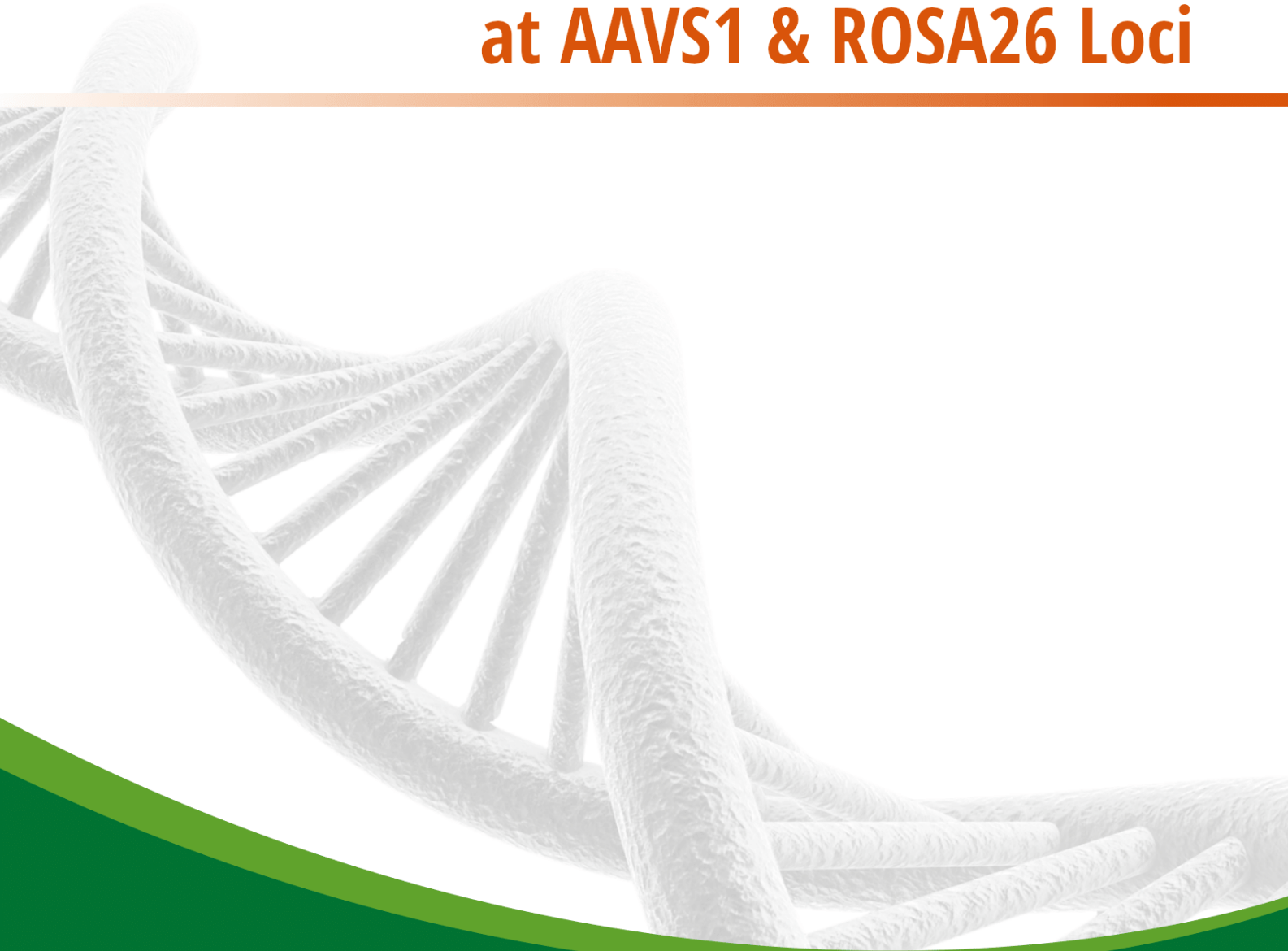


CRISPR Transgene Knockin at AAVS1 & ROSA26 Loci



CRISPR Transgene Knockin at AAVS1 and ROSA26 loci

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Introduction

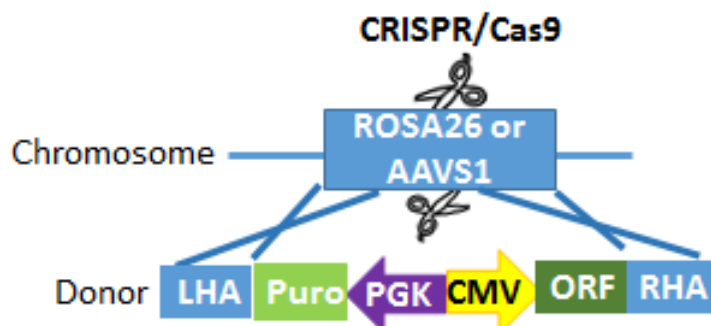
Many research projects require inserting functional transgenes and other genetic elements into a host cell genome. In such studies, the integration site of the transgene is very critical. When the transgene is integrated into random position of host cell genome, the expression of the transgene can be silenced or unpredictable depending on the integration site. Random integration can also cause insertional mutagenesis, which can possibly alter expression levels of neighbor genes. Genomic safe harbor sites are transcriptionally active genomic region, therefore allowing robust and stable gene expression. They are also safe insertion site. A transgene inserted at genomic safe harbor site does not have adverse effect on the host cell genome. The adeno-associated virus site 1 (AAVS1) locus on human chromosome 19, and mouse ROSA26 locus, are the two safe harbor sites that have been widely used for transgene insertion.

Taking advantage of recently discovered CRISPR technology, we have developed AAVS1 and ROSA26 safe harbor knockin systems for transgene integration. The AAVS1 or ROSA26 targeting sequence is cloned in the CRISPR all-in-one pCas-Guide vector, which can generate double stranded genomic break at AAVS1 or ROSA26 locus. The donor vector contains the AAVS1 or ROSA26 homologous arms flanking the gene of interest which will be integrated at AAVS1 or ROSA26 site via homologous recombination.

Notice to purchaser

The products included in this manual are 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.

Figure 1. Transgene insertion at AAVS1 or ROSA26 loci via CRISPR

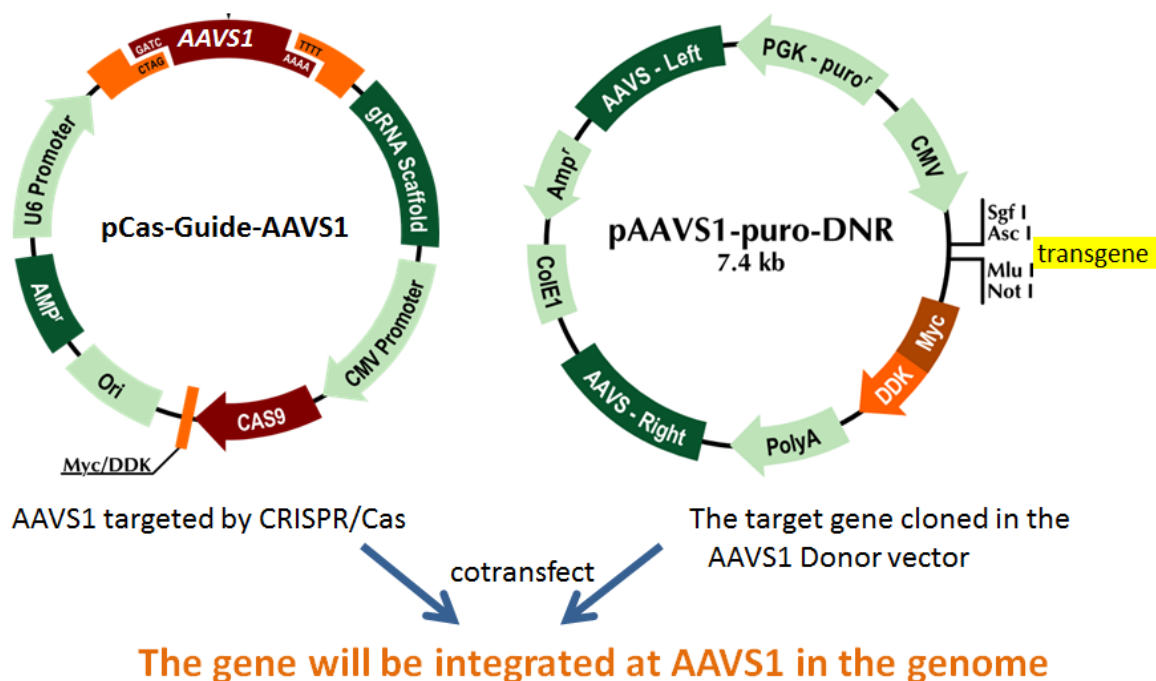


AAVS1 Transgene knockin via CRISPR

The AAVS1 site on human chromosome 19 is the most popular genomic safe harbor site for human cell. It has been shown to support transgene expression in many human cell types. However, AAVS1 locus could also be silenced by DNA methylations.

OriGene has developed a series of AAVS1 CRISPR products to help researchers insert their gene of interest in the AAVS1 genomic locus through homologous recombination (Figure 2).

Figure 2. Transgene insertion at AAVS1 using OriGene’s pre-designed gRNA and donor plasmid



1. AAVS1 gRNA vector, pCas-Guide-AAVS1 (SKU GE100023)

The pCas-Guide-AAVS1 vector (SKU GE100023) is a pre-designed and pre-validated gRNA vector, which can be transfected into human host cells and generate a double strand break at AAVS1 locus.

Package contents

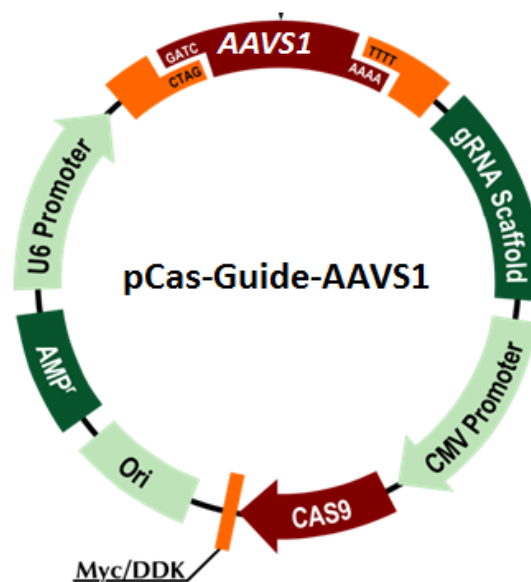
- One vial of 10 µg lyophilized pCas-Guide-AAVS1 (SKU: GE100023) plasmid DNA.
- Certificate of Analysis

* 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

- pCas-Guide-scramble (SKU GE100003)
- AAVS1 donor vectors (SKU GE100024, GE100035, GE100046, GE100048)
- Predesigned AAVS1 donor controls with different combinations of transgene and drug resistant marker (SKU GE100037, GE100039, GE100026, GE100063, GE100064, GE100065, GE100066, GE100068, GE100069, GE100070, GE100071, GE100072, GE100073)
- AAVS1 Transgene knockin vector kit (puro) (SKU GE100027)
- AAVS1 transgene knockin vector kit (BSD) (SKU GE100036)
- AAVS1 Transgene knockin vector kit (EF1a-puro) (SKU GE100046)
- AAVS1 transgene knockin vector kit (EF1a-BSD) (SKU GE100048)
- AAVS1 Cas9 insertion vector kits, Puro (SKU GE100038) and BSD (SKU GE100040)

Figure 3. The vector map of pCas-Guide-AAVS1



2. AAVS1 CRISPR Donor Plasmids

2.1 AAVS1 donor vectors

OriGene has developed a series of donor vectors around human AAVS1 locus (Table 1). Every vector contains AAVS1 left and right homologous arm to integrate the transgene cassette into AAVS1 locus through homologous recombination. Flanked by the two arms, there is a CMV or EF1a promoter driven expression cassette for a transgene expression and a PGK driven puromycin or blasticidin resistant gene for mammalian selection (Figure 4). A multiple cloning site downstream of the CMV/EF1a promoter is designed to be compatible with OriGene's precision shuttle vector system, so that the ORF clones can be easily shuttled to the AAVS1 donor vector by a simple "cut and ligate" process (Figure 5).

** The AAVS1 CRISPR donor vectors don't contain your gene of interest (GOI). The gene of interest needs to be cloned in order to insert your GOI into AAVS1 locus.*

Table 1 Comparison of different AAVS1 donor vectors

SKU	Vector Name	Promoter	Cell Selection
GE100024	pAAVS1-Puro-DNR	CMV	Puromycin
GE100035	pAAVS1-BSD-DNR	CMV	Blasticidin
GE100046	pAAVS1-EF1a-Puro-DNR	EF1a	Puromycin
GE100048	pAAVS1-EF1a-BSD-DNR	EF1a	Blasticidin

Package contents

- One vial of 10 µg lyophilized donor vector plasmid. Reconstitute in 100 µL dH₂O to make a final concentration of 100 ng/ µL.
- One vial of dried 5' primer (100 picomoles). Reconstitute in 10 µL dH₂O to make a 10 µM solution. VP1.5 for GE100024 and GE100035. EF51 for GE100046 and GE100048.
- One vial of dried 3' (XL39) primer (100 picomoles). Reconstitute in 10 µL dH₂O to make a 10 µM solution.
- Certificate of Analysis

** 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

- pCas-Guide-AAVS1 (SKU GE100023)
- pCas-Guide-scramble (SKU GE100003)
- AAVS1 Transgene knockin vector kit (puro) (SKU GE100027)
- AAVS1 transgene knockin vector kit (BSD) (SKU GE100036)
- AAVS1 Transgene knockin vector kit (EF1a-puro) (SKU GE100046)
- AAVS1 transgene knockin vector kit (EF1a-BSD) (SKU GE100048)
- AAVS1 Cas9 insertion vector kits, Puro (SKU GE100038) and BSD (SKU GE100040)

Figure 4. The vector maps of AAVS1 donor vectors

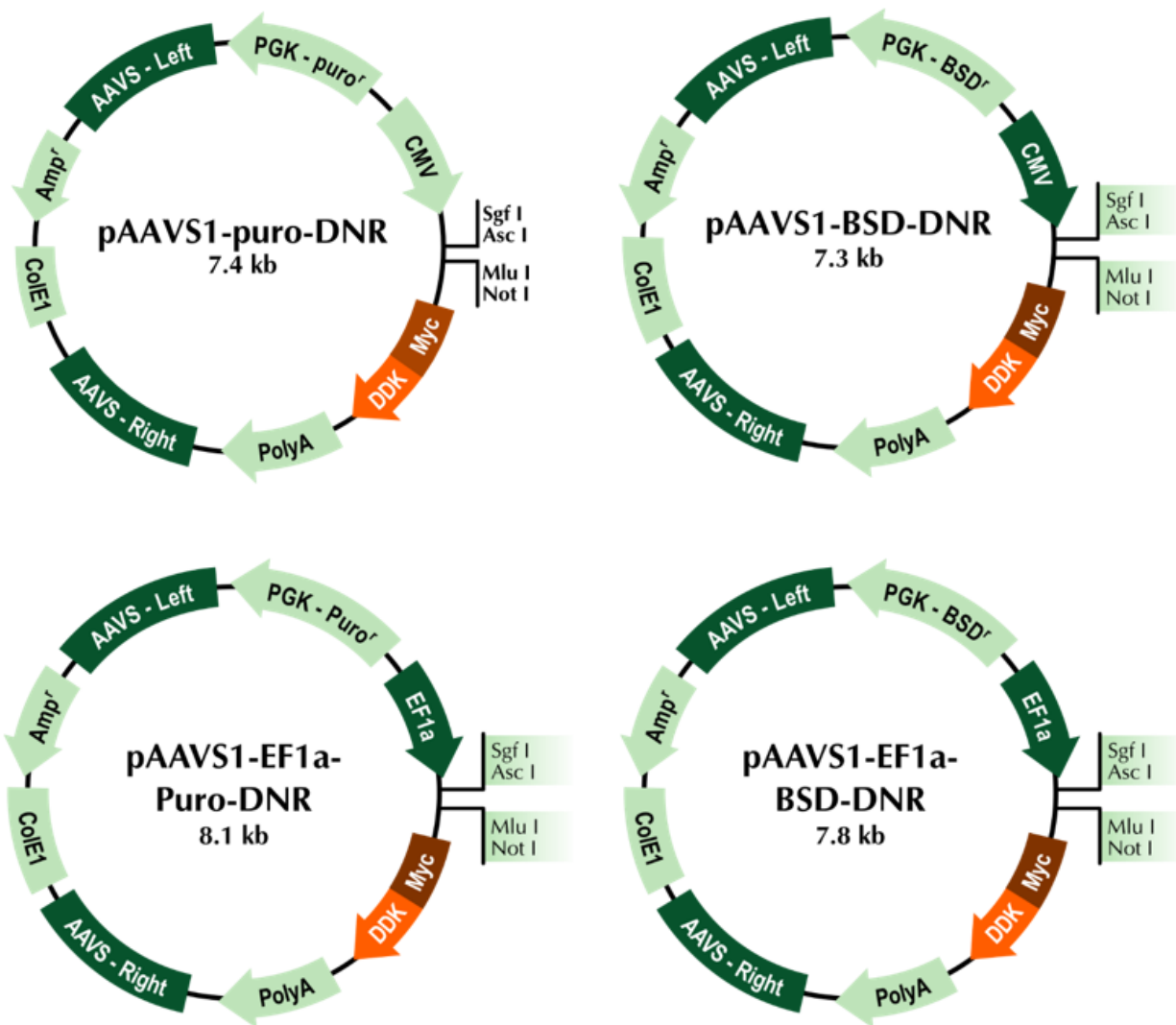
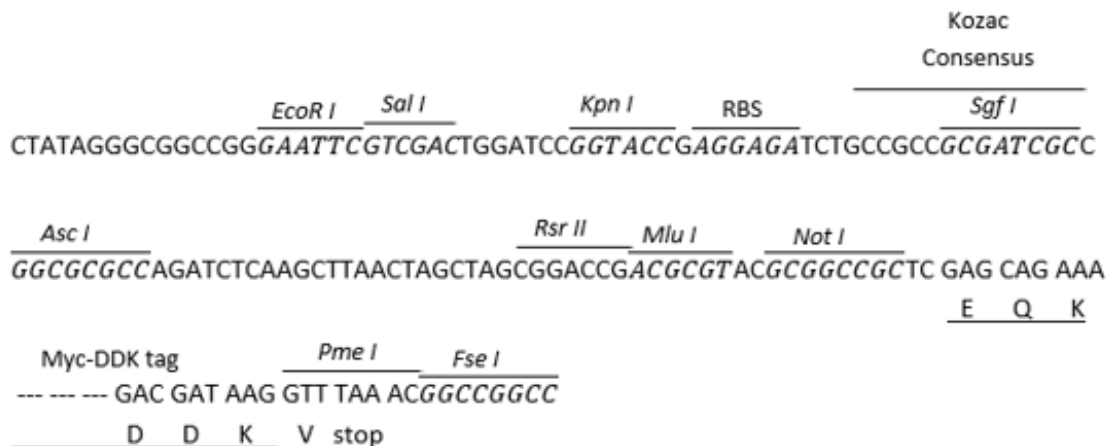
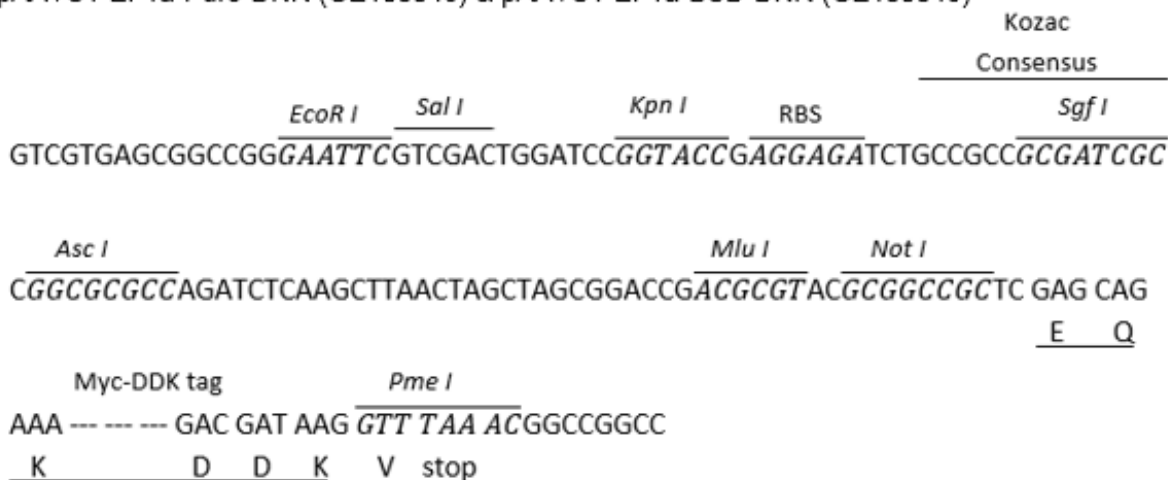


Figure 5 The Multiple cloning sites (MCS) of AAVS1 donor vectors

pAAVS1-Puro-DNR (GE100024) & pAAVS1-BSD-DNR (GE100035)



pAAVS1-EF1a-Puro-DNR (GE100046) & pAAVS1-EF1a-BSD-DNR (GE100048)



2.2 Predesigned AAVS1 donor controls

In addition to the simple AAVS1 donor vectors, OriGene also offers many pre-designed and pre-validated AAVS1 donor plasmids, containing different combinations of drug resistance marker and reporter gene (Table 2). These donor plasmids can be used as positive controls to label the cells with different reporter genes. They can also be used to clone your gene of interest (GOI) either with fluorescent protein (FP) tag or without the FP tag (replacing the original FP reporter).

Table 2 Comparison of predesigned AAVS1 donor controls

SKU	Vector Name	Promoter	Transgene /Selection Marker
GE100037	pAAVS1-Cas9-Puro-DNR	CMV	Cas9/Puromycin
GE100039	pAAVS1-Cas9-BSD-DNR	CMV	Cas9/Blasticidin
GE100026	pAAVS1-RFP-DNR	CMV	tRFP/Puromycin
GE100063	pAAVS1-mGFP-Puro-DNR	CMV	mGFP/Puromycin
GE100064	pAAVS1-mRFP-Puro-DNR	CMV	mRFP/Puromycin
GE100065	pAAVS1-mGFP-BSD-DNR	CMV	mGFP/Blasticidin
GE100066	pAAVS1-mRFP-BSD-DNR	CMV	mRFP/Blasticidin
GE100068	pAAVS1-EF1a-tGFP-puro-DNR	EF1a	tGFP/Puromycin
GE100069	pAAVS1-EF1a-tGFP-BSD-DNR	EF1a	tGFP/Blasticidin
GE100070	pAAVS1-EF1a-tRFP-puro-DNR	EF1a	tRFP/Puromycin
GE100071	pAAVS1-EF1a-tRFP-BSD-DNR	EF1a	tRFP/Blasticidin
GE100072	pAAVS1-EF1a-Luc-puro-DNR	EF1a	Luciferase/Puromycin
GE100073	pAAVS1-EF1a-Luc-BSD-DNR	EF1a	Luciferase/ Blasticidin

Package contents

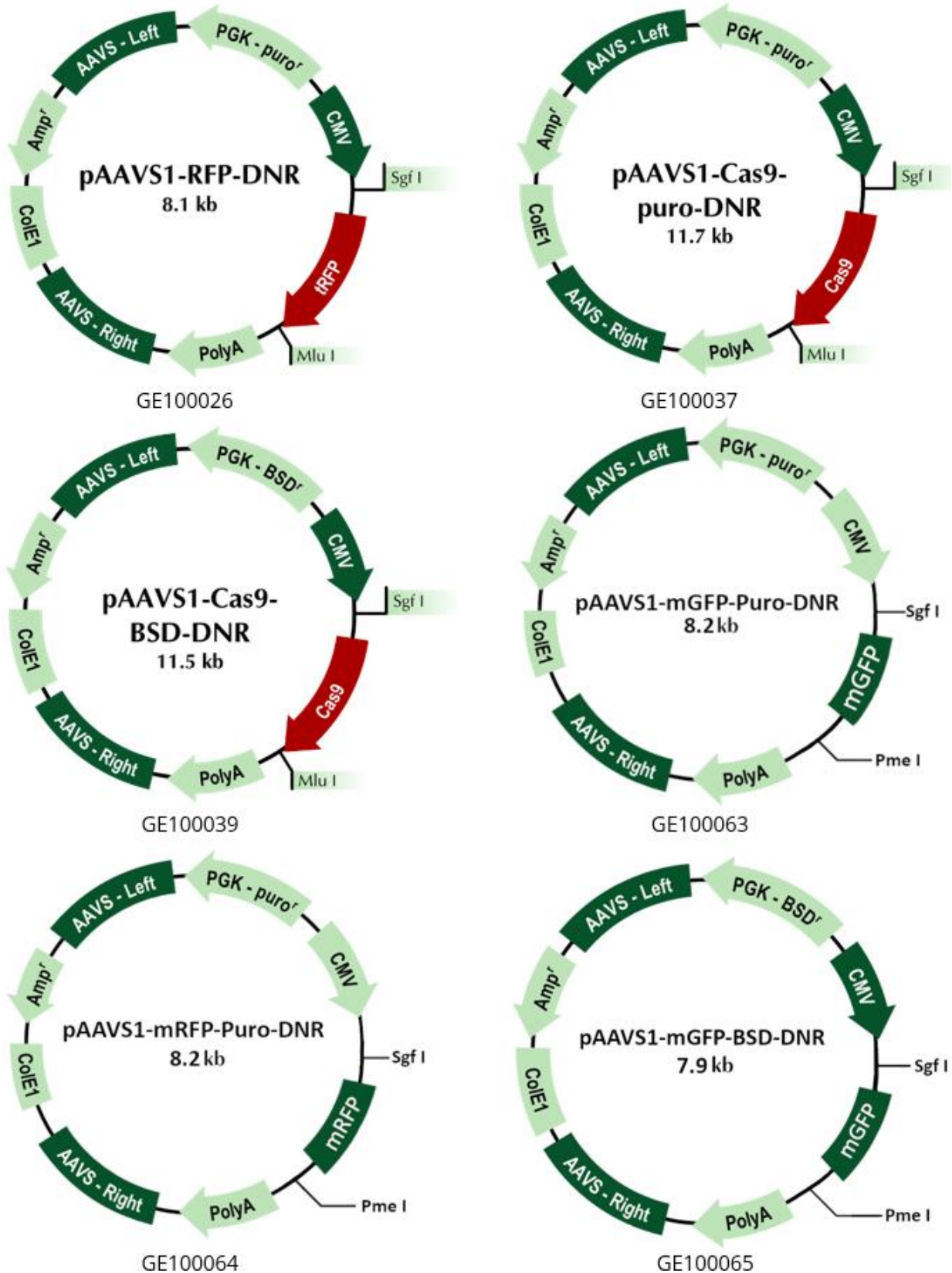
- One vial of 10 µg lyophilized AAVS1 donor control. Reconstitute in 100 µL dH₂O to make a final concentration of 100 ng/µL.
- Certificate of Analysis

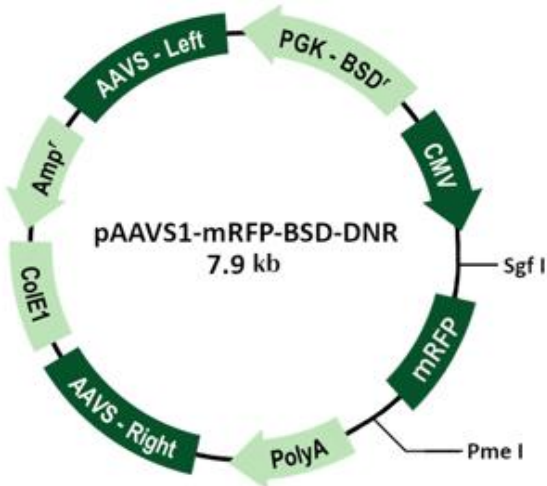
**The DNA is shipped at room temperature, but should be kept at -20°C for long-term storage. If properly stored, DNA is guaranteed to be stable for 12 months.*

Related OriGene Products

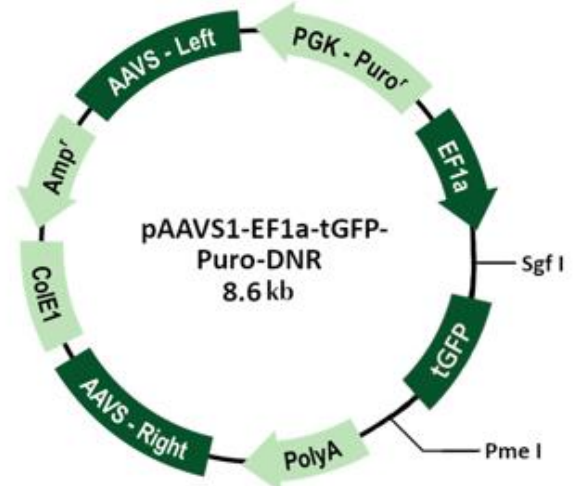
- pCas-Guide-AAVS1 (SKU GE100023)
- pCas-Guide-scramble (SKU GE100003)
- AAVS1 Transgene knockin vector kit (puro) (SKU GE100027)
- AAVS1 transgene knockin vector kit (BSD) (SKU GE100036)
- AAVS1 Transgene knockin vector kit (EF1a-puro) (SKU GE100046)
- AAVS1 transgene knockin vector kit (EF1a-BSD) (SKU GE100048)
- AAVS1 Cas9 insertion vector kits, Puro (SKU GE100038) and BSD (SKU GE100040)

Figure 6. The vector maps of predesigned AAVS1 donor controls

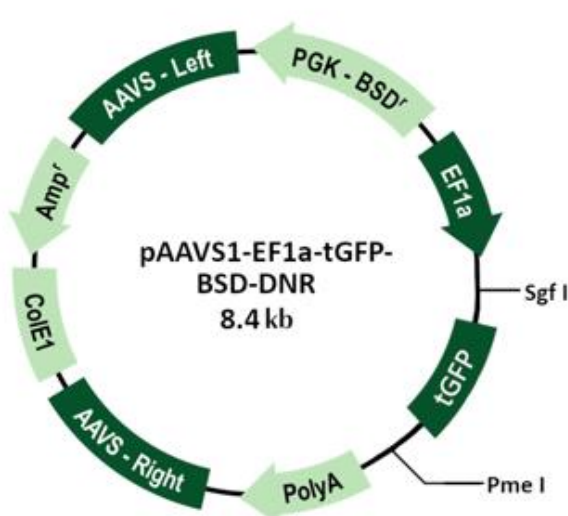




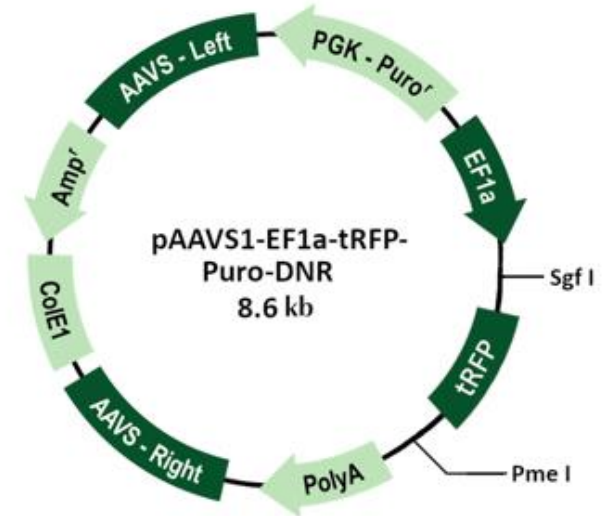
GE100066



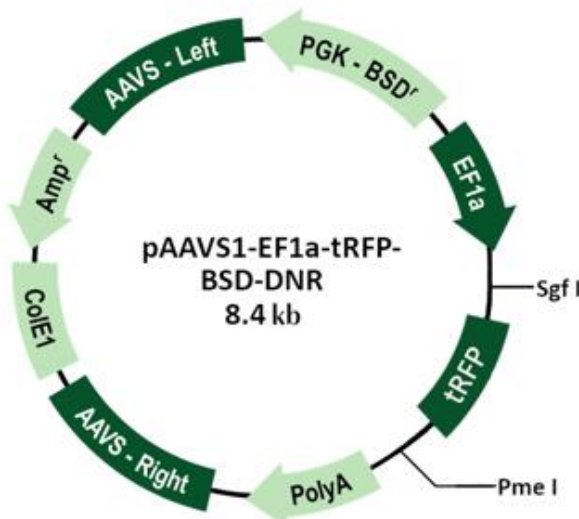
GE100068



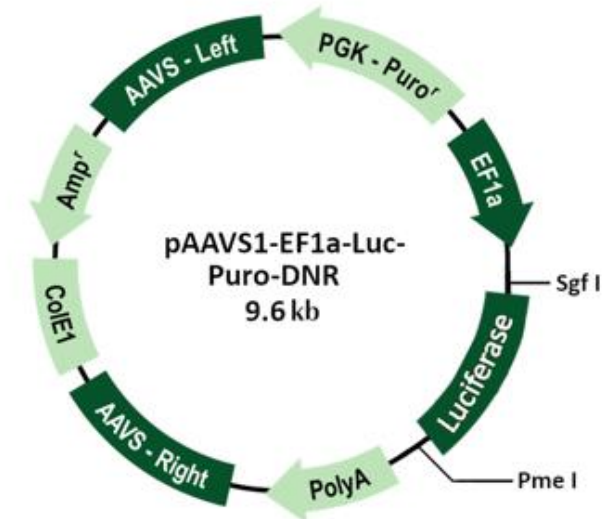
GE100069



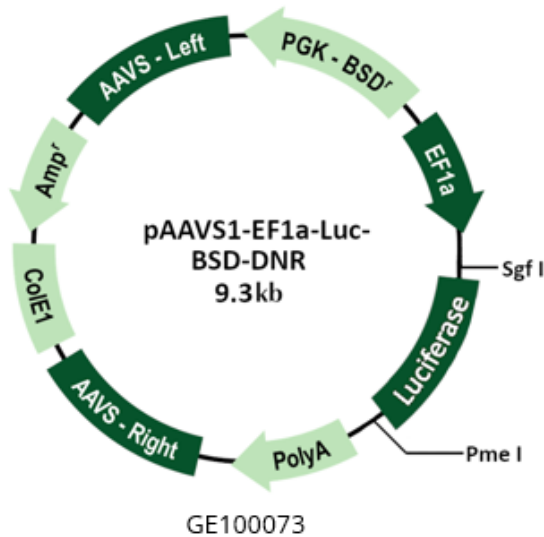
GE100070



GE100071



GE100072



3. AAVS1 Transgene knockin vector kit, puro (SKU GE100027, GE100047) or BSD (GE100036, GE100049)

The AAVS1 transgene knockin vector kits are complete vector kits to knock in your gene of interest in AAVS1 locus in human cells for robust and stable expression.

The kit contains the following three plasmids:

1. pCas-Guide-AAVS1 (SKU GE100023): This two-in-one vector will generate a double strand breakage in the human host cells at AAVS1 locus.
2. pCas-Guide-Scrambled control (SKU GE100003): Served as the negative gRNA control containing a non-specific gRNA sequence in pCas-Guide vector.
3. AAVS1 donor vector: An AAVS1 donor vector contains multiple cloning sites downstream of the CMV or EF1a promoter to clone your gene of interest. The plasmid also has AAVS1 left and right homologous arms to insert the transgene cassette into AAVS1 locus through homologous recombination. The multiple cloning sites are compatible to OriGene's ORF clones, which can be easily shuttled to this integration vector by a simple "cut and ligate" process.

**AAVS1 donor vectors don't contain your gene of interest. It needs to be cloned.*

Package Contents

1. One vial of 10 µg lyophilized pCas-Guide-AAVS1 (SKU GE100023) DNA.
2. One vial of 10 µg lyophilized pCas-Guide-scramble (SKU GE100003) negative control.
3. One vial of 10 µg lyophilized AAVS1 donor vector (SKU GE100024, or GE100035, or GE100046, or GE100048).
4. One vial of lyophilized forward sequencing primer (100pmols). VP1.5 forward primer for GE100027 and GE100036; EF51 forward primer for GE100047 and GE100049. Reconstitute in 10 µL dH₂O to make a 10 µM solution.
5. One vial of lyophilized reverse (XL39) sequencing primer (100pmols). Reconstitute in 10 µL dH₂O to make a 10 µM solution.
6. Certificate of Analysis

** Sequencing Primers are for donor vectors only, not for pCas-Guide-AAVS1 or pCas-Guide-scramble.*

**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. Reconstitute in 100 µL dH₂O to make a final concentration of 100 ng/ µL.*

Related OriGene Products

- [Genome-wide OriGene ORF clones](#)
- [Transfection reagent](#)
- [CRISPR/Cas9 products](#)

4. AAVS1 Cas9 insertion vector kits, Puro (SKU GE100038) and BSD (SKU GE100040)

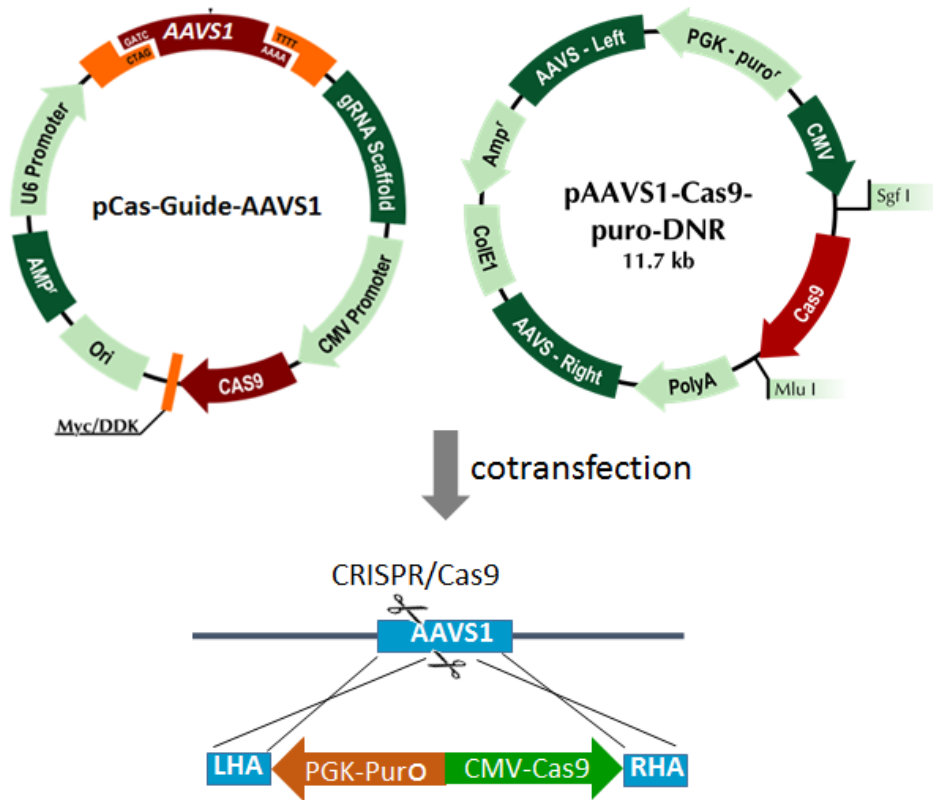
Package Contents:

1. One vial of 10 µg lyophilized pCas-Guide-AAVS1 (SKU GE100023).
2. One vial of 10 µg lyophilized pCas-Guide-scramble (cat# GE100003) negative control.
3. One vial of 10 µg lyophilized pAAVS1-Cas9-Puro-DNR (SKU GE100037) or pAAVS1-Cas9-BSD-DNR (SKU GE100039).
4. Certificate of Analysis

**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. Reconstitute in 100 µL dH₂O to make a final concentration of 100 ng/ µL.*

Using AAVS1 gRNA (GE100023) and Cas9 donor control (GE100037 or GE100039), you can create a stable cell line for Cas9 protein in which Cas9 expression cassette is inserted in AAVS1 locus. This stable cell line provides a powerful tool for CRISPR based genome editing (Figure 7).

Figure 7. Diagram of how Cas9 is inserted at AAVS1 locus in human cells



5. Experimental Protocol

5.1 Cloning a transgene into AAVS1 donor vector

To clone an ORF insert into the AAVS1 donor vectors, the first step is to select appropriate restriction enzymes. There are a few rare restriction enzymatic sites in the multiple cloning site (MCS) regions of AAVS1 donor vectors. Normally we suggest the following four pairs of enzymatic sites for cloning: SgfI/MluI, Asc/MluI, SgfI/NotI and Asc/NotI. Among them, SgfI/MluI is suitable for over 95% ORF insert.

OriGene's TrueORF clones have compatible sites with the AAVS1 donor vectors. The ORF sequence from OriGene clones can be easily shuttled into the AAVS1 donor vectors via a simple "cut-and-paste" procedure. The following protocol is for shuttling an ORF insert to the donor vectors using Sgf1/MluI enzymatic sites.

1. Digest an ORF insert from TrueORF clone in pCMV6-Entry vector

<u>Component</u>	<u>Volume</u>
10X restriction buffer	2 μ l
Sgf I (10 U/ μ l)	0.6 μ l
Mlu I (10 U/ μ l)	0.6 μ l
nuclease-free water	13.8 μ l
<u>TrueORF clone (500 ng)</u>	<u>3 μl</u>
Total volume	20 μ l

Incubate at 37°C for 3 hrs.

2. Digest AAVS1 donor vectors:

<u>Component</u>	<u>Volume</u>
10X restriction buffer	2 μ l
Sgf I (10 U/ μ l)	0.6 μ l
Mlu I (10 U/ μ l)	0.6 μ l
nuclease-free water	14.8 μ l
<u>AAVS1 or ROSA26 donor vector (200ng)</u>	<u>2 μl</u>
Total volume	20 μ l

* Around 4% of human genes have internal Sgf I or Mlu I sites. For these genes, please use the appropriate combination of restriction sites as recommended by OriGene.

Incubate at 37°C for 3 hrs. Add 0.5 μ l antarctic phosphatase (units used according to the manufacturer's protocol) to the digestion, and continue to incubate at 37°C for an additional 30 minutes.

3. Purify the digestion using a commercial PCR purification column and elute in 20 μ l 10 mM Tris buffer.

4. Set up a ligation reaction:

Component	Volume
10 x T4 DNA ligation buffer	1 μ l
T4 DNA Ligase (4U/ μ l)	0.75 μ l
Nuclease-free water	3.25 μ l
ORF insert (step 1)	2 μ l
digested vector (Step 2)	3 μ l
Total volume	10 μ l

Incubate the ligation reaction at room temperature for 1 hour.

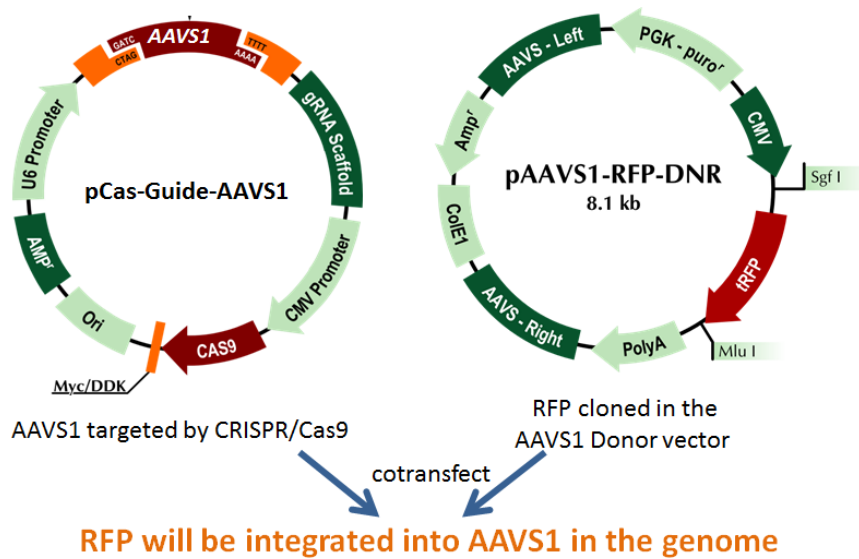
5. Transform the ligation reaction using high-efficiency competent *E. coli* cells ($\geq 1 \times 10^8$ CFU/ μ g DNA) following the appropriate transformation protocol. Plate the transformants on LB-agar plates containing 100 μ g/ml ampicillin.
6. Pick at least four colonies for subsequent DNA purification and screening. Amplify and purify the selected clone(s) by growing overnight in liquid LB containing 100 μ g/ml ampicillin, then isolate the DNA using standard plasmid purification procedures.
7. Confirm the insert by restriction digestion and/or vector primer sequencing using the provided V1.5 for 5' end sequencing and XL39 for 3' end.

5.2 Knockin the transgene into AAVS1 locus

The pAAVS1-RFP-DNR (SKU GE100026) donor plasmid is a predesigned donor DNA containing tRFP expression cassette. It is used here as an example for AAVS1 CRISPR knockin.

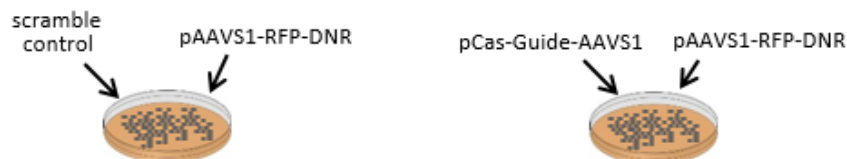
The pCas-Guide-AAVS1 vector generates double stranded break at AAVS1 site, and pAAVS1-RFP-DNR provides repair template via homologous recombination. Co-transfect both pAAVS1-RFP-DNR and pCas-Guide-AAVS1 can lead to the insertion of RFP-puro expression cassette in AAVS1 locus (Figure 8).

Figure 8. Knockin RFP at AAVS1 site using OriGene AAVS1 knockin kit



*The following protocol is for experiments performed in 6-well plates using [TurboFectin](#) (cat# TF81001) as transfection reagent. Please scale up or down the reagents accordingly based on the relative surface area of your plate (Table 3).

1. Approximately 18-24 hours before transfection, plate $\sim 3 \times 10^5$ adherent cells in 2 ml culture media into each well of a 6-well plate or $\sim 5 \times 10^5$ 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. Set up two transfections 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.

- a. Dilute 1 μg of pCas-Guide-AAVS1 (or scramble control) in 250 μL of Opti-MEM I (Life Technologies), vortex gently. Then add 1 μg of the RFP-donor DNA into the same 250 μL of Opti-MEM I. Vortex gently.
- b. Add 6 μL of Turbofectin 8.0 to the diluted DNA (not the reverse order) and pipette gently to mix completely.
- c. Incubate the mixture 15 minutes at room temperature.

Note: We recommend starting with the ratios of Turbofectin 8.0 and DNA listed in table 3; however, subsequent optimization may further increase the transfection efficiency.

Table 3. Recommended starting transfection conditions for Turbofectin 8

Tissue Culture Vessel	Growth area, cm ² /well	µg of DNA	Ratio of Turbofectin: DNA
96-well plate	0.35	0.1-0.15	3:1
24-well plate	2	0.5-1	3:1
12-well plate	4	1-2.5	3:1
6-well plate	9.5	1-5	3:1
35 mm plate	8	1-5	3:1
60 mm plate	20	2-10	3:1
100 mm plate	60	5-15	3:1

- d. Add the mixture above drop-wise to the cells plated in step 1 (no need to change the media). Gently rock the plate back-and-forth and side-to-side to distribute the complex evenly.
 - e. Incubate the cells in a 5% CO₂ incubator.
3. Passage cells around 3 weeks before puromycin selection or RFP sorting (Figure 9). 48hr post transfection, split cells 1:10, grow additional 3 days; then split the cells again 1:10. Split cells 7 times in total. Since puromycin resistant gene in the donor vector contains PGK promoter, RFP is driven by CMV promoter, the plasmid donor DNA before genomic integration will also provide puromycin resistance and express RFP. The reason to passage cells for around 3 weeks before puromycin selection is to dilute out cells containing the donor as episomal form.

Timelines of genome editing

- ✓ CRISPR targeted gene knockout / knockin--- 1 week post transfection
- ✓ Episomal donor vector dilution with cell passaging--- 3 weeks post transfection

Note. Since stable cell selection takes time, you can try to analyze the cells at P2 to detect genomic integration using genomic PCR (Figure 10). When designing primers for genomic PCR, one primer should be outside of the homologous arm region in donor DNA and one primer is in the functional cassette.

4. Apply puromycin selection or RFP sorting. After ~3 week cell passaging, you can use RFP to do cell sorting or use puromycin selection to enrich edited cells.

Note: We recommend you keep growing or freeze some of the transfected cells without selection; just in case, you need to perform the puromycin selection again.

5. The puromycin resistant cells are ready to be analyzed for genome editing.
 - Use microscope to observe RFP expression.
 - WB with anti-tRFP antibody (cat# [TA150061](#)) to detect RFP expression
 - Genomic PCR or sequencing to verify the integration of the functional cassette.

Note: Scramble control and donor vector will also give you some puromycin resistant cells as donor vector alone can randomly integrate into the genome too; however, the efficiency should be a lot lower than with a specific gRNA. Therefore, you should get more colonies with gene specific gRNA than scramble control if the gene specific gRNA cleaves efficiently.

6. Isolate individual cell colonies.

Two main methods, limiting dilution and cloning rings /cylinder, can be used to isolate individual cell colonies.

 - 1) Limiting dilution
This method is better to be used after puromycin selection. Dilute cells to seed about 1-2 cells/well in 96-well plate, after 1-2 weeks, observe under the microscope and select the wells only containing one cell colony, then further expand them to 6-well plate when they are confluent in the 96-well plate and so on.
 - 2) Cloning rings / cylinder
This method can be used at the same time with puro selection. Seed cells at lower density, such as 5% confluence in a larger cell culture dish, such as 10cm dish, apply puromycin selection when seeding.

Figure 9. Diagram of AVVS1 knockin process

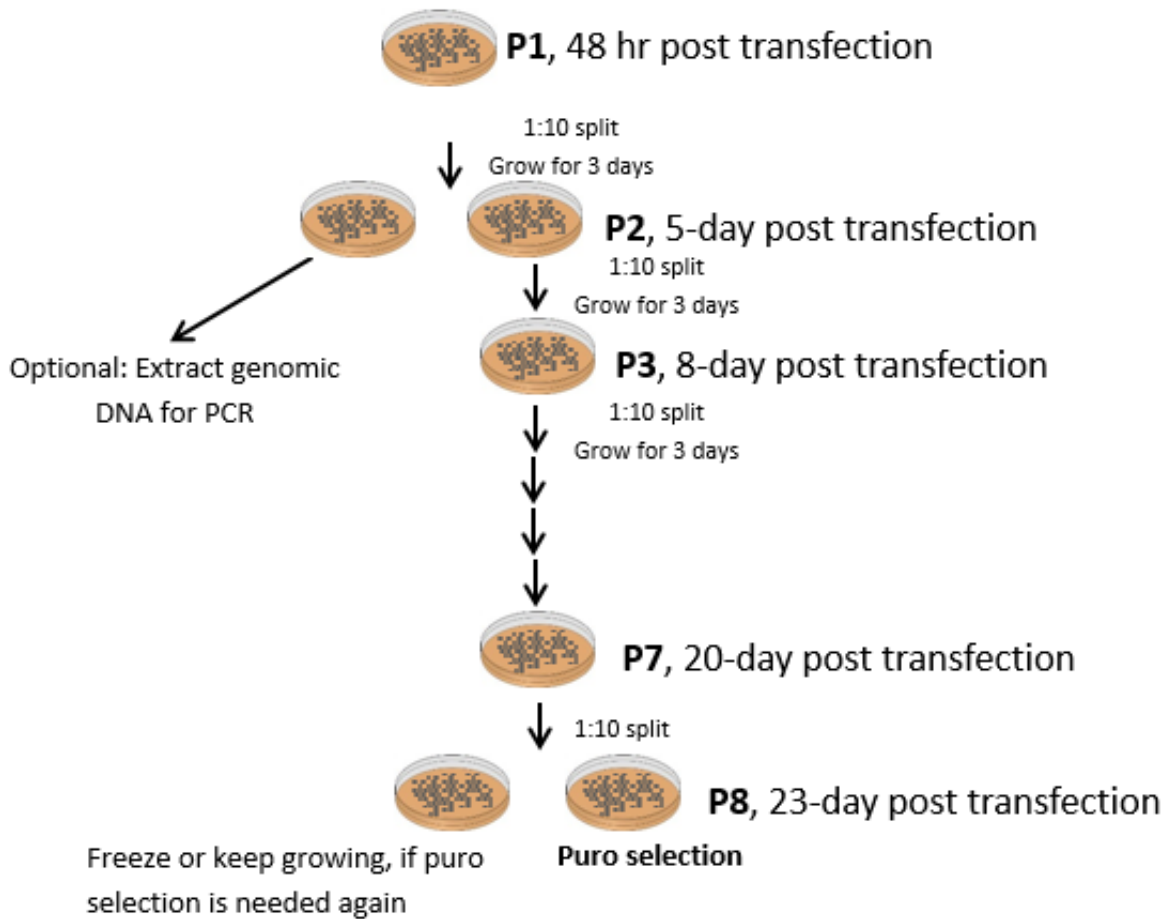
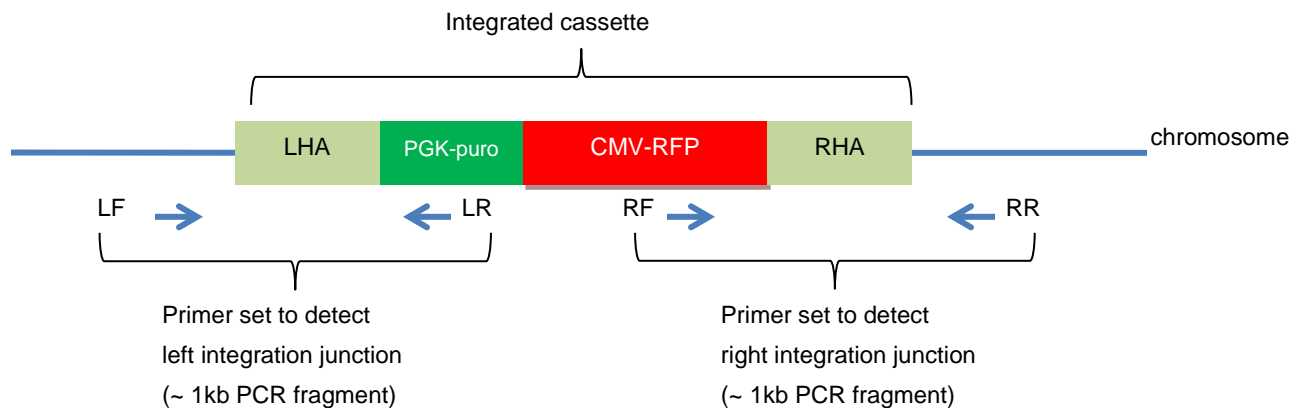


Figure 10. Diagram of genomic PCR Primer design.



LF, LR: Forward and reverse PCR primer to amplify the left integration junction

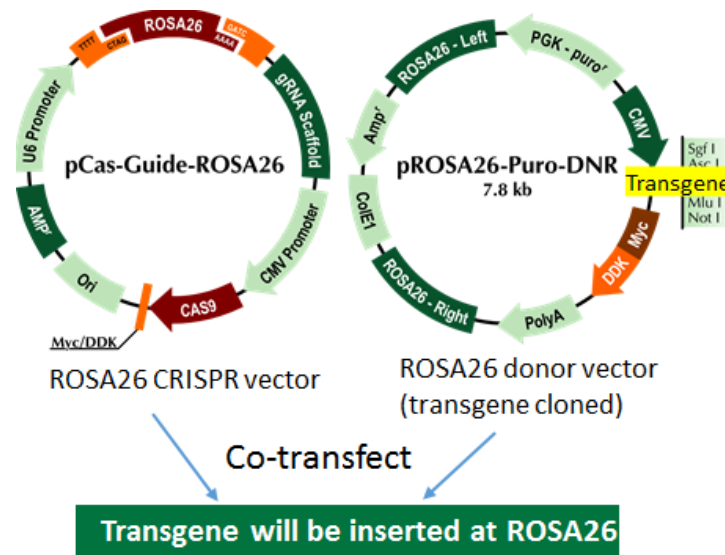
RF, RR: Forward and reverse PCR primer to amplify the right integration junction

ROSA26 Transgene knockin via CRISPR

The ROSA26 site is the most popular genomic safe harbor site for mouse cell. It has been used to create more than a hundred mouse knock-in cell lines. The transgene inserted in this locus showed a constitutive and strong gene expression in mice.

OriGene has developed a series of ROSA26 CRISPR products to help researchers insert their gene of interest in the ROSA26 genomic locus through homologous recombination (Figure 11).

Figure 11. Diagram of ROSA26 targeted insertion via CRISPR.



1. ROSA26 gRNA Vector, pCas-Guide-ROSA26 (SKU GE100050)

The pCas-Guide-ROSA26 (SKU GE100050) is a pre-designed and pre-validated gRNA construct, which can be transfected into mouse host cells and generate a double strand break at ROSA26 locus (Figure 12).

Package contents

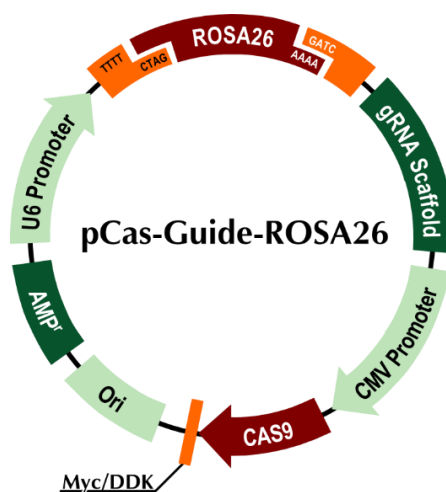
- One vial of 10 µg lyophilized pCas-Guide-ROSA26 (SKU: GE100050) plasmid DNA. Reconstitute in 100 µL dH₂O to make a final concentration of 100 ng/ µL.
- Certificate of Analysis

* 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

- pROSA26-Puro-DNR ([GE100051](#))
- pRosa26-tGFP-Puro-DNR (SKU GE100067)
- ROSA26 transgene knockin vector kit (puro) (SKU [GE100052](#))

Figure 12. The Vector map of pCas-Guide-ROSA26



2. ROSA26 donor vector, pROSA26-Puro-DNR (SKU GE100051)

Similar to AAVS1 donor vector, OriGene has developed a special donor vector around mouse ROSA26 locus. This vector contains ROSA26 left and right homologous arm to integrate the transgene cassette into ROSA26 locus through homologous recombination. Flanked by the two arms, there is a CMV promoter driven expression cassette for a transgene expression and a PGK driven puromycin resistant gene for mammalian selection (Figure 13). A multiple cloning site downstream of the CMV promoter is designed to be compatible with OriGene’s precision shuttle vector system, so that the ORF clones can be easily shuttled to the AAVS1 donor vector by a simple “cut and ligate” process (Figure 14).

**pROSA26-Puro-DNR is a donor vector without your gene of interest; the gene of interest needs to be cloned.*

Package contents

- One vial of 10 µg lyophilized pROSA26-Puro-DNR plasmid DNA. Reconstitute in 100 µL dH₂O to make a final concentration of 100 ng/ µL.
- One Vial of dried 5' (VP1.5) primer (100 picomoles), reconstitute in 10 µL dH₂O to make a 10 µM solution.
- One Vial of dried 3' (XL39) primer (100 picomoles), reconstitute in 10 µL dH₂O to make a 10 µM solution.
- Certificate of Analysis

* 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

- pCas-Guide-ROSA26 (GE100050)
- pRosa26-tGFP-Puro-DNR (SKU GE100067)
- ROSA26 transgene knockin vector kit (puro) (SKU [GE100052](#))

Figure 13. The vector map of pROSA26-Puro-DNR

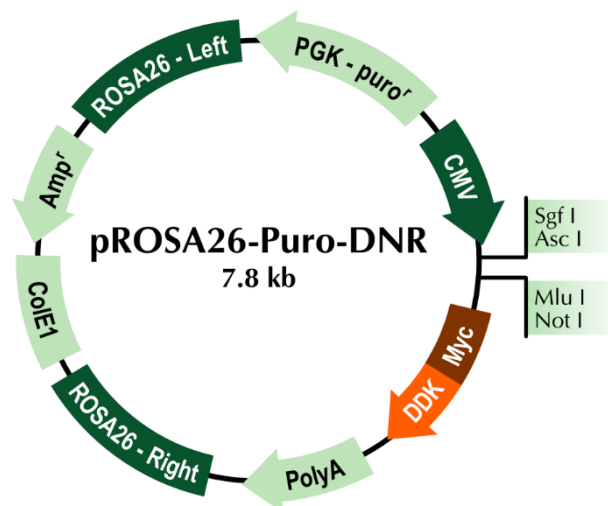
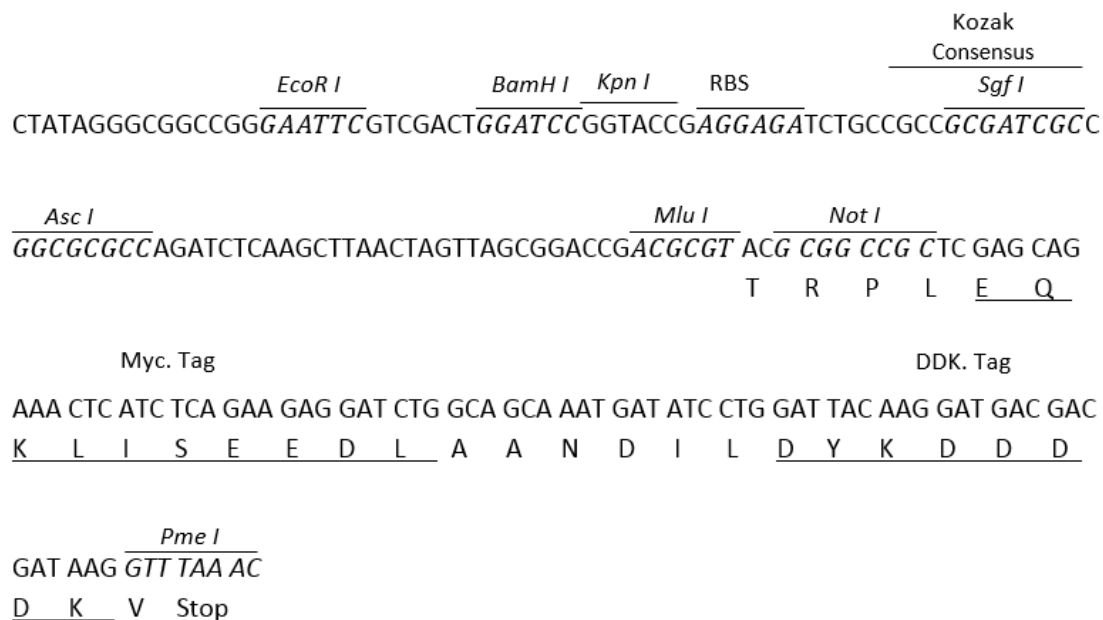


Figure 14. The Multiple cloning sites (MCS) of pROSA26-Puro-DNR (GE100051) vector



3. ROSA26 donor control, pRosa26-tGFP-Puro-DNR (SKU GE100067)

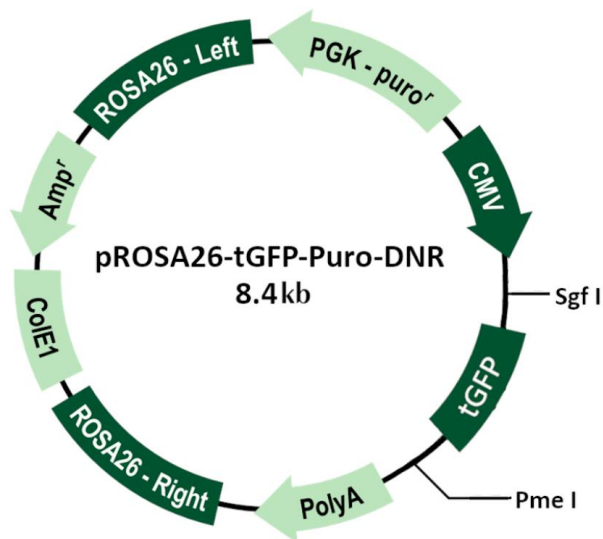
In addition to the empty ROSA26 donor vector, OriGene also offers a pre-designed and pre-validated donor control, containing a tGFP transgene in the ROSA26 donor vector (Figure 15). This donor control can be used as positive control to label the cells with green fluorescence. It can also be used to clone your gene of interest (GOI) either with C-terminal tGFP tag or without the tGFP tag (replacing the original tGFP reporter).

Package contents

- One vial of 10 µg lyophilized pRosa26-tGFP-Puro-DNR (SKU GE100067). Reconstitute in 100 µL dH₂O to make a final concentration of 100 ng/ µL.
- Certificate of Analysis

**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.*

Figure 15 The vector map of pRosa26-tGFP-Puro-DNR (SKU GE100067)



Kozak
Consensus

EcoRI BamHI KpnI RBS SgfI

CTATAGGGCGGCCGGGAATTCGTCGACTGGATCCGGTACCGAGGAGATCTGCCGCCGCGATCGCC

AscI MluI NotI

GGCGCGCCAGATCTCAAGCTTAAGTGTAGCGGACCGACGCGT ACG CGG CCG CTC GAG ATG

T R P L E M

tGFP Tag PmeI

GAG AGC GAC --- --- --- GGT GAA GAA AGA GTT TAA AC

E S D - - - G E E R V Stop

4. ROSA26 Transgene knockin vector kit, puro (SKU GE100052)

The ROSA26 transgene knockin vector kit is a complete vector kit to knockin your gene of interest in ROSA26 locus in mouse genome for robust and stable expression.

Package Contents

- One vial of 10 µg lyophilized pCas-Guide-ROSA26 (SKU GE100050).
- One vial of 10 µg lyophilized pCas-Guide-scramble (SKU GE100003) negative control.
- One vial of 10 µg lyophilized pRosa-Puro-DNR (SKU GE100051).
- Forward (VP1.5) and reverse (XL39) sequencing primers, 100pmols each, dried onto the bottom of screw cap tubes. Reconstitute each in 10 µL dH₂O to make a 10 µM solution. **Primers are for donor vector (pROSA26-Puro-DNR) only, not for pCas-Guide-ROSA26 or pCas-Guide-scramble.*
- Certificate of Analysis

**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. The DNA can be reconstitute in 100 µL dH₂O to make a final concentration of 100 ng/ µL.*

5. Experimental Protocol

The protocol is similar to AAVS1 transgene insertion via CRISPR. Please follow the experimental protocol on page p11 of this manual, use transgene cloned pROSA-Puro-DNR instead of pAAVS1-Puro-DNR.