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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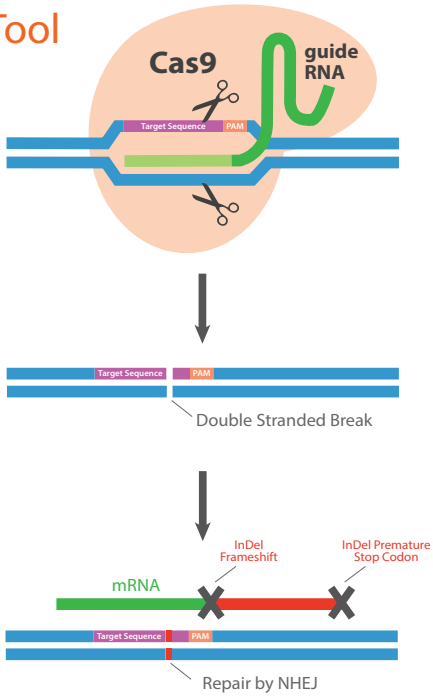
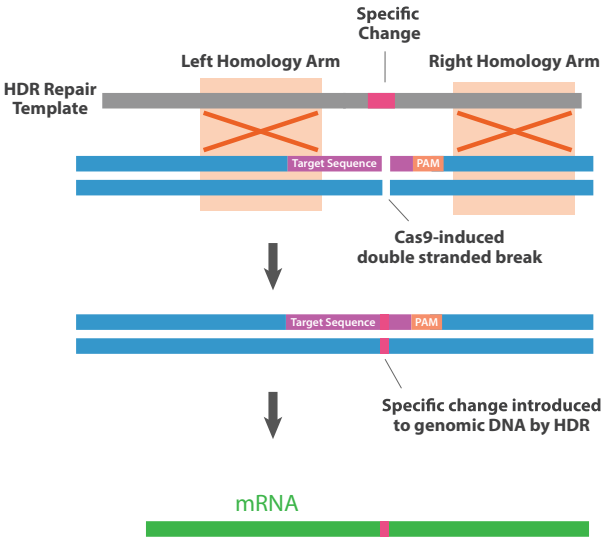
A Versatile and Fully-Customizable Genome Editing Tool

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.



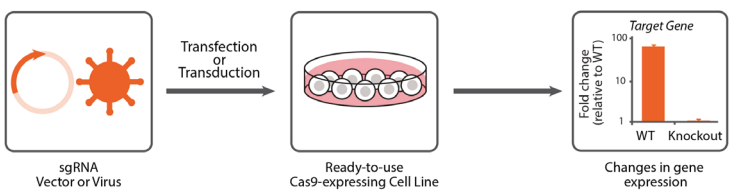


Gene Knock-In with CRISPR/Cas9

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.

Cas9-Expressing Cell Lines

abm offers a wide variety of Cas9-expressing cell lines that require only the introduction of sgRNA from: our genome-wide sgRNA library, a custom sgRNA vector/virus, or through transfection of *in vitro* transcribed sgRNA. Cas9 expression in our cell line collection has been pre-validated by qPCR and/or Western blot.



Cell Line	Cat.No.
293	T3252
293T	T3251
A375	T3262
A549	T3253
HCT116	T3263
HeLa	T3254
HepG2	T3256
HK-2	T3271
HL-60	T3264
HT1080	T3260
Astrocyte	T3452
Cardiomyocyte	T3454
Microglia	T3451
Skeletal Muscle	T3450
INS1E	T3289
Jurkat	T3261

Cell Line	Cat.No.
K562	T3258
L3.6	T3272
MCF7	T3257
Ovcar3	T3268
PC3-M	T3279
RL95-2	T3265
SKUT-1	T3266
SNU-387	T3267
T24	T3270
THP-1	T3274
U-87	T3259
NIH3T3	T3275
RCS	T3290
MDCK	T3299
DF1	T3453

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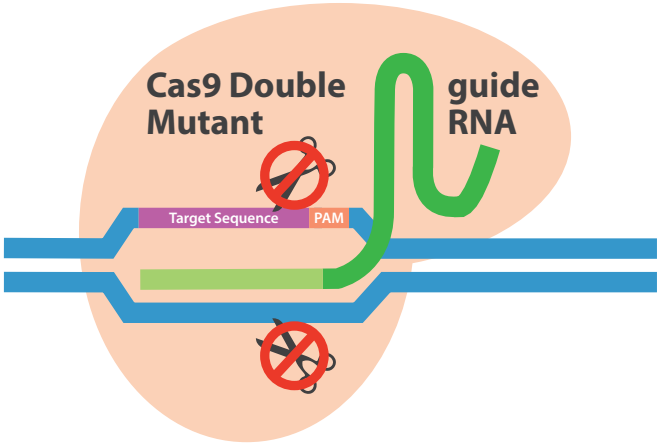
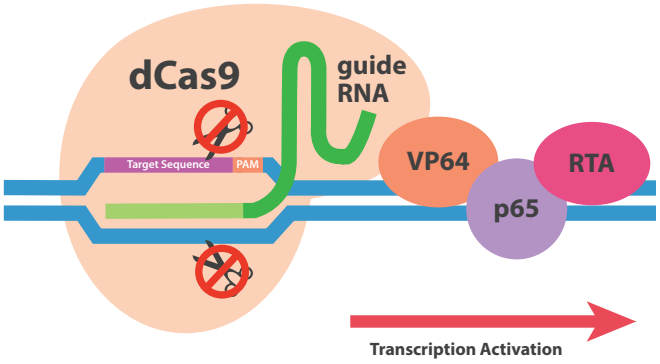
Custom Genomic Locus Targeting by dCas9

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)

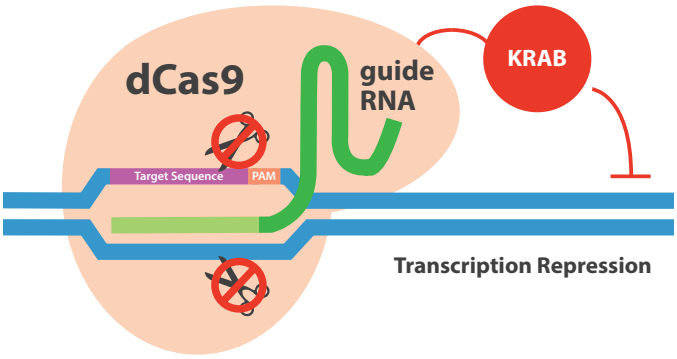


Transcription Activation by dCas-SAM

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.



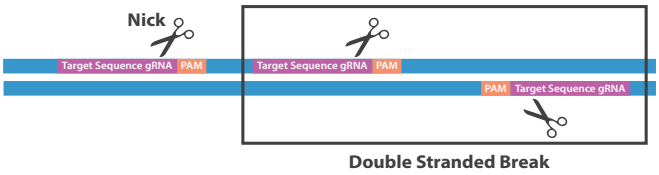
sgRNA-guided DNA targeting

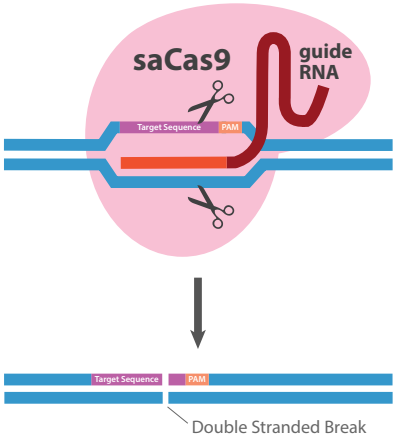
dCas9 Variant	Application	Product Type	Cat.No.
dCas9	Any genome targeting experiment	Lentiviral vector	K012, K014
		Lentivirus	K013
		Protein	K040, K042, K086
dCas9 - SAM	Transcription activation	Lentiviral vector	K015
		Lentivirus	K016
dCas9 - KRAB	Transcription repression	Lentiviral vector	K203
		Lentivirus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



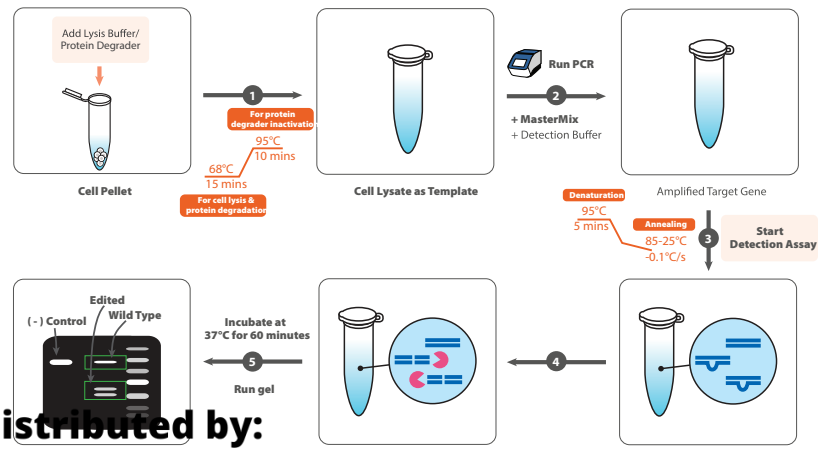


saCas9 Nuclease for *in vivo* applications

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

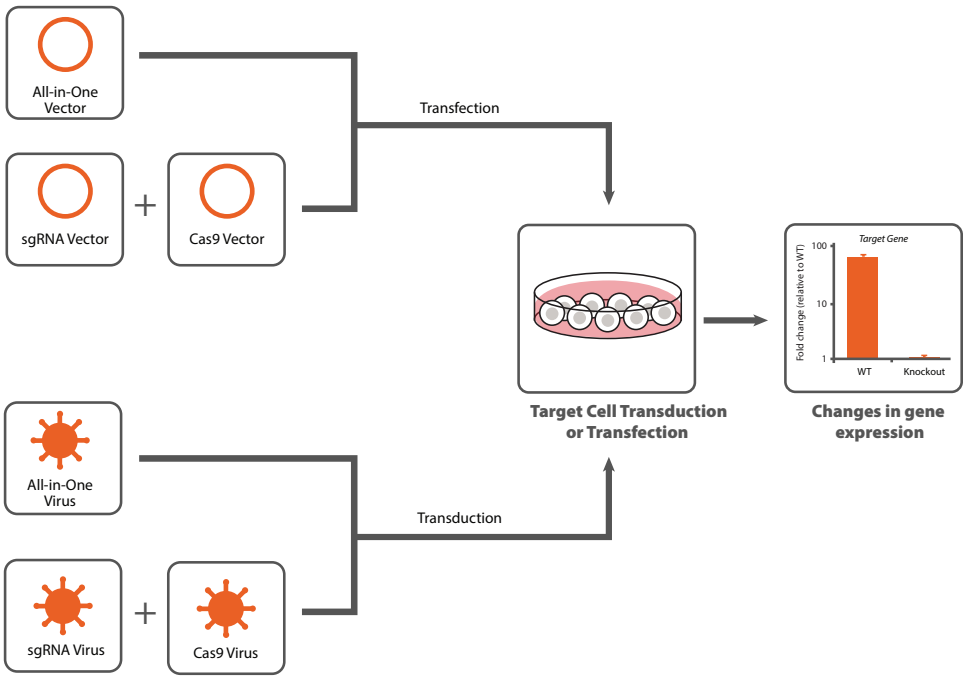
Designed as an easy, effective way to verify your genomic editing process, **abm**'s ready-to-use CRISPR Genomic Cleavage Detection Kit conveniently contains all the necessary reagents required, including a set of control template and primers to ensure reliable results. With a rapid 4 hour processing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

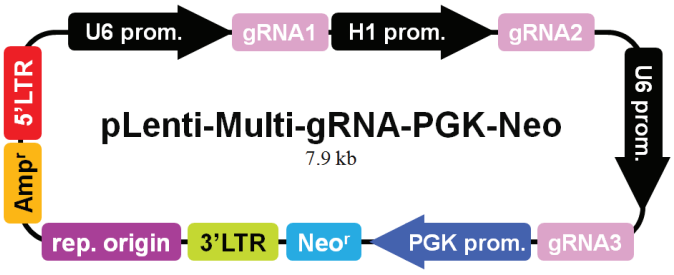
As well, choose from saCas9 or spCas9 sgRNA or All-In-One constructs. **abm**'s comprehensive sgRNA Library allows for unparalleled flexibility in experimental setup. And the best part? All sgRNAs are designed by our CRISPR experts!



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

CliniSciences Group

Austria
Company: CliniSciences GmbH
Address: Sternwartestrasse 76, A-1180 Wien - Austria
Telephone: +43 720 115 580
Fax: +43 720 115 577
Email: oesterreich@clinisciences.com
Web: https://www.clinisciences.com

Belgium
Company: CliniSciences S.R.L
Address: Avenue Stalingrad 52, 1000 Brussels - Belgium
Telephone: +32 2 31 50 800
Fax: +32 2 31 50 801
Email: belgium@clinisciences.com
Web: https://www.clinisciences.com

Denmark
Company: CliniSciences ApS
Address: Oesterbrogade 226, st. 1, Copenhagen, 2100 - Denmark
Telephone: +45 89 888 349
Fax: +45 89 884 064
Email: danmark@clinisciences.com
Web: https://www.clinisciences.com

Finland
Company: CliniSciences ApS
Address: Oesterbrogade 226, st. 1, Copenhagen, 2100 - Denmark
Telephone: +45 89 888 349
Fax: +45 89 884 064
Email: suomi@clinisciences.com
Web: https://www.clinisciences.com

France
Company: CliniSciences S.A.S
Address: 74 Rue des Suisses, 92000 Nanterre- France
Telephone: +33 9 77 40 09 09
Fax: +33 9 77 40 10 11
Email: info@clinisciences.com
Web: https://www.clinisciences.com

Germany
Company: Biotrend Chemikalien GmbH
Address: Wilhelm-Mauser-Str. 41-43, 50827 Köln - Germany
Telephone: +49 221 9498 320
Fax: +49 221 9498 325
Email: info@biotrend.com
Web: https://www.biotrend.com

Iceland
Company: CliniSciences ApS
Address: Oesterbrogade 226, st. 1, Copenhagen, 2100 - Denmark
Telephone: +45 89 888 349
Fax: +45 89 884 064
Email: island@clinisciences.com
Web: https://www.clinisciences.com

Ireland
Company: CliniSciences Limited
Address: Ground Floor, 71 lower Baggot street Dublin D02 P593 - Ireland
Telephone: +353 1 6971 146
Fax: +353 1 6971 147
Email: ireland@clinisciences.com
Web: https://www.clinisciences.com

Italy
Company: CliniSciences S.r.l
Address: Via Maremmana inferiore 378 Roma 00012 Guidonia Montecelio - Italy
Telephone: +39 06 94 80 56 71
Fax: +39 06 94 80 00 21
Email: italia@clinisciences.com
Web: https://www.clinisciences.com

Netherlands
Company: CliniSciences B.V.
Address: Kraijenhoffstraat 137A, 1018RG Amsterdam, Netherlands
Telephone: +31 85 2082 351
Fax: +31 85 2082 353
Email: nederland@clinisciences.com
Web: https://www.clinisciences.com

Norway
Company: CliniSciences AS
Address: c/o MerVerdi Munkerudtunet 10 1164 Oslo - Norway
Telephone: +47 21 988 882
Email: norge@clinisciences.com
Web: https://www.clinisciences.com

Poland
Company: CliniSciences sp.Z.o.o.
Address: ul. Rotmistrza Witolda Pileckiego 67 lok. 200 - 02-781 Warszawa -Poland
Telephone: +48 22 307 0535
Fax: +48 22 307 0532
Email: polska@clinisciences.com
Web: https://www.clinisciences.com

Portugal
Company: Quimigen Unipessoal LDA
Address: Rua Almada Negreiros, Lote 5, Loja 14, 2615-275 Alverca Do Ribatejo - Portugal
Telephone: +351 30 8808 050
Fax: +351 30 8808 052
Email: info@quimigen.com
Web: https://www.quimigen.pt

Spain
Company: CliniSciences Lab Solutions
Address: C/ Hermanos del Moral 13 (Bajo E), 28019, Madrid - Spain
Telephone: +34 91 269 40 65
Fax: +34 91 269 40 74
Email: espana@clinisciences.com
Web: https://www.clinisciences.com

Sweden
Company: CliniSciences ApS
Address: Oesterbrogade 226, st. 1, Copenhagen, 2100 - Denmark
Telephone: +45 89 888 349
Fax: +45 89 884 064
Email: sverige@clinisciences.com
Web: https://www.clinisciences.com

Switzerland
Company: CliniSciences AG
Address: Fracht Ost Flughafen Kloten CH-8058 Zürich - Switzerland
Telephone: +41 (044) 805 76 81
Fax: +41 (044) 805 76 75
Email: switzerland@clinisciences.com
Web: https://www.clinisciences.com

UK
Company: CliniSciences Limited
Address: 11 Progress Business center, Whittle Parkway, SL1 6DQ Slough- United Kingdom
Telephone: +44 (0)1753 866 511
or +44 (0) 330 684 0982
Fax: +44 (0)1753 208 899
Email: uk@clinisciences.com
IWeb: https://www.clinisciences.com

USA
Company: Biotrend Chemicals LLC
Address: c/o Carr Riggs Ingram, 500 Grand Boulevard, Suite 210 Miramar Beach, FL 32550- USA
Telephone: +1 850 650 7790
Fax: +1 850 650 4383
Email: info@biotrend-usa.com
Web: https://www.biotrend-usa.com

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