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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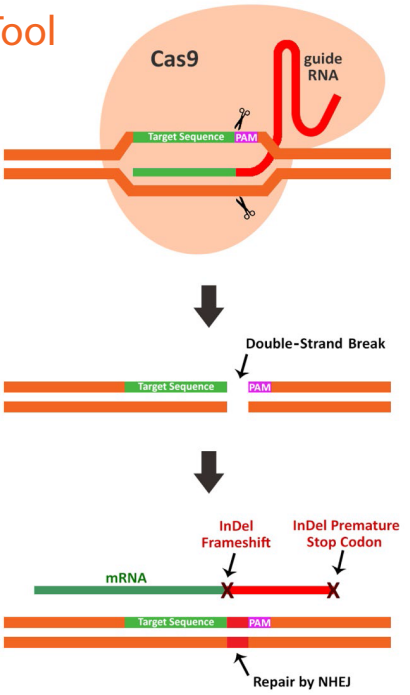
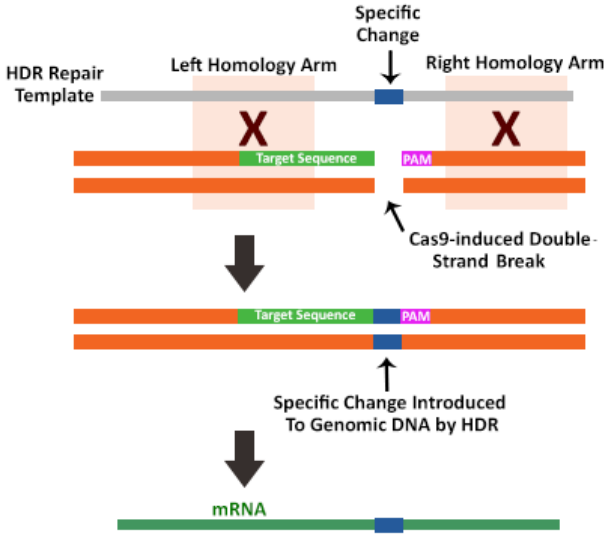
## A Versatile and Fully-Customizable Genome Editing Tool

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.



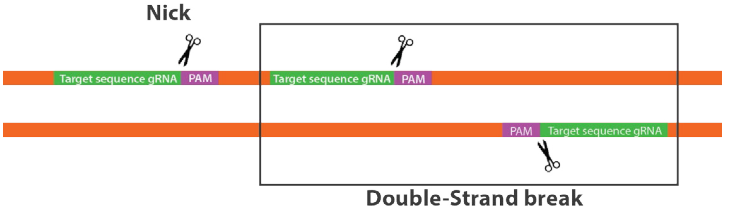




## Homology Directed Repair (HDR) with Cas9

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 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, effectively boosting the recognition sequence to 40 nucleotides.

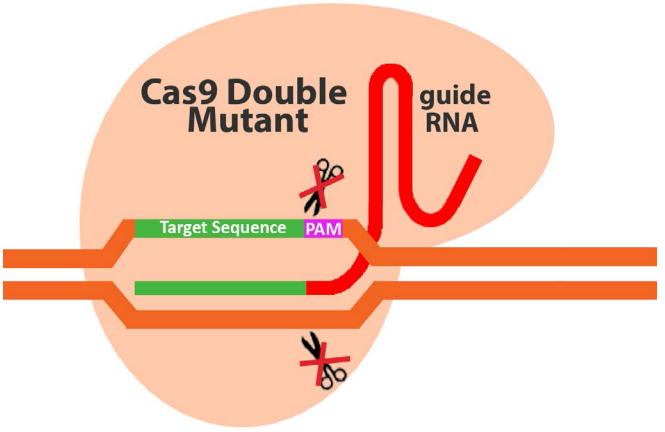


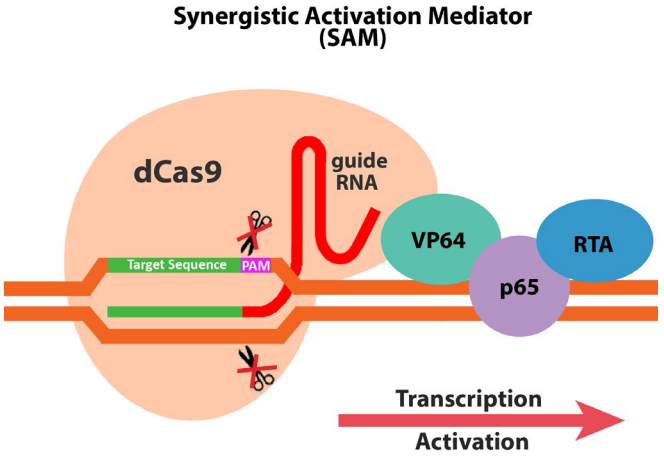

| Cas9 Type | Product Type | Cat.No. |
|---|---|---|
| Nuclease (wild-type) | Lentiviral vector / Lentivirus | K002, K003 |
| | Cumate-Inducible Lentivirus / Lentivirus | K017, K022, K024, K021, K023, K025 |
| | Protein | K008, K009, K030, K031 |
| Nickase (modified) | Lentiviral vector / Lentivirus | K005, K006 |
| | Adenovirus | K007 |
| | Protein (NLS, D10A, H840A) | K032, K033, K034, K035, K036, K037, K038, K039 |

# 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.



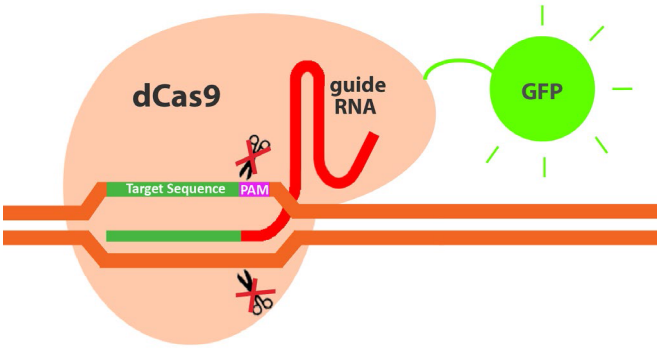




## Transcription Activation by dCas-SAM

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!

## Genomic Locus Visualization by dCas9-GFP

By the use of a dCas9-GFP fusion, it is now possible to visualize genomic sequences of interest directly within living cells. Tagging of individual chromosomes, monitoring of the packaging state of euchromatic DNA regions and identification of mutations are just some of the potential applications of this system.



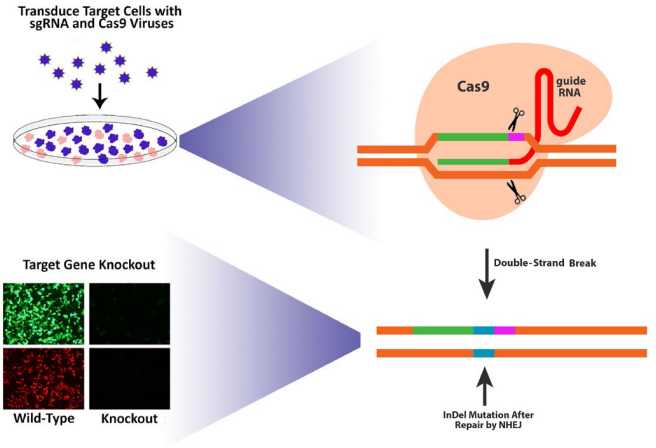

| dCas9 Variant | Application | Product Type | Cat.No. |
|---|---|---|---|
| dCas9 | Any genome targeting experiment | Lentiviral vector | K012, K014 |
| | | Lentivirus | K013 |
| | | Protein | K040, K041, K042, K043 |
| dCas9 - SAM | Transcription activation | Lentiviral vector | K015 |
| | | Lentivirus | K016 |
| dCas9 - GFP | Genomic Locus Imaging | Lentiviral vector | K026 |
| | | Lentivirus | K027 |

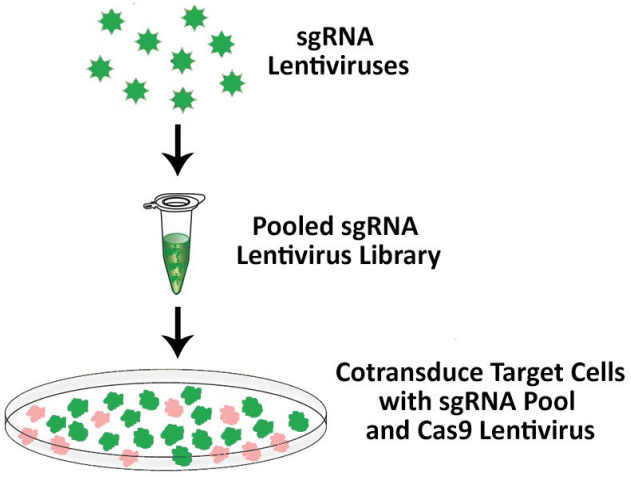
# CRISPR Services and Toolbox

## 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.



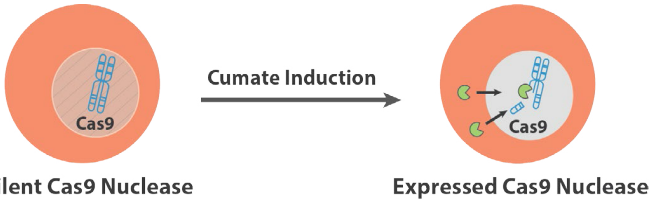

## CRISPR Custom Targeted Lentiviral sgRNA Library

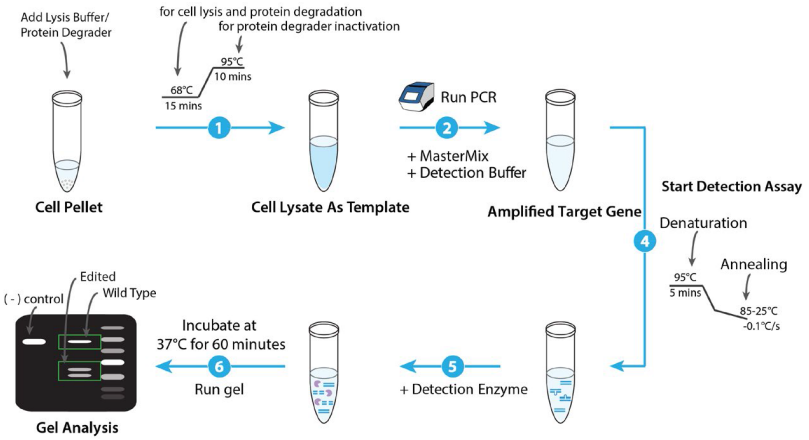
Cat. No. C209

Knockout up to 100 target genes with a single custom targeted sgRNA lentiviral pool! This custom product can be tailored to your specific experiments and is especially useful for the knockout of gene families and pathways. The pooled lentiviral vector constructs or pre-packaged lentivirus will be provided.

## CRISPR-ready Inducible Cas9 Custom Stable Cell Lines

**abm** now offers stable cell lines carrying cumate-inducible Cas9, offering robust and tight control over its activity. By limiting expression of Cas9 to only when it is needed, you also limit the chance of unwanted off-target effects, a significant improvement over constitutive expression. Simply add sgRNAs targeting your genes of interest, and trigger CRISPR at will!




## CRISPR Genomic Cleavage Detection Kit

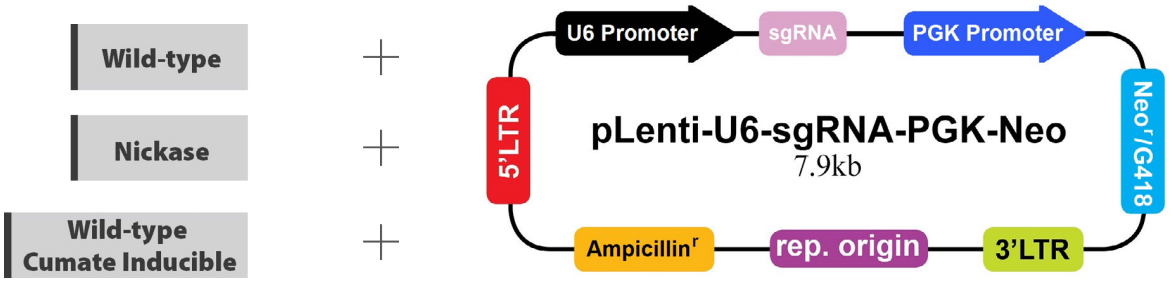
Cat. No. G932

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.

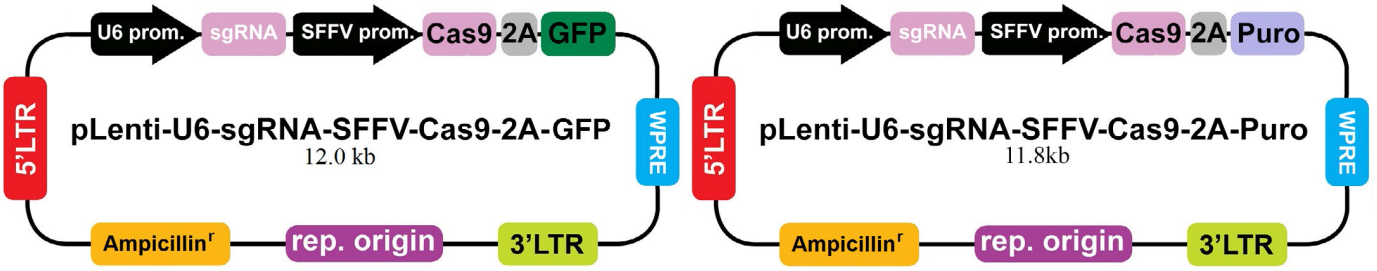
# Genome-wide sgRNA Libraries at Your Fingertips!

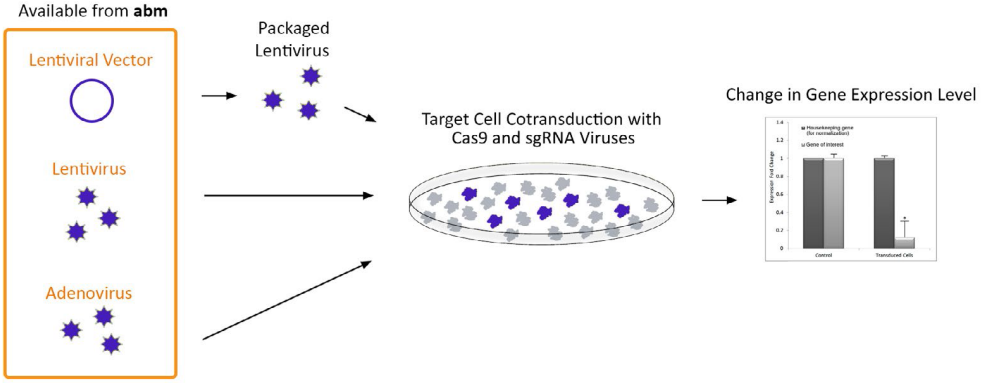
**abm** offers genome-wide CRISPR sgRNA libraries for targeting any human, mouse, or rat gene with the use of lentiviral vectors or ready-to-use lentiviruses and adenoviruses. Our lentiviral sgRNA vectors and viruses are provided as individual constructs or in a set of 3, both separate from Cas9 and as an All-In-One System. They can be used individually or pooled together to achieve optimal gene knockout. This allows for unparalleled flexibility in experimental setup. In addition, our sgRNA adenoviruses are useful for non-integrating, highly efficient genome editing in a wide range of target cells, especially cell types that are harder to transduce.

## Two Vector System




## All-In-One Vector System




| CRISPR sgRNA Format | Individual or Set of 3 | Product Type |
|---|---|---|
| sgRNA only (Cas9 required separately) | Individual sgRNA | Lentiviral vector |
| | | Lentivirus |
| | | Adenovirus |
| | Set of 3 sgRNA | Lentiviral vector |
| | | Lentivirus |
| All-In-One (sgRNA and Cas9 in a single vector) | Individual sgRNA | Lentiviral vector |
| | | Lentivirus |
| | | Adenovirus |
| | Set of 3 sgRNA | Lentiviral vector |
| | | Lentivirus |

## More Resources

**For more information about CRISPR-Cas9, visit our Knowledge Base and YouTube Channel!**

**Knowledge Base:**
https://www.abmgood.com/marketing/knowledge_base.php

**YouTube Channel:**
http://www.youtube.com/c/abmgood

***CRISPR Cas9 - A Brief Introduction***
https://youtu.be/1aJxXWkE3Ek

***CRISPR Cas9 - Methods and Tools***
https://youtu.be/INC_kdr7I34

***CRISPR Cas9 - gRNA Design***
https://youtu.be/dXPDefej0Ps

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