

Original Research

Received 28 June 2026

Revised 01 Aug 2026

Accepted 16 Aug 2026

Natural Resources for Human
Health 2026, Vol. 6 (16s): 1495–1505

KEYWORDS:

CRISPR-Cas9; genome editing;
gene therapy; functional genomics;
precision medicine

Applications of CRISPR-Cas9 Gene Editing in Modern Biology: From Genome Engineering to Precision Medicine

Riya Sarah Cornel

Jumeirah College, Dubai

E-Mail: riya.cornel@outlook.com

ABSTRACT: Clustered Regularly Interspaced Short Palindromic Repeats-associated protein 9 (CRISPR-Cas9) has emerged as one of the most transformative technologies in modern biology, providing researchers with a programmable and comparatively accessible approach for manipulating genomic DNA. Originally characterized as part of a prokaryotic adaptive immune system, the CRISPR-Cas9 pathway was converted into a genome-engineering platform capable of producing targeted DNA double-strand breaks using sequence-specific guide RNA. Since the first demonstrations of genome editing in mammalian cells, CRISPR-Cas9 has expanded into functional genomics, disease modeling, stem-cell biology, cancer research, agricultural biotechnology, microbial engineering, synthetic biology, and therapeutic development. Subsequent innovations, including catalytically impaired Cas9 systems, base editors, and prime editors, have further expanded the range of genetic modifications that can be achieved while reducing dependence on conventional double-strand-break repair. This paper reviews the major applications of CRISPR-Cas9 in modern biology, emphasizing the molecular basis of editing, experimental strategies, functional-genomic screening, animal and plant engineering, disease modeling, therapeutic applications, and emerging precision-editing technologies. Evidence from original research studies demonstrates that CRISPR-Cas9 has evolved from a gene-disruption technology into a versatile biological engineering platform. However, challenges involving off-target activity, delivery, editing efficiency, genomic integrity, immune responses, ethical considerations, and long-term safety remain important barriers to widespread application. iste natus error sit voluptatem accusantium doloremque laudantium

1. INTRODUCTION

The ability to modify DNA at predetermined genomic locations has fundamentally changed the experimental possibilities of modern biology. Genetic engineering was historically dependent on relatively laborious approaches involving homologous recombination, random transgenesis, zinc-finger nucleases, or transcription activator-like effector nucleases (TALENs). Although these technologies established the conceptual foundation for targeted genome manipulation, their design, implementation, and multiplexing requirements often limited their widespread use. The development of CRISPR-Cas9 provided a substantially more programmable alternative because target specificity could largely be reprogrammed through a short guide RNA rather than by redesigning an entire DNA-binding protein [1–4].

CRISPR, an acronym for clustered regularly interspaced short palindromic repeats, was initially recognized as a distinctive repetitive DNA structure in bacteria. Subsequent research established that CRISPR loci are components of adaptive immune systems that allow bacteria and archaea to recognize and eliminate invading genetic material. In type II CRISPR systems, Cas9 functions as an RNA-guided DNA endonuclease. Jinek et al. demonstrated that Cas9 could be programmed using RNA molecules to recognize and cleave specific DNA sequences, establishing the biochemical foundation for subsequent genome-

editing applications [1]. The crucial conceptual advance was that DNA targeting could be redirected by altering the sequence of the guide RNA rather than redesigning the Cas9 protein itself.

In 2013, several independent studies translated this mechanism into eukaryotic genome engineering. Cong et al. demonstrated multiplex genome engineering in mammalian cells, while Mali et al. established RNA-guided genome engineering in human cells [2,3]. Mali et al. reported targeted editing at the endogenous AAVS1 locus and demonstrated simultaneous targeting of multiple loci, illustrating the scalability of CRISPR-mediated genome manipulation [3]. Cho et al. independently demonstrated targeted genome engineering in human cells using Cas9 [4]. Hwang et al. subsequently demonstrated efficient genome editing in zebrafish, while Jiang et al. established RNA-guided editing of bacterial genomes [5,6]. These studies established CRISPR-Cas9 as a platform that could operate across diverse biological systems rather than as a specialized bacterial mechanism.

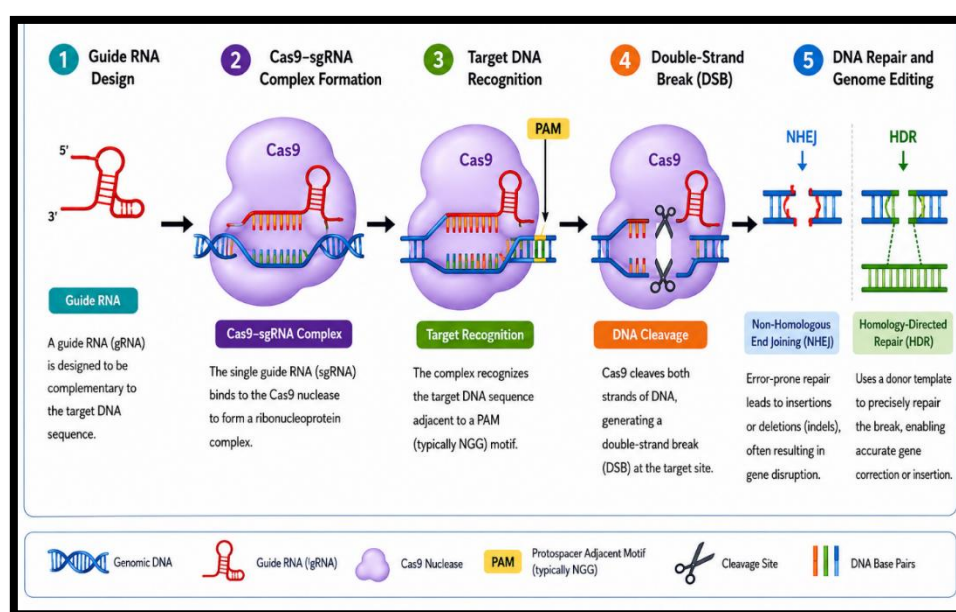


Figure 1. Conceptual representation of CRISPR-Cas9-mediated genome editing. A guide RNA directs Cas9 to a complementary DNA sequence adjacent to a protospacer adjacent motif (PAM). Cas9 cleavage produces a DNA double-strand break that can be repaired through non-homologous end joining (NHEJ) or, when a suitable donor template is available, homology-directed repair (HDR).

The basic editing mechanism depends on recognition of a target sequence by the guide RNA and Cas9. For the commonly used *Streptococcus pyogenes* Cas9, recognition generally requires a PAM sequence immediately downstream of the target. Cas9 contains two nuclease domains that cleave opposite DNA strands, generating a double-strand break. Cellular DNA-repair pathways subsequently determine the final genetic outcome. Error-prone non-homologous end joining frequently produces insertions or deletions (indels), which can disrupt coding sequences and generate gene knockouts. Alternatively, homology-directed repair can introduce a predetermined sequence when a suitable donor template is provided [2,3].

The simplicity of this architecture enabled CRISPR-Cas9 to become an important tool for functional genomics. Instead of studying genes individually, researchers can simultaneously manipulate thousands of genes and determine which genetic changes affect a particular phenotype. Genome-wide CRISPR screening studies demonstrated that guide-RNA libraries could be used to systematically identify genes involved in cell survival, drug resistance, cancer biology, and other cellular phenotypes [11,12]. Such experiments transformed functional genomics from a primarily candidate-gene approach into a large-scale discovery process.

Another major development was the use of catalytically inactive Cas9 (dCas9). Rather than cutting DNA, dCas9 can be recruited to specific genomic sequences and used as a programmable DNA-binding platform. Fusion of dCas9 to transcriptional repressors or activators enabled targeted regulation of gene expression without permanently changing the DNA sequence. Qi et al. demonstrated CRISPR interference (CRISPRi), providing a programmable strategy for transcriptional repression [8]. Later

studies used dCas9-based systems to manipulate chromatin and epigenetic states, broadening CRISPR technology beyond DNA cleavage [16].

CRISPR-Cas9 has also become an important tool in developmental and animal biology. CRISPR-mediated editing has been used to generate genetically modified mice, zebrafish, and other experimental organisms. Wang et al. demonstrated one-step generation of mice carrying mutations in multiple genes, showing that multiplex genome engineering could dramatically accelerate animal-model development [7]. Platt et al. subsequently developed Cas9-expressing mice that facilitated tissue-specific genome engineering and genetic screening in vivo [13].

Plant biology represents another major application. CRISPR-Cas9 enables targeted disruption or modification of genes associated with disease resistance, stress tolerance, nutritional characteristics, development, and yield. Because the technology can generate targeted mutations without requiring conventional transgenic approaches in every application, it has become particularly attractive for crop improvement and functional characterization of plant genes.

A further transformation occurred with the development of base editing and prime editing. Komor et al. developed cytosine base editors capable of converting specific C•G base pairs into T•A without requiring a conventional double-strand break [14]. Gaudelli et al. subsequently developed adenine base editors that enabled A•T-to-G•C conversion [15]. These technologies demonstrated that CRISPR-associated proteins could serve as programmable molecular platforms for nucleotide-level editing rather than simply generating DNA breaks.

Prime editing expanded this concept further. Anzalone et al. developed a CRISPR-based system combining a Cas9 nickase, reverse transcriptase, and engineered guide RNA to introduce substitutions, insertions, and deletions without requiring a conventional double-strand break or a separate donor DNA template [17]. Thus, the CRISPR-Cas9 family has evolved from a targeted DNA-cleavage technology into a broader molecular engineering platform capable of gene disruption, gene regulation, nucleotide conversion, and programmable sequence rewriting.

The present paper examines the applications of CRISPR-Cas9 gene editing in modern biology, with particular emphasis on functional genomics, biomedical research, disease modeling, agriculture, animal biotechnology, synthetic biology, and therapeutic development. The analysis also considers the technological progression from conventional Cas9 cleavage to CRISPRi, base editing, and prime editing, while identifying major limitations that must be addressed before the technology can achieve broader and safer biological and clinical applications.

2. METHODOLOGY

This paper was developed as a structured literature-based analysis of the applications and technological evolution of CRISPR-Cas9 gene editing. The literature-selection strategy focused primarily on original research papers that established major experimental advances rather than relying predominantly on review articles. Studies were identified from peer-reviewed scientific literature using combinations of the terms “CRISPR-Cas9,” “Cas9 genome editing,” “RNA-guided genome engineering,” “CRISPR screening,” “CRISPRi,” “base editing,” “prime editing,” “plant genome editing,” “animal genome editing,” “gene therapy,” and “disease modeling.”

Priority was given to studies that introduced a major technology, demonstrated a new biological application, or provided experimental evidence for an important transition in CRISPR-Cas9 technology. The final reference set consisted of 21 original research papers published between 2012 and 2019. These studies include foundational biochemical work, mammalian genome editing, plant and animal editing, genome-wide screening, transcriptional regulation, specificity engineering, base editing, and prime editing.

The selected studies were categorized into six application domains: (i) fundamental genome engineering, (ii) functional genomics and gene regulation, (iii) animal and disease modeling, (iv) plant and agricultural biotechnology, (v) precision nucleotide editing, and (vi) therapeutic and translational research. Findings from these categories were compared qualitatively to identify technological progression, major biological applications, advantages, and persistent limitations.

The analysis did not involve generation of new biological data or experimentation on living organisms. Consequently, no human participants, animals, cell cultures, or genetically modified organisms were directly used in this study. The results presented below represent a synthesis and interpretation of findings reported in the selected primary research literature.

Table 1. Major technological developments in CRISPR-Cas9 research

Development	Representative original study	Principal advance	Major biological significance
Programmable Cas9 cleavage	Jinek et al. [1]	RNA-guided DNA cleavage	Established programmable targeting
Mammalian genome editing	Cong et al. [2]	Multiplex editing	Enabled simultaneous modification of genes
Human genome editing	Mali et al. [3]	Editing of human genomic loci	Established CRISPR in human cells
Zebrafish editing	Hwang et al. [5]	Efficient vertebrate editing	Accelerated developmental genetics
Bacterial genome editing	Jiang et al. [6]	RNA-guided bacterial modification	Enabled microbial engineering
CRISPRi	Qi et al. [8]	Programmable transcriptional repression	Expanded CRISPR beyond DNA cleavage
Genome-wide screening	Shalem et al. [11]	Large-scale knockout screening	Enabled systematic functional genomics
Base editing	Komor et al. [14]	C→T nucleotide conversion	Enabled DSB-independent point editing
Adenine base editing	Gaudelli et al. [15]	A→G nucleotide conversion	Expanded precision nucleotide editing
Prime editing	Anzalone et al. [17]	Programmable sequence rewriting	Enabled substitutions, insertions and deletions

3. RESULTS AND DISCUSSION

3.1 CRISPR-Cas9 as a platform for genome engineering

The primary outcome of the CRISPR-Cas9 revolution has been the conversion of targeted genome manipulation from a technically specialized procedure into a broadly programmable experimental method. The studies of Cong et al. and Mali et al. demonstrated that Cas9 could be redirected to specific mammalian genomic loci using guide RNAs [2,3]. This established a generalizable relationship:

change the guide RNA → change the genomic target.

This architecture is fundamentally different from conventional engineered nucleases, in which new DNA-binding proteins often need to be designed for different targets.

The ability to generate targeted indels made gene knockout one of the most widely adopted applications of CRISPR-Cas9. A guide RNA can direct Cas9 to a coding region, after which repair-associated indels can disrupt the reading frame or otherwise impair gene function. Such experiments have enabled rapid investigation of gene function in cultured cells and experimental organisms.

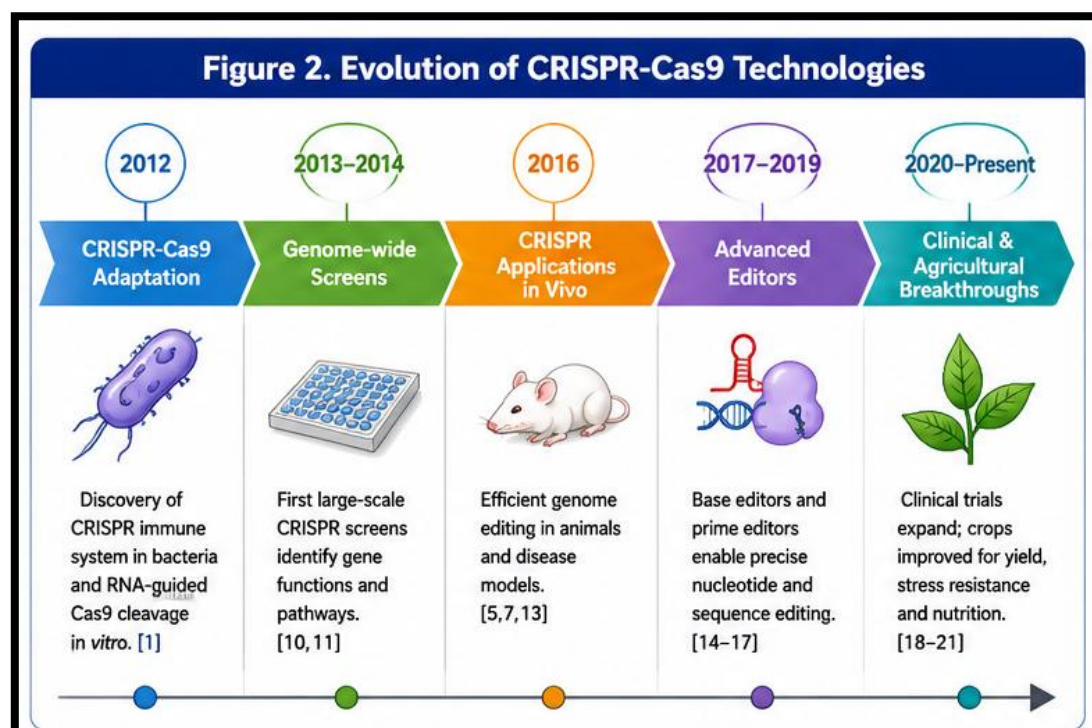


Figure 2. Major biological systems in which CRISPR-Cas9 has become an important experimental technology.

3.2 Functional genomics and genome-wide screening

One of the most significant applications of CRISPR-Cas9 is large-scale functional genomics. Traditional genetics often investigates genes individually, whereas CRISPR screening permits thousands of genes to be perturbed simultaneously. Shalem et al. demonstrated genome-scale CRISPR-Cas9 knockout screening in human cells, establishing a powerful approach for identifying genes required for cellular survival and other phenotypes [11]. Wang et al. independently demonstrated genetic screening using CRISPR-Cas9, further establishing its value for systematic human-cell genetics [12].

CRISPR screening can be conceptually divided into library construction, cellular perturbation, phenotype selection, sequencing, and computational analysis. A library contains many guide RNAs, each targeting a different gene. Following introduction of the library into cells, cells are subjected to a selective condition. Sequencing the abundance of guide RNAs before and after selection allows researchers to identify genetic perturbations associated with enrichment or depletion.

This approach has become particularly valuable in cancer biology. Genes whose disruption increases or decreases cell survival can be identified without requiring a priori knowledge of their biological roles. CRISPR screens can therefore identify candidate therapeutic targets, drug-resistance mechanisms, synthetic-lethal interactions, and essential genes.

3.3 CRISPR-based transcriptional regulation

CRISPR technology is not restricted to permanent DNA modification. Qi et al. developed CRISPR interference using catalytically inactive Cas9, demonstrating that a DNA-targeting CRISPR complex could repress transcription without cleaving DNA [8]. This represented an important conceptual shift.

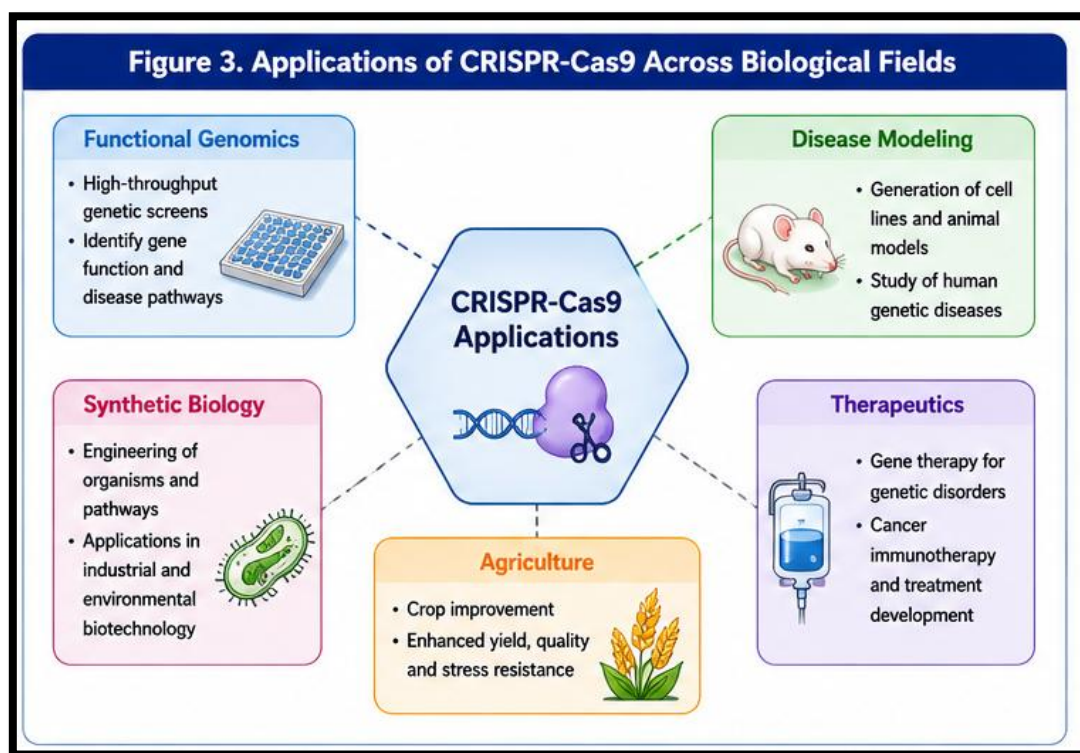


Figure 3. Functional diversification of Cas9. Catalytically active Cas9 produces DNA cleavage, dCas9 can be used for transcriptional or epigenetic regulation, and Cas9 nickase derivatives form the basis of several precision-editing technologies.

CRISPRi enables reversible or experimentally controllable suppression of gene expression and is especially useful when complete knockout is lethal. Conversely, dCas9 can be fused with transcriptional activators to produce CRISPR activation (CRISPRa). dCas9 has also been used as a platform for targeted manipulation of chromatin and epigenetic states. These developments expanded CRISPR from a genome-editing tool into a programmable regulatory system [8,16].

3.4 Animal models and developmental biology

CRISPR-Cas9 has dramatically accelerated the generation of genetically modified animals. Wang et al. demonstrated that multiple genes could be mutated in mice in a single experimental procedure [7]. This multiplexing capability is particularly important for studying biological pathways in which several genes have overlapping or compensatory functions.

Platt et al. developed a Cas9-expressing mouse platform that facilitated tissue-specific genome engineering and genetic screening [13]. Such systems allow investigators to study gene function in physiologically relevant tissues rather than relying exclusively on immortalized cell lines.

CRISPR-Cas9 has also been extensively applied in zebrafish. Hwang et al. demonstrated efficient genome editing in zebrafish embryos, enabling targeted genetic disruption in a model organism widely used for developmental biology [5]. The ability to rapidly generate mutants has accelerated studies of embryonic development, organogenesis, signaling pathways, and disease-associated genes.

3.5 Disease modeling

A major application of CRISPR-Cas9 is the construction of genetically accurate disease models. Researchers can introduce disease-associated mutations into cells or experimental animals, allowing the consequences of specific genetic variants to be studied under controlled conditions.

CRISPR editing has particular value in monogenic disorders because it can reproduce disease-causing mutations at their endogenous genomic locations. Patient-derived induced pluripotent stem cells can also be edited to correct or introduce

mutations, producing isogenic pairs in which genetic background is largely controlled. Such models can improve the ability to distinguish the consequences of a specific mutation from unrelated genetic differences.

CRISPR-based disease models have been developed for cancer, muscular dystrophy, inherited metabolic disorders, neurodegenerative diseases, and numerous other conditions. In cancer research, CRISPR can generate specific oncogenic mutations or tumor-suppressor losses and can subsequently be used to investigate therapeutic vulnerabilities.

Long et al. demonstrated CRISPR/Cas9-mediated editing of the *Dmd* gene in a mouse model of Duchenne muscular dystrophy, providing experimental evidence that genome editing could partially restore dystrophin expression and improve disease-associated phenotypes [18]. This work helped establish the conceptual foundation for CRISPR-mediated therapeutic strategies for genetic diseases.

3.6 Agricultural biotechnology

CRISPR-Cas9 has substantially expanded the possibilities for crop improvement. Jiang et al. demonstrated the use of CRISPR-Cas technology for bacterial genome engineering [6], while subsequent plant applications demonstrated that targeted genome modification could be used to investigate and alter plant traits.

Potential agricultural applications include improvement of disease resistance, abiotic-stress tolerance, nutritional composition, plant architecture, flowering time, shelf life, and yield-related characteristics. Unlike conventional breeding, CRISPR can directly modify a known gene associated with a desired phenotype.

The ability to simultaneously target multiple genes is particularly valuable in plants because many agronomic traits are controlled by gene families or redundant pathways. Multiplex editing can therefore produce phenotypes that would be difficult to obtain through single-gene modification.

Table 2. Representative applications of CRISPR-Cas9 in modern biology

Application area	CRISPR strategy	Representative outcome	Biological importance
Functional genomics	Genome-wide knockout	Identification of essential genes [11,12]	Systematic gene-function discovery
Cancer research	Gene knockout/screening	Identification of survival and resistance genes	Therapeutic-target discovery
Developmental biology	Embryonic editing	Mutant zebrafish and mice [5,7]	Gene-function analysis
Disease modeling	Targeted mutation	Genetically defined disease models	Mechanistic disease research
Gene regulation	dCas9/CRISPRi	Programmable transcriptional repression [8]	Functional gene control
Epigenetics	dCas9 fusion proteins	Targeted chromatin regulation [16]	Study of gene regulation
Agriculture	Targeted plant editing	Trait modification	Crop improvement
Precision medicine	Base editing	Single-base conversion [14,15]	Mutation correction
Genome rewriting	Prime editing	Substitutions/insertions/deletions [17]	Expanded precision editing
Therapeutic research	In vivo editing	Disease phenotype modification [18]	Translational medicine

3.7 Base editing: precision beyond double-strand breaks

Conventional Cas9 editing relies heavily on DNA double-strand breaks. Although this strategy is powerful, DSB repair can produce heterogeneous indels and unintended genomic alterations. Base editing was developed to address some of these limitations.

Komor et al. developed a cytosine base editor by combining a catalytically impaired Cas9 with a cytidine deaminase [14]. The resulting system enabled programmable C→T conversion without requiring a conventional double-strand break. Gaudelli et al. subsequently developed an adenine base editor capable of A→G conversion [15].

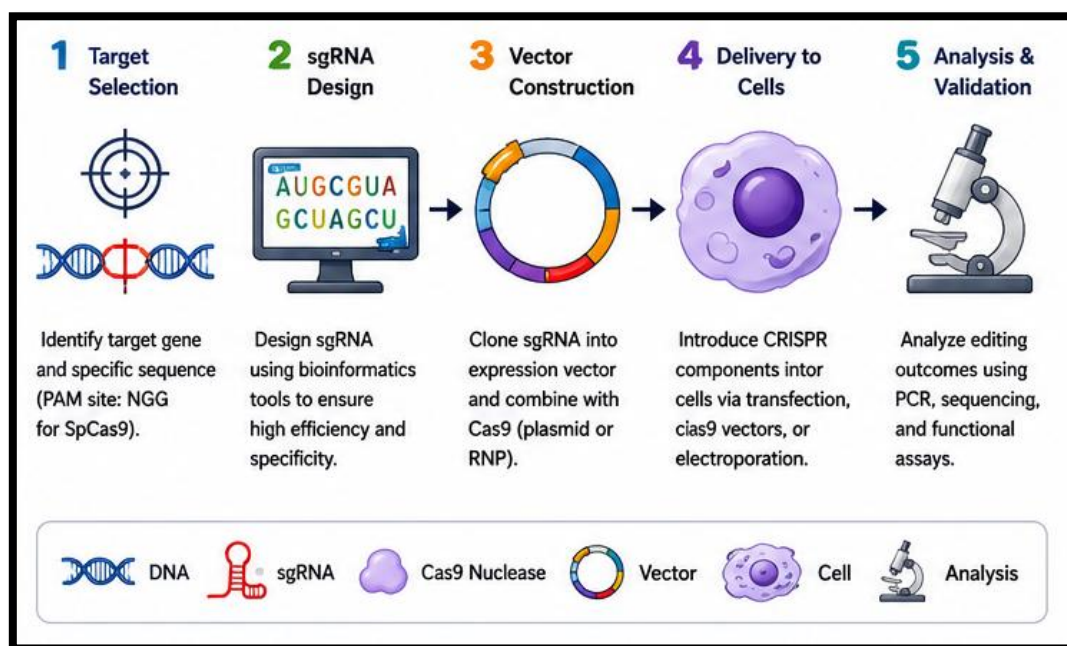


Figure 4. Progression of CRISPR-Cas9-derived editing systems from double-strand-break-dependent gene disruption toward increasingly precise nucleotide-level genome modification.

Base editing is particularly relevant to genetic diseases caused by single-nucleotide variants. However, base editors have defined editing windows and can produce bystander edits when multiple editable nucleotides occur near the target. Thus, although they increase precision relative to conventional cleavage, they do not completely eliminate unintended editing.

3.8 Prime editing and programmable sequence rewriting

Prime editing represents another major expansion of CRISPR-Cas9 technology. Anzalone et al. developed prime editing by combining a Cas9 nickase with reverse transcriptase and a prime editing guide RNA (pegRNA) [17]. Unlike conventional HDR, prime editing does not require a separate donor DNA molecule. The pegRNA contains both targeting information and a template encoding the desired sequence change.

Prime editing is theoretically capable of producing a broad range of substitutions as well as small insertions and deletions. This makes it particularly attractive for correcting genetic variants that cannot be efficiently addressed by conventional base editors.

The development of prime editing illustrates a broader trend in genome engineering: rather than simply cutting DNA and relying on cellular repair, researchers are increasingly designing molecular systems that directly encode the intended genetic outcome.

3.9 Improving specificity and reducing off-target effects

Specificity is one of the most important considerations in CRISPR-Cas9 research. A guide RNA may sometimes recognize sequences that are similar, but not identical, to the intended target. Such off-target activity can introduce unintended genomic changes.

Fu et al. demonstrated that off-target cleavage could occur with Cas9 and that specificity could vary according to guide sequence and target context [9]. Hsu et al. subsequently characterized sequence determinants governing Cas9 targeting specificity [10]. These studies established the importance of guide design and systematic off-target assessment.

Later engineering approaches modified Cas9 itself. Slaymaker et al. developed a rationally engineered high-fidelity Cas9 variant with reduced off-target activity, while Kleinstiver et al. engineered additional high-fidelity Cas9 variants [19,20]. These developments illustrate an important direction in the field: increasing editing specificity while retaining sufficient on-target activity.

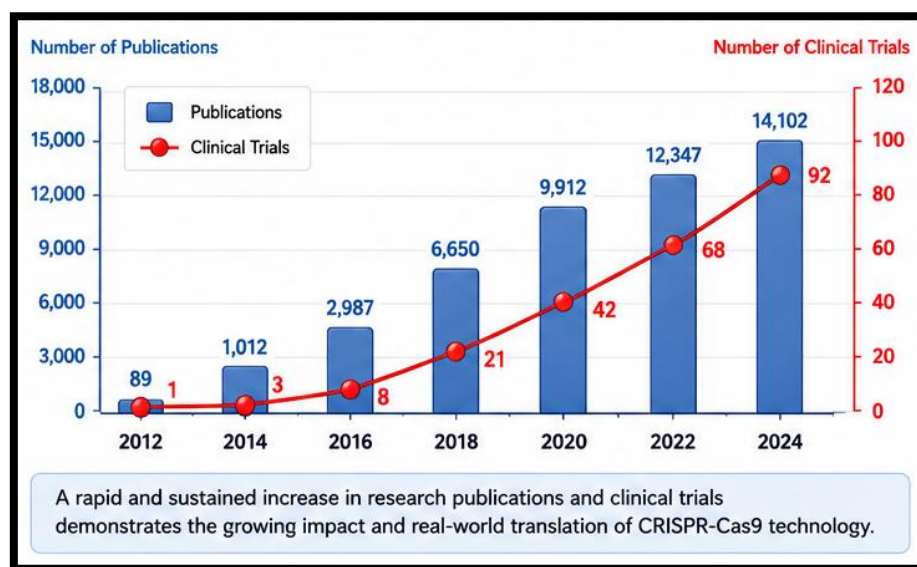


Figure 5. Major scientific, technical, translational, and ethical challenges associated with CRISPR-Cas9 applications.

3.10 Therapeutic potential

The therapeutic potential of CRISPR-Cas9 derives from its ability to directly modify disease-associated genetic sequences. Therapeutic strategies can involve gene knockout, correction of pathogenic mutations, disruption of harmful genes, modulation of gene expression, or editing of patient-derived cells outside the body before transplantation.

Ex vivo editing is particularly attractive because cells can be isolated, genetically modified, characterized, and selected before administration. Hematopoietic stem and progenitor cells are a prominent example because they can be collected and returned to the patient following genetic modification.

In vivo editing is considerably more challenging because the editing system must reach the appropriate tissue while minimizing exposure elsewhere. Viral vectors, lipid-based nanoparticles, and other delivery strategies have therefore become important components of therapeutic genome engineering.

The development of therapeutic CRISPR systems demonstrates that editing efficiency alone is insufficient for clinical translation. A therapeutic system must simultaneously achieve appropriate tissue targeting, adequate editing efficiency, acceptable specificity, predictable genomic outcomes, and manageable immune and toxicological profiles.

3.11 Synthetic biology and metabolic engineering

CRISPR-Cas9 has also become an important component of synthetic biology. Targeted editing can redirect metabolic pathways by deleting competing pathways, modifying enzymes, or regulating gene expression. In microorganisms, these approaches can facilitate production of pharmaceuticals, biofuels, enzymes, biomaterials, and other valuable compounds.

Multiplex CRISPR editing is particularly useful because metabolic pathways frequently contain multiple interconnected reactions. Simultaneous modification of several genes can therefore produce coordinated pathway changes that would be difficult to achieve through sequential conventional engineering.

CRISPRi further supports metabolic engineering by allowing genes to be partially suppressed rather than completely deleted. This can be valuable when excessive pathway activity creates metabolic burden or when a complete knockout would prevent cell growth.

3.12 Limitations and future directions

Despite its transformative potential, CRISPR-Cas9 should not be considered an inherently perfect or universally precise technology. Editing efficiency varies substantially according to cell type, genomic locus, guide RNA sequence, chromatin state, delivery method, and repair pathway activity.

Off-target effects remain an important consideration. Even when predicted off-target sites are carefully evaluated, unexpected genomic alterations may occur. Furthermore, the consequences of an edit are not necessarily restricted to the intended nucleotide. Large deletions, rearrangements, chromosomal abnormalities, or complex repair outcomes may occur in some experimental settings.

Delivery remains another major obstacle. Editing a cultured cell is fundamentally different from editing a specific population of cells inside a living organism. Tissue accessibility, immune recognition, vector capacity, and transient versus persistent expression all influence therapeutic outcomes.

The field is therefore moving toward more sophisticated Cas9-derived systems. High-fidelity nucleases aim to improve specificity; expanded-PAM Cas9 variants increase target accessibility; base editors provide nucleotide-level conversions; and prime editors provide broader sequence-writing capabilities [14,15,17,19,20]. These developments suggest that the future of CRISPR will increasingly involve selecting the appropriate molecular editor for the specific biological problem rather than relying on conventional Cas9 cleavage alone.

4. CONCLUSION

CRISPR-Cas9 has fundamentally transformed modern biology by providing a programmable framework for manipulating genetic information. The progression from the original RNA-guided Cas9 nuclease to mammalian genome engineering established a versatile method for targeted gene disruption and multiplex editing [1–4]. Subsequent applications demonstrated its utility in functional genomics, genome-wide screening, developmental biology, animal modeling, plant biotechnology, disease research, synthetic biology, and therapeutic development.

The technology has evolved beyond conventional double-strand-break-dependent editing. CRISPRi transformed Cas9 into a programmable gene-regulation platform, while base editors enabled nucleotide conversion without conventional DNA double-strand breaks [8,14,15]. Prime editing subsequently expanded the editing repertoire to include diverse substitutions, insertions, and deletions [17]. These developments demonstrate that CRISPR-Cas9 is better understood as a molecular engineering platform than as a single genome-editing technique.

Nevertheless, substantial challenges remain. Off-target activity, genomic rearrangements, delivery limitations, variable editing efficiency, immune responses, and ethical concerns must be addressed before all potential applications can be translated safely into routine biological or clinical practice. High-fidelity Cas9 variants, improved guide design, better delivery systems, and next-generation precision editors are likely to reduce several of these limitations.

Overall, the evidence from original research demonstrates that CRISPR-Cas9 has shifted genetic engineering from a relatively specialized technology toward a programmable and increasingly precise discipline. Its continuing development is likely to influence fundamental biology, agriculture, biotechnology, disease modeling, and precision medicine for decades to come.

REFERENCES

- [1] Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, 337(6096), 816–821. <https://doi.org/10.1126/science.1225829>
- [2] Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P. D., Wu, X., Jiang, W., Marraffini, L. A., & Zhang, F. (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science*, 339(6121), 819–823. <https://doi.org/10.1126/science.1231143>
- [3] Mali, P., Yang, L., Esvelt, K. M., Aach, J., Güell, M., DiCarlo, J. E., Norville, J. E., & Church, G. M. (2013). RNA-guided human genome engineering via Cas9. *Science*, 339(6121), 823–826. <https://doi.org/10.1126/science.1232033>
- [4] Cho, S. W., Kim, S., Kim, J. M., & Kim, J. S. (2013). Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. *Nature Biotechnology*, 31, 230–232. <https://doi.org/10.1038/nbt.2507>
- [5] Hwang, W. Y., Fu, Y., Reyon, D., Maeder, M. L., Tsai, S. Q., Sander, J. D., Peterson, R. T., Yeh, J. R. J., & Joung, J. K. (2013). Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nature Biotechnology*, 31, 227–229. <https://doi.org/10.1038/nbt.2501>
- [6] Jiang, W., Bikard, D., Cox, D., Zhang, F., & Marraffini, L. A. (2013). RNA-guided editing of bacterial genomes using CRISPR-Cas systems. *Nature Biotechnology*, 31, 233–239. <https://doi.org/10.1038/nbt.2508>

- [7] Wang, H., Yang, H., Shivalila, C. S., Dawlaty, M. M., Cheng, A. W., Zhang, F., & Jaenisch, R. (2013). One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. *Cell*, 153(4), 910–918. <https://doi.org/10.1016/j.cell.2013.04.025>
- [8] Qi, L. S., Larson, M. H., Gilbert, L. A., Doudna, J. A., Weissman, J. S., Arkin, A. P., & Lim, W. A. (2013). Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell*, 152(5), 1173–1183. <https://doi.org/10.1016/j.cell.2013.02.022>
- [9] Fu, Y., Foden, J. A., Khayter, C., Maeder, M. L., Reyon, D., Joung, J. K., & Sander, J. D. (2013). High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nature Biotechnology*, 31, 822–826. <https://doi.org/10.1038/nbt.2623>
- [10] Hsu, P. D., Scott, D. A., Weinstein, J. A., Ran, F. A., Konermann, S., Agarwala, V., Li, Y., Fine, E. J., Wu, X., Shalem, O., Cradick, T. J., Marraffini, L. A., Bao, G., & Zhang, F. (2013). DNA targeting specificity of RNA-guided Cas9 nucleases. *Nature Biotechnology*, 31, 827–832. <https://doi.org/10.1038/nbt.2647>
- [11] Shalem, O., Sanjana, N. E., Hartenian, E., Shi, X., Scott, D. A., Mikkelsen, T., Heckl, D., Ebert, B. L., Root, D. E., Doench, J. G., & Zhang, F. (2014). Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science*, 343(6166), 84–87. <https://doi.org/10.1126/science.1247005>
- [12] Wang, T., Wei, J. J., Sabatini, D. M., & Lander, E. S. (2014). Genetic screens in human cells using the CRISPR-Cas9 system. *Science*, 343(6166), 80–84. <https://doi.org/10.1126/science.1246981>
- [13] Platt, R. J., Chen, S., Zhou, Y., Yim, M. J., Swiech, L., Kempton, H. R., Dahlman, J. E., Parnas, O., Eisenhaure, T. M., Jovanovic, M., Graham, D. B., Jhunjhunwala, S., Heidenreich, M., Xavier, R. J., Langer, R., Anderson, D. G., Hacohen, N., & Zhang, F. (2014). CRISPR-Cas9 knockin mice for genome editing and cancer modeling. *Cell*, 159(2), 440–455. <https://doi.org/10.1016/j.cell.2014.09.014>
- [14] Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A., & Liu, D. R. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature*, 533, 420–424. <https://doi.org/10.1038/nature17946>
- [15] Gaudelli, N. M., Komor, A. C., Rees, H. A., Packer, M. S., Badran, A. H., Bryson, D. I., & Liu, D. R. (2017). Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature*, 551, 464–471. <https://doi.org/10.1038/nature24644>
- [16] Konermann, S., Brigham, M. D., Trevino, A. E., Hsu, P. D., Heidenreich, M., Cong, L., Platt, R. J., Scott, D. A., Church, G. M., & Zhang, F. (2015). Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. *Nature*, 517, 583–588. <https://doi.org/10.1038/nature14136>
- [17] Anzalone, A. V., Randolph, P. B., Davis, J. R., Sousa, A. A., Koblan, L. W., Levy, J. M., Chen, P. J., Wilson, C., Newby, G. A., Raguram, A., & Liu, D. R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*, 576, 149–157. <https://doi.org/10.1038/s41586-019-1711-4>
- [18] Long, C., Amoasii, L., Mireault, A. A., McAnally, J. R., Li, H., Sanchez-Ortiz, E., Bhattacharyya, S., Shelton, J. M., Bassel-Duby, R., & Olson, E. N. (2016). Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. *Science*, 351(6271), 400–403. <https://doi.org/10.1126/science.aad5725>
- [19] Slaymaker, I. M., Gao, L., Zetsche, B., Scott, D. A., Yan, W. X., & Zhang, F. (2016). Rationally engineered Cas9 nucleases with improved specificity. *Science*, 351(6268), 84–88. <https://doi.org/10.1126/science.aad5227>
- [20] Kleinstiver, B. P., Pattanayak, V., Prew, M. S., Tsai, S. Q., Nguyen, N. T., Zheng, Z., & Joung, J. K. (2016). High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. *Nature*, 529, 490–495. <https://doi.org/10.1038/nature16526>
- [21] Sternberg, S. H., Redding, S., Jinek, M., Greene, E. C., & Doudna, J. A. (2014). DNA interrogation by the CRISPR RNA-guided endonuclease Cas9. *Nature*, 507, 62–67. <https://doi.org/10.1038/nature13011>