

Next-Generation CRISPR Editing: Base Editing, Prime Editing, and the Transition Beyond Double-Strand Breaks

Mazhar Ismail*

BS Biotechnology, Institute Of Biochemistry & Biotechnology, University, PMAS Arid Agriculture University, Rawalpindi, Pakistan
Email: mazharismail511@gmail.com

Eesha Sajjid

MS Biotechnology, Department Biological Sciences, National University of Medical Science, Rawalpindi, Pakistan

Abstract

Genome editing technologies have advanced rapidly since the introduction of CRISPR-Cas9, which enables targeted genetic modification through double-strand DNA breaks. Despite its effectiveness, double-strand break-dependent editing is limited by imprecise repair outcomes, off-target effects, and activation of DNA damage responses, raising concerns regarding precision, reproducibility, and long-term genomic stability. These constraints have driven the development of next-generation CRISPR-based strategies that enable precise genome modification without inducing DNA cleavage. Among these approaches, base editing and prime editing represent major conceptual advances. Base editing enables efficient single-nucleotide conversion through programmable chemical modification of DNA bases, whereas prime editing expands editing scope by facilitating precise insertions, deletions, and substitutions via reverse transcription-mediated DNA rewriting. This review provides a mechanism-centered comparison of base editing and prime editing, synthesizing their molecular architectures, editing capabilities, efficiencies, and technical limitations. By explicitly contrasting break-dependent and break-free editing strategies, this review highlights emerging applications across biomedical

research, plant biotechnology, and functional genomics, while outlining remaining technical and translational challenges that inform context-dependent selection of precision genome engineering approaches.

Introduction

Genome editing has transformed modern biology by enabling targeted manipulation of genetic sequences across a wide range of organisms. Among available platforms, CRISPR-Cas9 has emerged as the most widely adopted technology due to its simplicity, programmability, and high editing efficiency. By introducing site-specific double-strand DNA breaks (DSBs), CRISPR-Cas9 leverages endogenous DNA repair pathways to generate insertions, deletions, or targeted sequence modifications, thereby accelerating advances in functional genomics, crop improvement, and

Author Details

Keywords: CRISPR Genome Editing; Base Editing; Prime Editing; Double-Strand Break-Free Editing; Precision Genome Engineering

Received on 25 Nov 2025

Accepted on 30 Dec 2025

Published on 16 Jan 2026

Corresponding E-mail & Author*:

Mazhar Ismail*

BS Biotechnology, Institute Of Biochemistry & Biotechnology, University, PMAS Arid Agriculture University, Rawalpindi, Pakistan
Email: mazharismail511@gmail.com

biomedical research (**Katti & Mitchell, 2022; Pickar-Oliver & Gersbach, 2024**).

Despite its transformative impact, DSB-dependent genome editing is constrained by fundamental biological limitations. DNA cleavage activates cellular damage-response pathways and frequently produces unpredictable repair outcomes, particularly in applications requiring single-nucleotide accuracy (**Xiong et al., 2023; Hsu et al., 2024**).

Recognition of these limitations has driven the development of next-generation CRISPR-based technologies designed to bypass double-strand breaks altogether. Among these, base editing and prime editing represent major conceptual and technical advances. Rather than relying on DNA cleavage and stochastic repair, these systems enable targeted genetic modification through programmable chemical conversion or RNA-templated DNA rewriting. This shift marks a fundamental transition in genome engineering—from break-and-repair strategies toward precision sequence rewriting (**Zhang et al., 2022; Anzalone et al., 2022**).

Base editing enables efficient and predictable single-nucleotide substitutions by coupling catalytically impaired CRISPR nucleases with nucleotide deaminases, allowing direct base conversion within defined editing windows. Prime editing further expands editing versatility by enabling precise substitutions, insertions, and deletions without donor DNA templates or double-strand break formation. Together, these technologies substantially broaden the scope of achievable genome modifications while reducing the risks associated with DSB-mediated repair (**Anzalone et al., 2022; Veillet et al., 2023**).

Although base editing and prime editing have been rapidly adopted across biological disciplines, existing reviews often focus on individual technologies, specific organisms, or application-driven case studies. A mechanism-centered synthesis that directly compares these approaches and situates them within the broader transition beyond DSB-dependent genome editing remains limited (**Scholefield & Harrison, 2021; Xiong et al., 2023**). Such an integrative perspective is essential for understanding the trade-offs between precision, efficiency, flexibility, and scalability that will define the future of genome engineering.

This review addresses this gap by systematically examining the molecular principles underlying base editing and prime editing, critically comparing their capabilities, limitations, and application potential across biological systems. By framing these technologies as complementary components of a precision-first genome editing paradigm, this work aims to clarify their roles in shaping the next phase of CRISPR-based innovation (**Pickar-Oliver & Gersbach, 2024**).

Limitations of Double-Strand Break–Based Genome Editing

The success of CRISPR-Cas9 has firmly established programmable nucleases as powerful tools for targeted genome modification. By introducing site-specific double-strand DNA breaks (DSBs), CRISPR-Cas9 enables genetic alterations through endogenous DNA repair pathways. Despite its widespread utility, this reliance on DNA cleavage imposes fundamental biological constraints that limit precision, predictability, and safety in genome engineering applications (**Katti & Mitchell, 2022; Pickar-Oliver & Gersbach, 2024**).

Following the induction of DSBs, cellular repair is dominated by non-homologous end joining (NHEJ), an efficient but inherently error-prone pathway. NHEJ frequently produces heterogeneous insertions and deletions at the target locus, resulting in variable editing outcomes even under identical experimental conditions. This stochastic nature of repair presents a major challenge for applications requiring single-nucleotide resolution or reproducible genetic outcomes. In contrast, homology-directed repair (HDR) enables precise sequence modification but occurs at low efficiency and is restricted to specific phases of the cell cycle, thereby limiting its applicability in non-dividing cells, plant tissues, and many microbial systems (**Xiong et al., 2023; Hsu et al., 2024**).

Beyond imprecise editing outcomes, DSB-dependent genome editing can compromise genomic integrity. Studies have reported unintended large deletions, chromosomal rearrangements, and translocations arising from both on-target and off-target cleavage events. Such genomic perturbations raise significant concerns for applications involving long-term genetic stability, including therapeutic genome editing and agricultural trait development. Furthermore, activation of DNA damage response pathways following DSB induction can affect cell viability, proliferation, and developmental processes, adding additional layers of complexity to downstream analyses (**Kosicki et al., 2018; Xiong et al., 2023**).

Another important limitation of DSB-based editing is its strong dependence on cellular context. Editing efficiency, repair pathway choice, and mutation profiles vary widely among species, cell types, and developmental stages. This variability reduces reproducibility and complicates the translation of CRISPR-Cas9 technologies across diverse biological systems. Repeated induction of DNA breaks may also impose selective pressures, potentially leading to unintended genetic or epigenetic consequences over time (**Pickar-Oliver & Gersbach, 2024; Hsu et al., 2024**).

Collectively, these challenges underscore a fundamental trade-off inherent to DSB-dependent genome editing: while CRISPR-Cas9 remains highly effective for gene disruption and large-scale genomic alterations, its reliance on DNA cleavage constrains its suitability for applications demanding high-fidelity sequence control. This limitation is particularly consequential in contexts where precise, predictable, and stable genome modifications are required. Recognition of these limitations has driven the development of next-generation CRISPR technologies that seek to bypass double-strand breaks altogether, enabling precise genome modification through alternative molecular mechanisms (**Zhang et al., 2022; Anzalone et al., 2022**).

Collectively, these limitations have catalyzed a fundamental shift from break-and-repair genome editing strategies toward precision genome rewriting approaches, as illustrated in Figure 1.

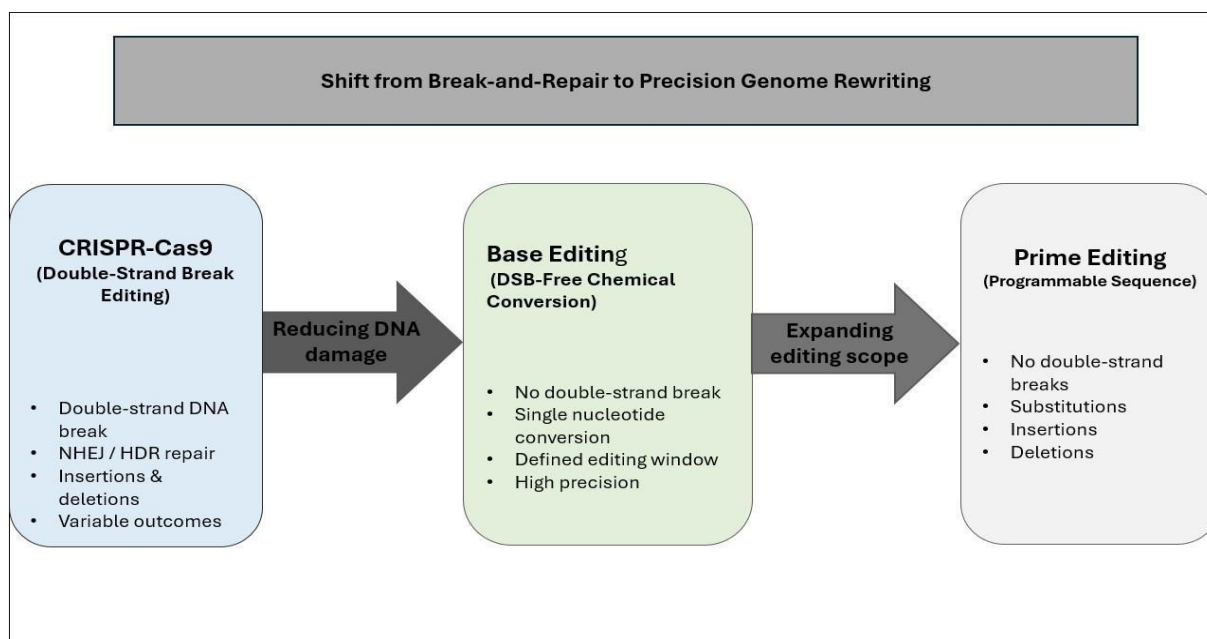


Figure 1. Evolution of CRISPR genome editing strategies from double-strand break-dependent CRISPR–Cas9 systems to precision, DSB-free genome editing approaches. Conventional CRISPR–Cas9 relies on DNA cleavage and endogenous repair pathways, resulting in heterogeneous and often unpredictable editing outcomes. Base editing enables precise single- nucleotide conversion through programmable chemical

modification without inducing double-strand breaks, whereas prime editing further expands editing capabilities by enabling targeted substitutions, insertions, and deletions via RNA-templated sequence rewriting. This progression illustrates a broader shift from break-and-repair strategies toward precision-first genome engineering. Figure created by the authors for conceptual illustration.

Base Editing: Precision Genome Modification Without DNA Cleavage Molecular Architecture and Editing Principle

Base editing emerged in response to the limitations of genome editing strategies that rely on the induction of double-strand DNA breaks. Unlike conventional CRISPR-Cas9 systems, base editing enables direct and programmable nucleotide conversion without physically cleaving the DNA backbone. This conceptual shift allows precise sequence modification while minimizing genomic disruption and cellular stress responses.

Base editors are chimeric protein complexes composed of three core components: (i) a catalytically impaired CRISPR-associated nuclease, typically a Cas9 nickase or dead Cas9; (ii) a nucleotide deaminase enzyme; and (iii) a guide RNA that directs the complex to a specific genomic locus. Upon target recognition, the deaminase catalyzes a chemical modification of a nucleotide within a defined editing window on the exposed single-stranded DNA. Cellular DNA repair and replication processes subsequently convert this modified base into a stable base substitution, without generating a double-strand break (Rees et al., 2018; Zhang et al., 2022).

By decoupling sequence modification from DNA cleavage, base editing offers improved precision and reduced genomic instability compared with DSB-dependent approaches, particularly for applications involving single-nucleotide variants (Katti & Mitchell, 2022; Pickar-Oliver & Gersbach, 2024).

Classes of Base Editors

Two major classes of base editors have been developed, each enabling a specific type of nucleotide transition.

Cytosine Base Editors (CBEs) catalyze the deamination of cytosine to uracil, which is subsequently resolved to thymine during DNA repair or replication. This process enables targeted C•G-to-T•A conversions. CBEs have been widely applied for correcting pathogenic point mutations and for precise genetic manipulation in plant, microbial, and mammalian systems (Komor et al., 2016; Veillet et al., 2023).

Adenine Base Editors (ABEs) convert adenine to inosine, which is interpreted as guanine by the cellular machinery, resulting in A•T-to-G•C substitutions. ABEs are particularly valuable due to their high product purity and relevance to a large fraction of disease-associated single-nucleotide variants (Koblan et al., 2021; Richter et al., 2021).

Together, CBEs and ABEs enable four transition mutations, covering a substantial proportion of biologically and clinically relevant point mutations (Zhang et al., 2022).

Advantages of Base Editing Over DSB-Dependent Approaches

Base editing directly addresses several limitations inherent to double-strand break-based genome editing strategies. First, the absence of DSB formation substantially reduces the risk of large deletions, chromosomal rearrangements, and activation of DNA damage response pathways. Second, base editing exhibits high efficiency in non-dividing cells and recalcitrant tissues where homology-directed repair is inefficient or inactive. Third, editing outcomes are comparatively predictable, with reduced allelic heterogeneity relative to NHEJ-mediated repair. Finally, base editing has demonstrated broad compatibility across diverse biological systems, including plants, microbes, and mammalian cells (Zhang et al., 2022; Veillet et al., 2023;

Pickar-Oliver & Gersbach, 2024).

These properties make base editing particularly suitable for applications requiring precise and reliable single-nucleotide corrections.

Technical Constraints and Limitations

Despite its advantages, base editing is subject to several intrinsic limitations. The scope of achievable edits is restricted to specific nucleotide transitions, excluding transversions and larger sequence modifications. Editing is confined to a narrow activity window, which can result in unintended bystander edits when multiple editable bases are present within the target region. In addition, target site selection remains constrained by protospacer-adjacent motif (PAM) requirements (**Zhang et al., 2022; Xiong et al., 2023**).

Off-target deamination events have also been reported at both DNA and RNA levels, underscoring the need for continued optimization of editor specificity and expression control. These limitations indicate that, while base editing represents a major advance in precision genome engineering, it does not constitute a universal solution for all genome modification needs (**Zuo et al., 2020; Pickar-Oliver & Gersbach, 2024**).

Positioning Base Editing in the Genome Editing Landscape

Base editing demonstrates that accurate genetic modification can be achieved without inducing DNA breaks, redefining genome editing as a chemically mediated process rather than a break- and-repair mechanism. While its precision and efficiency make it highly effective for single- nucleotide substitutions, its limited editing scope highlights the need for more versatile systems capable of performing broader sequence alterations. These unmet needs directly motivated the development of prime editing as a complementary genome editing strategy (**Katti & Mitchell, 2022; Anzalone et al., 2022**).

Prime Editing: Search-and-Replace Genome Editing Without Double-Strand Breaks

Molecular Architecture and Editing Mechanism

While base editing demonstrated that precise genome modification can be achieved without inducing double-strand breaks, its editing scope is inherently limited to specific nucleotide transitions. Prime editing was developed to overcome these constraints by enabling a broader range of programmable genetic modifications without requiring double-strand DNA cleavage or donor DNA templates (**Anzalone et al., 2022; Katti & Mitchell, 2022**).

Prime editing is based on a fusion between a catalytically impaired CRISPR-associated nuclease, typically a Cas9 nickase, and a reverse transcriptase enzyme. This complex is guided by a specialized prime editing guide RNA (pegRNA), which not only directs the editor to the target genomic locus but also encodes the desired genetic modification within an extended RNA template (**Anzalone et al., 2019; Anzalone et al., 2022**).

Upon target recognition, the Cas9 nickase introduces a single-strand break in the DNA. The reverse transcriptase then uses the pegRNA as a template to synthesize a new DNA strand containing the programmed edit. Cellular repair mechanisms subsequently incorporate this newly synthesized sequence into the genome, resulting in precise sequence modification with minimal genomic disruption (**Chen et al., 2021; Nelson et al., 2022**).

Importantly, unlike homology-directed repair-based CRISPR approaches, prime editing does not depend on exogenous donor DNA or cell-cycle-restricted repair pathways, thereby expanding its applicability across diverse cell types and organisms (**Anzalone et al., 2022; Scholefield & Harrison, 2021**).

Editing Capabilities and Scope

Prime editing substantially expands the range of achievable genome edits compared with base editing. In addition to supporting all twelve possible base substitutions, prime editing enables precise small insertions, deletions, and combination edits involving multiple sequence changes within a single editing event (**Anzalone et al., 2022; Xiong et al., 2023**).

This versatility allows prime editing to target a large fraction of pathogenic mutations and functional genomic variants that are inaccessible to base editors. As a result, prime editing has been widely regarded as the most flexible CRISPR-based genome editing platform developed to date, capable of addressing diverse genetic alterations with high theoretical precision (**Katti & Mitchell, 2022; Pickar-Oliver & Gersbach, 2024**).

Advantages of Prime Editing

Prime editing offers several key advantages that position it as a next-generation precision genome editing tool. First, the absence of double-strand breaks reduces the risk of large deletions, chromosomal rearrangements, and activation of DNA damage response pathways. Second, its broad editing scope allows substitutions, insertions, and deletions to be introduced with a single programmable system. Third, the elimination of donor DNA templates simplifies experimental design and reduces the risk of unintended genomic integration. Finally, prime editing exhibits reduced dependence on cell-cycle stage, increasing its potential utility in non-dividing cells and complex biological systems (**Anzalone et al., 2022; Liu et al., 2023**).

These properties make prime editing particularly attractive for applications requiring flexible and highly customized sequence modifications.

Technical Challenges and Current Limitations

Despite its conceptual elegance and broad editing potential, prime editing currently faces several technical challenges that limit its widespread application. Editing efficiencies are generally lower than those observed with base editors or conventional CRISPR-Cas9 systems, and performance varies substantially across cell types, organisms, and genomic loci (**Nelson et al., 2022; Liu et al., 2023**).

Successful prime editing is highly dependent on pegRNA design parameters, including primer binding site length, reverse transcription template configuration, and local sequence context. Optimization of these variables remains non-trivial and can significantly influence editing outcomes (Chen et al., 2021; Kim et al., 2023).

Delivery represents another major obstacle. The relatively large size of prime editing constructs complicates their packaging and delivery using viral and non-viral vectors. In addition, low- frequency unintended edits and incomplete editing outcomes have been reported, highlighting the need for further refinement of editor specificity and delivery strategies (Hsu et al., 2024; Pickar-Oliver & Gersbach, 2024).

Prime Editing in the Context of Precision Genome Engineering

Prime editing represents a critical milestone in the evolution of CRISPR technologies by demonstrating that programmable, flexible genome rewriting can be achieved without inducing double-strand DNA breaks. Whereas base editing excels at efficient single-nucleotide conversions, prime editing prioritizes editing versatility and sequence diversity. Together, these technologies illustrate a broader paradigm shift in genome engineering—from break-and-repair strategies toward controlled, precision-first sequence rewriting. Rather than replacing earlier CRISPR approaches, prime editing complements base editing and DSB- dependent systems, expanding the genome editing toolkit and enabling context-dependent selection of the most appropriate editing strategy (**Katti & Mitchell, 2022; Xiong et al., 2023; Pickar-Oliver & Gersbach, 2024**).

The distinct molecular architectures and editing mechanisms underlying base editing

and prime editing are illustrated in Figure 2.

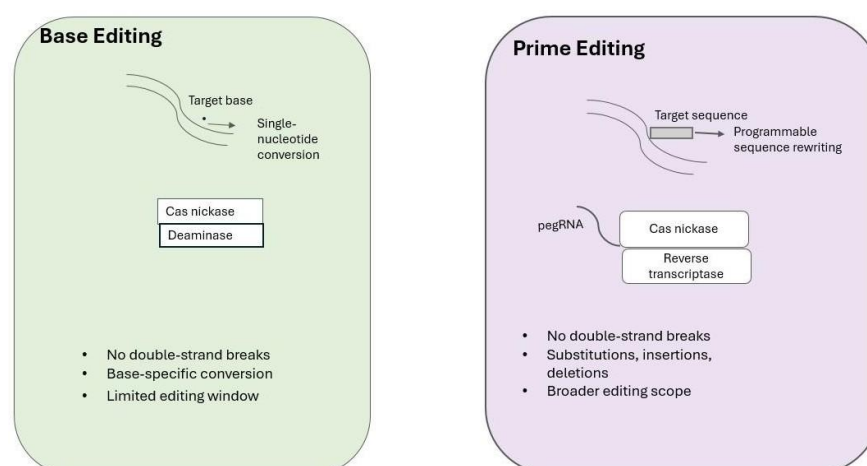


Figure 2. Molecular architectures and editing mechanisms of base editing and prime editing.

Base editors enable precise single-nucleotide conversion through deaminase-mediated chemical modification without inducing double-strand DNA breaks. Prime editors combine a Cas9 nickase, reverse transcriptase, and prime editing guide RNA (pegRNA) to facilitate programmable DNA sequence rewriting, including targeted substitutions, insertions, and deletions. These mechanistic differences account for the distinct editing scopes and applications of the two DSB-free genome editing strategies. Figure created by the authors for conceptual illustration.

Base Editing versus Prime Editing: Mechanistic and Functional Comparison

The development of base editing and prime editing marks a decisive transition in genome engineering from double-strand break-dependent modification to precision sequence rewriting. Although both technologies eliminate the need for DSB formation, they differ fundamentally in their molecular mechanisms, editing scope, efficiency, and practical deployment. A direct comparison is essential for understanding how these systems complement one another and for guiding context-dependent tool selection in future genome editing applications (**Katti & Mitchell, 2022; Pickar-Oliver & Gersbach, 2024**).

Mechanistic Distinctions

Base editing relies on enzymatic deamination to induce predictable chemical conversions of target nucleotides within a defined editing window. This mechanism is relatively simple and efficient, enabling rapid and precise transition mutations without extensive cellular processing. However, its reliance on deaminase chemistry inherently restricts the diversity of achievable edits (**Zhang et al., 2022; Koblan et al., 2021**).

In contrast, prime editing employs an RNA-templated DNA synthesis mechanism mediated by reverse transcription. By encoding the desired genetic modification within the pegRNA, prime editing enables programmable rewriting of genomic sequences. Although this approach introduces additional layers of molecular complexity, it substantially expands the range of possible edits, including substitutions, insertions, and deletions (**Anzalone et al., 2022; Chen et al., 2021**).

These mechanistic differences define a fundamental trade-off between efficiency and versatility that distinguishes base editing from prime editing (**Xiong et al., 2023**).

Editing Scope, Precision, and Predictability

Base editors are optimized for high-efficiency single-nucleotide transitions and typically yield predictable editing outcomes when target sites fall within compatible sequence contexts. This predictability makes base editing particularly suitable for correcting known point mutations with minimal allelic variability. Nevertheless, base editors are intrinsically limited in their capacity to generate diverse genetic alterations (Zhang et al., 2022; Veillet et al., 2023).

Prime editing overcomes these constraints by enabling all twelve base substitutions, as well as precise insertions and deletions. This expanded editing scope allows prime editing to address a broader spectrum of genetic variants, including mutations inaccessible to base editors. However, editing outcomes can be more heterogeneous due to variability in reverse transcription efficiency and cellular repair processes (Chen et al., 2021; Hsu et al., 2024).

Both technologies substantially reduce unintended genomic alterations relative to DSB-based editing, although bystander edits remain a concern for base editors, and incomplete or low-frequency unintended edits have been reported for prime editing (Xiong et al., 2023).

A comparative overview of double-strand break-dependent and DSB-free genome editing strategies is summarized in Table 1.

Table 1. Comparative overview of double-strand break-dependent and DSB-free genome editing strategies.

The table summarizes key mechanistic features, editing capabilities, efficiency, predictability, and practical considerations that inform context-dependent selection of genome editing approaches.

Feature	CRISPR–Cas9 (DSB-based editing)	Base Editing	Prime Editing
Induction of double-strand breaks	Required	Not required	Not required
Core editing mechanism	DNA cleavage followed by endogenous repair via non-homologous end joining (NHEJ) or homology-directed repair (HDR)	Enzymatic base conversion mediated by nucleotide deaminases	Reverse transcription-mediated programmable sequence rewriting
Types of genetic modifications	Insertions, deletions, large rearrangements	Single-nucleotide substitutions (transition mutations)	Substitutions, insertions, and deletions
Editing precision	Moderate; outcomes strongly depend on repair pathway choice	High for compatible target sites	High with broader editing scope
Dependence on cellular DNA repair pathways	High	Limited	Limited
Editing efficiency	Generally high	High in many biological systems	Variable and context-dependent
Editing outcome predictability	Variable	High	Moderate to high
Off-target or unintended effects	Off-target cleavage and large deletions reported	Possible bystander base edits	Low-frequency unintended edits reported

Delivery complexity	Relatively low	Moderate	High, owing to larger construct size
Suitability for non-dividing cells	Limited (HDR-dependent inefficient)	High	Moderate
Representative applications	Gene knockouts, genome-wide screens, large genomic alterations	Precise correction of single-nucleotide variants	Complex and customizable sequence modifications

Efficiency, Complexity, and Delivery Considerations

In practical applications, base editing generally exhibits higher editing efficiencies across a wide range of organisms and cell types. Its relatively compact molecular architecture facilitates delivery using existing viral and non-viral systems, making it amenable to large-scale and in vivo applications (**Koblan et al., 2021; Veillet et al., 2023**).

Prime editing, by comparison, often demonstrates lower and more variable editing efficiencies. The larger size and increased complexity of prime editing constructs pose significant challenges for delivery, particularly in contexts where vector capacity is limited. These practical constraints currently restrict the scalability of prime editing relative to base editing, despite its superior versatility (**Nelson et al., 2022; Liu et al., 2023; Pickar-Oliver & Gersbach, 2024**).

Context-Dependent Tool Selection

Rather than representing competing technologies, base editing and prime editing should be viewed as complementary components of an expanding genome editing toolkit. Base editing is optimally suited for applications requiring rapid, efficient, and predictable correction of single-nucleotide variants within compatible sequence contexts. In contrast, prime editing is better suited for applications demanding greater flexibility, such as the introduction of complex substitutions, small insertions, or deletions (**Katti & Mitchell, 2022; Xiong et al., 2023**).

The choice between these tools should be guided by the nature of the intended genetic modification, the target sequence environment, delivery constraints, and acceptable trade-offs between efficiency and editing breadth. Context-aware tool selection is therefore critical for maximizing both precision and practicality in genome engineering applications (**Pickar-Oliver & Gersbach, 2024**).

Conceptual Implications for the Future of Genome Engineering

The contrast between base editing and prime editing reflects a broader conceptual shift in genome engineering toward precision-first and control-oriented strategies. Rather than relying on DNA damage and stochastic repair, next-generation CRISPR systems emphasize programmable, predictable sequence modification.

As these technologies continue to mature, their integration is likely to redefine genome engineering as a modular discipline in which multiple editing strategies are deployed based on biological context and experimental objectives. This pluralistic framework—rather than the dominance of any single editing technology—will shape the future of precision genome engineering across biomedical, agricultural, and synthetic biology domains (**Katti & Mitchell, 2022; Pickar-Oliver & Gersbach, 2024; Hsu et al., 2024**).

Applications Across Biological Systems

From Precision Editing to Practical Implementation

The transition from double-strand break–dependent genome editing to precision sequence rewriting has significantly expanded the applicability of CRISPR technologies across diverse biological systems. Base editing and prime editing have enabled genetic modifications in contexts where sequence accuracy, genomic stability, and reproducibility are critical. Rather than providing an exhaustive catalogue of applications, this section highlights representative examples that illustrate how next-generation CRISPR editors address distinct biological and technical challenges across biomedical, plant, and microbial systems (**Pickar-Oliver & Gersbach, 2024; Hsu et al., 2024**).

Biomedical and Disease-Modeling Applications

A substantial proportion of genetic diseases arise from single-nucleotide variants, making base editing particularly well suited for biomedical applications. Base editors have been successfully employed to correct pathogenic point mutations in cellular and animal models with predictable outcomes and reduced unintended genomic alterations, owing to their ability to introduce precise base transitions without generating double-strand breaks (**Koblan et al., 2021; Richter et al., 2021**). These advantages are especially pronounced in non-dividing cells, where homology-directed repair–based approaches are inefficient or inactive (**Zhang et al., 2022; Katti & Mitchell, 2022**).

Prime editing further expands the therapeutic landscape by enabling correction of mutations that cannot be addressed through base conversion alone. Its ability to introduce small insertions, deletions, and complex substitutions allows modeling and potential correction of a broader spectrum of disease-associated variants (**Anzalone et al., 2022; Liu et al., 2023**). Although clinical translation remains in early stages, prime editing provides a flexible and powerful platform for precision disease modeling and functional interrogation of pathogenic mutations (**Hsu et al., 2024**).

Plant Biotechnology and Crop Improvement

In plant genome engineering, the avoidance of double-strand breaks is particularly advantageous due to the prevalence of error-prone repair pathways and regulatory constraints associated with transgene integration. Base editing has been widely applied to generate targeted point mutations that modulate agronomic traits such as stress tolerance, yield, and disease resistance, often without introducing foreign DNA (**Butt et al., 2021; Veillet et al., 2023**). Prime editing offers greater versatility in plant systems by enabling precise insertions and deletions in addition to base substitutions. Although current efficiencies in plants are generally lower than those achieved with base editors, continued optimization of editor design and delivery strategies is expected to enhance its utility (**Lin et al., 2022; Veillet et al., 2023**). Together, base editing and prime editing support the development of precision-bred crops that align with regulatory frameworks favoring minimal genomic disruption (**Pickar-Oliver & Gersbach, 2024**).

Microbial and Functional Genomics Applications

Microbial systems serve as valuable platforms for testing and deploying next-generation CRISPR technologies due to their genetic tractability and relevance to biotechnology. Base editing enables efficient introduction of targeted mutations for pathway optimization, metabolic engineering, and functional genomics, while preserving genome integrity—an important consideration for essential genes where disruptive mutations may be lethal (**Zhang et al., 2022; Xiong et al., 2023; Katti & Mitchell, 2022**).

Prime editing further enhances microbial genome engineering by enabling precise sequence rewriting of regulatory and coding regions, allowing fine control over gene expression and protein function **(Anzalone et al., 2022; Liu et al., 2023)**. As delivery and efficiency challenges are addressed, prime editing is likely to play an increasingly prominent role in microbial synthetic biology and systems-level genome engineering **(Pickar-Oliver & Gersbach, 2024)**.

Generalizable Principles of DSB-Free Genome Editing

Across biomedical, plant, and microbial systems, several general principles emerge from the application of base editing and prime editing. First, elimination of double-strand breaks consistently improves editing predictability and genomic stability, particularly in applications requiring precise sequence control **(Hsu et al., 2024; Xiong et al., 2023)**. This shift reduces reliance on endogenous DNA repair pathways that generate heterogeneous outcomes.

Second, a trade-off between efficiency and versatility governs tool selection. Base editing excels in contexts requiring rapid and efficient correction of compatible single-nucleotide variants, whereas prime editing prioritizes flexibility by enabling diverse sequence modifications at the cost of increased molecular complexity and reduced efficiency **(Koblan et al., 2021; Zhang et al., 2022; Nelson et al., 2022; Liu et al., 2023)**.

Finally, cross-system studies underscore that next-generation CRISPR technologies function best as complements rather than replacements for existing genome editing approaches. The continued coexistence of base editing, prime editing, and DSB-dependent systems reflects the diverse biological requirements of genome engineering across applications. The future of precision genome editing therefore lies in informed, context-dependent integration of multiple editing strategies rather than reliance on a single dominant technology **(Katti & Mitchell, 2022; Pickar-Oliver & Gersbach, 2024)**.

Delivery, Efficiency, and Scalability Challenges

Barriers to the Broad Deployment of Next-Generation CRISPR Editors

Despite the substantial conceptual and technical advances represented by base editing and prime editing, their widespread adoption remains constrained by challenges related to delivery, editing efficiency, scalability, and long-term safety. These limitations are particularly critical in translational, agricultural, and in vivo applications, where genome editing systems must perform reliably across diverse biological contexts while maintaining precision and reproducibility **(Pickar-Oliver & Gersbach, 2024; Hsu et al., 2024)**.

Delivery Constraints of Next-Generation Editors

Efficient delivery remains one of the most significant obstacles to the implementation of next-generation CRISPR technologies. Both base editors and prime editors are substantially larger than conventional CRISPR-Cas9 systems due to the incorporation of additional enzymatic domains, such as deaminases and reverse transcriptases. This increased molecular size complicates packaging into commonly used viral vectors, particularly adeno-associated viruses (AAVs), which have strict cargo size limitations **(Li et al., 2023; Pickar-Oliver & Gersbach, 2024)**.

Alternative delivery strategies, including lipid nanoparticles, electroporation, and ribonucleoprotein-based approaches, can circumvent some of these constraints but often suffer from reduced efficiency, limited tissue penetration, or restricted applicability to specific cell types. Delivery challenges are especially pronounced in plant systems and in vivo therapeutic contexts, where efficient tissue targeting and uniform editor expression remain major bottlenecks **(Raitskin et al., 2022; Hsu et al., 2024)**.

Editing Efficiency and Outcome Variability

Base editing generally achieves high editing efficiencies across a broad range of biological systems. However, editing outcomes are strongly influenced by target sequence context, editing window positioning, and cellular repair pathways. Even under optimized conditions, variability in editing efficiency and the occurrence of bystander edits can complicate multiplexed editing and limit reproducibility at larger scales (**Zhang et al., 2022; Xiong et al., 2023**).

Prime editing faces additional efficiency challenges due to its more complex editing mechanism. Editing success depends on multiple parameters, including pegRNA design, primer binding site length, reverse transcription template configuration, and local chromatin environment. As a result, prime editing efficiencies are often lower and more variable than those observed with base editing or conventional CRISPR-Cas9 systems (**Nelson et al., 2022; Liu et al., 2023**).

Scalability and Standardization Challenges

Scalability represents a critical barrier to the broader adoption of next-generation CRISPR technologies, particularly for high-throughput or genome-wide applications. Reliable large-scale deployment requires predictable editing outcomes across multiple targets, experimental conditions, and biological systems. The extensive optimization required for each target—especially in prime editing—currently limits scalability and complicates cross-study comparisons (**Xiong et al., 2023; Pickar-Oliver & Gersbach, 2024**).

In addition, the absence of standardized benchmarking frameworks for evaluating editing efficiency, specificity, and off-target effects hinders objective comparison between studies and laboratories. Establishing consistent evaluation metrics will be essential for improving reproducibility and enabling systematic assessment of next-generation genome editing platforms (**Hsu et al., 2024**).

Safety, Specificity, and Long-Term Considerations

Although base editing and prime editing avoid many risks associated with double-strand break–based genome editing, they are not entirely free from unintended effects. Reports of off-target deamination, incomplete editing outcomes, and low-frequency unintended sequence alterations underscore the need for continued refinement of editor specificity and comprehensive genomic assessment (**Zuo et al., 2020; Liu et al., 2023**).

Long-term safety considerations are particularly relevant in therapeutic and agricultural applications, where prolonged editor expression or repeated editing events may introduce cumulative genomic or epigenomic changes. Rigorous long-term studies will therefore be required to evaluate durability, safety, and potential downstream consequences prior to widespread clinical or agricultural deployment (**Pickar-Oliver & Gersbach, 2024; Hsu et al., 2024**).

Outlook: Overcoming Technical Barriers

Ongoing advances in protein engineering, guide RNA design, delivery technologies, and computational optimization are expected to address many of the current limitations of base editing and prime editing. The development of compact editors, improved delivery systems, and standardized evaluation pipelines will be central to translating these technologies from experimental tools into robust platforms for routine application (**Li et al., 2023; Pickar-Oliver & Gersbach, 2024**).

Overcoming these technical barriers will not only broaden the applicability of next-generation CRISPR editors but also reinforce the broader shift toward precision-first genome engineering—an approach that emphasizes control, predictability, and context-dependent tool selection as defining criteria for technological success (**Hsu et al., 2024**).

Future Directions: Beyond Double-Strand Break–Dependent Genome Editing

The emergence of base editing and prime editing represents more than a technical refinement of existing CRISPR technologies; it reflects a broader conceptual shift in genome engineering away from damage-induced DNA modification toward programmable sequence rewriting. By demonstrating that precise genetic alterations can be achieved without inducing double-strand breaks, next-generation CRISPR systems challenge long-standing assumptions regarding the necessity of DNA cleavage for effective genome editing (**Katti & Mitchell, 2022; Pickar-Oliver & Gersbach, 2024**).

Continued advances in editor engineering are expected to further improve the efficiency, specificity, and versatility of DSB-free genome editing platforms. Ongoing efforts to develop compact editors, expand PAM compatibility, and optimize guide RNA design are likely to alleviate current constraints related to delivery and target accessibility. In parallel, integration of computational design tools with high-throughput screening approaches will accelerate editor optimization and reduce unintended editing outcomes across diverse biological systems (**Hsu et al., 2024**).

Despite these advances, it is unlikely that double-strand break–dependent genome editing will be entirely displaced in the foreseeable future. Conventional CRISPR-Cas9 systems remain highly effective for gene knockouts, large-scale genomic rearrangements, and genome-wide functional screening in both prokaryotic and eukaryotic organisms. Rather than a replacement, the evolution of genome editing technologies is better understood as an expansion of the available toolkit, in which new precision editors complement existing cleavage-based approaches (**Pickar-Oliver & Gersbach, 2024**).

A key future priority will be the establishment of clear, context-dependent frameworks for tool selection. In this paradigm, base editing is best suited for efficient correction of compatible single-nucleotide variants, whereas prime editing enables flexible and customizable sequence modifications that extend beyond the capabilities of base conversion. Double-strand break–based systems will continue to play a role in applications where precision at the nucleotide level is not essential or where large genomic alterations are desired (**Katti & Mitchell, 2022; Hsu et al., 2024**).

Collectively, the gradual reduction in reliance on DNA cleavage signals a maturation of genome engineering as a discipline. Future innovations will increasingly prioritize control, predictability, and genomic integrity over maximal disruption. This precision-first framework is likely to shape the next phase of CRISPR development, influencing biomedical research, agricultural biotechnology, and synthetic biology by promoting rational, context-aware deployment of genome editing technologies.

Conclusions: Toward Precision-First Genome Engineering

The rapid evolution of CRISPR-based genome editing technologies reflects a broader shift in biotechnology toward prioritizing precision, predictability, and genomic integrity alongside editing efficiency. While CRISPR-Cas9 has revolutionized biological research through programmable DNA cleavage, its dependence on double-strand breaks inherently limits precise sequence control and raises concerns related to genomic stability. These limitations have catalyzed the development of next-generation CRISPR systems that enable accurate genome modification without inducing DNA breaks.

Base editing and prime editing exemplify this transition by demonstrating that targeted genetic alterations can be achieved through controlled nucleotide conversion and programmable sequence rewriting rather than damage-induced repair. Base editing provides an efficient and reliable platform for precise single-nucleotide substitutions, whereas prime editing expands the editing landscape by enabling flexible substitutions, insertions, and deletions with minimal genomic disruption.

Together, these technologies redefine genome editing as a precision-driven molecular process.

Importantly, the emergence of DSB-free genome editing does not render cleavage-dependent approaches obsolete. Instead, base editing, prime editing, and traditional CRISPR-Cas9 systems should be viewed as complementary tools within an increasingly diverse genome engineering toolkit. Effective application of these technologies requires context-aware selection based on the desired genetic outcome, biological system, delivery constraints, and acceptable risk profiles.

Despite substantial progress, key challenges—including delivery efficiency, editing variability, scalability, and standardized assessment of specificity and long-term safety—continue to limit the widespread deployment of next-generation editors. Addressing these challenges through continued advances in protein engineering, guide RNA design, delivery technologies, and computational optimization will be critical for translating precision genome editing from experimental systems into routine applications.

In summary, the ongoing shift away from DNA cleavage toward programmable sequence rewriting marks a maturation of genome engineering. Rather than maximizing genomic disruption to achieve genetic change, future innovations will increasingly emphasize control, predictability, and biological compatibility. The integration of next-generation CRISPR technologies into a flexible, context-dependent framework will ultimately define the future of genome editing across biomedical, agricultural, and synthetic biology applications.

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