BIHAR SHODH SAMAAGAM
Multidisciplinary, Multi-Lingual, Peer Reviewed Open Access Journal
issn : 3048-6971
Vol. 3, Issue 1, January-March 2025
Available online : https://www.biharshodhsamaagam.com/

Effects of CRISPR-Cas9 on Gene Expression in Model Organisms

Dr. Suman Swaraj
Assistant Professor
Department of Zoology
S N S R K S College ,Saharsa
B N Mandal University Madhepura

Introduction

CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats–CRISPR-associated protein 9) is a revolutionary gene-editing technology that enables scientists to make highly precise changes to the DNA of living organisms. At its core, CRISPR-Cas9 functions like molecular scissors: it can cut DNA at a desired location, allowing genes to be added, removed, or altered with unprecedented accuracy. This powerful tool is derived from a natural defense mechanism used by bacteria and archaea to combat invading viruses. These microbes incorporate segments of viral DNA into their own genome within the CRISPR array, creating a memory of past infections. When the virus attacks again, the bacteria transcribe the stored sequences into RNA, which guides the Cas9 enzyme to the matching DNA of the virus, enabling its destruction.

In 2012, researchers Jennifer Doudna and Emmanuelle Charpentier successfully adapted this bacterial immune system into a programmable genome-editing tool that could be used in virtually any organism. This adaptation made it possible to selectively edit genes in animals, plants, and even humans, revolutionizing genetic research and biotechnology.

The CRISPR-Cas system was first identified in the 1980s in Escherichia coli as an unusual arrangement of repetitive DNA sequences. In 2007, researchers established that CRISPR-Cas functions as an adaptive immune system in bacteria, enabling them to defend against viral infections. In 2012, Jennifer Doudna and Emmanuelle Charpentier demonstrated the potential of the CRISPR-Cas9 system for programmable gene editing in vitro, a breakthrough that earned them the Nobel Prize in Chemistry in 2020.Since then, CRISPR has been rapidly adopted in eukaryotic genome editing, enabling targeted manipulation of genes in animals, plants, and even humans.

Importance of Gene Editing in Zoological Research

Gene editing is a powerful approach to study gene function and regulation, allowing scientists to observe phenotypic outcomes of specific genetic changes. In zoological research, gene editing is instrumental in:

- Understanding developmental pathways
- Investigating gene-disease relationships
- Uncovering evolutionary and behavioral mechanisms
- Exploring adaptive traits and biodiversity

With CRISPR-Cas9, researchers can create animal models of human diseases, elucidate gene networks, and even explore synthetic biology avenues by designing organisms with novel traits.Before CRISPR, techniques like zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) were used for genome editing. However, these methods were often labor-intensive, expensive, and technically complex. CRISPR-Cas9 quickly became the preferred gene-editing method because of its simplicity, cost-effectiveness, and flexibility.CRISPR allows scientists to:

- Silence or knock out genes to study their function.
- Insert or repair genes to correct genetic disorders.
- Control gene expression levels by modulating promoter regions.
- Study non-coding RNA functions and epigenetic regulation.

Because of these capabilities, CRISPR has transformed fields like genetics, developmental biology, oncology, immunology, neuroscience, and agriculture.Zoology, the scientific study of animals, has been greatly enhanced by CRISPR technology. Animals are complex organisms with intricate interactions between their genes, physiology, and environment. Understanding these interactions at the molecular level requires the ability to manipulate genes and observe resulting changes.

In zoology, gene editing with CRISPR allows for:

- Functional genomics: Identifying and understanding the role of specific genes in animal development, behavior, and physiology.
- Disease modeling: Creating animal models that mimic human diseases, including cancer, neurological disorders, and genetic syndromes.
- Behavioral genetics: Studying how genes influence behavior, cognition, and neurobiology.
- Evolutionary biology: Exploring how gene variations lead to adaptive traits and speciation.
- Developmental biology: Investigating how genes control embryonic development and organ formation.

CRISPR is also pivotal in conservation zoology, where it is being explored as a means to combat extinction, restore endangered species, and reduce the impact of invasive organisms.

In biology, model organisms are species that are widely studied to understand particular biological processes. These organisms are selected because they are:

- Easy to breed and maintain in laboratory conditions.
- Genetically tractable (i.e., their genomes can be easily manipulated).
- Well-annotated genetically with existing tools and databases.
- Representative of broader biological principles.

Model organisms have played a crucial role in virtually every major discovery in genetics and molecular biology. The advent of CRISPR-Cas9 has enhanced their utility even further.

Key Model Organisms:

- Drosophila melanogaster (fruit fly)
- Danio rerio (zebrafish)
- Mus musculus (mouse)
- Caenorhabditis elegans (nematode worm)

Each of these organisms provides unique advantages, and CRISPR-Cas9 allows researchers to precisely alter their genomes to investigate the role of specific genes. By targeting genes and observing the resulting phenotypic changes, researchers can draw conclusions about gene function and regulation, not only in the model organism but also in higher animals and humans due to evolutionary conservation of genetic pathways.

Gene expression is the process by which information from a gene is used to synthesize functional gene products such as proteins or regulatory RNAs. Changes in gene expression can alter cell behavior, development, physiological functions, and disease states.

CRISPR-Cas9 can be used to:

- Knock out genes and study the resulting loss of expression.
- Insert genes or regulatory elements and study gain-of-function effects.
- Modify promoter regions to alter transcription levels.
- Investigate epigenetic changes and transcriptional regulation.

In model organisms, such manipulations help uncover the mechanisms that control tissue differentiation, organ development, behavior, aging, and disease progression.Model organisms serve as simplified systems to investigate complex biological phenomena. CRISPR has opened new avenues to study gene expression dynamically and precisely in these models, leading to groundbreaking insights into fundamental biological processes.

- **CRISPR-Cas9 Mechanism**

The CRISPR-Cas system originated as an adaptive immune response in prokaryotes—mainly bacteria and archaea—to defend against viral infections, especially bacteriophages. When a bacterium is attacked by a virus, it can capture fragments of the invader's DNA and integrate them into its own genome within the CRISPR loci, forming what are known as spacers. These spacers are interspersed between short repeat sequences, creating a pattern that gives CRISPR its name.When the same virus invades again, the bacterium transcribes the spacer DNA into small RNA fragments, known as crRNAs (CRISPR RNAs). These crRNAs, in combination with tracrRNAs (trans-activating CRISPR RNAs), guide the Cas (CRISPR-associated) protein, particularly Cas9, to the viral DNA. The Cas9 protein then cleaves the viral DNA at the targeted location, rendering the virus harmless.This precise, programmable defense mechanism inspired scientists to repurpose the system for gene editing in eukaryotic organisms.

- Components of the CRISPR-Cas9 System

The laboratory-adapted CRISPR-Cas9 system consists of two primary components:

- Cas9 Enzyme

Cas9 is an RNA-guided DNA endonuclease enzyme that introduces double-strand breaks (DSBs) at specific locations within the DNA. It is derived from Streptococcus pyogenes and has become the most commonly used Cas protein. Cas9 contains two active domains (RuvC and HNH) responsible for cleaving each strand of the DNA.

- Guide RNA (gRNA)

The guide RNA is a synthetic fusion of two RNA molecules (crRNA and tracrRNA) into a single structure known as single-guide RNA (sgRNA).

- PAM Sequence

The Protospacer Adjacent Motif (PAM) is a short DNA sequence immediately downstream of the target site. Cas9 recognizes the PAM sequence (typically 5′-NGG-3′ for SpCas9), and only cuts DNA where PAM is present, ensuring specificity.

- Targeting and Cutting DNA

The gene editing process using CRISPR-Cas9 occurs as follows:

1. Target Site Identification

The sgRNA binds to the target DNA by base pairing. This occurs only if a PAM sequence is present next to the target site.

2. DNA Binding and Cleavage

Cas9, guided by sgRNA, binds to the target DNA and creates a double-strand break at the desired location.

3. Cellular Repair Mechanisms

Once the DNA is cut, the cell attempts to repair the break using one of two pathways:

- Non-Homologous End Joining (NHEJ):

This is an error-prone process that often introduces insertions or deletions (indels) at the cut site. These indels can disrupt the gene, leading to gene knockout.

- Homology-Directed Repair (HDR):

If a repair template (donor DNA) is supplied, the cell can use this template to precisely repair the break. This allows for gene knock-in, where new DNA sequences can be introduced.

➢ **CRISPR Variants and Advanced Systems**

Several advancements have enhanced the versatility of CRISPR-Cas9:

▪ dCas9 (Catalytically Inactive Cas9):

Binds DNA but does not cut. Used to block transcription or recruit transcriptional regulators.

▪ CRISPRa/CRISPRi (CRISPR activation/interference):

Fusion of dCas9 with transcriptional activators (e.g., VP64) or repressors (e.g., KRAB) to modulate gene expression levels.

▪ Base Editing:

Directly converts one DNA base to another (e.g., C→T) without double-strand breaks.

▪ Prime Editing:

A more precise and flexible editing technique that allows insertions, deletions, and base substitutions with fewer off-target effects.

➢ **Delivery Methods**

Introducing CRISPR-Cas9 components into the cells of model organisms requires efficient delivery systems. Common delivery methods include:

▪ Microinjection:

Direct injection of Cas9 mRNA/protein and sgRNA into embryos (used in mice, zebrafish, etc.).

▪ Electroporation:

Uses an electric field to introduce CRISPR components into cells.

▪ Viral Vectors (e.g., Adeno-Associated Virus, Lentivirus):

Used for in vivo delivery, especially in mammalian models.

▪ Lipid Nanoparticles and Liposomes:

Non-viral, chemical methods for delivering CRISPR components.

Each method has advantages and limitations, often depending on the organism, tissue type, and editing goal.

- **Limitations of the Mechanism**

Despite its power, CRISPR-Cas9 is not without flaws:

- Off-target effects: Unintended cuts at genomic locations with similar sequences can cause mutations.
- Mosaicism: In multicellular organisms, especially when editing embryos, not all cells may receive the same genetic change.
- Incomplete editing: Not all target cells may be successfully edited, especially in somatic tissues.

Researchers continue to refine CRISPR technologies to reduce these limitations and increase precision.

- **Impact on Gene Expression**

The primary utility of CRISPR-Cas9 in biological research lies in its ability to precisely manipulate gene expression. By introducing targeted mutations or regulatory changes, scientists can either silence, enhance, or modify gene activity. This section explores how CRISPR-Cas9 affects gene expression at various molecular levels and what implications it has for functional genomics, disease modeling, and therapeutic innovation.

- **Gene Knockout and Loss-of-Function Studies**

CRISPR-Cas9 introduces double-strand breaks (DSBs) at specific genomic sites. When the cell repairs these breaks via the non-homologous end joining (NHEJ) pathway, small insertions or deletions (indels) often occur at the target site. These indels can disrupt the open reading frame (ORF), leading to a premature stop codon or frameshift mutation that results in a loss of gene function.

Impact:

- Complete silencing of the gene allows researchers to observe the phenotypic consequences of gene inactivation.
- Knockout models in organisms like mice, zebrafish, and Drosophila help in identifying essential genes and disease pathways.
- Conditional knockouts allow for tissue-specific or stage-specific gene expression studies.

- **Gene Activation and CRISPRa Systems**

Modified CRISPR systems such as CRISPR activation (CRISPRa) use a catalytically inactive version of Cas9 (dCas9) fused to transcriptional activators like VP64, p65, or Rta. When guided to a promoter or enhancer region, these complexes upregulate endogenous gene expression without modifying the DNA sequence.

Impact:

- Allows gain-of-function studies by enhancing the transcription of target genes.
- Used to explore the dose-dependent effects of gene expression.
- Ideal for drug target validation and identifying genes that suppress or promote disease states.

- **Gene Repression and CRISPRi Systems**

Conversely, CRISPR interference (CRISPRi) employs dCas9 fused to transcriptional repressors like KRAB (Krüppel-associated box). This complex binds to promoters or enhancers to block transcription initiation or elongation, thereby downregulating gene expression.

Impact:

- Offers reversible and tunable gene silencing without creating permanent mutations.
- Minimizes off-target effects seen in traditional knockouts.
- Useful in epigenetic research and non-coding RNA studies.

➢ **Fine-Tuning Gene Expression**

CRISPR can be engineered to modulate gene expression rather than switching genes fully on or off. This nuanced control helps in:

- Studying gene dosage effects.
- Analyzing regulatory thresholds in signaling pathways.
- Simulating heterozygous mutations found in many human diseases.
- Tools and Techniques:
- Use of titrated guide RNA expression.
- Integration of inducible systems like Tet-on/off or heat shock promoters.
- Employing chemical or light-sensitive dCas9 variants.

➢ **Alternative Splicing and Isoform Regulation**

CRISPR can target splice sites, intron-exon junctions, or regulatory elements controlling splicing. This allows:

- Switching between isoforms of a gene.
- Mimicking splicing mutations associated with genetic disorders (e.g., spinal muscular atrophy, cancer).
- Investigating the functional role of different transcript variants.

➢ **Modulating Non-Coding RNAs**

Beyond protein-coding genes, CRISPR-Cas9 can also regulate non-coding RNA genes, including:

- Long non-coding RNAs (lncRNAs): Implicated in gene regulation, chromatin remodeling, and development.
- MicroRNAs (miRNAs): Small RNAs that fine-tune gene expression post-transcriptionally.
- Enhancer RNAs (eRNAs): Transcripts from enhancer regions that influence nearby gene expression.

By editing these elements or regulating their expression with dCas9, researchers can study how non-coming regions orchestrate gene networks.

➢ **Epigenetic Regulation**

Fusing dCas9 with epigenetic modifiers such as:

- DNA methyltransferases (DNMT3A) for adding methylation marks.
- TET demethylases for removing them.
- Histone acetyltransferases (HATs) or deacetylases (HDACs) to control chromatin accessibility.

These tools enable precise epigenome editing, which can lead to heritable changes in gene expression without altering the DNA sequence.

➢ **Off-Target Effects on Gene Expression**

One of the major concerns with CRISPR-Cas9 is its potential to bind and cut non-targeted genomic regions, especially those with sequences similar to the sgRNA. These off-target edits may unintentionally:

- Disrupt nearby genes or regulatory elements.

- Activate oncogenes or silence tumor suppressor genes.
- Cause epigenetic shifts or transcriptional noise.

Mitigation Strategies:

- Designing highly specific sgRNAs.
- Using Cas9 variants with higher fidelity (e.g., eSpCas9, SpCas9-HF1).
- Employing off-target prediction algorithms and deep sequencing to verify accuracy.

➢ **Transcriptomic and Proteomic Changes**

Gene editing can produce ripple effects on the transcriptome and proteome. Even a single-gene knockout may lead to:

- Compensatory upregulation or downregulation of related genes.
- Changes in protein interaction networks.
- Alterations in metabolic pathways or signaling cascades.

Detection Tools:

- RNA-seq for whole-transcriptome analysis.
- Mass spectrometry and Western blotting for proteomic profiling.
- ChIP-seq to identify changes in transcription factor binding and chromatin state.

➢ **Functional Genomics and Systems Biology**

CRISPR is revolutionizing systems-level studies of gene expression. Researchers can:

- Perform CRISPR screens to identify essential genes for specific pathways.
- Map gene regulatory networks by perturbing transcription factors and cofactors.
- Model genetic interactions (epistasis) by multiplex editing of related genes.

These approaches provide a comprehensive view of cellular functions and enable predictive modeling of gene expression outcomes.

- **Challenges and Ethical Considerations**

While CRISPR-Cas9 has revolutionized gene editing and functional genomics, it also brings forth numerous scientific challenges and deep ethical concerns. As its applications expand, especially in modifying gene expression in living organisms, it becomes crucial to critically evaluate its limitations, potential risks, and moral implications. This section examines the main challenges and the ethical landscape surrounding the use of CRISPR-Cas9 in model organisms and beyond.

➢ **Technical Challenges**

- Off-Target Effects

One of the most significant limitations of CRISPR-Cas9 is its potential for unintended DNA cleavage at sites that resemble the target sequence. These off-target mutations can:

- Disrupt other genes or regulatory elements.
- Cause unexpected changes in gene expression.
- Lead to phenotypes unrelated to the intended gene knockout.

- Mitigation Strategies:

Use of high-fidelity Cas9 variants (e.g., eSpCas9, SpCas9-HF1).

Careful guide RNA (gRNA) design using bioinformatic tools.

Whole-genome sequencing to verify specificity.

- Mosaicism
- Delivery Efficiency

➢ **Biological Limitations**

- Functional Redundancy

Knocking out a gene does not always produce a clear phenotype due to functional redundancy—where related genes compensate for the loss. This complicates:

- Interpretation of gene function.
- Understanding of gene expression dynamics in networks.

- **Genetic Compensation**

Some organisms initiate transcriptional compensation mechanisms when a gene is knocked out, which can:

- Mask the loss-of-function effects.
- Lead to overexpression of related genes.
- Obscure the direct relationship between the edited gene and phenotype.

- **Epigenetic Unpredictability**

Epigenetic changes introduced via CRISPR (e.g., methylation or histone modifications) may be:

- Context-dependent (varying by cell type or developmental stage).
- Transient or unstable, leading to inconsistent gene expression patterns.
- Difficult to validate, as global transcriptomic shifts may not be directly linked to the targeted region.

➢ **Ethical Considerations in Researc**h

CRISPR-Cas9 is often used in vertebrate models such as mice, zebrafish, and non-human primates. This raises questions about:

- Pain, distress, or suffering due to genetic modifications.
- The moral status of animals in research.
- Limits on creating models with severe cognitive or physical impairments.
- Ethical Guidelines: Researchers must adhere to institutional and international standards (e.g., 3Rs: Reduction, Refinement, Replacement).
- Germline Editing

Editing the germline (heritable cells) in model organisms is common for studying gene function across generations. However, this practice has implications for:

- The stability of the genome in breeding lines.
- Potential unintended ecological effects if organisms are released into the environment.
- Setting a precedent for human germline editing, which remains controversial.

➢ Societal and Philosophical Questions

- Defining Naturalness

CRISPR blurs the line between "natural" and "artificial" life. Altering gene expression patterns raises philosophical questions:

- What does it mean for an organism to be "natural"?
- Are we creating organisms for human benefit or for knowledge?
- Should we impose limits on how extensively we modify living beings?

▪ Dual-Use Concerns

CRISPR has dual-use potential—the same technology used for beneficial purposes can also be misused, such as:

- Creation of harmful organisms (biosecurity threats).
- Unsanctioned enhancement in animals for sports, performance, or commercial traits.
- This raises the need for international oversight and bioethical consensus.

➢ **Regulatory and Legal Frameworks**

There is an urgent need to:

- Harmonize global regulations on CRISPR use in research and biotechnology.
- Develop species-specific guidelines for model organisms.
- Ensure transparency and peer oversight in research protocols.

In many regions, regulatory bodies like the NIH (USA), EMA (Europe), and CPCSEA (India) oversee CRISPR experiments involving animals.

➢ **Public Perception and Trust**

The public's acceptance of gene-editing research depends on:

- Transparency in communication about benefits and risks.
- Clear distinction between research and clinical use.
- Ethical treatment of animals and alignment with cultural values.

Misinformation or unethical use of CRISPR in animals can lead to:

- Public backlash.
- Funding cuts or policy restrictions.
- Delays in scientific progress.

➢ **Educational and Scientific Responsibility**

Scientists bear the responsibility to:

- Educate the public and policy-makers about CRISPR.
- Ensure that gene expression studies do not reinforce biases (e.g., linking genes to behavior or intelligence).
- Promote open-access data and collaborative ethics discussions.

➢ **Balancing Innovation and Responsibility**

As CRISPR-Cas9 continues to evolve, the scientific community must strive to:

- Balance innovation with restraint.
- Ensure that animal welfare, ecological integrity, and human ethics are not compromised.
- Encourage cross-disciplinary collaboration among scientists, ethicists, and policy-makers.

Conclusion

The emergence of CRISPR-Cas9 has revolutionized the field of genetic engineering, marking a transformative era in molecular biology and particularly in the study of gene expression within model organisms. What began as a bacterial defense mechanism has rapidly evolved into one of the most precise, efficient, and versatile tools for genome editing known to science. This system has fundamentally altered how researchers approach the complexities of gene regulation, enabling unprecedented control over when, where, and how genes are turned on or off.In the realm of model organisms, CRISPR-Cas9 has unlocked the ability to edit genes in species such as mice, zebrafish, fruit flies, and nematodes with remarkable specificity. These organisms, long valued for their genetic similarities to humans and their utility in laboratory settings, now serve as powerful platforms for exploring the intricate dynamics of gene expression. Through targeted gene knockouts, knock-ins, and transcriptional modulation, scientists can observe the functional consequences of gene activity across developmental stages and physiological processes. As a result, our understanding of gene networks, regulatory elements, and the epigenetic factors influencing transcription has expanded dramatically.

Beyond the technical achievements, the impact of CRISPR-Cas9 resonates across multiple disciplines. In medicine, it is shaping the future of gene therapy by offering new hope for the treatment of genetic disorders through precise correction or regulation of faulty genes. In agriculture, it is enhancing crop resilience and nutritional value by manipulating gene expression related to growth and stress responses. In fundamental science, it has catalyzed a wave of discovery, allowing for high-throughput screening of gene function and interactions. These developments are not confined to the lab—they are influencing global health, environmental sustainability, and the broader societal understanding of biology.

However, this technological progress is accompanied by challenges and ethical considerations. Off-target effects, mosaicism, and unexpected transcriptional outcomes continue to raise concerns, necessitating rigorous experimental validation and improved design of guide RNAs. Ethical debates surrounding germline editing, animal experimentation, and equitable access to gene-editing technologies must also be addressed with transparency, inclusivity, and care. The power to alter gene expression comes with a profound responsibility to ensure that scientific advancement is aligned with ethical standards and public trust.

Looking ahead, the future of CRISPR-based gene expression research appears both promising and expansive. Innovations such as RNA-targeting systems like Cas13, epigenome editing tools, and live-cell imaging techniques are refining our ability to study gene regulation in real time and in precise cellular contexts. Integration with artificial intelligence and single-cell technologies is enhancing our predictive capabilities and opening new avenues for personalized medicine and synthetic biology. As these tools continue to evolve, they will undoubtedly deepen our understanding of the genetic code and its regulation in diverse biological systems.

CRISPR-Cas9 has fundamentally redefined how we explore and manipulate gene expression in model organisms. It has bridged gaps between genotype and phenotype, enabled the modeling of complex human diseases, and opened new frontiers in biology, biotechnology, and medicine. As we continue to harness this technology, our journey into the regulatory blueprint of life promises not only scientific breakthroughs but also the possibility of transformative applications that improve the human condition. The legacy of CRISPR will be measured not only in the knowledge it uncovers but also in the ethical and responsible ways we choose to use it.

Bibliography

1. Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. Science, 346(6213), 1258096. https://doi.org/10.1126/science.1258096

2. Hsu, P. D., Lander, E. S., & Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. Cell, 157(6), 1262–1278. https://doi.org/10.1016/j.cell.2014.05.010

3. Adli, M. (2018). The CRISPR tool kit for genome editing and beyond. Nature Communications, 9(1), 1911. https://doi.org/10.1038/s41467-018-04252-2

4. Komor, A. C., Badran, A. H., & Liu, D. R. (2017). CRISPR-based technologies for the manipulation of eukaryotic genomes. Cell, 168(1-2), 20–36. https://doi.org/10.1016/j.cell.2016.10.044

5. 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

6. Zhang, X. H., Tee, L. Y., Wang, X. G., Huang, Q. S., & Yang, S. H. (2015). Off-target effects in CRISPR/Cas9-mediated genome engineering. Molecular Therapy - Nucleic Acids, 4, e264. https://doi.org/10.1038/mtna.2015.37

7. Bae, S., Park, J., & Kim, J. S. (2014). Cas-OFFinder: a fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases. Bioinformatics, 30(10), 1473–1475. https://doi.org/10.1093/bioinformatics/btu048

8. Hwang, W. Y., Fu, Y., Reyon, D., Maeder, M. L., Tsai, S. Q., Sander, J. D., … Joung, J. K. (2013). Efficient genome editing in zebrafish using a CRISPR-Cas system. Nature Biotechnology, 31(3), 227–229. https://doi.org/10.1038/nbt.2501

9. Niu, Y., Shen, B., Cui, Y., Chen, Y., Wang, J., Wang, L., … Ji, W. (2014). Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. Cell, 156(4), 836–843. https://doi.org/10.1016/j.cell.2014.01.027

10. Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., … Zhang, F. (2013). Multiplex genome engineering using CRISPR/Cas systems. Science, 339(6121), 819–823. https://doi.org/10.1126/science.1231143

11. National Human Genome Research Institute (NHGRI). (2022). What are model organisms? Retrieved from https://www.genome.gov

12. Carroll, D. (2017). Genome Engineering with Targetable Nucleases. Annual Review of Biochemistry, 86, 409–439. https://doi.org/10.1146/annurev-biochem-060815-014607

13. Pickar-Oliver, A., & Gersbach, C. A. (2019). The next generation of CRISPR–Cas technologies and applications. Nature Reviews Molecular Cell Biology, 20(8), 490–507. https://doi.org/10.1038/s41580-019-0131-5