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

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

PO315
1. KEY MESSAGE

Combining the Chinese hamster ovary (CHO) - K1 draft genome\(^2\), identified CHO glycosyltransferases\(^3\) 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 6, 8 and 9 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 proteins\(^4\), non-engineered CHO cell lines display a broad variety of N-glycans which often includes non-N-glycan structures, that have an undesired effect on e.g. efficacy, antibody-dependent cytotoxicity (ADCC) or lecithin-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 modulation, apoptosis and glutamine synthesis.

3. Experimental Overview

Characterization of sgRNA efficiency

Characterization of:
- Genotype: Sequencing of target regions
- Pheno-type: N-glycan profile, growth & viability in batch cultivation

Figure 2: Target transcript levels and workflow of cell sorting after transfection to enrich transfected cells via GFP or non-transfected cells using fluorescence-activated cell sorting (FACS).

4. Sequencing of pools and clones

A. Clone Level Sequencing

B. Pool Level Sequencing

Figure 3: Out of 84 screened clones, 20 clones harbor at least one genetic modification after multiplexing with 10 sgRNAs (top 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 GFP-positive cells representing Cas9/ΔGFP expressing populations. The frequency of initial generation increased at least one-fold for all sgRNA targets after FACS and range from 1-60%.

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

A. Growth and Viability in Batch Experiment

Figure 5: The three KO clones display heterogenous titers and productivity, where the 6x KO has the lowest and the 8x KO the highest titer. Within the control group, the two non-engineered clones reveal similar titers and specific Rituximab productivities.

B. Rituximab quantification

Figure 6: The three KO clones display heterogenous titers and productivity, where the 6x KO has the lowest and the 8x KO the highest titer. Within the control group, the two non-engineered clones reveal similar titers and specific Rituximab productivities.

C. Secretome N-glycan analysis

Figure 7: Microarrays of the secretome from (i) a non-engineered control cell line (ii) the 6x KO clone with insert in Box B, B4GalT2, GUS, Target 14 and (iii) the 9x KO clone with insert in BOX A.

References:

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