

Advances and challenges in the application of CRISPR technology for environmental biological pollution control

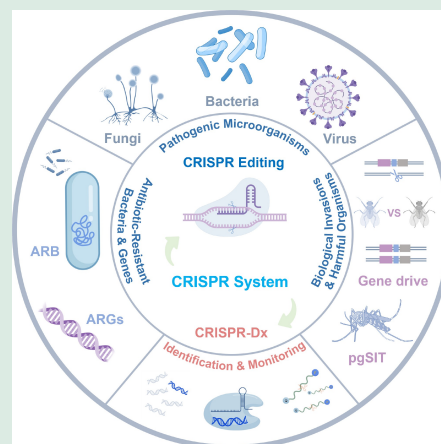
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HIGHLIGHTS

- CRISPR enables precise, programmable control of environmental biological pollution.
- Dual-use platforms allow both pathogen detection and genetic intervention.
- Applications target crop diseases, antibiotic resistance, and invasive insects.
- Key hurdles include delivery, off-target effects, and regulatory frameworks.
- Future directions include AI-designed editors and smart delivery systems.



ABSTRACT: Biological pollution, including pathogenic microorganisms, antibiotic resistance, invasive species, and harmful organisms, poses increasing threats to ecosystems, food security, and public health. In recent years, CRISPR-based technologies have emerged as powerful tools for biological pollution control due to their high precision, programmability, and versatility. In this Review, we summarize recent advances in CRISPR gene-editing applications for mitigating biological pollution, including the control of pathogenic microorganisms, antibiotic-resistant bacteria, and resistance genes, as well as invasive and harmful species through targeted genetic interventions. We also discuss the role of CRISPR diagnostic platforms in environmental surveillance as complementary tools for identifying and tracking biological contaminants. Furthermore, we critically examine key technical, ecological, and governance challenges that constrain the translation of CRISPR-based strategies from laboratory studies to real-world environmental applications. Finally, we highlight emerging directions in high-precision editing, intelligent delivery systems, and responsible governance frameworks that will be essential for the safe and sustainable deployment of CRISPR technologies in environmental pollution control.

KEYWORDS: CRISPR-Cas, Gene editing, Biological pollution, Antibiotic resistance, Invasive species

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1 Introduction

Biological pollution refers to the presence of harmful organisms and their biological products in environmental systems. It has become one of the most pressing global environmental challenges, affecting agriculture, public health, and industrial processes. Across diverse environmental compartments, including soil, freshwater, wastewater, and urban ecosystems, biological contaminants such as pathogenic microorganisms (Okesanya et al., 2025), antibiotic-resistant bacteria associated with human diseases (Cheng et al., 2025c), and invasive species that disrupt ecological stability pose substantial ecological and societal risks (Yadav et al., 2023). These forms of biological pollution can alter ecosystem functions, compromise ecosystem services, drive biodiversity loss, and generate significant economic burdens worldwide (Goda et al., 2025), while simultaneously threatening food security and human health (Garvey, 2019). Traditional counter-measures, including broad-spectrum chemical pesticides, antibiotics, and physical removal strategies, can suppress pollution to some extent but exhibit increasingly evident limitations (Miller and Arias, 2024). In environmental contexts, these approaches often suffer from low target specificity, poor long-term effectiveness, secondary environmental contamination, and unintended impacts on non-target organisms and beneficial microbial communities (Karaoglan et al., 2024). Consequently, there is an urgent need to develop next-generation biological pollution control technologies, referring to advanced, programmable, and target-specific strategies that can precisely detect, suppress, or eliminate harmful biological contaminants while reducing secondary pollution, minimizing impacts on non-target organisms, and maintaining ecosystem-level protection.

In recent years, clustered regularly interspaced short palindromic repeats (CRISPR) gene-editing technology has emerged as one of the most transformative breakthroughs in life sciences (Lu and Zhu, 2017). This technology provides unprecedented tools for understanding and mitigating biological pollution in environmental systems. Originally derived from the adaptive immune systems of archaea and bacteria that protect against invading genetic elements such as bacteriophages and plasmids, CRISPR-based mechanisms have been repurposed into versatile genome-editing platforms (Ishino et al., 2018). In environmental and microbial contexts, the programmability of CRISPR systems enables targeted genetic intervention

within complex biological communities (de Lorenzo, 2017). This capability offers opportunities to selectively manipulate specific organisms or genes while preserving overall ecosystem structure and function. At the molecular level, CRISPR systems rely on guide RNA (gRNA) molecules to direct Cas nucleases to complementary genomic sequences, where site-specific DNA cleavage occurs (Abudayyeh et al., 2016). Subsequent endogenous DNA repair processes can be exploited to achieve gene knockout, insertion, or precise sequence modification. As CRISPR technologies have evolved, the original double-strand break (DSB)-dependent Cas9 platform has been progressively expanded to include base editing and prime editing systems that operate without generating DSBs, thereby enhancing editing precision and reducing unintended genomic damage (Gasiunas et al., 2012; Eid et al., 2018; Anzalone et al., 2020; Gao, 2021). These advances are particularly important for environmental applications, where minimizing off-target effects and genomic instability in non-target organisms is essential for maintaining ecological safety.

In addition to genome editing, CRISPR systems have been repurposed as sensitive and programmable molecular diagnostic tools (Razavi et al., 2026). Cas12- and Cas13-based platforms can recognize specific DNA or RNA sequences and activate collateral cleavage of reporter molecules, thereby enabling rapid nucleic acid detection through fluorescence, lateral-flow, or electrochemical readouts (Hu et al., 2025a). Compared with conventional culture- or PCR-based methods, CRISPR diagnostics provide high specificity, rapid detection, compatibility with isothermal amplification, and potential for portable field deployment (Low et al., 2026). These advantages are particularly relevant to environmental monitoring, where pathogens, antibiotic resistance genes, invasive species-derived environmental DNA, and other biomarkers often occur at low concentrations in complex matrices such as wastewater, soil, surface water, and biofilms (Li et al., 2023). Thus, CRISPR diagnostics can support early warning, source tracking, ecological risk assessment, and post-treatment evaluation, complementing CRISPR-based biocontrol strategies in environmental systems.

The central advantage of CRISPR systems lies in their highly programmable targeting capability. In contrast to conventional environmental control strategies that rely on broad-spectrum suppression or non-specific removal, CRISPR enables the rational design of targeted interventions. These interventions can be directed at pathogen-specific virulence genes, antibiotic resistance determinants, or key genetic regulators

controlling the reproduction and persistence of invasive organisms (Tyagi et al., 2021; Anu et al., 2024; Zhang et al., 2025). Such precision enables interventions at the level of individual genes or pathways, addressing biological pollution at its source while minimizing impacts on non-target organisms and ecosystems.

Accordingly, this review provides a systematic overview of recent advances in the application of CRISPR gene-editing technologies for the control of environmental biological pollution. We first summarize the fundamental principles of CRISPR systems and their functional diversity, followed by an overview of emerging CRISPR-based molecular diagnostic technologies enabled by Cas protein-mediated collateral cleavage activity. We then review representative applications of CRISPR-based strategies in major biological pollution scenarios, encompassing the management of pathogenic microorganisms, the mitigation of antibiotic resistance genes and resistant bacteria in environmental reservoirs, and the genetic control of invasive and vector-borne species. Finally, this review examines the key challenges constraining the environmental application of CRISPR-based biocontrol, including delivery efficiency, ecological risks, resistance evolution, and regulatory considerations, and discusses their implications for sustainable biological pollution control. By integrating current advances and future perspectives, this review seeks to provide a foundation for further research and responsible implementation of CRISPR technology in global biological pollution management.

2 Overview of CRISPR technology

At the mechanistic level, the CRISPR–Cas system operates as an RNA-guided genome-editing platform that enables precise and programmable manipulation of target DNA sequences (Abudayyeh et al., 2016). Central to this process is a gRNA, which forms a ribonucleoprotein complex with a Cas nuclease and confers sequence specificity through base pairing with complementary genomic loci (Doudna and Charpentier, 2014). Upon recognition of the target site, the activated Cas nuclease introduces site-specific DNA cleavage (Jiang and Doudna, 2017), which can be resolved by endogenous DNA repair pathways to achieve targeted gene knockout, sequence insertion, or precise genetic modification (Liang et al., 2015; Cheng et al., 2022; Liu et al., 2024). The separation of targeting specificity (gRNA) from catalytic activity (Cas nuclease) constitutes the molecular basis for the versatility and

adaptability of CRISPR-based genome-editing technologies (Jang et al., 2025).

CRISPR–Cas systems are generally divided into two major classes based on the structural characteristics of their effector complexes (Koonin et al., 2017). Class 1 systems (including types I, III, and IV) rely on multi-subunit effector complexes composed of several Cas proteins. Although widely distributed in nature, their complex multi-component architecture makes engineering applications in eukaryotes more challenging (Koonin et al., 2017). Class 2 systems (including types II, V, and VI), in contrast, use single, multi-domain effector proteins, making them easier to manipulate and deliver (Makarova et al., 2019). Consequently, they constitute the primary source of modern genome-editing tools (Table 1).

Among these systems, the type II CRISPR–Cas9 derived from *Streptococcus pyogenes* is currently the most widely used and well-established gene-editing platform (Hu et al., 2018). Cas9 contains two nuclease domains—HNH and RuvC—and, under the guidance of gRNA, recognizes target DNA sequences and their adjacent protospacer adjacent motif (PAM; typically NGG), introducing a DSB at the target site (Jinek et al., 2012). Representative type V systems such as Cas12a require only a single crRNA, recognize distinct PAM sequences (e.g., TTTN), and generate staggered DNA ends upon cleavage, a feature advantageous for multiplex gene editing and molecular cloning applications (Zalatan et al., 2015). In addition to its site-specific DNA cleavage activity, Cas12a also exhibits collateral (*trans*) cleavage activity toward non-target single-stranded DNA following target recognition (Li et al., 2018), a property that has been widely exploited for sensitive nucleic acid detection (Cheng et al., 2024, 2025c). Type VI systems (e.g., Cas13) are unique in their ability to target single-stranded RNA. Cas13 proteins contain HEPN domains and similarly display collateral cleavage activity, whereby nonspecific RNA degradation is triggered upon recognition and cleavage of a complementary target RNA (Apostolopoulos et al., 2024; Chen et al., 2024). Collectively, the collateral cleavage activities of Cas12 and Cas13 form the mechanistic basis of CRISPR-based diagnostic platforms, enabling highly sensitive and specific detection of DNA- and RNA-based targets. These properties underpin their growing application in environmental pathogen surveillance, microbial monitoring, and early warning systems for biological contamination (Apostolopoulos et al., 2024; Cheng et al., 2025a, 2025b).

Early CRISPR–Cas9 editing strategies relied on DSB induction, with subsequent repair through non-

Table 1 Characteristics of CRISPR-Cas nucleases

Nucleases	Class	Type	Nuclease domain	Guide RNA	PAM/PFS	Substrate	Ref.
Cascade-Cas3	Class I	Type I	HD	crRNA	Diversify	dsDNA	Xiao et al. (2018)
Cas7–11	Class I	Type III	RRM	crRNA	no PFS bias	RNA	Hu et al. (2022)
Cas9	Class II	Type II	HNH and RuvC	crRNA and tracrRNA	G-rich	dsDNA	Jinek et al. (2012)
Cas12	Class II	Type V	RuvC	crRNA	T-rich	Mostly target dsDNA	Chen et al. (2018)
Cas13	Class II	Type VI	HEPN	crRNA	no PFS bias	RNA	Abudayyeh et al. (2017)

homologous end joining (NHEJ) or homology-directed repair (HDR) pathways to achieve gene knockout or insertion ([Fig. 1](#)) ([Gasiunas et al., 2012](#); [Gao, 2021](#)). However, DSBs may lead to unpredictable insertions or deletions, limiting their utility in applications requiring high precision ([Liu et al., 2022](#)). In microbes, site-specific DNA cleavage can cause cellular toxicity, such as growth inhibition or lethality, due to DNA damage or unrepaired breaks, limiting DSB-dependent genome editing ([Cui and Bikard, 2016](#); [Arazoe et al., 2018](#)). To address these limitations, more refined editing tools have been developed. Base editing enables targeted single-nucleotide conversions without introducing DSBs ([Eid et al., 2018](#)). Cytosine base editors (CBEs), such as BE3, enable the precise conversion of C•G to T•A at target sites by coupling a cytidine deaminase to a Cas9 nickase, avoiding double-strand breaks and minimizing indels ([Gaudelli et al., 2017](#); [Hu et al., 2025b](#)). Adenine base editors (ABEs), such as

ABE7.10, similarly mediate the conversion of A•T to G•C through a fused evolved adenine deaminase and Cas9 nickase, offering high-efficiency single-base editing with reduced genomic toxicity ([Gaudelli et al., 2017](#); [Anzalone et al., 2020](#)). These approaches substantially enhance editing precision and biosafety, features particularly relevant for environmental and microbial applications.

Prime editing further extends the capabilities of genome modification. Through the combination of a reverse transcriptase with a prime editing guide RNA (pegRNA), this method enables all 12 types of base substitutions, as well as small insertions and deletions, without inducing DSBs ([Anzalone et al., 2019](#)). Optimized editors such as aPE, augmented by the anti-CRISPR protein AcrIIA5 through inhibition of re-nicking and reduction of indels, achieve higher desired edit frequencies with fewer byproducts ([Chen et al., 2025](#)).

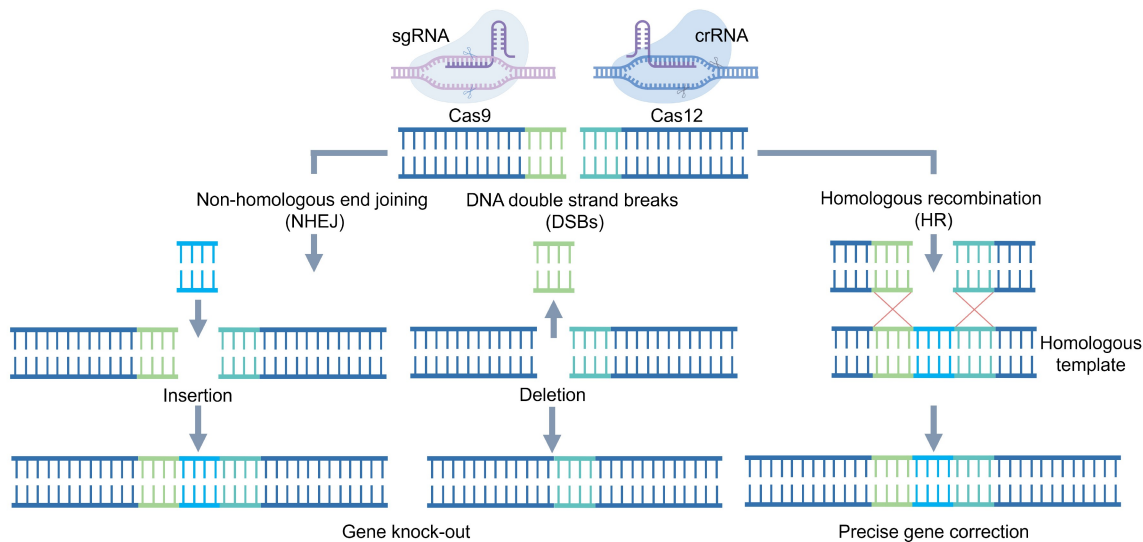


Fig. 1 Molecular principles of CRISPR genome editing. CRISPR genome editing relies on RNA-guided nucleases such as Cas9 and Cas12a for site-specific recognition and cleavage of target DNA. Upon target binding, these nucleases catalyze DNA cleavage, generating a double-strand break. Cellular DNA repair pathways then process the DSB to introduce genetic modifications (edits). Repair via non-homologous end joining and related pathways typically results in short insertions or deletions, while homologous recombination—utilizing an exogenous DNA repair template—enables precise, targeted modifications through homology-directed repair.

Collectively, these advances shift CRISPR-based genome engineering from conventional DSB-dependent editing toward more precise, flexible, and lower-toxicity genetic modification. Such improvements are particularly important for environmental and microbial applications, where editing precision, reduced genomic damage, and biosafety are critical for practical implementation.

3 CRISPR-based diagnostics for the identification and surveillance of biological contamination

Environmental matrices such as wastewater, surface water, and soil serve as important reservoirs for pathogenic microorganisms and antibiotic resistance genes (ARGs), posing significant ecological and public health risks (Cheng et al., 2025d). Effective monitoring of these environments is therefore critical for early warning, outbreak prevention, and ecological management. Traditional laboratory-based detection methods, however, are often labor-intensive, time-consuming, and limited in their ability to provide real-time insights across diverse environmental sites (Table 2).

CRISPR-diagnostic (CRISPR-Dx) platforms have emerged as transformative tools for environmental surveillance (Fig. 2). Leveraging the sequence-specific cleavage and collateral activity of Cas12a and Cas13, these systems enable rapid, highly sensitive, and programmable detection of DNA and RNA targets in complex environmental matrices (Apostolopoulos et al., 2024; Cheng et al., 2025a, b). Innovations such as one-pot amplification reactions, engineering of crRNA sequences, and portable, field-deployable devices further enhance their applicability for decentralized monitoring (Cheng et al., 2025a, c; Han et al., 2025). In particular, CRISPR-Dx platforms have been successfully applied to detect viral pathogens such as

SARS-CoV-2 in wastewater systems (Cheng et al., 2025a, b). They have also been used to track the occurrence and dynamics of antibiotic resistance genes across urban water cycles (Cheng et al., 2024), providing quantitative data that can inform environmental risk assessment and intervention strategies.

For wastewater-based surveillance, paper-based CRISPR/Cas12a devices coupled with reverse-transcription loop-mediated isothermal amplification (RT-LAMP) have been developed for the detection of SARS-CoV-2 in wastewater, allowing simultaneous detection of multiple viral genes, such as the *N*, *E*, and *S* genes, with reported limits of detection in the low-copy range (Cao et al., 2022). For example, one wastewater-oriented paper device achieved limits of detection of 25, 310, and 10 copies/mL for the *N*, *E*, and *S* genes, respectively, and showed high sensitivity in blind wastewater sample analysis (Cao et al., 2022). In addition to viral pathogens, CRISPR-Dx has been applied to monitor ARGs in aquatic environments. A portable biosensor integrating LAMP with CRISPR/Cas12a was reported for on-site detection of the macrolide resistance gene *ermB* in wastewater, combining magnetic-bead-based nucleic acid extraction with fluorescence and lateral-flow readouts (Mao et al., 2024). CRISPR-based assays have also been extended to wildlife- and aquaculture-related pathogens. For instance, recombinase polymerase amplification (RPA) coupled with Cas12a was used for the detection of *Renibacterium salmoninarum* in environmental DNA samples, illustrating the potential of CRISPR-Dx for pathogen surveillance in natural water systems and wildlife-associated environments (Zhao et al., 2024). These examples indicate that the detection scope of CRISPR-Dx has expanded from viral RNA to bacterial pathogens, ARGs, and environmental DNA biomarkers, with detection formats ranging from laboratory fluorescence assays to paper-based or lateral-flow devices suitable for decentralized monitoring.

By integrating high sensitivity, environmental adaptability, and rapid turnaround, CRISPR-based diagnostics offer a powerful approach for the proactive

Table 2 Comparison of qPCR, isothermal amplification, and CRISPR-Dx

Method	Advantages	Limitations	Ref.
qPCR	High sensitivity; accurate quantification; well established and widely used.	Needs a thermal cycler, stable power supply, trained staff, and laboratory conditions.	Li et al. (2023)
LAMP/RPA	Fast; works at a constant temperature; does not need a thermal cycler; useful for simple field testing.	May produce non-specific amplification or false-positive results, especially in complex environmental samples.	Yang et al., (2023); Pataer et al. (2024)
CRISPR-Dx	Fast and portable; adds guide RNA-based target recognition; can improve specificity while keeping simple equipment needs.	Less mature than qPCR for accurate quantification; performance may be affected by sample quality and environmental inhibitors.	Hu et al. (2025a)

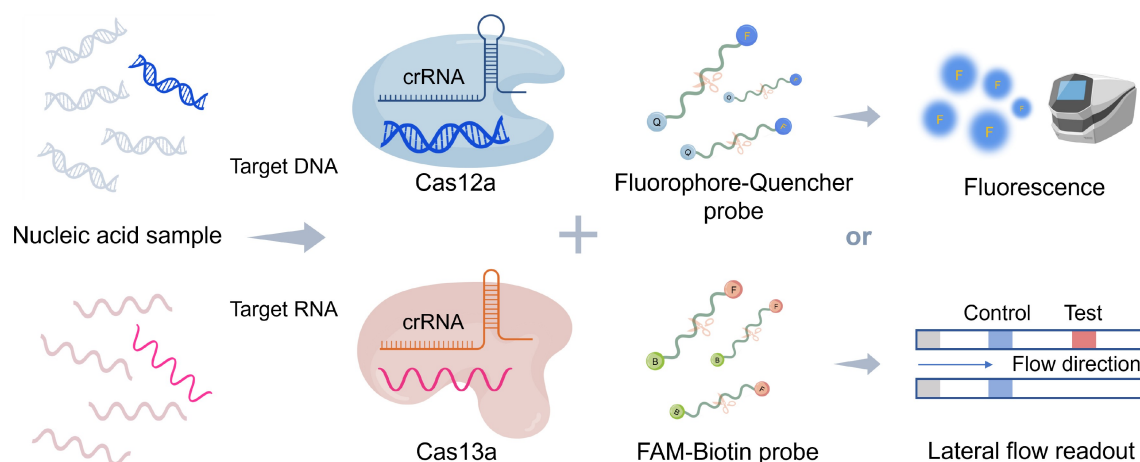


Fig. 2 Schematic illustration of CRISPR-Dx for nucleic acid detection. The figure illustrates the basic workflow of CRISPR-based diagnostics using Cas12a and Cas13a systems. In nucleic acid samples, target DNA is specifically recognized by the Cas12a-crRNA complex, whereas target RNA is recognized by the Cas13a-crRNA complex. Upon target binding, Cas12a or Cas13a is activated and exhibits collateral cleavage activity toward reporter probes. In the fluorescence-based readout, cleavage of fluorophore-quencher probes separates the fluorophore from the quencher, thereby generating a detectable fluorescence signal. Alternatively, in the lateral flow readout, cleavage of FAM-biotin-labeled probes produces a visible signal on the test strip, enabling rapid interpretation through the control and test lines.

management of biological contamination. Their deployment in environmental surveillance supports timely identification of ecological and public health threats. It also enables evidence-based mitigation strategies to reduce the spread of pathogens and resistance determinants across environmental reservoirs.

4 CRISPR gene editing in the control of pathogenic microorganisms

Following the advancement of CRISPR-Dx tools for environmental surveillance, effective strategies for mitigating detected biological contamination are critically needed. The advent of CRISPR-based genome-editing technologies has opened new avenues for the precise and targeted control of pathogenic microorganisms, offering a potential paradigm shift in biological pollution management.

Compared with conventional pathogen control approaches, including chemical pesticides, antibiotics, fungicides, resistant cultivar breeding, biological control agents, and physical sanitation measures, CRISPR-mediated host resistance provides a more genetically precise and potentially durable intervention strategy. Chemical pesticides and fungicides can rapidly suppress disease outbreaks and remain indispensable for large-scale agricultural production;

however, their repeated use may lead to environmental residues, non-target toxicity, disruption of beneficial microbiota, and the emergence of resistant pathogen populations (Carvalho, 2017). Conventional breeding has generated many disease-resistant cultivars, but it is often time-consuming and constrained by available resistance germplasm, linkage drag, and pathogen race specificity (Rato et al., 2021). Biological control agents, such as antagonistic bacteria, fungi, offer environmentally friendly alternatives, yet their efficacy is frequently influenced by environmental conditions, host specificity, and field stability (Manzoor et al., 2024). In this context, CRISPR-based editing of host susceptibility genes offers a complementary strategy by directly modifying genetic factors required for pathogen colonization or replication. Rather than broadly eliminating microbial communities, this approach aims to reduce host permissiveness to infection, thereby providing a sequence-defined and host-directed route for biological pollution control (Fig. 3).

In agricultural environments, pathogenic bacteria are major contributors to biological pollution, causing extensive crop diseases that threaten food security and agroecosystem stability (Tyagi et al., 2021; Ayaz et al., 2023). For instance, bacterial spot disease caused by *Xanthomonas* species severely affects tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*) production (Schwartz et al., 2015; Ortega et al., 2024). Ortega et al. demonstrated that CRISPR/Cas9-mediated knockout of the tomato susceptibility genes *SLBs5* and

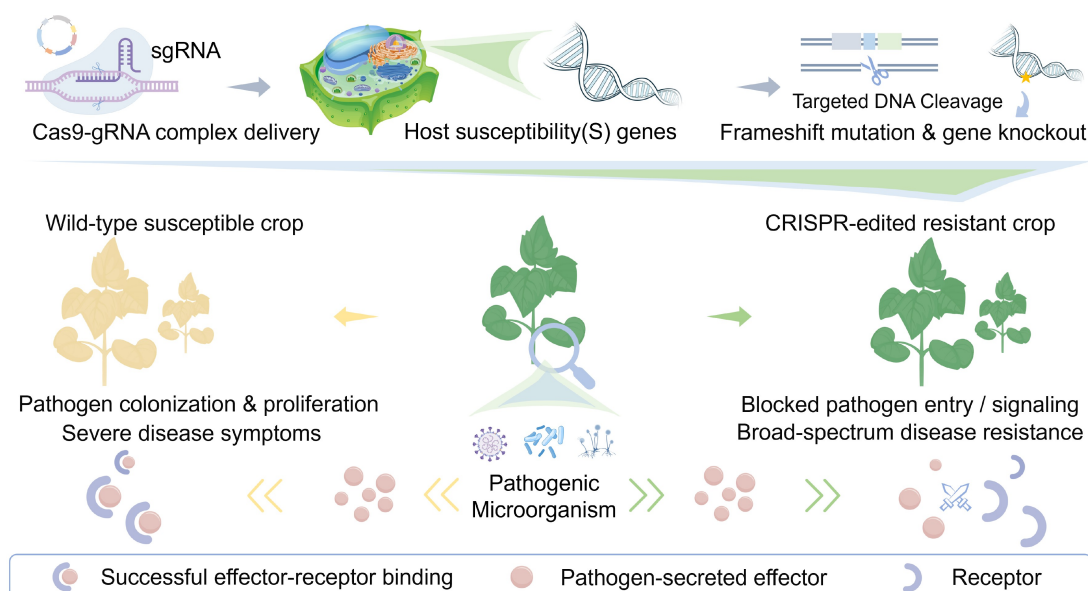


Fig. 3 General workflow for developing disease-resistant crops using the CRISPR-Cas9 system. This schematic illustrates a general strategy for generating disease-resistant crops through CRISPR-Cas9-mediated editing of host susceptibility genes. The Cas9-sgRNA complex, or its expression cassette, is delivered into plant cells, where the sgRNA guides Cas9 to specific host susceptibility (S) genes. Cas9-induced targeted DNA cleavage generates double-strand breaks, which are subsequently repaired by endogenous DNA repair pathways, often resulting in frameshift mutations and functional gene knockout. In wild-type susceptible crops, pathogen-secreted effectors can successfully interact with host receptors or susceptibility factors, promoting pathogen colonization, proliferation, and severe disease symptoms. In contrast, CRISPR-edited resistant crops carrying disrupted S genes exhibit impaired pathogen entry, effector recognition, or downstream susceptibility signaling, thereby restricting pathogen proliferation, and conferring broad-spectrum disease resistance.

SIBs5L—which are hijacked by pathogens during infection—effectively prevented pathogen invasion without introducing exogenous resistance traits (Ortega et al., 2024). Similarly, bacterial blight caused by *Xanthomonas oryzae* represents a persistent challenge to rice production systems worldwide (Kayastha et al., 2025). Li et al. (2024) disrupted the key gene *OsETR* in the rice cultivar *Nipponbare* using CRISPR/Cas9, generating three homozygous mutant lines with significantly enhanced resistance to multiple virulent strains, without yield penalties. In citrus production systems, *Xanthomonas citri*, the causal agent of citrus canker, poses a long-term threat to orchard sustainability (Su et al., 2023). Editing the promoter region of *CsLOB1* to block transcription activator-like effector (TALE) binding conferred high-level resistance to citrus canker, demonstrating the feasibility of promoter-targeted editing for disease control (Jia et al., 2016; Tyagi et al., 2021).

Beyond bacterial pathogens, fungal diseases are also major drivers of agricultural biological pollution, leading to persistent yield losses and increased reliance on fungicides (Paul et al., 2021). In soybean production, powdery mildew caused by *Erysiphe diffusa* severely compromises both yield and seed

quality (Bui et al., 2023). Thao et al. employed CRISPR/Cas9 to disrupt multiple *MLO* susceptibility genes (*GmMLO2*, *GmMLO19*, *GmMLO20*, and *GmMLO23*), revealing that triple and quadruple mutants exhibited progressively enhanced resistance, with quadruple mutants showing the strongest disease suppression (Bui et al., 2023). These results highlight susceptibility gene editing as an effective and environmentally favorable strategy for controlling fungal pathogens.

Viral pathogens further exacerbate biological pollution in agroecosystems by rapidly spreading across host populations and causing substantial economic losses (Jones, 2021). Tobacco mosaic virus (TMV), a positive-sense RNA virus, infects a wide range of solanaceous crops, including tobacco, tomato, and pepper (Jogam et al., 2023). For RNA viruses, CRISPR systems such as Cas13—which directly target RNA rather than DNA—offer unique advantages. Cas13a-mediated cleavage of viral RNA has been shown to reduce symptom severity and viral RNA accumulation in plants infected with cucumber mosaic virus (CMV) and TMV (Tyagi et al., 2021; Manzoor et al., 2024). In addition, host-directed genome editing has also proven effective against viral infection. Phanikanth et al.

disrupted the host susceptibility gene *TOM1*, which is essential for TMV replication, thereby conferring enhanced resistance to TMV in tobacco plants (Jogam et al., 2023).

Overall, CRISPR gene-editing technologies represent transformative tools for mitigating biological pollution caused by pathogenic bacteria, fungi, and viruses. Their precision and programmability enable targeted disruption of pathogen life cycles or reinforcement of host resistance through susceptibility gene deletion, offering environmentally sustainable alternatives to conventional chemical controls. However, the practical deployment of CRISPR-based disease-resistance strategies still face several challenges, including potential off-target effects, for example, the risk of unintended cleavage at highly homologous loci when designing gRNAs against conserved susceptibility-gene families such as *MLO* or *SWEET*, genotype-dependent editing efficiency, uncertain durability of resistance under field conditions, pathogen evolutionary escape, and regulatory or public-acceptance constraints. Future efforts should therefore combine improved gRNA design algorithms, high-fidelity Cas variants, and genome-wide off-target assessment to minimize unintended edits and improve the safety and reliability of CRISPR-based resistance breeding. Collectively, the studies summarized here demonstrate robust disease resistance across diverse crop systems and provide a conceptual framework for integrating CRISPR-based strategies into sustainable environmental and agricultural pollution management (Table 3).

5 Applications of CRISPR gene editing in the control of antibiotic-resistant bacteria and antibiotic resistance genes

The proliferation of antibiotic-resistant bacteria (ARB)

and the widespread dissemination of ARGs in environmental reservoirs constitute a major component of biological pollution and pose escalating threats to both ecosystem stability and global public health (Qiao et al., 2018; Nie et al., 2025). Conventional antibiotic-based interventions lack environmental specificity and often exacerbate selective pressure, accelerating the emergence of multidrug-resistant pathogens and further amplifying resistance dissemination (Muñoz et al., 2024; Peng et al., 2024). In this context, CRISPR-based gene-editing systems, characterized by high programmability and sequence-level precision, offer a transformative strategy for addressing ARB and ARG pollution at its genetic source (Ran et al., 2013; Knott and Doudna, 2018).

Rather than indiscriminately eliminating microbial populations, current CRISPR applications primarily focus on gene-centered resensitization strategies (Fig. 4), in which ARGs located on plasmids or chromo-somes are selectively disrupted. By inactivating ARGs while preserving host viability, this approach restores bacterial susceptibility to conventional antibiotics and reduces the ecological disturbance associated with broad-spectrum bactericidal treatments (Asgarali et al., 2009; Vivekanandan et al., 2025). To date, CRISPR-based interventions have been successfully designed to target multiple clinically and environmentally relevant resistance determinants, including *mecA* (methicillin resistance), *ermB* (erythromycin resistance), and *mcr-1* (colistin resistance) (Allemailem, 2024). Importantly, by removing resistance cassettes without exerting strong bactericidal pressure, CRISPR-based resensitization reduces the selective forces that typically drive the emergence and environmental persistence of multidrug-resistant bacteria. From an environmental perspective, this targeted genetic intervention offers a promising framework for mitigating the accumulation and spread of ARGs across wastewater systems, agricultural runoff, and natural aquatic environments, while maintaining the functional integrity of resident microbial communities (Uribe et al., 2021; Wan et al.,

Table 3 Applications of CRISPR gene-editing technology for plant resistance to pathogenic microorganisms

Pathogenic microorganisms	Microbial categories	Target gene	Disease resistance	Ref.
<i>Xanthomonas</i>	Bacteria	<i>SIBs5</i> and <i>SIBs5L</i>	Effectively prevent pathogen invasion	Ortega et al. (2024)
<i>Xanthomonas oryzae</i>	Bacteria	<i>OsETR</i>	Enhance resistance to bacterial wilt	Li et al. (2024)
<i>Xanthomonas citri</i>	Bacteria	<i>CsLOB1</i>	Effectively prevent pathogen invasion	Tyagi et al. (2021)
<i>Erysiphe diffusa</i>	Fungi	<i>MLO</i>	Effectively prevent pathogen invasion	Bui et al. (2023)
<i>Oidium neolycopersici</i>	Fungi	<i>SIPMR4</i>	Enhanced resistance to <i>O. neolycopersici</i>	Santillán Martínez et al. (2020)
<i>Phytophthora parasitica</i>	Fungi	<i>AtERF019</i>	Enhanced resistance to <i>P. parasitica</i>	Lu et al. (2020)
Tobacco mosaic virus (TMV)	Virus	<i>TOM1</i>	Enhanced resistance to TMV	Jogam et al. (2023)

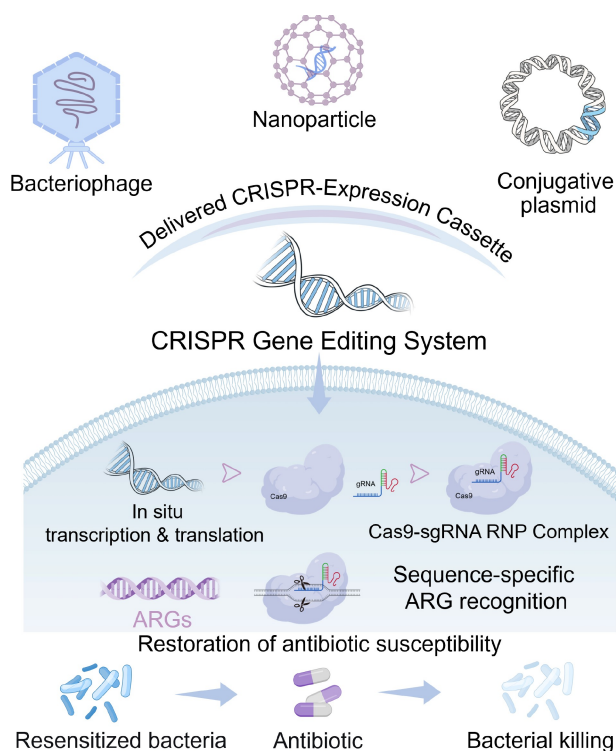


Fig. 4 General Workflow of “Gene-Focused Resensitization” against drug-resistant bacteria using the CRISPR-Cas9 system. This schematic illustrates a CRISPR-Cas9-based gene-focused resensitization strategy for combating drug-resistant bacteria. CRISPR-expression cassettes are delivered into resistant bacterial cells through different delivery vehicles, including bacteriophages, nanoparticles, and conjugative plasmids. After intracellular delivery, the CRISPR cassette undergoes *in situ* transcription and translation to generate Cas9 protein and sequence-specific sgRNA, which assemble into the Cas9-sgRNA ribonucleoprotein complex. Guided by the sgRNA, Cas9 specifically recognizes antibiotic resistance genes (ARGs) and induces targeted DNA cleavage, leading to ARG disruption, removal, or functional inactivation. As a result, resistant bacteria are converted into antibiotic-resensitized cells. Subsequent antibiotic treatment can then efficiently eliminate these resensitized bacteria. Certain graphical elements in this figure were obtained from BioGDP (Jiang et al., 2025).

2021).

For example, CRISPR-Cas9 has been successfully employed to induce cell death in highly resistant pathogens such as *Staphylococcus aureus* and *Clostridioides difficile* (Allemailem, 2024; Zhang et al., 2025). In particular, polymer-assisted, nonviral delivery of Cas9-sgRNA complexes has enabled efficient targeting of resistance genes like *mecA*, achieving potent genome editing and bacterial killing in methicillin-resistant *Staphylococcus aureus* (Kang et al., 2017). Importantly, this strategy exhibits strong species specificity, allowing for the selective elimination of target pathogens while preserving

beneficial and functionally important microbiota within complex environmental microbial communities.

Compared with other targeted elimination approaches such as bacteriocins, CRISPR-based antimicrobials offer greater programmability and sequence-level specificity. Bacteriocins can also display potent and often narrow-spectrum antibacterial activity and have been proposed as precision antimicrobials or antibiotic alternatives (Cotter et al., 2013; Heilbronner et al., 2021). However, their specificity is typically governed by naturally evolved peptide-receptor interactions or membrane-associated mechanisms, whereas CRISPR systems can be rationally redirected toward defined resistance genes, virulence factors, or strain-specific loci. Nevertheless, CRISPR-based killing remains more dependent on efficient intracellular delivery, which is a major limitation compared with diffusible bacteriocins.

Beyond targeting individual resistance genes, CRISPR systems have also been applied to disrupt complex phenotypic resistance mechanisms, including biofilm formation and quorum sensing (Katoch et al., 2020). Biofilms—structured bacterial consortia embedded within an extracellular polymeric matrix—play a critical role in chronic infection persistence, environmental survival, and enhanced antibiotic tolerance. CRISPR-Cas systems can be programmed to knock down key genes involved in biofilm development (e.g., *icaA* in *Staphylococcus aureus*) or quorum-sensing regulation (e.g., *lasR* in *Pseudomonas aeruginosa*). This targeted intervention leads to pronounced reductions in biofilm biomass, structural integrity, and collective resistance phenotypes (Saffari Natanzi et al., 2025). Such interventions are particularly relevant for environmental reservoirs, including wastewater treatment systems, where biofilms contribute to the long-term persistence and dissemination of ARB and ARGs.

The effectiveness of CRISPR-based antimicrobial strategies critically depends on the efficiency and robustness of delivery systems. To date, bacteriophage-based vectors and conjugative plasmids have been extensively explored for delivering CRISPR components to target bacteria (Hupfeld et al., 2018; Allemailem, 2024; Zhang et al., 2025). More recently, nanoparticle-mediated delivery has attracted increasing attention due to its capacity to protect CRISPR cargos from environmental degradation, enhance penetration through physical barriers such as biofilms, and improve targeting specificity (Fletcher et al., 2023). For example, liposomal Cas9 formulations reduced *Pseudomonas aeruginosa* biofilm biomass by more than 90% *in vitro*, while gold nanoparticle carriers increased genome-editing efficiency by up to 3.5-fold

compared with non-carrier systems (Saffari Natanzi et al., 2025). The integration of CRISPR technology with nanomaterials is thus driving the development of advanced “seek-and-destroy” platforms capable of functioning across heterogeneous environmental matrices to precisely eliminate ARB and ARGs.

In summary, CRISPR-based gene editing provides a powerful and highly precise toolkit for addressing pollution associated with antibiotic-resistant bacteria and resistance genes. By enabling pathogen-specific killing, genetic resensitization to antibiotics, and disruption of resistance-associated phenotypes, CRISPR-based approaches hold substantial promise. Nevertheless, translating these advances from laboratory systems to open environmental settings remains challenging. Key issues include efficient and selective delivery across heterogeneous microbial communities, limited access to ARGs carried on mobile genetic elements or extracellular DNA, the emergence of CRISPR-resistant bacterial subpopulations, and biosafety or regulatory uncertainties associated with engineered delivery vehicles. When appropriately validated and integrated with existing control systems, these approaches may help restore antimicrobial efficacy and mitigate one of the most critical challenges in contemporary environmental and public health protection.

6 Key applications of CRISPR gene editing in the control of biological invasions and harmful organisms

CRISPR-based genome editing technologies have fundamentally reshaped strategies for managing invasive species and harmful organisms. Compared with conventional physical, chemical, or biological control methods, CRISPR enables highly specific interventions at the genetic level, primarily through population suppression or population replacement (Table 4). These approaches offer the prospect of species-specific, potentially self-sustaining solutions to mitigate the profound ecological and economic impacts caused by biological invasions and harmful organisms.

One prominent application is the precision-guided sterile insect technique (pgSIT) (Kandul et al., 2019), which harnesses CRISPR to induce female-specific lethality and male sterility, thereby suppressing target populations. Beyond proof-of-concept laboratory studies, mosquito-based biological control programs have demonstrated substantial real-world impact (Apte

et al., 2024). In 2024, such vector-control strategies were recognized among the Top Ten Breakthroughs in Life Sciences, exemplified by the large-scale *Wolbachia*-based mosquito release campaign led by Luciano Moreira in Brazil. By establishing the world’s largest mosquito production facility and releasing *Wolbachia*-infected *Aedes aegypti*, this program achieved significant reductions in dengue transmission in endemic regions, underscoring the translational potential of precision biological control for vector-borne disease management (Lenharo, 2025).

In addition, pgSIT has been extended to the control of highly invasive and destructive pests, such as the spotted-wing drosophila. By crossing a Cas9-expressing line with sgRNA lines targeting female viability genes (e.g., *doublesex*, *dsx*) and male fertility genes (e.g., *tssk2*), researchers successfully generated sterile males capable of suppressing population growth in cage trials (Yadav et al., 2023). For the highly invasive and polyphagous agricultural *Spodoptera frugiperda*, CRISPR has been used to validate the role of *tssk2* in pgSIT. Knockout of *tssk2* induces male sterility without impairing other physiological functions, making it an ideal genetic target for generating sterile males (Anu et al., 2024; Salum et al., 2024).

For more sustained population control, CRISPR-based gene drive systems represent a powerful and highly engineered class of tools (Hammond et al., 2016). Unlike conventional Mendelian inheritance, in which a heterozygous allele is transmitted to approximately 50% of offspring, CRISPR-based homing gene drives can copy themselves onto the homologous chromosome and thereby achieve super-Mendelian inheritance (Fig. 5). These “selfish” genetic elements bias their own inheritance, enabling the rapid spread of desired genetic payloads through wild populations within a few generations (Jones, 2023). In invasive species management, suppression-type gene drives can cause population collapse by targeting genes essential for reproduction or sex determination (Kyrou et al., 2018). A landmark achievement is the homing gene drive targeting *doublesex* in the malaria vector *Anopheles stephensi*. By disrupting a female-specific exon, the drive induced intersex phenotypes and female sterility. In cage trials, the gene drive spread persistently and ultimately resulted in population collapse, demonstrating its potential for eliminating malaria vectors (Larrosa-Godall et al., 2025).

Beyond terrestrial pests, CRISPR gene editing has also been applied to control aquatic harmful organisms, particularly harmful algal blooms (Hwang et al., 2024). Current CRISPR-based approaches focus primarily on

Table 4 Simplified comparison of conventional and CRISPR-based control strategies

Method	Control principle	Specificity	Persistence	Key limitation	Ref.
Physical control	Removal, trapping, barriers, or habitat management	Low to moderate	Low	Labor-intensive and difficult to scale	Simberloff et al. (2013)
Chemical control	Pesticides, herbicides, or toxicants	Low	Low to moderate	Non-target effects, residues, and resistance	Simberloff et al. (2013)
Biological control	Natural enemies, pathogens, or competitors	Moderate	Moderate to high	Ecological uncertainty and non-target risks	Simberloff et al. (2013)
Conventional genetic control	Sterile or incompatible individuals	Moderate to high	Low without repeated releases	Requires mass rearing and continuous release	Alphey (2014)
CRISPR-based suppression	Disruption of genes required for survival or reproduction	High	Potentially high	Delivery, resistance, and biosafety concerns	Kyrou et al. (2018)
CRISPR-based replacement	Introduction or spread of engineered traits	High	Potentially high	Ecological, regulatory, and governance concerns	Noble et al. (2019)

engineering biological control agents rather than directly editing algal species. For example, CRISPR-Cas9 metabolic engineering of the yeast *Starmerella bombicola* has been used to enhance the overproduction of specific sophorolipid types, a class of biosurfactants. By knocking out or overexpressing key enzymes, researchers generated strains producing predominantly acidic or lactonic sophorolipids. Lactonic sophorolipids exhibit strong antimicrobial activity and have been shown to rapidly lyse cyanobacteria within harmful algal blooms, providing a biodegradable and environmentally friendly alternative to chemical algicides ([Xia et al., 2023](#)). This represents a novel application of CRISPR for enhancing natural product biosynthesis to support targeted bioremediation.

Overall, CRISPR technologies offer a versatile and powerful toolkit for managing invasive species and harmful organisms. From controllable pgSIT systems to self-propagating gene drives, and from agricultural pests to aquatic cyanobacteria, these genetic strategies provide unprecedented precision and technical potential for the targeted control of biological pollution. However, their environmental deployment remains limited by ecological uncertainty, resistance evolution, non-target risks, governance challenges, and the need for long-term monitoring and public acceptance.

7 Key technical issues and challenges

CRISPR-based biocontrol technologies continue to face critical technical hurdles related to delivery efficiency, functional robustness, and biosafety, which collectively represent the primary bottlenecks for real-world deployment ([Wan et al., 2021](#)). Among these, achieving efficient and target-specific delivery of CRISPR components within complex environmental matrices remains one of the most persistent challenges. For

strategies aimed at directly targeting pathogens in soil, aquatic systems, or environmental microbial communities, the use of engineered bacteriophages, nanoparticles, or conjugative plasmids as delivery vehicles is often severely constrained ([Wan et al., 2021](#); [Yang et al., 2021](#); [Allemailem, 2024](#); [Saffari Natanzi et al., 2025](#)). The heterogeneous chemical and physical properties of environmental media can impede vector diffusion, reduce payload stability, and promote non-specific uptake, thereby diminishing editing efficiency and increasing unintended exposure. Concurrently, off-target effects remain a significant concern, as unintended genetic modifications in non-target microorganisms or host species may disrupt beneficial microbiomes, induce unforeseen phenotypic changes, and potentially destabilize ecological networks ([Tyagi et al., 2021](#); [Paul et al., 2021](#); [Saffari Natanzi et al., 2025](#); [Zhang et al., 2025](#)).

Another major limitation lies in ensuring the durability of engineered resistance in the face of pathogen evolution ([Tyagi et al., 2021](#)). Although host-directed editing of susceptibility genes can confer strong resistance, the high genetic plasticity of many pathogens enables rapid adaptation through the emergence of novel virulence factors ([Gomaa et al., 2014](#)). This challenge is particularly acute for viral pathogens with high mutation rates, which can readily generate escape variants through alterations in PAM sequences or target sites ([Wu et al., 2021](#); [Allemailem, 2024](#)). In addition, the widespread occurrence of naturally encoded anti-CRISPR (Acr) proteins on mobile genetic elements represents an underappreciated ecological risk ([Pinilla-Redondo et al., 2020](#)). These Acr proteins can directly inhibit Cas nuclease activity, and environmental deployment of CRISPR systems may inadvertently select for and enrich Acr-harboring bacteria. Such selection pressure could compromise CRISPR efficacy and, in some contexts, even facilitate the persistence or dissemination of antibiotic resistance genes ([Kadkhoda et al., 2024](#)). Although multiplex

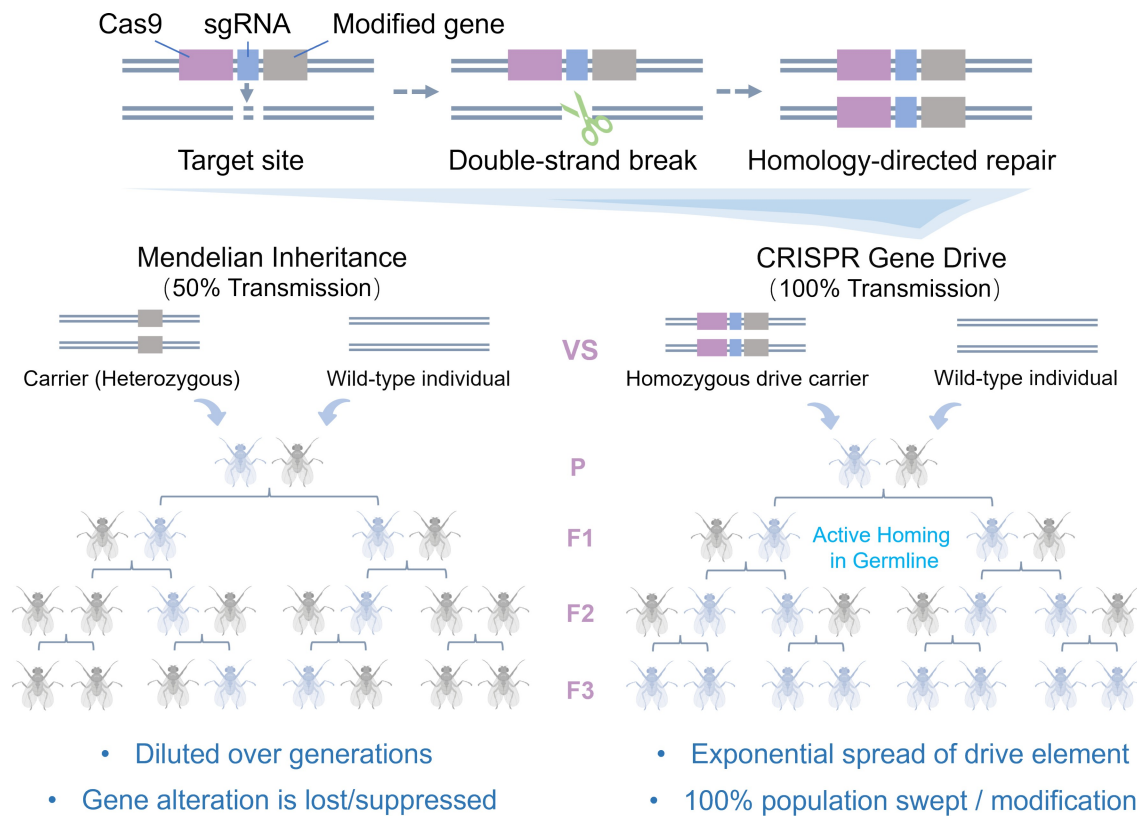


Fig. 5 Schematic diagram of gene drive inheritance utilizing CRISPR-Cas9. This figure illustrates the molecular basis and inheritance pattern of a CRISPR-Cas9-based gene drive system. The gene drive cassette typically contains the Cas9 nuclease, sgRNA, and a modified gene integrated at a specific genomic target site. In a heterozygous drive carrier, Cas9 guided by the sgRNA cleaves the wild-type allele on the homologous chromosome, generating a double-strand break. The break is then repaired through homology-directed repair (HDR) using the drive-containing chromosome as a template, thereby copying the entire drive cassette into the cut site, and converting the heterozygote into a homozygote. This process, referred to as “homing,” enables super-Mendelian inheritance of the modified allele. The lower panel compares Mendelian inheritance with CRISPR gene drive inheritance. Under standard Mendelian transmission, a heterozygous carrier passes the modified allele to only approximately 50% of the offspring, resulting in dilution or loss of the genetic modification over generations. In contrast, CRISPR gene drive systems promote active homing in the germline, allowing the drive element to be transmitted to nearly 100% of progeny. As a result, the drive allele can spread exponentially across successive generations, potentially sweeping through and modifying an entire population.

targeting of conserved genomic regions has been proposed as a mitigation strategy, this approach substantially increases design complexity and raises the risk of pleiotropic effects (Khalid and Poh, 2023).

Beyond technical and biological constraints, socio-ethical and governance challenges constitute some of the most complex barriers to the application of CRISPR-based biocontrol (Ali et al., 2026). Public perception, strongly influenced by the longstanding controversy surrounding genetically modified organisms (GMOs), continues to shape the societal acceptance of CRISPR-edited organisms. Despite the fact that many CRISPR-edited crops do not contain foreign DNA and instead harbor small deletions or point mutations comparable to natural genetic variation,

they are frequently regulated and perceived in a manner similar to conventional GMOs (Martínez-Fortún et al., 2022). This persistent “GMO stigma” can discourage research investment, slow regulatory approval, and hinder market adoption, even when such technologies offer clear environmental benefits, including reduced pesticide inputs and lower ecological footprints. Moreover, existing regulatory frameworks are often ill-equipped to address the unique characteristics of gene-editing technologies, particularly self-propagating systems such as gene drives. Current regulations may fail to adequately capture organisms that fall outside traditional GMO definitions. They also frequently lack comprehensive mechanisms for evaluating long-term ecological risks, socio-economic implications, and

ethical considerations associated with environmental release.

8 Perspectives

The emergence of CRISPR-based gene-editing technologies has introduced a transformative paradigm shift in the management of biological pollution. Unlike conventional strategies that rely on broad-spectrum suppression or population-level elimination, CRISPR enables precise genetic interventions targeting the molecular determinants underlying biocontamination. These targets include pathogen virulence genes, bacterial antibiotic resistance cassettes, and key regulatory genes governing the reproduction or persistence of harmful organisms. This unprecedented level of programmability and specificity supports environmentally compatible control strategies with minimal collateral impacts on non-target organisms and ecosystem functions. As a result, these approaches align well with sustainable development goals in agriculture, public health, and environmental protection.

Despite these advances, translating CRISPR technologies from laboratory settings to robust environmental and clinical applications remains challenging. From a technical perspective, the efficient, safe, and targeted delivery of CRISPR components within complex biological and ecological matrices continues to represent a major bottleneck. In addition, unintended off-target effects and rapid evolutionary escape of target organisms, particularly microbial pathogens, represent major challenges for genome-editing interventions. Furthermore, the widespread presence of natural anti-CRISPR mechanisms may undermine their long-term stability and effectiveness. Addressing these limitations is essential to ensure both functional efficacy and ecological biosafety under real-world conditions.

Encouragingly, ongoing technological innovation is rapidly expanding the CRISPR toolbox (Fig. 6). High-precision editing strategies, such as base editing and prime editing, enable targeted nucleotide substitutions and small insertions or deletions without inducing DNA double-strand breaks, thereby reducing genomic toxicity and off-target mutations (Gaudelli et al., 2017; Eid et al., 2018; Anzalone et al., 2019). In parallel, the rapid advancement of artificial intelligence (AI) and deep learning is accelerating progress by optimizing gRNA design, predicting on- and off-target editing outcomes, and facilitating the discovery and engineering of novel Cas proteins with diverse PAM requirements and expanded targeting scopes. Beyond

molecular design, AI-based modeling and simulation frameworks are expected to be increasingly integrated to predict the environmental behavior and ecological impacts of CRISPR-based strategies. Such approaches will enable the evaluation of intervention outcomes under application scenarios that more closely reflect real-world conditions (Xiang et al., 2021; Pacesa et al., 2024; Wang et al., 2025). Together, these developments substantially broaden the editable genomic landscape and enhance the capacity to respond to dynamic and evolving biological threats.

Equally critical to future success is the evolution of delivery technologies (Fig. 6). Next-generation delivery platforms are anticipated to move beyond passive cargo transport toward the development of environmentally responsive “smart vectors” capable of spatiotemporally controlled and conditional gene-editing activation (Wan et al., 2021; Saffari Natanzi et al., 2025). Biological vectors such as bacteriophages are entering an “Engineering 2.0” phase, in which synthetic biology approaches enable expanded host range, improved immune evasion, resistance to anti-CRISPR inhibition, and the integration of genetic circuits governing conditional CRISPR expression or lysis (Uribe et al., 2021; Kadkhoda et al., 2024). Such strategies are essential for minimizing off-target effects, preserving microbiome integrity, and enhancing biosafety in complex environments.

Beyond technical considerations, the environmental application of CRISPR technologies raises profound ethical, societal, and governance challenges (Fig. 6). Central issues include the controllability of ecological risks—such as the spread of edited traits in wild populations, impacts on non-target species, and potentially irreversible ecosystem-level changes—necessitating long-term monitoring and adaptive risk assessment frameworks. Equally important are questions of governance equity, including who controls gene-editing technologies, whom they benefit, and how risks are distributed globally. Without inclusive international governance mechanisms, these technologies risk exacerbating existing ecological and technological inequalities. Finally, broader philosophical debates concerning the boundaries between the natural and artificial will intensify, underscoring the need for interdisciplinary dialogue that integrates scientific evidence, ethical reasoning, public engagement, and indigenous knowledge systems.

In summary, CRISPR gene-editing technology provides an unprecedented toolkit for the precise and sustainable management of biological pollution. Its responsible deployment will depend on the synergistic advancement of high-precision editing tools, intelligent and controllable delivery systems, AI-assisted

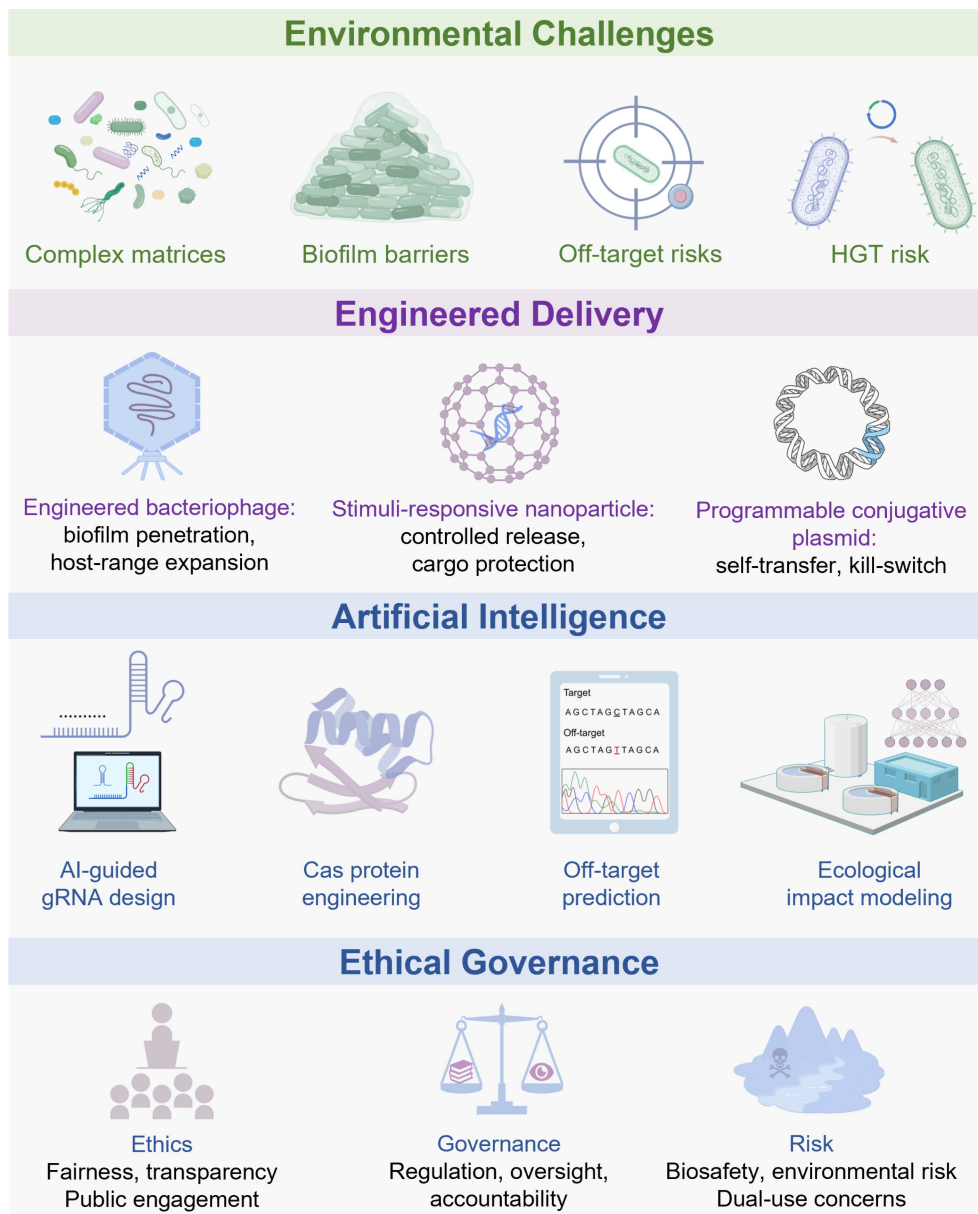


Fig. 6 Conceptual framework for the future development of CRISPR-based environmental biocontrol. The diagram illustrates the integration of high-precision genome-editing tools with advanced delivery systems, AI-assisted molecular design and application simulation, and robust ethical and governance considerations. Efficient delivery strategies, including bacteriophages, nanoparticles, and conjugative plasmids, facilitate precise targeting of pathogens, while AI supports both protein engineering and predictive modeling of ecological impacts. Ethical oversight and governance frameworks ensure responsible deployment, collectively enabling safe, sustainable, and effective management of biological pollution. Certain graphical elements in this figure were obtained from BioGDP (Jiang et al., 2025).

optimization, and robust, transparent governance frameworks (Fig. 6). Only through sustained scientific innovation, rigorous environmental risk assessment, and inclusive societal dialogue can CRISPR be guided toward realizing its full potential in ecological conservation, agricultural resilience, and human health protection.

CRediT Authorship Contribution Statement

Zhou-Hua Cheng and Dong-Feng Liu designed this work. Xiao-Fei Zheng, Zhou-Hua Cheng, Han-Qing Yu, and Dong-Feng Liu wrote and edited. All the authors contributed to discussion of the results and the manuscript.

Conflicts of interest Dong-Feng Liu is a Youth Editorial Board Member of *ENGINEERING Environment*. Han-Qing Yu is an Advisory

Board Member of *ENGINEERING Environment*. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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