# CRISPR in Livestock and Food Safety:
## Beyond Genome Editing

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## Background

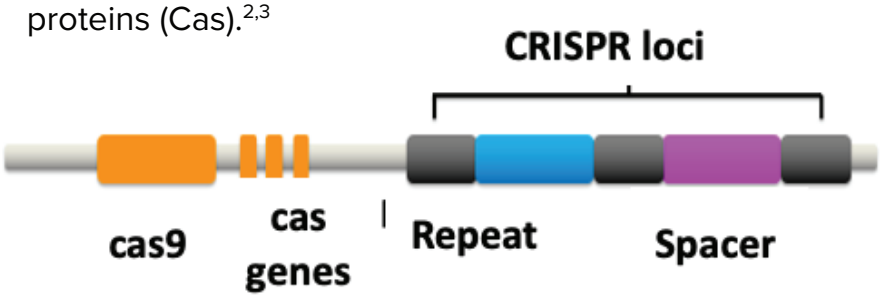
The CRISPR-Cas9 system is a novel biotechnology that is currently under diversified development as a gene editing tool for its application in humans, animals, plants, microorganisms, etc. In this fact sheet, we focus on its potential applications as "smart antimicrobials" in livestock for bacterial pathogen control, and rumen and gut microbiome editing to improve food safety and animal health.

## Brief introduction of the CRISPR-Cas9 system

### What is the CRISPR-Cas9 system?

Clustered regularly interspaced short palindromic repeats or CRISPR/Cas systems have been found in many species of bacteria and archaea, and act as defense mechanisms against invasion of foreign nucleic acid by recognizing and degrading these foreignelements.[1]

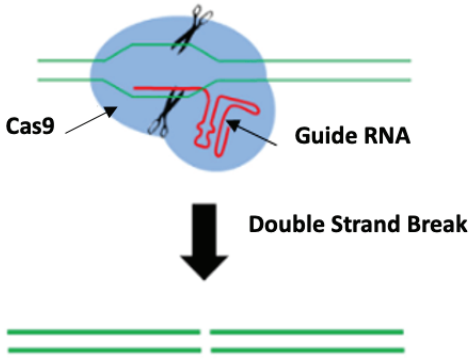
CRISPR/Cas9 systems are composed of CRISPR arrays consisting of spacers, such as gene-targeting sequences, interspersed with identical repeats and cas genes organized in operons, and cas genes encoding CRISPR-associated proteins (Cas).[2,3]




### The CRISPR-Cas9 system functions like a pair of "genetic scissors"

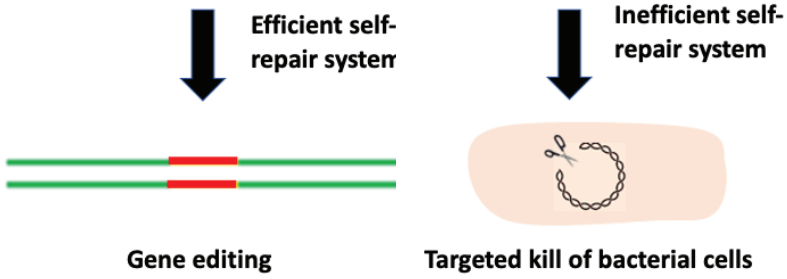
**Guide-RNA** (gRNA) from CRISPR arrays: finds target DNA sequences by complementary base-pairing.

**Cas9 protein:** carries out DNA cleavage to introduce double-strand breaks (DSBs) in the target DNA.



Gene editing tools for eukaryotes (like plants, animals, and human cells) – when resolving DSBs in DNA, the repair pathways result in small insertions, deletions, or replacements of DNA, thereby being able to introduce the designed gene edits. **To function as antimicrobials for bacterial cells** – different from eukaryotes, a DSB in the bacterial genome can be lethal to bacterial cells because most of these cells have an inefficient self-repair system.[4,5]

**Different repair systems between eukaryotes and prokaryotes lead to their different potential applications**




# How the CRISPR-Cas9 system can be used in livestock to improve food safety and animal health

## Advantages

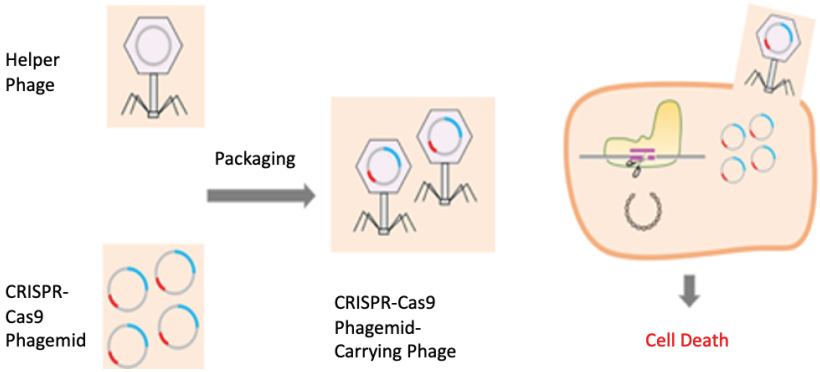
The CRISPR-Cas9 system is **sequence-specific**, which means it will selectively destroy microorganisms containing the target gene(s) in a mixed bacterial population while leaving the non-target bacterial populations unaffected.

The activity spectrum of CRISPR-Cas9 systems can be defined by the design and combination of gRNAs.

The CRISPR-Cas9 system can be easily programmed to kill specific bacterial cells by selecting specific genes as targets

## Combat foodborne pathogens

The CRISPR-Cas9 system could be designed to selectively kill foodborne pathogens by targeting virulence genes (genes found in disease-causing bacteria)



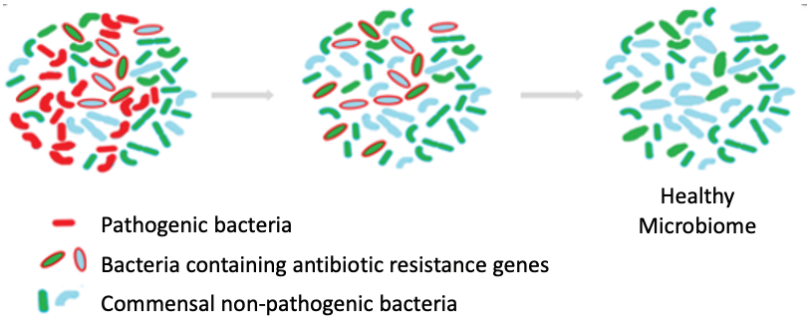

For example, researchers at Colorado State University conducted studies to develop a CRISPR-Cas9 system for the control of Shiga toxin-producing *E. coli* (STEC), which can cause severe human illness, by targeting their Shiga toxin genes (*stx1* and *stx2*) simultaneously. They constructed a phage-mediated system for efficient delivery of the CRISPR-Cas9-based targeted killing system into STEC cells inoculated in cattle rumen fluid, in vitro.[6,7]

## Mitigate antibiotic resistance

Eradicate antibiotic-resistant bacteria by targeting antibiotic resistance genes. Re-sensitize antibiotic-resistant bacteria and prevent the spread of antibiotic resistance genes by targeting transferrable genetic elements.[8,9]

## Improve animal health and performance by establishing and maintaining healthy microbiomes in livestock

Because of its sequence-specific targeting, the CRISPR-Cas9 system could be utilized as "smart" antimicrobials to remodel the rumen or gut microflora by sequentially knocking down undesired bacterial populations while maintaining the beneficial microflora.




# Challenges ahead for widespread application of the CRISPR-Cas9 system

## Consumer perception regarding the CRISPR-Cas9 System

Consumers need more information to help them understand the application of this technology in livestock and determine their willingness to accept products derived from livestock subjected to this novel technology

## Regulation of CRISPR biotechnologies

Biotechnology, in general, is regulated by the Coordinated Framework for Regulation of Biotechnology, formed in 1986 and updated in 2017, comprising of the U.S. Food and Drug Administration (FDA), United States Department of Agriculture (USDA), and Environmental Protection Agency (EPA).[10]

Use of the CRISPR-Cas9 system in the pre-harvest environment for foodborne pathogen control would primarily be regulated by FDA's Center for Veterinary Medicine (CVM) as a new animal drug, based on the Food, Drug, and Cosmetic Act (FD&C Act) and Guide 1240.3605.

New regulations that are not currently in documentation may arise for regulating meat products derived from animals being treated with the CRISPR-Cas9 system for bacterial control and/or microbiome editing purposes.[11]

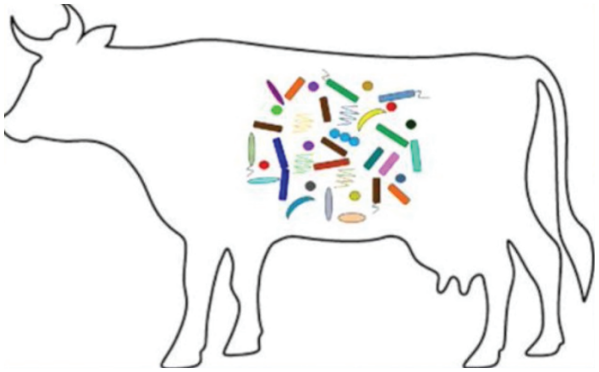
# Biosafety of the use of the CRISPR-Cas9 system in live animals and their environments

## The biosafety risk of CRISPR appliedfor bacterial control could be low

Recently, researchers at Colorado State University conducted a study to estimate the potential biosafety risk associated with the CRISPR-Cas9 system when applied to kill STEC in a bovine cell line model system, using next-generation sequencing for DNA comparison and proteomic analyses for protein identification and quantification. Their primary results indicated that the biosafety risk of the CRISPR-Cas9 system applied for pathogen control could be low because the CRISPR-Cas9 system was designed (i) to make a cleavage in target bacterial genomes and not in bovine genomes, and (ii) to be delivered into bacterial cells and not into bovine cells.[12]

## Different levels of biosafety risk when CRISPR is used as a gene editing tool vs. a bacterial control method

Gene editing CRISPR tools directly alter genomes of live animals, while the CRISPR-Cas9 system only manipulates the microbiome residing in the host animal.



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