A CHO MEDIA PLATFORM TO FACILITATE CELL LINE DEVELOPMENT

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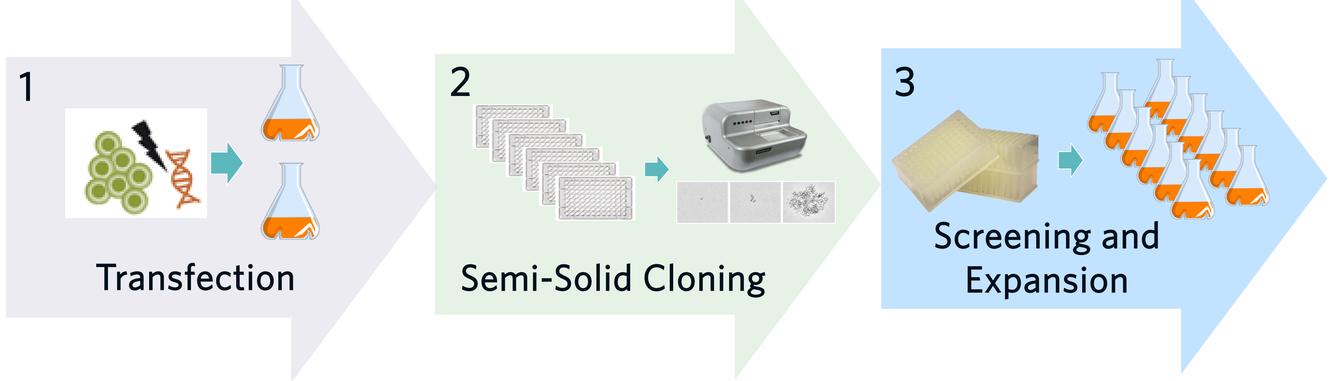
Introduction

Chinese hamster ovary (CHO) cells continue to be the workhorse host cell line for manufacturing protein therapeutics. The development of a top performing clone entails multiple activities which include transfection, clone selection, and productivity assessment. Irvine Scientific has developed the chemically-defined, animal component-free BalanCD® CHO media platform to allow media consistency from development to manufacturing. Implementing this media platform throughout cell line development can help simplify workflow and shorten development time by reducing the need to optimize the media at each step. In this work, we demonstrate how we used rational design to develop 1) the BalanCD Transfectory™ CHO medium that allows for high transfection efficiency of CHO cells ensuring productive yields of recombinant protein, 2) the CloneMedia CHO Growth A semi-solid medium to promote rapid and robust growth of single cell colonies and 3) the BalanCD CHO Feed 4 medium with enriched formulation to support high performing CHO growth and productivity during fed-batch production. Overall, the compatibility of Irvine Scientific's BalanCD CHO media platform provides a seamless, time-saving solution from gene to lead clones within 5 months as demonstrated through case studies.

Overview of Cell Line Development with the BalanCD CHO Media Platform



The ability to use a single, chemically-defined medium mitigates the risk involved with switching media throughout development, and shortens development time into and beyond production.

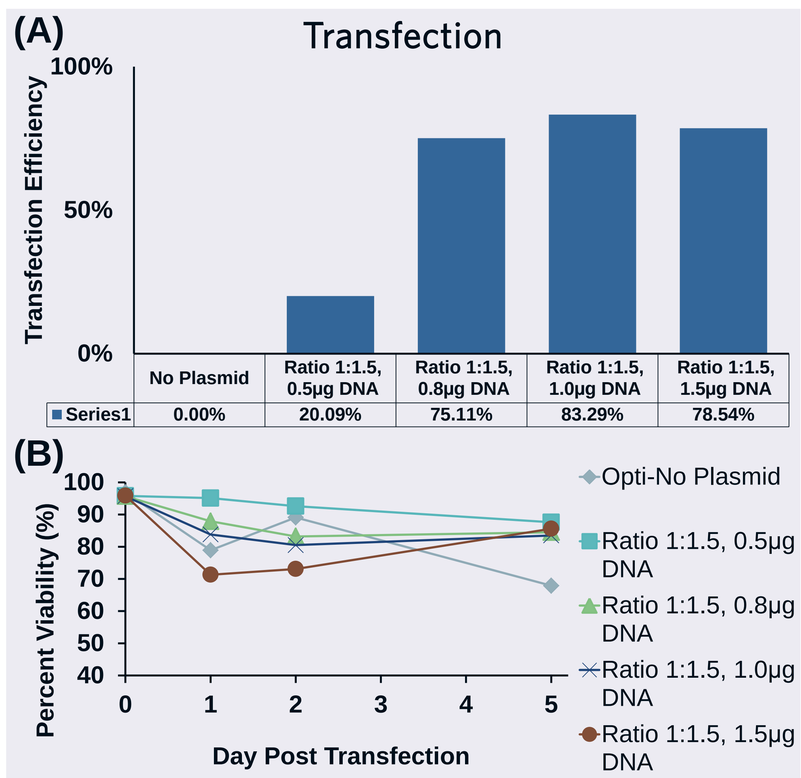
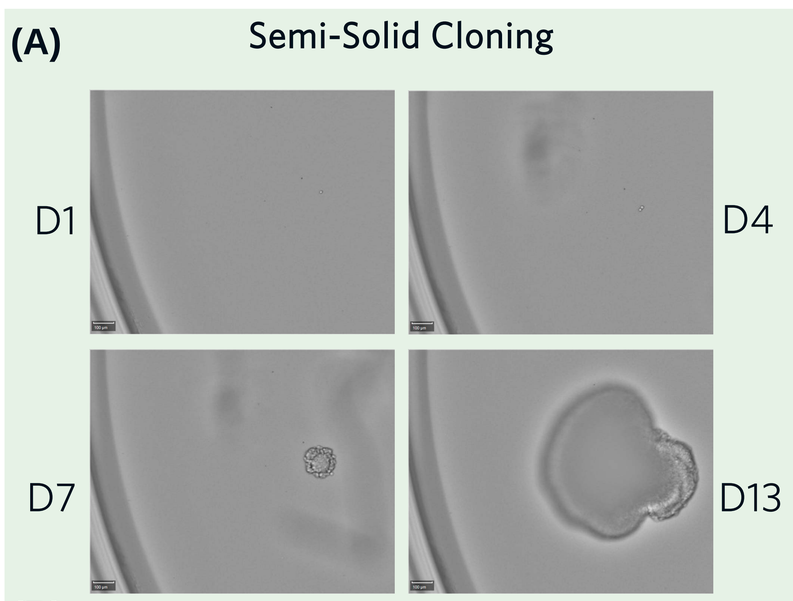


Figure 1: CHO transfection optimization. (A) GS-CHO cells adapted to BalanCD CHO Growth A were transfected with increasing amounts of DNA to determine optimal ratios of DNA (µg) to ml of media. Transfection efficiency is determined by the fraction of GFP positive cells as determined by flow cytometry. (B) Viability of transfected cells were determined using ViCell for 5 days.

➢ Transfectory CHO supports high transfection and viability of CHO cells.



(B)

Antibody	Total wells	1 cell/well	Most likely 1 cell/well	> 1 cell/well	False Negative	Cloning Efficiency
Ab #1	1600	169	115	64	8	10.6%
Ab #2	1560	167	91	68	13	10.7%

Figure 2: Semi-solid cloning of GS-CHO cells in 96-well plates. (A) CloneMedia CHO Growth A supports growth of clonal CHO cells. Images taken with the CloneSelect Imager are representative of clonal CHO growth over the course of 13 days. (B) Table represents clonal yields in two example campaigns.

➢ CloneMedia allows for rapid screening and identification of promising clones paired with high resolution imaging for monoclonality.

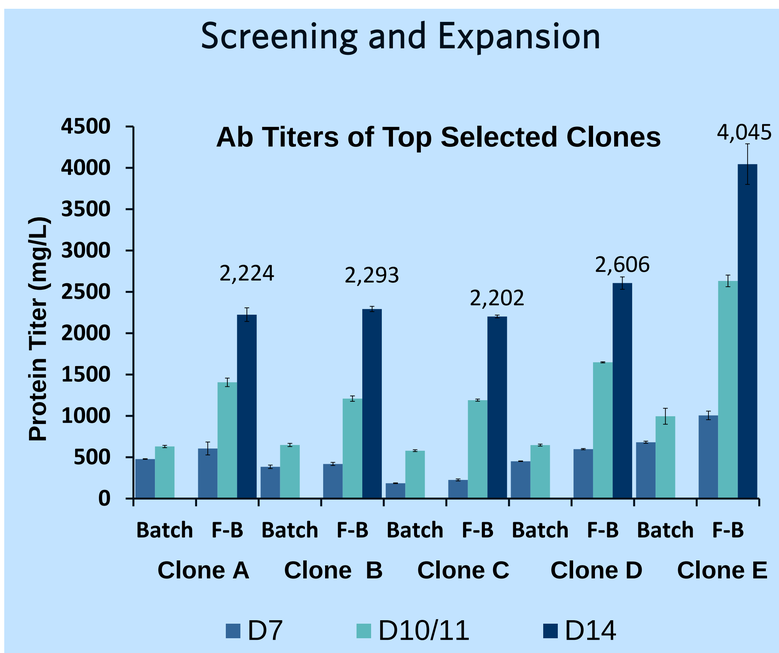


Figure 3: Fed Batch Production of Clones. Top clones were evaluated under platform batch and fed-batch strategy, grown in BalanCD CHO Growth A. BalanCD CHO Feed 4 was used in the fed-batch schedule. Cultures were terminated when viability dropped below 70%. Antibody titers are shown for days 7, 10/11, and 14 (mean ± STD; n=2).

➢ Amplifying from single colonies to fed batch is seamless with the BalanCD CHO platform of growth and feed media, and provides top clones ready for production.

Methods

Transfection

- GS-CHO host cell line (Horizon Discovery) adapted to BalanCD CHO Growth A medium (Irvine Scientific Cat#91128) with 4mM Glutamine. Cultured at 37°C, 5% CO_2 with 120 rpm agitation
- Cells transfected using FectoPro reagent (Polyplus) in BalanCD Transfectory CHO Growth A (Irvine Scientific Cat#91147) without Glutamine with MSX
- Viable cell density and viability measured using Vi-Cell XR (Beckman Coulter)

Semi-Solid Cloning

- CloneMedia CHO Growth A with no Glutamine (Molecular Devices Part# K8830)
- Stable transfected cells were seeded into 96wp on Day 0
- Images were captured using the CloneSelect Imager on Days 0, 1, 8, and 13

Platform Fed-Batch Strategy

- 30 ml cultures in BalanCD CHO Growth A medium in 125 ml shake flasks
- Initial seeding density at $3x10^5$ cells/ml
- Feed: BalanCD CHO Feed 4 (Irvine Scientific, Cat#94134)
- Feed Schedule: 20% v/v over 4 events
- Temp shift to 33°C on Day 6
- Antibody titers in supernatant measured using ForteBio Octet Qk^e with Protein A biosensors
- Cultures were harvested when viability was below 70%

Summary

- Irvine Scientific products provide media support through all stages of cell line development. The BalanCD CHO platform can simplify a CLD campaign through completion, alleviating the need for media adaptation and growth media screening. Using the BalanCD CHO platform simplifies feed optimization with scale-up and production
- BalanCD Transfectory CHO medium supports efficient transient and stable transfection of CHO cells with a simple, scalable protocol that uses the same medium before and after transfection
- CloneMedia provides excellent support for cloning single cells into robust colonies in semi-solid medium, ready for expansion
- BalanCD CHO Growth A and BalanCD CHO Feed 4 support fed-batch production providing a seamless transition to scale-up.

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