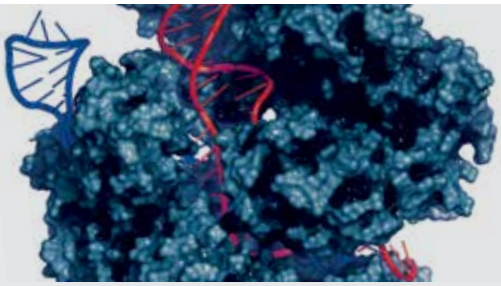
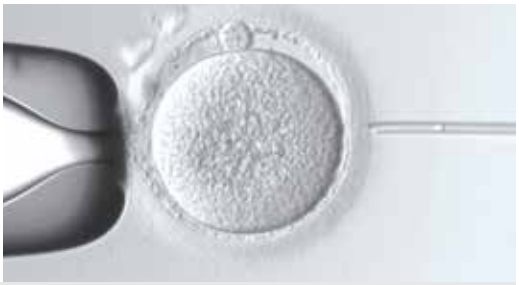
CRISPR/Cas9

CRISPR/Cas9

GENOME ENGINEERING THAT CATALYZES CHANGE



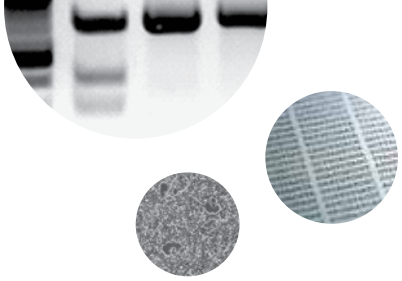


SYSTEMBIO.COM

SBI System Biosciences
Harnessing innovation to drive discoveries

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.



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 and AAV
- spCas9 protein

HR Donors to mediate

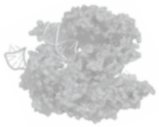
- Knock-outs
- Knock-ins
- Gene editing
- Gene tagging
- Knock-ins at AAVS1

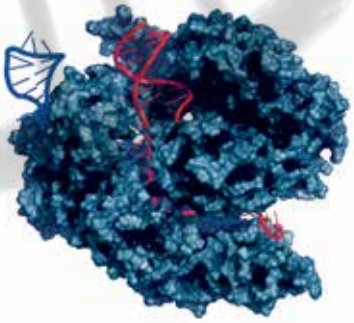
14

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

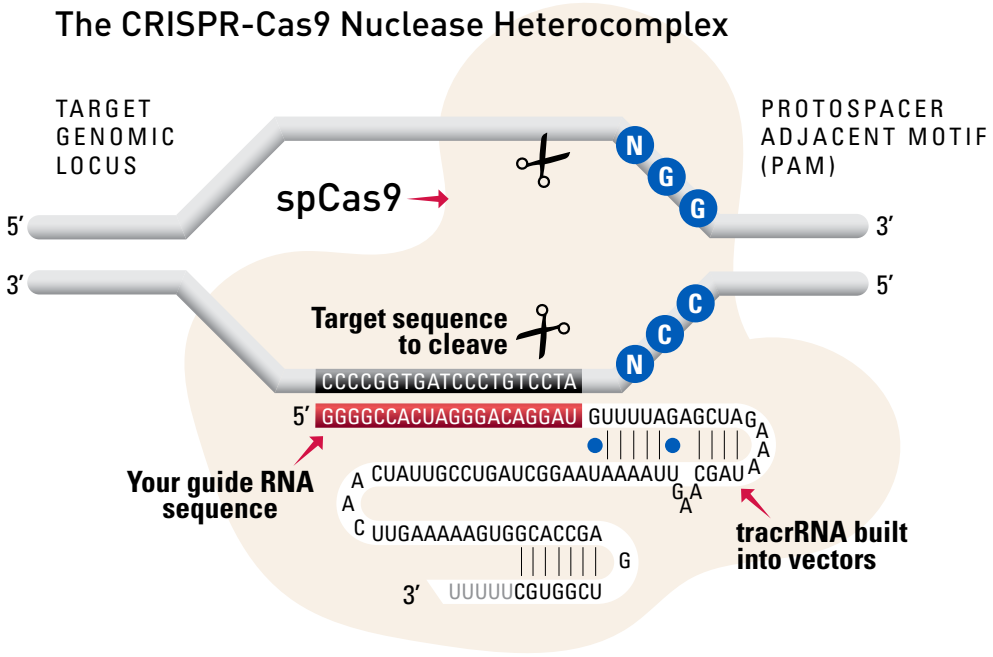
Through careful selection of the target sequence and design of a donor plasmid for homologous recombination, you can achieve efficient and highly targeted genomic modification.

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 spCas9 will cut upstream from if directed to by the gRNA.



WORKFLOW

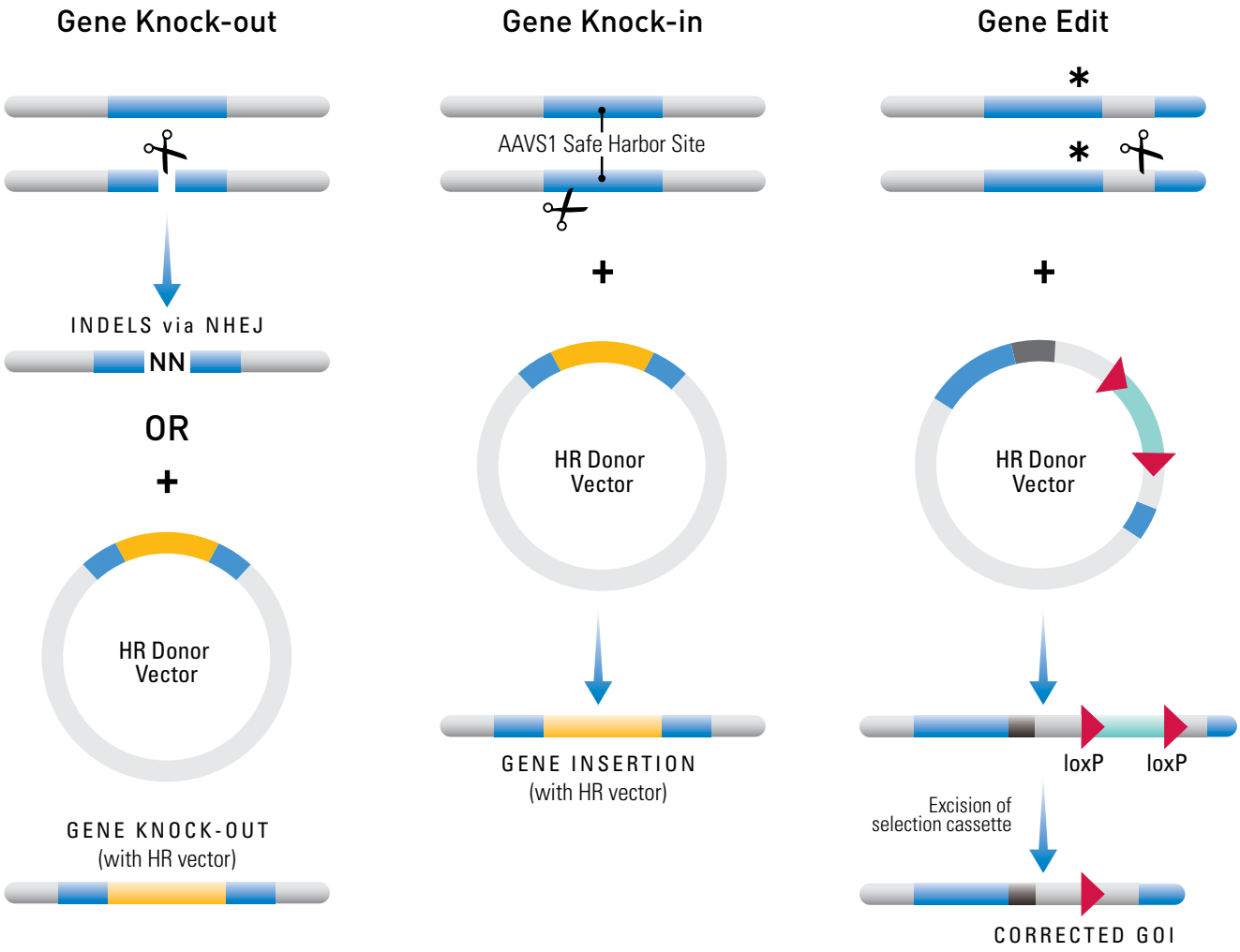
DESIGN: Select gRNA and HR donor plasmids. Choice of gRNA site and design of donor plasmid determines whether the homologous recombination event results in a knock-out, knock-in, edit, or tagging.

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.

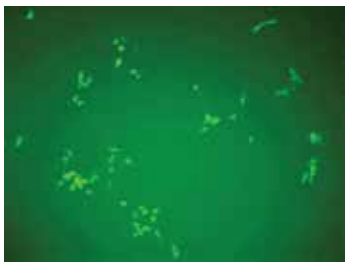
VALIDATE: Genotype or sequence putative mutants to verify single or biallelic conversion.



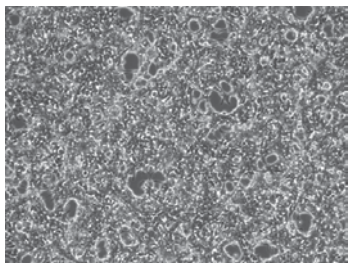
HR Integration at the AAVS1 Safe Harbor Site

Surveyor Nuclease Assays

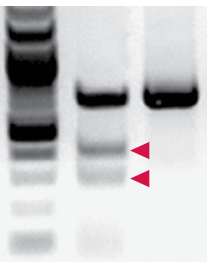
EF 1-hspCas9-H1-AAVS1-gRNA



GFP Fluorescence



Bright Field Phase



1 2 3

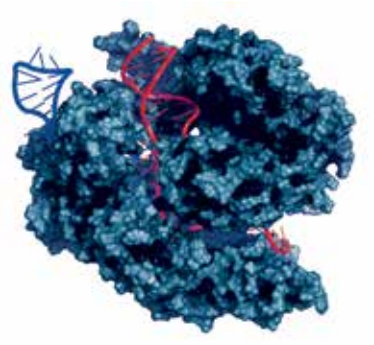
1. DNA marker
2. EF 1-hspCas9-H1-AAVS1-gRNA
3. Negative control

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



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 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.

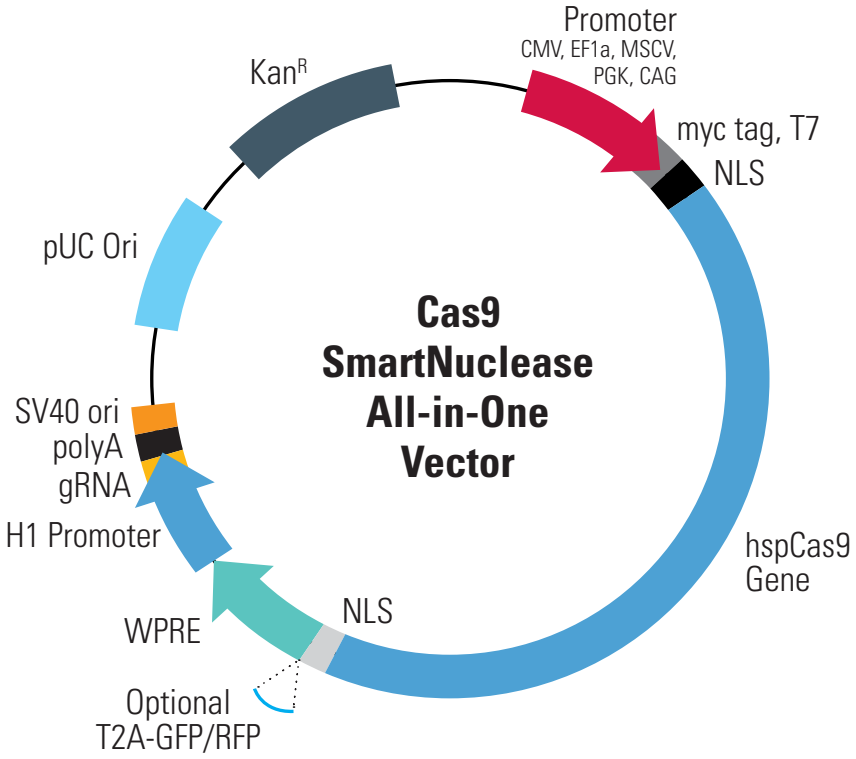
The **mutant PrecisionX Cas9 SmartNickase** (D10A mutation) creates nicks in genomic DNA instead of DSBs. Creating nicks favors the higher-fidelity homologous recombination process over non-homologous end joining (NHEJ), with paired nicking shown to reduce off-target activity by 50- to 1,500-fold in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

*Learn more about genome engineering with SBI—**email tech@systembio.com***

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***

Plasmid Vectors



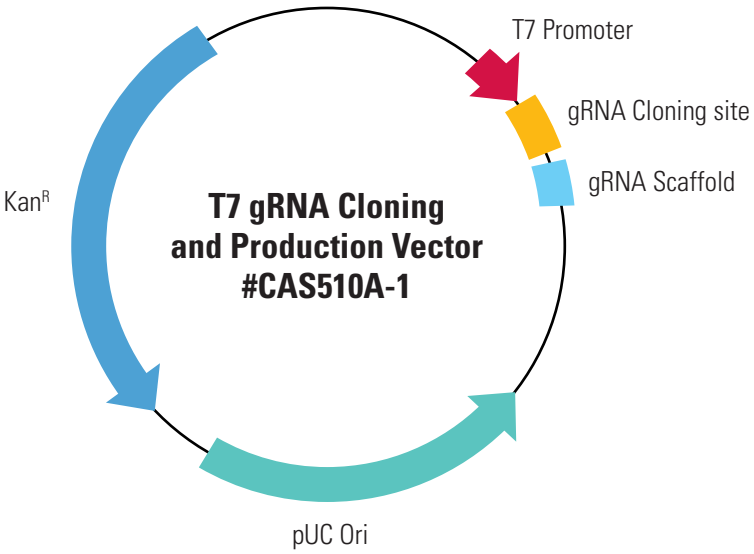
FOR THIS APPLICATION		USE THESE PRODUCTS
MODIFYING ORGANISMS ▪ Gene tagging ▪ Transgenic organism generation ▪ Model organism engineering	Creating transgenic animals	Injectable Cas9 mRNA & gRNA synthesis kits Page 8
	In vivo genome editing in animal models	AAV-Cas9 vectors Page 8
MODIFYING CELL LINES ▪ Stable KO, KI, and genome editing of somatic cells ▪ Transgenic cell line generation ▪ Cell-based disease models	Cells that are transfectable	Cas9 plasmids Page 6
	Difficult to transfect cell lines, ▪ Primary cells ▪ Hematopoietic cells ▪ Stem cells	AAV-Cas9 vectors Page 8 Lenti-Cas9 system Page 9
SCREENING ▪ Genome-wide surveys ▪ gRNA library screens ▪ Functional screens	All applications requiring stable Cas9 overexpression	Lenti-Cas9 System Page 9 AAVS1 Safe Harbor Cas9 Knock-In System Page 11
PRE-CLINICAL APPLICATIONS ▪ Off-target events are of highest concern	All applications	Cas9 Nickase, available in all delivery formats Page 6
SIMULTANEOUS ENGINEERING OF MULTIPLE MUTATIONS	All applications	Multiplex gRNA cloning kit, compatible with all Cas9 delivery options Page 10

Microinjectable mRNAs

To make the RNA-directed spCas9 system more efficient and convenient for *in vivo* applications, SBI has developed an injection-ready CRISPR/Cas9 mRNA system:

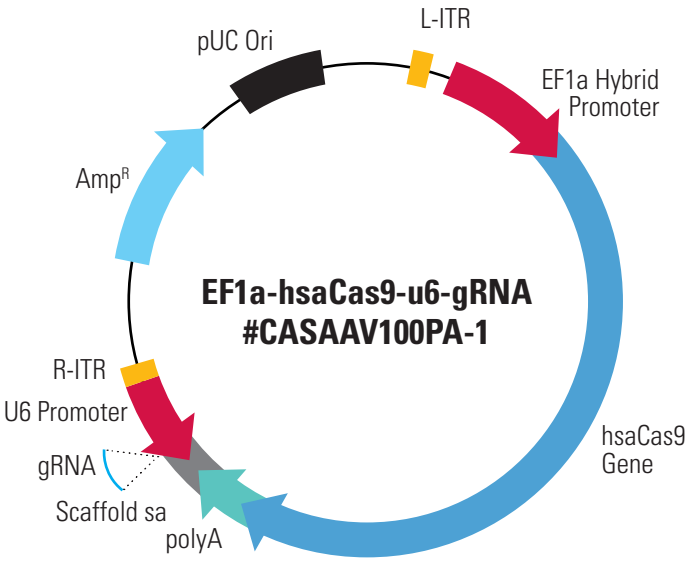
- Functionally-validated Cas9 mRNA – ready to use
- T7 gRNA cloning vector
- T7 gRNA production kit

Want faster Cas9 activity for creating transgenic organisms and cell lines? Try our new, transfectable Cas9 protein—learn more at ***systembio.com/cas9-protein***



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.



Cas9 AAV Vectors

For non-integrating introduction of Cas9 to cells, SBI offers Cas9 AAV vectors. With higher titers and higher multiplicity of infection than lentiviral vectors, Cas9 AAV expands your options for cell line engineering.

- Non-integrating Cas9 delivery
- High-titer AAV system
- Excellent for *in vivo* use—transgenic animals, animal models

Note that to fit the small insert requirements of the AAV vectors, our Cas9 AAV vectors employ a human codon-optimized Cas9 gene from Staphylococcus aureus.

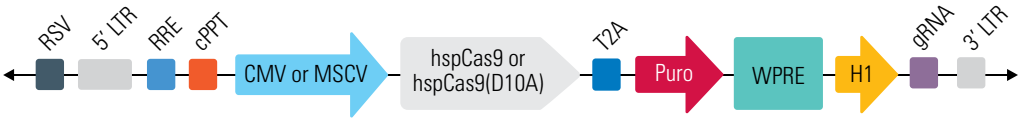
SBI simplifies use of AAV delivery systems with our AAVanced Concentration Reagent, a one-step, lysis-free way to concentrate AAV virus. Learn more at ***systembio.com/AAVanced.***

Cas9 Lentivectors

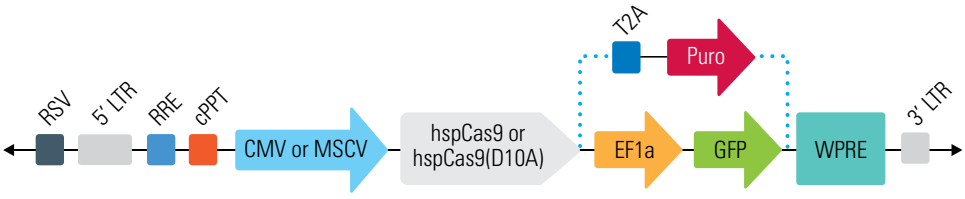
With SBI's Lenti-Cas9 SmartNuclease system, you can more easily perform genome engineering for a variety of applications.

- Use with hard-to-transfect cell lines
- Create stably expressing Cas9 cell lines
- Perform genome-wide gRNA library screens

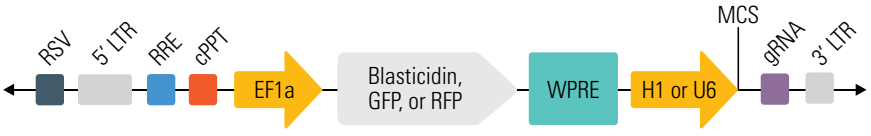
The all-in-one systems express Cas9 and gRNA from the same vector, streamlining your workflows. These vectors are pre-linearized and ready for cloning your gRNA sequence of choice, and come with two different promoter options—CMV or MSCV—and two different Cas9 variant options—wild type Cas9 or Cas9 nickase.



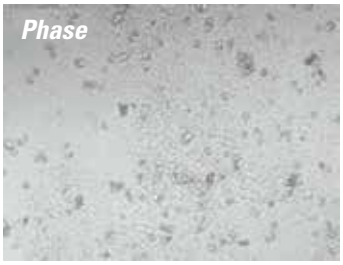
Our separate two-vector systems express Cas9 and gRNAs on different vectors, with the Cas9-expressing vector available either as plasmid or pre-packaged, ready-to-transduce lentivirus. In addition to the CMV or MSCV promoter options and Cas9 variant options with T2A-Puro selection, we also offer vectors that include constitutive GFP expression driven by the EF1 promoter, for easier identification of transduced cells.

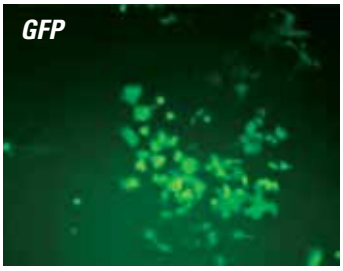


The gRNA vectors in the two-vector system come pre-linearized and ready to clone into your gRNA sequence of choice. Options include two different promoters—H1 or U6—and choice of positive selection—blasticidin, GFP, or RFP.



Sample Lenti-Cas9 data—transduce stem cells with Lenti-Cas9.





MSCV-hspCas9-EF1-copGFP
Cat #CASLV125VA-1

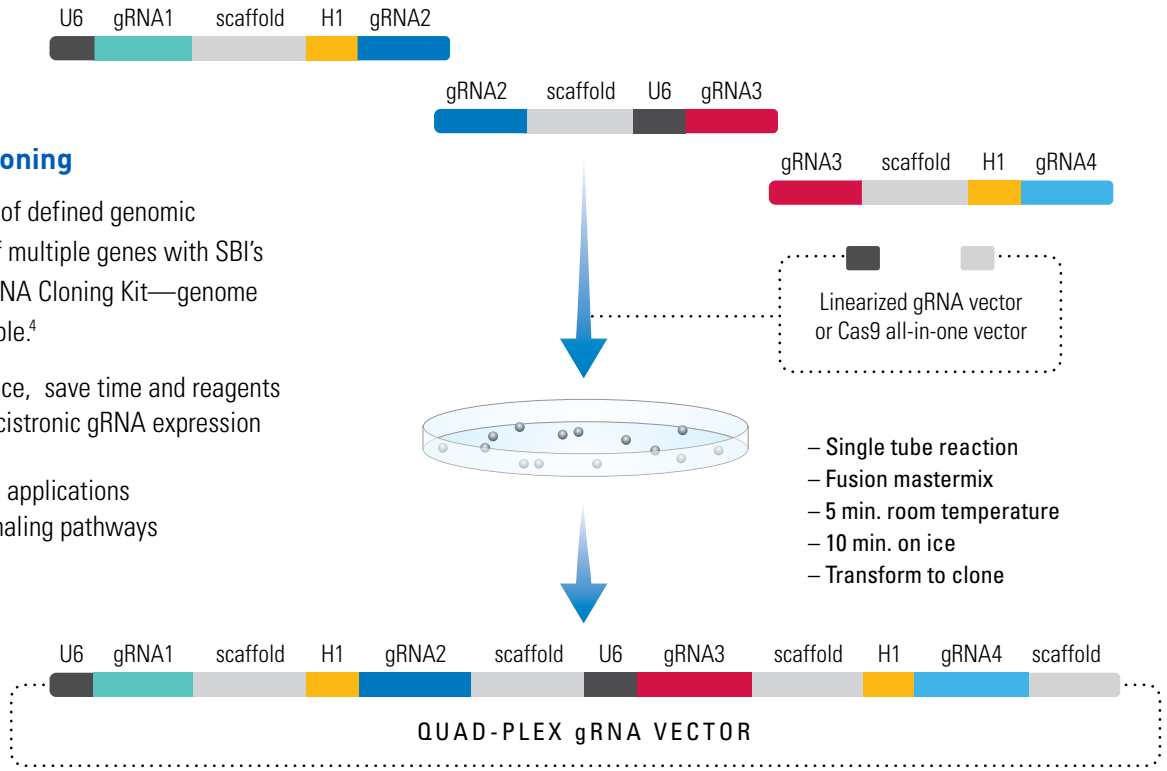
Fluorescence image of Human iPSC cell line infected with pseudoviral particles of MSCV-hspCas9-EF1-copGFP (Cat #CASLV125VA-1) at MOI = 60. Image was taken 6 days after virus transduction.

Find the most up-to-date list of Lenti-Cas9 SmartNuclease vectors online today at:
systembio.com/cas9-lentivirus

Multiplex gRNA Cloning

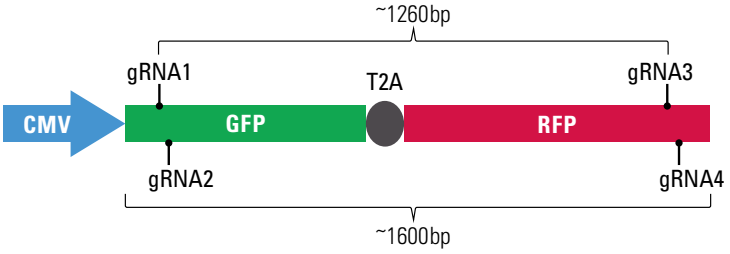
Enable precise deletion of defined genomic segments and control of multiple genes with SBI's PrecisionX Multiplex gRNA Cloning Kit—genome manipulation made simple.[4]

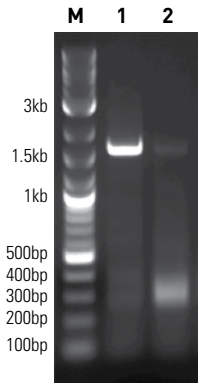
- Edit multiple loci at once, save time and reagents
- Easily generate multi-cistronic gRNA expression constructs
- Ideal for Cas9 Nickase applications
- Great for studying signaling pathways

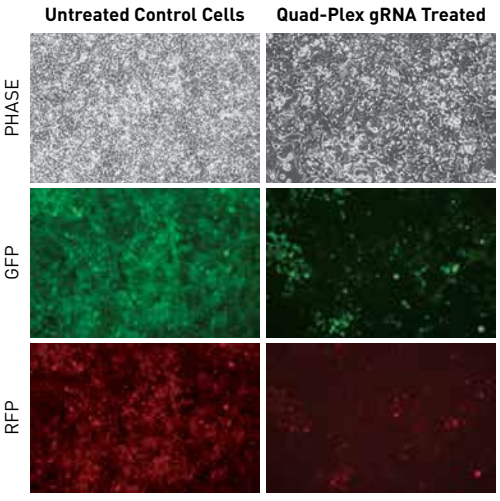


Sample Quad-Plex gRNA Targeting Data

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***

HARNESS THE POWER OF THE AAVS1 SAFE HARBOR SITE

Delivering consistent, robust transgene expression, the AAVS1 safe harbor site is a preferred target for gene knock-ins. Insertion at the site has been shown to be safe with no phenotypic effects reported, and the surrounding DNA appears to be kept in an open confirmation, enabling stable expression of a variety of transgenes.

With SBI's AAVS1 safe harbor site products, you get:

- Easy knock-in of any gene
- Precise, site-specific gene integration
  - Consistent, robust transgene expression
  - Simplified construction of isogenic cell lines—facilitates comparisons by removing variation due to different integration sites
- Minimal off-target integration
- Streamlined gRNA library screening

Use Cat # GE622A-1 to constitutively express any gene of interest at the AAVS1 site.

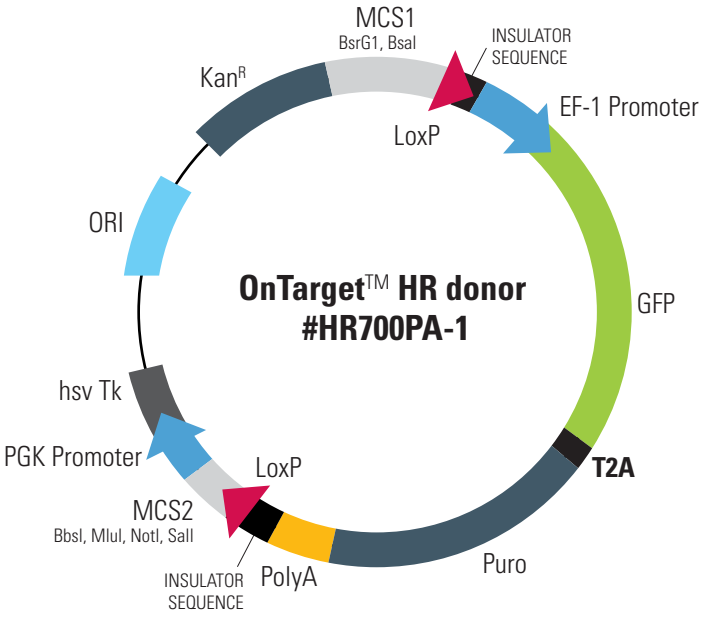
Use Cat # GE624A-1 for reporter construction, such as for tissue-specific expression.

Use Cat # CAS620A-1 to stably express Cas9 from the AAVS1 site.

Our AAVS1 safe harbor site products include HR donor vectors designed to work in tandem with our AAVS1 gRNA/Cas9 expression vector (Cat #CAS601A-1). The AAVS1 gRNA/Cas9 expression vector encodes a pre-validated gRNA specific for AAVS1 and the PrecisionX™ Cas9 SmartNuclease®—simply co-transfect cells with the AAVS1 gRNA/Cas9 expression vector and your AAVS1 donor construct of choice for targeted integration.

Purchase individual AAVS1 donor vectors or an AAVS1 Cas9-mediated integration kit (Cat #s GE620A-KIT, GE622A-KIT or GE624A-KIT), which comes with any of our three AAVS1 donor vectors, AAVS1 gRNA/Cas9-expression construct, and junction PCR primers to confirm the correct integration site (Cat # GE640PR-1).

The clever design of the AAVS1 donor vectors limits off-target integration for highly-specific targeting of the AAVS1 site. Taking advantage of the AAVS1's location within an intron, the puromycin marker has only a splice acceptor site and no promoter. Expression of puromycin can only occur when the construct integrates within an intron, reducing the probability of recovering off-target integrants in the presence of puromycin selection.



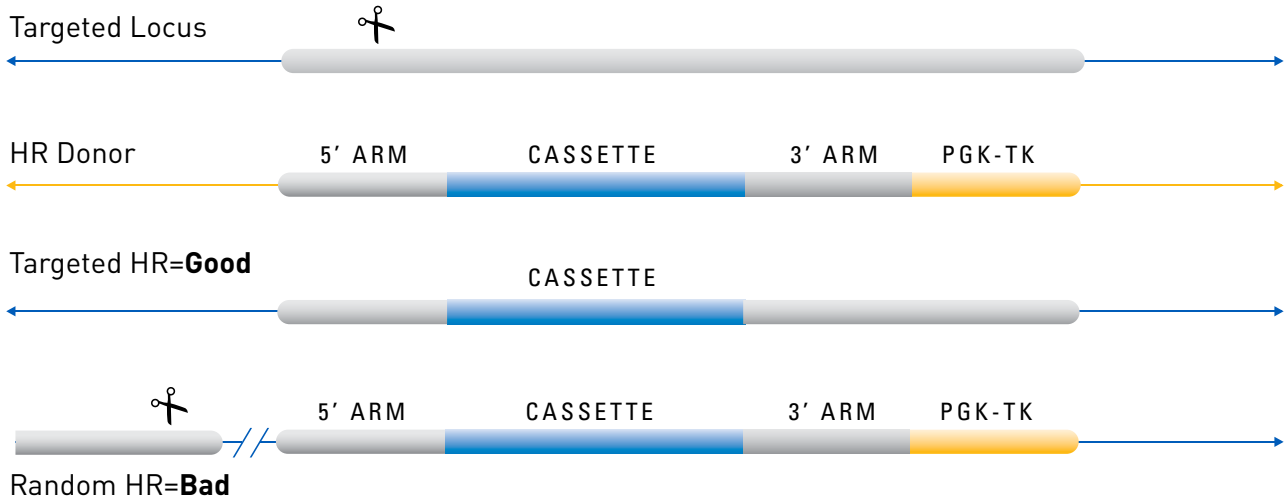
HR DONORS

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 selection cassette after integration, insulator sequences for maximal expression, and a range of promotors, GFP or RFP options, and positive and/or negative selection elements.

*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.

*Visit **systembio.com/hr-donors** to see the most up-to-date list of available HR donors.*

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), GE620A, GE622A, GE624A

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

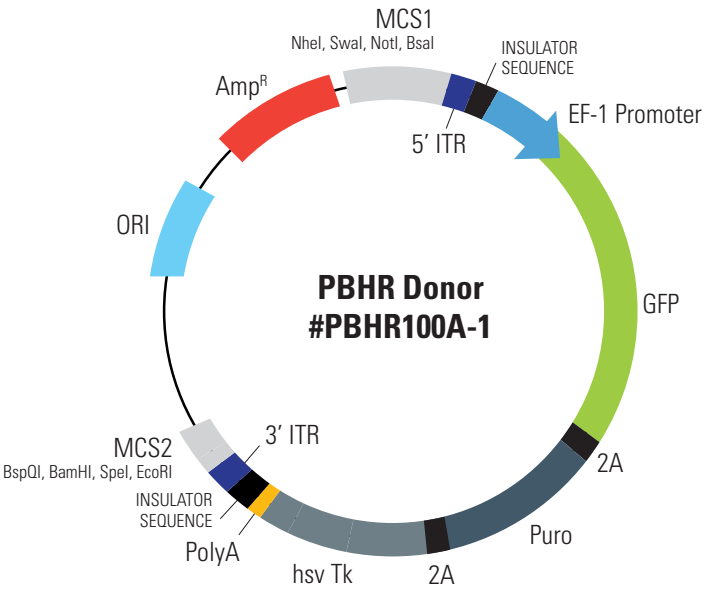
Choose a target site that creates an in-frame insertion towards the end of the coding region to create a protein fusion, or use a T2A or IRES element for a co-expressed marker under control of the endogenous promoter.

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

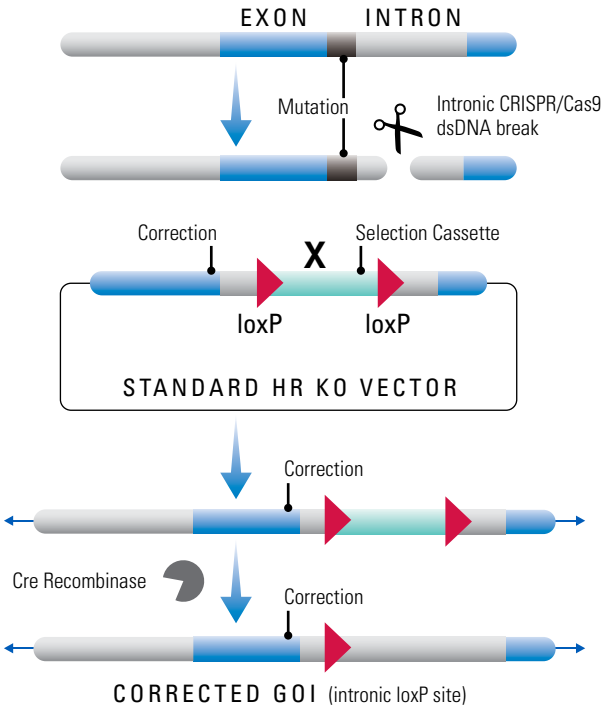
Have questions about HR donor technology? From protocols to troubleshooting, our technical support scientists are ready to help, at ***info@systembio.com****.*

Gene editing without a trace

PBHR100A enables seamless gene editing with no residual footprint.



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 Palo Alto, 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 homozygous modification (7/34), demonstrating the power of the Cas9 SmartNuclease system when coupled with an HR donor."

From start to finish, we offer services covering your entire genome engineering workflow. Our experienced staff understand the intricacies of using Cas9 and know how to overcome many of the common pitfalls that can make Cas9 challenging. Because each project is different, we can customize our offering to meet your specific project needs.

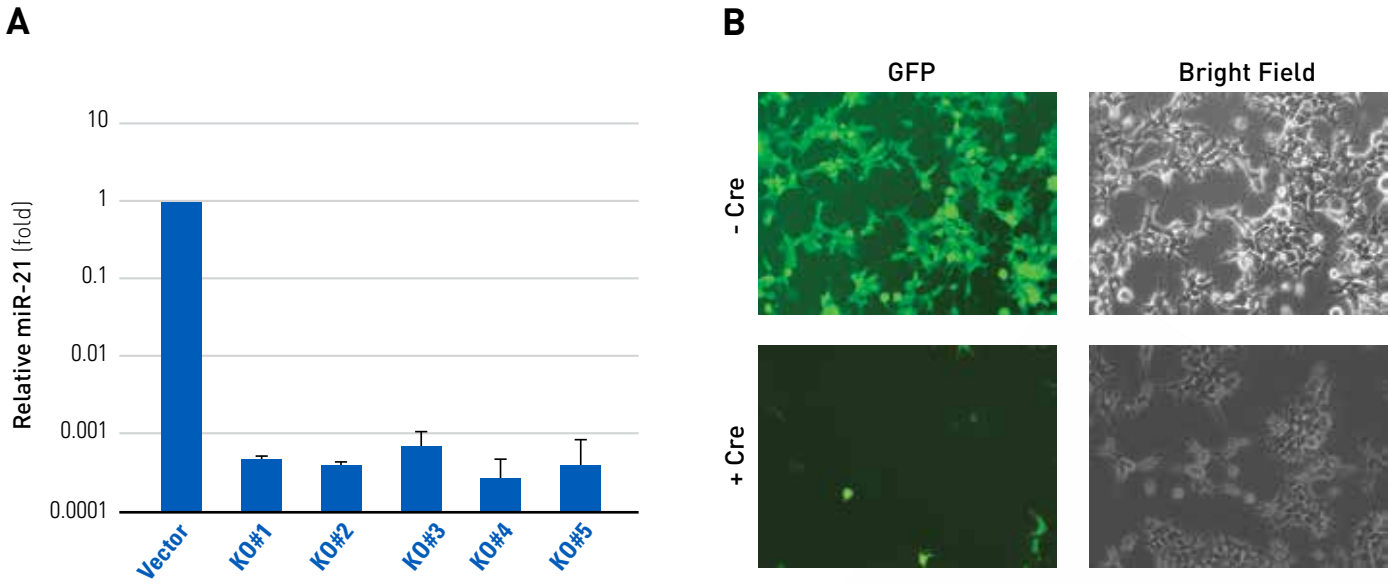
Tier 1: Design and cloning of custom gRNA and HR donors

SBI will design and clone custom gRNA and/or HR donor vectors. For gRNAs, SBI will design and clone a single gRNA (Cat# CS700A-1) or multiplex gRNAs (Cat# CS705A-1) against a target locus into any SBI SmartNuclease or SmartNickase vector (or customer provided gRNA cloning vector). For HR donors (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 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.

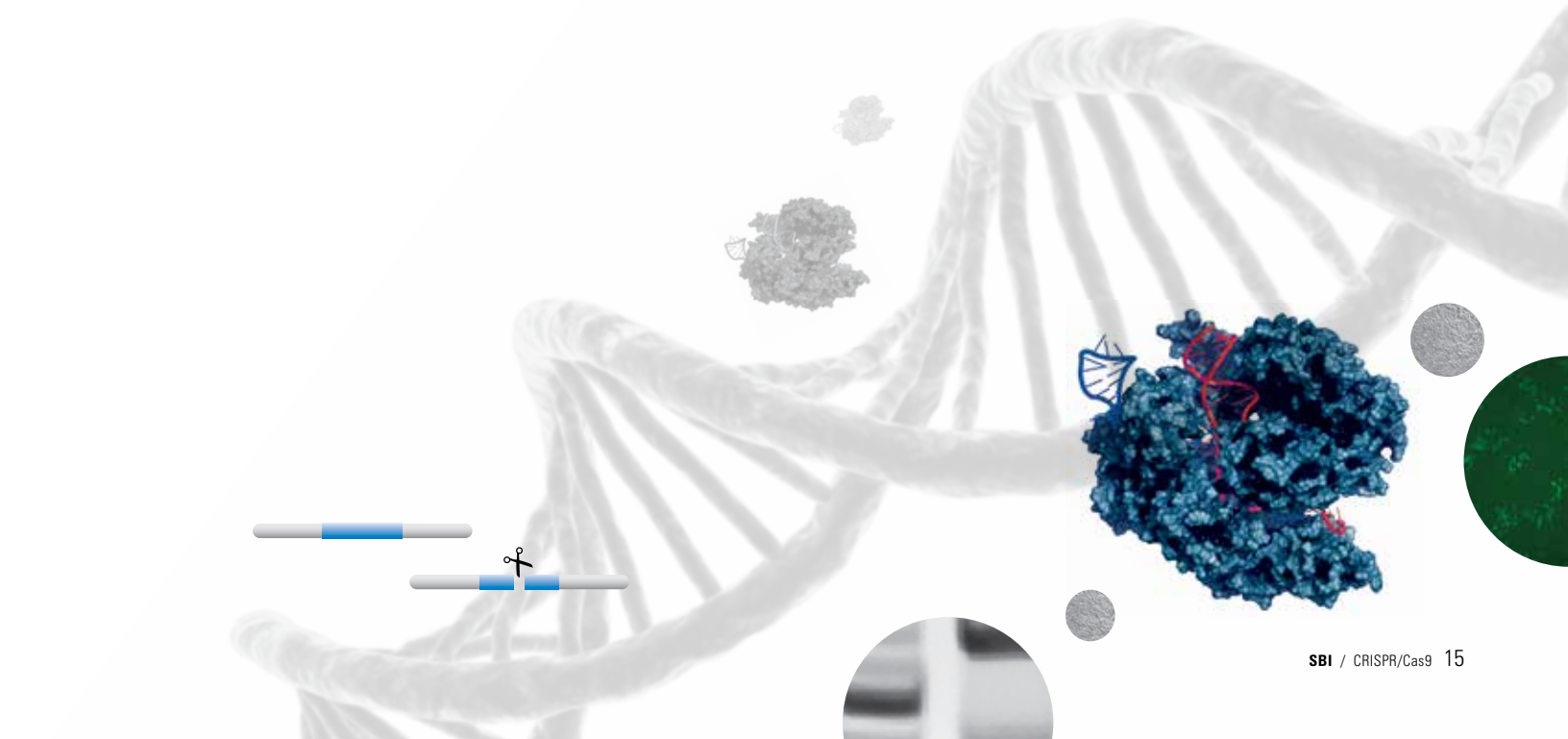
Tier 2: Full service custom cell line engineering

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 usually required, and is typically ordered as a package with two gRNA constructs. SBI can screen resistant cells to identify a clonal line with the desired modification (Cat# CS715B-1) or deliver a mixed population of resistant cells for further characterization (Cat# CS715A-1).

Find out more about our custom genome engineering services—contact us at ***services@systembio.com***



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 and Puro markers are efficiently excised, leaving only a single LoxP site from the HR Donor.



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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.

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