User Manual

CRISPR/Cas9 AAV(Adeno-associated virus) Production

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Safe Use of AAV (Adeno-associated virus)

1. AAV related experiments should be conducted in biosafety level 2 facilities (BL-2 level).

2. Please equip with lab coat, mask, gloves completely, and try your best to avoid exposing hand and arm.

3. Be careful of splashing virus suspension. If biosafety cabinet is contaminated with virus during operation, scrub the table-board with solution comprising 70% alcohol and 1% SDS immediately. All tips, tubes, culture plates, medium contacting virus must be soaked in chlorine-containing disinfectant before disposal.

4. If centrifuging is required, a centrifuge tube should be tightly sealed. Seal the tube with parafilm before centrifuging if condition allowed.

5. AAV related animal experiments should also be conducted in BL-2 level.

6. AAV associated waste materials need to be specially collected and autoclaved before disposal.

7. Wash hands with sanitizer after experiment.

Storage and Dilution of AAV(Adeno-associated virus)

Storage of AAV

Virus can be stored at 4°C for a short time (less than a week) before using after reception. Since AAV viruses are sensitive to freeze-thawing and the titer drops with repeated freeze-thawing, aliquot viral stock should be stored at -80°C freezer immediately upon arrival for long-term usage. While virus titer redetection is suggested before using if the AAV viruses have been stored for more than 12 months.

Dilution of AAV

Dissolve virus in ice water if virus dilution is required. After dissolving, mix the virus with medium, sterile PBS or normal saline solution, keeping at 4°C (using within a week).

Precautions

· Avoid AAV exposure to environmental extremes (pH, chelating agents like EDTA, temperature, organic solvents, protein denaturants, strong detergents, etc.)

· Avoid introducing air into the AAV samples during vortexing, blowing bubbles or similar operations, which may result in protein denaturation.

· Avoid repeated freezing and thawing.

· Avoid exposing to “regular” plastics (especially polystyrene or hydrophobic plastics) for prolonged periods in liquid phase. Most AAV viruses are very sticky and loss can occur if exposed to regular plastics, including tubes, cell culture plates, pipette tips, if not frozen. It is best to store AAV in siliconized or low protein binding tubes. Pluronic F-68 used at 0.01%-0.1% in the formulation buffer will minimize sticking if regular plastics are used.

· Avoid diluting AAV into low salt solution. Some AAV serotypes, such as AAV2, aggregates in low salt solution, which will be non-infectious.

Introduction of AAV(Adeno-associated virus)

In Genemedi Biosciences, recombinant adeno-associated Virus (rAAV) Expression Systems are utilized in delivering and expressing shRNA, human ORF, CRISPR *in vitro* and *in vivo*.

Adeno-associated virus (AAV) is a small single strand DNA virus infecting human and some other primate species. Currently, AAV has not known to cause disease and only induces very mild immune responses. As a member of the family Parvoviridae, wild type AAV requires the assistance of adenovirus or herpesvirus to complete the duplication, which is the reason why it's called adeno-associated virus [1,2]. The wild-type AAV2 genome consists of the viral rep and cap genes (encoding replication and capsid genes, respectively), flanked by inverted terminal repeats (ITRs) that contain all the cis-acting elements necessary for replication and packaging. The genome of typical AAV2 is about 4800bp, consisting of two upstream and downstream open read frames (ORFs) which are between two inverted terminal repeats (ITR) comprising Rep and Cap (Figure 1). ITR is required for synthesis of complementary DNA strand, while Rep and Cap can be translated into various proteins, including AAV virus cycle essential protein Rep78, Rep68, Rep52, Rep40 and enveloped protein VP1, VP2, VP3, etc. [3].

The present recombinant AAV (rAAV) vectors are generated by replacing all of the viral genome between the ITRs with a transcriptional cassette of less than 5 kilobases in length. The resulting construct is then co-expressed with two other plasmids: 1) an AAV-RC plasmid that provides the Rep and Cap genes in trans (separate from the ITR/Transgene cassette) and 2) an AAV helper plasmid that harbors the adenoviral helper genes. AAV-293 cells are used as the packaging cell line since they provide the E1a protein in trans as well. By modifying the Rep and Cap genes, scientists can control the serotypes to guide the recombinant AAV infection towards certain tissues. This 3-plasmid co-transfection system liberates the need for adenovirus during AAV production, which greatly simplifies the purification process.

To date, a total of 12 serotypes of AAV have been described with their own unique traits and tropisms [4]. Concerning high safety, low immunogenicity, long-term expression of exogenous genes, AAV is thought to be the best gene delivery tool for gene function research *in vivo*.

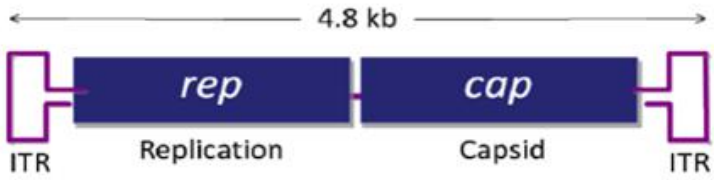

Figure 1. Schematic diagram of AAV2 genome structure.

Advantages of AAV for Gene Delivery and Expression

1. Superior biosafety rating

AAV is a naturally defective virus, requiring provision of several factors in trans for productive infection and has not been associated with any human disease. In our AAV production system, the AAV2 ITR sequences and rep/cap genes are present on separate plasmids that lack homology, preventing production of recombinant wild-type virus. These features give AAV a superior biosafety rating among gene delivery and expression vectors of viral origin.

2. Broad range of infectivity

AAV viruses infect a broad range of mammalian cells and have been used successfully to express human and non-human proteins. In contrast with other vectors of viral origin, AAV vectors have proven to be competent for gene expression in immunocompetent hosts.

3. High titer
Recombinant AAV can be produced at high titers of $\geq 10^7$ viral particles/ml with this protocol. Titers up to 10^{13} viral particles/ml after concentration have been published.

4. Infection does not require an actively dividing host cell
AAV can infect both dividing and non-dividing cells.

5. Long-term gene transfer potential
Recombinant AAV (rAAV) can be maintained in human cells, creating the potential for long-term gene transfer. In most cell populations, the viral genome typically remains epichromosomal, often forming concatemers, which are stable in slowly dividing or non-dividing cells, leading to long-term gene transfer and expression. Whereas in rapidly dividing cell populations, the AAV viruses can integrate into the host genome but not form concatemers, resulting in long-term gene expression in dividing cells, but this is a rare event. The integration occurs more frequently if an extremely high multiplicity of infection (MOI) of AAV is used or if infection occurs in the presence of adenoviral replicase, potentially supplied by the use of wild-type adenovirus. However, it will reduce the biosafety of the AAV system to increase integration events by using wild-type adenovirus.

AAV Serotypes and Native Tropism-AAV Selection Guide

Over the past decades, numerous AAV serotypes have been identified with variable tropism. To date, 12 AAV serotypes and over 100 AAV variants have been isolated from adenovirus stocks or from human/nonhuman primate tissues. Different AAV serotypes exhibit different tropisms, infecting different cell types and tissue types. So, selecting the suitable AAV serotype is critical for gene delivery to target cells or tissues.

Due to the exhibition of natural tropism towards certain cell or tissue types, rAAV has garnered considerable attention. Highly prevalent in humans and other primates, several AAV serotypes have been isolated. AAV2, AAV3, AAV5, AAV6 were discovered in human cells, while AAV1, AAV4, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12 in nonhuman primate samples [5,6]. Genome divergence among different serotypes is most concentrated on hypervariable regions (HVRs) of virus capsid, which might determine their tissue tropisms. In addition to virus capsid, tissue tropisms of AAV vectors are also influenced by cell surface receptors, cellular uptake, intracellular processing, nuclear delivery of the vector genome, uncoating, and second strand DNA conversion [7].

In order to better improve the infection efficiency and specificity of AAV to target tissues, scientists have genetically modified the viral capsid, and generated mosaic vectors to create chimeric AAV by swapping domain's or amino-acids between serotypes [8,9], which may allow researchers to specifically target cells with certain serotypes to effectively transduce and express genes in a localized area [10].

Meanwhile, the ability of AAV to penetrate the blood-brain barrier in animals is greatly limited or improved. Traditionally, AAV could only be injected into the brain tissue by surgery for scientific research in the central nervous system, which greatly increased the difficulty of the experiment and affected the experimental results. Now the modified AAV serotype of PHP.B and PHP.eB can infect the whole brain through the blood-brain barrier by peripheral blood injection [11]. Most popular rAAV serotypes and their tropisms are listed in the following table1.

Table 1. rAAV serotypes and their tropisms.

AAV Serotype	Tissue tropism							
	CNS	Retina	Lung	Liver	Pancreas	Kidney	Heart	Muscle
AAV1	√	√			√		√	√
AAV2		√		√		√		
AAV3		√	√	√			√	
AAV4	√	√					√	
AAV5	√	√	√		√			
AAV6	√		√	√			√	√
AAV7				√				√
AAV8		√		√	√			√
AAV9	√		√	√			√	√
AAV-DJ		√	√	√		√		
AAV-DJ/8		√		√				√
AAV-Rh10	√		√	√			√	√
AAV-retro	√	√						√
AAV-PHP.B	√						√	√
AAV-PHP.eB	√							√
AAV-PHP.S	√						√	√

CRISPR/Cas9 System

CRISPR (clustered regularly interspaced short palindromic repeats) is a family of DNA sequences derived from viruses that have previously infected the prokaryotes and has the ability to recognize invasive homologous DNA sequences from similar viruses, then directing Cas9 to specifically cleave them during subsequent infections, thus playing a key role in the antiviral defense system of prokaryotes [12]. Cas9 (CRISPR-associated 9) is an enzyme guided by CRISPR sequences to recognize and cleave specific strands of DNA which are complementary to the CRISPR sequence [13]. CRISPR sequences together with Cas9 enzymes make up the basis of a CRISPR/Cas9 technology which is a versatile genome-editing platform within organisms.

By 2010, three CRISPR/Cas9 systems have been identified in bacteria: Type I, II and III. Due to the relative simplicity, Type II CRISPR system from Streptococcus pyogenes have been eventually adapted for genome editing in mammalian cells [14]. This simpler CRISPR system is made up of four components, that is, the Cas9 endonuclease, trans-activating CRISPR RNA (tracrRNA) and two small RNA molecules named CRISPR RNA (crRNA) [15]. In this system, the mature crRNA base-paired to trans-activating crRNA (tracrRNA) forms a two-RNA structure, guiding Cas9 protein to induce double-stranded breaks in target DNA. At sites complementary to the crRNA-guide sequence, the Cas9 HNH nuclease domain cleaves the complementary strand, whereas the Cas9 RuvC-like domain cleaves the noncomplementary strand. To further apply this system to mammalian cells, Jennifer Doudna et.al. re-engineered the CRISPR/Cas9 system into a more manageable two-component system by fusing dual-tracrRNA and crRNA molecules into a "single-guide RNA (sgRNA)", in which Cas9 endonuclease could search and cleave the target DNA specified by the sgRNA (Figure 2). By manipulating the nucleotide sequence of the sgRNA, the artificial Cas9 system could be programmed to target any DNA sequence for cleavage [16].

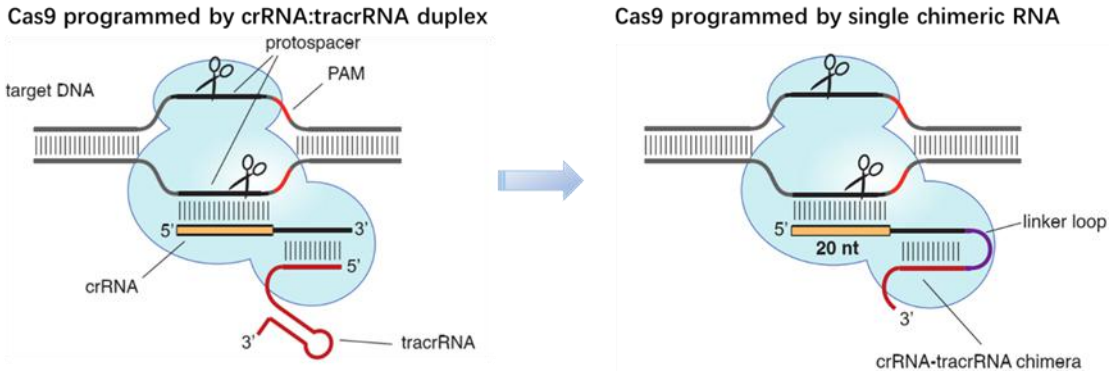

Figure 2. Cas9 can be programmed using a single engineered RNA molecule covering tracrRNA and crRNA features. Left, Cas9 is directed by a dual RNA structure formed by activating tracrRNA and targeting crRNA to cleave site-specifically–targeted dsDNA in type II CRISPR/Cas systems. Right, a chimeric RNA generated through fusion of crRNA to tracrRNA [16].

Advantages of CRISPR/Cas9 System

Remarkable versatility. The artificial CRISPR/Cas9 system can be programmed to target any DNA sequence for cleavage by manipulating the nucleotide sequence of the sgRNA and displays remarkable versatility from cells *in vitro* to animals or plants *in vivo*.

Simple to design and easy to operate. Compared to the previous genome editing tools, such as Zinc Finger

Nucleases (ZFN) and Transcription activator-like effector nucleases (TALEN), the CRISPR/Cas9 system doesn't need to rely on protein design, just requires a few simple DNA constructs to encode Cas9 protein and sgRNA, which is much more simple and easier.

Low cost. Compared to genome editing tools, such as ZFN and TALEN, the CRISPR/Cas9 system only requires less time and resources.

Table 2. Comparison among multiple gene editing tools.

Gene editing tool	ZFNs	TALENs	CRISPR/Cas9
Essential components	Zinc finger proteins + Fork1 fusion protein	TALE and Fork1 fusion protein	Guide RNA + Cas9 protein
DNA Binding Domain	Cys-His2 DNA Binding Protein	Conserved Amino Acid Repeated Motif	Single Stranded sgRNA
DNA Cleavage Domain	ForkI Restriction Endonuclease	ForkI Restriction Endonuclease	Cas9 Endonuclease
Work mode (Pair)	Pair	Pair	No
Guiding Mechanism	Protein Guided	Protein Guided	RNA Guided
Requirements	Two large protein constructs	Two large protein constructs	Simple 20nt change to construct
Easy to design	Difficult	Moderate	Easy
Feasibility	Difficult	Difficult	Easy
Efficiency	Low	Low	High
Specificity	18-36nt	30-36nt	23nt
Targeting	Poor	Average	Good
Off-target effects	Low	Low	High
Multiple gene mutation	Difficult	Difficult	Easy
Cost	High	Moderate	Low
Time consumption	Long (5-7 days)	Long (5-7 days)	Short (1-3 days)

AAV-SaCas9-gRNA System

To date, CRISPR/Cas9 system have been found in several microbial species, among which, Streptococcus pyogenes Cas9 (SpCas9), is the most robust and widely used Cas9, primarily recognizes NGG PAMs and is consequently restricted to sites containing this motif. However, genome editing in somatic tissues of postnatal animals, has been restricted in part by the challenge of delivering SpCas9 *in vivo*. To address this problem, recombinant AAV vectors are attractive vehicles due to their low immunogenic potential, no integration into host genome, and broad range of serotype specificity. Nevertheless, the restrictive cargo size (~4.5kb) of AAV presents an obstacle for packaging the commonly used SpCas9 and its single guide RNA (sgRNA) in a single vector.

In 2015, Kleinstiver et.al discovered one smaller-size Cas9 orthologues, Staphylococcus aureus Cas9 (SaCas9), which is 25% smaller than SpCas9, efficient and broadly targeting, thus can be packaged into AAV for *in vivo* genome editing (Figure 3) [17].

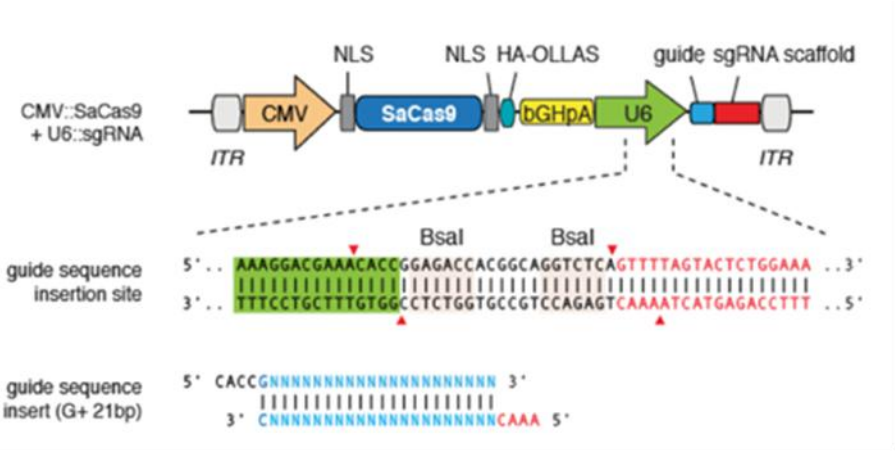

Figure 3. Single-vector AAV system [17]

Compared to SpCas9, SaCas9 is smaller, and can be effectively packaged into AAV vectors, leaving extra space for gRNA, which makes CRISPR-based gene editing in living organisms more feasible and endows SaCas9 great potential applications in the biomedical research field. In addition, SpCas9 recognizes a PAM sequence of 5'-NGG-3', while SaCas9 recognizes 5'-NNGRRT-3'. The greater variety in PAM sequences of SaCas9 makes an increased number of loci available for genome editing, which is of particular benefit in precise editing of genes with homology directed repair. In the meantime, the longer PAM sequence of SaCas9 occurs less frequently in the genome than that of SpCas9, which may present a restriction factor in its potential utility. Of course, this greater specificity of SaCas9 can also help prevent off-target effects.

Table 3. Comparison between SpCas9 and SaCas9.

Cas9	SpCas9	SaCas9
Gene size	~4.3kb	~3.3kb
PAM sequence	5'-NGG-3'	5'-NNGRRT-3'
Target sequence	20nt	24-21nt
Application	Research field	Virus packaging

To date, AAV-SaCas9 has been reported to be a potential therapeutic approach for a number of diseases, such as Duchenne muscular dystrophy (DMD), a severe hereditary disease, mainly resulting from dystrophin gene mutation, leading to a reading frameshift which abrogates dystrophin protein synthesis. El Refaey M et.al found that *in vivo* genome editing with systemically delivery of AAV-SaCas9 targeting dystrophin gene restores dystrophin expression and cardiac function in dystrophic mice [18]. Duchêne BL delivered AAV9 containing SaCas9 and pairs of sgRNAs targeting exons 47 and 58 of dystrophin into myoblasts in muscle biopsies of 4 DMD patients with different exon deletions, resulting in restore of a normal reading frame of dystrophin gene. Restoration of dystrophin expression was also obtained *in vivo* in the heart of the humanized mice model del52hDMD/mdx [19].

Recently, efforts have been tried to develop variants of SaCas9 by relaxing the PAM recognition specificity of SaCas9 through molecular evolution. One variant has been identified, referred to as KKH SaCas9, displaying robust genome editing activities at target sites with NNNRRT PAMs, thus garnering a larger range of potential editing targets than that of wild type SaCas9. GUIDE-seq results also show that comparable numbers of off-target effects in human cells are induced between wild-type and KKH SaCas9 [20].

sgRNA Design and AAV-SaCas9-gRNA Vector Construction

sgRNA Design

SaCas9-gRNA design principles

1. The PAM sequence that SaCas9 recognizes is NNGRRT (NNGAAT, NNGAGT, NNGGAT, NNGGGT) or NNGRRN, for sgRNA, the length is about 21 or 22 nucleotides.

2. 'TTTT' termination signal should be avoided in the sequences of sgRNA, and the GC content in 40%~60% is better.

3. If the sgRNA is driven by U6 or T7 promoter, the 5' end of sgRNA can be designed as G or GG to improve transcription efficiency, which should be considered.

4. The binding site of sgRNA should be as close to the coding region in the downstream of ATG as possible to induce frameshift mutation, the first or second exons is better.

5. SNPs should be checked in the binding site of sgRNA.

6. The distance between paired sgRNA should be taken into consideration before designing paired-gRNAs, if using Cas9 single nickase.

7. Whole genome off-target effect analysis is suggested. At least 5 bases can be allowed for the base mismatch and whether the off target is located in the gene encoding regions need to be confirmed. What's more, base insertion or deletion in off targets should be detected.

SaCas9-gRNA design

Based on the above principles, SaCas9-gRNA can be designed on the following website: https://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design. Human TRF3 gene was taken as an example for illustration (Gray, the first exon; Yellow, the second exon).

ATGGCCTCTGCGCCCTGGCCGGAGCGGGTTCCGAGGCTGCTCGCTCCGCGCTTACCCTCTTACCCGCCCCCACCCC
CAACAGTGGGATTACGGTCCATGGAGCAGGAGGAGACCTACCTGGAGCTCTACCTGGACCAGTGCGCCGCTCAG
GATGGCCTTGCCCCACCCAGGTCTCCCCTGTTCAGCCCAGTTGTACCTTATGATATGTACATACTGAATGCATCCA
ATCCGGATACTGCATTTAATTCGAACCCTGAAGTCAAAGAAACATCTGGTGATTTCTCATCTGTGGATCTTAGCTT
CCTACCAGATGAAGTTACCCAGGAAAATAAAGACCAGCCTGTCATTAGCAAACACGAAACTGAAGAAAATTCTG
AAAGCCAAAGTCCACAAAGTAGGTTGCCATCACCCAGCGAACAGGACGTTGGGCTGGGCTTAAACAGCAGCAGT
TTGTCAAATTCCCATTCACAGCTGCACCCTGGTGATACTGACTCAGTCCAGCCCTCTCCTGAGAAACCAAACTCCG
ACTCCTTGTCTCTGGCATCCATAACTCCCATGACACCAATGACCCCTATTTCAGAATGTTGTGGAATTGTACCTCA
ACTACAGAATATAGTTTCCACTGTAAACCTGGCCTGTAAGTTGGATCTGAAGAAAATAGCTTTGCATGCAAAAAA
TGCAGAATATAACCCAAAGAGGTTTGCTGCTGTCATAATGAGGATCCGAGAGCCCAGGACAACAGCCCTTATATT
TAGCTCTGGGAAGATGGTCTGCACGGGAGCCAAAAGTGAAGAGCAGTCTCGACTTGCAGCAAGAAAATATGCTC
GTGTGGTGCAGAAGCTTGGGTTCCCTGCCAGATTCCTCGATTTTAAAATTCAGAACATGGTTGGAAGCTGTGATGT
GAGATTTCCCATCAGGCTGGAAGGTTTGGTGCTAACCCATCAGCAGTTCAGTAGTTACGAGCCTGAACTGTTTCCT
GGTCTTATTTATAGAATGGTAAAACCACGAATTGTGTTGCTTATCTTTGTATCTGGAAAAGTTGTGTTGACAGGTG

CCAAAGAACGTTCTGAGATCTATGAAGCATTTGAAAACATCTATCCTATTCTAAAAGGTTTTAAAAAAGCCTGA
The confirmed sgRNAs are as follows.

SaCas9-gRNA1T: 5' CACCGGACCCCTATTTCAGAATGTTG 3' (Sense strand)
SaCas9-gRNA1B: 5'AAACCAACATTCTGAAATAGGGGTCC 3'

SaCas9-gRNA2T: 5' CACCGGTCATTGGTGTCATGGGAGTT3' (antisense strand)
SaCas9-gRNA2B: 5'AAACAACTCCCATGACACCAATGACC 3'

SaCas9-gRNA 3T: 5' CACCGGCTGGACTGAGTCAGTATCAC 3' (antisense strand)
SaCas9-gRNA3B: 5'AAACGTGATACTGACTCAGTCCAGCC 3'

AAV-SaCas9-gRNA Vector Construction

sgRNA target sequences are synthesized, annealing into a double chain and cloned into pAAV-SaCas9 vector, which is confirmed by sequencing.

Overall Experiment Procedure of AAV-SaCas9 Virus Production and Titer Detection

The AAV-SaCas9 virus can be packaged using AAV-293 cells, purified with iodixanol gradient ultracentrifugation method, and titer is detected by real-time quantitative PCR (QPCR) using primers targeting the AAV ITR. The detailed protocol can be consulted in the AAV Protocol.

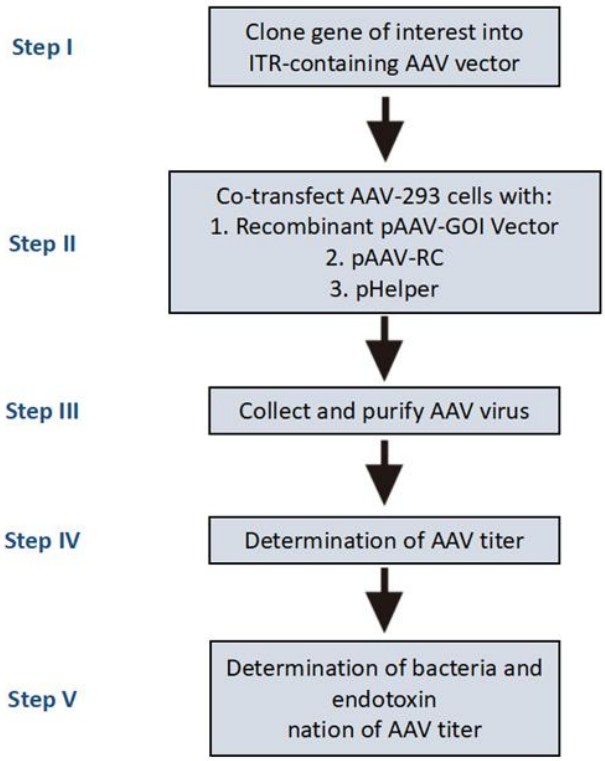

Figure 4. AAV packaging experiment flow chart.

Target Validation of AAV-SaCas9 in vitro

After AAV-SaCas9 titer detection, the cleavage effects of SaCas9 can be validated in 293T cells. The detailed recommended protocol for *in vitro* cell transduction can be consulted from AAV Protocol. Infect 293T cells with AAV-SaCas9 virus at confluency about 40%-60%. 24h post infection, change the medium. 96h post infection, harvest cells and extract the genome. Perform PCR using primers flanking target sequence, and sequence to confirm whether knockout effects exist by the appearance of emerged peaks.

Target Validation of AAV-SaCas9 *in vitro* by Sanger Sequencing

1. AAV-SaCas9-gRNA1: emerged peaks appeared in sequences around the target sequence, so there existed knockout effects.

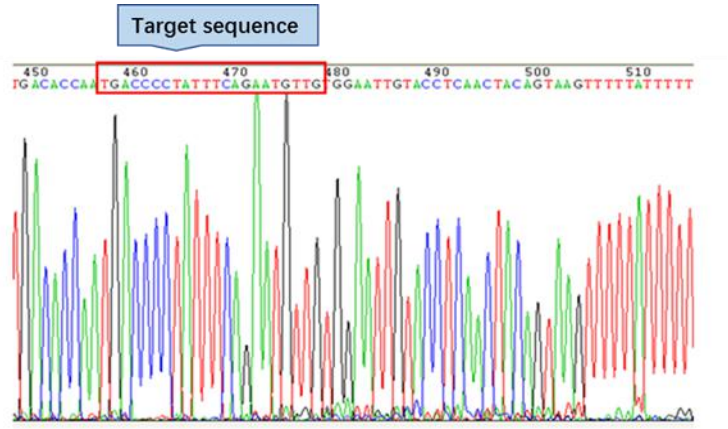

Figure 5. Sanger sequencing results of genome, from cells infected with AAV-SaCas9-gRNA1.

2. AAV-SaCas9-gRNA2: emerged peaks did not appeared in sequences around the target sequence, so there did not exist knockout effects.

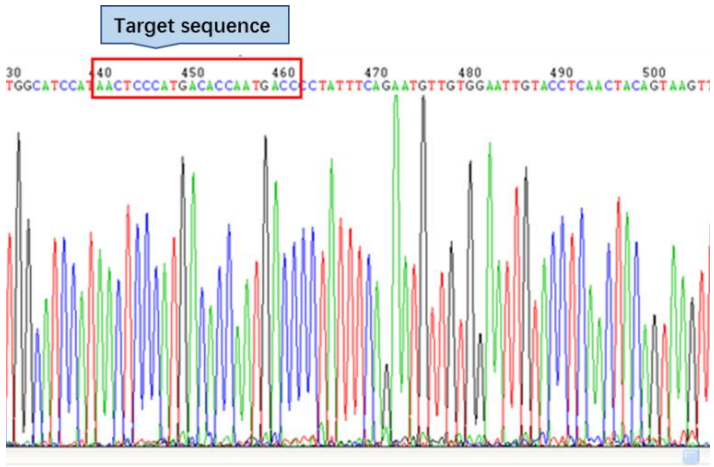

Figure 6. Sanger sequencing results of genome, from cells infected with AAV-SaCas9-gRNA2.

3. AAV-SaCas9-gRNA3: clear emerged peaks appeared in sequences around the target sequence, so there existed great knockout effects.

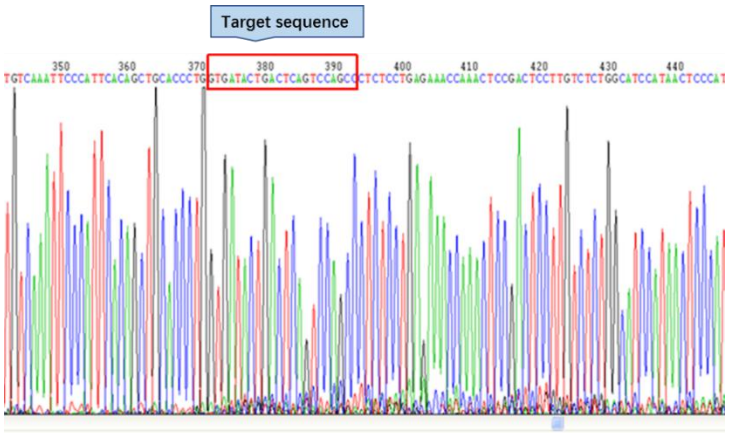

Figure 7. Sanger sequencing results of genome, from cells infected with AAV-SaCas9-gRNA3.

Conclusion

1. The results show better knockout effects of AAV-SaCas9-gRNA3 virus in 293T cells compared to the other two AAV-SaCas9 viruses.

2. These results only display the effectiveness of AAV-SaCas9-gRNA3, further experiments to get monoclonal cell line with frameshift mutation close to the start codon, such as monoclonal cell lines selection and screening by sequencing, need to be carried out.

Target Validation of AAV-SaCas9 in vivo

The successful validated AAV-SaCas9-gRNA3 can be delivered *in vivo*. Genemedi systematically organizes the corresponding optimal AAV serotype, gene delivery methods and injection volume for mouse and rat tissue infection, which can be found in the AAV User Manual.

About 3 weeks post infection, get the target tissues or organs, extract the genome and perform PCR and Sanger sequencing to confirm the knockout effects, which are the same as target validation of AAV-SaCas9 *in vitro*.

You can also extract protein from target tissues or organs 3 weeks post infection, and carry out Western blot to make sure the target protein is knocked out.

References

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Virus CRISPR/Cas9 Service

• For AAV CRISPR/Cas9 service, please visit:
https://www.genemedi.net/i/aav-sacas9-packaging

• For lentivirus CRISPR/Cas9 service, please visit:
https://www.genemedi.net/i/crispr-cas9-knockout-lentivirus-production-service

• For Adenovirus CRISPR/Cas9 service, please visit:
https://www.genemedi.net/i/adenovirus-cas9-packaging

Other protocols

• Crispr/cas9 mediated Gene knockout in Mammalian Cells - Protocol
https://www.genemedi.net/pdf/Genemedi-Crispr-cas9%20Protocol.pdf

• Recombinant Adenovirus-CRISPR/Cas9 Knockout System-User Manual
https://www.genemedi.net/pdf/Genemedi-Adenovirus-crispr%20User%20Manual.pdf

• Recombinant Lentivirus-CRISPR/Cas9 Knockout System-User Manual
https://www.genemedi.net/pdf/Genemedi-Lentivirus-crispr%20User%20Manual.pdf

• Adeno-associated Virus (AAV) Production Protocol:
https://www.genemedi.net/pdf/AAV%20production%20protocol-packaging%20concentration%20and%20purification-GeneMedi.pdf

• Lentivirus Production Protocol:
https://www.genemedi.net/pdf/lentivirus%20production%20protocol-packaging%20concentration%20and%20purification-GeneMedi.pdf

• Adenovirus Production Protocol:
https://www.genemedi.net/pdf/Adenovirus%20production%20protocol-packaging%20concentration%20and%20purification-GeneMedi.pdf

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