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

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**1. KEY MESSAGE**

Combining the chicken embryo kidney (CEK) -K1 draft genome\(^1\), identified CHO glycosyltransferases\(^2\) 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 transferases are 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 matured 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 proteins\(^3\), 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 lectin-mediated clearance of the glycoprotein. In this work, we investigate the limitations of targeting up to 10 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**

**4. Sequencing of pools and clones**

**A. Clone Level Sequencing**

<table>
<thead>
<tr>
<th>sgRNA Efficiency</th>
<th>Bulk Sorting of Cell Pools</th>
<th>Single Cell Cloning</th>
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</thead>
<tbody>
<tr>
<td>Characterization of sgRNA efficiency</td>
<td>Characterization of:</td>
<td>Phenotype:</td>
</tr>
<tr>
<td>N-glycan profile, growth &amp; viability in batch cultivation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**B. Pool Level Sequencing**

<table>
<thead>
<tr>
<th>Pool</th>
<th>Clone 1</th>
<th>Clone 2</th>
<th>Clone 3</th>
<th>Clone 4</th>
<th>Clone 5</th>
<th>Clone 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x A</td>
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<td>90</td>
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<td>70</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
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</tr>
<tr>
<td>3x A</td>
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<td>50</td>
<td>40</td>
<td>30</td>
</tr>
</tbody>
</table>

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

**A. Growth and Viability in Batch Experiment**

![Figure 2: Target transcript levels and workhorse of cell sorting after transfection to enrich transfected cells via GFP or generate single cell clones in 384 well format.](image)

**B. Rituximab quantification**

![Figure 6: The three KO clones display heterogenous titers and productivity, where the 1x 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.](image)

**C. Secretome N-glycan analysis**

![Figure 7: N-glycan profile of the secretome from (i) a non-engineered cell line and (ii) the 9x KO clone with insert in Bax.](image)

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**References**: