An overview of CRISPR-artificial intelligence theranostics: Current and emerging applications

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ABSTRACT

Clustered regularly interspaced short palindromic repeats (CRISPR)-based diagnostics are revolutionizing precision medicine by enabling highly sensitive detection of nucleic acid and protein biomarkers. Building on these capabilities, CRISPR-based theranostics now aim to unify real-time disease detection with targeted therapeutic interventions. However, traditional CRISPR diagnostics face several limitations, including restricted multiplexing, off-target effects, and challenges in delivery efficiency. To overcome these issues, artificial intelligence (AI) has significantly enhanced CRISPR platforms by enabling intelligent guide RNA (gRNA) design, interpretation of complex biosensor outputs, and facilitation of rapid clinical decision-making. Machine learning tools such as DeepCRISPR, Azimuth 2.0, DeepHF, and CRISPRpred support the development of highly specific gRNAs, reduce off-target events, and personalize genome-editing strategies based on individual genomic profiles. Recently, by combining CRISPR systems with nanomaterials, fluorescence-based detection, and electrochemical sensing, researchers have developed advanced biosensors capable of detecting a broad spectrum of disease biomarkers, from cancer-associated nucleic acids to viral and genetic disorders. These advances support both diagnostics and gene therapy, enabling accurate, low-cost testing at home, in point-of-care settings, and in resource-limited environments. Together, the integration of AI and CRISPR is accelerating biomarker discovery and the development of intelligent, adaptive therapeutic platforms. New point-of-care diagnostic tests (POCTs) based on CRISPR-AI are essential for early screening of high-mortality diseases, and CRISPR-based diagnostic assays have emerged as powerful, versatile alternatives to traditional nucleic acid tests, offering rapid, programmable, and portable diagnostic solutions. This review explores the evolution of CRISPR-AI theranostic systems, current and emerging POCT applications. It highlights the technological, clinical, and ethical challenges shaping their translation into next-generation precision diagnostics.

Keywords:
CRISPR-Cas; Biosensing; Gene editing; Diagnostics; Precision medicine

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1. Introduction

The clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein (CRISPR-Cas) system, a prokaryotic adaptive immune mechanism discovered in bacteria and archaea, has emerged as a powerful and programmable platform for genome manipulation.[1] Over the past two decades, its integration with advances in molecular biology and engineering has reshaped the landscape of precision medicine, addressing persistent challenges in disease modeling, diagnostics, and therapeutics.[2] Characterized by its RNA-guided nuclease (NUC) activity, CRISPR enables site-specific, efficient, and cost-effective genomic manipulation.[3] Recent advancements in CRISPR technology have met the World Health Organization's "REASSURED" criteria for optimal diagnostic tools, emphasizing

real-time efficiency, affordability, high sensitivity, specificity, user-friendly design, rapidity, equipment-free usability, and direct application for end users.[4]

While earlier genome-editing tools such as zinc finger NUCs and transcription activator-like effector NUCs laid the groundwork for targeted DNA modifications,[5] their widespread use was limited by the need for labor-intensive protein engineering, relatively high costs, and variable efficiency with significant off-target risks.[6] In contrast, CRISPR relies on a simple RNA-guided recognition (REC) mechanism, making it far more versatile, programmable, and scalable.[7] These features, coupled with lower costs and rapid adaptability to diverse targets, have positioned CRISPR as a transformative advance over earlier NUC-based systems. Subsequently, the introduction of CRISPR-Cas9 marked a transformative milestone by simplifying the targeting mechanism through guide RNA (gRNA)-directed DNA cleavage.[8] This leap in accessibility revolutionized modern molecular biology and was recognized with the 2020 Nobel Prize in Chemistry awarded to Charpentier and Doudna. However, Feng Zhang, a researcher at the Broad Institute of Massachusetts Institute of Technology (MIT) and Harvard, was the first to adapt and demonstrate the CRISPR-Cas9 system for genome editing in eukaryotic cells.[9] A timeline illustrating the evolution of genome editing technologies, from early programmable NUCs to modern CRISPR-based systems, is shown in **Figure 1**.

Beyond genome editing, CRISPR systems have been rapidly adapted for molecular diagnostics owing to the collateral cleavage activity exhibited by certain effector proteins, such as Cas12 and Cas13.[10] Upon target REC, these enzymes non-specifically cleave surrounding nucleic acids, a property used for signal amplification in diagnostic assays.[11] This mechanism underlies the design of CRISPR-based diagnostics (CRISPR-Dx) platforms such as Specific High-Sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK), DNA endonuclease targeted CRISPR trans reporter (DETECTR), 1-h low-cost multipurpose highly efficient system (HOLMES), and FnCas9 editor linked uniform detection assay (FELUDA), which have demonstrated high sensitivity and specificity for detecting viral, bacterial, and cancer-associated nucleic acids. These platforms fulfill the World Health Organization's REASSURED criteria and offer decentralized, rapid, and cost-effective alternatives to conventional nucleic acid testing.[12] Furthermore, integration with nanomaterials, fluorescent reporters, and electrochemical biosensors has enabled multiplexing, improved signal transduction, and enhanced detection sensitivity.[13] As a result, CRISPR-Dx systems are now being developed for point-of-care test (POCT) and even at-home applications, with increasing relevance in infectious disease surveillance, oncology, and resource-limited clinical settings.[14]

In parallel with their diagnostic applications, CRISPR technologies have diversified beyond double-stranded DNA (dsDNA) cleavage to enable precise and programmable control over gene expression.[15] Catalytically inactivated Cas9 (dCas9) fused to transcriptional repressors or activators forms the basis of CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa), respectively—tools that allow programmable gene silencing or upregulation without altering the underlying genomic sequence.[16] These systems have been instrumental in elucidating gene function and modeling disease phenotypes in a reversible and non-mutagenic manner.[17] Further advances, such as base editors that enable single-nucleotide substitutions without double-stranded breaks (DSBs), extend precision to targeted insertions, deletions, or sequence replacements,[18] thereby enhancing the safety and specificity of genome engineering while opening new avenues in synthetic biology, biosensor development, and theranostic circuit design.[19] Ultimately, these advances expand the functional landscape of gene manipulation and set the stage for context-responsive therapeutic interventions.

Despite their transformative potential, CRISPR-based technologies face persistent challenges such as off-target effects, suboptimal gRNA design, and context-specific variability in editing efficiency.[20] To overcome these limitations, artificial intelligence (AI) and machine learning (ML) are increasingly being integrated with CRISPR systems, offering data-driven solutions for enhancing precision, scalability, and personalization.[21] AI-based platforms such as DeepCRISPR, Azimuth 2.0, CRISPRpred, and Deep post-Hartree-Fock (DeePHF) have demonstrated high performance in predicting gRNA activity, minimizing off-target risks, and enabling rational design of genome-editing strategies across diverse biological contexts.[22] These tools analyze vast genomic datasets, epigenetic landscapes, and sequence-structure features to generate optimized gRNA candidates tailored to specific cell types and disease states.[23] Furthermore, AI-driven analytics are being incorporated into biosensing platforms to automate signal interpretation, noise reduction, and threshold calibration, thus enhancing diagnostic accuracy and decision-making.[24] The integration of AI not only addresses the inherent complexity of biological systems but also lays the foundation for next-generation CRISPR platforms that are intelligent, adaptive, and clinically actionable. The association of CRISPR with AI, nanotechnology, and biosensor engineering is giving rise to a new generation of programmable theranostic platforms, capable of both detecting disease biomarkers and executing context-specific therapeutic interventions.[25] These CRISPR-AI-integrated systems are now being explored for use in POCT, offering rapid, portable, and programmable diagnostic solutions for high-mortality diseases such as cancer, infectious disorders, and genetic syndromes.[26] By combining AI's predictive capabilities with CRISPR's molecular precision, such platforms hold the potential to enable closed-loop therapeutic circuits that can sense, diagnose, and respond in real time.[27] However, the path toward clinical adoption is shaped by significant translational challenges, including delivery

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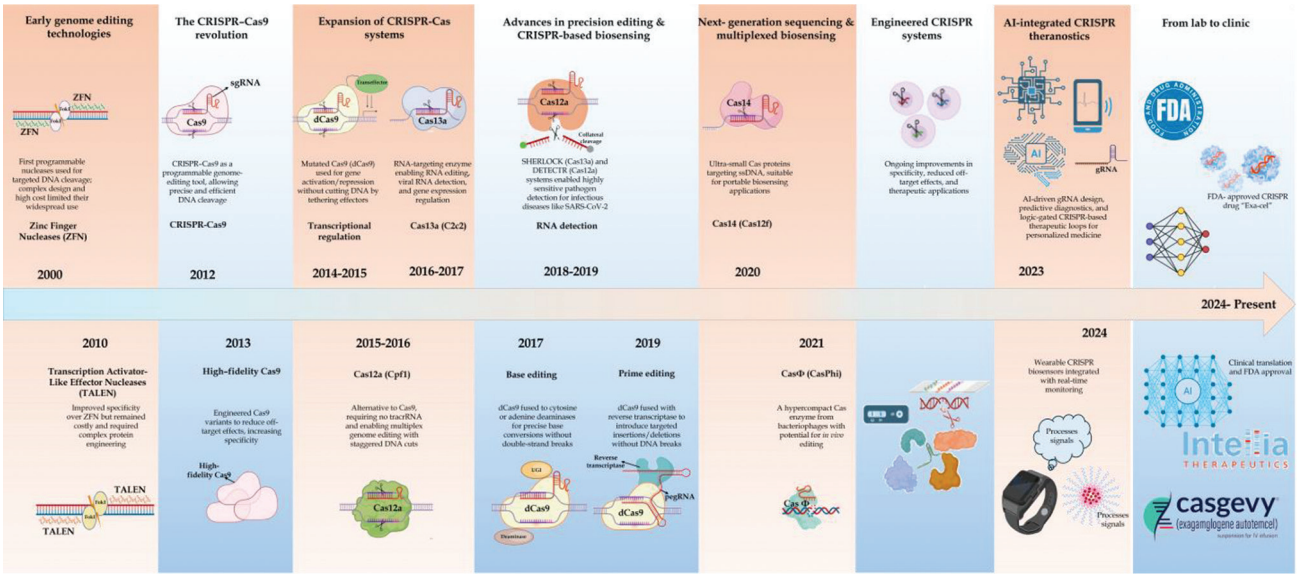

Figure 1. Timeline of genome editing technologies, highlighting significant advancements from early programmable nucleases to modern CRISPR-based systems. Figure created by the authors.

Abbreviations: AI: Artificial intelligence; Cas: CRISPR-associated protein; CRISPR: Clustered regularly interspaced short palindromic repeats; dCas: Catalytically inactivated Cas; DETECTR: DNA endonuclease targeted CRISPR trans reporter; Fok1: *Flavobacterium okeanokoites* restriction endonuclease; gRNA: Guide RNA; pegRNA: Prime editing guide RNA; SHERLOCK: Specific High-sensitivity Enzymatic Reporter unLOCKing; ssDNA: Single-stranded DNA; tracrRNA: Trans-activating CRISPR RNA; UGI: Uracil-DNA glycosylase inhibitor.

efficiency, biomolecular stability, regulatory complexity, and ethical concerns surrounding genome manipulation and data use.

In this review, we present a comprehensive synthesis of current CRISPR-AI applications in diagnostics and emerging theranostic systems. We explore their underlying principles, the role of biosensors as key enabling technologies, and their real-world potential in point-of-care and personalized medicine. Furthermore, we critically examine the technical, clinical, and ethical challenges that must be addressed to ensure their successful integration into future precision medicine frameworks.

2. The CRISPR-Cas toolkit

CRISPR-Cas systems consist of several key components, including a Cas protein, CRISPR RNA (crRNA), and trans-activating CRISPR RNA (tracrRNA).[28] The Cas protein, such as Cas9, functions as a molecular scissor, directed by the crRNA to cleave the targeted sequence.[29] tracrRNA is essential for the pre-processing of precursor CRISPR RNA (pre-crRNA) into the mature functional crRNA form, facilitating the assembly of a ribonucleoprotein (RNP) complex that carries out genome targeting.[30] Together, these elements have enabled scientists to precisely modify the genetic code, interrogate gene functionality, develop new treatments for hereditary diseases, and create genetically modified organisms with specific traits.[31,32] Importantly, variations in these core components have led to the development of various CRISPR systems with unique functionalities applicable to diagnostics, biosensing, and next-generation theranostics.[33] Thereby, understanding the role of each component in the CRISPR-Cas system is crucial to fully utilizing its potential in genetic engineering. A detailed summary of these components, their molecular roles, and diverse applications is presented in **Table 1**. While the toolkit outlines the structural components of CRISPR, understanding how these parts cooperate in natural immunity provides context for their repurposing in genome editing, as described in the following section.

2.1. Core components

2.1.1. Cas proteins

CRISPR-associated proteins are the catalytic core of the CRISPR-Cas system, functioning as molecular scissors to recognize and cleave nucleic acid sequences.[7] Cas proteins recognize specific protospacer adjacent motifs (PAM), as explained further in Section 2.1.4, enabling adaptability in targeting different genomic regions.[40] Cas9, derived from *Streptococcus pyogenes*, is the most widely used Cas variant due to its precision and ease of use in genome editing.[41] Cas12 and Cas14 possess collateral cleavage activity, allowing them to degrade non-target single-stranded DNA (ssDNA) after recognizing a target, an essential property of biosensors to amplify detection signals.[42] Cas13 specifically targets RNA, broadening the applications of CRISPR to transcriptomic regulation and RNA virus diagnostics.[43] The recently characterized CasΦ, a compact NUC encoded by bacteriophages, offers high gene editing efficiency, particularly in resource-limited or viral vector-constrained environments.[44] In addition, modified Cas proteins with expanded or altered PAM specificities have increased their applicability, enabling multiplexed targeting of various genomic regions.[45] Cas proteins also serve as effectors in base editing and prime editing systems, two advanced technologies that allow precise nucleotide conversion without inducing DSBs.[46] The incorporation of Cas proteins into biosensors presents significant potential for point-of-care diagnostics, enabling swift and accurate identification

Table 1. Key components of CRISPR-Cas systems and their applications

CRISPR-Cas component	Description	Key application (s)	References
Cas protein (e.g., Cas9, Cas12, Cas13)	"Molecular scissors" that cleave nucleic acids (DNA or RNA). Different Cas proteins have distinct cleavage mechanisms and target-specific types of nucleic acids	Gene knockout, insertion, or correction via DNA/RNA cleavage	34
Guide RNA (gRNA)/ single guide RNA	A synthetic RNA molecule that directs the Cas protein to a specific target sequence in the genome. It consists of a spacer sequence (complementary to the target DNA/RNA) and a scaffold sequence (binds to the Cas protein)	Ensures site-specific cleavage	35
Protospacer adjacent motif	A short, specific DNA sequence located adjacent to the target (protospacer) sequence. Recognized by Cas proteins and essential for their binding and cleavage activity	Determines where Cas can bind and cut	36
Dead Cas (dCas) variants (e.g., dCas9, dCas12, dCas13)	Catalytically inactive Cas proteins (mutated to prevent DNA/RNA cleavage) that retain their ability to bind to specific DNA/RNA sequences when guided by gRNA	Gene regulation, imaging, epigenetic editing	37
Base editors (e.g., CBE, ABE)	Fusion proteins combining a dCas protein with a deaminase enzyme. These systems enable precise single-nucleotide changes without introducing double-strand breaks	Correct point mutations without DNA breaks	38
Prime editors	Advanced gene editing tools combining a Cas9 nickase (which cuts only one DNA strand), a reverse transcriptase, and a prime editing guide RNA	Precise insertions, deletions, or base changes	39

Abbreviations: ABE: Adenine base editor; CBE: Cytosine base editors; CRISPR-Cas: Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein.

of diseases and pathogens, thereby improving personalized medicine and environmental monitoring.

2.1.2. crRNA and tracrRNA

crRNA and tracrRNA are central to the RNA-guided mechanism of most Class 2 CRISPR systems.[47] The crRNA contains a sequence complementary to the target DNA (the spacer) and a repeat-derived sequence that binds tracrRNA.[48] The tracrRNA is essential for crRNA maturation and for recruiting Cas proteins to form a functional RNP complex.[49] In native systems, the tracrRNA and crRNA operate as two distinct molecules; however, they are often fused into a single gRNA (sgRNA) in laboratory applications to streamline genome editing protocols.[50] This programmable dual-RNA complex directs the Cas protein to its target with high sequence specificity, forming the molecular basis for genome and transcriptome editing.[51]

2.1.3. gRNA

In most applications, crRNA and tracrRNA (discussed in Section 2.1.2) are fused into a sgRNA, simplifying design for genome editing and diagnostics. It consists of a ~20-nucleotide spacer sequence that is complementary to the target DNA or RNA, and a scaffold region that forms the necessary secondary structures for Cas binding.[52] The gRNA-Cas complex scans the genome for PAM-adjacent regions. It induces cleavage or modulation only on successful base-pairing with the target.[53] Precision in gRNA design is critical for minimizing off-target activity and improving editing efficiency. AI-driven tools such as Benchling, CRISPR-DO, and sgRNA Designer are increasingly used to predict gRNA activity and specificity, optimizing their application in both therapeutic and diagnostic platforms, as listed in **Table 2**.[54]

2.1.4. PAM

The PAM is a short, conserved DNA sequence flanking the target site that is essential for Cas protein binding and initiation of the editing process.[81] The PAM sequence varies across Cas proteins, such that Cas9 recognizes NGG, whereas Cas12a recognizes TTTV. PAMs play a vital role in distinguishing self from non-self DNA, thereby preventing autoimmune targeting of the host genome.[46] Identifying and utilizing the appropriate PAM sequences can significantly enhance the efficiency and accuracy of CRISPR-based genome editing. In biosensing, PAM-dependent activation of Cas12 ensures signal specificity, which increases the potential for multiplexed detection of nucleic acid biomarkers in CRISPR-Dx platforms.[42] Despite their essential role, PAM requirements can also restrict CRISPR applications by limiting the number of accessible genomic sites, particularly in AT- or GC-rich regions.[82] To overcome this bottleneck, researchers have engineered Cas variants with relaxed or altered PAM REC, such as SpRY, SpCas9-NG, and evolved Cas12a proteins, which expand the range of editable targets.[83] In addition, PAM-independent approaches such as base editors, prime editors, and CRISPR-associated transposases bypass conventional PAM constraints, thereby broadening the versatility of CRISPR-based genome engineering.[84] These innovations collectively enhance the flexibility, precision, and translational applicability of CRISPR systems across diverse biological contexts.

Taken together, these components not only operate in coordination but also differ in ways that define their application potential. For example, Cas9 remains the standard for dsDNA cleavage, whereas Cas12 extends functionality through collateral ssDNA cleavage for diagnostic assays, and Cas13 uniquely targets RNA, enabling transcriptome editing and RNA virus detection.[85] Similarly, while natural systems rely on separate crRNA and tracrRNA molecules, the laboratory-engineered sgRNA simplifies experimental workflows by merging these roles into a single guide molecule. PAM further distinguishes Cas9 and Cas12 systems by constraining targetable loci, in contrast to Cas13, which does not require PAM sequences.[86] These distinctions shape the choice of effector systems for specific tasks such as genome engineering, transcriptome regulation, or rapid biosensing, highlighting the adaptability of the CRISPR toolkit.

2.2. CRISPR array

The CRISPR array is a locus within the microbial genome consisting of short, repetitive DNA sequences interspersed

Table 2. Tools for CRISPR guide RNA design and editing

Resource	Supported genomes	CRISPRi/a functionality	Enzymes	Link and references
Benchling	Many	No	SpCas9, SaCas9, NmeCas9, StCas9, TdCas9, Cas12a	https://www.benchling.com[55]
CHOPCHOP	Many	Yes	Cas9 (custom PAM), Cas12a, CasX, Cas13	https://chopchop.cbu.uib.no/[56]
CRISPick	Human, mouse, rat	Yes	SpCas9, SaCas9, AsCas12a, enAsCas12a	https://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design[57]
CRISPOR	Many	No	Cas9 (various PAMs), CasX, Cas12a	http://crispor.tefor.net/[58]
E-CRISP	Many	Yes	SpCas9	http://www.e-crisp.org/E-CRISP/[59]
FlashFry	Any reference genome	No	SpCas9, Cas12a	https://github.com/mckennalab/FlashFry[60]
GUIDES	Human, mouse	No	SpCas9	https://guides.sanjanalab.org/[61]
GuideScan	Human, mouse, *Drosophila*, yeast, zebrafish, *Caenorhabditis elegans*	No	SpCas9, AsCas12a	http://www.guidescan.com/[62]
RGEN Cas-Designer	Many	No	SpCas9 (variants), SaCas9, Cas12a, Cas12b	http://www.rgenome.net/cas-designer/[63]
Vienna Bioactivity CRISPR (VBC) score	Human, mouse, *Drosophila*, C. elegans, rat, *Xenopus tropicalis*	No	SpCas9	https://viennabioactivity.org/[64]
Cas13design	Human, mouse, zebrafish, *Drosophila*, *C. elegans*, *Arabidopsis*, RNA viruses	NA	RfxCas13d	https://cas13design.nygenome.org/[65]
BE-Hive	Human, mouse, zebrafish, *Drosophila*, *C. elegans*, *Arabidopsis*, RNA viruses	NA	CBE, ABE base editors	https://behive.broadinstitute.org/[66]
DeepCRISPR	Human	No	SpCas9	https://github.com/biomedBit/DeepCRISPR[67]
PAVOOC	Human	Yes	SpCas9	https://pavooc.shinyapps.io/pavooc/[68]
CRISPRitz	Custom genomes	No	Cas9, Cas12a, Cas13	https://github.com/pinellolab/CRISPRitz[69]
Cas-OFFinder	Custom genomes (via local install)	No	Any Cas	http://www.rgenome.net/cas-offinder/[70]
CCTop	Human, mouse, zebrafish, and others	No	Cas9	https://cctop.cos.uni-heidelberg.de: 8043/[71]
CRISPR-DO	Human, mouse	No	SpCas9	http://cistrome.org/CRISPR-DO/[72]
sgRNA Scorer	Human, mouse	No	SpCas9	https://crispr.med.harvard.edu/sgRNAScorer/[73]
CRISPRater	Human	No	SpCas9	https://sourceforge.net/projects/crisprater/[74]
Cas-Designer 2.0	Human, mouse, plant genomes	No	Cas9, Cas12a, variants	https://www.rgenome.net/cas-designer-2/[70]
Deskgen	Human, mouse, custom	No	SpCas9, SaCas9, Cas12a	https://www.deskgen.com/[75]
Geneious	Custom	No	SpCas9	https://www.geneious.com/[76]
Horizon Discovery	Human, mouse	No	SpCas9	https://horizondiscovery.com/[77]
IDT	Human, mouse, zebrafish, others	Yes	SpCas9, Cas12a	https://www.idtdna.com/pages/tools[77]
Off-Spotter	Human (custom genomes)	No	SpCas9	http://cm.jefferson.edu/Off-Spotter/[78]
Synthego	Human, mouse	Yes	SpCas9	https://www.synthego.com/[79]
TrueDesign	Human	Yes	SpCas9, Cas12a	https://www.thermofisher.com/truedesign[80]

Abbreviations: ABE: Adenine base editor; AsCas12a: *Acidaminococcus* sp.-derived Cas12a nuclease; Cas: CRISPR-associated protein; CBE: Cytosine base editors; CRISPRa: CRISPR activation; CRISPRi: CRISPR interference; CRISPR: Clustered regularly interspaced short palindromic repeats; enAsCas12a: Enhanced AsCas12a; NA: Not available; NmeCas9: *Neisseria meningitidis* Cas9; PAM: Protospacer adjacent motif; SaCas9: *Staphylococcus aureus* Cas9; SpCas9: *Streptococcus pyogenes* Cas9; StCas9: *Streptococcus thermophilus* CRISPR3-Cas; TdCas9: *Treponema denticola* Cas9 endonuclease deficient Cas9.

with unique spacer sequences derived from previously encountered genetic elements, such as viruses or plasmids. This array acts as a genetic memory system, enabling the CRISPR-Cas machinery to recognize and respond to recurring foreign sequences.[87] Understanding the interactions between the CRISPR array, Cas proteins, and gRNA helps scientists optimize the system for various applications in genetic engineering. Recent advances in CRISPR technology have led to the development of more precise gene-editing tools, such as CRISPR-Cas9, which offer enhanced specificity and reduced off-target effects.[88] In addition, researchers have developed new variants, such as CRISPR-Cas12 and CRISPR-Cas13, which expand the range of applications by targeting RNA sequences, and

these innovations hold promise for the treatment of genetic disorders.[89]

2.2.1. Spacer sequences

Spacer sequences are short DNA segments within the CRISPR array, derived from foreign genetic material encountered during previous infections, and serve as a molecular memory of past attacks.[90] Following the transcription of these spacer sequences into gRNA, the Cas protein is directed to the target DNA for precise editing.[91] The gRNA provides the necessary instructions for the Cas protein to identify and bind to the target DNA sequence.[46] This molecular memory enables sequence-specific targeting and high-fidelity editing or interference.[92] This specificity enables researchers to make targeted modifications without affecting other parts of the genome, making spacer sequences crucial for CRISPR-Dx platforms.

2.2.2. Repeat sequences

Repeat sequences are highly conserved, palindromic DNA elements that flank each spacer within the CRISPR array and are essential for the system's proper functioning, as they serve as REC sites for the Cas protein.[28] During crRNA processing, the repeats facilitate the correct folding and cleavage of the long precursor transcript into individual guide units.[93] By understanding the interplay between spacer and repeat sequences, further optimization of artificial CRISPR systems can be achieved for a broad set of applications, ranging from gene therapy to biotechnology.

2.3. Classification of CRISPR-Cas systems

The classical classification of CRISPR-Cas proteins was originally based on factors such as evolutionary origin, structural composition, cleavage domains, and nucleic acid targets.[94] However, with growing structural and functional insights, these systems are now broadly categorized into two major subclasses: Class 1 and Class 2, each exhibiting distinct characteristics and functions, as illustrated in **Table 3**. Class 1 systems are characterized by multi-protein effector complexes and can target a broad range of DNA sequences. This adaptability makes them vital tools for genome editing, RNA interference, and microbial defense applications.[95] In contrast, Class 2 systems are generally defined by their simplicity, comprising a single large effector protein, with CRISPR-Cas9 being the most prominent example.[96] The simplicity, efficiency, and precision of Cas9 establish it as the predominant tool in gene editing. Within these two main categories, further subdivisions are defined based on functional features and molecular components: Type I, Type III, and Type IV in Class 1, and Type II, Type V, and Type VI in Class 2.[97] Each subtype exhibits variations in components, mechanisms of interference, and target specificity, offering a diverse array of tools for genome manipulation. Type II systems (e.g., Cas9) are well-recognized for their ability to introduce DSBs, whereas Type V (e.g., Cas12) systems possess collateral cleavage activity and target DNA with high precision.[50,98] Type VI systems, represented by Cas13, primarily function in RNA targeting, facilitating transcriptome modulations.[99] Notably, Cas14 (also termed Cas12f), classified under Type V,

Table 3. Classification of CRISPR-Cas immune systems

Class	Type	Key effector protein (s)	Target nucleic acid	Key characteristics	Common subtypes/notes (examples)
Class 1	Multi-component effector complexes				
	Type I	Cas3 (helicase/nuclease), cascade complex (CasA-E, Cas5-8)	DNA	Most abundant; Cas3 unwinds and cleaves DNA. Requires a protospacer adjacent motif (PAM)	I-A, I-B, I-C, I-D, I-E, I-F
	Type III	Cas10 (cyclase/nuclease), Csm/Cmr complex	DNA and/or RNA	Can target both DNA and RNA. Exhibits unique "collateral" RNA cleavage activity upon target binding	III-A, III-B, III-C, III-D, III-E, III-F
	Type IV	Cas5, Cas7, Csf1 (small Cas8-like)	DNA (likely)	Less understood; often plasmid-encoded. Lacks Cas1/Cas2 for adaptation. Simpler architecture	IV-A, IV-B, IV-C
Class 2	Single-component effector proteins				
	Type II	Cas9	DNA	Widely used for genome editing; single effector protein. Requires crRNA and tracrRNA (often fused as sgRNA). PAM-dependent	II-A (SpCas9), II-B (Cas4-containing), II-C (minimal systems like FnCas9)
	Type V	Cas12 (e.g., Cas12a/Cpf1, Cas12b, Cas12c, Cas12d, Cas12e, Cas12f, Cas12g, Cas12h, Cas12i, Cas12u)	DNA	Creates staggered DNA cuts; diverse PAM requirements; exhibits collateral ssDNA cleavage	V-A (Cas12a/Cpf1), V-B (Cas12b/C2c1), V-C (Cas12c/C2c3), V-D (Cas12d/CasY), V-E (Cas12e/CasX), V-F (Cas12f/CasRx), V-G, V-H, V-I, V-U
	Type VI	Cas13 (e.g., Cas13a/C2c2, Cas13b, Cas13c, Cas13d)	RNA	Exclusively targets RNA. Possesses collateral RNA cleavage activity	VI-A (Cas13a), VI-B (Cas13b), VI-C (Cas13c), VI-D (Cas13d/CasRx)
	New discoveries	SubCas9 (from *Streptococcus uberis*)	DNA	Smaller than traditional Cas9; potentially less immunogenic. Targets different genetic sequences	Yet to be fully classified; candidate for inclusion in Type II

Abbreviations: Cas: CRISPR-associated protein; CRISPR-Cas: Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein; crRNA: CRISPR RNA; Csm: Type III-A complexes; Cmr: Type III-B complexes; sgRNA: Single guide RNA; tracrRNA: Trans-activating CRISPR RNA.

has emerged as a hypercompact effector with high specificity for ssDNA, expanding the CRISPR toolkit for applications requiring minimal off-target activity and compatibility.[100] Current CRISPR research continues to focus on refining the classification of interference mechanisms, as advancements in these areas can significantly enhance the specificity and efficiency of gene-editing technologies. While this section has outlined the core components of the CRISPR toolkit, the following section (Section 3) focuses on how these components operate together through the processes of adaptation, expression, and interference.

3. Mechanism of the CRISPR-Cas system

Building on the description of individual components in Section 2, this section discusses the coordinated mechanisms—adaptation, expression, and interference—through which the CRISPR-Cas system functions. These stages collectively enable prokaryotes to recognize and neutralize foreign genetic elements and serve as the foundation for the system's adaptation in gene editing, diagnostics, and biosensing.[101-103] Furthermore, this system relies on essential defense mechanisms, including transcription, crRNA biogenesis, and target degradation.[94] The mechanism is illustrated in **Figure 2** and described in detail in **Table 4**.

3.1. Adaptation

The adaptation phase initiates the CRISPR-Cas immune response by facilitating the acquisition and genomic integration of foreign DNA fragments, termed protospacers, derived from invading mobile genetic elements such as plasmids or bacteriophages.[104] These protospacers are inserted into the CRISPR array as new spacer sequences, flanked by conserved repeat elements. The integration process is carried out by the Cas1-Cas2 protein complex, which selectively captures foreign DNA and inserts it near the leader sequence of the CRISPR array.[107] These spacers function as genetic repositories, allowing the system to identify and target exogenous DNA during subsequent infections.[108] In engineered systems, the principle of spacer acquisition has inspired the design of synthetic gRNAs to direct Cas proteins to specific genomic loci for targeted editing.[109] The ability to define multiple spacers in CRISPR arrays has enabled multiplexed gene editing, allowing for the simultaneous modification of several genes in a single experiment.[110] Moreover, this programmable specificity is fundamental to the creation of sensitive and programmable biosensors, providing high sensitivity, cost-effectiveness, and rapid detection of genetic material, essential for point-of-care diagnostics and real-time mutation tracking.[98] In addition, the adaptation phase is crucial for the long-term evolution of bacterial immunity, as it enables the system to respond to evolving genetic threats.[111] Collectively, this phase ensures both the adaptability of CRISPR-Cas systems in nature and their flexibility as tools in synthetic biology, diagnostics, and precision therapeutics.

3.2. Expression

The expression phase involves transcribing the CRISPR array into a long pre-crRNA, containing alternating repeat

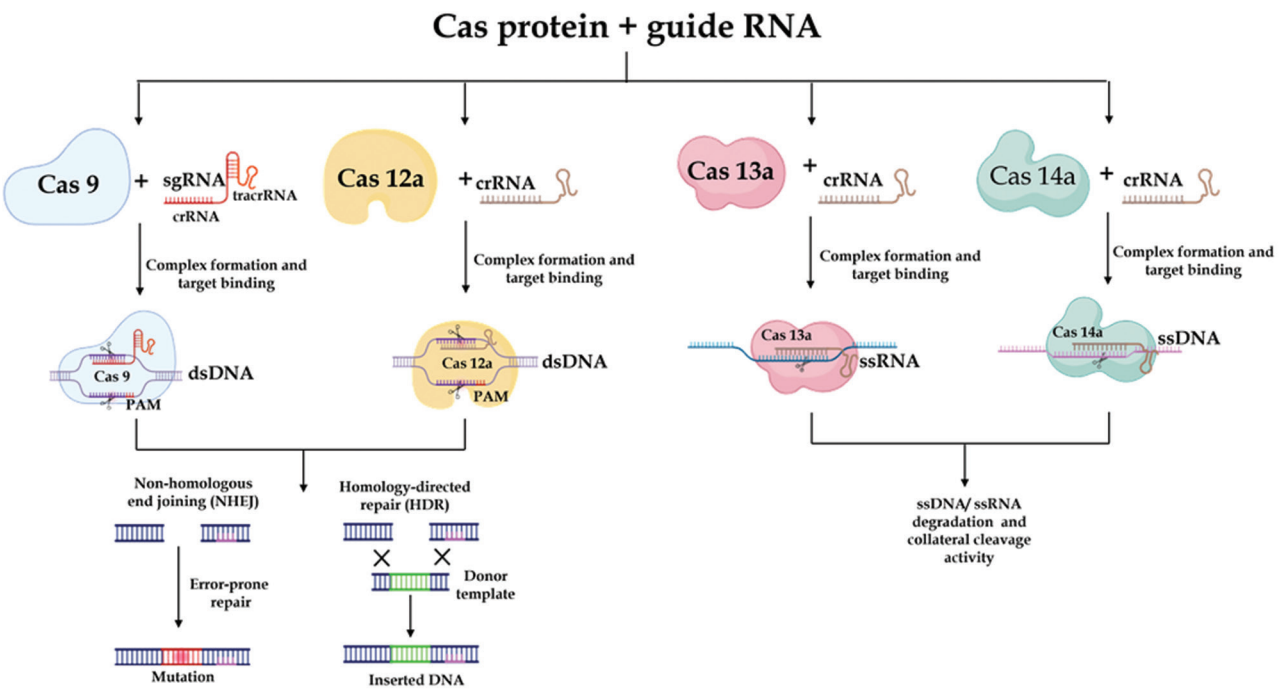

Figure 2. Comparative mechanisms of different Cas proteins in genome and transcriptome editing. Cas9 and Cas12a target dsDNA, inducing double-strand breaks that are repair via non-homologous end joining or homology-directed repair. Cas13a targets ssRNA, leading to its degradation and collateral RNA cleavage. Cas14a targets ssDNA with collateral cleavage activity, making it useful for biosensing applications. Figure created by the authors.

Abbreviations: Cas: CRISPR-associated protein; crRNA: CRISPR RNA; dsDNA: Double-stranded DNA; PAM: Protospacer adjacent motif; sgRNA: Single guide RNA; ssRNA: Single-stranded RNA; ssDNA: Single-stranded DNA; tracrRNA: Trans-activating CRISPR RNA.

Table 4. Molecular mechanism of CRISPR-Cas system: Three main stages

Stage	Description	Key molecular players and steps	Outcome/function	References
1. Adaptation (spacer acquisition)	The prokaryotic cell "remembers" a foreign invader by acquiring a small piece of its DNA and integrating it into its own genome. This creates a genetic "memory of past infections	(i) Recognition of foreign DNA: When a phage or plasmid invades, specific Cas proteins (primarily Cas1 and Cas2) identify and capture a short segment of the foreign DNA, known as a "protospacer." This process is often influenced by sequence motifs on the foreign DNA (ii) Integration into CRISPR array: The protospacer is then inserted as a new "spacer" into the CRISPR locus of the host genome. New spacers are typically added at one end of the CRISPR array, creating a chronological record of infections. This integration is accompanied by the duplication of an adjacent "repeat" sequence	Genetic memory: The host genome gains a new, unique spacer sequence, effectively "immunizing" the cell against future attacks by the same or similar invaders	104-106
2. Expression (crRNA biogenesis/ maturation)	The integrated genetic memory (CRISPR array) is transcribed and processed into small functional RNA molecules that will guide the Cas proteins	(i) Transcription of CRISPR array: The entire CRISPR locus (repeats and spacers) is transcribed into a long precursor CRISPR RNA (pre-crRNA) (ii) Processing of pre-crRNA: This transcript is cleaved by specific Cas ribonucleases (e.g., Cas6 in some systems or Cas9 aided by tracrRNA in Type II systems) into individual mature CRISPR RNAs (crRNAs), each containing a single spacer sequence flanked by partial repeat sequences (iii) Assembly of effector complex: The mature crRNAs associate with one or more Cas proteins to form a functional ribonucleoprotein (RNP) complex, also called a surveillance complex (e.g., cascade in Type I, or Cas9 alone in Type II)	Formation of guided surveillance complexes: Ready-to-use "search-and-destroy" complexes are formed, pre-programmed with genetic information from past invaders	
3. Interference (targeting)	Upon reinvasion by the same foreign nucleic acid, the pre-programmed Cas-crRNA complex recognizes and degrades the invader. This is the "active defense" phase	(i) Target scanning and recognition: The Cas-crRNA complex continuously scans the cell for foreign nucleic acids that match its spacer sequence. Recognition also requires the presence of a protospacer adjacent motif (PAM) sequence (in DNA-targeting systems) for efficient binding (ii) Base pairing: If a match is found and the PAM is present, the crRNA base-pairs with the complementary sequence on the foreign DNA or RNA (iii) Cleavage of target: The Cas protein's nuclease domain is activated, leading to the precise cleavage and degradation of the invading nucleic acid, thereby neutralizing the threat	Immunity and invader elimination: The foreign genetic material is destroyed, preventing successful infection or replication by the pathogen or plasmid	

Abbreviations: CRISPR-Cas: Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein; pre-crRNA: Precursor CRISPR RNA; tracrRNA: Trans-activating CRISPR RNA.

and spacer sequences.[112] This precursor is subsequently processed into individual crRNAs, each encoding the sequence information of a single spacer corresponding to a previously acquired protospacer.[121] In many systems, this processing requires a tracrRNA, which hybridizes to the repeat region and recruits RNase III to cleave the pre-crRNA. The resulting mature crRNAs associate with Cas proteins to form an active RNP complex capable of recognizing target nucleic acids.[122] To streamline the system for biotechnological and therapeutic applications, many platforms use synthetic gRNAs, which are crucial for directing Cas proteins to specific DNA or RNA sequences.[46] This process significantly improves the specificity and efficiency of genome editing, enabling targeted knockouts, insertions, and gene corrections through homology-directed repair (HDR) or base editing.[115] Well-designed gRNAs help reduce off-target effects, which is crucial for therapeutic applications. In recent years, an expanding ecosystem of computational and AI-driven platforms has leveraged the expression and modification of gRNAs to control gene expression, build genetic circuits, and reprogram cells, supporting precise genetic alterations with controlled timing and frequency.[116] These tools analyze factors such as sequence context, secondary structure, chromatin accessibility, and mismatch tolerances to predict gRNA activity.

3.3. Interference

The interference phase is the effector stage of the CRISPR-Cas immune system, during which the CRISPR-derived RNA guides the Cas protein to locate and neutralize target nucleic acids.[117] Following the expression and processing of gRNAs, the mature crRNA (or engineered sgRNA) forms an RNP complex with a Cas effector, enabling site-specific surveillance of invading DNA or RNA.[118] The REC process depends on the sequence complementarity between the gRNA and the protospacer region of the target, typically adjacent to a PAM in DNA-targeting systems.[119] Upon binding, the CRISPR-Cas complex undergoes structural activation and initiates site-specific cleavage of the target nucleic acid. This molecular interference is central not only to natural immunity but also to the programmable functions of CRISPR in synthetic applications.[120] In DNA-targeting systems, interference involves double-stranded cleavage mediated by conserved NUC domains, while RNA-targeting systems use RNA-guided cleavage without the requirement for PAM REC.[121] Some systems also exhibit collateral cleavage activity, wherein non-target ssDNA or single-stranded RNA (ssRNA) molecules are degraded on activation.[122] This property has been repurposed in CRISPR-Dx to amplify detection signals

in real-time biosensing assays. Through this programmable interference process, CRISPR-Cas systems have emerged as precision genome engineering tools, unlocking a wide range of possibilities in research, medicine, and biotechnology.[16]

3.3.1. REC of target DNA sequences

Target REC is a critical step in CRISPR-Cas interference, during which the gRNA-Cas complex locates a specific nucleic acid sequence based on base-pair complementarity.[7] In DNA-targeting systems, REC typically begins with the identification of a PAM, which signals the Cas effector to initiate local DNA unwinding.[123] The gRNA then probes for sequence complementarity, leading to the formation of an R-loop structure if a match is found. This interaction ensures specificity and triggers the downstream cleavage mechanism.[124] In RNA-targeting systems, where PAM REC is not required, target REC is driven solely by sequence pairing and target accessibility.[125] The precision of this step is essential for both genome editing fidelity and the sensitivity of CRISPR-Dx.

3.3.2 Binding and cleavage by Cas Protein

Once the gRNA-Cas complex has successfully recognized its target, stable binding induces a conformational change in the Cas protein, activating its endonuclease activity.[126] This activation leads to the site-specific cleavage of the target nucleic acid. In DNA-targeting systems, both strands of the DNA are typically cleaved, while RNA-targeting systems cleave ssRNA targets.[127,128] The precision of cleavage depends on stable guide-target pairing and proper alignment of the catalytic site with the cleavage region. This tightly regulated process forms the basis for programmable gene editing, allowing for the insertion, deletion, or replacement of genetic material with high specificity. In diagnostic platforms, cleavage activity is often coupled with signal amplification to enable sensitive and rapid detection.[129]

3.3.3 Mechanisms of repair and gene editing

Following DNA cleavage by the CRISPR-Cas complex, the host cell activates endogenous DNA repair pathways to resolve the break. The two major mechanisms are non-homologous end joining (NHEJ) and HDR.[130] NHEJ directly ligates the broken DNA ends and often introduces insertions or deletions (indels), resulting in gene disruption. In contrast, HDR utilizes a homologous DNA template to precisely insert or correct genetic sequences at the cleavage site.[131] The balance between these pathways determines the outcome of gene editing and is influenced by cell type, cell cycle stage, and template availability. Harnessing these repair mechanisms allows CRISPR to be used for both gene knockouts and precise genomic modifications.

3.3.4. CRISPRa/CRISPRi regulation

Beyond genome editing, catalytically inactive variants of Cas proteins have enabled transcriptional regulation through CRISPRi and CRISPRa.[132] In CRISPRi, a deactivated Cas protein (dCas) is guided to a gene promoter or coding region to sterically block transcription, effectively silencing gene expression.[133] Conversely, CRISPRa employs dCas fused to transcriptional activators to enhance gene expression without altering the underlying DNA sequence.[134] These systems offer precise, reversible, and multiplexed control of gene networks, allowing researchers to interrogate gene function and disease pathways without introducing permanent genetic alterations. As a result, CRISPRa/i approaches are widely used in functional genomics, cell reprogramming, and therapeutic gene modulation.[135]

4. Diagnostic utility of CRISPR effectors

CRISPR-Cas systems have emerged as powerful tools not only for genome editing but also for next-generation molecular diagnostics. The programmable nature of Cas effectors, combined with their high specificity and sensitivity, has enabled the development of novel detection platforms capable of identifying nucleic acid targets with remarkable precision, as outlined in **Table 5**.[136] Unlike traditional diagnostics that rely on extensive thermal cycling or probe hybridization, CRISPR-based assays can operate under isothermal conditions and exploit the unique cleavage activities of Cas proteins to generate easily detectable signals.[137] These features make them ideal candidates for POCT and portable diagnostics, especially in resource-limited settings. This section explores the diagnostic applications of key CRISPR effectors such as Cas9, Cas12, Cas13, and Cas14, focusing on their underlying mechanisms, molecular properties, and real-world implementations in nucleic acid detection platforms. Beyond genome manipulation, the unique collateral activities of Cas proteins have rapidly expanded their use into molecular diagnostics and biosensing, as discussed below.

4.1. Cas9

The CRISPR-Cas9 system is the most widely characterized Class 2 CRISPR effector and remains the cornerstone of CRISPR-Dx. Cas9 is a dual-lobed endonuclease composed of a REC lobe and a NUC lobe, which consists of the RuvC and HNH domains responsible for cleaving the non-complementary and complementary DNA strands, respectively.[160] Furthermore, the target engagement requires a 5'-NGG-3' PAM, following which an sgRNA directs Cas9 to form an RNA-DNA hybrid (R-loop) and initiates site-specific DSBs.[161] Hence, this molecular specificity enables its application in diagnostics, where both catalytically active Cas9 and its NUC-deactivated variant (dCas9) are repurposed for nucleic acid sensing.[162] In addition, in cleavage-based formats, Cas9 generates specific DSBs following amplification of the target sequence, releasing detectable reporters through fluorescent or lateral flow readouts. For example, Zhu *et al.*[163] developed a Cas9-based lateral flow biosensor capable of detecting *Mycoplasma pneumoniae* with a sensitivity of three copies and 100% accuracy in 123 clinical samples within 30 min.

In contrast, dCas9 enables non-cleaving, binding-based platforms such as CRISPR/dCas9-mediated enzyme-linked immunosorbent assay (ELISA) or Bio-SCAN, where sequence-specific DNA REC is coupled with optical or electrochemical signal generation.[164,165] **Figure 3** illustrates two such diagnostic formats: a dCas9-mediated ELISA and a Cas9-mediated lateral flow assay. These platforms are adaptable across targets ranging

Table 5. Commercially available CRISPR-Cas-based diagnostic kits

Nuclease	Technique	Preamplification	Detection method	Targeted pathogens/ organisms	LOD (copies per mL)	References
Cas9a	NASBACC	NASBA	Electronic optical reader	Zika/dengue	6.0×10^5	138
	CRISDA	PCR	Fluorescence spectrophotometer	SNPs	1.5×10^2	139
	CAS- EXPAR	EXPAR	Real-time fluorescence monitoring	Sensing of methylated DNA; *Listeria monocytogenes* mRNA	4.9×10^2	140
	CASLFA	PCR	Paper-based LF device	African swine fever virus	20 copies/reaction	141
	FELUDA	RPA/PCR	Paper-based LF device	SARS-CoV-2 virus	10^4	142
	CRISPR- chip	-	Electrochemical	gDNA from cell lines and DMD patients	1.4 x 10^5	143
	FLASH	PCR	NGS	Antimicrobial resistance	1.1×10^3	144
	Cas9nAR	Strand- displacing DNA polymerase	Fluorescence	*Escherichia coli*, *Saccharopolyspora erythraea;* KRAS SNPs in cell lines	1.0×10^2	145
Cas12a	DETECTR	LAMP	Paper-based LF device	HPV16/18	6.0×10^2	146
	HOLMES	PCR	Fluorescence spectrophotometer	Japanese encephalitis virus, pseudorabies virus	6.6×10^3	147
	E-CRISPR	-	Electrochemical	DNA: HPV16, parvovirus B19; protein: TGFβ1	3.0×10^{10}	148
	CRISPR- MTB	RPA	Fluorescence or μPAD	*Mycobacterium tuberculosis*	1–100 copies/ reaction	149
Cas12b	CDetection	RPA	Fluorescence	HPV, ABO blood genotyping, BRCA1, and TP53 SNPs	6.0×10^2	150
	HOLMES v2	LAMP	Fluorescence	RNA virus, human mRNA, circRNA, and DNA methylation	6.0×10^3	151
	STOPCovid	LAMP	Fluorescence, LF device	SARS-CoV-2	2.0×10^3	386
Cas12f (Cas14)	Cas14-DETECTR	PCR	Fluorescence	HERC2 SNPs in human samples	6.0×10^3	152
Cas13a	SHERLOCK	NASBA or RPA	Fluorescence spectrophotometer/ Paper-based LF device	Virus/bacteria/cancer mutation	1.2×103	153
	SHERLOCKv2	RPA	LF device	Virus/bacteria/cancer mutation	4.8	154
	CREST	RPA/PCR	Fluorescence spectrophotometer/ Paper-based LF device	SARS-CoV-2	100 copies/μL	155
	CARMEN	PCR	Fluorescence spectrophotometer	HIV, influenza A strains	5.4×102	156
	CARVER	PCR	Fluorescence spectrophotometer	Viral RNA	500 attomole (~3.01×10^5 copies/mL)	157
	SHINE	RPA	Fluorescence or lateral flow	SARS-CoV-2	5.0×103	158
	APC- Cas	DNA polymerase	Fluorescence	*Salmonella enteritidis*	-	140
	PECL- CRISPR	EXPAR	Electrochemiluminiscence	miRNA	6.0×10^5	159

Abbreviations: APC-Cas: Allosteric Probe-Initiated Catalysis and CRISPR-Cas13a system; BRCA1: BReast CAncer gene 1; CARMEN: Combinatorial arrayed reactions for multiplexed evaluation of nucleic acids; CARVER: Cas13-assisted restriction of viral expression and readout; CASLFA: Cas9-mediated lateral flow nucleic acids assay; CAS-EXPAR: CRISPR/Cas9-triggered isothermal exponential amplification reaction; CDetection: Cas12b-mediated DNA detection; circRNA: Circular RNA; CREST: Cas13-based, rugged, equitable, scalable testing; CRISDA: CRISPR-Cas9-triggered nicking endonuclease-mediated strand displacement amplification method; CRISPR-Cas: Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein; DETECTR: DNA endonuclease targeted CRISPR trans reporter; DMD: Duchenne muscular dystrophy; EXPAR: Exponential amplification reaction; E-CRISPR: CRISPR-Cas12a (cpf1)-based electrochemical biosensor; FELUDA: FnCas9 editor linked uniform detection assay; FLASH: Finding low abundance sequences by hybridization; gDNA: Genomic DNA; HERC2: Giant E3 ubiquitin protein ligase; HOLMES: One-hour low-cost multipurpose highly efficient system; HPV: Human papillomavirus; KRAS: Kirsten rat sarcoma virus; LAMP: Loop-mediated isothermal amplification; LF: Lateral flow; LOD: Limit of detection; mRNA: Messenger RNA; miRNA: MicroRNA; MTB: *Mycobacterium tuberculosis*; NASBA: Nucleic acid sequence-based amplification; NASBACC: Nucleic acid sequence-based amplification-CRISPR cleavage; PCR: Polymerase chain reaction; PECL-CRISPR: CRISPR/Cas13a powered portable emitter coupled logic chip; RPA: Recombinase polymerase amplification; SHERLOCK: Specific High-sensitivity Enzymatic Reporter unLOCKing; SHINE: Streamlined Highlighting of Infections to Navigate Epidemics; SNPs: Single nucleotide polymorphisms; STOP: SHERLOCK testing in one pot; TGFβ1: Transforming growth factor beta 1; TP53: Tumor protein p53.

from SARS-CoV-2 and foodborne pathogens to antimicrobial resistance genes, highlighting Cas9's diagnostic versatility.[166-168] Notably, to address PAM constraints and off-target concerns, engineered variants such as *Francisella novicida*-derived Cas9 (FnCas9) and high-fidelity variant of *S. pyogenes* Cas9 (SpCas9-HF1) have been developed with relaxed PAM compatibility and enhanced mismatch discrimination, improving sensitivity for single-nucleotide polymorphism

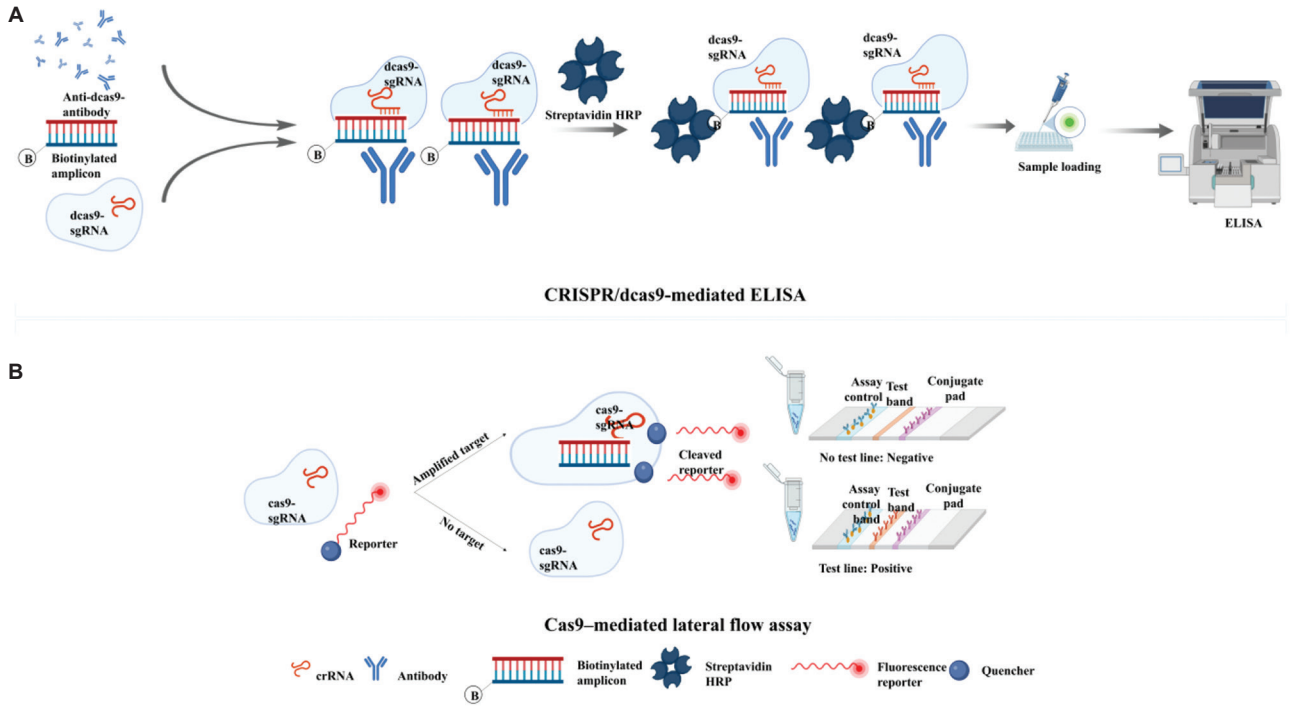

Figure 3. CRISPR-based nucleic acid detection methods: (A) dCas9-mediated ELISA and (B) Cas9-mediated lateral flow assay. In the dCas9-mediated ELISA, the dCas9–sgRNA complex binds specifically to a biotinylated target amplicon, which is captured by anti-dCas9 antibodies immobilized on a plate. Signal amplification occurs through streptavidin–HRP binding to biotin, producing a colorimetric readout upon substrate addition, which is measured using an ELISA plate reader. This method provides high specificity by leveraging the CRISPR–dCas9 system for target recognition and enzyme-linked detection for signal enhancement. In contrast, the Cas9-mediated lateral flow assay utilizes an active Cas9–sgRNA complex to detect and cleave a fluorescently labeled reporter upon target recognition. If the target is present, the reporter is cleaved, allowing detection at the test line, indicating a positive result. In the absence of the target, the uncleaved reporter flows past the test line, resulting in a negative outcome. The bottom legend defines the key molecular components involved, including crRNA, antibodies, biotinylated amplicons, streptavidin–HRP, fluorescent reporters, and quenchers. By combining the specificity of CRISPR-based detection with enzyme-linked and lateral flow-based readouts, these methods offer robust and versatile diagnostic tools for nucleic acid detection. Figure created by the authors. Abbreviations: Cas: CRISPR-associated protein; CRISPR: Clustered regularly interspaced short palindromic repeats; crRNA: CRISPR RNA; dCas9: Catalytically inactivated Cas9; ELISA: Enzyme-linked immunosorbent assay; HRP: Horseradish peroxidase; sgRNA: Single guide RNA.

detection and rare variant screening.[169,170] Recent diagnostic studies have applied FnCas9 in lateral flow and fluorescent biosensors with attomolar sensitivity, demonstrating strong potential for clinical translation.[171,172] Importantly, Cas9's modular architecture allows rapid reprogramming of sgRNAs, making it highly suitable for multiplexed detection and point-of-care formats.[173] These properties, along with their compatibility with low-cost amplification systems such as nucleic acid sequence-based amplification (NASBA), polymerase chain reaction (PCR), strand displacement amplification (SDA), and finding low-abundance sequences by hybridization, have led to their incorporation in emerging diagnostic workflows for infectious diseases, cancer mutation profiling, and even food safety monitoring.[174,175] Together, the mechanistic precision, engineering flexibility, and real-world clinical performance of Cas9-based diagnostics solidify its foundational role in CRISPR-driven molecular sensing platforms. Altogether, these advances position Cas9 as both a pioneering genome editor and a versatile molecular sensor at the forefront of CRISPR-Dx.

4.2. Cas12

The CRISPR-Cas12 system has emerged as a pivotal tool for nucleic acid diagnostics due to its unique dual-mode NUC activity. Upon REC of a target DNA sequence flanked by a 5'-TTTV-3' PAM, Cas12a undergoes conformational activation and initiates DSBs via its RuvC domain.[176] Notably, this activation also triggers a robust trans-cleavage mechanism, whereby Cas12a indiscriminately cleaves nearby ssDNA. This property enables signal amplification in diagnostic platforms through cleavage of labeled reporter molecules, a feature not present in Cas9, and is highly advantageous for sensitive and rapid detection.[177,178]

Furthermore, Cas12a's molecular versatility has been harnessed in a range of detection formats. The DETECTR assay, developed during the COVID-19 pandemic, combined reverse transcription loop-mediated isothermal amplification (LAMP) with Cas12a-mediated detection of SARS-CoV-2 in clinical nasopharyngeal samples, producing results in under 30–45 min, with 95% positive predictive agreement and 100% negative predictive agreement, directly visualized through lateral flow readouts.[179] The schematic in **Figure 4** illustrates a CRISPR-Cas12a-based diagnostic approach that integrates nucleic acid detection with a lateral flow assay for rapid and sensitive diagnostics. In oncology, Yu *et al.*[180] engineered an amplification-free Cas12a-based electrochemiluminescent biosensor incorporating DNA tetrahedron nanostructures

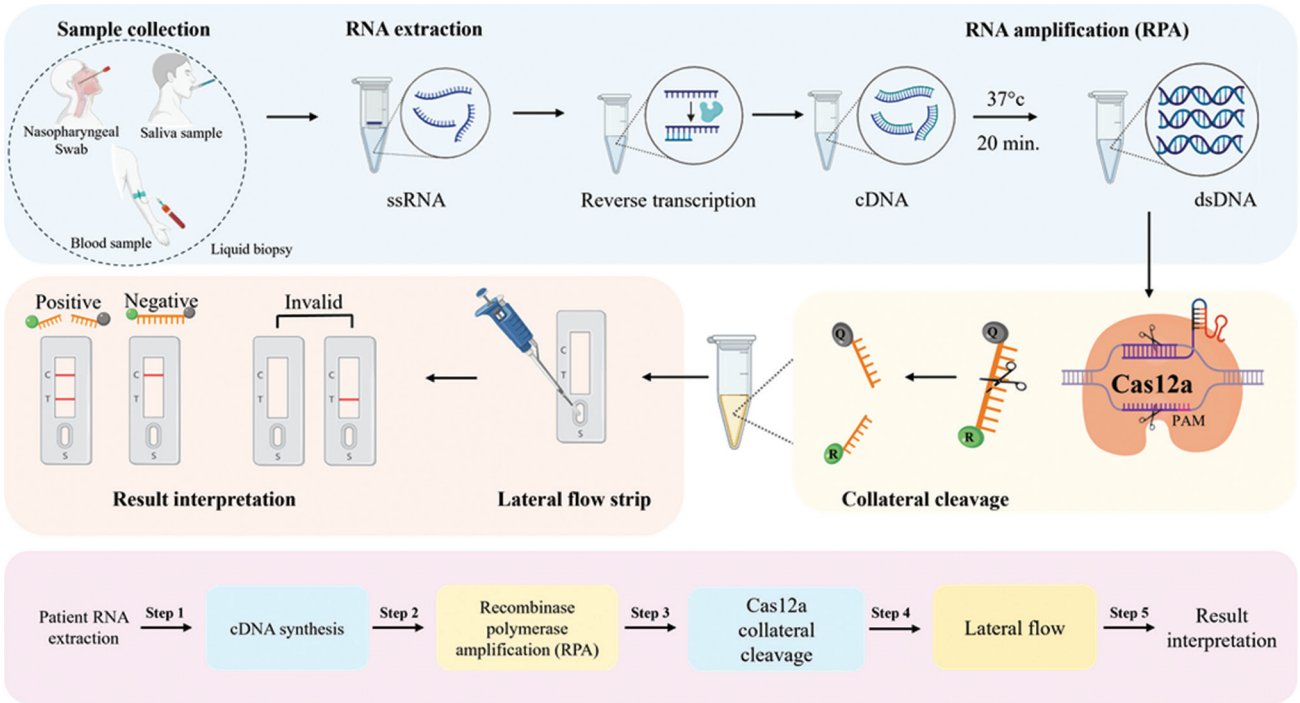

Figure 4. Schematic representation of a CRISPR–Cas12a-based diagnostic approach that integrates nucleic acid detection with a lateral flow assay for rapid and sensitive diagnostics. The process begins with sample collection, where nasopharyngeal swabs, saliva, or blood—part of a liquid biopsy—are obtained from patients. Step 1 involves RNA extraction to isolate ssRNA, which is then reverse transcribed into cDNA. In Step 2, the cDNA undergoes RPA at 37°C for 20 minutes, generating dsDNA to enrich the target sequence. Step 3 introduces the amplified DNA to Cas12a, which, upon recognizing the specific target sequence, activates its collateral cleavage activity, indiscriminately cutting nearby single-stranded reporter probes labeled with a fluorophore and quencher. In Step 4, the cleaved reporter probes are applied to a lateral flow strip, initiating visual detection. Finally, Step 5 involves result interpretation, where the test strip is analyzed based on the appearance of test and control lines, to determine whether the outcome is positive, negative, or invalid. This approach provides an efficient and accurate nucleic acid detection platform, making it ideal for point-of-care diagnostics. Figure created by the authors.
Abbreviations: Cas: CRISPR-associated protein; cDNA: Complementary DNA; CRISPR: Clustered regularly interspaced short palindromic repeats; dsDNA: Double-stranded DNA; PAM: Protospacer adjacent motif; ssRNA: Single-stranded RNA.

for the amplification-free detection of human papillomavirus (HPV)-16, achieving a detection limit of 8.86 fM within a 100-min workflow. Similarly, Luo *et al.*[181] reported a nanobeacon-driven Cas12a sensor for direct microRNA (miRNA)-21 detection within five minutes, bypassing the need for reverse transcription and illustrating Cas12a's ability to detect non-coding RNAs in complex samples.

Beyond these applications, Cas12a has been used to detect bacterial and environmental pathogens. A dual-mode biosensor integrating fluorescence and electrochemical readouts successfully identified *Staphylococcus aureus* using a Cas12a-triggered hybridization chain reaction, with a detection limit of 5.7 CFU/mL.[182] Similarly, for tuberculosis diagnostics, an electrochemical Cas12a sensor targeting the *IS6110* gene of *Mycobacterium tuberculosis* achieved a detection limit of 14.5 nM through voltammetry within 60 min.[183] In addition, Cas12a-based biosensors have been adapted to detect resistance-associated mutations in *M. tuberculosis*, enabling genotypic identification of drug-resistant strains such as multidrug-resistant tuberculosis.[184] Companies like Mammoth Biosciences have integrated Cas12-based DETECTR platforms into pathogen-specific diagnostics, enabling rapid, point-of-care detection of infectious agents, including tuberculosis (**Table 6**). These biosensors facilitate rapid treatment stratification in clinical settings and address critical gaps left by slow, culture-based drug susceptibility tests. Moreover, Cas12a-based biosensing has also been applied to antibiotic monitoring; a fluorescence aptasensor for ampicillin, using Cas12a-cleavable ssDNA reporters, reached a detection threshold of 0.01 nM within 30 min, even in spiked food samples.[185] In several contexts, Cas12a has been coupled with recombinase polymerase amplification (RPA) and rolling circle amplification (RCA) for the sensitive detection of *Escherichia coli* in water and food samples. For instance, a dual fluorescence/lateral flow RPA-Cas12a assay detected *E. coli* O157:H7 down to 1.8 fg/μL (≈ 2.4 CFU/mL) within 40 min, while an RCA-Cas12a electrochemical biosensor achieved attomolar-level predictive sensitivity.[186]

Mechanistically, Cas12a's trans-cleavage activity allows for single-molecule detection, high turnover rates, and flexibility in signal design. Its compatibility with isothermal amplification (RPA, LAMP) and tolerance to variable reaction conditions make it highly suited for point-of-care and field-deployable diagnostic systems.[207] Importantly, the programmability of its gRNAs and its broad modality compatibility—fluorescent, electrochemical, and lateral flow—position Cas12a as a diagnostic effector of exceptional breadth, enabling the detection of viral RNA, tumor DNA, bacterial

Table 6. Leading CRISPR-Cas companies: Focus areas in diagnostics, prognostics, and biosensing

Company name	CRISPR variant (s)	Primary diagnostic focus/ platforms	Detection method	Key features/notes	References
Diagnosis					
SHERLOCK Biosciences	Cas13a, Cas12a	Infectious diseases (e.g., SARS-CoV-2, tuberculosis, malaria), genetic mutations	Fluorescence, lateral flow	Pioneers of the SHERLOCK platform; high sensitivity; POC ready	https://sherlock.bio[187]
Mammoth Biosciences	Cas12a, Cas14, CasΦ	Infectious diseases (e.g., SARS-CoV-2), genetic conditions	Lateral flow, fluorescence	Co-founded by Nobel laureate Jennifer Doudna; develops the DETECTR platform; supports multiplexing and portable diagnostics	https://mammoth.bio[188]
CrisprBits	Cas12a, Cas13a	Infectious diseases (e.g., SARS-CoV-2 Omicron variant, other GI tract infections), antimicrobial resistance genes	Lateral flow, electrochemical	Indian company developing affordable and accessible CRISPR-based diagnostics; low-cost; community surveillance and environmental testing	https://crisprbits.com[189]
Caspr Biotech	Cas12a, Cas13a	Molecular diagnostics (various applications), COVID-19	Paper-based strip, RT-RPA	Portable and accurate CRISPR-based diagnostics; diverse applications	https://sosv.com/company/caspr-biotech/[190]
Scope Biosciences	Cas12a	Molecular diagnostics	Fluorescence, LAMP	Dutch biotech firm; high specificity; pathogen and mutation detection	https://scopebio.com[191]
Locus Biosciences	Cas3	Precision antibacterial (bacterial infections)	Bacteriophage-mediated	Uses CRISPR-Cas3 bacteriophage (crPhage) technology to degrade bacterial DNA	https://www.locus-bio.com[192]
Medic Life Sciences	Cas9, Cas12a	Cancer biomarkers, drug discovery	Fluorescent, CRISPR screens	Functional genomics to identify and validate cancer targets	https://www.medic-life-sciences.com[193]
Integrated DNA Technologies (IDT)	Cas9, Cas12a, Cas13a	Reagents and tools for CRISPR diagnostics	Enabling reagents	Supports R&D; major reagent supplier for diagnostic developers	https://sg.idtdna.com/page/products/crispr-genome-editing[194]
Thermo Fisher Scientific	Cas9, Cas12a	Reagents, kits, and platforms for CRISPR research and diagnostics	Multiplex PCR, qPCR, LAMP	Supplies instruments and kits for CRISPR R&D workflows	https://www.thermofisher.com/in/en/home/life-science/genome-editing/crispr-nuclease-vector.html[195]
Takara Bio Inc.	Cas9, Cas12a	Molecular diagnostics, gene therapy	LAMP, RT-qPCR	Japanese biotechnology company specializing in genome editing, gene therapy, and diagnostics; Asia-Pacific leader	https://www.takarabio.com/?srsltid=AfmBOooCQFJ7Nuv1BZef22CUGhEXW_jNCJnuSuoi36BrSTTcaw1aDne0[196]
Prognosis					
Academic/research institutions (e.g., Broad Institute, UC Berkeley)	Cas9, Cas12a	Discovery of prognostic gene signatures, drug resistance biomarkers	Genome-wide knockout/ activation screens	Pioneers in CRISPR-based prognostic screening	https://www.broadinstitute.org/crispr/information-about-crispr-cpf1-cas12a-systems[197]
Companies with strong diagnostic platforms (e.g., SHERLOCK Biosciences, Mammoth Biosciences)	Cas13a, Cas12a	Prognostic biomarker detection, monitoring disease progression	SHERLOCK assay, isothermal amplification	Enables detection of relapse and recurrence; focuses on real-time, low-abundance biomarker detection	https://sherlock.bio https://mammoth.bio[187,188]

(Cont'd...)

Table 6. *(Continued)*

Company name	CRISPR variant (s)	Primary diagnostic focus/ platforms	Detection method	Key features/notes	References
Companies focused on precision oncology/ drug discovery (e.g., Repare Therapeutics, KSQ Therapeutics)	Cas9	Identification of drug response biomarkers, overcoming drug resistance	Functional CRISPR screens	Precision oncology targeting vulnerabilities	https://www.reparerx.com https://ksqtx.com[198,199]
Crown Bioscience	Cas9	Predictive biomarker identification for drug discovery	CRISPR-edited tumor models	Identifies treatment response profiles in oncology	https://www.crownbio.com[200]
Takara Bio Inc.	Cas9, dCas9	Tools and services for biomarker discovery	Custom screen libraries	Supports multiplexed CRISPR-based diagnostics	https://www.takarabio.com/?srsltid=AfmBOooCQFJ7Nuv1BZef22CUGhEXW_jNCJnuSuoi36BrSTTcaw1aDne0[196]
Companies Developing Liquid Biopsy Solutions (CRISPR-enhanced)	Cas12a, Cas13a	Detection of circulating prognostic biomarkers	Microfluidics-integrated CRISPR detection	Focused on non-invasive prognosis solutions	https://www.freenome.com https://guardanthealth.com https://www.natera.com[201-203]
Biosensing					
Mammoth Biosciences	Cas12a, Cas14, CasΦ	Broad molecular diagnostics (healthcare, environmental)	DETECTR, isothermal amplification	Leading player in CRISPR diagnostics; aims to create a "programmable diagnostic platform" for any biomarker	https://mammoth.bio[188]
SHERLOCK Biosciences	Cas13a, Cas12a	Infectious disease diagnostics (POC)	SHERLOCK, LFA, RPA	Rapid and affordable POC diagnostics using RNA/DNA sensing; strong COVID-19 and STI testing portfolio; acquired by OraSure Technologies (2024)	https://sherlock.bio[187]
Cardea Bio	Cas9, dCas9	Integrated biosensors (CRISPR-Chip)	CRISPR-Chip (electronic detection)	Label-free, real-time sensing of nucleic acids; integrates CRISPR with electronics; useful in continuous monitoring and environmental biosensing	https://www.serraventures.com/cardea-bio-1[204]
CrisprBits	Cas12a	Affordable diagnostics (developing nations focus)	OmiCrisp (paper-based), fluorescence	Focused on SARS-CoV-2 variants and AMR detection; cost-effective platform for India; exploring food/agriculture applications	https://crisprbits.com[189]
Proof Diagnostics	Cas12a	Rapid COVID-19 diagnostics	Fluorescent LFA	Developed during the pandemic for at-home testing; demonstrates CRISPR's agility in emergency diagnostic development	https://www.ginkgo.bio[205]
Caribou Biosciences	Cas9, chRDNA	Broader genomics and potential diagnostics	CRISPR-Cas screens	Primarily therapeutics-focused; exploring diagnostics using engineered Cas proteins for broad detection and high-throughput applications	https://www.cariboubio.com[206]

(Cont'd...)

Table 6. *(Continued)*

Company name	CRISPR variant (s)	Primary diagnostic focus/ platforms	Detection method	Key features/notes	References
Thermo Fisher Scientific	Cas9, Cas12a	CRISPR reagents and platforms (enabler)	qPCR-compatible, isothermal platforms	Supplies critical reagents and kits; not a biosensor firm per se, but supports CRISPR-based detection R&D globally	https://www.thermofisher.com/in/en/home/life-science/genome-editing/crispr-nuclease-vector.html[195]

Abbreviations: AMR: Antimicrobial resistance; CRISPR-Cas: Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein; dCas9: Catalytically inactivated Cas9; DETECTR: DNA endonuclease-targeted CRISPR trans reporter; GI: Gastrointestinal; LAMP: Loop-mediated isothermal amplification; LFA: Lateral flow assay; POC: Point-of-care; qPCR: Quantitative polymerase chain reaction; RPA: Recombinase polymerase amplification; RT: Real-time; R&D: Research and development; SHERLOCK: Specific High-sensitivity Enzymatic Reporter unLOCKing; STI: Sexually transmitted infection.

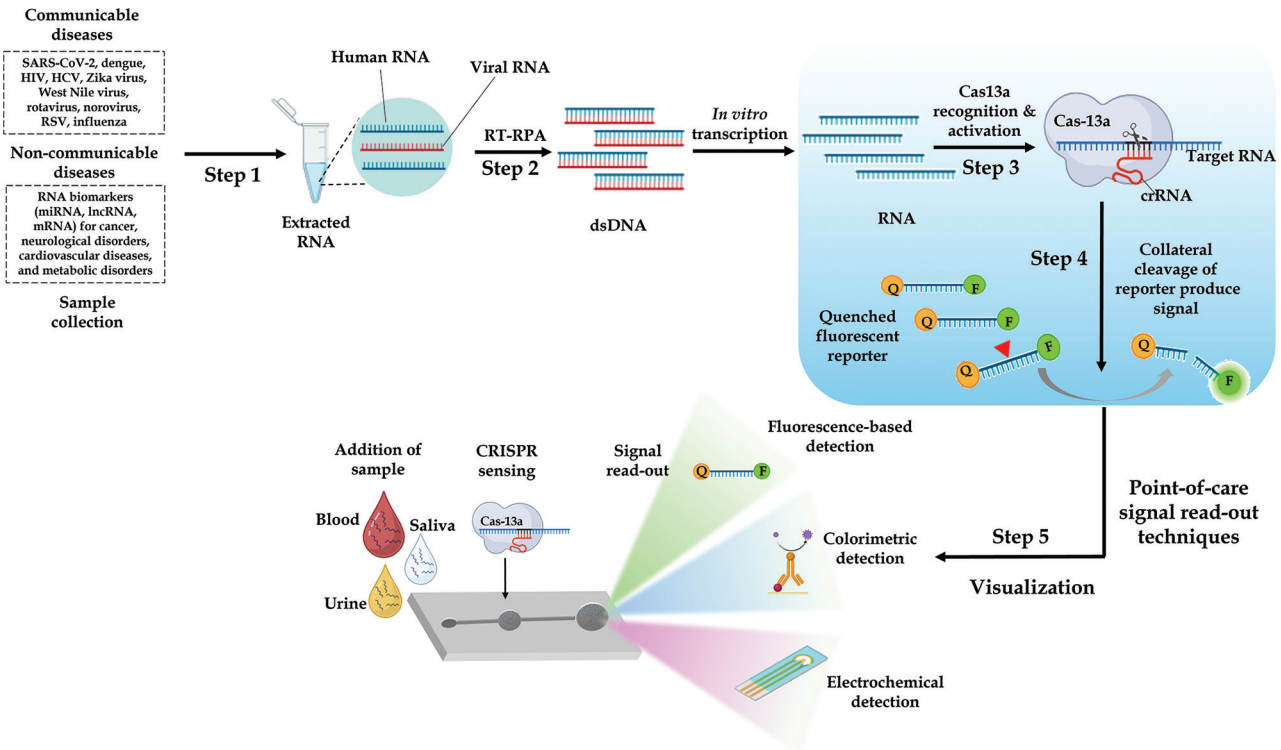

Figure 5. CRISPR–Cas13a-based nucleic acid detection workflow. Step 1 involves nucleic acid extraction from the sample. In Step 2, isothermal amplification methods, such as RPA, enhance detection sensitivity by increasing the target RNA concentration. Step 3 illustrates the Cas13a–crRNA complex binding to the target RNA, resulting in its activation. In Step 4, the activated Cas13a enzyme exhibits collateral cleavage activity, cutting not only the target RNA but also surrounding RNA, including a quenched fluorescent reporter. Step 5 highlights the detection phase, where the cleavage of the reporter RNA generates a detectable signal. This signal can be visualized using various modalities, including fluorescence-based detection, colorimetric assays, and electrochemical sensors—offering versatile, sensitive, and specific diagnostic capabilities. Figure created by the authors.

Abbreviations: Cas: CRISPR-associated protein; CRISPR: Clustered regularly interspaced short palindromic repeats; crRNA: CRISPR RNA; dsDNA: Double-stranded DNA; HCV: Hepatitis C virus; lncRNA: Long non-coding RNA; mRNA: Messenger RNA; miRNA: MicroRNA; RPA: Recombinase polymerase amplification; RSV: Respiratory syncytial virus.

genomes, miRNAs, and chemical contaminants across clinical, environmental, and industrial domains.

4.3 Cas13

The CRISPR-Cas 13 system is unique in targeting RNA rather than DNA, using crRNA-guided REC to activate its dual higher eukaryotes and prokaryotes nucleotide-binding NUC domains for both specific target cleavage and non-specific trans-cleavage of surrounding ssRNA.[208] This collateral cleavage activity accounts for its remarkable diagnostic sensitivity, often reaching attomolar limits, making Cas13 a powerful tool for RNA pathogen detection, miRNA profiling, and transcriptomic diagnostics.[209] Cas13-centered diagnostics are exemplified by the SHERLOCK platform, which integrates real-time RPA amplification and Cas13a-mediated reporter cleavage.[210] **Figure 5** illustrates the workflow of nucleic acid detection based on CRISPR-Cas13a, emphasizing its versatility and specificity. It was quickly adapted for SARS-CoV-2 detection and later extended to other pathogens. Notably, SHERLOCK has been refined to detect infectious bursal disease

virus with a 5 aM limit, delivering superior sensitivity to real-time PCR across 70 field samples.[211] Sherlock Biosciences, which pioneered the SHERLOCK system, continues to expand CRISPR-Cas13 applications to cover a broad spectrum of infectious and antimicrobial resistance diagnostics (**Table 6**). For mosquito-borne illnesses, a malaria-specific SHERLOCK assay using LwCas13a enables simultaneous *Plasmodium* species identification and drug-resistance single-nucleotide variant (SNV) genotyping in a multiplexed reaction, demonstrating field-compatible precision.[212] Similarly, Cas13-based diagnostics have been employed to detect resistance-associated mutations in HIV, offering rapid genotypic insights for guiding antiretroviral therapy.[213] By targeting specific RNA regions linked to drug resistance, these assays outperform conventional PCR in detecting low-abundance variants, making them valuable for real-time treatment decisions in clinical and low-resource settings. Notably, a rapid isothermal Cas13a-based kit for genital herpes simplex virus achieved near-perfect sensitivity and specificity (96.2% and 100%, respectively), with a 1 copy/μL detection threshold in a 194-sample clinical cohort.[214]

In oncology, the Cas-catalyzed hairpin DNA circuit electrochemical biosensor (COMET) utilizes Cas13a collateral cleavage followed by a catalytic hairpin DNA circuit to reliably quantify multiple non-small cell lung carcinoma-associated RNAs, including miR-17, miR-155, miR-19b, and miR-210, with a dynamic range from 50 aM to 5 nM.[215] It differentiated early-stage cancer patients from controls within 36 min using only 10 μL of serum. Furthermore, for direct, amplification-free miRNA profiling, photoelectrochemical and microfluidics-enabled electrochemical sensors incorporated Cas13a to detect miRNA-21 and other non-coding RNAs in <10 min with picomolar sensitivity.[216] Such modalities highlight Cas13's adaptability to highly sensitive RNA-targeted detection. Mechanistically, Cas13-based diagnostics thrive due to collateral cleaving of ssRNA reporters, precise single-base mismatch discrimination (especially for SNV detection), PAM-independent targeting flexibility, and multiplexing options through orthogonal reporter designs.[217] Combined with isothermal amplification techniques like real-time RPA/LAMP or even in amplification-free formats, Cas13 assays combine high specificity, rapid performance, and simple hardware compatibility, making them prime candidates for both field-based infectious disease surveillance and precision diagnostics in oncology and genetic research.[218]

4.4. Cas14

Cas14, also referred to as Cas12f, represents one of the most compact CRISPR-associated NUCs, measuring only ~400–700 amino acids.[100] First discovered by Jennifer Doudna's group in 2018, Cas14 exhibits a unique combination of features that distinguish it from Cas9, Cas12, and Cas13 effectors. It targets ssDNA with high specificity and performs both cis- and trans-cleavage without requiring a PAM, offering exceptional flexibility in sequence REC.[152] These attributes make it an attractive candidate for diagnostic and genome engineering applications, particularly where space-constrained delivery systems or highly sensitive detection are required.[219]

Figure 6 illustrates Cas14a-mediated DNA cleavage mechanisms, showing its cis- and trans-cleavage activities for enhanced genome-editing precision.

Mechanistically, Cas 14 is guided by a crRNA to recognize ssDNA targets, on which it activates trans-cleavage of surrounding non-target ssDNA molecules, a property similar to Cas12a and Cas13 but executed with higher substrate selectivity and in a PAM-independent manner. This molecular precision and low background activity make Cas14 highly suitable for integration into biosensors that depend on signal amplification through reporter cleavage. Its minimal size further facilitates incorporation into point-of-care and microfluidic diagnostic devices, where compact delivery is essential.[220,221] Several biosensing strategies have successfully employed Cas14a for nucleic acid and small molecule detection. For example, Wang *et al.*[222] developed a photoelectrochemical biosensor combining Cas14a with upconversion nanoparticles and SDA to detect T-2 toxin in oats and protein kinase PTK7 in human serum. This sensor achieved femtogram-level sensitivity and demonstrated high specificity in complex biological matrices. Similarly, a primer exchange reaction (PER)-Cas14a electrochemical biosensor has been engineered for circulating tumor DNA detection, including epidermal growth factor receptor (EGFR) *L858R* mutations associated with non-small cell lung cancer.[223] This platform achieved a detection limit of 0.34 fM and a broad dynamic range, showing strong potential for liquid biopsy applications. Cas14a has also been applied in pathogen diagnostics. In one study, an electrochemical sensor integrating Cas14a with PtPd@PCN-224 nanoenzymes enabled ultra-sensitive detection of *Burkholderia pseudomallei*, with a detection limit of just 12.8 aM.[224]

In a different context, the highly sensitive Aptamer-Regulated R-loop for bioanalysis system utilized Cas14a's collateral activity for aptamer-guided detection of various small molecules, including adenosine triphosphate (ATP), histamine, cadmium, and thrombin. This system provided nanomolar sensitivity in a label-free format and demonstrated broader versatility than comparable Cas12a-based approaches.[225,226] Importantly, Cas14a's diagnostic potential has attracted the attention of industry, with Mammoth Biosciences actively engineering Cas14 variants for portable, rapid, and equipment-free diagnostic platforms.[227] Their development focus includes lateral flow systems and CRISPR-integrated paper devices that can operate in decentralized, low-resource settings—features crucial for global infectious disease surveillance and mobile diagnostics. Despite its advantages, Cas14 still faces several translational challenges. Its activity can be sensitive to temperature fluctuations, and its ssDNA-targeting nature limits broader genomic applications unless modified or paired with upstream amplification strategies.[228] In addition, further studies are needed to evaluate off-target effects, enzyme kinetics, and real-world performance in clinical samples. Nonetheless, Cas14a's compact size, PAM independence, and collateral cleavage capability position it as a powerful next-generation CRISPR effector for precision diagnostics and synthetic biosensor systems.

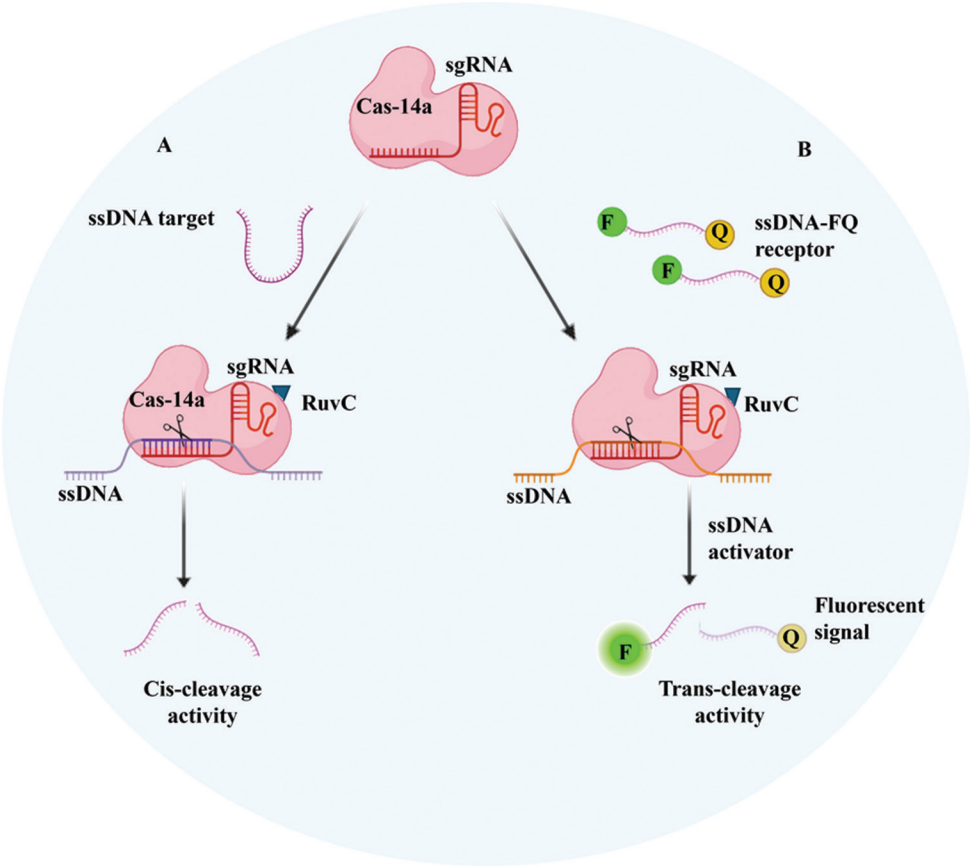

Figure 6. Cas14a-mediated DNA cleavage mechanisms. The Cas14a protein binds to an sgRNA and recognizes an ssDNA target. Upon binding, Cas14a exhibits two types of cleavage activities: (A) Cis-cleavage activity, where the target ssDNA is directly cleaved at the binding site by the RuvC domain; and (B) Trans-cleavage activity, which is triggered upon recognition of an ssDNA activator. This results in the cleavage of surrounding ssDNA substrates, including an FQ-labeled ssDNA reporter, leading to the release of a fluorescent signal. This signal serves as an indicator of Cas14a activation and target DNA recognition. Figure created by the authors.
Abbreviations: Cas: CRISPR-associated protein; FQ: Fluorescence-quencher; RuvC: RecU-like resolvase; sgRNA: Single guide RNA; ssDNA: Single-stranded DNA.

5. Programmable CRISPR-based biosensors

Biosensing is a multidisciplinary approach that detects biological molecules by combining a biorecognition element, such as enzymes or nucleic acids, with a signal transducer that converts biological interactions into measurable signals.[229] Among emerging technologies, CRISPR-Cas systems have rapidly gained prominence as programmable biosensing platforms, offering precise, portable, and adaptable solutions for molecular diagnostics.[101] In these biosensors, Cas effectors are directed by synthetic gRNAs to identify specific nucleic acid targets, whereupon signal generation occurs through mechanisms such as collateral cleavage, binding-induced changes, or enzymatic activation.[178] CRISPR-based biosensors differ fundamentally from conventional techniques such as PCR, ELISA, or NGS by providing amplification-free, isothermal, and real-time detection capabilities, often used at the point-of-care.[230] Platforms such as CRISPR-Chip, which integrates dCas9 with a graphene field-effect transistor, enable label-free and portable detection of DNA mutations, offering clinical applications in cancer and genetic disorders.[231] Cas effectors, including Cas9, Cas12, Cas13, and Cas14, have been tailored into biosensor designs, each contributing distinct molecular properties.[98] For example, Cas13-based SHERLOCK and Cas12-based DETECTR systems exploit collateral cleavage for sensitive, programmable nucleic acid detection.[232] **Figure 7** compares DETECTR and SHERLOCK technologies, showcasing their distinct enzymatic activities and diagnostic applications. Beyond nucleic acids, CRISPR biosensors are being adapted to detect proteins and small molecules through aptamer fusion and hybrid REC elements.[233] Miniaturization strategies such as microfluidic integration, paper-based assays, and smartphone-compatible readouts are driving the development of field-deployable CRISPR diagnostics.[234] These biosensors are being engineered to detect pathogens such as SARS-CoV-2 and Zika virus, cancer-associated mutations such as *EGFR* and *KRAS*, and metabolic biomarkers for diseases including diabetes and cardiovascular disorders.[235,236] Despite their promise, CRISPR biosensors face limitations, including off-target effects, scalability constraints, and the need for regulatory validation.[237] Continued advancements in enzyme engineering, gRNA optimization, and multiplex signal design are enhancing the specificity, sensitivity, and clinical robustness of these platforms.[238] Notably, integrating CRISPR with aptamer REC, nanostructures, electrochemical sensors, and fluorescent or bioluminescent reporters has led to a new generation of multiplexed, low-cost biosensing solutions.[239]

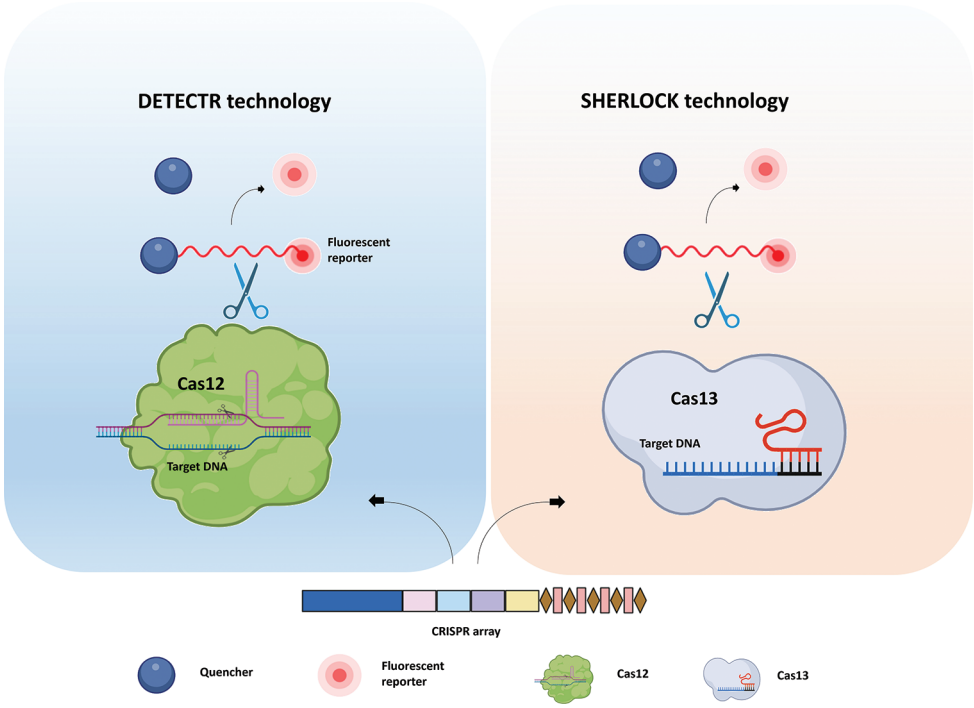

Figure 7. Comparison between DETECTR and SHERLOCK technologies. The left panel illustrates DETECR, which utilizes the Cas12 enzyme guided by a crRNA to bind to a specific target DNA sequence. Upon successful target recognition, Cas12 exhibits collateral cleavage activity on single-stranded DNA, leading to the degradation of a fluorescent reporter molecule and generation of a detectable signal. In contrast, the right panel depicts SHERLOCK, which is based on the Cas13 enzyme. Cas13 is similarly guided by crRNA to recognize a target RNA sequence. Upon binding, Cas13 activates its collateral RNA cleavage activity, resulting in the breakdown of a fluorescently labeled RNA reporter and the production of a detectable signal. Both technologies leverage the collateral cleavage activity of Cas enzymes to enable precise and sensitive nucleic acid detection. The bottom legend identifies key molecular components involved in both methods, including fluorescent reporters, target DNA, Cas enzymes (Cas12 for DETECTR and Cas13 for SHERLOCK), and guide RNA sequences. These CRISPR-based platforms have revolutionized molecular diagnostics by enabling rapid, precise, and portable detection of pathogens and genetic variations. Figure created by the authors.
Abbreviations: Cas: CRISPR-associated protein; CRISPR: Clustered regularly interspaced short palindromic repeats; crRNA: CRISPR RNA; DETECTR: DNA endonuclease targeted CRISPR trans reporter; SHERLOCK: Specific High-sensitivity Enzymatic Reporter unLOCKing.

This section provides a comprehensive overview of diverse CRISPR biosensor architectures, beginning with CRISPR-Cas12a (cpf1)-based electrochemical biosensors (E-CRISPR) and expanding through colorimetric, fluorescence-based, bioluminescent, aptamer-assisted, and nanotechnology-enabled platforms. These modular diagnostic systems exemplify how CRISPR effectors are redefining biosensing at the molecular level and enabling precision diagnostics in both laboratory and field settings. Several companies are actively developing these platforms. For example, Sherlock Biosciences has pioneered the SHERLOCK platform for rapid detection of infectious agents using Cas13 enzymes, while Mammoth Biosciences has developed the DETECTR system, leveraging Cas12 and Cas14 enzymes for portable, highly sensitive diagnostics. Companies such as CrisprBits, Caspr Biotech, and Scope Biosciences are also contributing to global CRISPR biosensing innovation, targeting both healthcare and environmental applications (**Table 6**).

5.1. E-CRISPR

E-CRISPR is an innovative biosensing platform that integrates the sensitivity and miniaturization potential of electrochemical transduction with the nucleic acid REC and cleavage capabilities of CRISPR to identify specific DNA or RNA sequences.[240] Unlike fluorescence- or colorimetric-based assays, which rely on optical signals, E-CRISPR detects biomolecular interactions by quantifying electrical changes—such as current, potential, or impedance—induced by CRISPR-mediated cleavage or binding events.[241] This strategy enables portable, low-cost, and label-free detection, making it particularly well-suited for point-of-care diagnostics in both clinical and field settings.[242] Mechanistically, an E-CRISPR platform typically incorporates a gold- or carbon-based working electrode modified with capture probes specific to the target nucleic acid.[243] Upon introduction of the CRISPR complex, most often Cas12a or Cas13a paired with a predesigned gRNA, the system recognizes the target sequence.[244] Furthermore, the binding induces activation of the NUC, triggering collateral or target-specific cleavage of nearby ssDNA reporter strands attached to the electrode surface.[245] This cleavage disrupts the electrochemical interface, leading to quantifiable signal shifts measured by differential pulse voltammetry, square wave voltammetry, or electrochemical impedance spectroscopy.[246,247] In one notable application, a Cas12a-based E-CRISPR biosensor was developed for the rapid detection of HPV-16, a high-risk oncogenic strain of HPV.[248] The sensor

achieved picomolar-level sensitivity in blood and cervical swab samples and delivered results within an hour, underscoring its potential for real-time, resource-limited diagnostics.[249] In another design, researchers engineered an amplification-free E-CRISPR system integrating DNA tetrahedron nanostructures and aptamer-conjugated electrodes, enabling signal amplification without enzymatic pre-processing.[250] The versatility of E-CRISPR has also been extended to detect small molecules and proteins by combining CRISPR REC with aptamer-based molecular switches.[251] These hybrid designs further expand the utility of electrochemical biosensors beyond nucleic acids, accommodating a broader range of diagnostic biomarkers. Importantly, electrochemical sensors can be miniaturized for integration into wearable or handheld devices, enhancing accessibility in remote or under-resourced regions. Despite their advantages, these platforms face challenges, including standardization of surface functionalization, potential nonspecific adsorption, and regulatory oversight regarding CRISPR usage.[252] However, ongoing innovations in nanostructured electrodes, microfluidic interfacing, and CRISPR enzyme engineering continue to improve signal fidelity and clinical translatability. As such, electrochemical CRISPR biosensors are emerging as powerful tools for rapid, accurate, and decentralized molecular diagnostics.

5.2. Colorimetry-CRISPR biosensors

Colorimetry-CRISPR biosensors are a class of diagnostic platforms that translate CRISPR-mediated nucleic acid REC into visible color changes, allowing rapid, equipment-free detection of specific genetic targets.[253] These systems leverage the cleavage activity of Cas12 or Cas13 effectors on ssDNA or RNA reporters labeled with chromogenic substrates, producing a colorimetric signal that can be seen with the naked eye.[254] This simplicity makes colorimetric CRISPR diagnostics especially well-suited for low-resource, point-of-care settings, where access to advanced fluorescence or electrochemical instrumentation is limited.[255] Mechanistically, colorimetric detection relies on collateral cleavage: when Cas12 or Cas13 is activated by binding to a target sequence, it nonspecifically cleaves nearby reporter molecules.[256] These reporters are often linked to gold nanoparticles (AuNPs), horseradish peroxidase, or G-quadruplex/hemin complexes, which generate a visible signal shift upon structural disruption.[257] For instance, in AuNP-based systems, cleaved DNA linkers result in nanoparticle dispersion or aggregation, causing a distinct color change from red to purple, or vice versa, depending on the assay design.[258] A well-established application of this technology is the detection of SARS-CoV-2 using Cas12a, where viral RNA is reverse transcribed and amplified using isothermal methods such as RPA.[259] The amplified product then activates Cas12a, which cleaves ssDNA-labeled reporters, producing a color change that correlates with viral presence.[260] Such assays have demonstrated comparable sensitivity to real-time PCR in field validation studies, with results visible within 30–60 min.[261] Beyond infectious diseases, colorimetric CRISPR platforms are also being explored for early cancer diagnostics.[262] For example, CRISPR-Cas13a-based colorimetric assays have been developed for miRNA detection in plasma samples, allowing identification of key biomarkers such as miR-21, often overexpressed in non-small cell lung carcinoma.[209] These platforms use catalytic hairpin assembly and G-quadruplex-based color generation, offering high sensitivity without the need for fluorescent probes.[263] The portability, low cost, and ease of interpretation offered by colorimetric CRISPR biosensors make them especially attractive for decentralized diagnostics, including community health programs, rural clinics, and emergency outbreak response.[264,265] However, challenges remain in terms of signal quantification, multiplexing capability, and the shelf-stability of reagents under varied environmental conditions.[252] As research progresses, improvements in nanozyme integration, stabilized chromogenic substrates, and multiplex color-code readouts are expected to broaden the clinical applicability of colorimetry-CRISPR biosensors. These developments position them as promising diagnostic tools in efforts to achieve universal, rapid, and affordable disease detection globally.

5.3. Fluorescence and bioluminescence-based biosensors

Fluorescence and bioluminescence-based CRISPR biosensors provide highly sensitive, real-time platforms for nucleic acid detection and molecular imaging.[266] These systems translate CRISPR-mediated target REC into light-based signals, using either externally excited fluorophores or internally generated bioluminescent reporters.[267] This capability allows researchers and clinicians to monitor dynamic biological events *in vitro* and *in vivo* with high precision, making these platforms valuable not only for molecular diagnostics but also for live-cell imaging and personalized therapy monitoring. Fluorescent CRISPR biosensors typically use Cas12 or Cas13 proteins coupled with reporter probes labeled with a fluorophore and quencher.[268] Upon activation by a target sequence, the Cas effector cleaves the reporter, separating the fluorophore from the quencher and producing a measurable fluorescent signal.[269] Furthermore, fluorogenic probes such as quantum dots, carbon dots, and fluorescence resonance energy transfer (FRET) pairs enable multiplexed detection and high signal-to-noise ratios.[270-272] Luminescence resonance energy transfer, an advanced variant of FRET, utilizes lanthanide donors for distance-based molecular measurements, offering improved photostability and reduced background interference, ideal for intracellular sensing applications.[273] To enhance sensitivity, these biosensors are often combined with isothermal amplification techniques, which enable nucleic acid amplification at a constant temperature without the need for thermal cycling.[274] Techniques such as RPA, LAMP, SDA, and NASBA are frequently integrated into CRISPR fluorescence workflows.[275-278] These combinations enable ultra-sensitive detection of genetic material even at attomolar levels. In addition, the SHERLOCK and DETECTR platforms are landmark examples of fluorescence-based CRISPR diagnostics.[279,280] SHERLOCK uses Cas13a with fluorescent RNA reporters to detect RNA viruses such as Zika and SARS-CoV-2, while DETECTR uses Cas12a with ssDNA-fluorescence-quencher probes for DNA-based pathogens and mutations, including HPV.[281,282] Notably, bioluminescence-based CRISPR biosensors differ from fluorescence-based systems in that they

do not require external excitation.[283] Instead, they employ luciferase-based systems that emit light through enzymatic oxidation reactions.[284] These platforms are gaining traction for *in vivo* imaging and cancer diagnostics, where real-time, non-invasive monitoring of tumor progression is essential. For instance, bioluminescent CRISPR reporters have been used to monitor tumor metastasis, immune cell infiltration, and therapeutic response in mouse models, providing dynamic insights into disease biology.[285] Furthermore, researchers have developed hybrid systems combining CRISPR-Cas9 with split luciferase complementation assays, enabling the detection of target sequences through reconstituted light emission.[286] These approaches have been applied in tracking gene-editing outcomes, validating gRNA activity, and imaging CRISPR dynamics within living tissues. Despite their advantages, fluorescence- and bioluminescence-based CRISPR biosensors face challenges in instrumentation requirements, signal stability, and background interference, particularly in complex biological matrices.[225] Nevertheless, continuous improvements in reporter chemistry, enzyme engineering, and signal amplification are addressing these limitations and paving the way for real-time diagnostics and therapy monitoring in clinical settings.

5.4. Aptamer-integrated CRISPR biosensor

Aptamers are short, single-stranded nucleic acids that adopt-specific three-dimensional conformations, enabling them to bind with high specificity and affinity to a diverse array of targets, including proteins, small molecules, metal ions, and even whole cells.[287] Integrating aptamers with CRISPR-Cas biosensing platforms significantly expands their functionality beyond nucleic acid detection, enabling CRISPR-mediated identification of a broader range of biological targets. Mechanistically, aptamer-CRISPR biosensors operate through a target-induced conformational change in the aptamer.[160] Upon recognizing its ligand (e.g., thrombin or ATP), the aptamer either releases or reveals a trigger DNA or RNA sequence that subsequently activates a CRISPR-Cas system.[288] The activated Cas effector then engages in collateral cleavage of reporter molecules, leading to a detectable signal such as fluorescence or an electrochemical readout.[238] One well-documented application involves the aptamer-Cas12a detection of thrombin, a critical biomarker in coagulation and cardiovascular diseases.[289] In this system, thrombin binding induces a structural switch in the aptamer, releasing a pre-hybridized DNA strand that activates Cas12a-mediated collateral cleavage.[290] This approach has demonstrated attomolar sensitivity in complex biological matrices, including plasma and serum, without the need for nucleic acid amplification.[291] Recent innovations have expanded this model to dual-target biosensors, such as those detecting both thrombin and ATP, by using orthogonal aptamers and corresponding Cas enzymes.[292] These multiplexed systems enable simultaneous monitoring of multiple biomarkers, enhancing diagnostic accuracy in complex diseases such as cancer and sepsis.[293,294] Furthermore, in infectious disease diagnostics, aptamer-Cas13a platforms have been developed to detect bacterial toxins such as Staphylococcal enterotoxin B.[295,296] Here, aptamers serve as the initial biorecognition elements, while the CRISPR component ensures signal amplification and transduction. These systems provide high specificity and reduce cross-reactivity, particularly important in pathogen-rich environments.[245] The major advantages of aptamer-CRISPR integration include their exceptional molecular REC specificity, which is comparable to that of antibodies but with greater chemical stability and ease of synthesis.[297] Aptamers can be custom-engineered to bind a virtually unlimited range of molecular targets, making the platform highly versatile. Furthermore, aptamer-CRISPR systems are compatible with various signal detection strategies, including colorimetric, fluorescent, electrochemical, and bioluminescent outputs, enabling their application across multiple diagnostic platforms and clinical settings.[245] Despite their advantages, challenges such as ensuring aptamer stability in physiological fluids, avoiding non-specific activation of Cas effectors, and scaling production for clinical deployment remain.[298] However, advances in Systematic Evolution of Ligands by Exponential Enrichment and aptamer engineering are addressing these limitations. As this synergy continues to evolve, it is likely to fuel breakthroughs in precision diagnostics, real-time monitoring, and even smart theranostic systems for personalized medicine.

5.5. Nanotechnology-enhanced CRISPR biosensors

Nanotechnology-CRISPR platforms integrate the molecular specificity of CRISPR-Cas systems with the unique physicochemical properties of nanomaterials to enhance diagnostic sensitivity, delivery efficiency, and therapeutic precision.[299] By leveraging nanocarriers such as AuNPs, liposomes, lipid nanoparticles, graphene oxide, and magnetic beads, researchers have significantly improved the stability, biocompatibility, and cellular targeting of CRISPR-based systems in both *in vitro* diagnostics and *in vivo* applications.[300-303] These nanomaterials protect CRISPR components, including Cas proteins and gRNAs, from NUC-mediated degradation while facilitating their cellular uptake, endosomal escape, and nuclear localization.[304] For example, an AuNP-based CRISPR delivery system has been developed to repair genetic mutations linked to muscular dystrophy, in which polyethylene glycol-coated nanoparticles deliver gRNA and Cas9 to facilitate cell repair.[305] The integration of magnetic nanoparticles with CRISPR has also enhanced the sensitivity and accuracy of biomolecular detection, allowing earlier disease detection and treatment.[306] In one platform, magnetic bead-Cas12a complexes were used for the isolation and detection of HPV DNA, improving both the signal-to-noise ratio and reproducibility of detection assays.[307] These systems also allow for pre-concentration of low-abundance biomarkers, enabling detection at attomolar levels without amplification. Beyond DNA/RNA detection, nanomaterial-CRISPR constructs have been applied in point-of-care immunodiagnostics, such as multiplexed lateral flow assays using AuNP-Cas conjugates, and even in sono-immunotherapy, where CRISPR-functionalized nanobubbles release their payload in response to ultrasound.[308-310] Furthermore, liposome-based CRISPR delivery vehicles are being optimized for non-invasive administration routes, including nasal and oral formulations,

which are crucial for decentralized healthcare models.[311] This combination of nanotechnology and CRISPR is not only enhancing therapeutic precision and minimizing off-target effects but also expanding the potential of biosensors in real-time, portable, and ultrasensitive diagnostics. As the field advances, nanomaterial-CRISPR hybrids will offer potential for enhancing diagnostic capabilities, increasing treatment precision, and supporting the development of personalized medicine.

5.6. Liquid biopsy biomarkers and CRISPR biosensing

Circulating biomarkers such as cell-free DNA (cfDNA), miRNAs, messenger RNAs, and exosomal RNA have become indispensable tools for non-invasive diagnostics.[312-314] CRISPR-based biosensors have demonstrated significant promise in detecting these biomarkers with high sensitivity and specificity, transforming liquid biopsies into powerful platforms for early disease detection, real-time monitoring, and prognosis.[302] One of the key strengths of CRISPR diagnostics is their adaptability to detect low-abundance nucleic acids in blood, plasma, saliva, and urine without the need for complex infrastructure.[45] Cas12a and Cas13a effectors are particularly suited for this application. Cas12a targets DNA-based markers such as cfDNA fragments from tumors or pathogens, while Cas13a excels at sensing small non-coding RNAs like miRNAs.[315] In oncology, cfDNA released by tumor cells harbors signature mutations that can be detected using CRISPR-Cas12a systems. These systems are capable of identifying mutations in the *EGFR*, *KRAS*, or *TP53* genes, allowing for earlier cancer detection or monitoring of treatment response.[316] Cas14-based platforms, with their ability to recognize ssDNA at high resolution, are also emerging as powerful tools for SNV detection in cfDNA.[152] miRNAs, such as miR-21, miR-155, and miR-20a, are well-established circulating markers in cancers and cardiovascular diseases.[207,317,318] CRISPR-Cas13a biosensors have demonstrated efficient detection of these miRNAs, benefiting from the enzyme's RNA-guided collateral cleavage activity that amplifies signal output.[207] These sensors can even be integrated into lateral flow strips or microfluidic chips for rapid bedside analysis. Exosomal RNAs, including messenger RNAs and miRNAs enclosed within extracellular vesicles, are gaining REC for their diagnostic value in neurological diseases and aggressive tumors.[342] Cas13-based biosensors, when combined with vesicle isolation techniques, have shown the ability to detect exosomal RNA cargo with precision, opening avenues for early-stage diagnostics in diseases such as glioblastoma or pancreatic cancer.[318] Furthermore, CRISPR biosensors are being developed to simultaneously detect multiple biomarkers from a single sample, enabling multiplex diagnostics. For example, triple detection of miR-21, miR-375, and miR-141 can differentiate between subtypes of prostate cancer,[319] while combined analysis of miR-122 and hepatitis B virus RNA provides insights into liver disease progression.[320] The ability to harness circulating biomarkers using programmable CRISPR tools brings the diagnostic process closer to real-time, patient-friendly testing. It also paves the way for predictive medicine, where disease risk can be assessed long before symptoms appear.

6. Clinical and translational applications

The integration of CRISPR-Cas systems with biosensing technologies has significantly advanced diagnostics, disease monitoring, and molecular medicine. The high specificity of CRISPR effectors in nucleic acid REC, coupled with their programmability and collateral cleavage capabilities, has established them as powerful platforms for next-generation biosensors.[42] These tools have shown exceptional sensitivity and specificity in detecting nucleic acid targets, biomarkers, and pathogens across various clinical and environmental settings. As illustrated in **Figure 8**, the complete translational pipeline, from molecular design to functional implementation, has matured to support real-world integration in both diagnostic and therapeutic frameworks. Furthermore, since its groundbreaking use in genome editing was demonstrated in 2013, CRISPR/Cas9 has been adapted for diagnostic platforms, enabling the development of portable, cost-effective, and fast detection systems.[242] Researchers have integrated Cas enzymes into microfluidic models, paper-based assays, and smartphone-compatible devices, enabling real-time analysis of biological signals even in resource-limited settings. These platforms are capable of identifying targets ranging from emerging viral pathogens to single-nucleotide mutations in oncogenes and rare genetic disorders.[221] As research continues to optimize gRNA design, enzyme engineering, and delivery systems, these platforms are becoming more robust and adaptable to complex biological samples.[321] In the following sections, we explore the expanding translational landscape of CRISPR diagnostics, covering POC innovations, AI-assisted platforms, emerging theranostic strategies, and broader applications in biotechnology, synthetic biology, and sustainable healthcare.

Recent advancements have explored the integration of CRISPR platforms with other functional nucleic acids, including aptamers, ribozymes, small interfering RNA (siRNAs), and peptide nucleic acids (PNAs), to enhance both diagnostic and therapeutic potential.[322] CRISPR-aptamer hybrids have been employed in biosensing platforms to improve sensitivity and enable rapid, point-of-care detection of disease biomarkers.[45] Similarly, ribozyme-controlled CRISPR switches provide tunable NUC activation, siRNA co-delivery strategies enhance gene-silencing efficiency, and PNA-assisted CRISPR approaches improve target REC and editing precision.[323-325] These hybrid systems represent promising next-generation CRISPR-based theranostic tools with improved specificity and clinical applicability.

6.1. Point-of-care CRISPR-Cas technology application

The development of point-of-care kits using CRISPR-Cas systems has been driven by the need for fast, accurate, affordable, and specific diagnostic tools that can be used outside traditional laboratory settings, especially in resource-limited areas.[238] Standard POCT, such as rapid strep tests, glucose monitoring, and pregnancy tests, provide instant results, enabling medical professionals to determine the appropriate treatment quickly.[326] For instance, a rapid strep test can instantly confirm the presence of strep throat, allowing the physician to prescribe the necessary antibiotics.[327] Similarly,

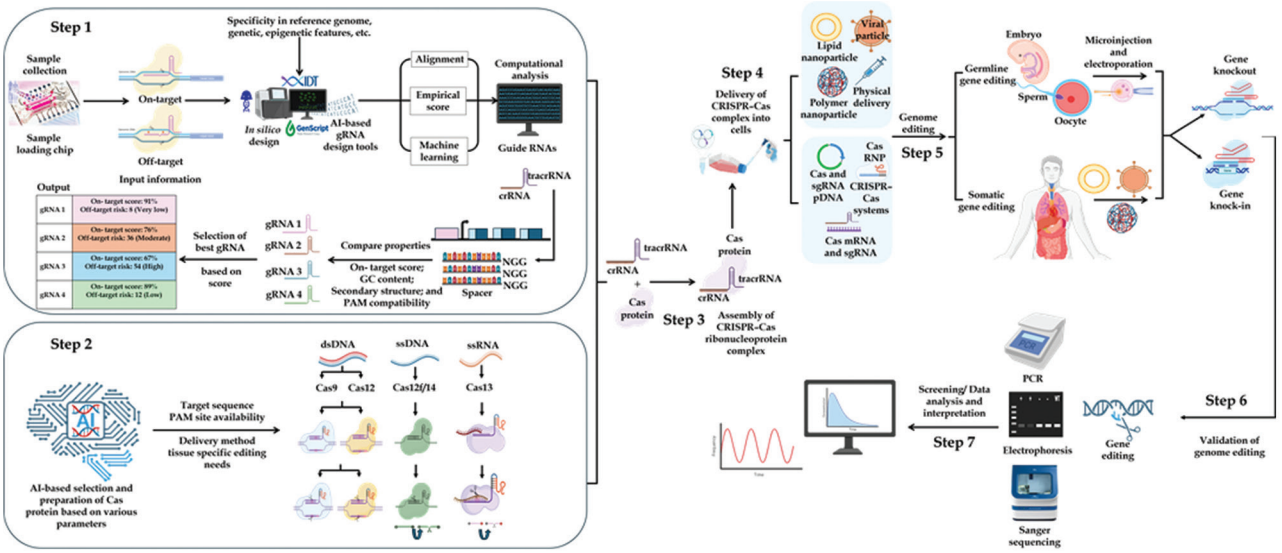

Figure 8. AI-assisted CRISPR–Cas genome editing workflow from sample to validation. This figure presents an AI-integrated CRISPR–Cas workflow, beginning with Step 1, where biological samples are collected, nucleic acids are extracted, and target regions are analyzed using AI-based *in silico* gRNA design tools. Machine learning models evaluate gRNAs based on on-target efficiency, off-target risk, and structural features to identify the most suitable candidates. In Step 2, AI assists in selecting the appropriate Cas variant based on PAM compatibility, target type, and delivery constraints. In Step 3, the selected gRNA and Cas protein are assembled into a CRISPR–Cas RNP complex. In Step 4, the complex is delivered into cells via physical or chemical methods. Step 5 involves genome editing in either germline or somatic cells, resulting in gene knockout or knock-in via NHEJ or HDR pathways. In Step 6, editing outcomes are validated using PCR, electrophoresis, and sequencing techniques. Finally, in Step 7, AI assists in the analysis and interpretation of results, providing feedback to enhance the precision of future editing cycles. Figure created by the authors.

Abbreviations: AI: Artificial intelligence; Cas: CRISPR-associated protein; CRISPR: Clustered regularly interspaced short palindromic repeats; crRNA: CRISPR RNA; dsDNA: Double-stranded DNA; gRNA: Guide RNA; HDR: Homology-directed repair; mRNA: Messenger RNA; NHEJ: Non-homologous end joining; PAM: Protospacer adjacent motif; PCR: Polymerase chain reaction; pDNA: Plasmid DNA; RNP: Ribonucleoprotein; sgRNA: Single guide RNA; ssDNA: Single-stranded DNA;; ssRNA: Single-stranded RNA; tracrRNA: Trans-activating CRISPR RNA.

glucose monitoring aids diabetics in managing their blood sugar levels in real time.[328] Key technological advancements, including SHERLOCK in 2017, DETECTR in 2018, and the incorporation of paper-based platforms and lateral flow assays in 2019, have significantly contributed to the development of CRISPR-Cas POC kits as mentioned in **Table 7**.[329] Using lateral flow assays, the first CRISPR-Cas kits for COVID-19 were based on SHERLOCK (Cas13-based RNA detection) and DETECTR (Cas12-based RNA-to-DNA detection), which provided a rapid (30–60 min) and low-cost method to detect SARS-CoV-2.[330,331]

Diagnostic methods with a lower limit of detection can detect diseases earlier, when the pathogen load is typically low, allowing more accurate and timely medical interventions.[337] The ability of next-generation CRISPR diagnostics to detect multiple targets simultaneously has greatly enhanced their versatility. Advances in smartphone-based devices and handheld fluorescence readers have also increased the portability of these technologies. One notable example is the Streamlined Highlighting of Infections to Navigate Epidemics platform, which combines SHERLOCK and Heating Unextracted Diagnostic Samples to Obliterate NUC technologies to enable real-time viral RNA detection on portable devices.[280] Building on this, the Minimally Instrumented SHERLOCK platform developed at MIT enables variant-level detection of SARS-CoV-2 and integrates seamlessly with a smartphone for real-time readouts, offering rapid, multiplexed, and cost-effective diagnostics in field settings.[338] The advantages of POCT include reduced wait times for patients, especially in emergencies, and the elimination of multiple appointments, which in turn reduces overall healthcare costs.[339] Moreover, POCT enables quicker adjustments to treatment plans, enhancing patient satisfaction and outcomes.

The integration of CRISPR detection with microfluidic platforms has further improved the automation of sample processing and increased test throughput.[340] Researchers are also working to optimize these kits for operation at ambient temperatures and to reduce sample preparation steps, such as extraction-free processing, thereby improving on-site usability.[341-343] These enhancements make CRISPR-Cas point-of-care kits more accessible, effective, and affordable, especially in low-resource environments. CRISPR-based liquid biopsy tests for genetic disorders and cancer biomarkers are becoming feasible, offering high-sensitivity electrochemical CRISPR biosensors that deliver rapid results without requiring expensive sequencing technologies.[344] Recent innovations, such as the INSPECTR platform developed by Mammoth Biosciences, demonstrate how synthetic DNA circuits and lyophilized reagents can enable instrument-free, paper-based POC diagnostics.[345] In addition, wearable biosensing devices, such as graphene-integrated microneedle patches, now allow real-time monitoring of circulating biomarkers, including

Table 7. CRISPR-Cas systems in point-of-care (POC) diagnostics: Features, applications, and challenges

Feature/ system	Description	Cas enzyme (s) used	Readout method	Key advantages for POC	Applications	Limitations/challenges for POC	References
SHERLOCK	(Specific High-sensitivity Enzymatic Reporter unLOCKing) - A foundational CRISPR-Cas POC platform	Cas13 (primarily Cas13a)	Fluorescence (plate reader, handheld fluorimeter, smartphone adapter); LFA	Ultrasensitive (attomolar), highly specific (single-base mismatch), direct RNA detection (no RT needed), multiplexing	RNA viruses (e.g., SARS-CoV-2, Zika, dengue, Lassa, Nipah, HIV), bacterial RNA, and cancer biomarkers (miRNAs)	Requires pre-amplification for ultra-low targets (adds steps). Fluorescence readouts require a device, though increasingly portable. LFA may have lower sensitivity	332
DETECTR	(DNA Endonuclease-Targeted CRISPR Trans Reporter) - Another foundational CRISPR-Cas POC platform	Cas12a (e.g., LbCas12a, AsCas12a)	Fluorescence (plate reader, handheld fluorimeter, smartphone adapter); LFA	High sensitivity (attomolar), highly specific (SNP detection), direct DNA detection, robust, and rapid	DNA virus detection (e.g., HPV, African swine fever virus), bacterial pathogens (e.g., *Mycobacterium tuberculosis*), and genetic mutations	Requires pre-amplification for low targets. Fluorescence readouts require a device. LFA may have lower sensitivity. Primarily targets dsDNA	232
HOLMES	(One-Hour Low-cost Multipurpose highly Efficient System) - Optimized versions of DETECTR for enhanced performance	Cas12a, Cas12b	Fluorescence, LFA	Improved speed and ease of use, reduced contamination risk with one-pot design, high sensitivity, and specificity	Pathogen detection, including DNA viruses, bacteria, and multiplexed assays	Still in development for widespread adoption. Optimization required for each new target	333
CARMEN	(Combinatorial Arrayed Reactions for Multiplexed Evaluation of Nucleic Acids) - A highly multiplexed platform	Multiple Cas enzymes (e.g., Cas13, Cas12)	Fluorescence imaging (specialized reader)	Extremely high multiplexing capability, comprehensive sample analysis	Pathogen surveillance, pan-viral screening, and complex diagnostic panels	Requires specialized array readers and more complex data analysis. Not a simple "strip" POC test	43
CRISPR-Chip/ E-CRISPR	Integrates Cas activity with electrochemical sensing for sensitive and rapid detection	dCas9 (binding), Cas12a, Cas13a (cleavage)	Electrochemical (potentiostat, custom chip readers)	High sensitivity, quantitative potential, rapid readout, potential for miniaturization and automation, direct electrical signal (no optics needed)	Pathogens, cancer biomarkers, and environmental contaminants.	Requires microfabrication for chip development. Still needs further validation in complex real-world samples	334
Cas14-based POC	Utilizes the miniature Cas14 enzyme for direct ssDNA detection	Cas14a	Fluorescence, LFA, colorimetric	Very small size (facilitates miniaturization), PAM-independent for ssDNA targeting (flexible design), high specificity (SNP detection)	ssDNA virus detection (e.g., parvoviruses), genotyping, and specific mutation detection	Requires conversion of dsDNA to ssDNA for many targets. Not as widely studied or commercialized compared to Cas12/13	268
CRISPR-Dx coupled with LFA	General category of CRISPR-Cas systems integrated into LFA formats	Cas12a, Cas13a	Visual (colorimetric band on a strip)	Extremely simple, low-cost, equipment-free, rapid (minutes), suitable for field use and resource-limited settings	Infectious diseases (e.g., COVID-19, influenza, tuberculosis, malaria), agricultural diagnostics, and food safety	Lower sensitivity compared to fluorescence-based methods, typically qualitative or semi-quantitative. Risk of false positives if not handled carefully (e.g., contamination)	335
CRISPR-Dx coupled with isothermal amplification	All CRISPR-Cas POC systems often rely on a pre-amplification step to boost sensitivity	Cas9, Cas12, Cas13, Cas14	Fluorescence, LFA, colorimetric, electrochemical	Eliminates the need for thermal cyclers (like PCR), enabling truly portable and battery-operated devices. Achieves extremely high sensitivity	Risk of contamination if not designed as a "one-pot" system	Essential for ultrasensitive detection in most real-world scenarios	336

Abbreviations: AsCas12a: *Acidaminococcus* sp. Cas12a; CRISPR-Cas: Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein; CRISPR-Dx: CRISPR-based diagnostics; dCas9: Catalytically inactivated Cas9; dsDNA: Double-stranded DNA; E-CRISPR: CRISPR-Cas12a (cpf1)-based electrochemical biosensor; HPV: Human papillomavirus; LbCas12a: *Lachnospiraceae* bacterium Cas12a; LFA: Lateral flow assay; miRNA: MicroRNA; PAM: Protospacer adjacent motif; PCR: Polymerase chain reaction; RT: Reverse transcription; SNP: Single-nucleotide polymorphism; ssDNA: Single-stranded DNA.

cfDNA. For instance, a CRISPR-Cas9 microneedle patch integrating graphene biointerfaces enabled *in vivo* monitoring of cfDNA in interstitial fluid with femtomolar sensitivity over 10 days in mice.[346]

Future updates include the discovery of smaller CRISPR proteins like Cas14, which targets ssDNA, to facilitate the development of miniaturized diagnostic platforms.[226] Digital platforms, including cloud data sharing, AI-driven analysis, and smartphone-based detection, are being integrated into CRISPR diagnostics to enable point-of-care kits to deliver real-time data analysis and support epidemiological tracking.[347] The integration of CRISPR-based biosensors into wearable devices allows real-time monitoring of disease biomarkers, opening new avenues for personalized medicine. With high accuracy and efficiency, CRISPR-Cas could transform diagnostic medicine and streamline treatment decisions.[348] Ongoing research and investment are essential to fully harness their potential in POCT applications.

6.2. AI-augmented CRISPR diagnostics

While CRISPR-based genetic medicine has shown great promise, its clinical use is still limited by challenges such as off-target effects, delivery inefficiencies, limited control over editing outcomes, and variability in patient responses. These challenges emphasize the need for smarter, adaptive systems that can improve both the accuracy and safety of genome editing. Building on the foundation of CRISPR-based genome editing, the integration of AI is now accelerating the development of precision and personalized medicine. The combination of AI and CRISPR-Cas biosensing is ushering in a new era of diagnostics that are more intelligent, faster, and more predictive.[45] While CRISPR provides the molecular precision required to detect specific nucleic acid targets, AI enhances the interpretation, accuracy, and adaptability of these diagnostic platforms, especially in high-throughput or point-of-care settings. One of the earliest and most important applications of AI in CRISPR diagnostics is in gRNA design. Beyond gRNA optimization, AI plays a crucial role throughout the entire CRISPR diagnostic workflow, from image interpretation in biosensors to the prediction of editing outcomes.[349]

Deep learning models such as convolutional neural networks (CNNs) and recurrent neural networks are employed to interpret complex fluorescence or electrochemical patterns generated by CRISPR-based biosensors.[350,351] For instance, CNN architectures trained on labeled fluorescence images from SHERLOCK assays enable real-time signal interpretation and noise discrimination in smartphone-integrated diagnostic platforms.[352] In addition, sequence-based prediction models, such as DeepCRISPR, utilize high-throughput datasets such as genome-wide, unbiased identification of DSBs enabled by sequencing (GUIDE-seq) to train deep neural networks for accurate on- and off-target cleavage prediction.[67] These models incorporate parameters such as chromatin accessibility, PAM proximity, and nucleotide content, resulting in improved precision over traditional heuristics. Such integrative methods enable context-aware gRNA optimization, enhancing editing efficiency across various genomic contexts. For instance, models such as DeepSpCas9 and DeePHF include chromatin state and thermodynamic stability to predict cell-type-specific gRNA performance, addressing differences between primary tissues and immortalized cell lines.[353,354]

Moreover, AI-assisted models are being adapted for CRISPRa/i applications, where predicting binding affinity and transcriptional impact is more complex than assessing cleavage efficiency. For example, in 2022, Xu *et al.*[14] developed MiCaR, a microfluidic CRISPR-Cas12a platform capable of multiplex detection of up to 30 viral targets (e.g., nine HPV subtypes and eight respiratory viruses) within 40 min, achieving ~0.26 attomole sensitivity with 97.8% accuracy. These advances underscore how AI integration enhances signal deconvolution and multiplex accuracy, complementing CRISPR's detection capabilities for field-ready diagnostics. These tools are particularly valuable for the development of multiplexed diagnostics and therapeutic strategies, where off-target effects, guide competition, and signal interference must be minimized through precision modeling.[355] **Table 8** summarizes the integration of AI across various CRISPR-Cas systems in gene editing, diagnostics, and therapeutic workflows. As these AI platforms continue to mature, their integration into closed-loop diagnostic-therapeutic systems is

Table 8. CRISPR-Cas systems and their applications in medical genetics, enhanced by artificial intelligence

CRISPR-Cas system type	Key features	Applications in medical genetics	How AI enhances applications	References
CRISPR-Cas9	Most widely studied and utilized; uses a single guide RNA (sgRNA) and the Cas9 enzyme	Gene editing, therapies for inherited diseases and cancer, disease modeling	Predicts optimal sgRNAs, classifies variants, and aids in therapy design	356
CRISPR-Cas12 (e.g., Cas12a/Cpf1)	Smaller than Cas9; cleaves DNA with staggered ends; exhibits collateral ssDNA cleavage activity	Diagnostics for pathogens, cancer, and mutations; supports multiplex detection	Analyzes signals, detects diagnostic patterns, and enables point-of-care applications	357
CRISPR-Cas13 (e.g., Cas13a)	Targets and cleaves RNA; exhibits collateral RNA cleavage activity	RNA virus detection, biomarker analysis, RNA editing	Optimizes RNA target selection and tracks expression changes	358
Base editing (e.g., ABEs, CBEs)	Employs a Cas protein (often dead Cas9) fused with a deaminase enzyme to change a single nucleotide	Precise correction of point mutations with minimal off-targets	Predicts off-targets and optimizes base editor design	359
Prime editing	Uses a reverse transcriptase fused to a Cas protein to directly insert or modify DNA sequences	Corrects a broad range of mutations with high accuracy; suitable for complex disorders	Assists in designing prime editors, improves prediction of editing outcomes, and enhances accuracy for complex genetic disorders	360

Abbreviations: ABEs: Adenine base editors; CBEs: Cytosine base editors; CRISPR-Cas: Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein.

expected to reduce the experimental burden, streamline clinical validation, and support adaptive CRISPR workflows in real-world patient settings. **Figure 8** offers a step-by-step overview of the AI-assisted CRISPR-Cas editing workflow, illustrating how AI models predict and rank gRNA candidates based on specificity, off-target risk, and structural suitability. This is followed by the formation, delivery, and editing outcomes of the RNP complex in both germline and somatic contexts. As shown in **Figure 8**, advanced AI-driven platforms extend beyond initial gRNA screening by incorporating large-scale genomic datasets to enhance gRNA performance predictions, taking into account thermodynamic stability, structural compatibility, chromatin accessibility, and sequence context. These improved designs enhance both sensitivity and on-target accuracy while reducing unintended effects across diverse genomic landscapes.[22] To achieve this, ML models are trained using labeled datasets such as GUIDE-seq and circularization for *in vitro* reporting of cleavage effects by sequencing (CIRCLE-seq), incorporating sequence motifs, secondary structures, and thermodynamic profiles to predict gRNA efficiency and specificity. These models consistently outperform traditional rule-based algorithms and enable the generation of highly optimized guides for both research and clinical applications.[354]

AI is also playing a crucial role in signal interpretation and noise reduction in CRISPR-based biosensors. For fluorescence-based and lateral flow platforms, CNNs and computer vision tools can differentiate true positive signals from background noise, thereby enhancing diagnostic confidence.[361] This is particularly useful for smartphone-integrated biosensors, where variations in lighting and environmental conditions can impact signal accuracy. In multiplexed biosensing setups, where multiple biomarkers are detected simultaneously using a single CRISPR platform, AI is employed to deconvolute complex signal patterns and correlate them with disease states. Moreover, AI is facilitating real-time disease forecasting and adaptive testing strategies. By integrating CRISPR diagnostic data with patient history, wearable sensor data, or environmental exposure metrics, AI models can predict disease onset, recommend follow-up tests, or dynamically tailor biomarker panels.[362] This real-time feedback loop is particularly valuable in managing epidemics or monitoring chronic diseases. Another promising frontier is the application of AI to design synthetic circuits in CRISPR-Dx. These bio-computational systems can execute logical functions (AND, OR, NOT) at the molecular level, enabling CRISPR biosensors to make autonomous decisions, such as releasing a therapeutic payload only when multiple disease markers are detected.[363,364]

These advances, however, raise important ethical concerns. The use of AI to interpret molecular diagnostics, particularly those involving patient-specific genomic data, necessitates robust safeguards to ensure privacy, data security, and informed consent.[365] Furthermore, the explainability of AI models becomes critical in a clinical context; patients and physicians must understand how diagnostic decisions are made, especially when these inform irreversible gene-editing actions.[366] Regulatory bodies increasingly recommend the incorporation of transparent AI pipelines and human-in-the-loop designs to mitigate algorithmic bias and ensure accountable decision-making in CRISPR-Dx.[367] Overall, AI not only boosts the diagnostic capabilities of CRISPR systems but also makes them more scalable, personalized, and adaptable to emerging clinical needs. The integration of AI across diagnostic, therapeutic, and prognostic domains has progressed to clinical trial stages, encompassing a broad spectrum of diseases and technologies. The future of CRISPR diagnostics lies at the intersection of molecular biology and computational intelligence. Recent breakthroughs have resulted in the development of innovative CRISPR-based biosensing platforms, ranging from wearable and implantable devices to AI-enabled diagnostics, as illustrated in **Figure 9**.

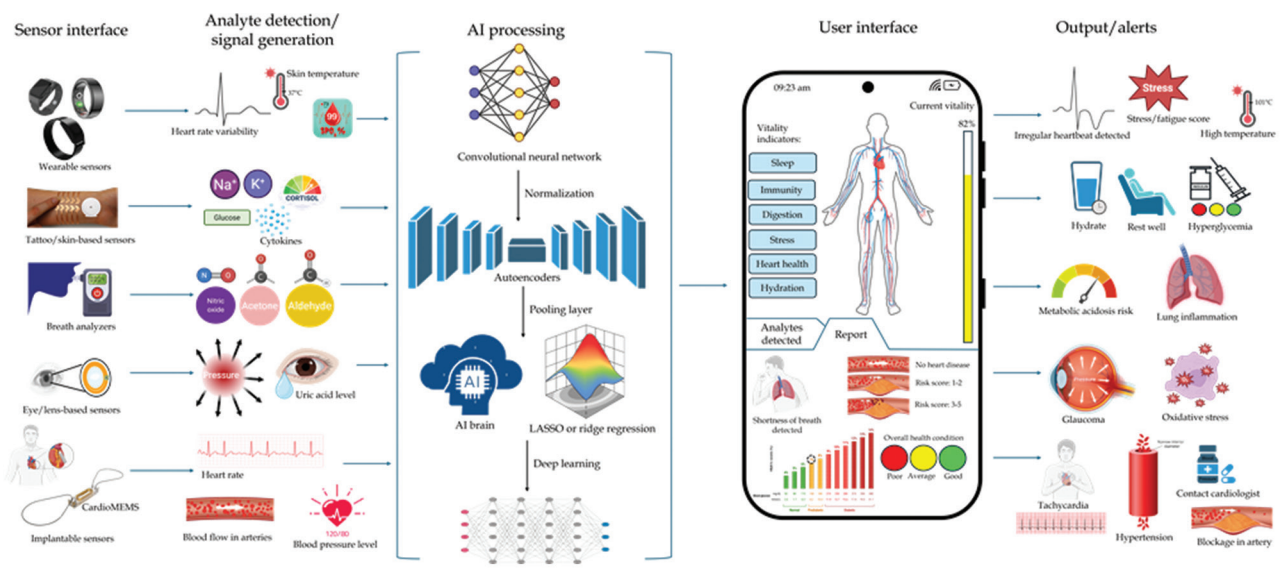

Figure 9. Next-generation biosensors. The figure depicts various innovative wearable, implantable, and biochemical biosensors enabling continuous, non-invasive monitoring of vital health parameters. Figure created by the authors.
Abbreviations: AI: Artificial intelligence; LASSO: Least Absolute Shrinkage and Selection Operator; K^+: Potassium ion; Na^+: Sodium ion.

6.3. Theranostic applications - The CRISPR loop

The fusion of diagnostics and therapeutics, known as theranostics, represents one of the most innovative and disruptive applications of CRISPR-Cas systems. This "CRISPR loop" closes the cycle between disease detection and personalized treatment using a single platform to identify a molecular signature and act on it, marking a shift toward precision and autonomous medicine.[368] In this approach, a CRISPR-based theranostic framework typically comprises three interconnected functional layers: a biosensor module (e.g., Cas12 or Cas13) that detects disease-specific biomarkers; a decision layer, often augmented by AI for signal interpretation and response modeling; and an effector layer that executes therapeutic interventions through gene editing or transcriptional regulation.[369-370]

Mechanistically, the loop begins with CRISPR-based biosensors identifying molecular anomalies—genetic, epigenetic, or transcriptomic—in patient samples. Once a target is validated, the system either directly activates a therapeutic CRISPR response or sequentially initiates downstream interventions using programmable Cas effectors, executing an autonomous sensing-response cycle, as exemplified by platforms like lnCasApt.[363] This closed-loop design enables real-time diagnostics and on-demand therapeutic delivery, pushing the boundaries of personalized medicine. The interplay between CRISPR and AI across each stage of the diagnostic workflow is illustrated in **Table 9**, which outlines how this innovation loop transforms precision diagnostics through continuous feedback, optimization, and automation.

In sickle cell disease, CRISPR-Cas9 has already been used therapeutically to edit the *BCL11A* enhancer and restore fetal hemoglobin expression. In a theranostic scenario, a CRISPR biosensor could first detect elevated levels of sickling-related biomarkers (e.g., circulating miRNAs or fetal hemoglobin transcript levels) and, upon validation, trigger Cas9-based genome editing.[417] In cancer theranostics, CRISPR-based biosensors can detect circulating tumor DNA mutations, such as those in *TP53*, *KRAS*, or *EGFR*, in blood.[375-377] Once a mutation is verified, CRISPR effectors, loaded into nanoparticles, viral vectors, or lipid carriers, can be delivered to tumor sites for gene knockout or repair.[378] Several preclinical models using Cas9 and Cas12a for such "edit-on-detect" protocols have demonstrated promising results. CRISPR-dCas systems fused to transcriptional regulators (CRISPRa/CRISPRi) are also being investigated for therapeutic gene modulation following the detection of abnormal gene expression.[379] For example, Lee *et al.*[380] successfully used dCas9-VPR delivered through peptide-imprinted nanoparticles to induce insulin production in glucose-stimulated HEK293T cells, highlighting the potential of this approach for diabetes management.

To improve specificity and prevent premature activation, researchers are developing logic-gated CRISPR systems that activate only when multiple disease-specific inputs are present, such as a combination of RNA biomarkers and protein markers.[381] This adds a safety buffer, ensuring that editing or silencing is triggered only under pathophysiologically relevant conditions. Some platforms, like CRISPR recorders, are being explored to log cellular events over time, providing a molecular timeline that could enhance personalized treatment response strategies.[382] Theranostic CRISPR loops also enable longitudinal health monitoring, where repeated sampling and molecular feedback allow the system to modify therapeutic

Table 9. CRISPR-Cas artificial intelligence (AI) in diagnostics: The innovation loop

Stage in the loop	CRISPR-Cas role	AI's role	Impact on medical genetics diagnostics	References
1. Target identification and discovery	CRISPR-Cas systems (e.g., Cas9, Cas12, Cas13) detect disease-related DNA/RNA sequences	Identifies key biomarkers and CRISPR targets from omics data	Accelerates the discovery of novel diagnostic targets for inherited diseases, infectious agents, and cancer, making diagnostics more comprehensive and precise	371-373
2. Assay design and optimization	Selects appropriate Cas types and designs guide RNAs for specific targets	Predicts optimal gRNAs and assay conditions to minimize off-targets	Reduces experimental trial-and-error, accelerating the development of highly accurate and reliable diagnostic tests. Improves the robustness and performance of CRISPR-based assays	
3. Diagnostic platform development	Integrates CRISPR into formats such as strips, chips, or portable devices	Designs POC-friendly, automated, and efficient platforms	Enables the development of rapid, portable, and cost-effective diagnostic tools suitable for diverse settings, from clinics to remote areas. Reduces reliance on centralized laboratories	
4. Data acquisition and processing	Generates raw detection signals (e.g., fluorescence, sequencing data)	Processes and extracts meaningful signals from assay outputs	Ensures high-quality data input for accurate diagnosis, even with complex or low-concentration samples. Speeds up data interpretation and minimizes human error	
5. Interpretation and reporting	Indicates the presence of genetic markers via cleavage or activation events	Generates specific, detectable readouts (e.g., fluorescence or colorimetric signals) that reflect underlying genetic variations	Delivers faster, more accurate, and nuanced diagnostic reports. Moves beyond simple binary outcomes (positive/negative) to provide deeper insights into patient health and disease prognosis. Facilitates personalized medicine	
6. Feedback and refinement	Uses real-world performance data to improve assay design	Learns from outcomes to refine target selection and optimize assay protocols	Drives continuous improvements in diagnostic accuracy, sensitivity, and efficiency. Supports adaptive learning, making the diagnostic systems more robust and responsive to new genetic variants and disease challenges	

Abbreviations: CRISPR-Cas: Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein; gRNAs: Guide RNAs; POC: Point-of-care.

strategies adaptively. This holds promise for diseases with dynamic molecular signatures, such as cancer, autoimmune disorders, and neurodegenerative diseases.[368] Clinically, theranostic approaches have already begun transforming disease management pathways. For example, in prostate cancer, prostate-specific membrane antigen (PSMA)-based radiotheranostics enable simultaneous diagnosis using ^{68}Ga-labeled tracers and therapy with ^{177}Lu-PSMA.[383]

In infectious diseases, CRISPR-Cas diagnostic platforms such as SHERLOCK have been coupled with programmable drug delivery systems to simultaneously detect and suppress viral infections.[384] In glioblastoma, magnetic resonance imaging-visible nanoparticles loaded with redox-activated therapeutic agents have demonstrated real-time imaging and localized treatment in preclinical models, exemplifying the potential of fully integrated diagnostic- therapeutic systems.[385] From a regulatory perspective, the dual-function nature of CRISPR-based theranostic platforms introduces unique challenges. Unlike traditional diagnostics or therapies, these systems operate as integrated, closed-loop architectures, often utilizing AI algorithms that interpret biosensor data and autonomously direct genetic interventions. Therefore, they must simultaneously comply with regulatory standards for *in vitro* diagnostics, such as the Clinical Laboratory Improvement Amendments.[386] Existing regulatory pathways are being challenged by the novelty of AI-guided, dynamically adapting gene-editing systems, prompting the development of new paradigms that consider software as a medical device, algorithm versioning, and continuous learning models.[387] Regulatory convergence on these hybrid platforms will be vital for their safe clinical deployment. Overall, these advances highlight the transformative potential of AI-integrated CRISPR theranostics in delivering adaptable, autonomous, and highly personalized healthcare solutions.

6.4. Beyond medicine

CRISPR-Cas technology, initially recognized for its transformative impact on genetic diagnostics and therapeutics, has also emerged as a powerful tool in non-medical sectors such as industrial biotechnology, synthetic biology, agriculture, and environmental management.[388] In healthcare, CRISPR has been deployed in clinical trials for treating monogenic diseases such as sickle cell anemia and β-thalassemia, where it facilitates the correction of disease-causing mutations by targeting the *BCL11A* enhancer to restore fetal hemoglobin expression.[389,390] The platform is also being used to develop next-generation immunotherapies, including CRISPR-edited chimeric antigen receptor T-cell (CAR-T) cells for improved cancer targeting, editing of oncogenes for tumor suppression, and immune system modulation for chronic infections.[391]

In agriculture, CRISPR is utilized to boost crop yields, improve disease resistance, and develop plants that can withstand harsh environmental conditions.[392] For instance, CRISPR has been applied to generate disease-resistant rice varieties, drought-tolerant maize, and non-browning mushrooms, offering more resilient and sustainable food systems. Livestock breeding programs are also adopting CRISPR to accelerate the development of disease-resistant or high-productivity animal strains, thereby improving food security and economic efficiency.[393] CRISPR-Cas systems are instrumental in industrial biotechnology, enabling the production of biofuels, bioplastics, and high-value biopharmaceuticals such as insulin, vaccines, and antibiotics in engineered microbial hosts with enhanced yields and reduced production costs.[394,395] Synthetic biology applications utilize CRISPR for genome-scale engineering, metabolic pathway optimization, and microbial strain development. In basic and translational research, CRISPR enables high-throughput functional genomics through knockout and activation screens, accelerating the identification of genes involved in drug response, tumor progression, and disease resistance.[396-399] Furthermore, epigenome editing with catalytically deactivated Cas proteins (dCas9/dCas12), fused to transcriptional regulators, allows for precise gene expression modulation without altering the underlying DNA sequence—a promising avenue for studying complex disorders such as autism, Alzheimer's disease, and depression.[400,401] CRISPR's potential also extends to environmental conservation and sustainability. Gene drives based on CRISPR-Cas9 are being designed to control invasive species, suppress disease-transmitting mosquito populations, and preserve biodiversity.[402] In microbial ecology, CRISPR tools help monitor and engineer microbiomes for soil remediation, wastewater treatment, and greenhouse gas mitigation.[403-404] Overall, the expansion of CRISPR-Cas technology into agriculture, industrial biotech, and environmental applications showcases its versatility and transformative potential across multiple sectors, offering robust solutions for global challenges ranging from food security to climate change.

7. Limitations and translational challenges

Despite rapid advances in CRISPR-based biosensing and therapeutics, several technical, biological, regulatory, and ethical challenges continue to limit their broad clinical implementation. Translating these systems from the laboratory to real-world healthcare environments requires not only solving scientific limitations but also addressing practical, societal, and governance-related concerns. A major technical limitation lies in off-target activity, especially in Cas9- and Cas13-based systems. Although various CRISPR-Dx rely on collateral cleavage, non-specific activation due to poor gRNA design or suboptimal assay conditions can lead to false positives or background noise.[406,407] Enhanced gRNA design algorithms and the development of high-fidelity Cas variants, particularly in Cas9 systems, are critical to improving specificity.[408] In addition, sample preparation remains a bottleneck, as many biosensors require high-purity nucleic acids, which are difficult to isolate from complex biofluids like saliva or plasma, particularly in low-resource settings.[409,410] This challenge necessitates innovations in extraction-free, direct-detection strategies and inhibitor-resistant assay chemistries. Field-deployable diagnostics are further constrained by the temperature sensitivity of reagents and reliance on isothermal amplification methods such as RPA and LAMP, which typically require cold chain storage and precise thermal conditions.[277] To overcome this, researchers are engineering thermostable

Cas proteins and lyophilized assay formulations that retain functionality at ambient temperatures.[411]

Multiplexing, while promising, introduces complications such as cross-reactivity and overlapping signal interference. Advanced signal encoding strategies, including fluorescent barcoding and AI-assisted signal deconvolution, offer potential solutions; however, their clinical validation remains limited.[412] Scalability presents another challenge. Manufacturing CRISPR-based biosensors at consistent quality levels demands strict standardization in assay design, production, and validation. Variability in reagents or device assembly can impede regulatory approval processes from authorities such as the United States Food and Drug Administration (FDA) or the European Medicines Agency.[173] Beyond technical issues, ethical, legal, and social implications are gaining prominence. CRISPR biosensors, especially wearable or autonomous formats, raise concerns about privacy, informed consent, biosurveillance, and data ownership.[413] Moreover, AI-integrated CRISPR systems bring new challenges regarding algorithmic transparency, patient comprehension, and responsibility in decision-making.[414] From a technical standpoint, AI-driven CRISPR platforms face significant bottlenecks beyond ethical concerns. Biological datasets often suffer from noise, batch effects, and limited sample diversity, which hinder robust model training and reproducibility.[415] Furthermore, models trained on one cell type, species, or assay condition frequently fail to generalize across contexts, limiting translational potential.[416] Recent approaches such as transfer learning, ensemble modeling, and multimodal data integration (e.g., combining genomic, transcriptomic, and imaging datasets) are being explored to mitigate these limitations [[356]. However, widespread clinical adoption will require extensive benchmarking across heterogeneous datasets and rigorous external validation. Regulatory agencies and clinical institutions must co-develop transparent, human-in-the-loop frameworks that ensure accountability while promoting innovation.[417] As these platforms become more embedded in everyday health infrastructure, the need for strong global ethical guardrails and cooperative policy frameworks becomes increasingly urgent. Policies must safeguard patient autonomy, guarantee transparent data governance, and promote equitable access, especially in underserved communities.[418] Inclusive design involving patients, clinicians, ethicists, and regulators is essential to avoid reinforcing health inequities. In parallel, international collaboration is necessary to align cross-border standards, ensuring safe and ethical use of CRISPR technologies globally. This includes harmonizing data privacy laws, clinical trial protocols, and equitable access models.[419] Such collaboration could facilitate a unified approach to managing both the risks and rewards of CRISPR-based interventions. Furthermore, theranostic applications, which integrate diagnostics and therapeutics in a single closed-loop system, present regulatory and safety complexities. Current frameworks are ill-equipped to evaluate dual-function platforms, especially those guided by AI.[420] As such, there is a pressing need for new regulatory paradigms that accommodate these hybrid technologies. These should address not only efficacy and safety, but also software updates, adaptive algorithms, and ethical deployment. Finally, practical limitations such as cost, manufacturing complexity, and the potential immunogenicity of bacterial Cas proteins must be addressed to ensure global access and patient safety.[421,422] Emerging tools, such as logic-gated Cas circuits and CRISPR recorders, offer dynamic control and molecular memory capabilities but face hurdles in biocompatibility, stability, and *in vivo* delivery.[423] In summary, while CRISPR biosensors and theranostics offer unprecedented potential for real-time, personalized, and decentralized medicine, achieving safe and equitable implementation requires overcoming a multilayered set of technical, ethical, and policy-related challenges. A concerted global effort, rooted in responsible innovation and cooperative governance, will be key to unlocking the full translational promise of CRISPR technologies.

8. Precision personalized medicine with CRISPR-Cas: Current impact and future horizons

Having outlined the mechanistic basis and diagnostic applications, it is important to consider how CRISPR may evolve in clinical and translational contexts. CRISPR-Cas technology is rapidly redefining personalized medicine by enabling precision-level interventions tailored to an individual's unique molecular and genetic makeup. The ability to detect, modulate, or correct disease-driving genomic alterations at their origin aligns CRISPR with the core tenets of precision healthcare. Clinical applications are no longer speculative, with real-world therapies emerging across oncology, hematology, infectious diseases, and rare genetic disorders. Notably, CRISPR-Cas9-based gene editing therapies for β-thalassemia and sickle cell disease have entered advanced clinical stages, with FDA-approved treatments like Exagamglogene Autotemcel (developed by CRISPR Therapeutics and Vertex) already marking the transition from bench to bedside. Beyond monogenic disorders, CRISPR's integration with CAR-T therapy has further amplified its translational scope. Engineered immune cells, modified via CRISPR to enhance tumor specificity or evade immune checkpoints, are now being deployed in leukemia and lymphoma trials. Likewise, CRISPR-based human leukocyte antigen (HLA) editing is helping to overcome histocompatibility barriers in allogeneic transplantation, bringing "off-the-shelf" immunotherapies closer to reality. In the realm of diagnostics, HLA genotyping using CRISPR-Cas systems is improving organ transplant matching and autoimmune disease stratification. These innovations are increasingly supported by AI, which enhances gRNA design, predicts off-target edits, and optimizes dosing strategies, driving a convergence of gene editing and computational biology. The growing momentum is also reflected in the economic landscape. According to Precedence Research, the global CRISPR gene editing market was valued at United States dollars (USD) 3.5 billion in 2023 and is projected to surpass USD 15.8 billion by 2032, growing at a compound annual growth rate of 18.5%. This surge is fueled by increasing clinical trials, rising demand for personalized therapies, and expanding applications in diagnostics, agriculture, and synthetic biology (https://www.precedenceresearch.com/

crispr-based-gene-editing-market). **Table 10** provides a historical overview of key capital investments and market developments that have shaped the CRISPR-Cas trajectory, highlighting the shift from fundamental discovery to FDA-approved therapies and commercial growth.

Several biotech firms, including CRISPR Therapeutics,[441] Editas Medicine,[442] Beam Therapeutics,[443] Intellia Therapeutics,[444] and Mammoth Biosciences, are now spearheading CRISPR innovations, with multiple candidates in preclinical and clinical pipelines. However, barriers remain, such as variability in patient responses, challenges in delivery (particularly to immune-privileged tissues like the brain), high treatment costs, and regulatory complexity, all of which must be addressed to ensure equitable access and safety. And therefore, public–private partnerships and government-backed subsidies will be vital in democratizing CRISPR-based personalized medicine. This convergence of molecular biology, computational tools, and patient-centered design is poised to catalyze a paradigm shift from reactive treatments to predictive, preventive, and adaptive interventions. Building on this foundation, the following subsections explore what CRISPR biosensing may look like by 2030 and how the diagnostic-therapeutic continuum is evolving through smart, scalable platforms.

8.1. CRISPR-integrated wearable biosensors

Miniaturization and hardware integration are likely to redefine how and where CRISPR biosensors are utilized. Wearable devices, such as smartwatches, clothing fabrics, or subdermal chips, embedded with CRISPR detection modules could continuously track biomarkers in sweat, saliva, or interstitial fluid, enabling non-invasive, real-time health monitoring.[421] For instance, smart patches may detect inflammatory cytokines and activate CRISPR-dCas circuits to suppress harmful gene expression, offering preventive modulation in chronic conditions. These personalized health dashboards, dynamically adjusting to each individual's physiological and genetic signals, represent a paradigm shift toward autonomous, precision-guided healthcare—revolutionizing preventive medicine by enabling early disease detection, timely intervention, and improved quality of life.

Table 10. CRISPR-Cas: Market capital investment trajectory (approximately 2012–2025)

Period	Key developments and investment landscape	Estimated market size/investment highlights (approximate)	References
2005–2011	Discovery and fundamental research phase: CRISPR-Cas was known as a bacterial immune system. Investment was primarily through academic grants and basic science research. No significant "market capital investment" in the commercial sense	Minimal commercial market; focus on basic scientific funding	424
2012–2014	CRISPR-Cas9 gene editing revolution: Key publications (Doudna/Charpentier, Zhang, Church) demonstrate CRISPR-Cas9's ability to edit mammalian cells, sparking intense commercial interest. Formation of early-stage biotech companies	Early VC funding: Millions of dollars in seed and Series A funding for foundational CRISPR companies such as Editas Medicine, CRISPR Therapeutics, and Intellia Therapeutics. For example, Editas Medicine received significant early investment	425-428
2015–2016	"CRISPR hype" and initial public offerings (IPOs): Major pharmaceutical partnerships and IPOs of leading CRISPR companies. Patent disputes began	IPO launches: Editas Medicine and CRISPR Therapeutics went public in 2016, raising hundreds of millions. Intellia Therapeutics also went public around this time. Strategic partnerships: Large pharma companies (e.g., Vertex, Bayer, Novartis, AstraZeneca) initiate collaborations worth hundreds of millions to billions. Example: Vertex-CRISPR Therapeutics deal valued up to $2.6 billion (2015)	429-432
2017–2019	Clinical trials and pipeline expansion: Lead candidates advanced into clinical trials for genetic disorders (e.g., sickle cell disease, β-thalassemia, eye diseases). Continued VC funding for new entrants and platform technologies	Continued VC and public offerings: Hundreds of millions to billions of dollars raised through additional funding rounds and follow-on public offerings by existing companies. Investor interest in gene editing continued to broaden during this period	433-435
2020	Nobel Prize recognition: Emmanuelle Charpentier and Jennifer Doudna were awarded the Nobel Prize in Chemistry for CRISPR, further legitimizing the field and boosting investor confidence	Increased investment and valuations: Renewed investor interest, often accompanied by higher valuations for CRISPR companies following the Nobel Prize recognition	436,437
2021–2023	Acceleration of clinical progress and first approval: Positive clinical trial data across indications. In December 2023, Casgevy (Vertex/CRISPR Therapeutics) became the first CRISPR-based therapy approved in the UK and US for sickle cell disease and β-thalassemia	Market size growth: The Global CRISPR market is valued at approximately US$3.2–US$3.3 billion in 2023 (various sources). Significant late-stage private rounds and public market investments	438,439
2024–2025 (current and near-future)	Post-approval momentum and diversification: Rollout of Casgevy. Ongoing progress in base editing and prime editing. Broader applications in agriculture, diagnostics, and other therapeutic areas gaining traction	Projected global CRISPR market: ~US$4.0–US$5.5 billion in 2024–2025 (various reports, including *CRISPR* and *Cas Gene Market, CRISPR-Based Gene Editing Market*). Continued investment in new technologies and expanded clinical pipelines	440
Beyond 2025 (forecast)	Sustained high growth: Strong CAGR (11–22% annually) projected, driven by emerging therapies, expanded applications, and improved delivery methods	Forecasted market size: US$10–US$17 billion by 2030–2034, indicating substantial future capital investment	439

Abbreviations: CAGR: Compound annual growth rate; CRISPR-Cas: Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein; UK: United Kingdom; US: United States; VC: Venture capital.

8.2. CRISPR-based liquid biopsy platforms

Liquid biopsies provide a minimally invasive approach for monitoring circulating tumor DNA, miRNAs, and exosomes in patient biofluids.[445] CRISPR-powered assays enhance sensitivity and specificity, supporting early cancer detection, therapy response monitoring, and relapse prediction.[446] When combined with AI-driven analytics, CRISPR-based liquid biopsies hold strong potential for precision oncology and real-time clinical decision-making.[494] ML algorithms can integrate multi-omic profiles (genomic, transcriptomic, and proteomic data) with CRISPR assay outputs, enabling patient stratification, therapy outcome prediction, and adaptive treatment decisions in oncology and other complex diseases.[447] Importantly, AI can also reduce noise in CRISPR signal readouts, minimizing false positives while identifying clinically relevant rare events with high accuracy.[448] Together, the integration of CRISPR diagnostics and AI-driven liquid biopsy analysis holds strong potential for next-generation precision oncology, enabling early detection and more personalized clinical treatments.

While this review emphasizes wearable biosensors and liquid biopsy platforms as particularly promising directions, it is important to note that other innovations, such as synthetic biology-driven gene circuits, advanced multiplexing strategies, and low-cost paper-based diagnostics, also hold significant potential. Together, these complementary approaches ensure that CRISPR technologies continue to advance toward a more accessible, intelligent, and globally equitable future for precision medicine.

9. Conclusions

AI-enhanced CRISPR technology holds great promise for future therapeutic advances. By promoting ethical research practices, fostering interdisciplinary collaboration, and establishing strong regulatory frameworks, the scientific community can ensure that the benefits of AI-enhanced CRISPR are fully and fairly realized. AI is being utilized in digital pathology, biomarker development, and the enhancement of treatment outcomes for patients across various applications. The seamless integration of AI-based solutions throughout the healthcare workflow will be a key driver of AI-enabled advancements in clinical oncology practices. CRISPR-AI is transforming precision medicine by turning CRISPR-Cas systems into powerful, programmable tools for real-time diagnostics, theranostics, and personalized treatments. This integration allows a shift from static testing to adaptive, closed-loop systems that sense molecular signals, interpret disease states, and autonomously guide therapy. AI supports these systems by enhancing gRNA design, predicting off-target effects, deciphering complex biosensor signals, and providing smart decision-making assistance across diverse clinical environments. The adoption of advanced ML techniques, including deep learning, reinforcement learning, generative adversarial networks, and transfer learning, is likely to further improve the accuracy and predictive power of AI models in genomics. Collaboration across disciplines is essential to CRISPR-AI innovation, combining expertise from bioinformatics, molecular biology, computer science, clinical research, and ethics. Establishing dedicated interdisciplinary research centers can create environments where experts from various fields collaborate on CRISPR-AI projects. In addition, hosting regular conferences and workshops that focus on bridging the gap between disciplines can foster collaboration and knowledge exchange. Encouraging joint academic programs and cross-disciplinary training initiatives will also help build a common language and understanding among researchers from different backgrounds.

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