Simplifying Progress

Evaluation of Targets for CHO Cell Line Engineering and Generation of Enhanced Host Cell Lines

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Introduction

To satisfy the growing demand for biopharmaceuticals, commercial production cell lines must express proteins at high yields with suitable product quality. Traditionally, mammalian expression systems have been optimized targeting expression vector, bioprocess regimen and media. With the advent of CRISPR tools, genome editing enables optimization of the host cell line for biopharmaceutical production. This study illustrates the process of screening for advantageous gene targets and the creation of an improved CHO host cell line, optimized for the production of recombinant proteins.

1. Experimental approach

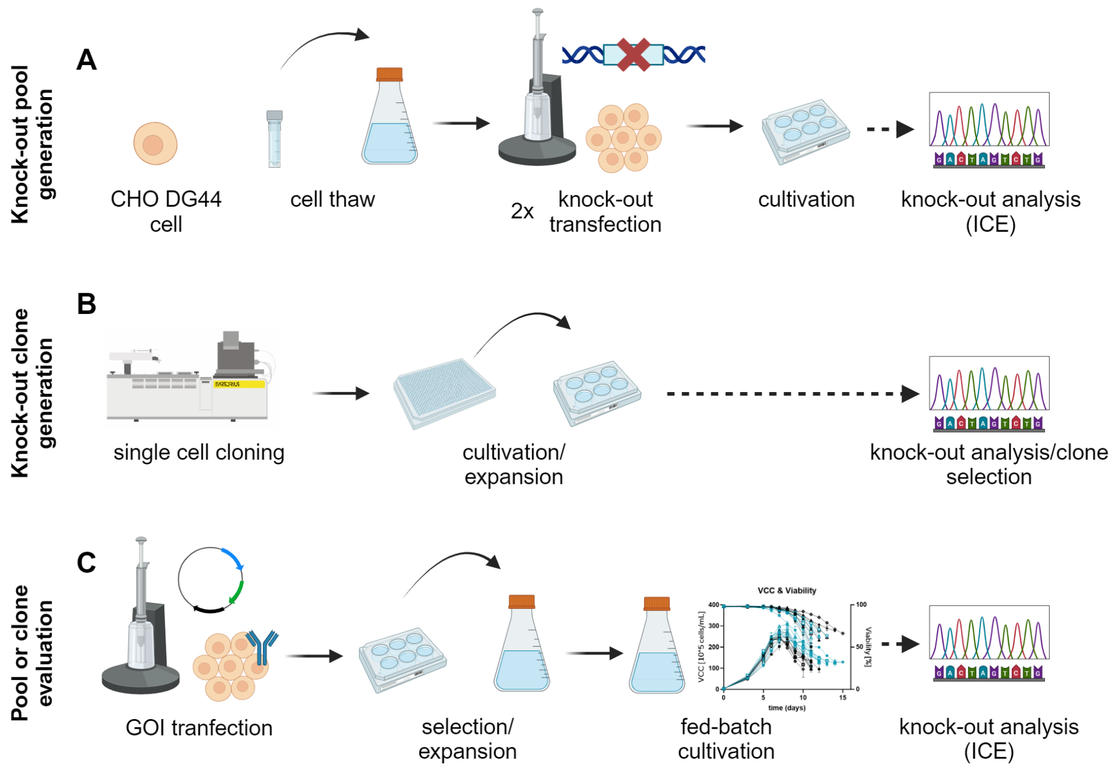


Figure 1: Schematic overview on gene knock-out cell line generation. A: CHO DG44 cells are transfected with a gRNA/Cas complex to induce mutations in the target gene. Sanger sequencing and Interference of CRISPR Edits (ICE, Synthego) analysis is applied to assess the percentage of frame-shift mutations (knock-out score). C: For initial knock-out (KO) effect evaluation, KO-pools were transfected with a gene-of-interest (GOI) and stable recombinant protein expressing cell pools were generated. These pools were evaluated for their process performance in a 25 mL shake flask fed-batch process. B + C: Knock-out host clone generation. Single cell clones were generated from KO-pools that showed superior bioprocess performance during the initial knock-out effect evaluation on pool level. Created with BioRender.com

2. Knock-out effect evaluation

Selected gene targets (gene 1-3) were knocked-out in CHO DG44 cells to assess the effect on bioprocess performance (Fig 2). Knock-out of gene 2 showed increased process time (+ 2 days) resulting in an increased integral of viable cell concentration (IVCC) with a 59% titer increase on average.

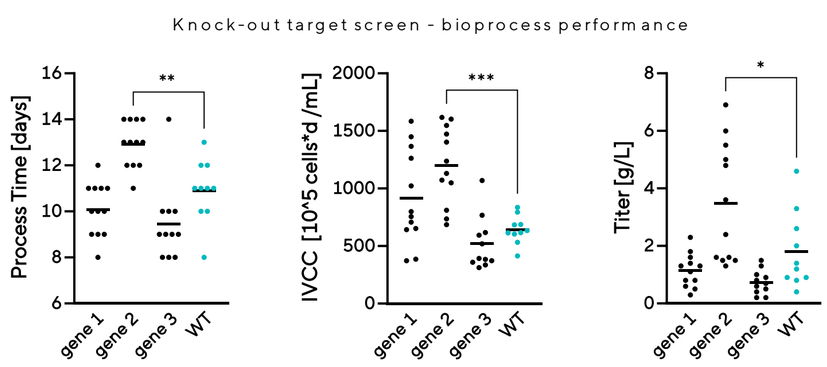


Figure 2: Fed-batch data of a target knock-out screen. Exemplary data for three gene targets is shown. Clonal CHO DG44 knock-out cell lines were generated and transfected to overexpress an IgG1 molecule (antibody 1). See Fig. 1 A-C for details. Lines indicate mean, each dot represents a knock-out host clone stably overexpressing antibody 1.

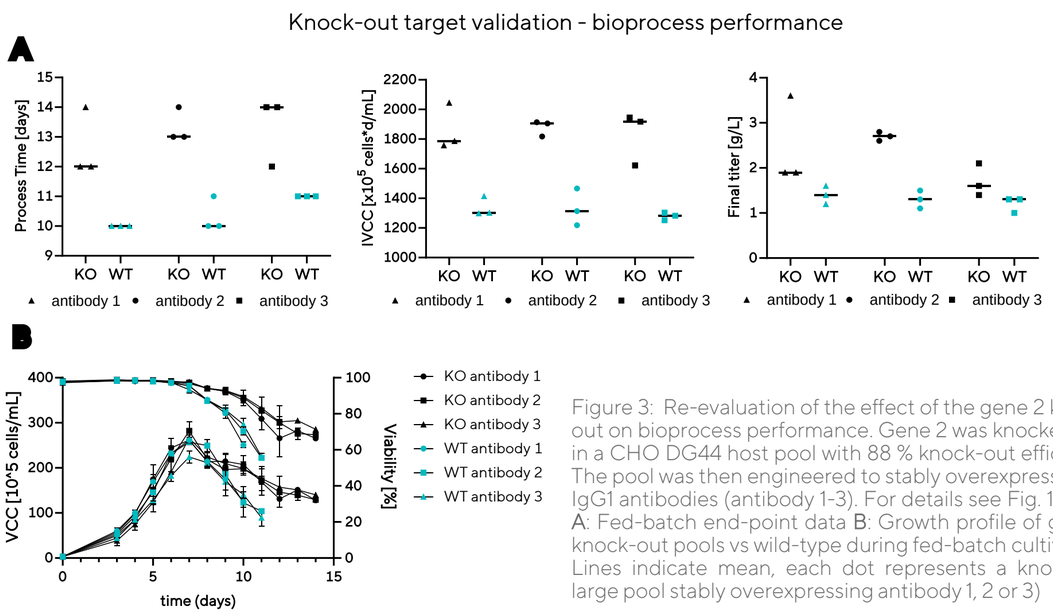


Figure 3: Re-evaluation of the effect of the gene 2 knock-out on bioprocess performance. Gene 2 was knocked-out in a CHO DG44 host pool with 88 % knock-out efficiency. The pool was then engineered to stably overexpress three IgG1 antibodies (antibody 1-3). For details see Fig. 1 A + C. A: Fed-batch end-point data B: Growth profile of gene 2 knock-out pools vs wild-type during fed-batch cultivation. Lines indicate mean, each dot represents a knock-out large pool stably overexpressing antibody 1, 2 or 3)

To validate the observed effects, gene 2 knock-out pools were generated overexpressing three IgG1 antibodies (antibody 1-3) and cultivated in fed-batch mode (Fig. 3). In line with the previous results, process time was increased (+2-3 days) elevating IVCC and titer by 39-43 % and 42-108 % respectively. Generally, the knock-out cells show a prolonged viability over the duration of cultivation (Fig. 3 B). These results indicate that gene 2 is a promising knock-out target for production cell line engineering.

3. Engineering a host cell line for higher productivity

To identify the knock-out clones with the best bioprocess characteristics, clonal cell lines with a knock-out in gene 2 were generated (Fig 1 A+ B) and evaluated in a fed-batch process (Fig.1 C). Out of the 30 knock-out clones generated, 9 clones showed higher titer, runtime and specific productivity compared to the wild-type cell line (Fig. 4). Additionally,, lactate dehydrogenase (LDH), host cell protein (HCP) and osmolality data was considered to select and rank the top 4 clones, clones 1-4 (Fig. 4 and Tab. 1). The same growth pattern with prolonged viability is visible for the knock-out clones as it was observed with the knock-out pools (compare Fig. 5 B to Fig. 3B). The top 4 clones showed a prolonged cultivation duration of + 3 days and an increase in titer of 30-110 % compared to the wild-type host cell line (Fig 5). Clone 4 showed the best combination of specific productivity, titer and growth (Fig. 5) and was selected for comprehensive bioprocess performance evaluation (data see poster #137).

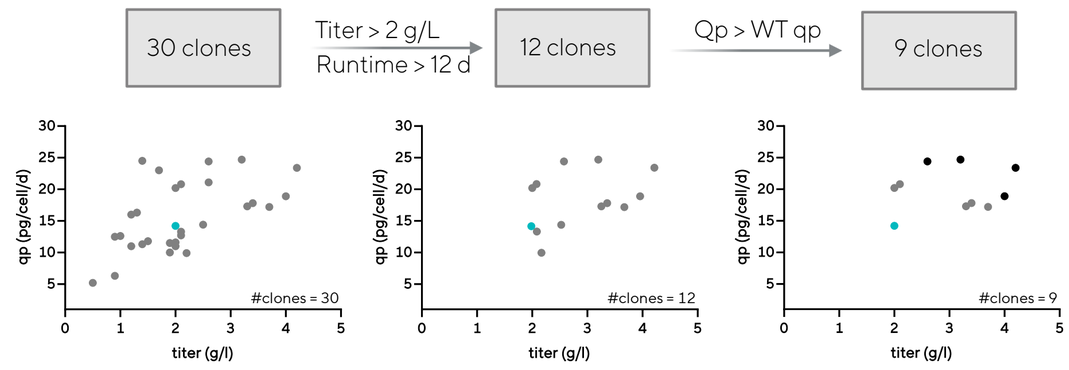
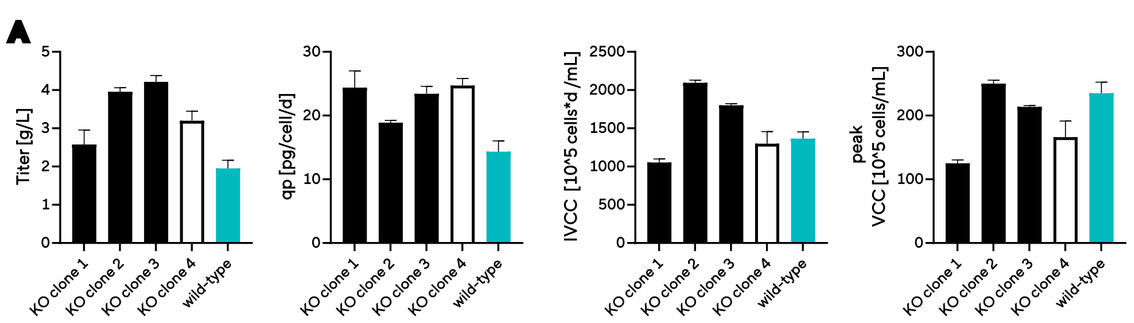
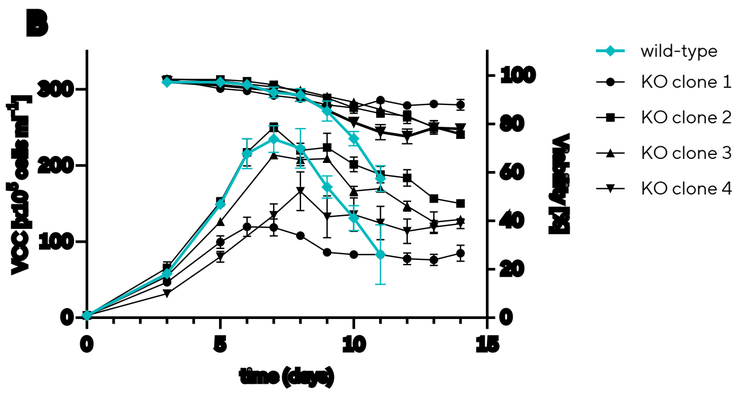


Figure 4: Fed-batch data of 30 knock-out clones overexpressing an IgG1 molecule (antibody 1) as stable cell pools. Top 9 clones were selected for high titer, runtime and specific productivity (qp). WT is depicted in blue; top 4 host clones are depicted in black. Each dot represents n = 2-3 biological replicates for knock-out host clones and n = 6 for the wild-type host.

Knock-out clone #	Titer [g/L]	Qp [pg/c/d]	IVCC [10^5 cells*d/mL]	final Viability [%]	Peak VCC [10^5 cells/mL]	Osmolality [mOSM/kg H2O]	LDH [U/L]	HCP [g/L]
1	2.6	**24.4**	**1053**	**88**	**125**	406	**1275**	0.32
2	**4.2**	**23.4**	1802	**76**	214	371	**1523**	**0.26**
4	3.2	**24.7**	**1298**	**78**	**166**	387	2134	0.27
9	2.0	20.2	**994**	**83**	**102**	407	**998**	0.47
3	**4.0**	18.9	2095	76	250	**339**	1651	**0.26**
8	2.1	**20.8**	**998**	68	**142**	388	**1559**	0.49
6	**3.7**	17.2	2140	68	264	**342**	1570	**0.23**
7	**3.4**	17.8	1870	67	256	**334**	2600	0.27
5	3.3	17.3	1884	69	256	358	1656	**0.26**
wild-type	2.0	14.2	1405	57	241	**357**	5787	0.33

Table 1: Fed- batch data of top 9 knock-out clones (Figure 4) and the wild-type cell line including growth data, osmolality (day 10), LDH (day 10) and HCP (day 7) analytics. The top 4 clones for each variable are marked in bold. The knock-out clones were ranked according to the number of top performance in each variable. Values are means of , n = 2-3 for knock-out host clones and n = 6 for the wild-type host.





Evaluation of the new engineered host cell line on poster #137

Figure 5: B: Fed-batch data of top 4 clones. A: end-point data; white bar indicates the top knock-out clone B: VCC and viability data over fed-batch runtime. Error bars indicate standard deviation, n = 2-3 for knock-out host clones and n = 6 for the wild-type host.

4. Conclusion

We described how large pools can be used to screen for effects of gene knock-outs on bioprocess performance. The screen revealed a target prolonging cell culture duration which resulted in increased titer during the bioprocess. Thirty clones with a knock-out in said target gene were generated and screened for their bioprocess performance. We generated a clonal knock-out host cell line that shows longer process time, higher titer and specific productivity compared to the wild-type host. This improved engineered host could be used to develop high performance cell lines for the production of therapeutic proteins.