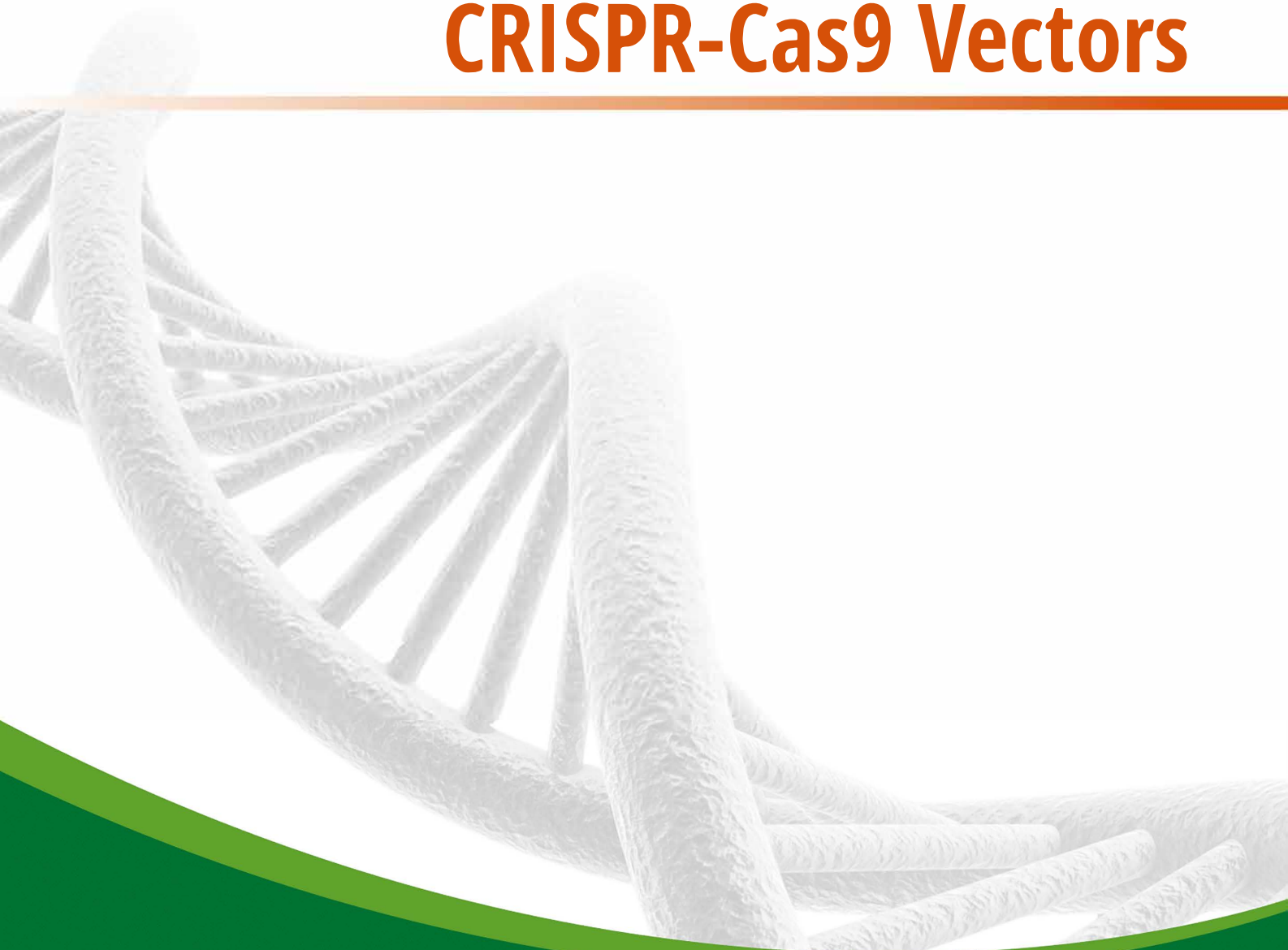


CRISPR-Cas9 Vectors



CRISPR Vector

Introduction	3
I. All-in-One SpCas9 gRNA cloning vector	3
Package contents	3
Related OriGene Products and Services.....	3
Notice to purchaser.....	4
Product Description	4
Figure 1. The vector maps of the All-in-One SpCas9 gRNA cloning vectors	5
Experimental Protocols	5
II. Cas9 Nickase Vectors	8
Package contents	8
Related OriGene Products.....	8
Product description	8
Experimental protocol	9
Figure 2. The vector maps of the Cas9 Nickase Vectors	9
III. gRNA only Vectors	10
Package contents	10
Related OriGene Products.....	10
Product Description	10
Figure 3. The vector maps of the gRNA only vectors	11
Experimental protocol	11
IV. Cas9 only vectors	11
Package contents	11
Related OriGene Products.....	11
Product Description	12
Lentiviral based Cas9 only vectors	12
Table 1. Comparison of different Lentiviral based Cas9 only vectors	12
Figure 4. The vector maps of Lenti Cas9 vectors	12
pAAVS1-Cas9-Puro-DNR and pAAVS1-Cas9-BSD-DNR vectors	13
Figure 5. Diagram of how Cas9 is inserted at AAVS1 locus.....	13
V. T7 driven CRISPR Vectors	14
Package contents	14
Related OriGene Products.....	14

Product Description 14

 Figure 6. The vector maps of T7 driven CRISPR Vectors 15

Experimental protocol 15

VI. CRISPR scramble controls..... 17

 Package contents 17

 Product description 17

 Figure 7. The vector maps of pCas-Scramble and pCas-Scramble-EF1a-GFP..... 17

VII. Cre expression vector for Cre-Lox recombination 18

 Package contents 18

 Related OriGene Products..... 18

 Product Description 18

 Figure 8. The vector map of pCMV6-Entry-Cre 18

VIII. FAQ..... 19

Introduction

The CRISPR/Cas9 system has been widely used for genome editing including gene disruption, site specific mutagenesis, and epigenetic regulation et al. *Streptococcus pyogenes* (SpCas9) is currently the most commonly used Cas9 protein. Genome editing via SpCas9 requires a “NGG” protospacer adjacent motif (PAM) sequence at the target site, which limit the editing scope of CRISPR/Cas9 system. To expand the scope of editing sites and optimize the editing specificity, various SpCas9 mutants have been studied and successfully used in CRISPR system.

OriGene is constantly working on adding new CRISPR vectors with various Cas9 variants and different functional cassettes.

Featured CRISPR Vectors

- **All-in-One SpCas9 gRNA cloning vector:** gRNA cloning vector containing SpCas9 expression cassette
- **Cas9 Nickase Vector:** Contain Cas9 Nickase mutations
- **gRNA only vector:** gRNA cloning vectors that don't contain Cas9 expression cassette
- **Cas9 only vector:** For the expression of Cas9; can't be used for gRNA cloning.
- **T7 driven CRISPR vector:** For in vitro production of gRNA and Cas9 mRNA

I. All-in-One SpCas9 gRNA cloning vector

pCas-Guide (SKU GE100002)
 pCas-Guide-EF1a-GFP (SKU GE100018)
 pCas-Guide-EF1a-CD4 (SKU GE100022)
 pLenti-Cas-Guide (SKU GE100010)
 pLenti-EF1a-Cas-Guide (SKU GE100045)
 pCas-Guide-EF1a-RFP (SKU GE100140)

Package contents

- One vial of CRISPR vector DNA (10 µg, lyophilized). Reconstitute in 100 µL dH₂O to make a final concentration of 100 ng/ µL.
- Certificate of Analysis
- Application Guide available online.

** OriGene plasmids are shipped at room temperature, but should be kept at -20 °C for long-term storage. If properly stored, DNA is guaranteed to be stable for 12 months.*

Related OriGene Products and Services

- Oligo annealing buffer, SKU GE100007
- CF3 sequencing primer, SKU GE100008

- Scramble controls: pCas-Scramble (SKU GE100003) and pCas-Scramble-EF1A-GFP (SKU GE100021)
- Custom donor DNA with one of the four predesigned functional cassettes
<https://www.origene.com/products/gene-expression/crispr-cas9/genome-editing-inquiry>
 - GFP-PGK-Loxp-Puro-LoxP
 - RFP-PGK-Loxp-BSD-LoxP
 - Luciferase-PGK-Loxp-Puro-LoxP
 - mBFP-PGK-Loxp-Neo-Loxp

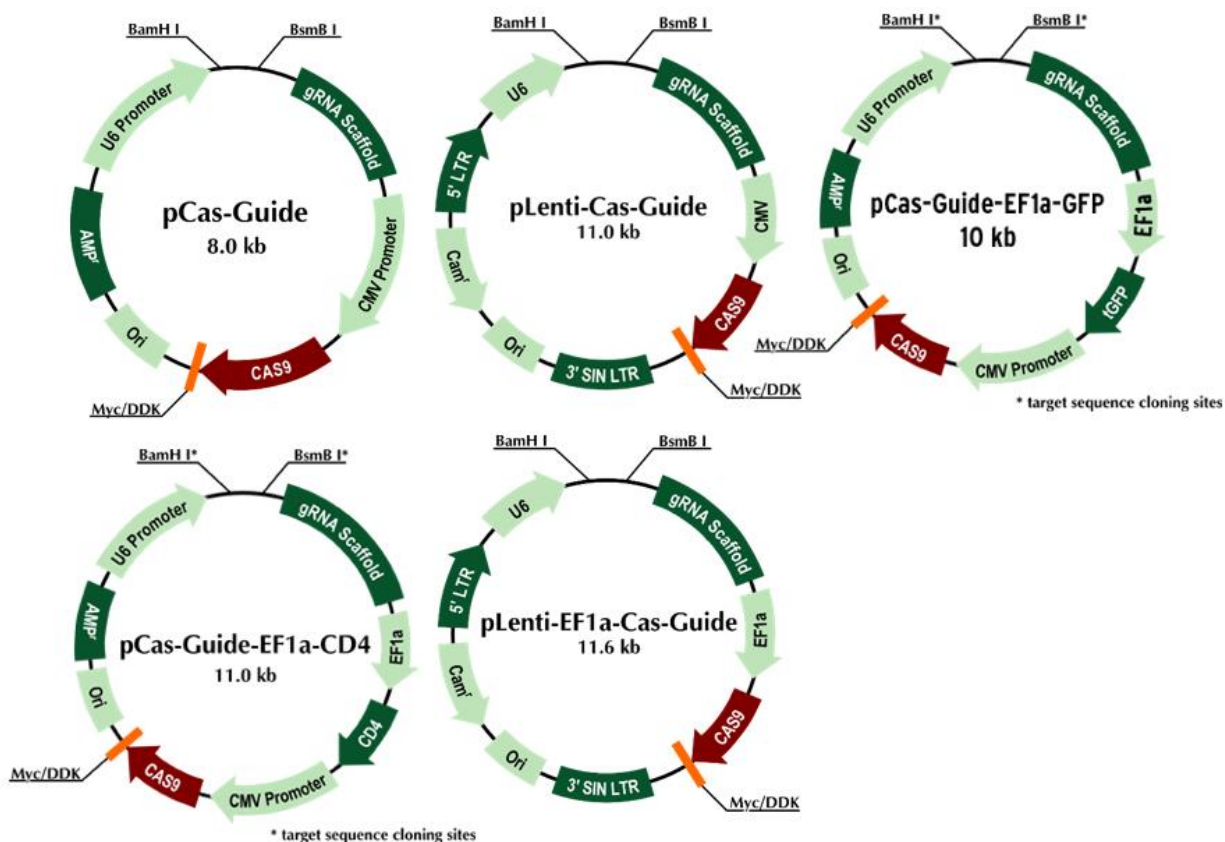
Notice to purchaser

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.

Product Description

The all-in-one SpCas9 gRNA cloning vectors are designed for the cloning of the guide RNA under U6 promoter. The vectors also express a CMV-driven codon-optimized SpCas9 protein, which can cut the desired targeted genome after cloning of specific gRNA sequence.

Figure 1. The vector maps of the All-in-One SpCas9 gRNA cloning vectors



Experimental Protocols

1. Digest the CRISPR all-in-one vector with BamH I and BsmB I

Resuspend 10 µg lyophilized vector 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.

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

2. Design and clone target sequence into CRISPR vector

Design the gRNA Oligos

Normally we recommend choosing at least 2-3 gRNA sequences for each CRISPR editing project. To facilitate cloning of the 20-bp target sequence into OriGene CRISPR vector, extra bases need to be added to the end of target sequence.

- 1) Select a desired 20-bp sequence as a target. The following is an example sequence:

Forward sequence: 5' ATGGGAGGTGGTATGGGAGG 3'
 Reverse complement sequence: 5' CCTCCCATACCCACCTCCCAT 3'

- 2) 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'

- 3) Add 'aaaac' to the 5' end of reverse complementary sequence and 'c' to its 3' end. The final reverse complementary sequence is

5' aaaacCCTCCCATACCCACCTCCCATc 3'

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

5' gatcgXXXXXXXXXXXXXXXXXXXXXXXXXg 3'
 3' cXXXXXXXXXXXXXXXXXXXXXXXXXcaaaa 5'

- 4) Order the two final oligos from a commercial oligo provider, such as IDT.

Cloning the double stranded oligos into CRISPR vector

1. Anneal the 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 ₂ O

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

94°C for 4min

75°C for 5 min
 65°C for 15 min
 25°C for 20 min

After annealing, transfer the solution to a 1.5 mL tube and add 360 µL of dH₂O.
 The double-stranded oligo DNA is ready for ligation.

2. Ligation and transformation

1) Prepare the ligation according to the following protocol

<u>Component</u>	<u>Volume</u>
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
dH ₂ O	6.5 µL
Total Volume	10 µL

- 2) Mix the solution and incubate the tube at 22 to 37°C or room temperature for two hours according to the manufacturer's recommendation.
- 3) Add 1 µL of the ligation mixture to 10 µL of competent cells (efficiency rated > 10⁶ cfu/µg DNA) on ice. Do the transformation according to the manufacturer's protocol.
- 4) Mix the tube gently and keep it on ice for 25 minutes.
- 5) Heat shock the tube for 30 seconds at 42°C.
- 6) Put the tube on ice for 2 minutes, then add 500 µL LB or SOC medium.
- 7) Rock the tube gently at 37°C for 1 hour.
- 8) 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).
- 9) 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.
- 10) Incubate the two plates at 37°C for 16 hours to allow colony formation.

3. Screening colonies

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.

II. Cas9 Nickase Vectors

pCas-Guide-Nickase(D10A) (SKU GE100019)
pT7-Cas9-Nickase (D10A) (SKU GE100020)
pCas-Guide-Nickase (H840A) (SKU GE100062)
pCas-Guide-Nickase (D10A, H840A) (SKU GE100061)

Package contents

- One vial of circular Nickase plasmid DNA, 10 µg, lyophilized. Reconstitute in 100 µL dH₂O to make a final concentration of 100 ng/ µL.
- Certificate of Analysis
- Application Guide

Related OriGene Products

- Oligo annealing buffer, SKU GE100007
- CF3 sequencing primer, SKU GE100008
- Custom donor DNA with one of the four predesigned functional cassettes
<https://www.origene.com/products/gene-expression/crispr-cas9/genome-editing-inquiry>
 - GFP-PGK-Loxp-Puro-LoxP
 - RFP-PGK-Loxp-BSD-LoxP
 - Luciferase-PGK-Loxp-Puro-LoxP
 - mBFP-PGK-Loxp-Neo-Loxp

Product description

The wild type SpCas9 has two active nuclease domains and it can produce double-stranded DNA breaks. By mutating one of two Cas9 nuclease domains, researchers created the CRISPR nickase that can only create a single-strand break. Nickase requires two different gRNAs (one on sense strand and the other on anti-sense strand) to cause double-stranded break, which significantly lower the off-target problem of genome editing.

pCas-Guide-Nickase (D10A) (SKU GE100019), pCas-Guide-Nickase (H840A) (SKU GE100062), and pCas-Guide-Nickase (D10A, H840A) (SKU GE100061) have the same vector backbone as pCas-Guide (SKU GE100001). They are also all-in-one vectors that can be used to clone gRNA target sequence and express the mutant Cas9 protein.

Similarly, pT7-Cas9-Nickase (D10A) (SKU GE100020) vector has the same vector backbone as pT7-Cas9 (SKU GE100014). It can be used for in vitro production of Cas9 D10A mutant mRNA.

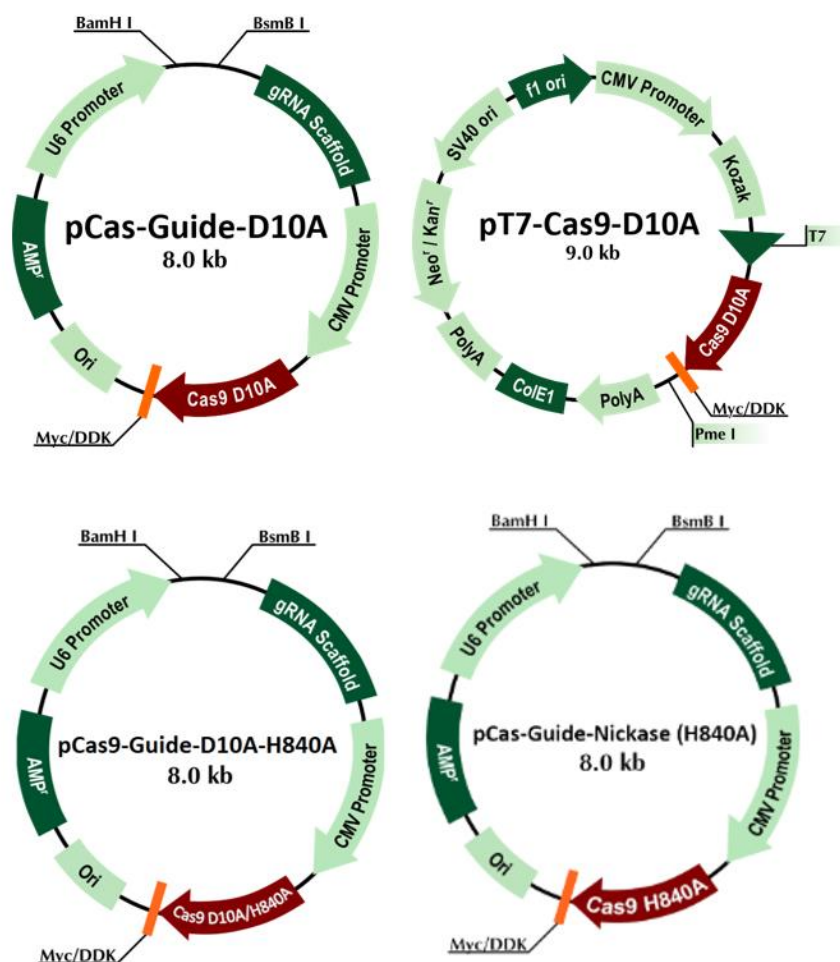
Experimental protocol

The experimental protocols for GE100019, GE100061 and GE100062 are similar to the above all-in-one Cas9 gRNA cloning vectors (page 5).

The experimental protocol for GE100020 is similar to pT7-Cas9 vector (page 15).

Since Cas9 D10A or H840A needs two different gRNAs to make double strand break, you will need to validate the cleavage efficiency of each gRNA using wild type Cas9 before using them together with Cas9 D10A or H840A vector.

Figure 2. The vector maps of the Cas9 Nickase Vectors



III. gRNA only Vectors

- pGuide (SKU GE100042)
- pGuide-EF1a-GFP (SKU GE100044)
- pLenti-Guide-Puro (SKU GE100032)

Package contents

- One vial of circular plasmid DNA, 10 µg, lyophilized. Reconstitute in 100 µL dH₂O to make a final concentration of 100 ng/ µL.
- Certificate of Analysis

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

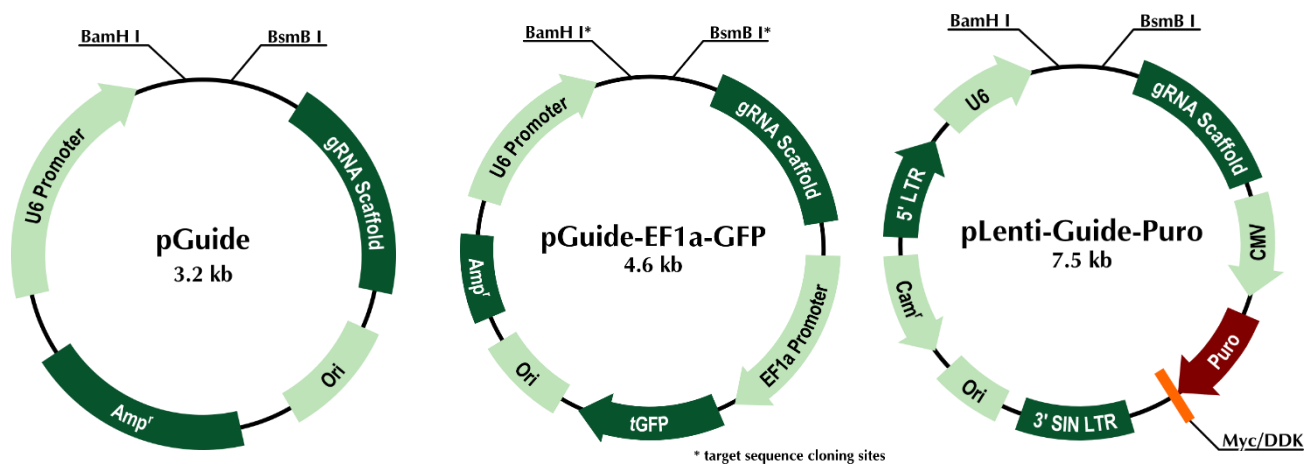
Related OriGene Products

- Oligo annealing buffer, SKU GE100007
- CF3 sequencing primer, SKU GE100008
- Cas9 only vectors,
 - pLenti-Cas9 (SKU GE100028),
 - pLenti-Cas9-IRES-Puro (SKU GE100029),
 - pLenti-EF1a-Cas9-IRES-Puro (SKU GE100030),
 - pLenti-Cas9-P2A-tGFP (SKU GE100031)
 - pAAVS1-Cas9-Puro-DNR (SKU GE100037)
 - pAAVS1-Cas9-BSD-DNR (SKU GE100039)
- Custom donor DNA with one of the four predesigned functional cassettes
 - <https://www.origene.com/products/gene-expression/crispr-cas9/genome-editing-inquiry>
 - GFP-PGK-Loxp-Puro-LoxP
 - RFP-PGK-Loxp-BSD-LoxP
 - Luciferase-PGK-Loxp-Puro-LoxP
 - mBFP-PGK-Loxp-Neo-LoxP

Product Description

gRNA only vectors can be used if your target cells already express Cas9. OriGene offer both regular mammalian vector (pGuide and pGuide-EF1a-GFP), and lentiviral vector (pLenti-Guide-Puro) for the gRNA cloning. Target sequence can be cloned into the vector via BamH I and BsmB I sites. pGuide and pGuide-EF1a-GFP vectors contain the ampicillin resistance gene for the selection of E. coli transformants. pLenti-Guide-Puro contains chloramphenicol resistance for E. coli selection.

Figure 3. The vector maps of the gRNA only vectors



Experimental protocol

The cloning protocol for the gRNA only vector is the same as the all-in-one CRISPR vector.

IV. Cas9 only vectors

- pLenti-Cas9 (SKU GE100028),
- pLenti-Cas9-IRES-Puro (SKU GE100029)
- pLenti-EF1a-Cas9-IRES-Puro (SKU GE100030)
- pLenti-Cas9-P2A-tGFP (SKU GE100031)
- pAAVS1-Cas9-Puro-DNR (SKU GE100037)
- pAAVS1-Cas9-BSD-DNR (SKU GE100039)

Package contents

- One vial of circular plasmid DNA, 10 µg, lyophilized. Reconstitute in 100 µL dH₂O to make a final concentration of 100 ng/ µL.
- Certificate of Analysis

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

Related OriGene Products

- gRNA only vectors, pGuide (GE100042), pGuide-EF1a-GFP (SKU GE100044), pLenti-Guide-Puro (SKU GE100032)

- Custom donor DNA with one of the four predesigned functional cassettes
<https://www.origene.com/products/gene-expression/crispr-cas9/genome-editing-inquiry>
 GFP-PGK-Loxp-Puro-Loxp
 RFP-PGK-Loxp-BSD-Loxp
 Luciferase-PGK-Loxp-Puro-Loxp
 mBFP-PGK-Loxp-Neo-Loxp

Product Description

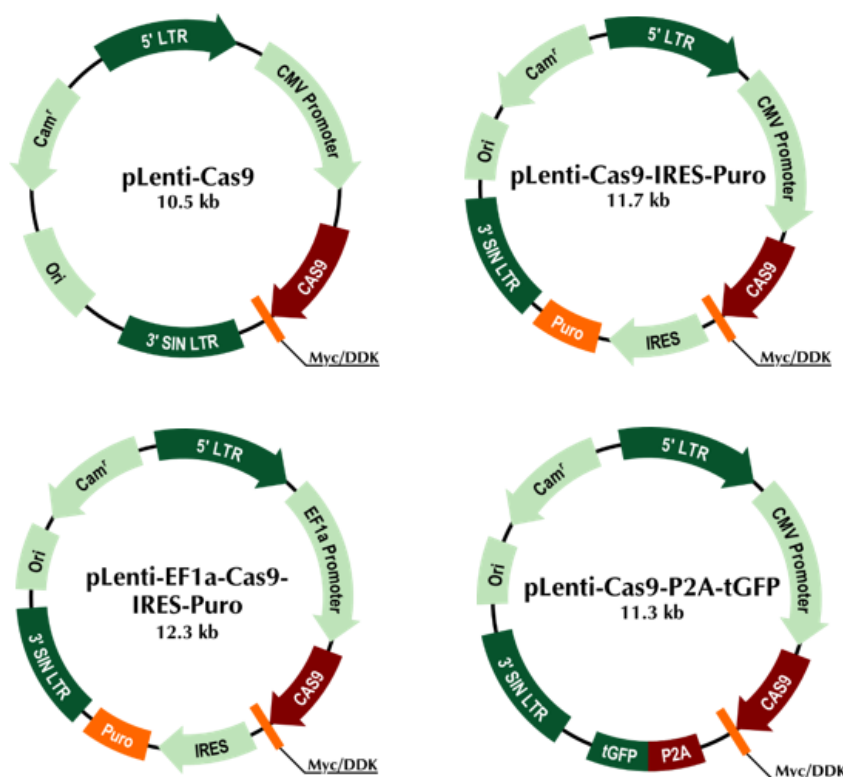
Lentiviral based Cas9 only vectors

OriGene offers a series of lentiviral based Cas9 only vectors. All these vectors can express Cas9 protein after introduction into target cells. They are 3rd generation lentiviral vectors that can be transfected or transduced into target cells to develop Cas9 stable cells. The genome of these cells can be easily edited when introduced with gene specific gRNA sequence.

Table 1. Comparison of different Lentiviral based Cas9 only vectors

	pLenti-Cas9	pLenti-Cas9-IRES-Puro	pLenti-EF1a-Cas9-IRES-Puro	pLenti-Cas9-P2A-tGFP
Cas9 Promoter	CMV	CMV	EF1a	CMV
Mammalian selection	none	puro	puro	tGFP

Figure 4. The vector maps of Lenti Cas9 vectors



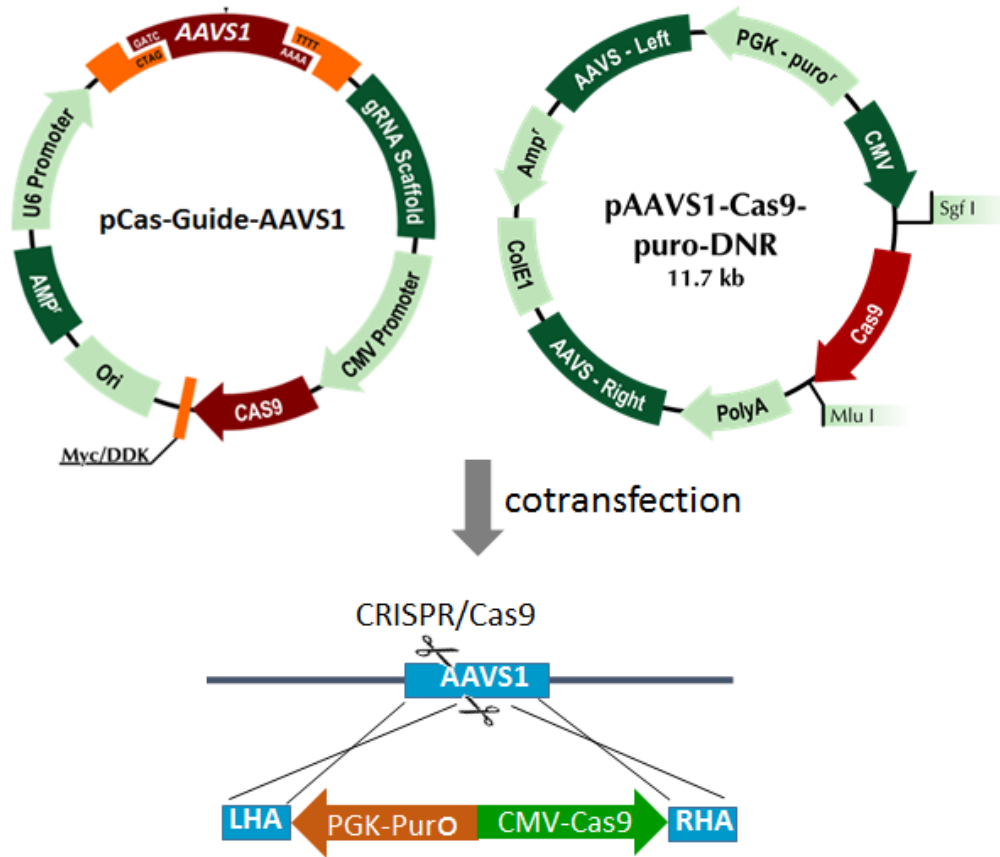
pAAVS1-Cas9-Puro-DNR and pAAVS1-Cas9-BSD-DNR vectors

These two vectors can be used as regular mammalian Cas9 expression vectors when transfected into cells. The expression of Cas9 is driven by CMV promoter.

The pAAVS1-Cas9-Puro-DNR and pAAVS1-Cas9-BSD-DNR vectors also contain AAVS1 homologous arm sequences, so both vectors can be used as donor vectors together with pCas-guide-AAVS1 (gRNA targeting AAVS1, SKU GE100023) to insert Cas9 at AAVS1 locus in human cells. AAVS1 locus has been proven to be a safe harbor location for exogenous insertion to achieve stable and robust gene expression.

More details can be found from the Diagram of how Cas9 is inserted at AAVS1 locus (figure 5) and CRISPR Transgene knockin at AAVS1 and ROSA26 loci product manual.

Figure 5. Diagram of how Cas9 is inserted at AAVS1 locus



V. T7 driven CRISPR Vectors

pT7-Guide-IVT (SKU: GE100025)

pT7-Cas9 (SKU: GE100014)

Package contents

- One vial of circular plasmid DNA, 10 µg, lyophilized. Reconstitute in 100 µL dH₂O to make a final concentration of 100 ng/ µL.
- Certificate of Analysis

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

Related OriGene Products

- Custom donor DNA with one of the four predesigned functional cassettes
<https://www.origene.com/products/gene-expression/crispr-cas9/genome-editing-inquiry>
 - GFP-PGK-Loxp-Puro-LoxP
 - RFP-PGK-Loxp-BSD-LoxP
 - Luciferase-PGK-Loxp-Puro-LoxP
 - mBFP-PGK-Loxp-Neo-LoxP

Product Description

To make gene knockout animals, Cas9 mRNA and gRNA are often injected into the embryos. The T7 driven CRISPR/Cas system will serve the purpose. Both gRNA and Cas9 mRNA can be produced *in vitro* using T7 *in vitro* transcription system and OriGene's T7 driven CRISPR Vectors.

isolate the appropriate band using a gel purification column. Elute the digested plasmid vector in 40 µL of 10 mM Tris buffer.

Note: Please do not dephosphorylate the vector as oligos are not phosphorylated.

2. Target sequence designing and cloning.
OriGene has developed a proprietary Cas9 guide RNA designing tool which is free to use, <http://www.blueheronbio.com/>. Design a target sequence of 20 bp, To facilitate cloning of the 20-bp target sequence, extra bases need to be added to the ends.

- a. Select a desired 20-bp sequence as a target. The following is an example sequence:
Forward sequence: 5' ATGGGAGGTGGTATGGGAGG 3'
Reverse complement sequence: 5' CCTCCCATACCCACCTCCCAT 3'

Add 'atagG' to the 5' end of the forward sequence and 'G' to its 3' end.
The final sense oligo in this example will be
5' atagG ATGGGAGGTGGTATGGGAGGg 3'

- b. Add 'aaaac' to the 5' end of reverse complementary sequence and 'C' to its 3' end.
The final reverse example complementary oligo:
5' aaaacCCTCCCATACCCACCTCCCATc 3'

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

5' atagGxxxxxxxxxxxxxxxxxxxxxxxxG 3'
3' CxxxxxxxxxxxxxxxxxxxxxxxxCaaaa 5'

- c. Order the two final oligos from a commercial oligo provider, such as IDT. The oligos are ready to be cloned into the BsmB I digested pT7-Guide-IVT vector. Follow the oligo cloning procedure on page 5 of this manual.

3. Sequencing the cloned target sequence in pT7-Guide-IVT can be done by the common M13 forward primer: 5' CGCCAGGGTTTTCCAGTCACGAC 3'

Producing gRNA and Cas9 mRNA using T7 in vitro transcription kits

To make gRNA using pT7-Guide-IVT vector, we recommend using MeGashortscript T7 kit (Life Technologies) and follow the manufacturer's protocol. pT7-Guide-IVT vector can be linearized using EcoR I, which cuts out T7 and gRNA fragment. You don't need to purify the fragment. You only need to clean it using PCR purification column, then follow the MeGashortscript T7 kit protocol to produce gRNA.

To make Cas9 mRNA using pT7-Cas9, we recommend using mMMESSAGE Mmachine T7 ULTRA kit (Life Technologies) and follow the manufacturer's protocol. pT7-Cas9 vector can be linearized using Pme I which is at the 3' end of Cas9 sequence. You can then clean up the Pme I digested reaction using a PCR purification column, and follow by the mMMESSAGE Mmachine T7 ULTRA kit protocol to produce capped and polyadenylated Cas9 mRNA.

VI. CRISPR scramble controls

pCas-Scramble (SKU GE100003)

pCas-Scramble-EF1A-GFP (SKU GE100021)

Package contents

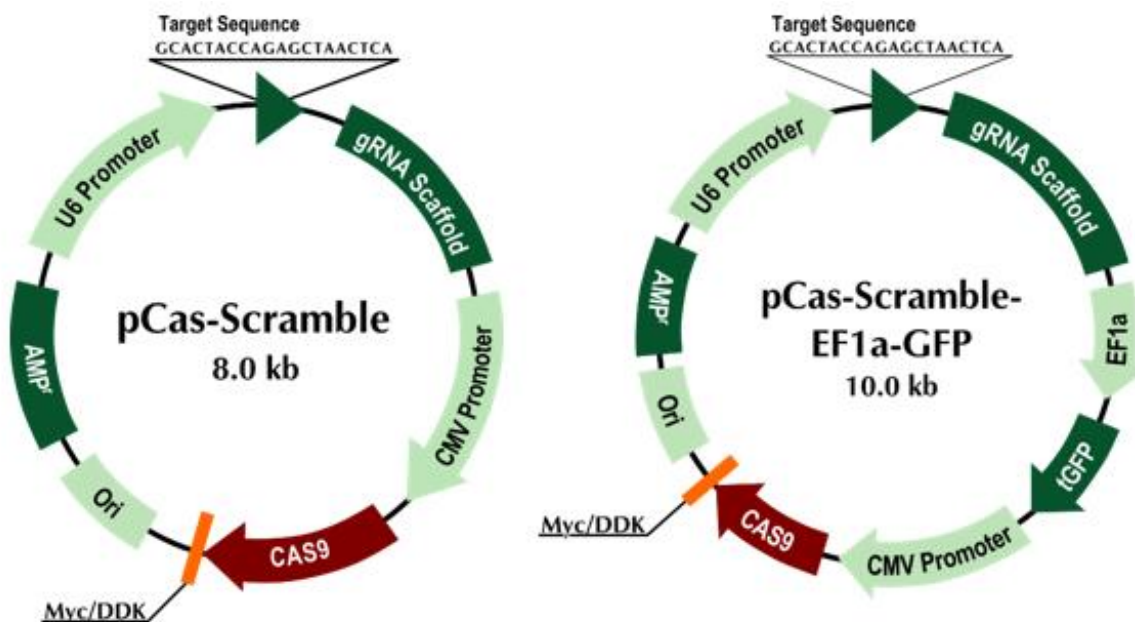
- One vial of circular plasmid DNA, 10 µg, lyophilized. Reconstitute in 100 µL dH2O to make a final concentration of 100 ng/ µL.
- Certificate of Analysis
- Application Guide available on line

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

Product description

Using CRISPR technology for genome editing, you need a negative scrambled control, a 20bp scrambled sequence cloned in CRISPR vectors. After transfecting the CRISPR scrambled control into cells, a guide RNA containing the scrambled sequence (which does not target any sequence) will be produced.

Figure 7. The vector maps of pCas-Scramble and pCas-Scramble-EF1a-GFP



VII. Cre expression vector for Cre-Lox recombination

pCMV6-Entry Cre (SKU: GE100017)

Package contents

- One vial of circular plasmid DNA, 10 µg, lyophilized. Reconstitute in 100 µL dH₂O to make a final concentration of 100 ng/ µL.
- Certificate of Analysis
- Application Guide available on line

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

Related OriGene Products

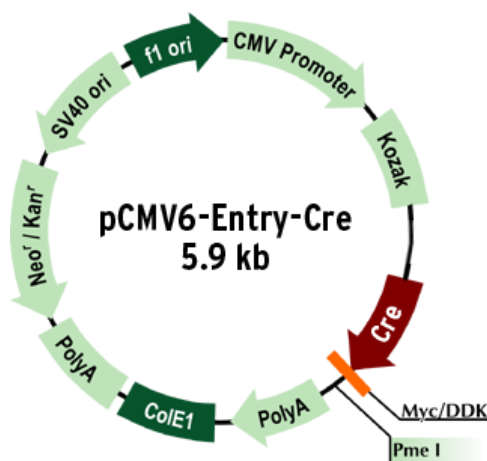
- Genome wide gene knockout/knockin kit via CRISPR, <https://www.origene.com/products/gene-expression/crispr-cas9/knockout-kits>
- Cloning the homologous arms in predesigned donor rescue construct harboring functional cassettes (service from <https://www.origene.com/products/gene-expression/crispr-cas9/grna-cloning-donor-construction>).

GFP-PGK-Loxp-Puro-LoxP
 RFP-PGK-Loxp-BSD-LoxP
 Luciferase-PGK-Loxp-Puro-LoxP
 BFP-PGK-Loxp-Neo-Loxp

Product Description

In our predesigned donor vector cassette, the PGK-puro cassette is flanked by two LoxP sites, which can be floxed out by Cre recombinase. pCMV6-Entry-Cre is a Cre recombinase mammalian expression vector in which Cre is under CMV promoter. Expressed Cre contains a C-terminal Myc-DDK tag (DDK is the same as Flag tag).

Figure 8. The vector map of pCMV6-Entry-Cre



Q9: Is there a method for isolating single cell colonies from the engineered pool of cells?

You can isolate single cell colonies through series of dilution or using cloning cylinders.

Q10: Do you need to linearize a donor template before transfection for efficient repair?

The donor template DNA is not preferred to be linearized as this will increase random integration.

Q11: How to screen the edited cells after transfecting the CRISPR/Cas9 vector?

For mutations, you can do genomic PCR and sequence it. If you do gene knockout, the selection marker in the donor template DNA will help the selection. If no donor DNA for gene knockout out, then genomic PCR and sequencing to confirm indels. If necessary, you can isolate individual cell colonies for introduction of specific mutations and other genome editing applications. You can do WB for gene knockout after isolating single cell colonies.

Q12: Does CRISPR/Cas9 system work for non-dividing cells?

NHEJ repair works in non-dividing cells; HDR is not active in non-dividing cells. OriGene's new KN2.0 CRISPR knockout kit is designed based on NHEJ-donor repairing mechanism. It works in both dividing and non-dividing cells. It also provides a selection cassette to help downstream screening of knockout cells.

Q13: Do you have the cas9 antibody?

Yes, We do have Cas9 antibody (cat# [TA190309](#)). In our CRISPR/Cas9 vectors, Cas9 has a C-terminal Myc-DDK tag, which can also be recognized by OriGene's anti-DDK antibody (SKU [TA50011-100](#)).

Q14: If I want to use CRISPR/Cas9 to knock down a certain gene, what kind of negative control should I use?

You can use a scramble control, pCas-Scramble, SKU GE100003, or pCas-Scramble-EF1a-GFP, SKU GE100021.

Q15: For gene targeting in mice, do you recommend transfecting ES cells or pronuclei?

You can do both. You can inject mRNA (gRNA and Cas9 mRNA) or plasmid DNA (target sequence cloned pCas-Guide) into the zygotes or ES cells.

Q16: What is the limit for multiple gene disruption?

You can do multiplexes using CRISPR/Cas9 system. You can co-transfect the gRNA vectors or co-inject several guide RNAs into your cells; thus, you will achieve multiple gene disruption or genome editing. The limit could be transfection efficiency.

Q17: How do you make sure that Cas9 will not integrate in genome if you use lentivector?

For screening purpose, for short term, integration of Cas9 into the genome for 2 weeks does not affect cells. You can also use the [integration-deficient lenti packaging kit \(cat# TR30036\)](#) to produce lentivirus that won't integrate into the cellular genome, acting just like plasmid.

Q18: What is the known CRISPR/Cas9 editing efficiency relative to other genome editing approaches?

In general, the genome editing efficiency of CRISPR/Cas9 is similar or higher than TALEN. However, CRISPR/Cas9 is much more simple and easy to do. You will need to engineer the protein to recognize new DNA sequence in TALEN system, while CRISPR/Cas9 is RNA based.

Q19: What is the sequence of CF3 sequencing primer?

5'-ACGATACAAGGCTGTTAGAGAG-3'

Q20: What is the scrambled sequence in pCas-Scramble and pCas-Scramble-EF1a-GFP?

5' GCACTACCAGAGCTAACTCA 3'

Q21: Do you provide gRNA cloning service and donor vector service?

Yes, you can order [gRNA cloning service and donor vector service](#) through the following website.

<https://www.origene.com/products/gene-expression/crispr-cas9/grna-cloning-donor-construction>

Q22: What is unique about the 3rd generation Lentiviral vectors?

The 3rd generation lentiviral vectors are safer than the 2nd generation vectors. The 3rd generation packaging systems express gag and pol from one packaging vector and rev from another vector. The 3rd generation packaging systems DO NOT express tat (Trans-Activator of Transcription).

Q23: Can I use a second generation packaging system with the pLenti vectors?

Yes, a second generation packaging system should work with OriGene's third generation pLenti vectors although we have not explicitly tested this. You can use OriGene's high efficient third generation lenti-packaging kit (cat# [TR30037](#)) for pLenti-vectors.

Q24: How can I sequence the target sequence cloned in pT7-Guide vector?

You can use the M13 forward primer to sequence the target sequence cloned in pT7-Guide vector.

M13F: 5' CGCCAGGGTTTTCCAGTCACGAC 3'