Genome editing and beyond

A Guide to CRISPR/Cas9

The latest advance in genomic DNA editing is the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)/Cas9 system. This simple-to-use and robust technique has had a paradigm-shifting impact on genome editing by allowing for highly specific targeting of DNA sequences, while bypassing the need for costly and time-consuming protein engineering. CRISPR/Cas9 has truly taken the scientific community by storm by offering a simple solution for gene silencing and activation, genome editing and more, all carried out within living cells. And now, all of these can be at your fingertips! **abm** is proud to offer an expanded line of CRISPR-related products and services. Look inside for further details!

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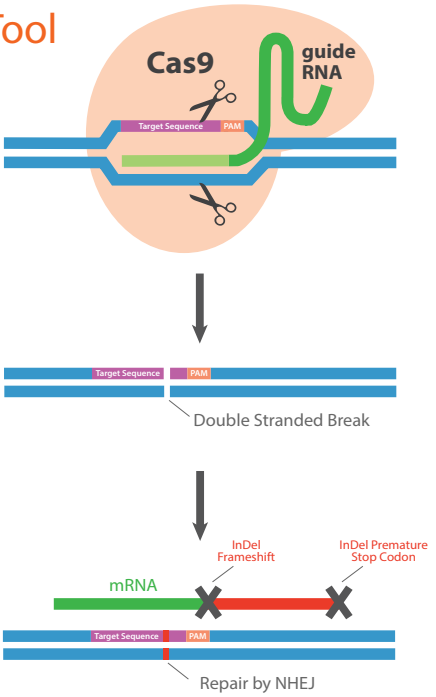
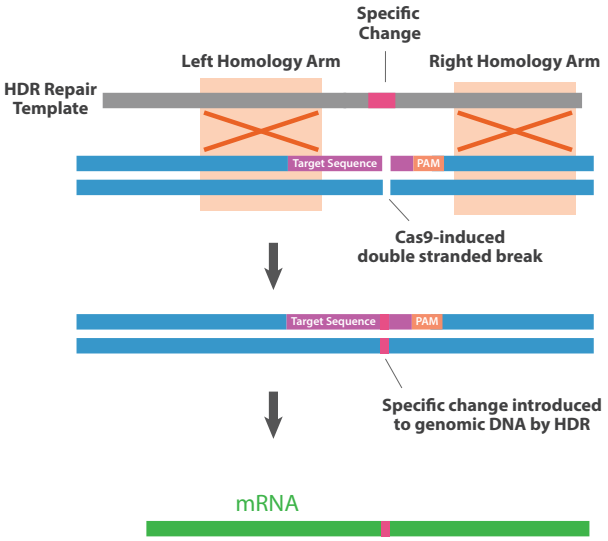
A Versatile and Fully-Customizable Genome Editing Tool

CRISPR/Cas9 allows for highly specific genomic modification and the silencing of genes of interest. This versatile system requires co-expression of two distinct components: (1) a nuclease, Cas9, and (2) a target-specific single guide RNA (sgRNA). *Streptococcus pyogenes* Cas9 interrogates the genome for sequences complementary to the 20 nucleotide target region of the sgRNA and adjacent to the protospacer-adjacent motif (PAM) "5'-NGG". The Cas9 nuclease introduces a double strand break, which is then repaired by a highly error-prone process called Non-Homologous End Joining (NHEJ). This can result in a frameshift insertion or deletion (InDel), thus effectively silencing the gene.





Gene Knock-In with CRISPR/Cas9

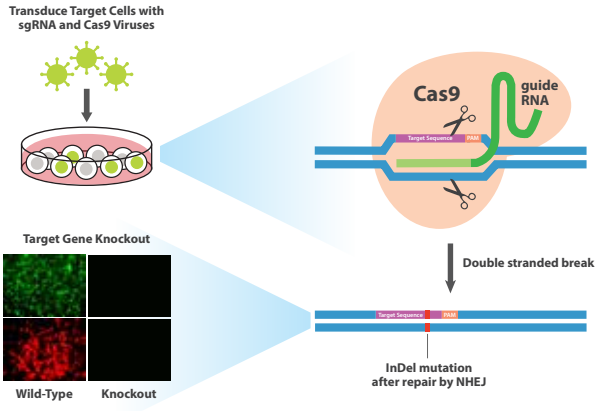
In addition to NHEJ, cells can utilize Homology Directed Repair (HDR), which can be exploited to introduce specific modifications to genomic DNA. If a repair template is provided containing the desired new sequence, flanked by homologous sequences immediately upstream and downstream of the double strand break, the new sequence will be permanently introduced into the genomic DNA via homology directed repair.

CRISPR Services

CRISPR Custom Knockout Service

Cat. No. C208

With this highly customized service, we can knockout any gene in any cell line. All you have to do is send us your desired target cells and the species, gene name, and accession number of the gene to be knocked out. The successfully genome-edited cells will be shipped back to you after strict quality control and verification of gene knockout. **Now available:** 100% Guaranteed CRISPR Knockout Service (C508).



E. coli Knockout/Knock-In Services

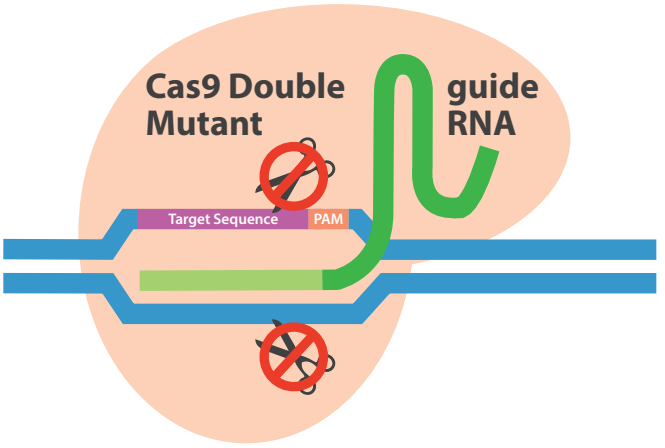
Cat. No. C424 & C425

CRISPR-assisted gene knockout and knock-in services are available for *E. coli*! Simply select an *E. coli* strain and the sequence to be knocked in or out, and receive your edited bacteria in as little as 8 weeks.

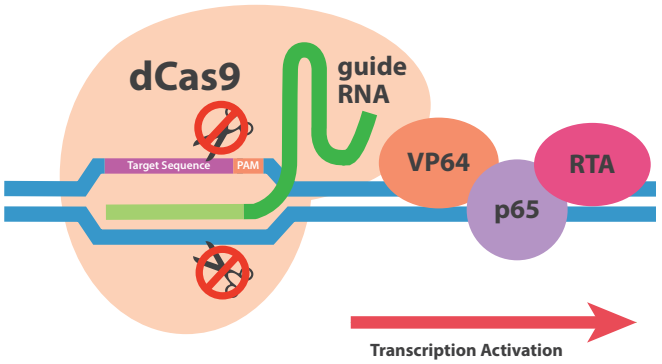
Custom Genomic Locus Targeting by dCas9

Double-Mutant Cas9

The Cas9 double-mutant (dCas9) is unable to cleave DNA, but has retained the unparalleled specificity of the wild-type enzyme. As such, it is ideally suited for targeting attached proteins of interest to specific genomic loci, bypassing the need to engineer a new construct for each target sequence. **abm** offers this system for a wide range of potential applications.



Synergistic Activation Mediator (SAM)

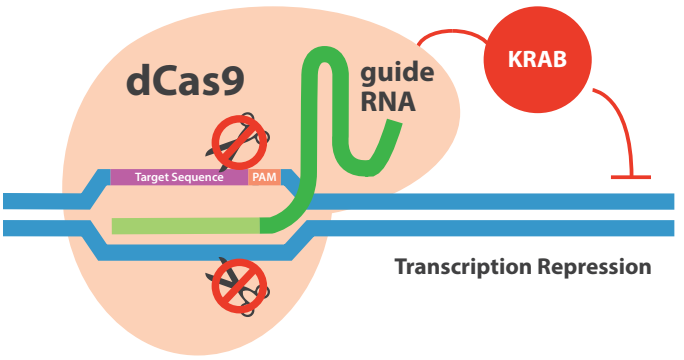


Transcription Activation by dCas-SAM

Synergistic activation mediators (SAM) linked to dCas9 are extremely effective at inducing expression of a gene of interest. We offer dCas9 fused to a tripartite SAM (VP64, p65 and RTA), a highly effective and easy-to-use design. Only two components are needed: the dCas9-SAM and the sgRNA. Easy!

Transcription Repression by dCas9-KRAB

dCas9 can be fused to a Krüppel-associated box (KRAB) domain for targeted gene repression at the transcriptional level. Simply deliver the dCas9-KRAB and an sgRNA targeting the gene of interest's promoter/enhancer region for easy, efficient gene repression.



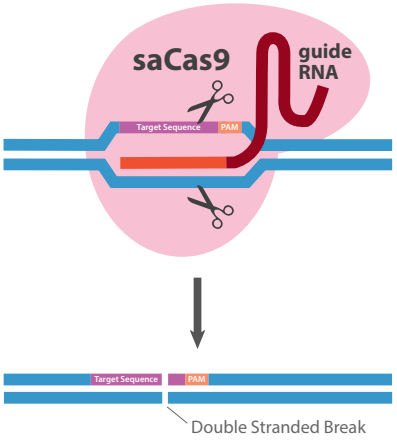
sgRNA-guided DNA targeting

dCas9 Variant	Application	Product Type	Cat.No.
dCas9	Any genome targeting experiment	Lentiviral vector	K012, K014
		Lentivirus	K013
		Protein	K040, K042, K086
dCas9 - SAM	Transcription activation	Lentiviral vector	K015
		Lentivirus	K016
dCas9 - KRAB	Transcription repression	Lentiviral vector	K203
		Lentivirus	K204

Cas9 Variants for any Application

Cas9 Nickase for Enhanced Specificity and Accuracy

By inactivating one of its catalytic domains, the Cas9 nuclease is turned into a "nickase" – nCas9. This modified enzyme introduces a single strand nick instead of a double strand break. In order to engage the NHEJ or HDR pathways, two nCas9/sgRNA complexes are needed, which cleave the DNA in close proximity (<20 nucleotides). This approach greatly reduces off-target effects caused by non-specific sgRNA binding by requiring two specific binding events.

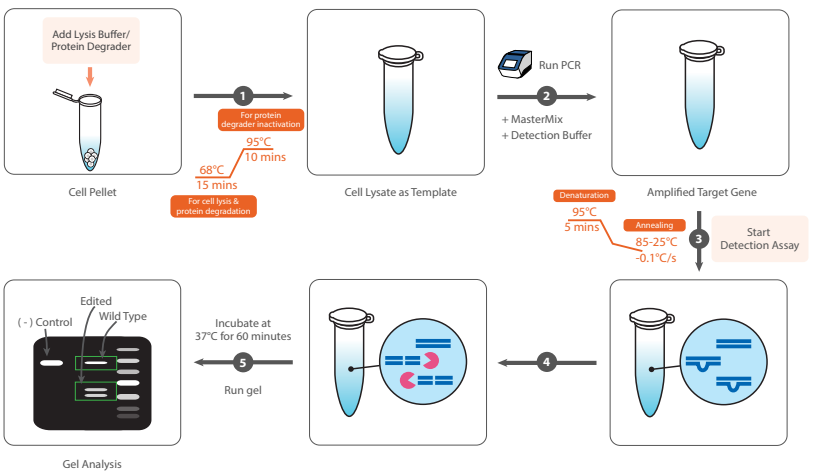


saCas9 Nuclease for *in vivo* applications

A miniature Cas9 isolated from *S. aureus*, saCas9 is ~1 kb smaller than spCas9, allowing it to be efficiently packaged into Adeno-Associated Virus (AAV). AAV is a preferred method of gene delivery for *in vivo* studies due to its low immunogenicity and ability to selectively infect certain tissue types. saCas9's PAM sequence is "5'-NNGRRT", so it can be used to target different regions of the genome than spCas9.

Cas9 Type	Product Type	Cat.No.
spCas9 Nuclease (wild-type)	Lentiviral vector / Lentivirus	K002 / K003
	Adenovirus	K004
	Protein	K008, K009, K030, K031
	Stable Cell Lines (293T, 293, A549, HeLa, etc.)	T3251, T3252, T3253, T3254, etc.
spCas9 Nickase (modified)	Lentiviral vector / Lentivirus	K005 / K006
	Adenovirus	K007
	Protein (D10A / H840A)	K032, K034 / K036, K038
saCas9 Nuclease	AAV Vector	K207
	AAV Virus (Serotypes 1 to 11)	K208 to K218
	Protein (wildtype / null mutant)	K044, K045 / K046, K047

CRISPR Verification



CRISPR Genomic Cleavage Detection Kit

Cat. No. G932

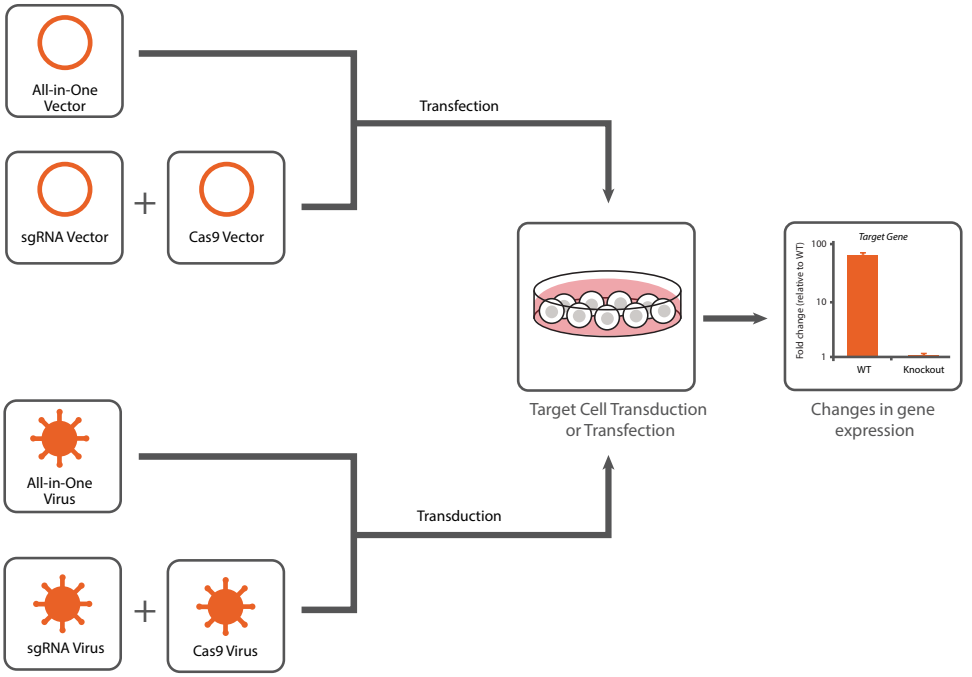
Designed as an easy, effective way to verify your genomic editing process, **abm**'s ready-to-use CRISPR Genomic Cleavage Detection Kit conveniently contains all the necessary reagents required, including a set of control template and primers to ensure reliable results. With a rapid 4 hour processing time, this qualitative assay will be a great addition to any genome-editing toolbox.

Genome-wide sgRNA Libraries at Your Fingertips!

abm offers genome-wide CRISPR sgRNA libraries for targeting any human, mouse, or rat gene with the use of non-viral plasmids, lentivirus, AAV, or adenovirus.

Our sgRNA vectors and viruses are provided as individual constructs or in a set of 3, both separate from Cas9 and as an All-In-One System. They can be used individually or pooled together to achieve optimal gene knockout.

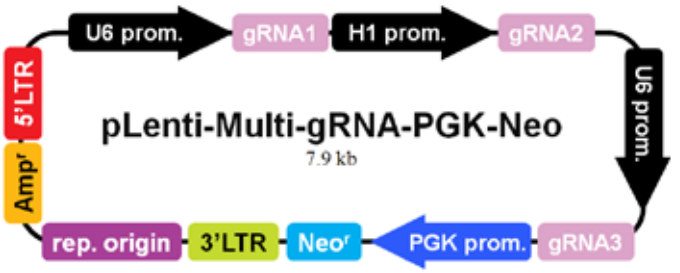
As well, choose from saCas9 or spCas9 sgRNA or All-In-One constructs. **abm**'s comprehensive sgRNA Library allows for unparalleled flexibility in experimental setup. And the best part? All sgRNAs are designed by our CRISPR experts!



CRISPR Multiplex sgRNAs

Cat. No. C420 to C423

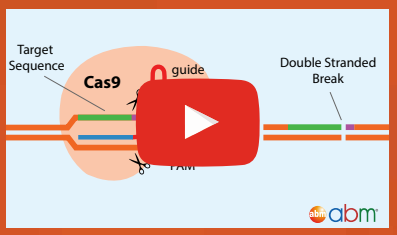
abm's CRISPR multiplex sgRNA system allows for optimal expression of multiple sgRNAs from alternating the U6 and H1 RNA pol III promoters on a single lentiviral vector. Ideal for use with Cas9 nickase, which requires 2 sgRNAs for double-stranded cleavage.



CRISPR sgRNA Format	Individual or Set of 3	Cas9 Type	Product Type
sgRNA only (Cas9 required separately)	Individual sgRNA	spCas9	Lentiviral vector / Lentivirus
			Adenovirus
			AAV vector / AAV
			Non-Viral Vector
		saCas9	AAV vector / AAV
	Set of 3 sgRNA	spCas9	Lentiviral vector / Lentivirus
			Non-Viral Vector
	2-4 Multiplexed sgRNAs	spCas9 / saCas9	Lentiviral vector
All-In-One (sgRNA and Cas9 in a single vector)	Individual sgRNA	spCas9	Lentiviral vector / Lentivirus
			Non-Viral Vector
		saCas9	AAV vector / AAV
	Set of 3 sgRNA	spCas9	Lentiviral vector / Lentivirus
			Non-Viral Vector

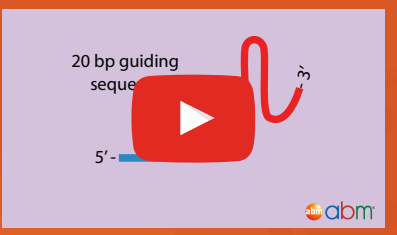
Knowledge Base and Videos

https://info.abmgood.com/CRISPR



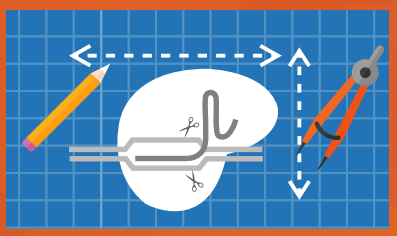
CRISPR Cas9:
A Brief Introduction

CRISPR Cas9:
Methods and Tools



CRISPR Cas9:
gRNA Design

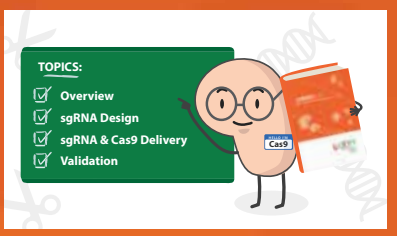
New Tools and Resources



CRSPR Project Design Tool

Get a tailored list of tools for your gene editing project

info.abmgood.com/myCRISPR



CRISPR Crash Course

Our FREE 4-week course teaches you how to do a CRISPR gene KO

info.abmgood.com/crispr-crash-course

CRISPR Knockout Handbook

Our FREE 39-page manual includes protocols & case studies

info.abmgood.com/crispr-KO

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