ADVANCING THE FRONTIERS OF BIOTHERAPEUTICS

CRISPR/Cas9 for Therapeutic Protein Production

Introduction

Monoclonal antibodies (mAbs), as well as their derivative products including antibody-drug conjugates, Fc-fusion proteins, and antibody fragments, are well recognized for their vast range of therapeutic and diagnostic uses (Mahmuda et al., 2017). The majority of recombinant proteins used in treating conditions including cancer, autoimmune disorders, cardiovascular ailments, etc. are mAbs (Wang C. et al., 2017). Due to their vast applications, mAbs and therapeutic proteins have become the market's dominating product category for biopharmaceuticals, with over 68 mAbs authorized by the US FDA as of the beginning of 2017 alone. (Cai, 2017)

To keep up with the market's rising demand, CRISPR/Cas9 can:

- Speed up the time to market
- Boost the output of therapeutic proteins
- Produce vast quantities of therapeutic protein
- Improve cost efficiency.

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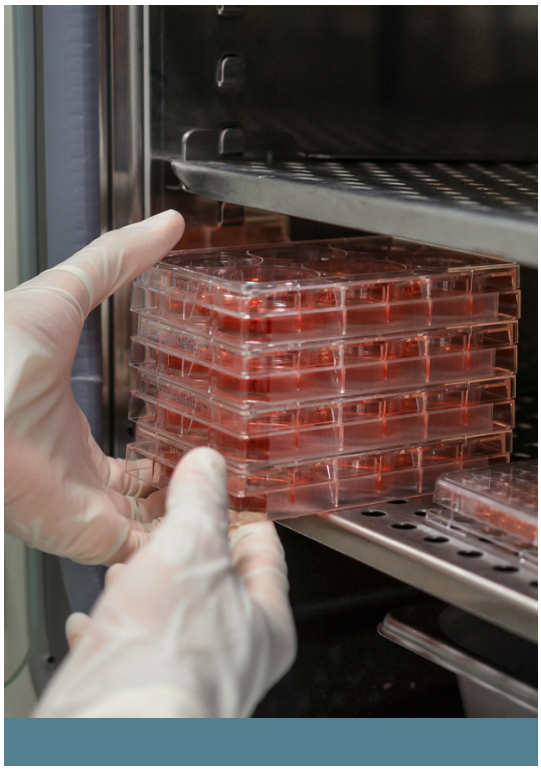
CRISPR/Cas9 Cell Engineering for Production of Novel Therapeutic Antibodies

Chinese hamster ovary (CHO), mouse myeloma-derived NS0 and Sp2/0 cells, human embryonic kidney cells (HEK293), and human embryonic retinoblast-derived PER.C6 cells are the most well-known mammalian host cell lines for recombinant mAb production (Tripathi and Shrivastava, 2019). CRISPR/Cas9 helps pioneer novel methods for the stable engineering of these host cells for antibody manufacturing.

CRISPR/Cas9 facilitates the engineering of the host cell such to:

- Enhance production efficiency
- Improve quality of antibodies from mammalian cell lines
- Prevent programmed cell death
- Regulate cell cycle progression
- Modify chaperones
- Alter cell metabolism
- Manipulate growth conditions.



Regulating Apoptosis and Cycle Progression in Cells

Apoptosis is the term for programmed cell death that occurs under cell stress. Preventing apoptosis in a cell line that expresses antibodies:

- Enhances cell viability
- Reduces cell death
- Lengthens the life of the cell culture
- Boosts productivity of the target antibody output (Fischer et al., 2015a).

Targeting genes such as Mcl-1 and Bax increases the synthesis of therapeutic proteins. CRISPR/Cas9 has been utilized to modify anti-apoptotic genes Mcl-1 and suppress pro-apoptotic genes like Bax in mammalian host cells. (Narimani, Sharifi and Jalili, 2019, Zhang et al., 2019). According to a recent study, co-transfecting CHO cells with Mcl-1 increased antibody production yield by 34% (Zhang et al., 2018).

Engineering of Chaperones

When mAbs are created by recombinant cell lines, chaperones and foldases play a crucial role in managing the folding process (Nishimiya et al., 2013). Using CRISPR/Cas9 to alter chaperones can affect the amount of therapeutic protein produced by changing how well the recombinant protein product can translate (Panting et al., 2021). By employing CRISPR/Cas9 for the overexpression of protein disulphide isomerase (an enzyme that catalyzes the creation of disulphide bonds) in CHO cells, the production of proteins from the cells was successfully elevated (Eisenhut et al., 2018).

Metabolic Engineering

It's typical for ammonia and lactate to build up during recombinant CHO cell growth. This often occurs as a result of the glutamine and glucose present in the culture media and has a negative impact on the developing cells and the secreted recombinant product. Through employing the CRISPR/Cas9 system to engineer cell metabolism, it is possible to prevent the buildup of such harmful metabolic byproducts significantly improving growth conditions and increasing cell viability & growth rate. Through the disruption of single amino acid catabolic genes in CHO cells, Let et al observed reduced lactate and ammonium secretion while specific growth rate and viable cell density were increased. (Ley et al., 2019)

Engineering Cells for Hypothermic Growth

It is well known that lowering the cell-culture temperature increases the yields of recombinant proteins in CHO cells. A lower temperature causes cell growth to slow down, extending cellular vitality and enlarging cell size. When mammalian cells are exposed to lower temperatures, the expression of cold stress genes, such as cold-inducible RNA-binding protein (CIRP), is changed. Tan et al demonstrated improvements in the productivity and yields of recombinant interferon in the CHO cell line through over-expression of CIRP. In 2015, Zhang et al successfully modulated the expression of CIRP in HTC75, HeLa, U2OS and 293T cells using the CRISPR/Cas9 system. (Zhang et al, 2015)

The CRISPR/Cas9 system has revolutionised genome engineering over the past decade making for **cost effective**, **rapid** and **simple** gene editing. With therapeutic proteins and monoclonal antibodies dominating the current biotherapeutic market, it's a requirement that companies have a competitive edge. By engineering the chosen cell line, CRISPR/Cas9 can **speed up** the time to market, **boost** the output of therapeutic proteins, and produce **rapid and large quantities** of therapeutic protein providing the artfulness required in this market.

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