PrecisionX™ Cas9 SmartNuclease Vector System

Catalog#s CAS8/9xx series

User Manual

Store at -20°C upon receipt

A limited-use label license covers this product. By use of this product, you accept the terms and conditions outlined in the Licensing and Warranty Statement contained in this user manual.

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I. Introduction

A. Overview of CRISPR system

In the past decade, a great deal of progress has been made in the field of targeted genome engineering. Technologies such as designer zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs), and homing meganucleases have made site-specific genome modifications a reality in many different model organisms ranging from zebrafish to mammalian cells. Based on the results to date, however, genome editing tools that are efficient, flexible, and cost-effective have remained elusive to the general research community. The recent discovery of the type II prokaryotic CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system, originally discovered in the bacterium *Streptococcus pyogenes* as a mechanism to defend against viruses and foreign DNA, has provided yet another tool for targeted genome engineering, this time taking advantage of a system that uses small RNAs as guides to cleave DNA in a sequence-specific manner. With its ease in designing guide sequences to target specific sequences (unlike ZFNs and TALENs where construct assembly can be laborious and time-consuming), as well as its targeting efficiency, this system has the potential to be a disruptive technology in the field of genome-engineering.

The CRISPR/CRISPR-associated (Cas) system involves 1) retention of foreign genetic material, called “spacers”, in clustered arrays in the host genome, 2) expression of short guiding RNAs (crRNAs) from the spacers, 3) binding of the crRNAs to specific portions of the foreign DNA called protospacers and 4) degradation of protospacers by CRISPR-associated nucleases (Cas). A well-characterized Type II CRISPR system has been

previously described in the bacterium *Streptococcus pyogenes*, where four genes (*Cas9, Cas1, Cas2, Csn1*) and two non-coding small RNAs (pre-crRNA and tracrRNA) act in concert to target and degrade foreign DNA in a sequence-specific manner [Jinek *et. al.* 2012]. The specificity of binding to the foreign DNA is controlled by the non-repetitive spacer elements in the pre-crRNA, which upon transcription along with the tracrRNA, directs the Cas9 nuclease to the protospacer:crRNA heteroduplex and induces double-strand breakage (DSB) formation. Additionally, the Cas9 nuclease cuts the DNA only if a specific sequence known as protospacer adjacent motif (PAM) is present immediately downstream of the protospacer sequence, whose canonical sequence *in S. pyogenes* is 5' - NGG -3', where N refers to any nucleotide.

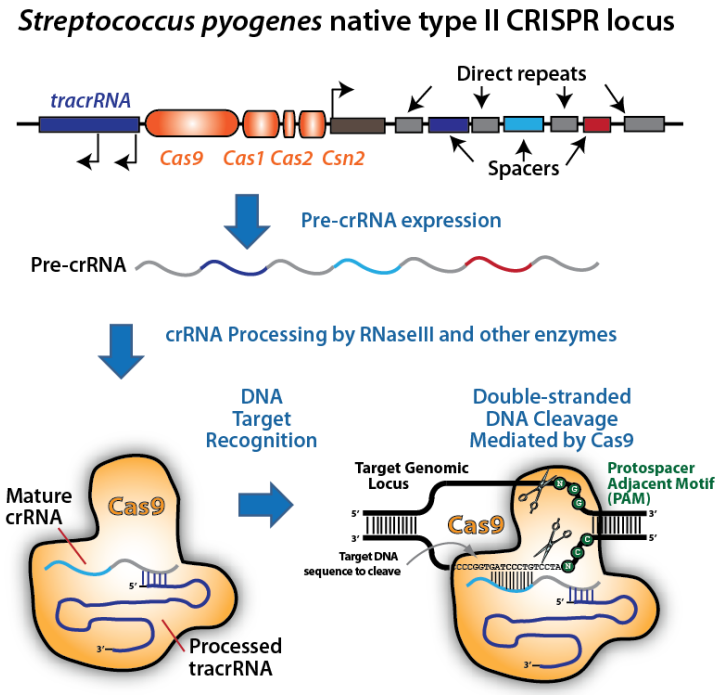


Figure 1: Overview of the CRISPR system. Figure adapted from Cong *et al*. “Multiplex Genome Engineering Using CRISPR/Cas Systems”.

Recently, it has been demonstrated that the expression of a single chimeric crRNA:tracrRNA transcript, which normally is expressed as two different RNAs in the native type II CRISPR system, is sufficient to direct the Cas9 nuclease to sequence-specifically cleave target DNA sequences. By adapting the endogenous type II CRISPR/Cas system in *S. pyogenes* for utility in mammalian cells, several groups have independently shown that RNA-guided Cas9 is able to efficiently introduce precise double stranded breaks at endogenous genomic loci in mammalian cells with high efficiencies and minimal off-target effects [Cong *et al.* 2013, Mali *et al.* 2013, Cho *et al.* 2013].

In addition, several mutant forms of Cas9 nuclease have been developed to take advantage of their features for additional applications in genome engineering and transcriptional regulation Biochemical characterization of a mutant form of Cas9 nuclease (D10A) functions as a nickase (Jinek *et al.* 2012), generating a break in the complementary strand of DNA rather than both strands as with the wild-type Cas9. This allows repair of the DNA template using a high-fidelity pathway rather than NHEJ, which prevents formation of indels at the targeted locus, and possibly other locations in the genome to reduce possible off-target/toxicity effects while maintaining ability to undergo homologous recombination (Cong *et al.* 2013). Recently, paired nicking has been shown to reduce off-target activity by 50- to 1,500 fold in cell lines and to facilitate gene knockout in mouse zygote without losing on-target cleavage efficiency (Ran et al., 2013). Finally, tandem knockout of both RuvCI and HNH nuclease domains (which control cutting of the DNA strands) shows that the null-nuclease mutant (double mutant) can act as a transcriptional repressor (Qi *et al.* 2013) with minimal off-target effects, which leads to possibilities for studying site-specific transcriptional regulation.

Taken together, the RNA-guided Cas9 system defines a new class of genome engineering tools, creating new opportunities for research across basic sciences, biotechnology and biomedicine.

B. Product Information and Vector Maps

To make the RNA-directed Cas9 system more efficient, affordable, and convenient to use, SBI has developed the all-in-one, programmable PrecisionX™ Cas9 SmartNuclease expression system, including a human codon optimized Cas9 (hspCas9) and custom guide RNA (gRNA) consisting of a chimeric crRNA-tracrRNA transcript expressed from a single construct (see vector map, Fig.2). SBI's all-in-one Cas9/gRNA SmartNuclease™ expression constructs include the following features:

1) The hspCas9 (and hspCas9 mutants) used in this system include two nuclear localization signals (NLS) at the N-terminus and C-terminus to ensure efficient import of the hspCas9 protein into the nucleus.

2) The expression vectors also contain a Myc-tag at the N-terminus for ease of detection and purification of the recombinant Cas9 protein.

3) To facilitate diverse applications of the system, hspCas9 may be expressed from a number of different commonly utilized promoters that are active in mammalian cells. (See Table 1).

4) The hspCas9 ORF is followed by a regulatory element called WPRE (Woodchuck virus post-transcriptional regulatory element) to boost gene expression and stabilize the mRNA transcript.

To avoid reconstituting the CRISPR/Cas9 RNA processing machinery, a custom gRNA (crRNA-tracrRNA chimeric transcript)

can be generated from the pre-cut, ready-to-use linearized vectors through the use of annealed oligonucleotide duplexes encoding the 20bp target sequence upstream of PAM, with the gRNA expressed under the control of a robust H1 polymerase III promoter. Our programmable, all-in-one vector format allows for highly flexible targeting of any genomic loci in the form of N_{20}NGG.

Table 1. List of available all-in-one Cas9 SmartNuclease™ expression vectors

Cat#	Description	Size
CAS900A-1	**EF1-hspCas9-H1-gRNA SmartNuclease vector**	**10 µg**
CAS920A-1	**CAG-hspCas9-H1-gRNA SmartNuclease vector**	**10 µg**
CAS940A-1	**CMV-hspCas9-H1-gRNA SmartNuclease vector**	**10 µg**
CAS960A-1	**MSCV-hspCas9-H1-gRNA SmartNuclease vector**	**10 µg**
CAS980A-1	**PGK-hspCas9-H1-gRNA SmartNuclease vector**	**10 µg**
CAS800A-1	**Cas9 Nickase: EF1-hspCas9 (D10A) nickase-H1-gRNA SmartNickase vector**	**10 µg**
CAS820A-1	**Cas9 Nickase: CAG-hspCas9 (D10A) nickase-H1-gRNA SmartNickase vector**	**10 µg**
CAS840A-1	**Cas9 Nickase: CMV-hspCas9 (D10A) nickase-H1-gRNA SmartNickase vector**	**10 µg**
CAS805A-1	**Cas9 Null Nuclease: EF1-hspCas9 DM-H1-gRNA NullNuclease vector**	**10 µg**

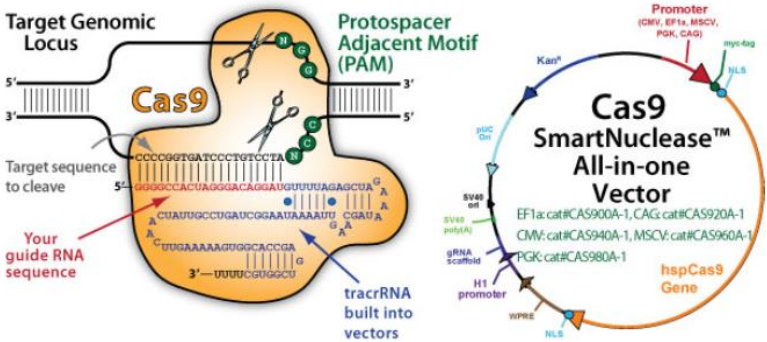


Fig. 2: RNA-directed Cas9 SmartNuclease Expression Vector (Cat # CAS9xxA-1)

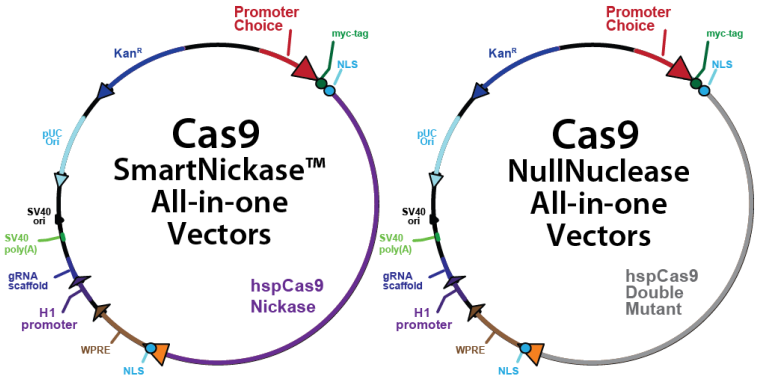


Fig. 3: RNA-directed Cas9 SmartNickase and Double Mutant NullNuclease Expression Vectors (Cat # CAS8xxA-1)

C. Validation Data for the Cas9 SmartNuclease™ System targeting the Human AAVS1 Locus

Since the CRISPR-Cas9 Nuclease system is relatively new and its efficacy has not yet been characterized to the extent of other competing technologies, we have compared head-to-head against

TALENs targeting the well-established human *AAVS1* locus. The TALEN pair (pZT-AAVS1 L1/R1, SBI cat. no. GE601A-1) has been previously validated by Dr. Jizhong Zou of the NIH for cleavage activity and HDR efficiency, with rates of ~25% for cleavage and 8.1% for HDR in 293T cells. Using our Cas9 SmartNuclease Expression System (EF1a version, Fig. 4A), we cloned in a guide RNA sequence per Mali *et al.* and compared its efficacy of cleavage and HDR efficiency to the TALEN pair targeting a stably integrated EGIP (Enhanced Green Fluorescent Inhibited Protein) cell line. This construct contains a stop codon in the middle of the coding region (thus truncation of full-length EGFP) as well as a 53bp sequence from the human *AAVS1* gene (Fig. 4B) for targeting via Cas9 SmartNuclease or TALENs.

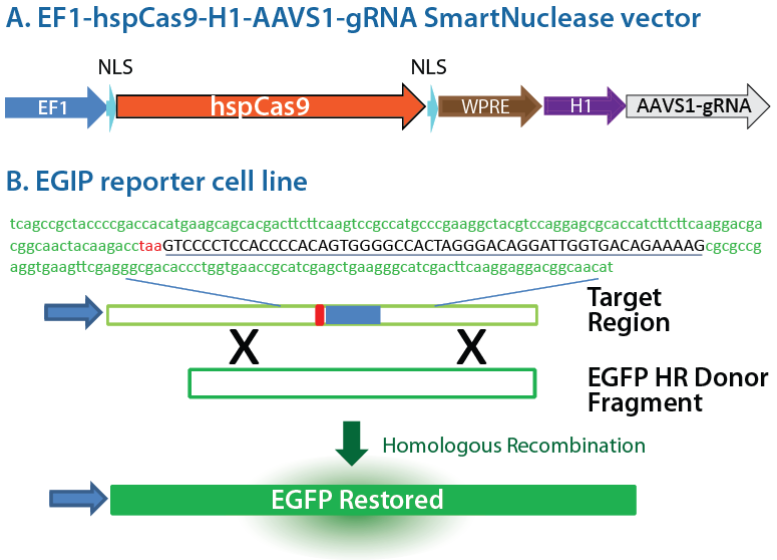


Fig. 4: A) Design of the Cas9 SmartNuclease construct targeting human AAVS1 locus and B) EGIP cell line for monitoring HDR efficiency of donor vector bearing EGFP fragment.

The Cas9 SmartNuclease with guide RNA targeting the AAVS1 Safe Harbor locus is available as **Catalog # CAS601A-1**.

For those new to Cas9 technology, we recommend use of the Cas9 SmartNuclease AAVS1 Positive Control Kit, **Catalog # CAS605A-1**, which includes the CAS601A-1 vector, EGIP 293T reporter line, AAVS1/GFP rescue donor, and primers for Surveyor assay.

The following data were generated using the AAVS1-targeting Cas9 SmartNuclease (Cat# CAS601A-1) and Positive Control Kit (CAS605A-1).

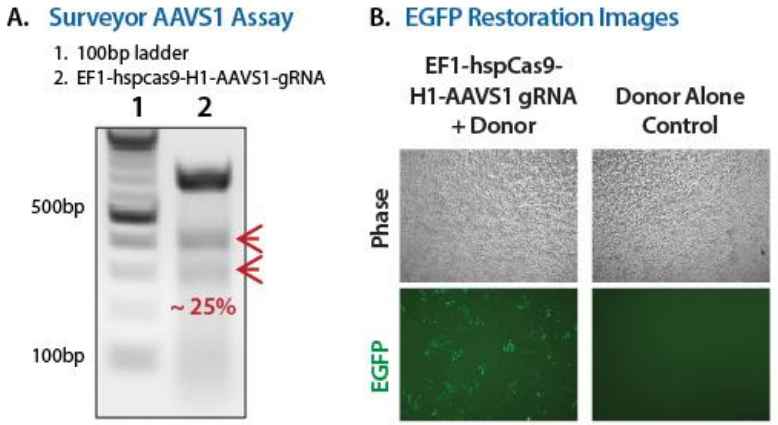


Fig. 5: A) Cleavage efficiency of the Cas9 SmartNuclease targeting the *AAVS1* locus measured by T7 endonuclease assay and B) HDR efficiency of donor EGFP fragment for Cas9 SmartNuclease system vs TALENs (pZT-AAVS1-L1/R1) as measured by GFP positive clones at day 2 (top panel) and 1 week post-transfection (bottom panel) using EGIP cell line.

Acknowledgements:

Design of the pZT-AAVS1-L1/R1 and EGIP 293T stable cell line are kindly provided by Dr. Jizhong Zou of the NIH Center for Regenerative Medicine, a Common Fund initiative of the U.S. National Institutes of Health.

D. Validation Data for the Cas9 SmartNuclease™ Expression System Targeting Luciferase Gene

To further validate our RNA-directed Cas9 SmartNuclease system, we designed and cloned two gRNAs which target the luciferase gene (Fig. 6A). In addition, we designed a donor vector (Fig. 6B) which contains homology sequences flanking the luciferase gene which was stably integrated into a reporter cell line and contains a red fluorescent protein (RFP) sequence. This allows measurement of 1) cleavage activity using either Surveyor Nuclease or luciferase assay and 2) efficiency of a homology-directed event (HDR) by monitoring the presence of RFP signal as the luciferase gene is replaced by homologous recombination.

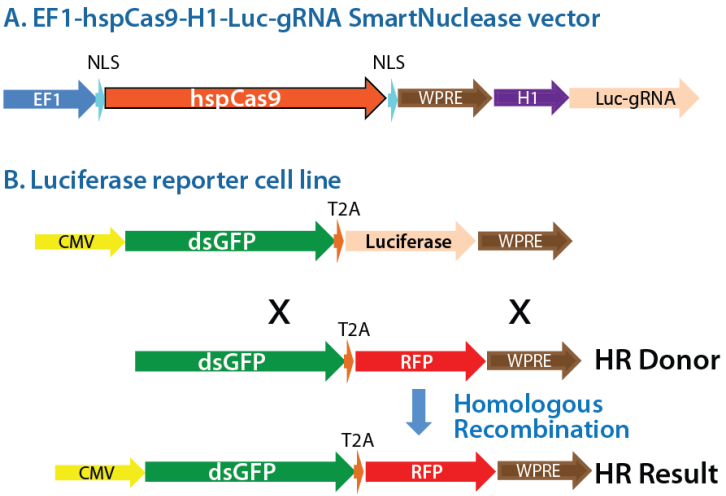


Fig. 6: Design of gRNA and donor vectors targeting a luciferase gene for functional validation of the RNA-directed Cas9 SmartNuclease Expression System.

Based on initial results, we have seen a reduction up to 40% in luciferase levels (Fig. 7A) using one of the gRNAs targeting luciferase (Luc-gRNA1) and ~30% cleavage via the Surveyor Nuclease assay (Fig. 7B), which illustrates the efficacy of the system.

To further demonstrate the utility of the system to effect homology-directed recombination, we show that we can obtain robust HDR efficiency (compared to donor vector only) when using the gRNAs expressed via the all-in-one SmartNuclease expression vector in conjunction with an RFP-bearing donor vector to replace the stably integrated luciferase gene in a reporter cell line (Fig. 8)

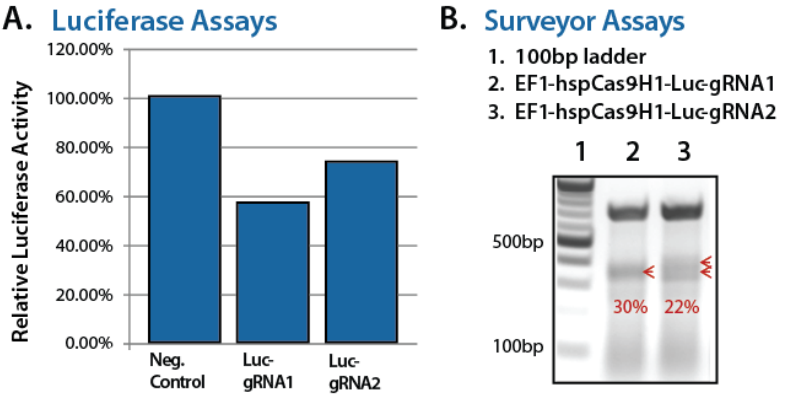


Fig. 7: Validation data showing cleavage efficiency of guide RNAs targeting Luciferase via A) Luciferase assays and B) Surveyor Nuclease Assays

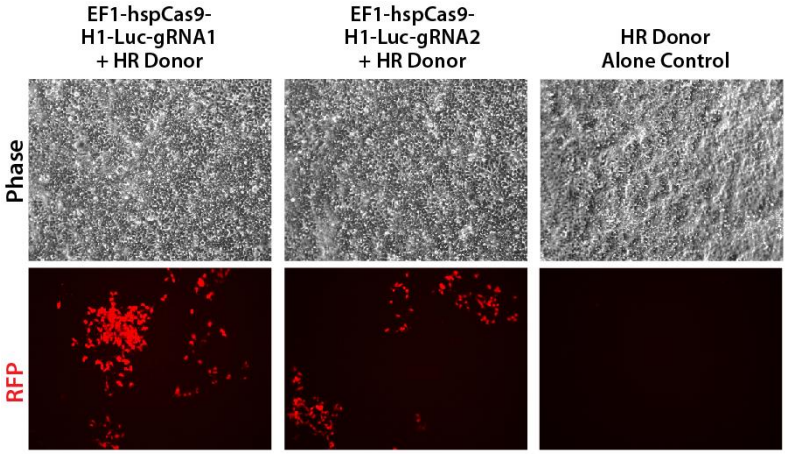


Fig. 8: Homologous recombination efficiencies of RFP donor vector using Luc-gRNA directed Cas9 SmartNuclease system to target Luciferase to RFP recombination (10X magnification).

E. Validation Data for the Cas9 SmartNickase and NullNuclease Vectors

We have validated the activity of the Cas9 Nickase and Null Nuclease (Double-Mutant) for inducing NHEJ compared to wild-type Cas9 nuclease using a validated guide RNA targeting the human AAVS1 locus (Section I.C). The results from the Surveyor Nuclease Assay (Fig. 9) indicates no detectable NHEJ-induced mutations.

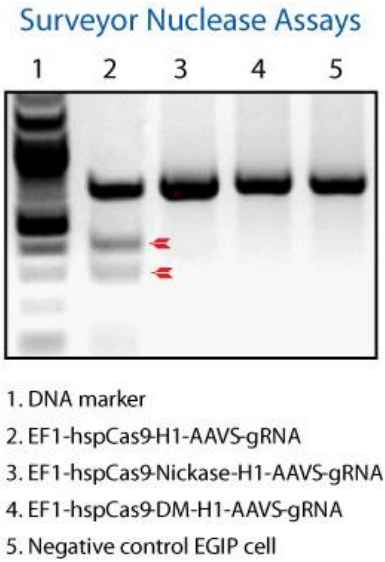


Fig. 9: Surveyor nuclease assay results comparing wild-type Cas9 with nickase and double mutant versions of Cas9

F. Key Advantages of the Cas9 SmartNuclease™ System

- All-in-one vector system combining codon-optimized hspCas9 and gRNA cloning and scaffolding sequences – no need for multiple plasmid constructs
- Cloning vector is fully propagatable, no limits on the number of gRNAs that can be cloned.
- Precise directional cloning of the gRNA insert into vector backbone
- Rapid, highly-efficient cloning with low background (~99% cloning efficiency)
- Multiplexing compatibility – Fully compatible with SBI's Multiplex gRNA cloning kit, allowing multiple gRNA sequences to be cloned into a single vector.

G. Applications of the Cas9 SmartNuclease™ Expression System

We have developed the all-in-one expression system to target a wide range of researchers who are interested in the following (but not limited to) research areas:

- Genome editing and engineering of model organisms
- Synthetic biology applications
- Gene/Cell-based therapy

H. List of Components

Table 2. List of components included in the Cas9 SmartNuclease™ Expression System (including SmartNickase and NullNuclease mutant versions):

Reagent	Amount
Cas9 SmartNuclease Vector	**10 µg**

H. Additional Materials Required

1) LB Agar and Broth containing 50µg/ml Kanamycin
2) Any high-transformation efficiency *E.coli* competent cells
3) Zyppy™ Plasmid MiniPrep Kit (Zymo Research, Cat. # D4019)
4) Qiagen EndoFree Plasmid Maxi Kit (Qiagen, Cat. # 12362)
5) PureFection Transfection Reagent (System Biosciences, Cat #LV750A-1) or equivalent

I. Related Products

SBI offers a number of Homologous Recombination (HR) Donor Vectors, including the popular piggyBac HR Donor for seamless excision: Cat# PBHR100A-1. The full selection of HR Donor vectors may be viewed on the following webpage:

http://www.systembio.com/genome-engineering-precisionx-HR-vectors/ordering.

J. Shipping and Storage Conditions for Kit

PrecisionX™ Cas9 SmartNuclease Expression System are shipped on room temp, blue ice or dry ice. Upon receiving, store the kit at -20°C. Shelf life of the product is 1 year after receipt if stored in -20°C.

II. Protocol for the Cas9 SmartNuclease™ Expression System

A. Quick Overview of the Protocol

The general workflow of the cloning, validation, and transfection of the gRNA/Cas9 SmartNuclease expression construct into cells is depicted in Fig. 10. Briefly, here are the steps involved in the process:

1) Design two DNA oligonucleotides that are sense and antisense sequences of the target DNA which is 20bp upstream of the PAM (5' - NGG - 3')

2) Anneal the two oligonucleotides to generate a duplex

3) Clone the duplex into a linearized Cas9 vector by ligation reaction

4) Transform into competent cells and grow in LB/ Kanamycin plate (50 µg/ml)

5) Confirm positive clones by direct sequencing

6) Transfect sequence-verified all-in-one construct into mammalian cells using standard transfection protocols

7) Perform Surveyor Nuclease assay (or other suitable mismatch cleavage assays) to check the site-specific genome cleavage, or perform homology recombination assays for genome modification using a suitable donor vector.

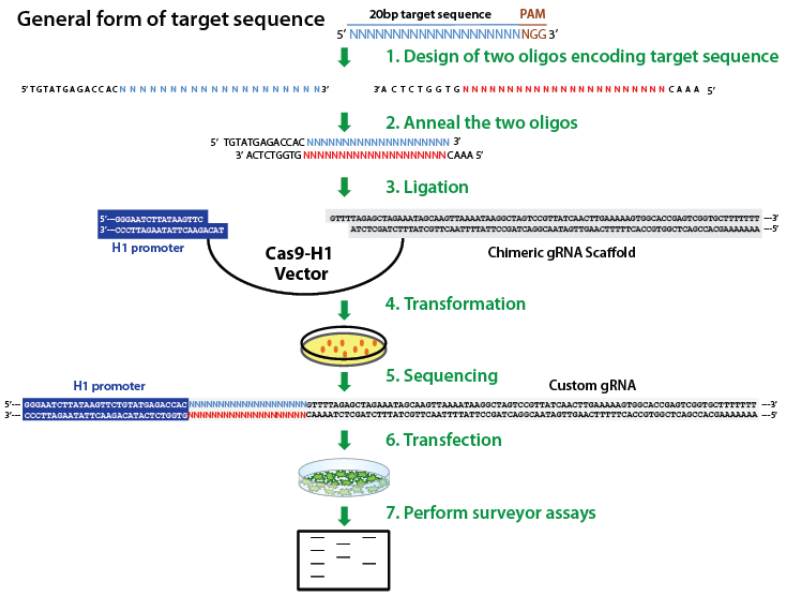


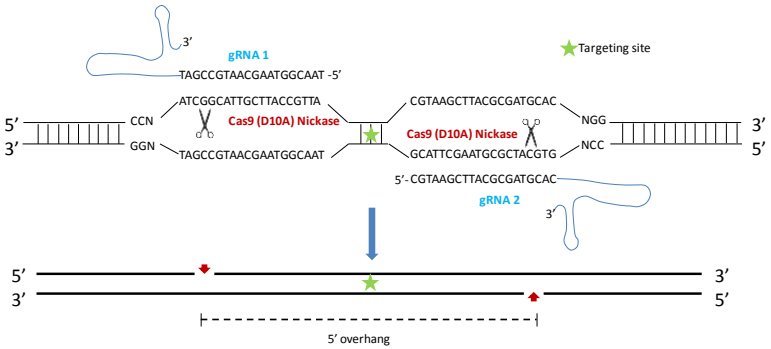
Fig. 10: General Workflow for RNA-Guided Cas9 SmartNuclease™ Expression System.

B. Selection of Target DNA Sequence

The selection of the target DNA sequence is not limited by any constraints, with exception of a PAM sequence in the form of 5' - NGG - 3' (where N = any base) immediately following the target sequence. The typical length of the target sequence is 20bp – as shown here:

5’ NNNNNNNNNNNNNNNNNNNNGG 3’

In order to enhance genome editing specificity, paired gRNA with hspCas9 (D10A) SmartNickase (CAS800A-1, CAS820A-1, CAS840A-1) can be used to generate double nicking with 5’ overhang. Please follow the guideline below for paired gRNA selection and design.



Choose your gRNA1 from the anti-sense strand upstream of your targeting site
Choose your gRNA2 from the sense strand downstream of your targeting site

Fig. 11: Schematic illustration of generating 5’ overhang double strand DNA breaks using paired gRNAs with hspCas9 (D10A) Nickase.

Please note that only gRNA pairs creating 5’ overhangs with less than 8bp overlap between the guide sequences were able to mediate detectable indel formation (Ran et al. 2013). To achieve high cleavage efficiency using Cas9 Nickase with paired gRNAs, make sure each gRNA is able to efficiently induce indels when coupled with wide-type Cas9.

C. Design of Guide RNA Oligonucleotides

Design two DNA oligonucleotides (a top strand and a bottom strand) according to the following structure shown below.

5’ TGTATGAGACCACNNNNNNNNNNNNNNNNNNN 3’
3’ ACTCTGGTGNNNNNNNNNNNNNNNNNNNCAAA 5’

The top strand has a TGTATGAGACCAC overhang at its 5’end, followed by the selected target sequence. The bottom strand has an AAAC overhang at its 5’ end followed by a target sequence complementary to the top strand and a GTGGTCTCA overhang at its 3’ end.

Example:

If your target sequence is AGCGAGGCTAGCGACAGCATAGG (AGG = PAM sequence), then the oligo sequences would be:

Top strand oligo:

5’ – TGTATGAGACCACAGCGAGGCTAGCGACAGCAT - 3’

Bottom strand oligo:

5’ – AAACATGCTGTCGCTAGCCTCGCTGTGGTCTCA - 3’

D. Cloning into the Cas9 SmartNuclease™ Vector

1) Anneal the two single-strand DNA oligonucleotides:

Dilute your stock primers to 10uM using 1x Annealing Buffer (10mM Tris pH7.5, 50mM NaCl, 1mM EDTA) and set up the annealing reaction as follows:

Materials	Amount
10uM Top strand oligo	5 µl
10uM Bottom strand oligo	5 µl
Total volume	10 µl

Incubate reaction mixture at 95°C for 5 minutes (can be done in PCR machine). Remove the tube and leave it on bench at room temperature to cool down to RT.

Alternatively, you can set a thermocycler program to cool down the oligos at a rate of 1°C/min (will take ~40min to 60min to complete)

2) Ligation of Oligo Duplex into Vector

a) Linearize the Cas9 H1 vector by BsaI
b) Set up the ligation reaction for linearized vector and Annealed oligo mix based on manual facture's protocol.

3) Transformation Reaction

a. Add a vial of competent cells to the ligation mix
b. Place cells on ice for 15 minutes
c. Heatshock cells at 42°C for 50 seconds, then immediately transfer cells to ice for 2 minutes
d. Add 250 µl SOC medium and incubate at 37°C for 1 hour with shaking
e. Spread 100 µl of cultured cells on a pre-warmed LB plate containing 50 µg/ml Kanamycin and incubate overnight at 37°C

4) Confirmation of Positive Clones

a. Pick 1 to 2 colonies, grow in LB/Kanamycin medium overnight at 37°C with shaking
b. Next day, miniprep plasmid DNAs and send for sequencing using the forward sequencing primer (not provided) (**5' GTCATCAACCCGCTCCAAGG 3'**)
c. Align your raw sequencing data with the top strand primer sequence.

E. Transfection of the Cas9 SmartNuclease™ Construct into Target Cells

1) Plate 100,000 to 200,000 of target cells (e.g. 293T cells) into a single well of a 12-well plate in 1 ml of appropriate growth medium. Include a single well of cells as negative control (which can be non-relevant plasmid DNA or linearized Cas9 SmartNuclease plasmid DNA)

2) Next day, or when cells are 50-60% confluent, transfect target cells with the Cas9 SmartNuclease vector (and donor vector if HDR is desired) using a suitable transfection reagent following the manufacturer's recommended protocol for 6-well plates. The use of reduced or serum-free media containing no antibiotics to dilute the vector/transfection complex is highly recommended.

   Note: For 293T cells, we transfected 0.5 µg of the Cas9 SmartNuclease vector into cells for cleavage of target luciferase gene (Fig. 4A/B) and used a 1:1 ratio of Cas9 SmartNuclease vector (0.5 µg) and HR donor vector (0.5 µg) for HDR applications (Fig. 5). We have also used a 1:1.5 ratio in 293T cells with good results. In general, we suggest optimizing the amounts and ratios of Cas9 SmartNuclease and donor vectors for optimal results in a target cell line.

3) Allow at least 12 hours before changing transfection media to complete growth media

4) Assay for cleavage activity using Surveyor Nuclease, mutation characterization by genotyping analysis, or HDR activity (if using donor vector in parallel) 48-72 hours after transfection

5) If assaying for HDR of donor vector, select cells with

targeted insertion of donor vector using FACS-based sorting of fluorescent marker or antibiotic selection (e.g. Puro/Neo) using a suitable concentration of antibiotics for the targeted cell line.

III. Frequently Asked Questions

Q. How many guide RNA constructs do you have to design to target a DNA sequence of interest?

Due to the unpredictable efficacy of a particular guide RNA construct, for optimal results we suggest designing multiple (2 or more) constructs targeting a particular DNA sequence of interest. By designing several constructs (following the simple design rules outlined in Section II. B and C), one has increased chances of finding a construct(s) to cleave target DNA with the highest efficiency.

Q. We designed a guide RNA construct to transfect into target cells and there is no evidence of activity. What are the possible reasons for this?

There are many possibilities of why a particular guide RNA does not show any measureable effects. Some of the possibilities include the following:

1) **Poor transfection efficiency of target cells:** For certain cell types (e.g. primary, stem, suspension cells), passive transfection may not be very efficient. In these cases, active transfection systems (e.g. NucleoFection) may provide better results.

2) **Errors in guide RNA design:** The sequences of oligo duplexes targeting the DNA should be carefully checked to follow design rules.

3) **Mutation(s) in DNA sequence targeted:** In certain cases, the DNA sequence targeted may contain mutations which affect recognition of the gRNA sequence, leading to failure of cleavage. It is difficult to know in advance, but if it happens repeatedly, it may be necessary to follow-up with another gRNA sequence or perhaps sequence-verifying the genomic target prior to design of gRNA constructs.

4) **Length of Time Before Assaying**: We suggest a minimum of 48 hours post-transfection to begin assaying for cleavage of a DNA target; however, in certain cases, it may be useful to wait up to 1 week to observe the full effect of cleavage.

Q. We want to perform HDR applications using the Cas9 SmartNuclease system, but we do not have the corresponding donor vectors. What are our options in this case?

There are several options for performing HDR of a donor vector into cells that have been targeted with the Cas9 SmartNuclease system.

Option #1 – Design an HDR donor vector containing the region of DNA to be inserted or corrected into target cells. Typically, this vector contains 5' and 3' arms homologous (~800bp) to the desired insert/correction region, and may contain selection or fluorescent markers for selection of cells after HDR.

Option #2 – SBI provides a full suite of off-the-shelf HDR cloning vectors containing multiple MCS for cloning in of homology arms and insert sequences, as well as selectable fluorescent and antibiotic selection markers. Please inquire for availability of these vectors.

Option #3 – SBI can build a custom HR donor vector targeting any sequence of interest as part of our custom cloning services. Please inquire with services AT systembio.com to discuss a custom project or request a quotation.

IV. References

Carr PA, Church GM. Genome engineering. Nat Biotechnol. 2009 Dec;27(12):1151-62. doi: 10.1038/nbt.1590

Bhaya D, et al. CRISPR-Cas systems in bacteria and archaea: versatile small RNAs for adaptive defense and regulation. Annu Rev Genet. 2011;45:273-97. doi: 10.1146/annurev-genet-110410-132430.

Terns MP, Terns RM. CRISPR-based adaptive immune systems. Curr. Opin. Microbiol. 14, 321 (2011) Curr Opin Microbiol. 2011 Jun;14(3):321-7. doi: 10.1016/j.mib.2011.03.005. Epub 2011 Apr 29.

Makarova KS et al. Evolution and classification of the CRISPR-Cas systems. Nat Rev Microbiol. 2011 Jun;9(6):467-77. doi: 10.1038/nrmicro2577. Epub 2011 May 9.

Wiedenheft B, et al., RNA-guided genetic silencing systems in bacteria and archaea. Nature. 2012 Feb 15;482(7385):331-8. doi: 10.1038/nature10886.

Jinek M, et al. A programmable Dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012 Aug 17;337(6096):816-21. doi:10.1126/science.1225829. Epub 2012 Jun 28.

Barrangou R. RNA-mediated programmable DNA cleavage. Nat Biotechnol. 2012 Sep;30(9):836-8. doi: 10.1038/nbt.2357.

Mali P, et al. RNA-guided human genome engineering via Cas9. Science. 2013 Feb 15;339(6121):823-6. doi: 10.1126/science.1232033. Epub 2013 Jan 3.

Cong L, et al. Multiplex genome engineering using CRISPR/Cas systems. Science. 2013 Feb 15;339(6121):819-23. doi: 10.1126/science.1231143. Epub 2013 Jan 3.

Jinek M, et al. RNA-programmed genome editing in human cells. Elife. 2013;2:e00471. doi: 10.7554/eLife.00471. Epub 2013 Jan 29

Qi LS et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell. 2013 Feb 28;152(5):1173-83.

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