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

PO315

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1. KEY MESSAGE

Combining the chinese hamster ovary (CHO) - K1 draft genome[1,2], identified CHO glycosyl-transferases[3] and the power of multiplexing gene knock-outs with CRISPR/Cas9[4] via co-transfection of Cas9 and one single guiding RNA (sgRNA) per target, we generated 20 Rituximab expressing CHO-S 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 viabilites, no changes in *N*-glycan galactose contents but an increase of matured and sialylated *N*-glycan 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[5], non-engineered CHO cells 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 **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, apoptosis and glutamine synthesis.

B. Hypothesis

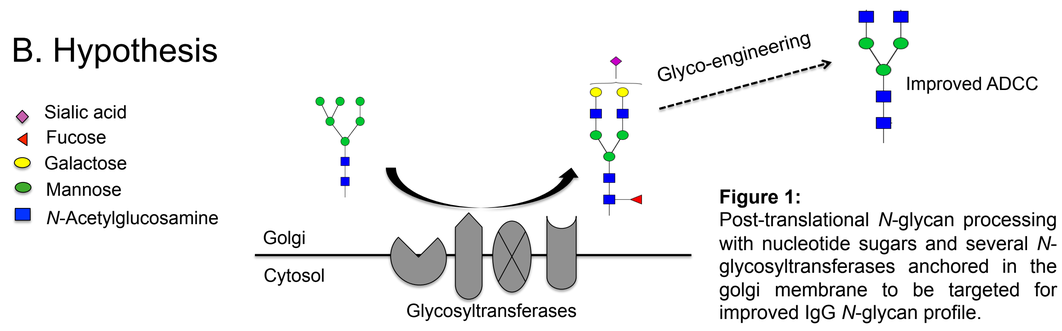


Figure 1:
Post-translational *N*-glycan processing with nucleotide sugars and several *N*-glycosyltransferases anchored in the golgi membrane to be targeted for improved IgG *N*-glycan profile.

3. Experimental Overview

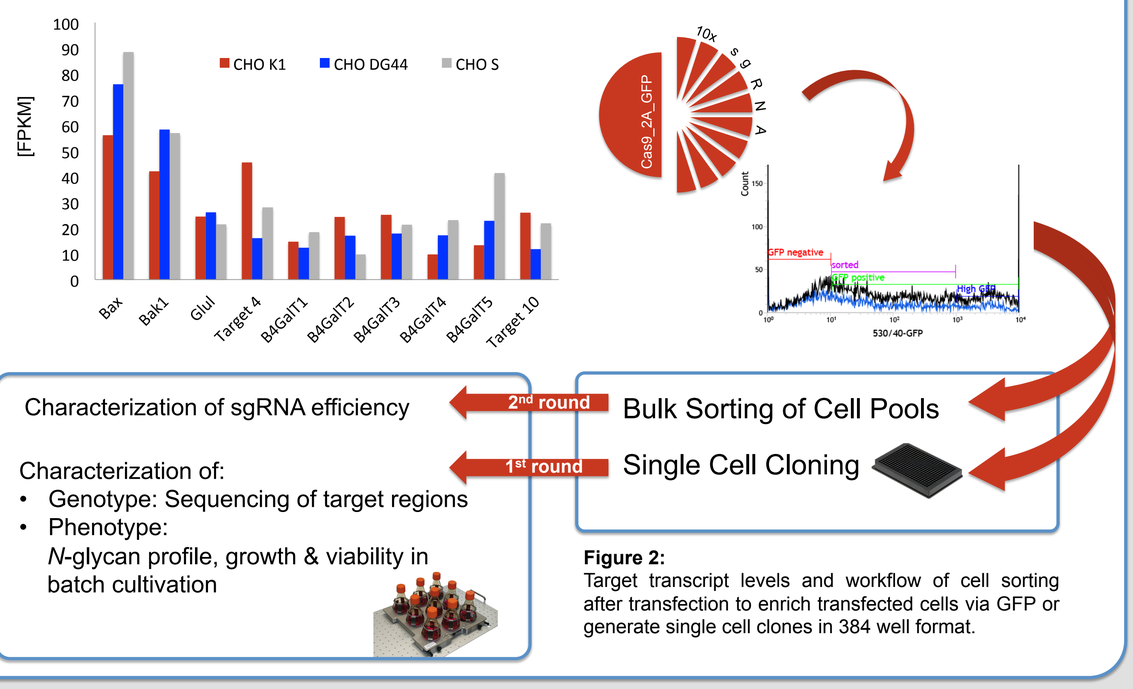


Figure 2:
Target transcript levels and workflow of cell sorting after transfection to enrich transfected cells via GFP or generate single cell clones in 384 well format.

4. Sequencing of pools and clones

A. Clone Level Sequencing

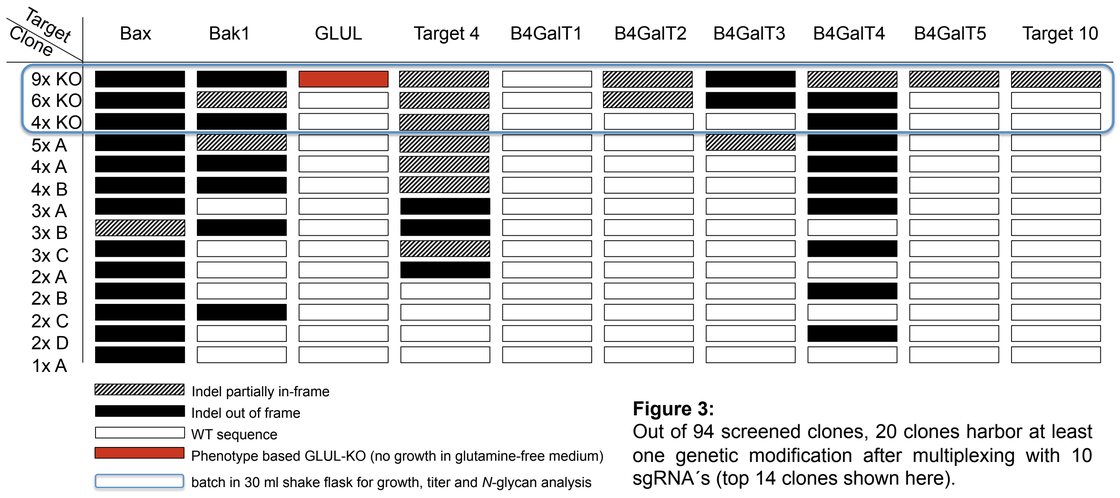
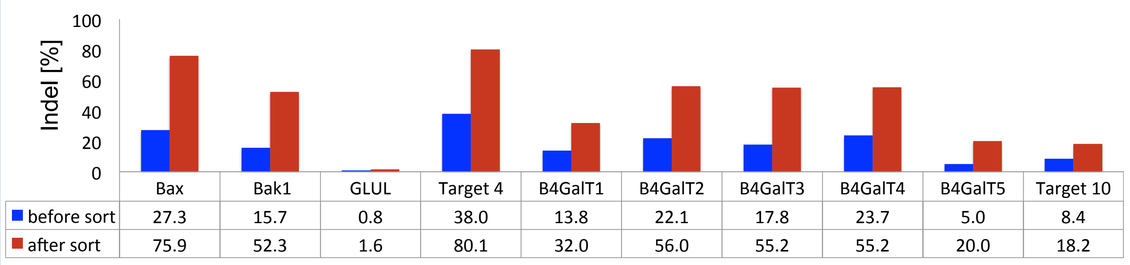


Figure 3:
Out of 94 screened clones, 20 clones harbor at least one genetic modification after multiplexing with 10 sgRNA´s (top 14 clones shown here).

B. Pool Level Sequencing



	Bax	Bak1	GLUL	Target 4	B4GalT1	B4GalT2	B4GalT3	B4GalT4	B4GalT5	Target 10
before sort	27.3	15.7	0.8	38.0	13.8	22.1	17.8	23.7	5.0	8.4
after sort	75.9	52.3	1.6	80.1	32.0	56.0	55.2	55.2	20.0	18.2

Figure 4:
Frequency of indel 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 indel generation increased at least one-fold for all sgRNA targets after FACS and range from 1-80%.

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

A. Growth and Viability in Batch Experiment

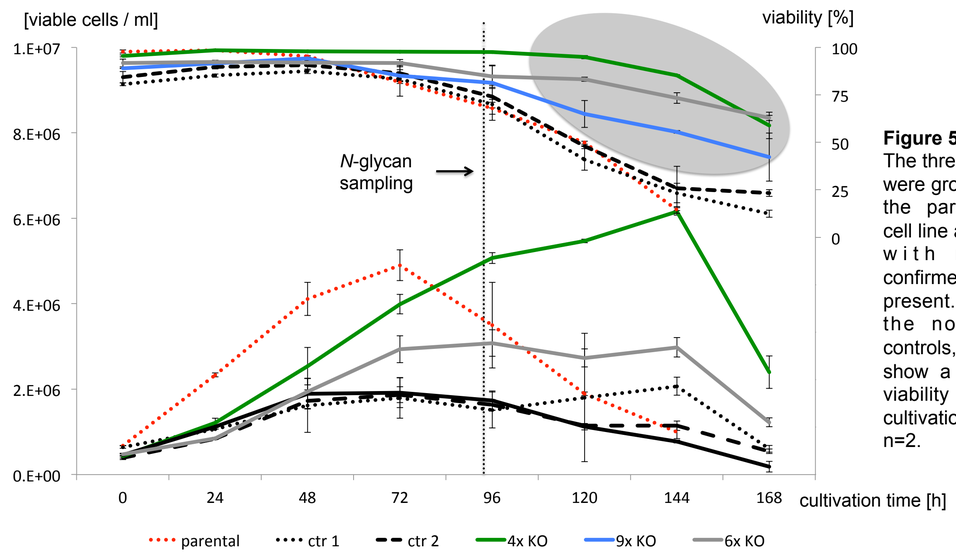


Figure 5:
The three top-KO clones were grown in parallel to the parental producer cell line and two controls with no MiSeq-confirmed indels being present. Compared to the non-engineered controls, the KO clones show a slower drop in viability after 120h of cultivation (grey area), n=2.

B. Rituximab quantification

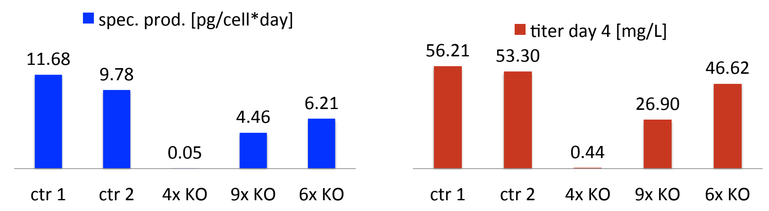


Figure 6:
The three KO clones display hetero-geneous titers and productivity, where the 4x KO has the lowest and the 6x KO the highest titer. Within the control group, the two non-engineered clones reveal similar titers and specific Rituximab productivities.

C. Secretome *N*-glycan analysis

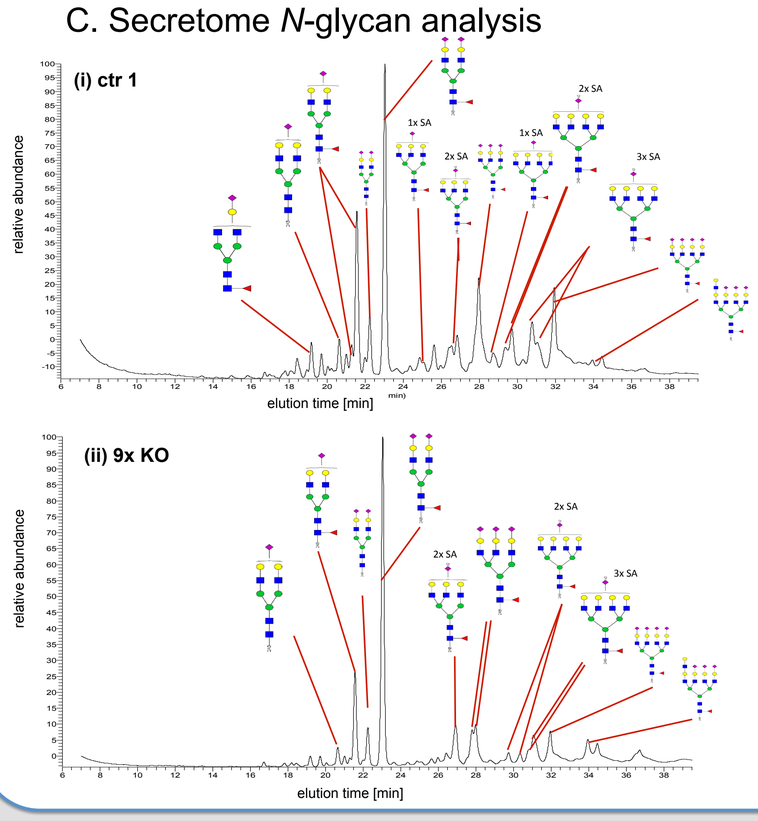


Figure 7:
N-glycan profiles of the secretome from (i) a non-engineered clone ctr 1 and (ii) the 9x KO clone with indels in Bax, Bak1, GLUL, Target 4, B4GalT2, 3, 4 & 5 and target 10 (SA = sialic acid).

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