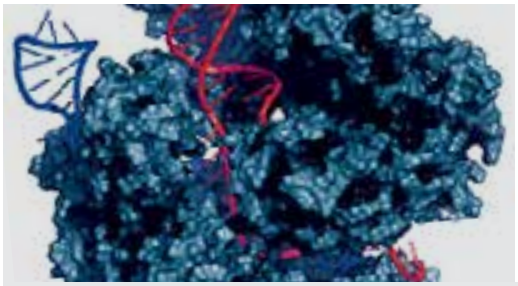
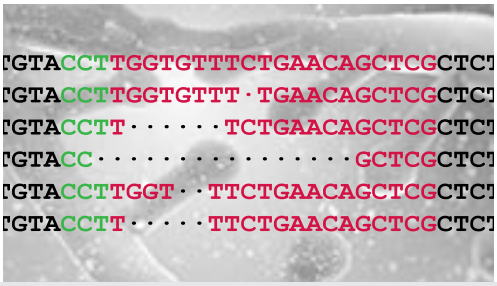
bioNova científica, s.l.

CRISPR/Cas9

CATALYZING CHANGE: GENOME ENGINEERING WITH CRISPR/Cas9





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SBI System Biosciences
Harnessing innovation to drive discoveries

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A SMART CHOICE FOR GENOME ENGINEERING

These days, it seems like CRISPR/Cas9[1,2] is everywhere. A simple yet powerful technology, CRISPR/Cas9 is already changing the way researchers are doing biology, and the work is only just beginning. But with so many companies flooding the market with products and services, how do you know which one is right for you? Because experience matters, we think your best choice is System Biosciences (SBI). We've been selling CRISPR/Cas9 systems longer than any other commercial vendor in the market, giving us an edge when it comes to direct experience with this game-changing technology. With a focus on easy-to-use systems that are as targeted as possible, we've been helping researchers successfully engineer genomes since our first sale in April 2013.

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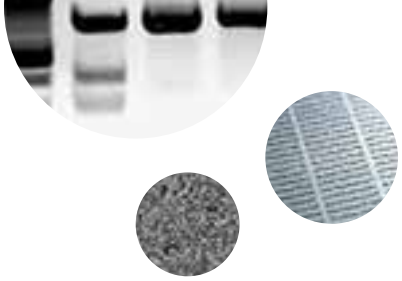


04

LEARN

Learn about genome engineering with the CRISPR/Cas9 system

06

USE

Everything you need for efficient, targeted genome engineering using CRISPR/Cas9

PrecisionX™ Cas9 SmartNuclease™, SmartNickase™

- Plasmids
- Injection-ready mRNA
- Multiplex gRNA
- Lentiviral vectors

HR Donors to mediate

- Knock-outs
- Knock-ins
- Gene editing
- Gene tagging

10

OUTSOURCE

Save time and resources with genome services from the experts that create our high-quality products.

- Custom gRNA design and cloning
- Custom homologous recombination (HR) donor design and cloning
- Custom cell line engineering—knock-outs, mutation correction or addition, and more

Efficient and successful—up to a 75% mutation rate was recently achieved at the NIH using SBI's Cas9 SmartNuclease system for direct genome editing in mouse zygotes, with 90% biallelic mutations

Genome Editing With CRISPR/Cas9 system

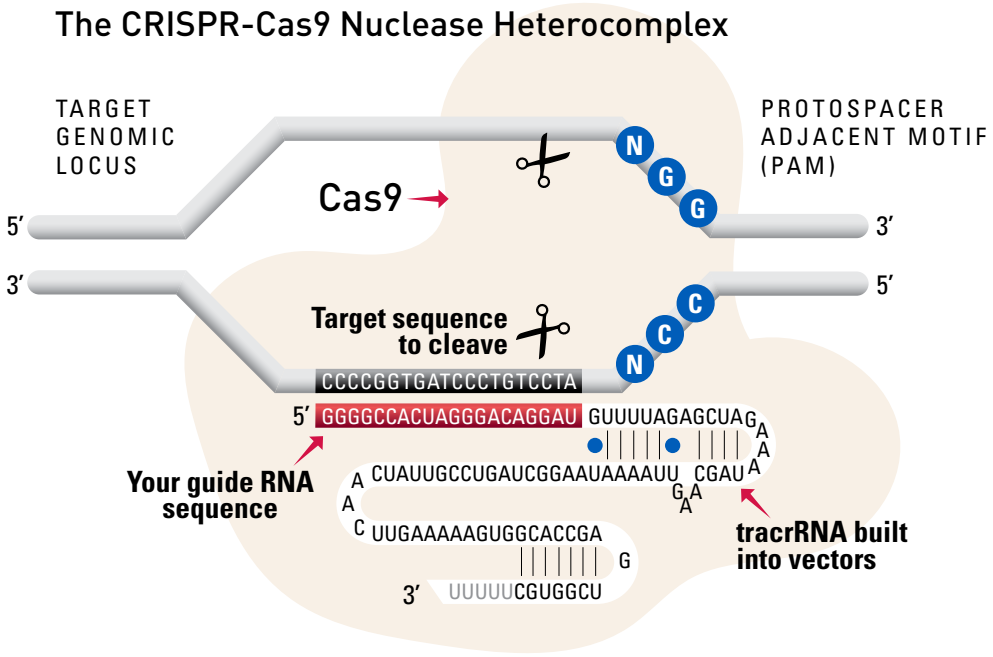
Through careful selection of the target sequence and design of a donor plasmid for homologous recombination, you can achieve efficient and highly targeted genomic modification.

GENE KNOCK-OUTS / GENE KNOCK-INS / GENE EDITING / GENE TAGGING

Cas9 protein—uses guide RNA (gRNA) to direct site-specific, double-strand DNA cleavage adjacent to a protospacer adapter motif (PAM) in the target DNA.

gRNA—RNA sequence that guides Cas9 to cleave a homologous region in the target genome. Efficient cleavage only where the gRNA homology is adjacent to a PAM.

PAM—protospacer adapter motif, NGG, is a target DNA sequence that Cas9 will cut upstream from if directed to by the gRNA.



WORKFLOW

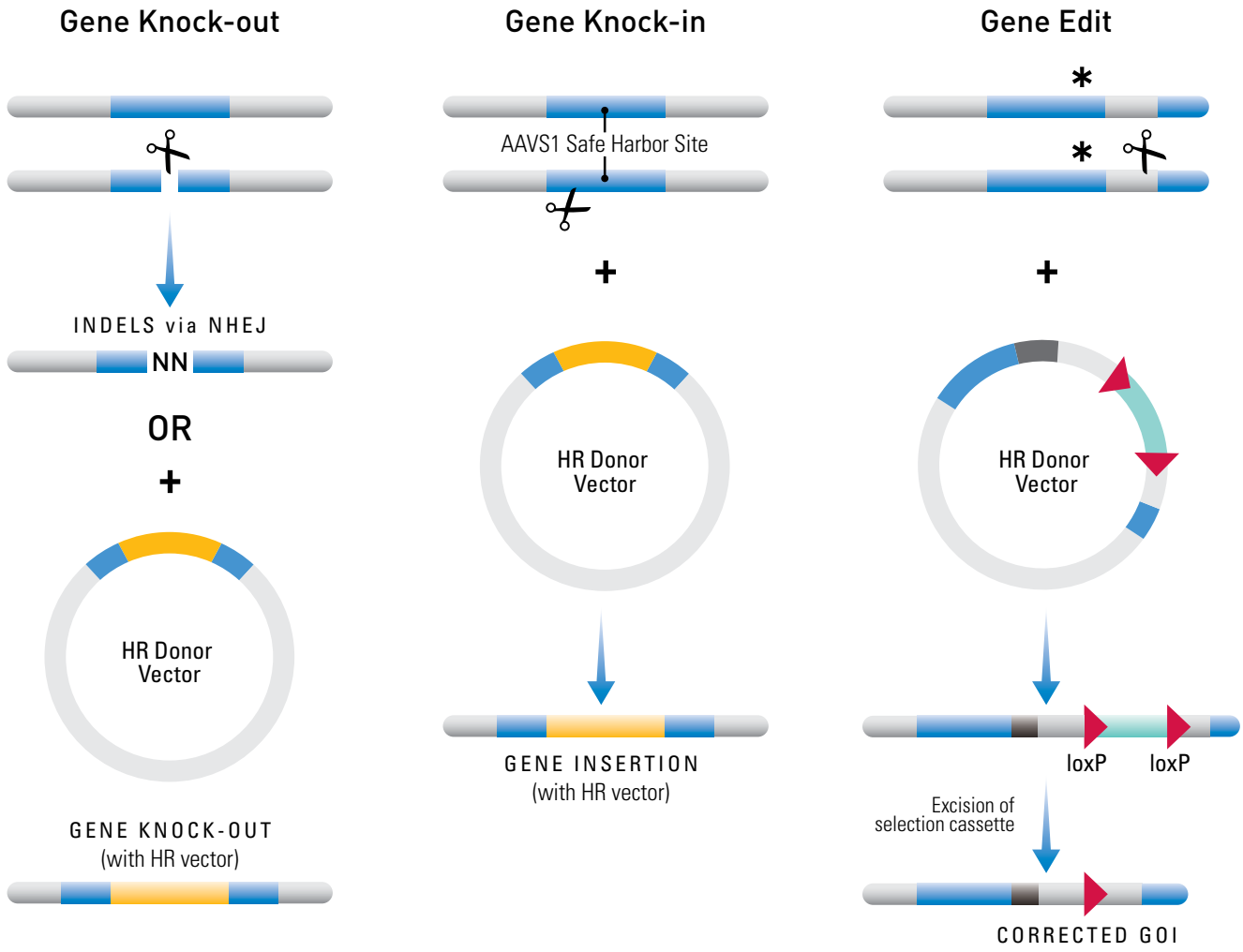
DESIGN: Select gRNA and HR donor plasmids. Choice of gRNA site and design of donor plasmid determines whether the homologous recombination event results in a knock-out, knock-in, edit, or tagging.

CONSTRUCT: Clone gRNA into all-in-one Cas9 vector. Clone 5′ and 3′ homology arms into HR donor plasmid. If creating a knock-in, clone desired gene into HR donor.

CO-TRANSFECT or **CO-INJECT:** Introduce Cas9, gRNA, and HR Donors into the target cells using co-transfection for plasmids, co-transduction for lentivirus, or co-injection for mRNAs.

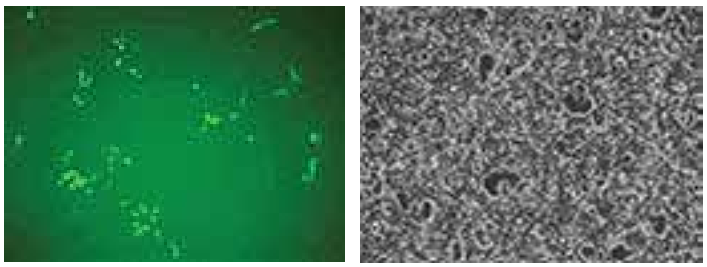
SELECT/SCREEN: Select or screen for mutants and verify.

VALIDATE: Genotype or sequence putative mutants to verify single or biallelic conversion.



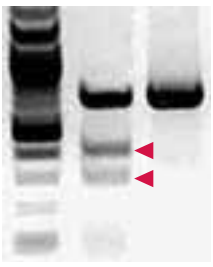
HR Integration at the AAVS1 Safe Harbor Site

EF 1-hspCas9-H1-AAVS-gRNA



GFP Fluorescence | Bright Field Phase

Surveyor Nuclease Assays



1 2 3

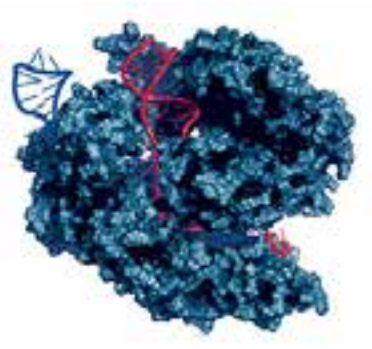
1. DNA marker
2. EF 1-hspCas9-H1-AAVS-gRNA
3. Negative control

Gene knock-in at the adeno-associated virus (AAV) safe harbor site in 293T cells using a GFP-expressing HR donor. GFP expression (left panel) and surveyor nuclease assays (right panel) show efficient knock-in and target site cleavage.

EFFICIENT, EASY-TO-USE CRISPR/Cas9 SYSTEMS

Use any of our CRISPR/Cas9 Systems for any genome engineering application:

- Gene knock-outs
- Gene knock-ins
- Gene editing
- Gene tagging



Free phone consultation with the purchase of any PrecisionX product.

Check the website to see the most up-to-date selection of Cas9 SmartNuclease, SmartNickase and null SmartNuclease plasmids—visit:

systembio.com/cas9-plasmids

With a focus on simplifying genome engineering, the team at SBI has created a wide range of options for delivering both Cas9 protein and gRNA. Human codon-optimized and with your choice of different mammalian promoters, you can deliver wild-type or mutant Cas9 and custom gRNA as plasmids, mRNA, lentivirus vectors, and, starting summer 2015, via adeno-associated virus (AAV) vectors.

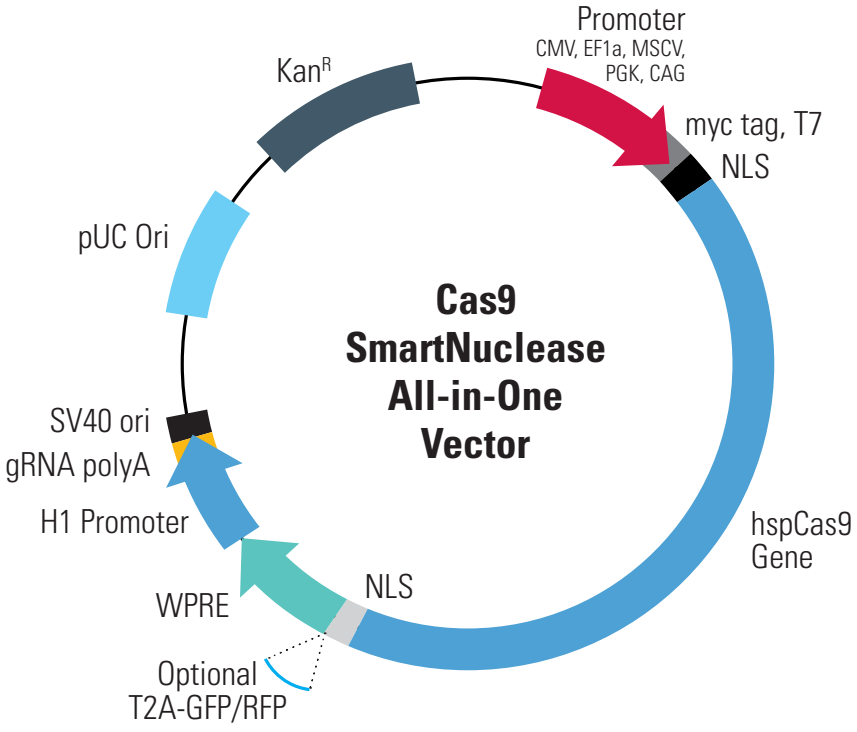
Wild-type or mutant—which Cas9 is right for you?

Wild-type PrecisionX Cas9 SmartNuclease generates double-strand breaks (DSBs), and can be used with an HR donor plasmid for efficient, targeted genome engineering.

The **mutant PrecisionX Cas9 SmartNickase** (D10A mutation) creates nicks in genomic DNA instead of DSBs. Creating nicks favors the higher-fidelity homologous recombination process over non-homologous end joining (NHEJ), with paired nicking shown to reduce off-target activity by 50- to 1,500 fold in cell lines, and to facilitate gene knockout in mice without losing on-target cleavage efficiency.[3]

SBI also offers a **null PrecisionX Cas9 SmartNuclease** (D10A and H840A mutations) for experimental controls. This mutant has both nuclease and nickase activities completely inactivated. *Learn more about genome engineering with SBI—**email tech@systembio.com***

Plasmid Vectors



Microinjectable mRNAs

To make the RNA-directed Cas9 system more efficient and convenient for in vivo applications, SBI has developed an injection-ready CRISPR/Cas9 mRNA system:

- Functionally-validated Cas9 mRNA – ready to use
- T7 gRNA cloning vector
- T7 gRNA production kit

Multiplex mutation generation

Enable precise deletion of defined genomic segments and control of multiple genes with SBI's PrecisionX Multiplex gRNA Cloning Kit—genome manipulation made simple.[4]

- Edit multiple loci at once, save time and reagents
- Easily generate multi-cistronic gRNA expression constructs
- Ideal for Cas9 Nickase applications

Cas9 Lentivectors

With SBI's Lenti-Cas9 SmartNuclease system, you can more easily perform genome engineering on cell types that are difficult to transfect with plasmids and/or create stable Cas9-producing cell lines. Choose from our all-in-one Lenti-Cas9 format that expresses both Cas9 and gRNA from a single construct, or a two vector system with separate Cas9 and gRNA expression vectors. *Find the most up-to-date list of Lenti-Cas9 SmartNuclease vectors at* ***systembio.com/cas9-lentivirus***

Detect Cas9 vectors or proteins with ready-to-go PCR primers or Cas9 antibody—wt or mutant. Learn more at ***systembio.com/detect-cas9***

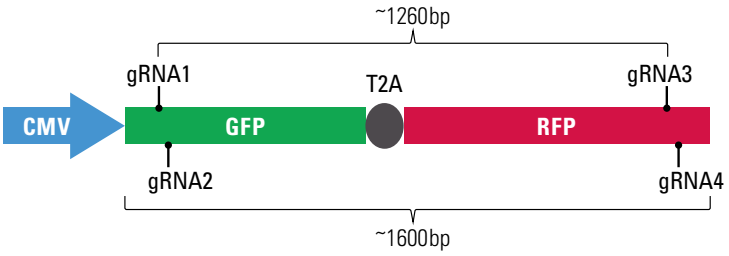
See how UC Davis scientist Angus Lee has used SBI's injection-ready Cas9 mRNA system for generating mouse models for the Mouse Biology Program at UC Davis—read the case study at *systembio.com/angus-lee-case-study.*

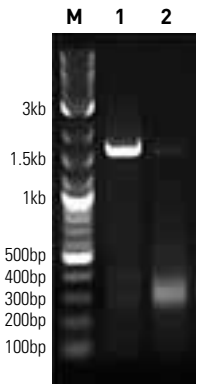
Lee, AY and Lloyd, KC. Conditional Targeting of Ispd Using Paired Cas9 Nickase and a Single DNA Template in Mice. *FEBS Open Bio.* 2014; **4**:637–642. PMCID: PMC4141200.

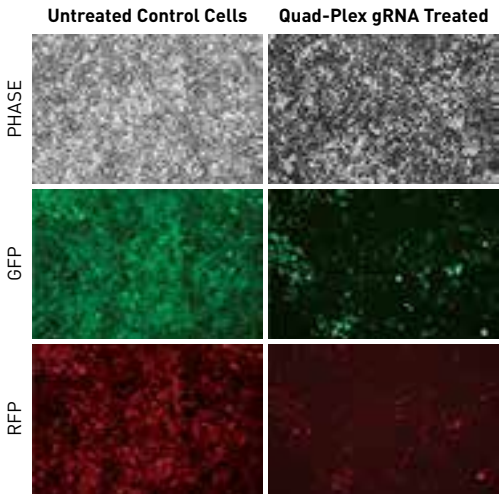
Sample Quad-Plex gRNA Targeting Data

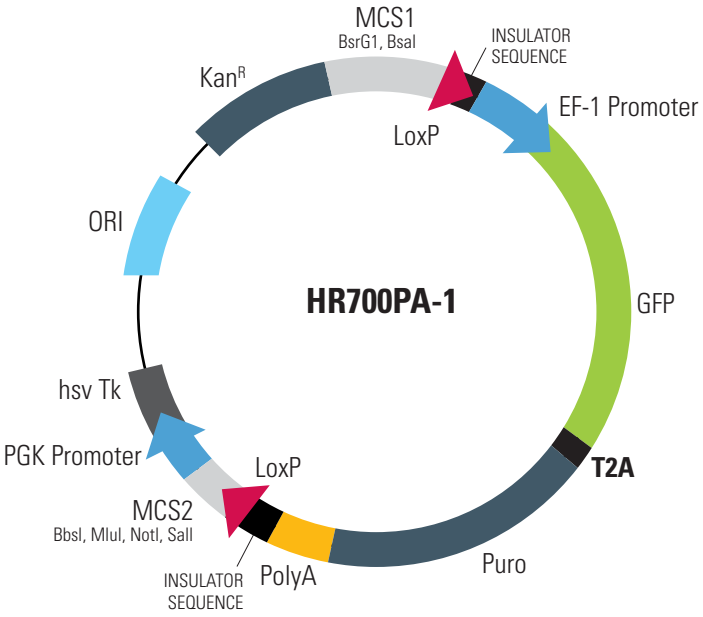
SBI's Multiplex gRNA cloning kit enables efficient removal of a 1260 bp GFP-T2A-RFP segment from a cell line with a stably integrated CMV-GFP-T2A-RFP expression cassette. We cloned four gRNAs into a Cas9 SmartNickase vector (EF1Nickase-H1-gRNA) to guide two double nicking events—one at the 5' end of the GFP and the other at the 3' end of the RFP gene. (Left panel) PCR assays with primers just outside of the GFP and RFP genes generate a 1600 bp fragment in the absence of the SmartNickase vector (lane 1), and a 340 bp fragment in the presence of the Cas9 SmartNickase-4 gRNA construct (lane 2), demonstrating the efficiency of SmartNickase-mediated paired double-nicking and GFP-T2A-RFP genomic deletion. (Right panel) Deletion of both GFP and RFP activities can also be seen in a functional assay, through reduction in both GFP and RFP fluorescence.

Learn more about our multiplex Cas9 systems at ***systembio.com/multiplex-grna***









HR DONORS

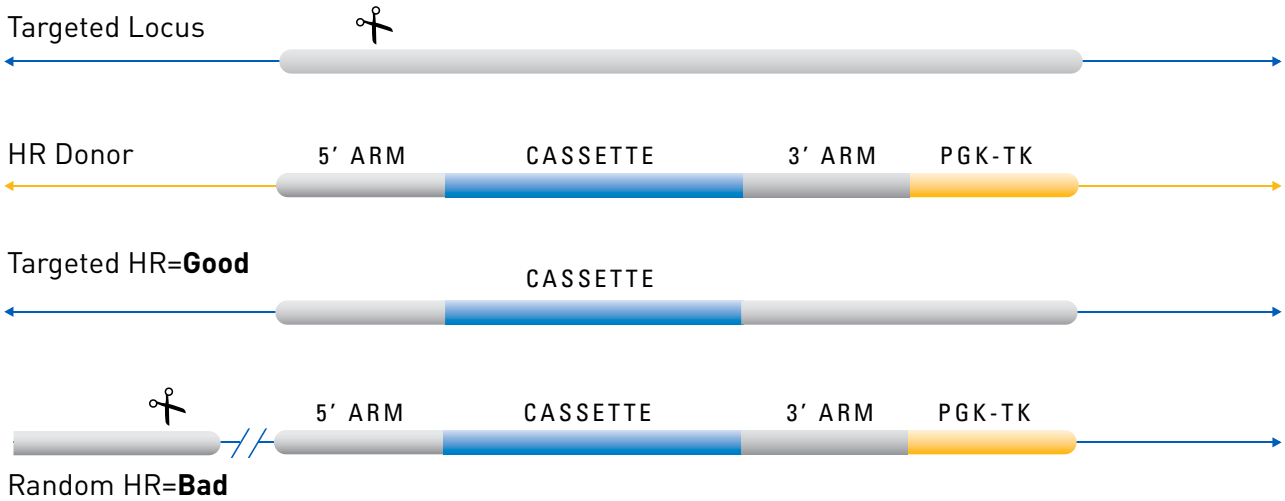
Even though gene knock-outs can result from double strand breaks (DSBs) caused by Cas9 alone, SBI recommends the use of HR donor plasmids for more efficient and precise mutation. HR donors can supply elements for positive or negative selection ensuring easier identification of successful mutation events. In addition, HR donors can include up to 6-8 kb of open reading frame for gene knock-ins or tagging, and, when small mutations are included in either 5′ or 3′ homology arms, can direct specific, targeted gene edits.

Ho, TT, *et al.* Targeting non-coding RNAs with the CRISPR/Cas9 system in human cell lines. *Nucleic Acids Res.* 2015 February 18; **43**(3): e17. PMCID: PMC4330338.

Most of SBI's HR donor plasmids come with two multiple cloning sites (MCS) for inserting the 5′ and 3′ homology arms, loxP sites for excision of the HR vector after integration, insulator sequences for maximal expression, and a range of promotors, GFP or RFP options, and positive and/or negative selection elements.

The HR100PA knock-in plasmid contains two additional MCS sites for insertion of the knock-in gene.

*Our newest HR donors—**OnTarget**™ **HR Donors**—include a PGK-hsvTK cassette outside of the homology arms, which can be used as a powerful selection marker against unwanted random integration events.*



Cells with random HR are selected out using the built in PGK-TK negative selection marker. Minimize your risk of unwanted integration events.

Gene Knock-outs

Choose a target site close to the start codon for maximal coding sequence disruption.

KO HR donors:
HR110PA, HR210PA, HR410PA, HR510PA, HR700PA, HR710PA, HR720PA

Gene Knock-ins

Choose a specific location in the genome such as a "safe harbor" locus[5] for stable expression with minimal context-dependent effects.

KI HR donors:
HR100PA, GE602A, GE603A (positive control for GE602A), HR600PA

Gene Edits

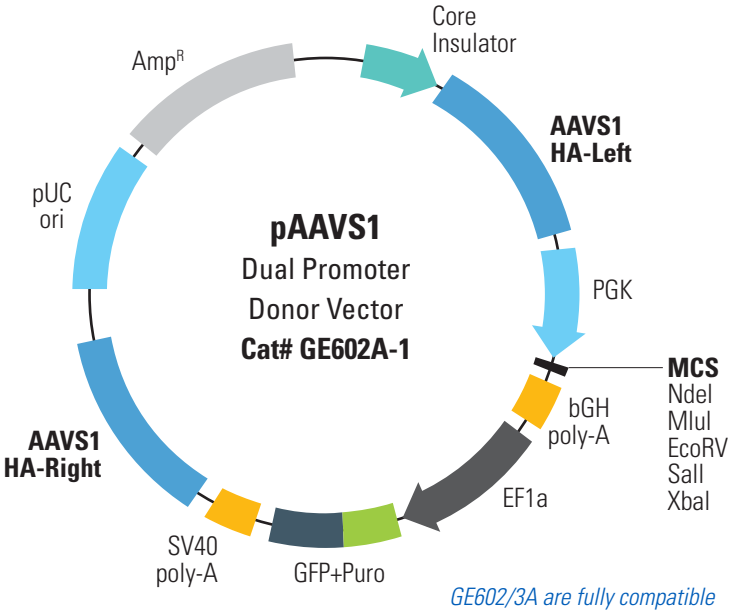
Choose a target site within an intron adjacent to the desired single nucleotide edit. Be sure not to disrupt the splice acceptor or splice donor sites. The edit will be included in either the 5′ or 3′ homology arm of the HR donor.

Gene editing HR donors:
PBHR100A, HR110PA, HR210PA, HR410PA, HR510PA, HR700PA, HR710PA, HR720PA

Gene Tagging

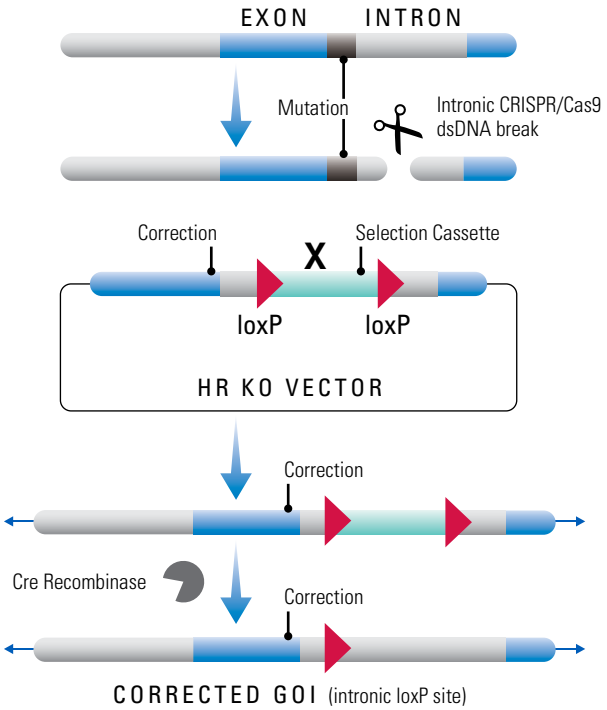
Choose a target site that creates an in-frame insertion towards the end of the coding region to create a protein fusion, or use a T2A or IRES element for a co-expressed marker under control of the endogenous promoter.

Gene tagging HR donors:
HR120PA, HR130PA, HR150PA, HR180PA, HR220PA



GE602/3A are fully compatible with Cas9 products.

Application—Gene Editing



GENOME ENGINEERING SERVICES

When you need to focus on your research and don't have the time to spend on designing genome engineering strategies and vectors, or cloning and cell line engineering, SBI offers an array of services conducted by the same experts that create our products.

Experienced team with dozens of successful genome engineering projects completed

- Well-versed in the latest techniques for efficient and effective CRISPR/Cas9 genome engineering
- Well-equipped with SBI's high-quality genome engineering products

State-of-the-art facility in Mountain View, CA

- All services completed on-site
- Ensures consistent quality, confidentiality, and timeliness of delivery

"In a recent in-house experiment to knock-out miR-21 in HCT116 cells using PrecisionX Cas9 SmartNuclease with an HR donor, we obtained a high rate of bi-allelic modification (7/34), demonstrating the power of the Cas9 SmartNuclease system when coupled with an HR donor."

Custom gRNA design and cloning (Cat# CS700A-1)

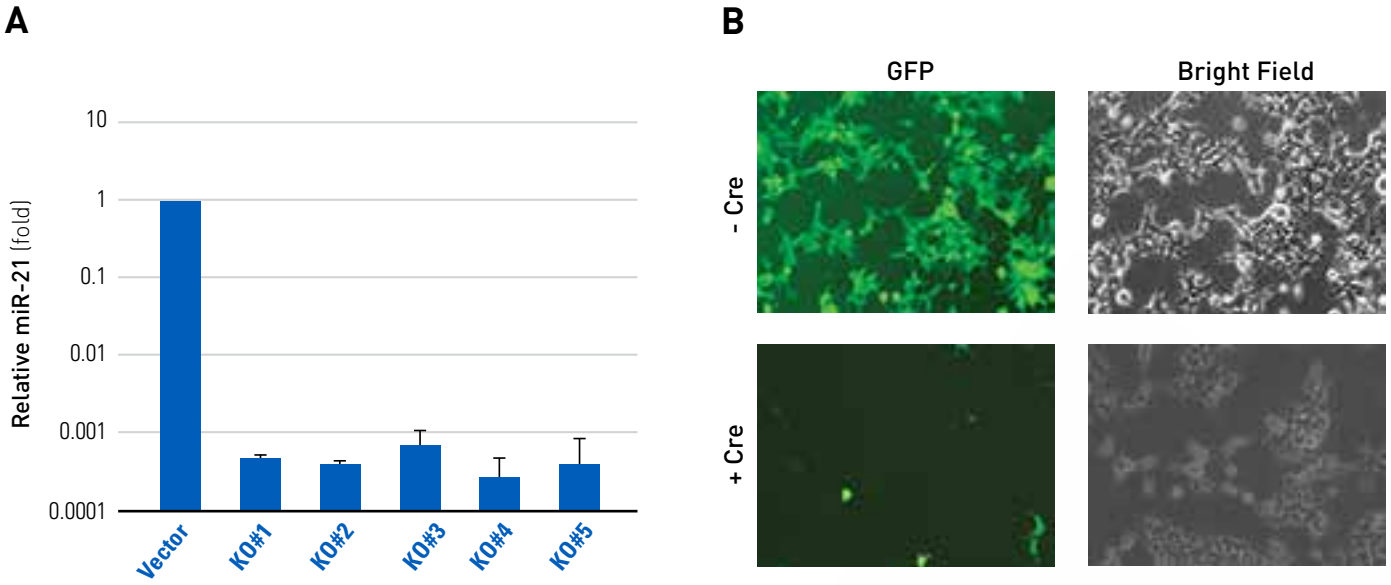
SBI will design and clone a gRNA against a target locus into any SBI SmartNuclease or SmartNickase vector (or customer provided gRNA cloning vector).

Custom HR donor plasmid design and cloning (Cat# CS600HR-1)

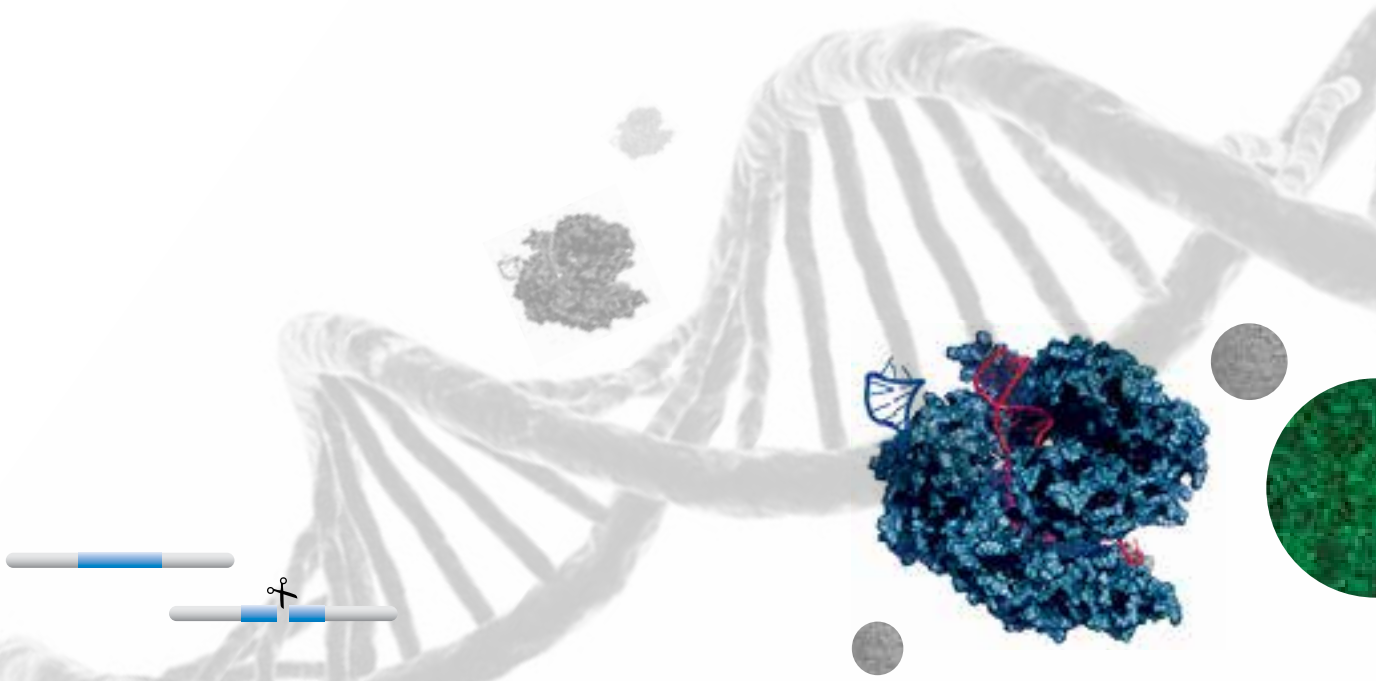
SBI will design and clone homology arms into any SBI HR donor plasmid for knock-out, knock-in, tagging, or single nucleotide modification genome engineering projects. When a custom HR donor plasmid design is ordered together with custom gRNA design and cloning, the donor vector will not contain full gRNA sequences in the homology arms to ensure full compatibility with gRNA.

Custom cell line engineering (Cat# CS715A-1, CS715B-1)

SBI will use custom Cas9 and HR donor constructs to engineer target cell lines for knock-out, knock-in, tagging, or single nucleotide modification applications. Use of HR Donor is required, and is typically ordered as a package with the gRNA construct. SBI can screen resistant cells to identify a clonal line with the desired modification (Cat# CS715B-1) or deliver unscreened cells (Cat# CS715A-1).



Effective, efficient knock-out of miR-21 in HEK293 cells.[4] gRNA, HR Donor design (with puro and GFP selection markers), implementation, and analysis performed by SBI's genome engineering services team. (A) Low relative levels of miR-21—as measured by qPCR in GFP-positive clones—demonstrate the effectiveness of the approach. (B) After excision with Cre recombinase, the inserted GFP marker is efficiently excised, leaving only a single LoxP site from the HR Donor.



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04. Ho, TT, *et al.* Targeting non-coding RNAs with the CRISPR/Cas9 system in human cell lines. *Nucleic Acids Res.* 2015 Feb **18**; 43(3):e17.

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About System Biosciences

Seeking out novel technologies and tomorrow's hot new research areas, the team at SBI accelerates research by striving to be the first company to develop and commercialize new inventions. From novel genome editing tools to exosome research, expression and imaging vectors, RNAi libraries, and stem cell tools, SBI harnesses today's innovations to drive tomorrow's discoveries.

SBI System Biosciences
Harnessing innovation to drive discoveries