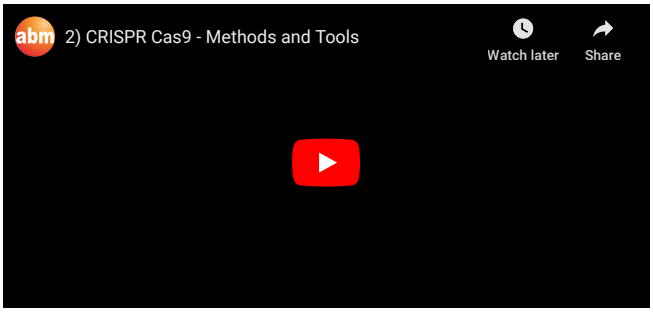
CRISPR Cas9

Methods and Tools

35 min Read

Video Summary

The CRISPR Cas9 system (**C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats CRISPR-Associated Proteins 9) allows scientists to efficiently knock-out or knock-in any gene of interest by the use of a 20 bp guiding RNA and a Cas9 endonuclease. Given its ease of use, this new genome-editing system offers a versatile platform and has potential to supplant the strenuous zinc finger and TALEN approaches. To utilize this platform, it is essential to efficiently introduce the gRNA and Cas9 nuclease into the living system. There are many tools and methods available to scientists for this purpose and here we will briefly summarize these available technologies.



CRISPR Design Tools

Gene Delivery Methods

Several methods exist for gene delivery, including viral and non-viral systems (1) (2) (3). Each of these approaches carry their own advantages and disadvantages, therefore, it is important to choose the most suitable vector depending on the nature of your experiment. While there is no 'perfect vector' that can be used for all applications, considering the following parameters will help in selecting the most appropriate gene delivery vehicle:

1. *in vitro* or *in vivo* research: the requirements for in vivo applications (i.e. as a gene therapy vector) is more extensive. For example, the vector should elicit minimal immune response after delivery and in many cases, the vector should have the ability to target specific tissue or cell types (3) (4).
2. Gene of interest size: the genetic material that a viral vector can deliver is often limited to its genome size. Non-viral delivery methods provide the solution for expressing genes with larger coding sequences.
3. Target cell type: some cell types such as neurons, hepatocytes and myocytes are post-mitotic, therefore vectors capable of delivering materials into non-dividing cells are necessary for those cells types (4).
4. Transient or permanent expression: some vectors offer stable long-term transgene expression while others are only capable of short-term expression. For example, many viral vectors can integrate into the host's genome thereby establishing permanent gene expression in target cells (4)

Viral Vectors

Over the years scientists have exploited viral vectors for their natural ability to penetrate into cells for successful gene delivery. For safety reasons, the pathogenic part of many viruses has been altered to function as carrier vehicles, including lentivirus, adenovirus and adeno-associated virus. One common feature between all viral mediated gene deliveries is the remarkable infection efficiency (1). Despite their notable infection efficiency, some viral vectors present the following drawbacks: (a) limited size of gene that can be delivered by the virus, (b) acute immune response associated with the viral vector and (c) production of viral vectors can be difficult (1).

a. Lentiviral Vectors

A subclass of retrovirues, lentiviruses differ from other retroviruses for their unique ability to infect both proliferating and quiescent cells (1). Lentiviruses integrate non-specifically into the host's genome following infection, allowing long-term stable expression of the transgene (1). As a medium sized virus, lentiviral vectors have the ability to delivery exogenous genetic material up to 5.0 kb. Lentivirus tropism can be re-established with different types of the envelope protein used in viral production, offering a relief for lentviruses on their dependence on CD4, the T-cell receptor protein required for natural lentivirus infection (4). The most commonly used heterologous envelope protein in recombinant lentiviral vectors today is the 'vesicular stomatitis virus glycoprotein (VSV-G)'. VSV-G pseudotyped lentiviruses have increased their host cell range as VSV-G is able to interact with the phospholipid component of a number of receptors on cell membrane (2) (4).

b. Adenoviral Vectors

Due to its large genome size, adenovirus can accommodate DNA particles up to 8-9 kb, providing an alternative method to deliver larger transgenes via viral-mediated gene transfer. The entry of adenoviruses into cells is mediated via the highly-expressed cell surface coxsackie virus B-adenovirus receptor (CAR), which makes this virus highly infective in many cell types (2). While adenovirus is able to infect a

broad range of dividing and non-dividing cells, this non-discriminating tropism can lead to transduction of untargeted cells in a living system. In contrast to other integrating viruses, adenovirus vectors provide transient gene expression only, as they remain as episomes inside the cell (1) (4). Adenovirus is notorious for causing acute inflammatory response in vivo thus limiting its clinical applications to localized tissues for gene therapy (1) (2) (5).

c. Adenovirus-Associated Viral Vectors (AAV)

Unlike adenoviruses, AAV lack in pathogenicity making them the ideal vector for in vivo applications (for a detailed descritpion of AAV please visit Adeno Associated Virus - An Introduction). AAV is capable of infecting both dividing and non-dividing cells and its entry into cells is mediated through heparin sulphate proteoglycans and integrans (5). Naturally occurring AAV can integrate in a site-specific location on chromosome 19 through the action of its Rep protein; however, present day recombinant AAV does not code for Rep and therefore persists as extra-chromosomal DNA (4). Differential tropism can be achieved with different AAV serotypes. By using different capsid protein during viral production, the recombinant AAV can be tailored towards infection of a certain type of cells. The drawback with AAV is its small genome size that can only allow incorporation of transgenes up to 3.4 kb. Table 1 highlights AAV serotype tropism towards specific tissue types.

Table 1 — Table of AAV Serotypes and their respective Tropisms.

AAV Serotype	CNS/Retina	Heart	Lung	Liver	Skeletal Muscle
AAV1	X	X	X		X
AAV2	X			X	X
AAV3	X	X		X	
AAV4	X	X			
AAV5	X		X		
AAV6		X	X	X	X
AAV7	X			X	X
AAV8	X			X	X
AAV9	X	X	X	X	X
AAV10	X		X		

Comparing the different Viral Methods

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Table 2 — Characteristics of Lentiviral, Adenoviral and AAV Vectors.

Features	Lentivirus	Adenovirus	AAV
Packaging Capacity	5Kb	8-9Kb	3.4Kb
Efficiency	***	****	****
Cell Type	Most Dividing/Non-Dividing Cells	Most Dividing/Non-Dividing Cells and High Transduction Rate Towards Primary Cells	All Cell Types
Integrating	Yes	No	90% Not, 10% May Integrate

Immune Response	***	*****	**

Non-viral Gene Delivery

Non-viral gene delivery can be further divided into two categories: chemical and physical (1) (2). When compared to viral-mediated methods, non-viral methods can be less efficient in gene delivery, however; they are by far more cost-effective, and most importantly, there is no restriction on transgene size and no concern with immunological responses (1).

We offer CRISPR Cas9 sgRNA expression vectors for use with non-viral gene transfer methods.

a. Physical Non-viral Gene Delivery

Physical methods, such as microinjection, electroporation and gene gun, uses physical force to disrupt the cell membrane in order to allow the gene to enter the cell (2). While these techniques are straightforward and easy to perform, specialized equipments are required for the procedures. In addition, since blunt force is applied, this method can cause tremendous cell death or tissue damage in some applications (1) (2).

b. Chemical Non-viral Gene Delivery

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Read more about the transfection reagents we offer here.

Planning Your Experiment

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1. Choose a CRISPR Cas9 System by asking yourself "What would you like to achieve?"

CRISPR applications vary from gene disruption (i.e. InDel mutations), gene activation or repression to precise editing of genomic information. Find out which Cas9 variant suits your requirement by reading our introduction to CRISPR Cas9 Introductory Guide.

Once you have identified the CRISPR system to use, the next step would be designing the sgRNA.

You can find a list of our Cas9 Variants here.

2. Design sgRNA for your Target Gene

The genome-modification ability of the CRISPR Cas9 system depends greatly on the specificity of the sgRNA, which guides the Cas9 endonuclease to the desired genome target. In order to minimize potential off-target effects, many considerations should be taken when designing the sgRNA and choosing a target sequence. Learn more about how to design with our CRISPR Cas9 - gRNA Design Guide.

Once you have designed the sgRNA to your target sequence, you can clone the sgRNA into an appropriate expression vector (see Gene Delivery Methods for more information).

Browse our genome-wide collection of sgRNA in Lentiviral Vectors here.

3. Delivery sgRNA and Cas9 into your Experimental Model

The best way to deliver the sgRNA and Cas9 components depends on the model system. For example, primary cell lines are more susceptible to viral infections than transfections and stem cells are prone to electroporation. Determine the best gene delivery method for your system.

We provide sgRNA and Cas9 expression in ready-to-use lentiviral, adenoviral, and AAV formats.

4. Evaluate Genome-editing Efficiency

Successful genome alteration requires validation via downstream verification experiments such as PCR, sequencing, endonuclease mismatch detection assay or the SURVEYOR assay. Find tips on how to evaluate your CRISPR Cas9 experiment with our CRISPR Cas9-Assessing Efficacy and Accuracy Guide (Coming Soon).

Our Experience with CRISPR Cas9 Gene Delivery

To knockout GFP expression in a stable GFP-expressing HEK293 cell line, we cloned the sgRNA into a lentiviral vector. The following protocol is used to package the lentiviral vector into viral particles, which are subsequently used to deliver the sgRNA into the cells.

Day 1:

1. In the afternoon, seed ~1.2 x 107 293T cells in a 10 cm dish

Day 2:

1. Check to make sure the cells are 70-80% confluent.
2. Prepare the transfection complex as follows:
    a. Solution A: Dilute 20 µg DNA plasmids (10 µg sgRNA expression vector and 10 µg of **abm**'s Second Generation (LV003) or Third Generation (LV053) Packaging Mix) in 1 mL serum-free, antibiotic-free medium.
    b. Solution B: Dilute 80 µL of LentiFectin™ Transfection reagent (G074) in 1 mL serum-free, antibiotic-free medium.
    c. Incubate both solutions at room temperature for 5 minutes.
    d. Mix Solutions A and B together well and incubate at room temperature for 20 minutes.
3. Add 4.5 mL serum-free medium to the transfection complex.
4. Remove medium from the cells in the 10 cm dish.
5. Add the complete transfection complex from step 4 to the cells and incubate at 37°C for 5-8 hours.
6. Add 0.65 mL FBS to the 10 cm dish and incubate at 37°C overnight

Day 3:

1. Remove the transfection medium from the cells.
2. Add 10 mL complete culture medium to the cells.
3. Incubate at 37°C for 24 hours.

Day 4:

1. Collect the supernatant medium from the culture dish.
2. Centrifuge the supernatant at 3000 rpm for 15 minutes at 4°C to pellet cell debris.
3. Transfer the cleared supernatant to a fresh tube. Filter the cleared supernatant with a low-protein binding 0.45 µM sterile filter.
4. Titer the cleared supernatant using abm's qPCR Lentivirus Titer Kit (LV900).

Human Embryonic Kidney 293 GFP positive cells were cultured in DMEM with 10% FBS and 1% Pen/Strep. The cells were seeded in 12-well plate at 100,000/well. The viruses were tested at MOI of 10. As control, the cells were infected with only sgRNA virus or cas9 virus. Adding both viruses is expected to lead to GFP knockout. The cells were incubated at 37C overnight in 5% CO2 incubator.

The virus was washed away next morning and fresh media was added and cells were incubated again for 2-3 days.

Substantial decrease in GFP expression was observed 3-4 days later. 6-7 days after transduction, GFP expression completely disappeared in >90% of cells. This was observed using FACS **(Figure 1)** and fluorescent microscopy **(Figure 2)**.

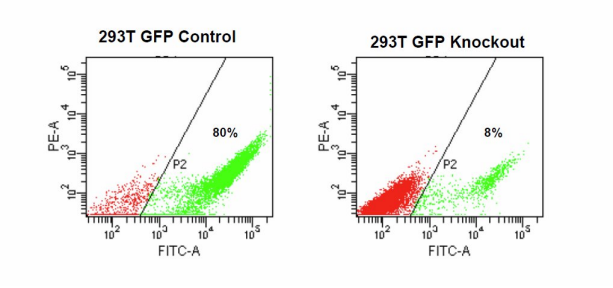

Figure 1 – GFP-expressing 293T cells were transduced with Lenti EF1a-Cas9 and Lenti U6-sgGFP at an MOI of 10. FACS analysis was conducted 7 days after transduction.

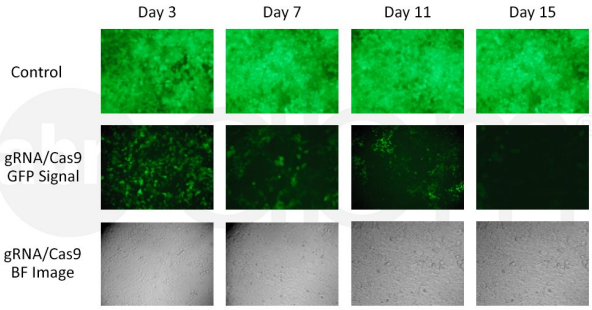

Figure 2 – GFP reporter gene knock-out in 293T cell line using the CRISPR Cas9 system.

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3. *Gene therapy and DNA delivery systems.* **Ibraheem, D, Elaissari, A and Fessi, H.** 1-2, s.l. : International Journal of pharmaceutics, March 17, 2014, Vol. 459.
4. *Gene therapy: trials and tribulations.* **Somia, N. and Verma, I.M.** s.l. : Nature Reviews Genetics, November 11, 2000, PLoS Computational Biology, Vol. 1, pp. 474-483.
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General Materials & Others

PCR, qPCR, and RT
PCR Polymerases
Molecular Biology Enzymes and Kits
RNA Mango
DNA & RNA Purification Kits
Expression Systems
Reagents and Chemicals
Equipment & Lab Consumables
Gels, Blots & ELISAs

SafeView DNA Stains
Cloning Vectors & Packaging Mixes

Antibodies

Secondary Antibodies
Tag Antibodies
Loading Control Antibodies
IHC Antibodies
Monoclonal Antibodies
Polyclonal Antibodies

Cellular Materials

Immortalized Cell Lines
Stable Cell Lines
Tumor Cell Lines
Primary Cells
Growth Factors & Cytokines
Media, Reagents, Kits & Plastics
Viruses for Cell Immortalization
Mycoplasma Detection & Elimination

Custom Services

Cell Biology Services
CRISPR Genome Editing Services
DNA & Cloning Services
Viral Packaging Services
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Genetic Materials

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CRISPR
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