SARTORIUS

Simplifying Progress

Strategies to remove Single Cell Cloning from Genetic Knock Out Screens in Host Cell Line Engineering

Jannis Marzluf[1,2], Daniela Kirchmeier[1], Jennifer Klein[1], Christoph Zehe[1], Ann-Cathrin Leroux[1]

[1] Sartorius Stedim Cellca GmbH, Ulm, Germany
[2]University of Ulm, Ulm, Germany
Corresponding author: jannis.marzluf@sartorius.com

Introduction

Chinese Hamster Ovary (CHO) cell lines are the most common used host system to produce biopharmaceuticals. Genetic engineering is utilized to optimize metabolic balances by adjusting gene expression levels or to remove adverse proteins by knocking out coding genes. These efforts improve performance, productivity, quality and facilitate downstream processing. While refined single gene knock out (KO) host cell lines can be easily generated using the CRISPR toolbox, throughput, timelines and screening of putative genetic targets still display significant challenges in host cell line engineering. Here, we present a highly efficient workflow utilizing CRISPR gene editing in stable CHO pools to perform a KO screen on candidate genes. We show that generated pools are genetically stable for a long period of time (>6 weeks) in single KO and up to 7x multiplexed KO approaches. These stable pools are able to capture the phenotype observed in monoclonal KO cells, reproducing previously confirmed performance effects on the bioprocess. Furthermore, the evaluation via KO pools can represent the heterogeneity observed in the host cell line without the need to generate tens of KO clones for each candidate gene. This enables a three times higher target screening capacity from 4 to 12 genes with the same workload. Additionally, the timeline was reduced from 11 to 6 weeks.

1. Optimized Platform to Knock Out desired Gene in Host Cell Line Engineering

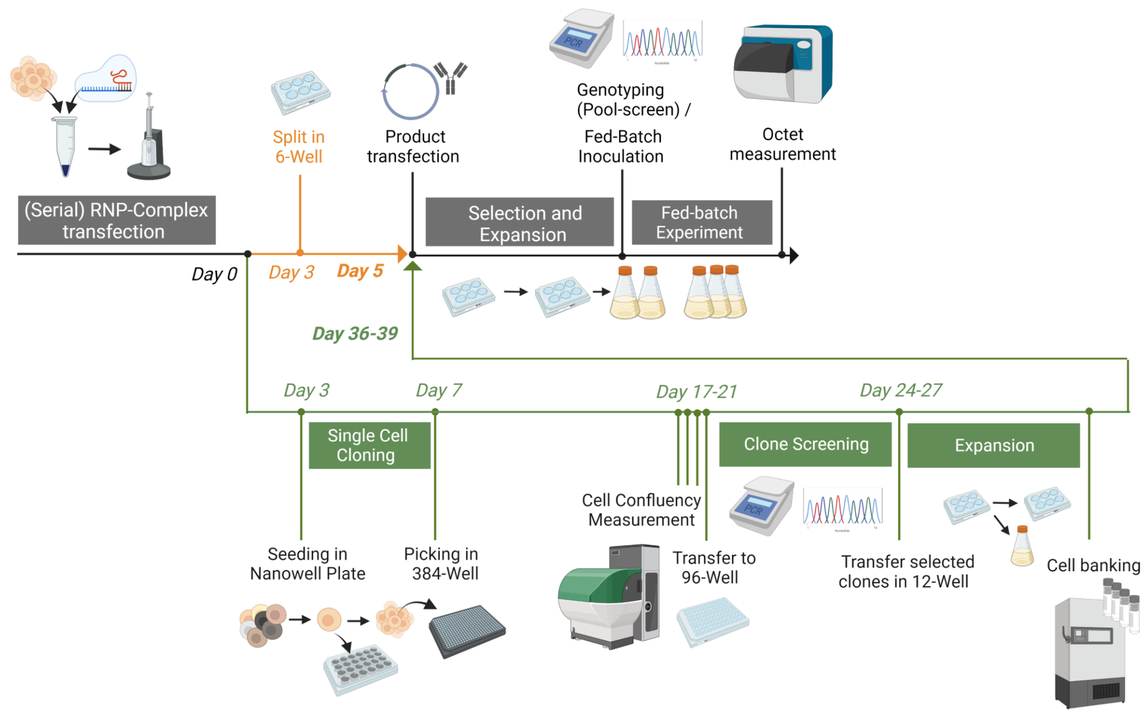
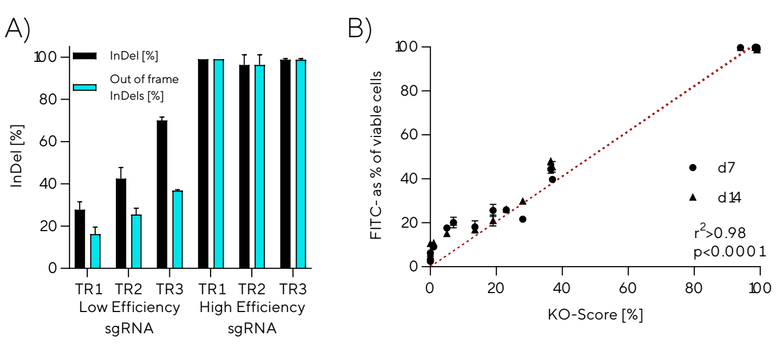


Figure 1: **Workflow for the generation of host cell protein KO clones and subsequent performance capture in a fed-batch bioprocess.** Further improvements in timeline and data integrity can be achieved by bypassing the Single Cell Cloning directly to pooled fed-batch evaluation. This reduces the workflow by 5-6 weeks. Furthermore, KO-Scores in bulk pools may be increased by employing serial transfections with the same RNP-complex.

2. Genotype – Phenotype relationships appear to be correlated in heterogenous Fucosyltransferase 8 KO CHO subpopulations

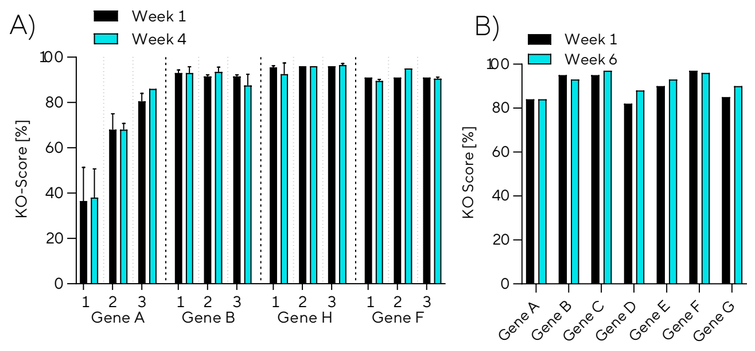


Key takeaways: CRIPSR edited Pools:

- Multiple transfections increase the KO-Scores and create stable subpopulations in bulk pools
- Edited pools show a high correlation between genotype and phenotype

Figure 2: **Multiple transfections to assess pooled KO strategies.** A: InDel percentages in FUT8 KO pools. The pools were generated by multiple transfection with RNP-complexes. [n=6; Mean ± SD] B: Correlation of FITC+ cell population as a percentage of viable cells plotted against FUT8 KO-efficiency in the heterogenous edited pools. [n=6; Mean ± SD] (LE=Low Efficiency; HE=High Efficiency; TR=Transfection)

3. Generated Heterogenous Knock Out Pools are stable in single and multiplex approaches



Key takeaways: long-term cultivation of heterogenous KO pools:

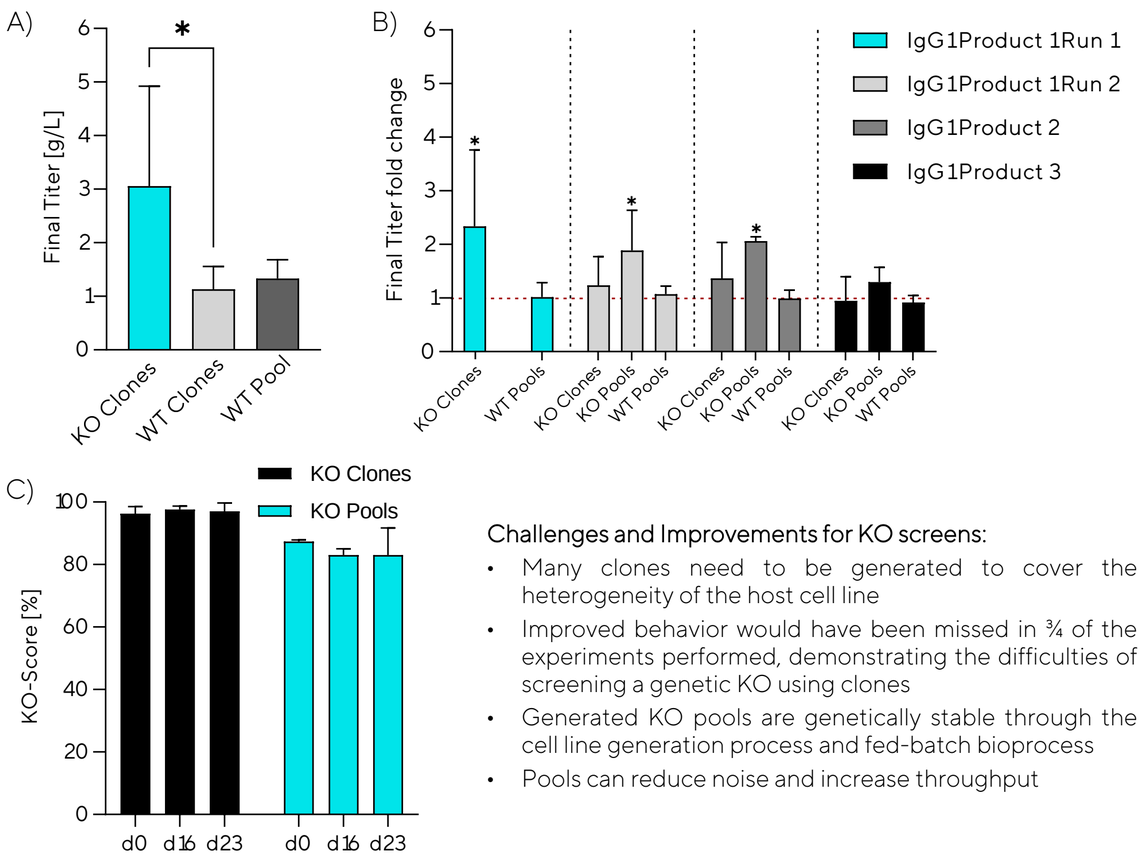
- Generated KO CHO pools are stable in single (>4 weeks) and multiplexed (>6 weeks) pools
- Stability and linearity of genotype – phenotype relationships may change in a target dependent manner

Figure 3: **Stability of single and multiplexed KO pools over multiple weeks.** A: KO-scores in single Gene KO pools after one, two and three sequential RNP-complex transfections. Each subsequent column pair (black + blue bars) represents two pools after the respective number of transfections in the first week (black bars) and after four weeks (blue bars). [n=2; Mean ± SD] B: KO-Score in one 7x KO pool after three multiplexed transfections in the first week and after six weeks. [n=1]

4. CHO KO Pools are better suited to connect genotype to phenotype in a bioprocess environment

Candidate genes identified from host cell protein analysis were knocked out:

- A KO in Gene A was shown to have productivity enhancing effects in 12 generated KO clones with a mean 3-fold increase in final titer in fed-batch experiment compared to wildtype clones and pools
- Mean 2-fold increase in final titer was also observed with other biotherapeutic proteins in heterogenous CHO KO pools, but not in re-transfected KO clones

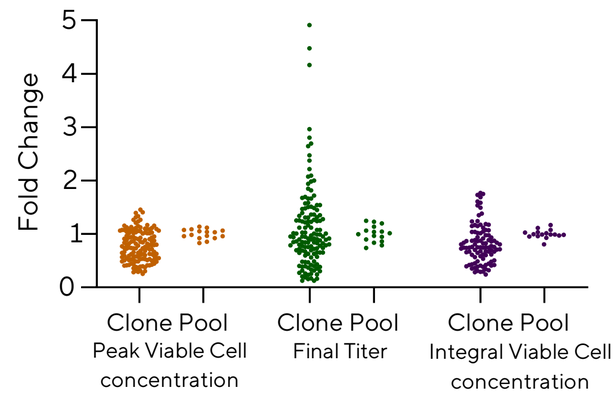


Challenges and Improvements for KO screens:

- Many clones need to be generated to cover the heterogeneity of the host cell line
- Improved behavior would have been missed in ¾ of the experiments performed, demonstrating the difficulties of screening a genetic KO using clones
- Generated KO pools are genetically stable through the cell line generation process and fed-batch bioprocess
- Pools can reduce noise and increase throughput

Figure 4: **KO of gene A increases final fed-batch titer in CHO clones and heterogenous KO pools.** A: Final titers of Gene A KO clones compared to wildtype clone and pool samples. [n=12 (Gene A KO), 10 (WT clones), 3 (WT pool); Mean ± SD]. B: KO clones from A and a CHO WT pool were transfected with three different antibody coding genes [n=12 (Gene A KO clones), 3 (Gene A KO Pools), 3 (WT Pools); Mean ± SD]. C: KO scores along the cell line generation process until fed-batch cultivation. [n=12 (Gene A KO clones), (Gene A KO Pools); Mean ± SD].

5. Using pools instead of clones for genetic KO screens significantly reduces timelines and increases data integrity



Better Data with less work:

- Generated clones show a large heterogeneity, hindering the discovery of performance increasing effects by skewing results through biased selection of few clones
- Pools enable the representation of all types of cells in one sample and therefore better show the effects resulting from genetic editing
- The timeline to evaluate a single gene KO is reduced from 11 to only 5 weeks with an additional 3x capacity increase in regard to the number of genes screened in parallel

Figure 5: **Empirical variance in fed-batch bioprocess behavior comparing clones and pools.** Performance parameters peak viable cell concentration, final titer and integral of viable cell concentration were normalized against WT samples in their respective experiment and then plotted for the fold change observed. [n=141 (Clone), 16 (Pool); Mean ± SD].

Outlook

Genetically engineered CHO cell lines play an integral part in the future of biopharmaceutical production. They promise improved process performance and superior product quality. At Sartorius, we have established an efficient pipeline for the generation of any desired KO CHO DG44 cell line. We have identified multiple candidate genes for KO in multiplexed engineered host cell lines. With the novel pooled evaluation for KO Screens, we could increase our efficiency 6-fold. These advancements allow us to deliver host cell lines with improved productivity, growth behavior and product quality. Furthermore, future engineering efforts are highly efficient , which will intensify the impact on process performance.

Overall, this platform allows the efficient creation of CHO DG44 knockout cell lines, which can be used to study their production characteristics and takes us one step forward to safer and more cost-efficient host cells.