System Biosciences
Accelerating discoveries through innovations

Genome Engineering

Cas9 SmartNuclease™ RNA System

Mammalian Genome Engineering with mRNA and gRNA

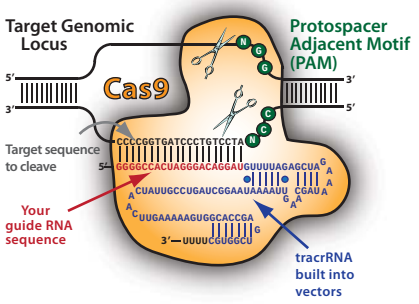
The CRISPR-Cas9 RNA System

The type II prokaryotic CRISPR (clustered regularly interspaced short palindromic repeats) adaptive immune system has been shown to facilitate RNA-guided sequence-specific DNA cleavage, which provides a new class of genome engineering tools. To make the RNA-directed Cas9 system more efficient, and convenient to use for in vivo applications, SBI has developed a CRISPR/Cas9 mRNA system, which includes functionally-validated Cas9 mRNA (cat# CAS500A-1), T7 gRNA cloning vector and T7 gRNA production kit. To avoid reconstituting the CRISPR/Cas9 RNA processing machinery, a custom gRNA (crRNA-tracrRNA chimeric transcript) can be generated from the ready-to-use linearized T7 gRNA cloning vector (cat# CAS510A-1) through the use of annealed oligonucleotide duplexes encoding the 20bp target sequence upstream of the Protospacer Adjacent Motif (PAM). As the custom gRNA is under the control of T7 promoter, it is ready for in vitro transcription (IVT) with the T7 gRNA synthesis kit (cat# CAS510A-KIT). The AAVS1 gRNA sequence was cloned into the CAS510A-1 T7 vector and scaffolded-gRNA targeting the AAVS1 site was generated in vitro. These gRNAs were co-transfected with the Cas9 synthetic mRNA in combination with the AAVS1 HR vector harboring a GFP marker. The activities of the Cas9 mRNA + AAVS1 gRNA transfection was compared with that of the EF1 Cas9 SmartNuclease-AAVS1 gRNA all-in-one vector system. Cells were imaged for GFP fluorescence after 3 days.

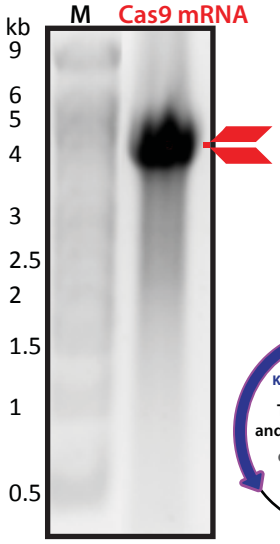
Highlights

- Specific genome cleavage guided by an RNA sequence
- Easy gene knockouts
- Efficient genome editing with homologous recombination
- No need to make a new protein effector for each new target
- All-in-one Cas9 -gRNA cloning Nickase and Null vectors

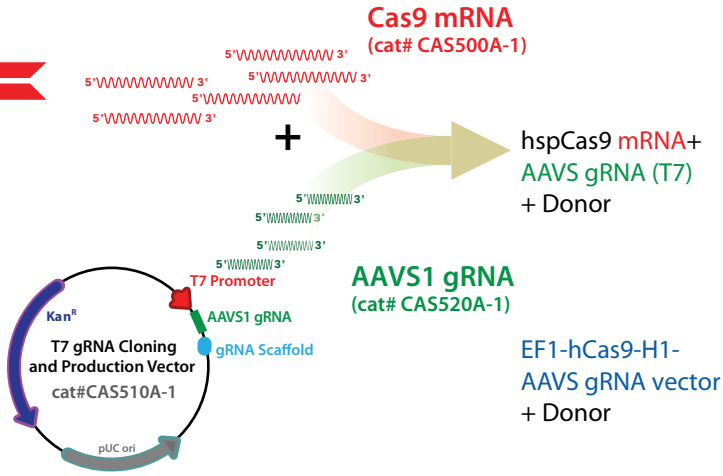
The CRISPR-Cas9 Nuclease Heterocomplex



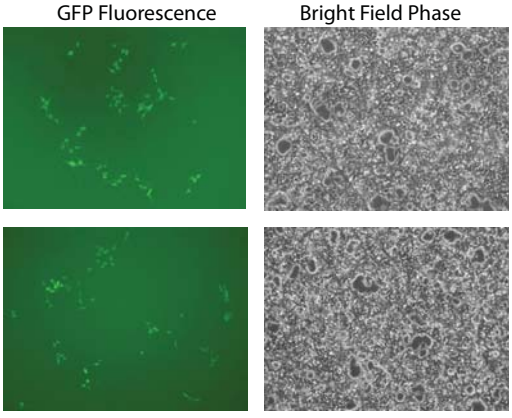
Cas9 Synthetic mRNA



Cas9 gRNA Production Vector



HR Integration at AAVS1 Images



www.systembio.com/cas9

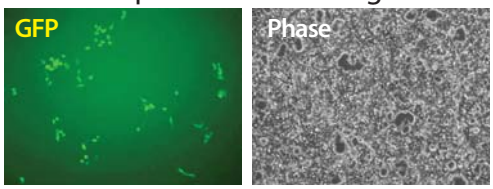
Mutant Cas9 SmartNucleases

The Cas9 SmartNuclease system for genomic targeting can be used in two ways. The wild-type hspCas9 vectors retain their nuclease domains and will produce double-stranded breaks in the target as directed by the gRNA. This system can be used with HR vectors or applied to mutating a targeted site via nonhomologous end joining (NHEJ) which lead to insertions/deletions at the site of DNA cleavage. One amino acid mutation at position D10A in Cas9 results in the inactivation of the nuclease catalytic activity and converts Cas9 to a "nickase" enzyme that makes single-stranded breaks at the target site. This Cas9 Nickase can be used at a targeted site to favor homologous recombination at the site with HR vectors and lowers the rate of NHEJ. The Cas9 double mutant (DM) with changes at amino acid positions D10A and H840A completely inactivates both the nuclease and nickase activities. SBI offers both of these mutant Cas9 versions, Cas9 Nickase: EF1-hspCas9-Nickase-H1-gRNA linearized SmartNickase vector (catalog# CAS800A-1) and the Cas9 Double Mutant: EF1-hspCas9-DM-H1-gRNA linearized NullNuclease vector (catalog# CAS805A-1). These Cas9 vectors were tested for the alteration of activity in assays compared to the wild-type hspCas9 vector with the AAVS1 gRNA in combination with an AAVS1 HR vector harboring a GFP marker.

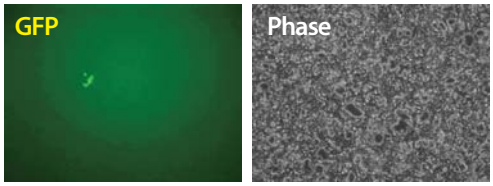
Comparison between hspCas9, Cas9 SmartNickase and Double Mutant Cas9 NullNuclease

HR Integration at AAVS1 Images

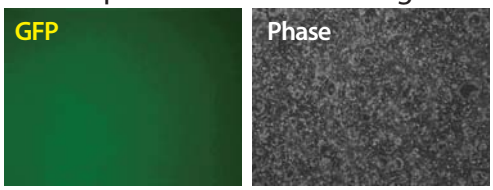
EF1-hspCas9-H1-AAVS1-gRNA



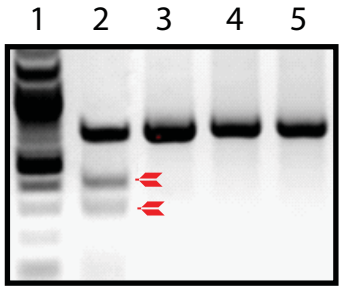
EF1-hspCas9-Nickase-H1-AAVS1-gRNA



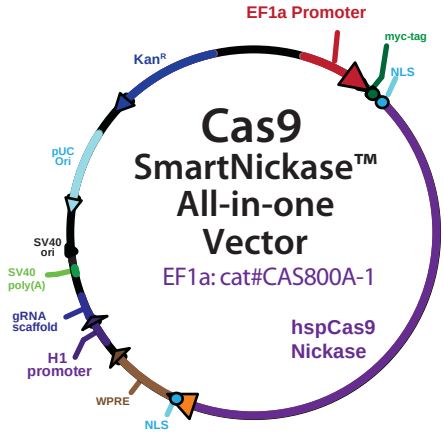
EF1-hspCas9-Null-H1-AAVS1-gRNA

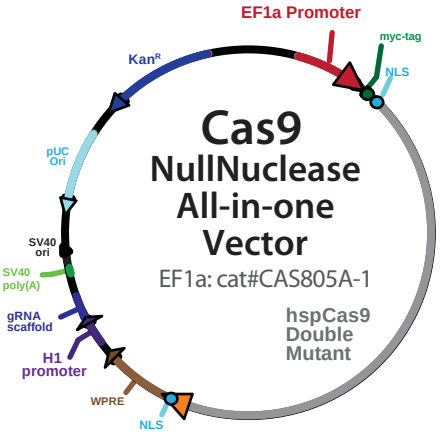


Surveyor Nuclease Assays



1. DNA Marker
2. EF1-hspCas9-H1-AAVS1 gRNA
3. EF1-hspCas9 Nickase-H1-AAVS1 gRNA
4. EF1-hspCas9 Null Nuclease-H1-AAVS1 gRNA
5. Negative Control EGFP Cells





We Also Offer Custom Services - *Have SBI Design and Build a Custom Cas9 SmartNuclease Vector.*

System Biosciences offers a wide-range of custom services to support your research, allowing you to spend less time making tools, and more time making discoveries. To learn more, visit our website at www.systembio.com/service or call us at 888-266-5066.

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