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

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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 saccharome N-glycosylation.

This resulted in clones with prolonged viabilities, no changes in N-glycetn 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 cell lines are the production of similar N-glycans 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. effcacy, antibody-dependent cell cytotoxicity (ADCC) or lent-mediated clearance of the glucocyan in vivo. In this work, we investigated 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, apoprosis and glutamine synthesis.

3. Experimental Overview

Characterization of sgRNA efficiency

Bulk Sorting of Cell Pools

Characterization of:
- Genotype: 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

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

A. Growth and Viability in Batch Experiment

B. Rituximab quantification

C. Secretome N-glycan analysis

References:

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

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

Figure 3: Out of the screened clones, 20 clones harbor at least one genetic modification after multiplexing with 10 sgRNAs in this 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 Cas9-2A-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: Phylogenetic tree of the secretome from (i) a non-engineered control cr 1 and (ii) the 9x KO clone with inserts in Bbt, Bgl2, LULS, Target 4, BgGI, T3, 4 & 5 and target 10 (SA = sialic acid).

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