# CRISPR activation (CRISPRa) & CRISPR interference (CRISPRi)

ORIGENE

www.origene.com

# CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi)





9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436

9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436

# CRISPRa SAM System

## 1. Introduction

CRISPR/Cas9 Synergistic Activation Mediator (SAM) system was first discovered by Dr. Zhang's group. It is a robust system to activate gene expression through CRISPR technology. This system consists of three major components, the enzymatically deficient Cas9 (dCas9), transcriptional activator VP64 fusion protein, a modified gRNA construct with MS2 RNA aptamer, and a MS2-p65-HSF1 transactivation enhancer. The dCas9 protein contains mutations in two active endonuclease domains, losing the capability to cut DNA. However, it can still bind to DNA when coupled with gRNA. When dCas9 is fused with transcriptional activators such as VP64, it can be used to activate the gene expression. VP64 is a transcriptional activator which contains four copies of VP16, a transcriptional factor of HSV virus. p65 and HSF1 are co-transactivation factors. They can be brought in proximity to dCas9-VP64 protein through the interaction of MS2 RNA aptamers between gRNA and enhancer. The combination of these three transactivators comprises the most effective transcription activation system, which can robustly activate the transcription of both coding and non-coding RNA (lincRNA) (Figure 1).

OriGene's CRISPR activation (CRISPRa) kits are designed based on CRISPR SAM system. Each kit contains three gene specific pCas-Guide-CRISPRa plasmids and a MS2-p65-HSF1 transactivation enhancer. The pCas-Guide-CRISPRa plasmid is a Two-in-One vector which encodes both dCas9-VP64 fusion protein and a gene specific gRNA with MS2 RNA aptamer. A free scramble control is also provided in the same vector in each kit. The gRNA sequence used in each CRISPRa kit is designed to target the promoter region for the target gene. It can bring the dCas9 / transcription activators complex to the promoter region of the gene to activate the endogenous gene expression.

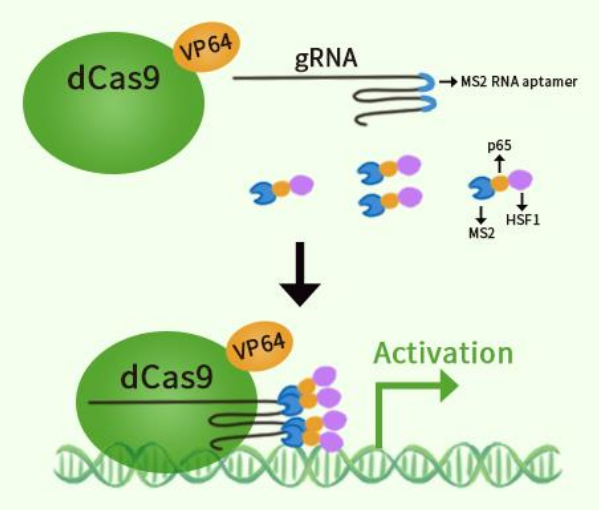
### Notice to purchaser

This product is for research use only. Use in and/or for diagnostics and therapeutics is strictly prohibited. By opening and using the product, the purchaser agrees to the following: The plasmids may not be distributed, resold, modified for resale or used to manufacture commercial products without prior written approval from OriGene Technologies, Inc. If you do not agree to the above conditions, please return the UNOPENED product to OriGene Technologies, Inc. within ten (10) days of receipt for a full refund by contacting customer service at custsupport@origene.com.



9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436

**Figure 1. CRISPRa SAM system activates gene expression**




## 2. Vectors used in CRISPRa system

### 2.1 gRNA cloning vector

- **pCas-Guide-CRISPRa vector (Cat# GE100055)**
- **pCas-Guide-GFP-CRISPRa vector (Cat# GE100074)**
- **pCas-Guide-Puro-CRISPRa vector (Cat# GE100081)**

pCas-Guide-CRISPRa (Cat# GE100055), pCas-Guide-GFP-CRISPRa (Cat# GE100074), and pCas-Guide-Puro-CRISPRa (Cat# GE100081) vectors are all-in-one CRISPR gRNA cloning vectors. All CRISPRa vectors contain the expression cassette for dCas9-VP64 fusion protein, and MS2 RNA aptamers in the gRNA scaffold loops. dCas9-VP64 itself can activate gene expression. However, with the help of p65-HSF1 (cat# GE100056), gene activation will be synergistically increased to thousands of folds.

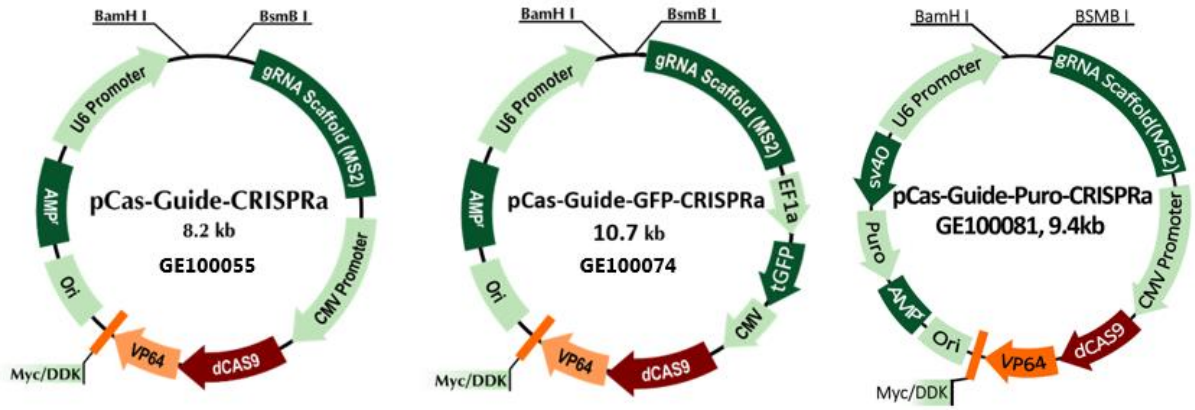
pCas-Guide-GFP-CRISPRa and pCas-Guide-Puro-CRISPRa are the upgraded vectors based on pCas-Guide-CRISPRa, which contain a tGFP reporter gene or a puro selection marker to help downstream screening or selection of target cells.

Please see the plasmid maps of these gRNA cloning vectors on Figure 2.



9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436

**Figure 2. The plasmid maps of All-in-one CRISPRa gRNA cloning Vectors.**




## 2.2 CRISPRa-Enhancer (Cat# GE100056)

The enhancer vector (figure 3) encodes a fusion protein of MS2 and the transactivation domains of p65 and HSF1. MS2 binds to MS2 RNA aptamer in the CRISPRa gRNA, thus bringing the three transactivation domains together, VP64, p65 and HSF1. This enhancer vector cannot activate gene expression by itself. It needs to work with CRISPRa gRNA construct.

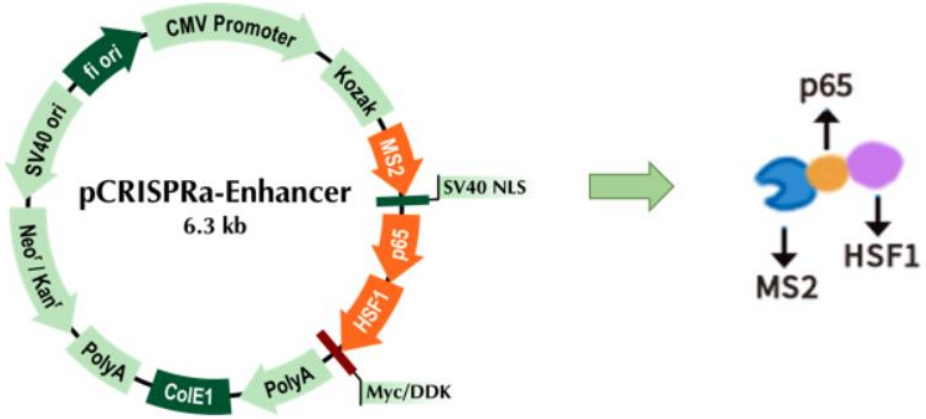
**Features:**

CMV driven MS2-p65-HSF1

Requires pCas-Guide-CRISPRa for gene activation

MS2-p65-HSF1 synergistically activate gene expression with dCas9-VP64

**Figure 3. The plasmid map of CRISPRa-Enhancer vector**

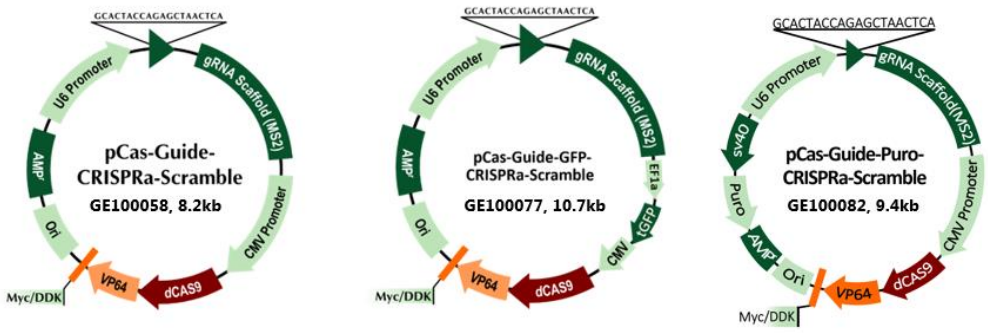


### 2.3 CRISPRa Scramble controls

- **pCas-Guide-CRISPRa-Scramble (Cat# GE100058)**
- **pCas-Guide-GFP-CRISPRa-Scramble (Cat# GE100077)**
- **pCas-Guide-Puro-CRISPRa-Scramble (Cat# GE100082)**

A scramble gRNA sequence is cloned in pCas-Guide-CRISPRa, or pCas-Guide-GFP-CRISPRa, or pCas-Guide-Puro-CRISPRa vector (Figure 4), which serves as a negative control for CRISPRa system.

**Figure 4. The plasmid maps of CRISPRa Scramble controls**




## 3. CRISPRa SAM vector kit for gene activation (SKU GE100057)

The CRISPRa SAM vector kit is a pre-assembled kit containing all the three vectors for CRISPRa gene activation, including gRNA cloning vector, CRISPRa-Enhancer, and CRISPRa scramble control.

### Package contents

- One vial of 10 μg lyophilized pCas-Guide-CRISPRa gene activation vector (Cat# GE100055).
- One vial of 10 μg lyophilized pCas-Guide-CRISPRa-Scramble (Cat# GE100058).
- One vial of 10 μg lyophilized pCRISPRa-Enhancer (Cat# GE100056).
- Certificate of Analysis
- Application Guide available online.

**The DNA is shipped at room temperature, but should be kept at -20°C for long-term storage. If properly stored, it should be stable for at least 12 months.*



9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436

### Related OriGene Products

- **pCas-Guide-GFP-CRISPRa** (Cat# GE100074): all-in-one CRISPRa vector with GFP expression cassette
- **pCas-Guide-Puro-CRISPRa** (Cat# GE100081): all-in-one CRISPRa vector with puromycin resistance marker
- **gRNA Cloning service**: https://www.origene.com/products/gene-expression/crispr-cas9/grna-cloning-service
- **qPCR Primer Pairs**: https://www.origene.com/qpcr/primers.aspx
- **Transfection Reagents**: https://www.origene.com/products/others/transfection-reagents

## 4. Genome-wide Gene Activation Kits using CRISPRa SAM

OriGene offers pre-designed genome-wide and locus specific gene activation kit using CRISPRa SAM system for human and mouse genes.

Each kit contains 3 individual gRNA constructs to ensure efficient target. To activate the expression of endogenous gene, each gRNA sequence is designed to target the promoter region of the target gene. The gRNA sequence will lead dCas9-VP64 to the promoter region. With the help of other two transactivation domains, p65-HSF1, from enhancer plasmid, the expression level will be greatly increased to up to thousand folds comparing the parent cell line.

### Package contents

- Three vials of gene specific gRNA constructs in pCas-Guide-GFP-CRISPRa vector (SKU GAxxxxxxG1, GAxxxxxxG2, GAxxxxxxG3), 3-5 µg DNA in TE buffer
- One vial of 10µg lyophilized pCas-Guide-GFP-CRISPRa-Scramble (Cat# GE100077)
- One vial of 10µg lyophilized pCRISPRa-Enhancer (Cat# GE100056)
- Certificate of Analysis
- Application Guide available online.

**The DNA is shipped at room temperature, but should be kept at -20°C for long-term storage. If properly stored, it should be stable for at least 12 months.*

### Related OriGene Products

- **pCas-Guide-CRISPRa** (Cat# GE100055): all-in-one CRISPRa vector without GFP expression cassette
- **gRNA Cloning service**: https://www.origene.com/products/gene-expression/crispr-cas9/grna-cloning-service
- **qPCR Primer Pairs**: https://www.origene.com/qpcr/primers.aspx
- **Transfection Reagents**: https://www.origene.com/ /cdna/transfection.mspx



9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436

# 5. Experimental protocol

## 5.1 Clone gRNA into CRISPRa gRNA cloning vector

1. Prepare the pre-cut pCas-Guide-CRISPRa or pCas-Guide-GFP-CRISPRa vector

   a. Digest CRISPRa vector with BamH I and BsmB I

   Resuspend the 10 µg lyophilized pCas-Guide-CRISPRa or pCas-Guide-GFP-CRISPRa DNA in 100 µL dH2O, and incubate for at least 30 min before use. Set up a digestion reaction as described below.

| Component | Volume |
|---|---|
| 10X restriction buffer | 3 µL |
| BamH I | 0.8 µL |
| BsmB I | 0.8 µL |
| Nuclease free water | 15.4 µL |
| Vector DNA | 10 µL |
| Total volume | 30 µL |

   Incubate the reaction at 37ºC for 3 hrs, then add 1 µL antarctic phosphatase (units used according to the manufacturer's protocol), and continue the incubation at 37ºC for another 30 min.

   **Dephosphorylation of the digested vector is essential to eliminate self-ligation.*

   b. Purify the desired pre-cut vector fragment

   Separate the digested vector on an agarose gel, and isolate the vector band using a gel purification column. Elute the digested vector in 40 µL of 10 mM Tris buffer.

2. Design the specific gRNA oligos

   a. Select a desired gene specific 20-bp sequence as a target sequence. The following is an example of 20bp target sequence:

   Forward sequence: 5' ATGGGAGGTGGTATGGGAGG 3'

   Reverse complement sequence: 5' CCTCCCATACCACCTCCCAT 3'

   b. Add 'gatcg' to the 5' end of the forward sequence and 'g' to its 3' end. The final sense oligo in this example will be

   5' gatcgATGGGAGGTGGTATGGGAGGg 3'

   c. Add 'aaaac' to the 5' end of reverse complementary sequence and 'c' to its 3' end.

   The final reverse complementary sequence is

   5' aaaacCCTCCCATACCACCTCCCATc 3'



9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436

**The two oligos should anneal to form the following double strand:*

5'gatcgxxxxxxxxxxxxxxxxxxxxg 3'
3'cxxxxxxxxxxxxxxxxxxxxcaaaa 5'

d. Order the two final oligos from a commercial oligo provider, such as IDT.

3. Clone the double stranded oligos into CRISR vector

a. Anneal the two oligos to form double-stranded duplexes

In a PCR tube, add the following:

| | |
|---|---|
| 2 µL | Forward oligo (100 µM stock) |
| 2 µL | Reverse oligo (100 µM stock) |
| 4 µL | 10X annealing buffer |
| 32 µL | $dH_2O$ |

Mix the solution and follow the steps to anneal the oligos in a PCR machine:

$94^0$C for 4min
$75^0$C for 5 min
$65^0$C for 15 min
$25^0$C for 20 min

After annealing, transfer the solution to a 1.5 mL tube and add 360 µL of $dH_2O$.
The double-stranded oligo DNA is ready for ligation.

b. Ligation and transformation

a) Prepare the ligation according to the following protocol

| Component | Volume |
|---|---|
| 10x Ligation buffer | 1 µL |
| Precut pCas-Guide vector (10 ng/ µL) | 1 µL |
| Annealed double-stranded oligos (diluted from step 1) | 1 µL |
| Ligase (0.5 u/ µL, Weiss unit) | 0.5 µL |
| dH2O | 6.5 µL |
| Total Volume | 10 µL |

b) Mix the solution and incubate the tube at 22 to $37^0$C or room temperature for two hours according to the manufacturer's recommendation.

c) Add 1 µL of the ligation mixture to 10 µL of competent cells (efficiency rated > $10^6$ cfu/µg DNA) on ice. Do the transformation according to the manufacturer's protocol.

d) Mix the tube gently and keep it on ice for 25 minutes.

e) Heat shock the tube for 30 seconds at $42^0$C.



9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436

f) Put the tube on ice for 2 minutes, and then add 500 µL LB or SOC medium.

g) Rock the tube gently at 37$^0$C for 1 hour.

h) Spread 50 µL of the *E. Coli* cells on an LB agar plate containing the corresponding antibiotics (most vectors are ampicillin resistant except lenti CRISPR vectors, which is chloramphenicol resistant).

i) Centrifuge the remaining *E. Coli* cells at 5K rpm for 5 minutes. Discard most supernatant (around 50 µL supernatant left) and resuspend the cell pellet in the remaining liquid. Spread all the *E. Coli* cells on a separate LB-agar plate.

j) Incubate the two plates at 37$^0$C for 16 hours to allow colony formation.

c. Screen positive CRISPRa construct

Typically, at least 95% of the colonies should contain the desired insert. Pick 6 to 10 colonies into 5 mL LB-ampicillin or LB- chloramphenicol (for Lenti vector) culture each, and culture overnight. Perform DNA purification and sequence the purified DNA. Analyze the sequencing data to identify a correct clone for proper insert identification and orientation.

## 5.2 Activate the gene expression using CRISPRa construct

A sample protocol listed below is for 6-well plates and using TurboFectin (cat# TF81001) as the transfection reagent.

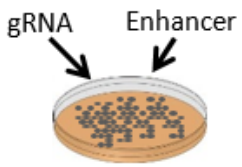
**Different types of cells require different transfection reagents. Please follow the manufacturer's protocol for your transfection.*

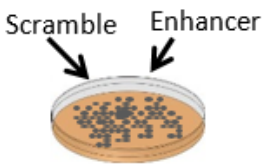
1. Seed the cells the day before transfection

Approximately 18-24 hours before transfection, plate ~3 X 10$^5$ adherent cells in 2 ml culture media into each well of a 6-well plate or ~5x10$^5$ suspension cells per well to obtain 50-70% confluence on the following day. The number of cells varies depending on the size of your cells.

2. Set up two transfection complexes in complete culture media

1) Target gene specific CRISPRa construct + pCRISPRa-Enhancer
2) CRISPRa-Scramble control and pCRISPRa-Enhancer






9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436

In a small sterile tube, combine the following reagents in the prescribed order. The order of reagent addition is important to achieve the optimal results.

- Dilute 1 µg of gRNA vector and 0.3ug Enhancer vector in 250 uL of Opti-MEM I (Life Technologies), vortex gently.
- Add 3.9 µL of Turbofectin 8.0 to the diluted DNA (not the reverse order) and pipette gently to mix completely.
- Incubate the mixture 15 minutes at room temperature.

** We recommend starting with the ratios of 3:1, Turbofectin: DNA. However subsequent optimization may be needed to increase the transfection efficiency.*

3. Add the DNA:Turbofectin complex drop-wise to the cells (no need to change the media). Gently rock the plate back-and-forth and side-to-side to distribute the complex evenly.

4. 48 hours post transfection, extract mRNA using Trizol method (following manufacturer's instructions)
5. Measure the gene expression with qPCR or WB analysis.

## 5.3 CRISPRa Validation Data

Target gene expression can be upregulated using only pCas-Guide-CRISPRa gRNA construct (with or without enhancer). To test our CRISPRa system, we have designed five gene specific gRNA sequences (table 1) based on the human ASCL1 gene promoter sequence, and cloned them into CRISPRa gRNA cloning vectors. The gRNA constructs and scramble control were co-transfected with CRISPRa enhancer into HEK293T cells using MegaTran 2.0 transfection reagent. All transfection showed comparable transfection efficiency under microscope (Figure 5). 48 hours after transfection, the expression level of ASCL1 was measured using specific qPCR reaction. All gRNA constructs have shown increased level of ASCL1 expression (Figure 6).

**Table 1 Five specific gRNA sequences designed for human ASCL1 gene activation**

| Name | Sequence | Promoter Position |
|---|---|---|
| ASCL1-g1 | TTGAAAAGGCGGACGCACTC | +31 |
| ASCL1-g2 | CGGGAGAAAGGAACGGGAGG | +204 |
| ASCL1-g3 | AATAAACAGGCGGCGCGCTC | -300 |
| ASCL1-g4 | AAGAACTTGAAGCAAAGCGC | -447 |
| ASCL1-g5 | TCCAATTTCTAGGGTCACCG | +583 |



9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436

**Figure 5 All gRNA constructs including the scramble control showed comparable transfection efficiency**

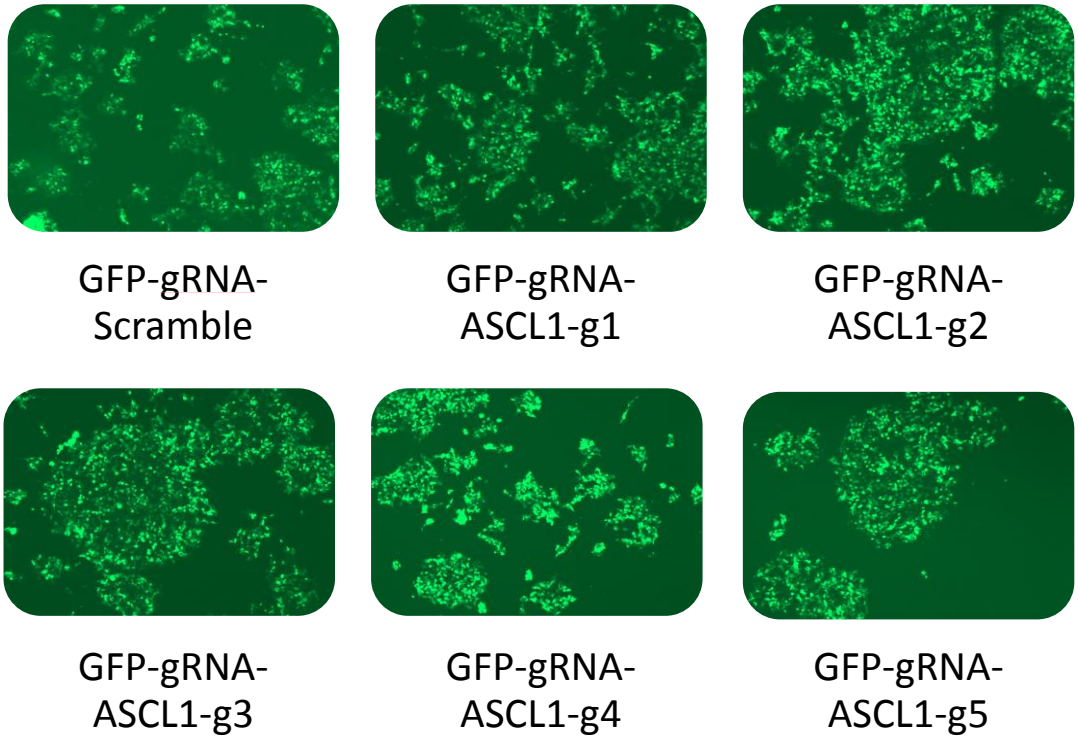
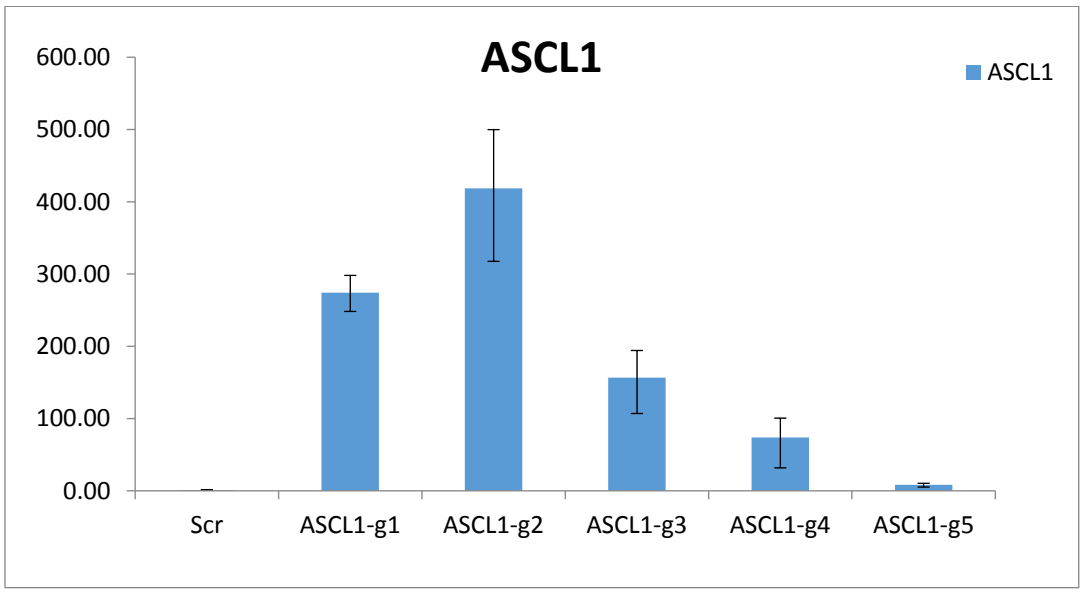



**Figure 6. All gRNAs can activate ASCL1 gene expression with the help of enhancer**




We have also compared the gene activation effects with and without enhancer using ASCL1-g1 and ASCL1-g3 constructs. Both ASCL1-g1 and ASCL1-g3 can activate the gene expression without the help of



9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436

enhancer vector comparing to the scramble control (Figure 7). But with enhancer, all gRNA constructs have shown hundred folds of increasing expression level of target gene (Figure 8). So pCRISPRa-Enhancer (Cat# GE100056) vector can greatly increase the expression level of target gene through the synergistic gene activation.

**Figure 7 Cas9-VP64 without p65-HSF1 can also activate gene expression**

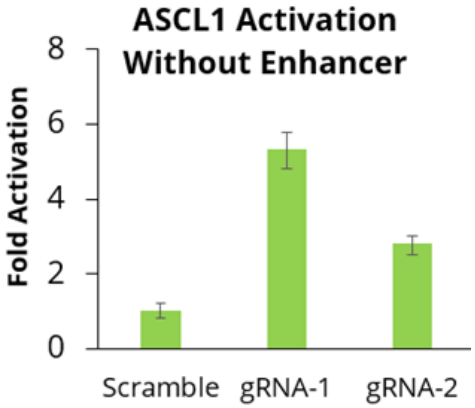
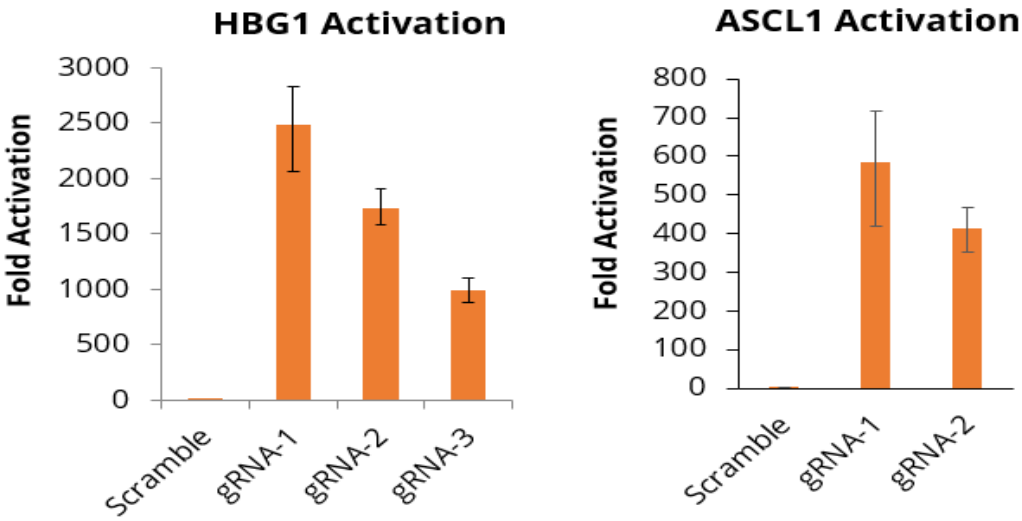



**Figure 8 CRISPRa SAM (VP64 with p65-HSF1) synergistically activates gene expression**






9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436

# CRISPR inhibitory (CRISPRi) System

## 1. Introduction

The dCas9 can also be fused with transcription repression domains to repress endogenous gene expression. Based on this CRISPR inhibitory (CRISPRi) System, we have designed all-in-one CRISPRi vectors using the KRAB and MeCP2 repression domains to carry out robust gene repression.

**Krüppel-associated box (KRAB) is a well-known transcriptional repressor domain. MeCP2 has been shown to bind to methylated DNA.*

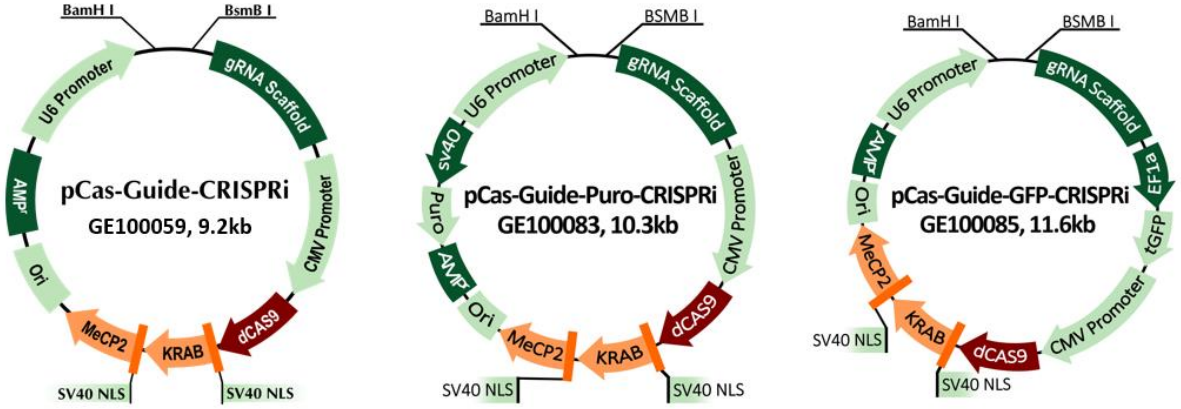
## 2. CRISPRi gene inhibitory system

### 2.1 All-in-one CRISPRi gRNA cloning vectors

- **pCas-Guide-CRISPRi vector (Cat# GE100059)**
- **pCas-Guide-Puro-CRISPRi vector (Cat# GE100083)**
- **pCas-Guide-GFP-CRISPRi vector (Cat# GE100085)**

The all-in-one CRISPRi gRNA cloning vectors contain gRNA cloning sites and CMV driven dCas9-KRAB-MeCP2 expression cassette. After cloning a specific gRNA targeting sequence, the vector can be transfected into target cells and lead to gene repression.

**Figure 9 The plasmid maps of pCas-Guide-CRISPRi vectors**






9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436

### Package contents

- One vial of 10 μg lyophilized CRISPRi vector.
- Certificate of Analysis

**The DNA is shipped at room temperature, but should be kept at -20°C for long-term storage. If properly stored, it should be stable for at least 12 months.*

### Related Optional Reagents

- Nuclease free water
- BamH I, BsmB I
- T4 DNA ligase and buffer
- Competent *E. coli* cells
- LB agar plates with ampicillin, 100 μg/mL; kanamycin, 25 μg/mL
- LB broth (10 g/L Tryptone, 5 g/L Yeast Extract, 10 g/L NaCl. Adjust pH to 7.0 with 1 N NaOH)
- DNA purification reagents

### Related OriGene Products

- gRNA Cloning - https://www.origene.com/crispr-cas9/grna-cloning-donor-construction.aspx
- qPCR Primer Pairs - https://www.origene.com/qpcr/primers.aspx
- Transfection Reagents - https://www.origene.com/products/others/transfection-reagents
- cDNA clones - https://www.origene.com/cdna/
- Validated Antibodies - https://www.origene.com/antibodies
- CRISPR/Cas9 products - https://www.origene.com/crispr-cas9

### Notice to purchaser

This product is for research use only. Use in and/or for diagnostics and therapeutics is strictly prohibited. By opening and using the product, the purchaser agrees to the following: The plasmids may not be distributed, resold, modified for resale or used to manufacture commercial products without prior written approval from OriGene Technologies, Inc. If you do not agree to the above conditions, please return the UNOPENED product to OriGene Technologies, Inc. within ten (10) days of receipt for a full refund by contacting customer service at custsupport@origene.com.

## 2.2 CRISPRi scramble controls

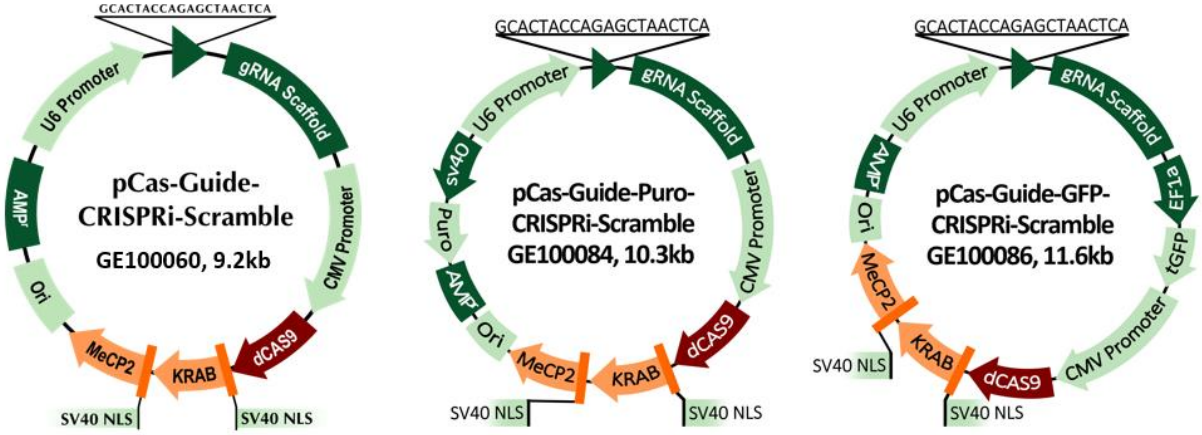
- **pCas-Guide-CRISPRi-Scramble (Cat# GE100060)**
- **pCas-Guide-Puro-CRISPRi-Scramble (Cat# GE100084)**
- **pCas-Guide-GFP-CRISPRi-Scramble (Cat# GE100086)**

CRISPRi scramble controls contain gRNA scrambled sequence in CRISPRi vectors, which serve as negative controls for CRISPRi inhibition.



9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436

**Figure 10. The plasmid maps of CRISPRi scramble controls**




## 3. Experimental protocol

1. Resuspend and digest the CRISPRi plasmid (Cat# GE100059, GE100083 and GE100085) with BamH I and BsmB I

   Resuspend the 10µg lyophilized DNA in 100 µL dH2O, and incubate for at least 30 min before use. Set up a digestion reaction as described below.

| Component | Volume |
|---|---|
| 10X restriction buffer | 3 µL |
| BamH I | 0.8 µL |
| BsmB I | 0.8 µL |
| Nuclease free water | 15.4 µL |
| Vector DNA | 10 µL |
| Total volume | 30 µL |

   Incubate the reaction at 37°C for 3 hrs, then add 1 µL antarctic phosphatase (units used according to the manufacturer's protocol), and continue the incubation at 37ºC for another 30 min.

   **Dephosphorylation of the digested vector is essential to eliminate self-ligation.*

   Purify the desired vector fragment by running the digestion reaction on an agarose gel, and isolate the appropriate band using a gel purification column. Elute the digested plasmid vector in 40 µL of 10 mM Tris buffer.

2. Target sequence designing and cloning into precut CRISPRi vector.

2.1 Design the specific gRNA oligos.
2.2 Clone the gRNA oligos into precut CRISRPi vector.



9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436

2.1 Design the specific gRNA oligos.

a. Select a desired gene specific 20-bp sequence as a target sequence.

Forward sequence: 5′ ATGGGAGGTGGTATGGGAGG 3′
Reverse complement: 5′ CCTCCCATACCACCTCCCAT 3′

b. Add 'gatcg' to the 5′ end of the forward sequence and 'g' to its 3′

end. The final sense oligo in this example will be

5′ gatcgATGGGAGGTGGTATGGGAGGg 3′

c. Add 'aaaac' to the 5′ end of reverse complementary sequence and 'c' to its 3' end for pCas-Guide-CRISPRi vector (Cat# GE100059).

**For pCas-Guide-Puro-CRISPRi (Cat# GE100083 and GE100085), add 'TAAAC' to the 5′ of the reverse complement.**

The final reverse complementary sequence is

5′ aaaacCCTCCCATACCACCTCCCATc 3′

**The two oligos should anneal to form the following double strand:*

5′gatcgxxxxxxxxxxxxxxxxxxxxg 3′
3′cxxxxxxxxxxxxxxxxxxxxcaaaa 5′

d. Order the two final oligos from a commercial oligo provider, such as IDT.

2.2 Clone the gRNA oligos into precut CRISRPi vector.

a. Anneal the two oligos to form double-stranded duplexes

In a PCR tube, add the following:

| | |
|---|---|
| 2 µL | Forward oligo (100 µM stock) |
| 2 µL | Reverse oligo (100 µM stock) |
| 4 µL | 10X annealing buffer |
| 32 µL | $dH_2O$ |

Mix the solution and follow the steps to anneal the oligos in a PCR machine:

$94^0$C for 4min
$75^0$C for 5 min
$65^0$C for 15 min
$25^0$C for 20 min

After annealing, transfer the solution to a 1.5 mL tube and add 360 µL of $dH_2O$.
The double-stranded oligo DNA is ready for ligation.

b. Ligation and transformation



9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436

Prepare the ligation according to the following protocol

| Component | Volume |
|---|---|
| 10x Ligation buffer | 1 µL |
| Precut pCas-Guide vector (10 ng/ µL) | 1 µL |
| Annealed double-stranded oligos (diluted from step 1) | 1 µL |
| Ligase (0.5 u/ µL, Weiss unit) | 0.5 µL |
| dH2O | 6.5 µL |
| Total Volume | 10 µL |

Mix the solution and incubate the tube at 22 to 37$^0$C or room temperature for two hours according to the manufacturer's recommendation.

Add 1 µL of the ligation mixture to 10 µL of competent cells (efficiency rated > $10^6$ cfu/µg DNA) on ice. Do the transformation according to the manufacturer's protocol.

c. Screen positive CRISPRi construct

Typically, at least 95% of the colonies should contain the desired insert. Pick 6 to 10 colonies into 5 mL medium and culture overnight. Perform DNA purification and sequence the purified DNA. Analyze the sequencing data to identify a correct clone for proper insert identification and orientation.

## 3. Transfection and CRISPRi inhibition

A sample protocol listed below is for 6-well plates and using TurboFectin (cat# TF81001) as transfection reagent. If your experiments require a different size of culture plates, just scale up or down the reagents accordingly based on the relative surface area of your plate. Different type of cells may need a different transfection reagent; please follow the manufacturer's corresponding protocol.

1) Approximately 18-24 hours before transfection, plate ~3 X $10^5$ adherent cells in 2 ml culture media into each well of a 6-well plate or ~5x$10^5$ suspension cells per well to obtain 50-70% confluence on the following day. The number of cells varies depending on the size of your cells.
2) Transfection in complete culture media.

   In a small sterile tube, combine the following reagents in the prescribed order. The order of reagent addition is important to achieve the optimal results.

3) Dilute 1 µg of CRISPRi plasmid in 250 uL of Opti-MEM I (Life Technologies), Vortex gently. A corresponding CRISPRi-Scramble in the same CRISPRi vector should be transfected separately as a negative control.
4) Add 3 µL of Turbofectin 8.0 to the diluted DNA (not the reverse order) and pipette gently to mix completely.
5) Incubate the mixture 15 minutes at room temperature.

   **Note:** We recommend starting with the ratios of 3:1, Turbofectin: DNA. However subsequent optimization may be needed to increase the transfection efficiency.

6) Add the mixture above drop-wise to the cells (no need to change the media). Gently rock the plate back-and-forth and side-to-side to distribute the complex evenly.



9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436

7) 24 hours post transfection, replace the media with puromycin (5ug/ml)
8) Incubate with puromycin for 3 days
9) Extract mRNA using Trizol method (following manufacturer's instructions)
10) Measure the gene expression with qPCR or WB analysis.

## 4. Validation Data

To test our CRISPRi system, we have designed some gene specific gRNA sequences based on the human ARPC2 and BRCA1 gene promoter sequences, and cloned them into CRISPRi gRNA cloning vector. The gRNA constructs and scramble control were transfected into HEK293T cells. 48 hours after transfection, the expression level of ARPC2 and BRCA1 were measured using specific qPCR reactions. All gRNA constructs have shown reduced level of gene expression comparing to the scramble control sample (Figure 11).

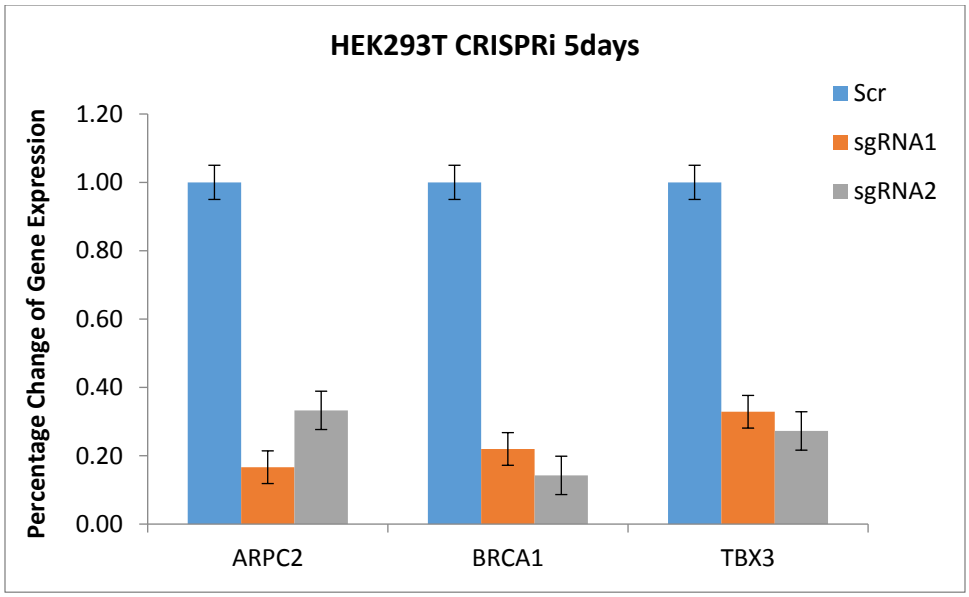
**Table 2 The gRNA sequences designed for specific genes activation**

| Name | Sequence | Position from TSS |
|---|---|---|
| ARPC2-g1 | TGTCGGTGAAGCGGCAGTGG | +51 |
| ARPC2-g2 | CAGGCGGGTTCAGGCTTCGG | +91 |
| BRCA1-g1 | GCTCGCTGAGACTTCCTGGA | +116 |
| BRCA1-g2 | GGATTTCCGAAGCTGACAGA | +281 |
| TBX3-g1 | TTCGCTCACGTTTTAGGACA | +191 |
| TBX3-92 | ATTGAAACCGAGACACCCTC | +254 |

**Figure 11 dCas9-KRAB-MeCP2 significantly repressed gene expression**



9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436




# FAQ

**Q1: How do CRISPRa SAM and CRISPRi work?**

CRISPRa/CRISPRi is an RNA-guided genome expression regulatory tool. Two mutations are introduced into Cas9 to silence the nuclease activity (called as dead Cas9 or dCas9). dCas9 can be recruited by gRNA to the target genomic loci, but don't cut the target DNA. dCas9 is used as anchor to recruit fusion partner-protein to the targeted promoter. In CRISPRa, activating factors like VP64 and VPR are fused with dCas9, while in CRISPRi, inhibitory factors like KRAB, MeCP2 are used.

Our engineered CRISPRa SAM belongs to the second generation of the CRISPRa family, which was originally reported by Dr. Feng Zhang's lab (Konermann et al. 2015). SAM stands for Synergistic Activation Mediator. The CRISPRa vector is an all-in-one vector, containing gRNA cloning sites (gRNA modified with MS2 RNA aptamers in the loop), and the expression cassette of dCas9-Vp64 fusion protein. Another vector, CRISPRa enhancer, encodes the co-activator MS2-P65-HSF1, which can be recruited by the MS2 RNA aptamers of the gRNA scaffold and synergistically activates gene expression with dCas9-Vp64.

Our CRISPRi system is engineered to fuse dCas9 with dual repressive domains, KRAB domain and MeCP2 repressive domain, which has shown stronger inhibition effects compared with classical CRISPRi-KRAB system (Yeo et al. 2018).

**Q2: Where should CRISPRa/CRISPRi gRNAs target?**

The CRISPRa SAM and CRISPRi system use the same PAM (protospacer-adjacent motif) specificity as wildtype Cas9 (NGG). However, the target genomic region should be optimized for CRISPRa and CRISPRi



9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436

systems. According to the article (Horlbeck et al. 2016), CRISPRa gRNA within the region from -550bp to -25bp (distance to TSS), and CRISPRi gRNA from -25bp to +500bp (distance to TSS) show the best regulation effect. OriGene's gene specific CRISPRa-SAM activation kit contains 3 gRNA sequences designed for same gene using our proprietary algorithm.

**Q3: Why doesn't my CRISPRa-SAM kit have dramatic effects?**

The effects of CRISPRa are affected by multiple factors, including transfection efficiency, gRNA targeting efficiency, and chromatin accessibility. We recommend you to first optimize the transfection efficiency according to your target cell type and reagents. CRISPRa is also affected by the accessibility of the target DNA. The factors including nucleosome occupancy and chromatin structure can influence the reach of gRNA and dCas9 to its target DNA. In addition, based on our experiments, the highly-expressed genes (such as MYC in HEK293T) won't show a significant response to further stimulation from exogenous CRISPRa SAM system.

**Q4: How to avoid off-target issues using CRISPRa/CRISPRi?**

You can blast your target sequences. If the off-target sequences don't have the PAM (NGG), then they won't be targeted by CRISPR/Cas9. You also want to choose target sequences with mismatches in the 8-14 bp at the 3' end of the target sequences. It has been reported that CRISPR-fusion protein-related products can increase off-targeting issues from the nonspecific binding of the fusion proteins (Zhou et al. 2019). To offset these effects, you can use scrambled sgRNA as negative control in your experiment.

**Q5: How many target RNA sequences should I use for a CRISPRa/CRISPRi project?**

Due to the unpredictable nature of gRNA, we recommend designing 3 gRNA against your target genes. In our CRISPRa activation kit, 3 gene specific sgRNAs, a scrambled sgRNA, and CRISPRa-Enhancer are provided. In addition, it has been observed that the co-transfection of 3 gRNAs can be more potent in stimulating the target genes (Chavez et al. 2016).

Updated on June 2021



9620 Medical Center Drive
Rockville MD, 20850
Tel 888.267.4436