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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 genome², 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, 8 and 6 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 structures 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, non-engineered CHO cells display a broad variability of N-glycans which often includes N-glycan structures, that have an undesired effect on e.g. efficacy, antibody-dependent cell cytotoxicity (ADCC) or lecin-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, Nglycosyltransferase modulation, apoptosis and glutamine synthesis.

3. **Experimental Overview**

**Characterization of sgRNA efficiency**

**Bulk Sorting of Cell Pools**

**Single Cell Cloning**

4. **Sequencing of pools and clones**

**A. Clone Level Sequencing**

Target transcript levels and workflow of cell sorting after transformation to enrich transfected cells via sGFP or the power of multiplexing gene knock-outs with CRISPR/Cas9.

**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**

6. **Acknowledgement**

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