Creating a robust cell line development platform starts with the host cell line

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Abstract

What do you need to create a robust cell line development platform? A robust host cell line – one that grows in suspension culture in the desired medium and has a consistent doubling time of < 24 h. We established such a host cell line, which can also seamlessly switch between cloning and production media.

Cytiva has adapted a serum-dependent CHO-K1 cell line to serum-free suspension culture in chemically defined and animal component-free HyClone™ media. To ensure success in the shortest time, we used a two-pronged, four-media adaptation strategy with eight adaptation trains. After growing the resulting GOCHO™ host cell line for 130 more generations, it doubled in < 20 h, displayed no cell clumping, and readily transitioned between cloning medium and HyClone™ ActiPro™ production medium. We then evaluated the GOCHO™ host cell line using model monoclonal antibodies. The cell line readily yielded stable clones that produced 3 to 5 g/L in a shake-flask, fed-batch process that was not optimized.

Materials and methods

CHO-K1 cells were thawed in α-MEM (Cytiva) + 10% fetal bovine serum (FBS; Cytiva). After a brief expansion in T-flasks, cells were seeded in shake flasks (SF) in media mixtures containing 50% of the desired end serum-free medium (SFM; Fig 1). Once success criteria were met at each stage, the cells were transitioned to the next media mixture containing less serum-containing medium (SCM). Cells were cryopreserved at each transition. Once cells were passaged into 100% SFM, a research cell bank (RCB) was generated and tested for mycoplasma. Cells were then thawed and passaged for an additional 100+ generations, with RCBs being generated every ~ 30 generations. Adaptation to multiple HyClone™ media was performed in parallel, with the goal of establishing a cell line that could transition seamlessly between cloning medium and the ultimate production medium, HyClone™ ActiPro™ (Cytiva). A seamless transition, defined as maintaining viability > 95% with no apparent growth lag, was tested first in SF (Fig 5), and later within the context of the cell line development workflow (see Fig 6 and 7 for representative clones).

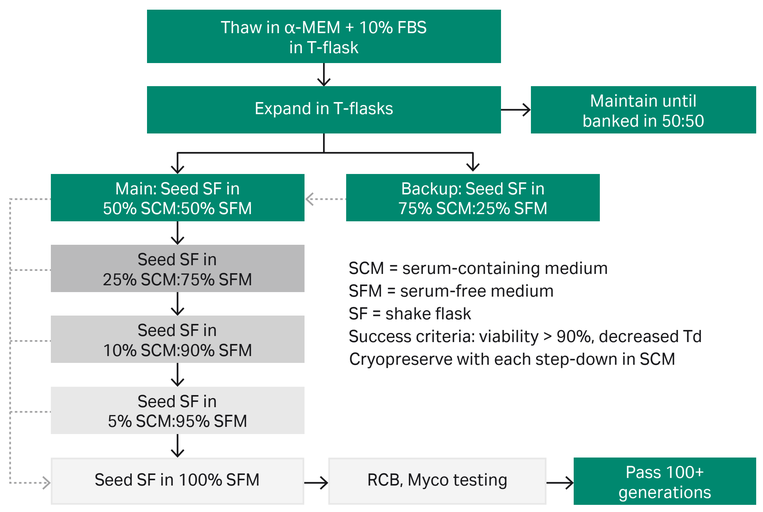

Fig 1. Simultaneous adaptation strategy: CHO-K1 cells thawed in serum in T-flasks were transferred to shake flasks with the first serum reduction step. Td is doubling time.

Summary

CHO-K1 cells were adapted from adherent culture in SCM (Fig 2A) to suspension culture in serum-free chemically defined (Fig 2B) without clumping.

(A) (B)

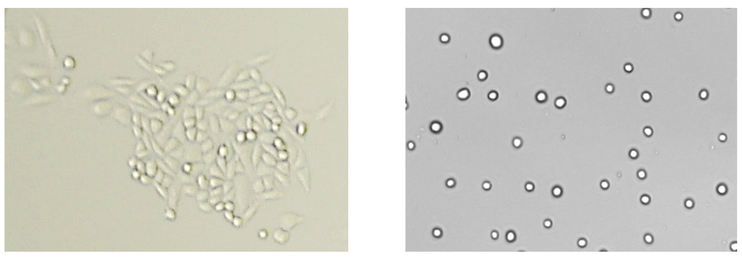

Fig 2. Images of CHO-K1 (A) pre-adaptation and (B) post-adaptation.

Results

Adaptation timeline

Cells were assessed every 3 to 4 d (Fig 3) and passaged in either existing media or into media with decreased serum per the serum reduction plan (Fig 1). Simultaneous adaptation to both suspension culture and serum-free growth took ~ 8 wk. Additional passage of the GOCHO™ host in SFM for ~ 130 generations decreased Td to < 20 h (Fig 5).

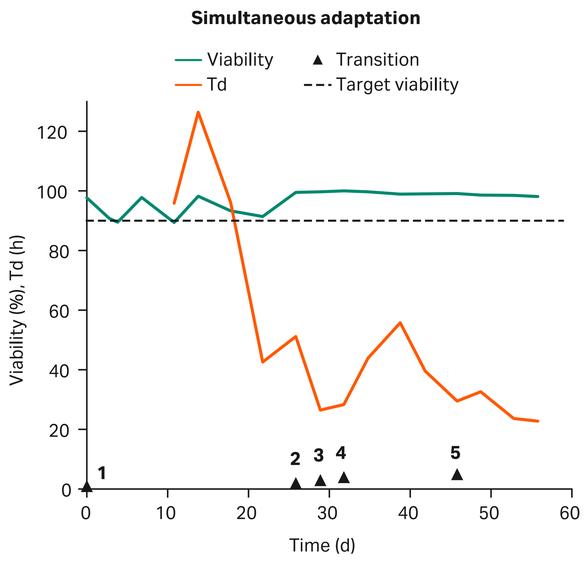

Fig 3. Successful simultaneous adaptation: Adaptation of CHO-K1 to serum-free growth in SF is shown for one of the four SFM used. After starting in 50:50 SCM:SFM (1), cells were passaged into 25:75 SCM:SFM (2), 10:90 SCM:SFM (3), and 5:95 SCM:SFM (4) before being seeded into 100% SFM (5). Viability (green) remained above the 90% target (black) from wk 3 onward. Td (orange) trended down over time.

Value of multiple parallel adaptations

In addition to the simultaneous adaptation strategy, a sequential adaptation strategy was also performed. As Figure 4 shows, after ~ 5 wk to successfully remove serum in T-flasks (transitions 1–4), cells failed to transition to SF (transition 5) as indicated by lost growth (negative Td not graphed), loss of viable cells, and no recovery of cell health to > 90% viability.

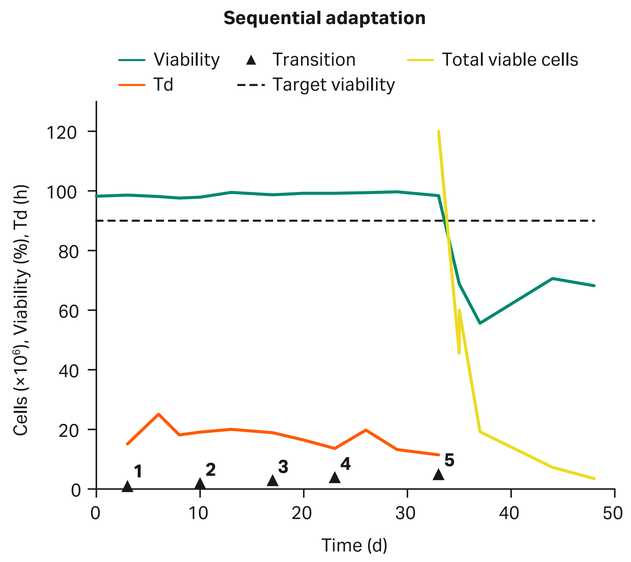

Fig 4. Failed sequential adaptation: CHO-K1 adapted to serum-free growth in T-flasks failed to transition to suspension growth in SF. After transition 5, total viable cells (yellow) decreased, viability (green) decreased, and Td (orange) went negative (not graphed).

Seamless media switching

One of the biggest challenges in cell line development (CLD) is the transition in/out of cloning media. To confirm that GOCHO™ cells were ready for CLD workflow testing, cells were grown in shake flasks and alternately passaged in either cloning media (CM) or ActiPro™ (AP) production media. The original train (green) was maintained in parallel for reference. Media switching was observed to be seamless as viability was maintained above 95% and there was no apparent growth lag. The differences in Td observed between CM and AP is consistent with the Td of GOCHO™ cells in CM and AP when not switching media.

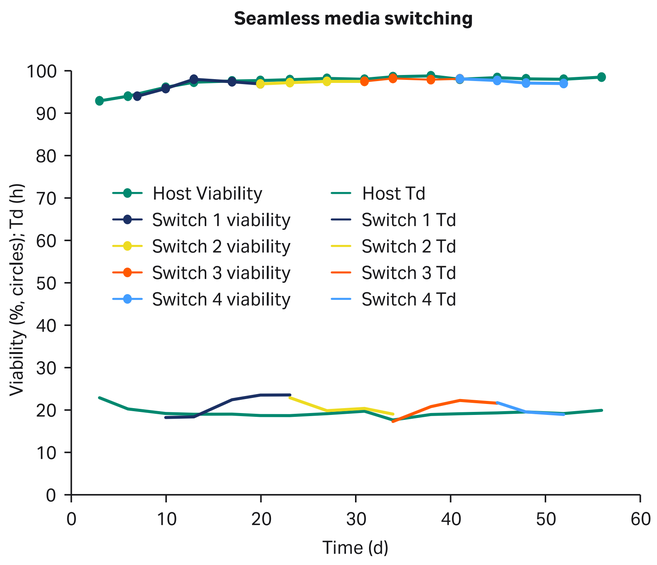

Fig 5. Seamless media switching: GOCHO™ cells were thawed and alternately passaged between cloning medium and ActiPro™ medium. Doubling times (Td, *bottom lines*) and viability (*circles*) were monitored over 2 mo. Switches: 1 to CM, 2 to AP, 3 back to CM, and 4, back to AP.

CLD workflow testing

GOCHO™ cells were transfected with three different model monoclonal antibodies, and clones were isolated using cloning medium and ActiPro™ medium in the CLD workflow. Top clones were assessed for fed-batch productivity (Fig 6) and production stability (Fig 7).

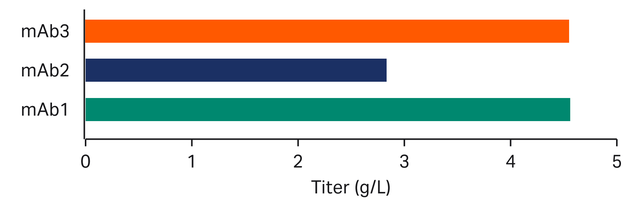

Fig 6. Top stable clone titers: Clones were evaluated for fed-batch titer in SF. The titer of the top stable clone from each molecule is shown from a standard Cell Boost™ 7a+7b feed strategy.

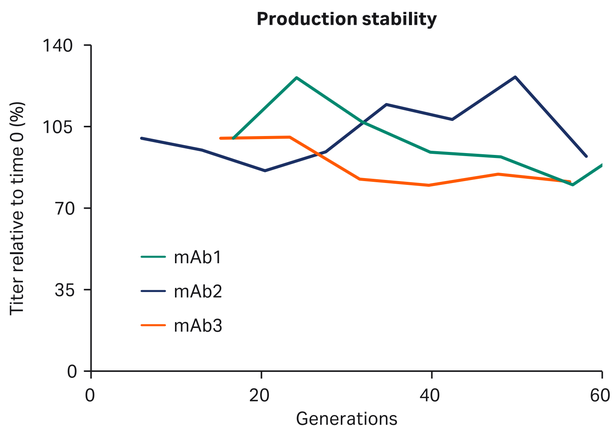

Fig 7. Top clone stability: Clones were evaluated for production stability in shake flasks. Weekly productivity assessments were used to establish productivity levels over time, and stable clones maintained titers within 30% of initial values.

Conclusion

CHO-K1 cells were successfully adapted out of adherent culture in SCM to suspension culture in SFM. The GOCHO™ host cell line was free of cell clumps (Fig 2), had a Td of < 20 h, and seamlessly switched between cloning media and ActiPro™ production media (Fig 5). GOCHO™ cell line performance within the context of the cell line development workflow was confirmed with the successful isolation of stable high-producing clones from three model monoclonal antibodies (Fig 6 and 7).

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