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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 glycosyltransferase3 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, 8 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 IgG glycoforms with nucleotide sugars and several N-glycan structures in the Golgi membrane to be targeted for glycolytic engineering, Post-translational modifications, apoptosis and glutamine synthesis.

3. Experimental Overview

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

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References: