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# CRISPR-Cas9-Driven Stable Expression of Exogenous Proteins in CHO Cells *via* Site-specific Integration

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## Introduction

The process of CRISPR-Cas9-mediated stable expression begins with the introduction of the Cas9 nuclease, which creates a Double-Strand Break (DSB) at a predetermined site in the genome. Along with this, a donor DNA template, which carries the desired exogenous gene, is introduced into the cell. The cell's DNA repair machinery then repairs the DSB, incorporating the donor gene into the target site. This method allows for the precise insertion of the gene into the desired locus, eliminating the randomness associated with traditional integration methods. In the case of CHO cells, this site-specific integration can be particularly valuable for creating high-yield, stable cell lines that express the target protein consistently over time. The use of CRISPR-Cas9 to achieve stable expression has the potential to overcome several key limitations associated with older techniques, such as random integration and poor expression stability, thereby improving the overall productivity of CHO cell-based systems. One of the key advantages of CRISPR-Cas9-mediated site-specific integration is the ability to control the integration site. In traditional gene insertion techniques, the transgene integrates randomly into the genome, often resulting in the silencing of the transgene or reduced expression due to integration into less favorable genomic regions [1]. The advent of CRISPR-Cas9 technology has revolutionized genetic engineering and molecular biology, offering unprecedented precision in genome editing. Among its many applications, the ability to achieve stable expression of exogenous proteins in mammalian cells stands out, particularly in the context of Chinese Hamster Ovary (CHO) cells, which are the workhorse of the biopharmaceutical industry. CHO cells are widely used for the production of therapeutic proteins, including monoclonal antibodies, enzymes, and other biologics. However, the process of incorporating foreign genes into the genome of CHO cells in a stable and efficient manner has traditionally been challenging. Conventional methods often rely on random integration of transgenes, which can lead to variable expression levels and instability of the product over time. In contrast, CRISPR-Cas9-mediated site-specific integration provides a more reliable and controlled means of achieving stable transgene expression. This approach offers significant advantages in terms of precision, efficiency, and the potential for streamlined production of biologics at a large scale.

## Description

Randomness can lead to the generation of cell populations with varying expression levels, making it difficult to identify clones that consistently produce the target protein at high levels. By contrast, site-specific integration ensures that the gene is inserted at a predetermined location within the genome, such as the safe harbor loci, which are known to support stable and high-level expression. This targeted approach increases the likelihood of achieving high and consistent protein expression in CHO cells, which is critical for the production of biopharmaceuticals. Moreover, site-specific integration reduces the chances of disrupting essential genes or regulatory regions in the host genome, which could lead to deleterious effects on cell growth or product quality. The incorporation of CRISPR-Cas9 into CHO cell line development represents a significant advancement over traditional methods such as viral transduction or plasmid-based systems. With viral systems, the insertion of the transgene is often inefficient and unpredictable, and there is also the risk of introducing unwanted mutations or alterations to the host genome. Plasmid-based methods, while relatively simple, also rely on random integration, which can lead to unstable transgene expression and low production yields. CRISPR-Cas9, on the other hand, provides a more streamlined and efficient approach by allowing for the precise and stable insertion of the gene at a known locus in the CHO genome. Additionally, CRISPR-Cas9 enables the use of multiple genetic modifications within a single cell, making it a powerful tool for engineering CHO cells with optimized properties for the production of complex proteins. This capability is particularly important for the production of biologics that require multiple modifications or post-translational modifications, such as glycosylation patterns, which can be fine-tuned in the host cell using CRISPR-based techniques [2].

In addition to its precision and efficiency, CRISPR-Cas9-mediated site-specific integration offers several other advantages that make it an attractive choice for the biopharmaceutical industry. One of these is the ability to select for cells that have successfully integrated the exogenous gene at the desired site. This is typically done by co-selecting for a selectable marker, which is included in the donor template along with the target gene. The selectable marker allows researchers to identify and isolate cells that have undergone successful integration, ensuring that only the desired clones are selected for further expansion and protein production. Furthermore, the use of CRISPR-Cas9 enables the creation of clonal populations of cells that express the target protein at high levels, eliminating the need for time-consuming screening and optimization steps that are often required with traditional methods. This can significantly reduce the time and cost associated with the development of stable CHO cell lines for large-scale protein production. Despite the numerous benefits, there are challenges that need to be addressed in order to fully realize the potential of CRISPR-Cas9-mediated stable expression in CHO cells. One of the main challenges is the efficiency of the genome editing process. While CRISPR-Cas9 has proven to be highly efficient in many model systems, the process of introducing exogenous genes into CHO cells can still be relatively inefficient, particularly when compared to other cell lines that are more amenable to genetic manipulation. The difficulty in achieving efficient delivery of the Cas9 and donor DNA template into CHO cells remains a significant barrier, and researchers are actively working on improving delivery methods, such as electroporation, lipid-based transfection, and viral vectors, to enhance the efficiency of genome editing in these cells [3].

Another challenge is the potential for off-target effects, which can occur when the Cas9 nuclease cleaves DNA at unintended sites, leading to unwanted genetic alterations. While advances in CRISPR technology have led to the development of more precise Cas9 variants with reduced off-target activity, the risk of off-target mutations remains a concern, particularly when working with complex mammalian genomes. To mitigate this risk, researchers are using high-fidelity Cas9 variants and employing careful design of the guide RNA to minimize off-target effects. Additionally, various screening methods, such as next-generation sequencing, can be used to identify and eliminate clones with off-target mutations, ensuring that only cells with the desired genetic modifications are selected for further development. The regulatory

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landscape surrounding CRISPR-Cas9-based genome editing in mammalian cells, particularly for biopharmaceutical applications, is another factor that must be carefully considered. Regulatory agencies, such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), have established guidelines for the use of genome-editing technologies in the development of biologics. These guidelines address concerns related to the safety and efficacy of genetically modified cell lines, including the potential for unintended genetic changes and the long-term stability of the cell lines. As CRISPR-Cas9 technology becomes more widely adopted in biopharmaceutical manufacturing, regulatory agencies will need to update and refine their guidelines to account for the specific challenges and risks associated with genome editing in CHO cells [4].

Looking forward, CRISPR-Cas9-mediated stable expression of exogenous proteins in CHO cells holds tremendous potential for improving the efficiency and productivity of biopharmaceutical manufacturing. As the technology continues to evolve, it is likely that CRISPR will become an indispensable tool for optimizing CHO cell lines and enabling the production of high-quality biologics. The ability to precisely manipulate the genome of CHO cells opens up new possibilities for improving protein yield, enhancing product quality, and reducing the time and cost associated with the development of therapeutic proteins. Furthermore, CRISPR-Cas9 could facilitate the production of more complex proteins, such as bispecific antibodies, therapeutic enzymes, and vaccines, by enabling precise modifications to the CHO genome that are tailored to the specific needs of the biologic [5].

## Conclusion

CRISPR-Cas9-mediated site-specific integration represents a groundbreaking approach to stable protein expression in CHO cells, offering significant advantages over traditional methods. Its precision, efficiency, and ability to create high-yield, stable cell lines make it an ideal tool for advancing the production of biopharmaceuticals. While there are challenges to overcome, including efficiency, off-target effects, and regulatory hurdles, the potential for CRISPR to revolutionize the biopharmaceutical industry is immense. As the technology matures and its applications expand, CRISPR-Cas9 will undoubtedly play a key role in the future of biologics manufacturing, driving innovation and enabling the production of life-saving therapies more efficiently and at a larger scale.

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## Conflict of Interest

None.

## References

1. Fischer, Simon, René Handrick and Kerstin Otte. "The art of CHO cell engineering: A comprehensive retrospect and future perspectives." *Biotechnol Adv* 33 (2015): 1878-1896.
2. Kim, Jee Yon, Yeon-Gu Kim and Gyun Min Lee. "CHO cells in biotechnology for production of recombinant proteins:Current state and further potential." *Appl Microbiol Biotechnol* 93 (2012): 917-930.
3. Wurm, Florian M. "Production of recombinant protein therapeutics in cultivated mammalian cells." *Nat Biotechnol* 22 (2004): 1393-1398.
4. Lee, Jae Seong, Thomas Beuchert Kallehauge, Lasse Ebdrup Pedersen and Helene Faustrup Kildegaard. "Site-specific integration in CHO cells mediated by CRISPR/Cas9 and homology-directed DNA repair pathway." *Sci Rep* 5 (2015): 8572.
5. Lee, Jae Seong, Lise Marie Grav, Lasse Ebdrup Pedersen and Gyun Min Lee, et al. "Accelerated homology-directed targeted integration of transgenes in Chinese hamster ovary cells *via* CRISPR/Cas9 and fluorescent enrichment." *Biotechnol Bioeng* 113 (2016): 2518-2523.

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