CHO glyco-engineering using CRISPR/Cas9 multiplexing for protein production with homogeneous N-glycan profiles

Amann, Thomas; Hansen, Anders Holmgaard; Pristovsek, Nusa; Singh, Ankita; Min Lee, Gyun; Andersen, Mikael Rørdam; Kildegaard, Helene Fastrup

Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

Citation (APA):
CHO-glyco-engineering using CRISPR/Cas9 multiplexing for protein production with homogeneous N-glycan profiles

Thomas Amann¹, Anders H. Hansen¹, Nuša Pristovšek¹, Ankita Singh¹, Gyun M. Lee¹,², Mikael R. Andersen³, Helene F. Kimega¹

¹The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Lyngby, Denmark
²Korea Advanced Institute of Science and Technology, Department of Biological Sciences, Daejeon, South Korea
³Institute for Biotechnology and Biomedicine, Technical University of Denmark, Lyngby, Denmark

¹Correspondence: thoam@sustain.dtu.dk

1. KEY MESSAGE

Combining the Chinese hamster ovary (CHO) - K1 draft genome², identified CHO glycosyltransferases³ and the power of multiplexing gene knock-outs with CRISPR/Cas9 via co-transfection of Cas9 and one single guiding RNA (sgRNA) per target, we generated 20 Rituximab expressing CHO cell lines differing in amount and combination of insertions or deletions (indels) in the targeted genes. Clones harboring 9, 6 and 4 indels were further investigated for growth, Rituximab productivity and secretome N-glycosylation.

This resulted in clones with prolonged viabilities, no changes in N-glycan galactose contents but an increase of matured and sialylated N-glycans in the secretome. Additionally we point out, that multiplexing an increasing amount of genes most likely results in clones only revealing a few of all possible combinations of the targets and is highly driven by the sgRNA efficiency which can differ from each other by factor 4, even after FACS sorting.

2. Introduction: N-glycan engineering

A. Background information

Although CHO cells strength is the production of similar Nglycans to what is found on glycosylated human proteins⁴, non-engineered CHO cell lines display a broad variety of N-glycans which often includes N-glycan structures, that have an undesired effect on e.g. efficacy, antibody-dependent cell cytotoxicity (ADCC) or lectin-mediated clearance of the IgG protein. In this work, we investigate the limitations of targeting up to ten gene targets via multiplexing in a Rituximab producing CHO cell line. The targets include N-glycosyltransferases, enzymes involved in nucleotide sugar synthesis, N-glycosyltransferase modulation, apoptosis and glutamine synthesis.

3. Experimental Overview

A. Clone Level Sequencing

B. Pool Level Sequencing

Characterization of sgRNA efficiency

Bulk Sorting of Cell Pools

Single Cell Cloning

Characterization of:

- Glycotype: Sequencing of target regions
- Phenotype: N-glycan profile, growth & viability in batch cultivation

4. Sequencing of pools and clones

A. Clone Level Sequencing

B. Pool Level Sequencing

Figure 3:

Out of the screened clones, 20 clones harbor at least one genetic modification after multiplexing with 10 sgRNAs (high 14 clones shown here).

5. Results: Growth, Rituximab titers and secretome N-glycosylation

A. Growth and Viability in Batch Experiment

B. Rituximab quantification

C. Secretome N-glycan analysis

Figure 7:

Mannose and Acetylglucosamine profiles of the secretome from (1) the parental producer cell line and two controls with and without Cas9 sgRNA, (2) the non-engineered clones reveal identical titers and specific Rituximab productivity.