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

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1. KEY MESSAGE

Combining the Chinese hamster ovary (CHO) - K1 draft genome1,2, 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 mature 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 N-glycans to what is found on glycosylated human proteins4, non-engineered CHO cell 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 glycoprotein.

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 modulations, apolipoproteins and glucose synthesis.

3. Experimental Overview

Characterization of sgRNA efficiency

Bulk Sorting of Cell Pools

Single Cell Cloning

Figure 2: Target transcript levels and workflow of cell sorting after transformation to enrich engineered cells via GFP or generate single cell clones in 384 well format.

Characterization of sgRNA efficiency

4. Sequencing of pools and clones

A. Clone Level Sequencing

Box 1

B. Pool Level Sequencing

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

Figure 4: Frequency of initial generation after pool level sequencing of the different target regions before (blue) and after (red) FACS sorting for GPF-positive cells representing Cas9 2A-GFP expressing populations. The frequency of initial generation increased at least one-fold for all sgRNA targets after FACS and range from 1-60%.

Figure 5: The three top KO clones were grown in parallel to the parental producer cell line and two controls with no M (grey) - confirmed indels being present. Compared to the non-engineered controls, the KO clones show a slower drop in viability after 120h of cultivation (grey area, n=2).

B: Figure 1: Phosphotransferase N-glycan processing with nucleotide sugars and several N-glycantransferases anchored in the golgi membrane to be targeted for improved IgG N-glycan profile.

C: Figure 7: N-terminal purification of the secretome from (i) a non-engineered control line (c1), (ii) the 9x KO clone with insert in Box Bait 1, GLUL, Target 4, B4GalT1, B4GalT3, 3, 4 & 5 and target 10 (SA = sialic acid).

Table: Comparison of KO clones' growth, titers, productivity, and N-glycan analysis with the non-engineered control. The three top-KO clones were grown in parallel to the parental producer cell line and two controls with no M (grey) - confirmed indels being present. Compared to the non-engineered controls, the KO clones show a slower drop in viability after 120h of cultivation (grey area, n=2).

References:

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