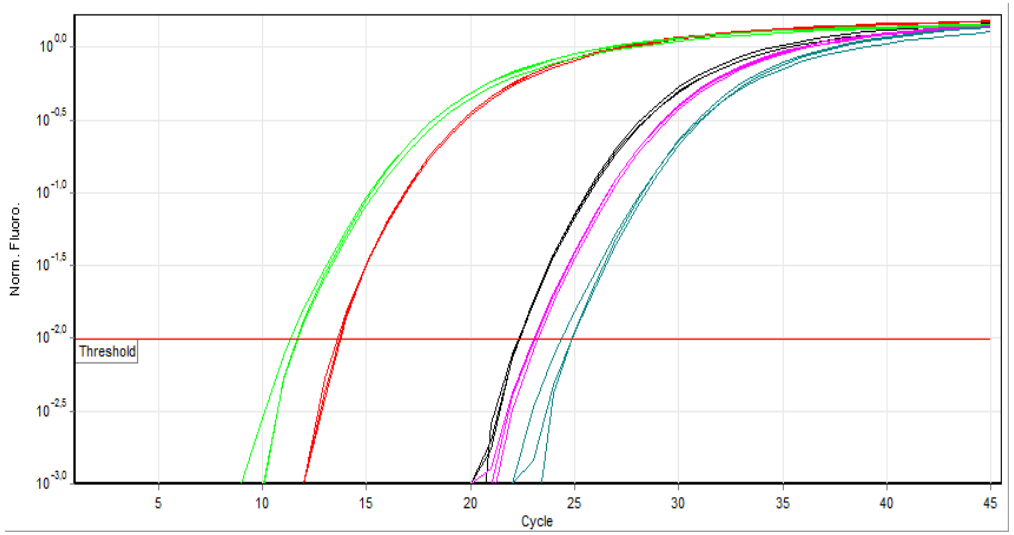




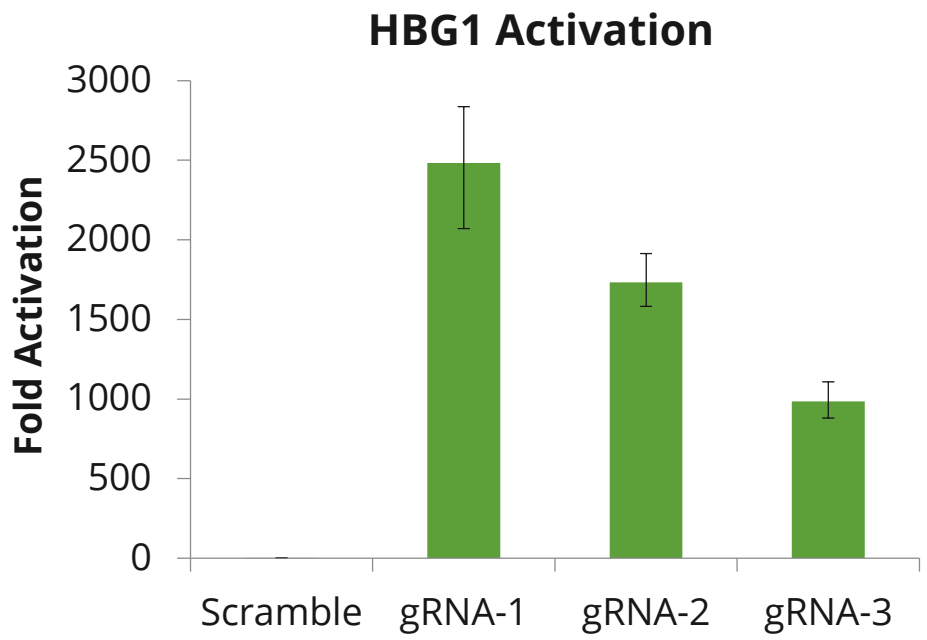
Transfection reagents
http://www.origene.com/cdna/transfection.mspx

Gene Activation Protocol -Continue

2. Transfected cells were harvested 48 hours post transfection. Gene expression level was measured using qPCR.



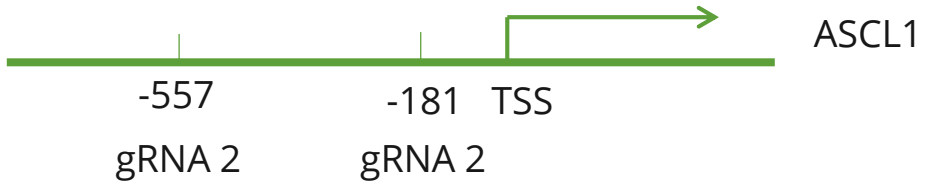
HBG1 Expression is Increased 2,000 Folds



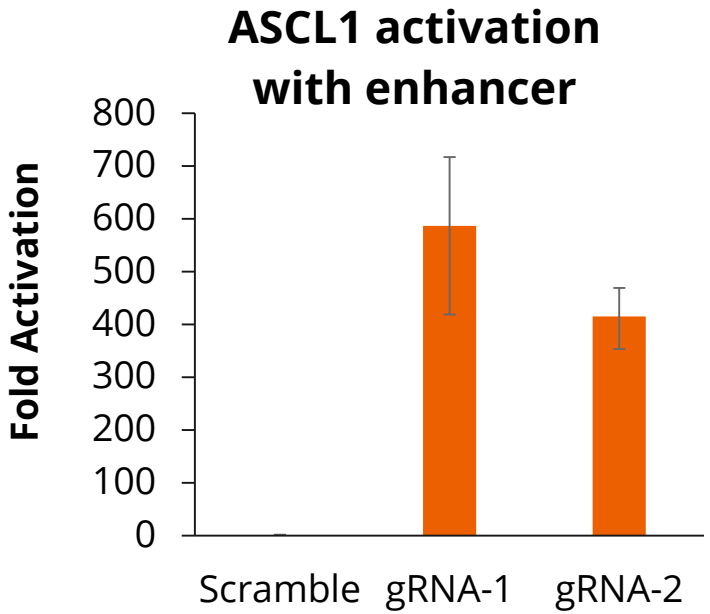
Human ASCL1 Activation

gRNA 1: CGGGAGAAAGGAACGGGAGG

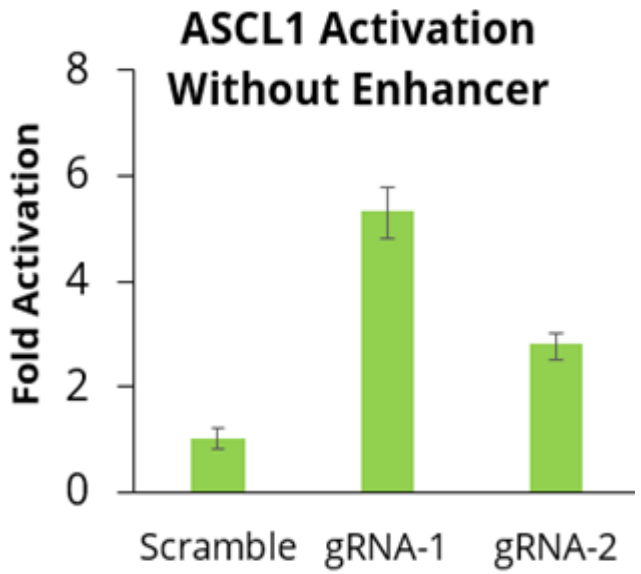
gRNA 2: TCCAATTTCTAGGGTCACCG



ASC1 Expression is Robustly Activated by CRISPRa



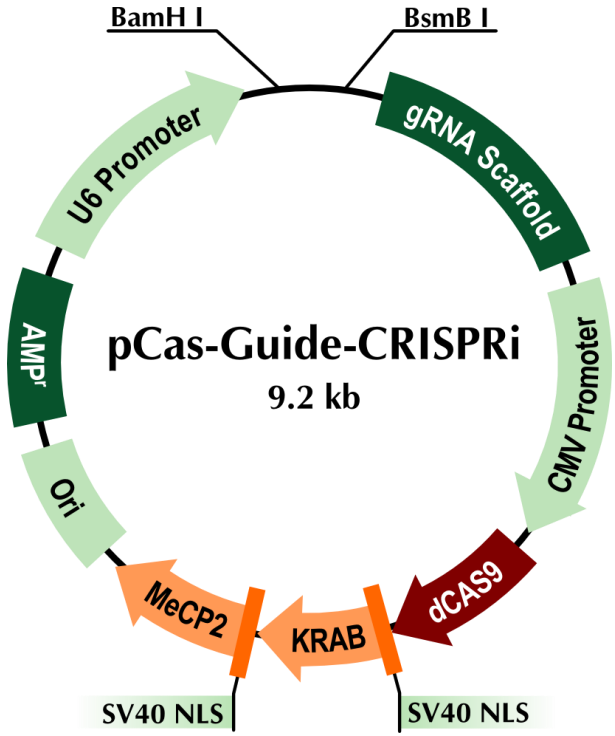
gRNA/dCas9-VP64 was cotransfected with the enhancer MS2-p65-HSF1 into HEK293 cells. Gene expression was measured 48 hrs post transfection.



gRNA/dCas9-VP64 was transfected into HEK293 cells without the enhancer MS2-p65-HSF1. Gene expression was measured 48 hrs post transfection.

CRISPRi Vector

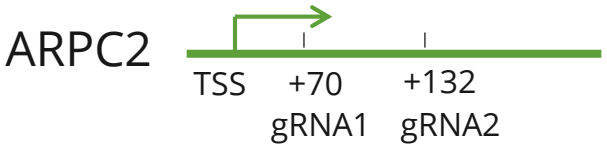
- All-in-one Vector
- gRNA cloning site
- CMV driven dCas9 -KRAB-MeCP2



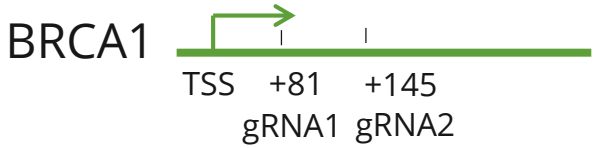
CRISPRi Gene Repression Validation

- ARPC2, BRCA1 target sequences were cloned into pCas-Guide-CRISPRi
- Gene repression was measured 48 hrs post transfection into HEK293 cells

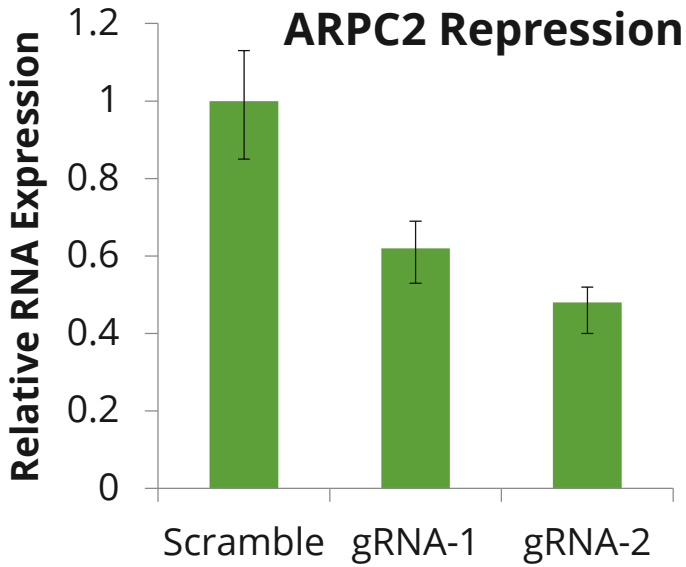
ARPC2 gRNA 1: TGTCGGTGAAGCGGCAGTGG
ARPC2 gRNA 2: CAGGCGGGTTCAGGCTTCGG

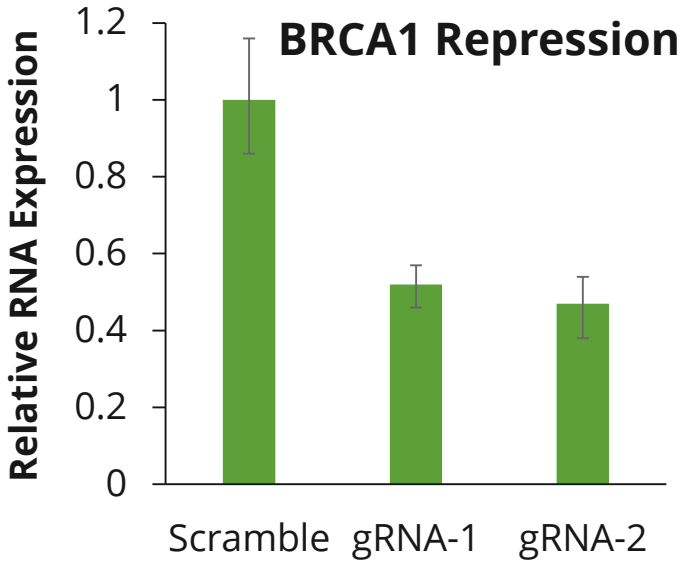


BRCA1 gRNA 1: GGATTTCCGAAGCTGACAGA
BRCA1 gRNA 2: GCTCGCTGAGACTTCCTGGA



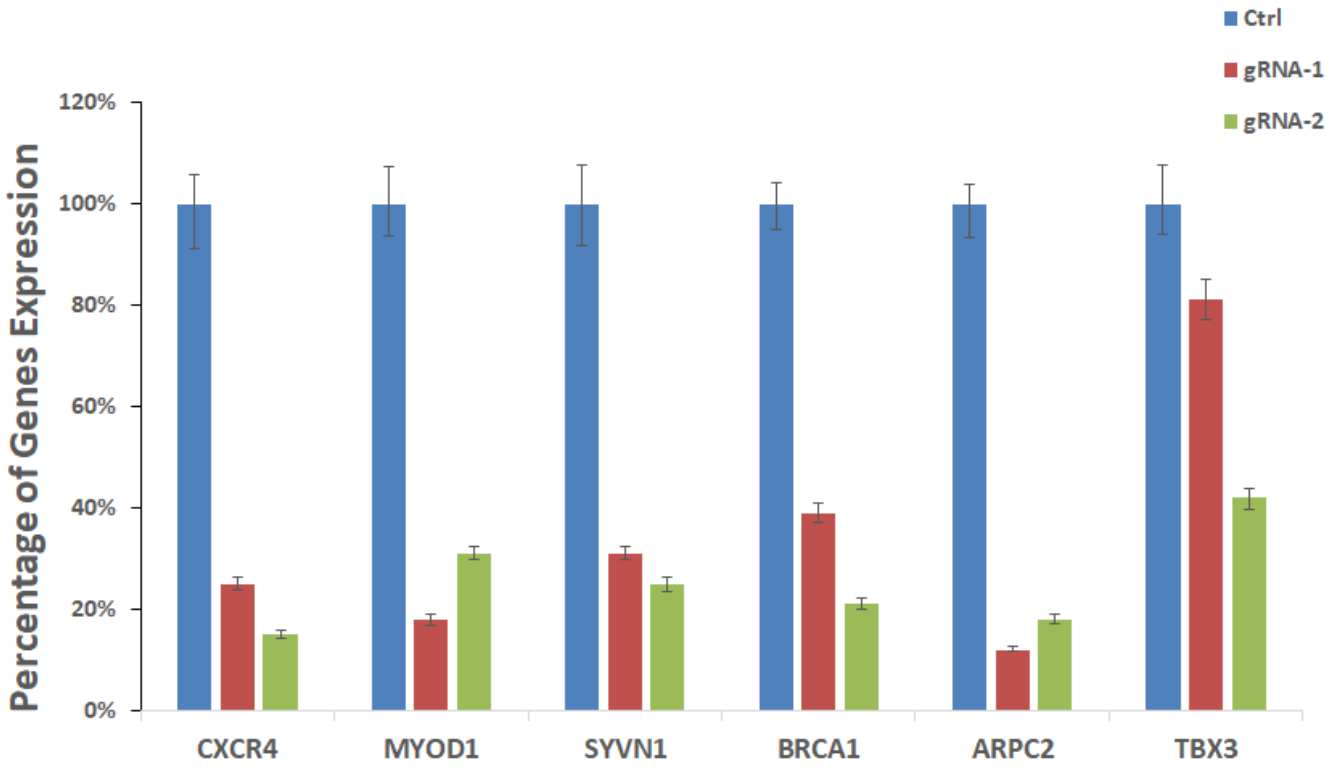
CRISPRi Repression Results





All-in-one CRISPRi vector containing gene specific gRNA and dCas9-KRAB-MeCP2 was transfected into HEK293T cells Using **MegaTran 2.0**. gRNA scramble control was used as a negative control. Cells were harvested 48 hrs post transfection and qPCR was performed to measure mRNA expression.

Repression by dCas9-KRAB-MeCP2



All-in-one CRISPRi vector containing gene specific gRNA and dCas9-KRAB-MeCP2 was transfected into HEK293T cells Using MegaTran 2.0. gRNA scramble control was used as a negative control. Cells were harvested 48 hrs post transfection and qPCR was performed to measure mRNA expression.

Gene expression repression was significant across all genes tested as shown above.

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