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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 galactosic 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 human proteins1, 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 leuko-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, apoptosis and glutamine synthesis.

3. Experimental Overview

Characterization of sgRNA efficiency

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

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B. Pool Level Sequencing

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Figure 2: Target transcript levels and work-flow of cell sorting after transformation to enrich transfected 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 (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 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%.

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 5: Plaque-transient N-glycan processing with nucleotide sugars and several N-glycosyltransferases anchored in the galact membrane to be targeted for improved IgG N-glycan profile.

Figure 6: The three top-KO clones display higher-glycosylated titers and productivity, where the 6x KO has the lowest and the 1x KO the highest titer. Within the control group, the two non-engineered clones reveal similar titers and specific Rituximab productivity.

Figure 7: Multiplexed titers of the secretome from (i) a non-engineered control, (ii) the 6x KO clone with intact Be L, Bak1, GLUL, Target 4, (iii) KO Bak1, GLUL, Target 4, (iv) KO Bak1, GLUL, Target 4 and (v) the 6x KO clone with intact Be L, Bak1, GLUL, Target 4, (vi) KO Bak1, GLUL, Target 4 and (vii) KO Bak1, GLUL, Target 4 and target 10 (SA = sialic acid).

Reference:

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