Utilizing Cell Imaging Technology to Shorten Timelines and Reduce Development Resources by Earlier Identification of High Producing Recombinant CHO Cell Lines

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Abstract

Reducing cell line development timelines and resources is a key goal in the generation of biomanufacturing cell lines. In traditional cell line development workflows, hundreds, or even thousands of clones are scaled up to large static or shaken culture volumes so that accurate cell counts and recombinant protein titer measurements can obtained. Not only does the scale up of these large quantities of clones require a significant amount of time, but it is also very resource heavy, often typically requiring multiple personnel and a significant amount of laboratory space. In this study, we employed the Solentim Cell Metric™ imaging technology in our cell line development workflow with the aim of reducing development timelines and resources by earlier identification of lead producing recombinant clones. A CHOZN® GS stable pool expressing a recombinant human IgG was single cell cloned by limiting dilution in 96 well plates. Clonality was verified by imaging on D0, D3, and D7 post plating. The clones expanded, and once they reached approximately 75% confluency, the verified single cell clones (SCCs) were consolidated onto a new plate. Post consolidation, the clones were split into replica 96 well plates, one for continued scale up and one for a 96 well assay. 7 days post plating, the confluencies of the clones in the assay plate were analyzed via the Cell Metric imager, and cell culture supernatants were analyzed for IgG titers via ForteBio® interferometry. Titers were normalized to confluency and the rank order of the clones was established. The same set of clones were scaled up into shake cultures and characterized for growth and productivity in both a 7 day batch assay and a 14 day fed-batch assay. The data indicates that the rank order is not necessarily conserved, however the lead clones in static scale up to be the lead clones in shake culture. Incorporation of this early normalized productivity screen into our cell line development process may prove to be invaluable, saving us a considerable amount of time and resources by significantly reducing the number of clones that need to be scaled up into shake cultures for further characterization.

Materials and Methods

Cell Culture

The CHOZN GS stable pool expressing a recombinant human IgG used in this study was cultured in EX-CELL® CD CHO Fusion growth medium (Sigma-Aldrich). Single cell cloning was performed by using limiting dilution in 96 well plates (Corning®) in 80% Cloning Medium (Sigma-Aldrich) + 20% conditioned CD CHO Fusion. Ten plates were generated, and a total of 80 clones were scaled up and analyzed at the 96 well stage. Sixty (60) clones were selected for scale-up into a 7 day TPP® assay using EX-CELL Advanced™ CHO Fed-batch Medium. 30 clones were included in a 14-day fed batch TPP assay using EX-CELL Advanced Medium and Feed. Cell culture titers were analyzed via interferometry by using a ForteBio instrument (Pall Life Sciences).

Cell Imaging

Clonality of single cell clones was verified by imaging on D0, D3, and D7 on the Cell Metric imager (Solentim, see **Figure 1**). Only wells that contained a single cell on D0 were scaled up for further characterization (**Figure 1**). Static 96 well assay plates were imaged on the day of plating (D0) and the day of titer analysis (D7, **Figure 2**). The Verify clonality program was used for all cell imaging.

Results and Discussion

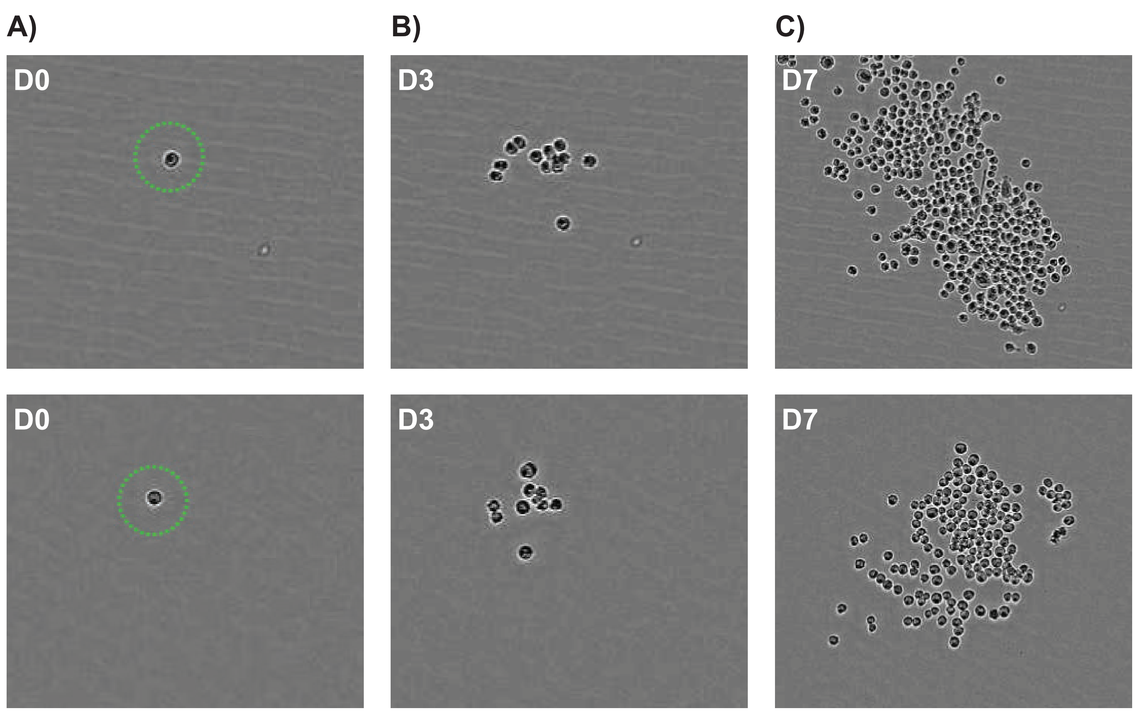

Figure 1. Representative images from single cell clones.

Images from two different single cell clones taken on the Cell Metric imager showing (A) a single cell on D0, (B) a small group of cells on D3, and (C) full colonies on D7. Only those wells containing a single cell on D0 were selected for scale-up.

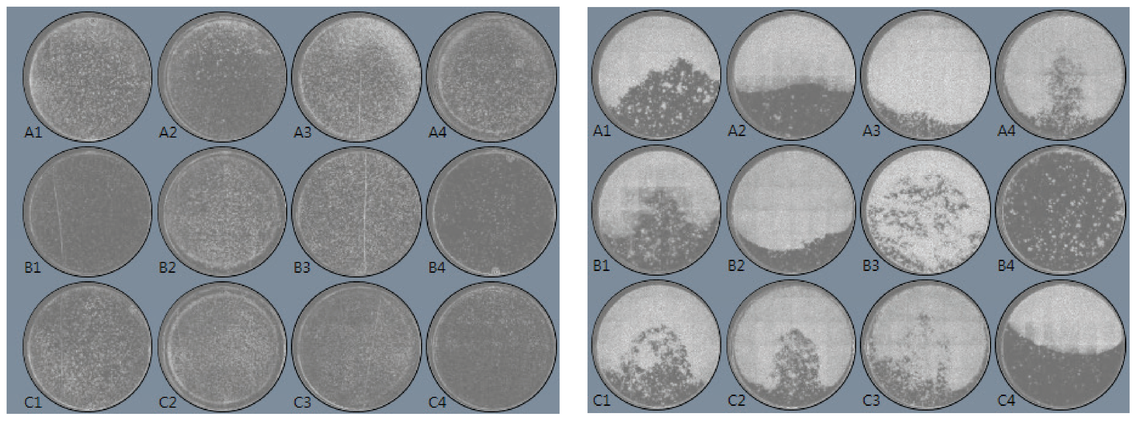

Figure 2. Plate image of static 96 well assay on D0 (plating) and D7 (harvest).

A representative image of various wells and their growth from (A) day of seeding and (B) day of harvesting of the 96 well static assay. Cells were imaged via the Cell Metric to assess confluency.

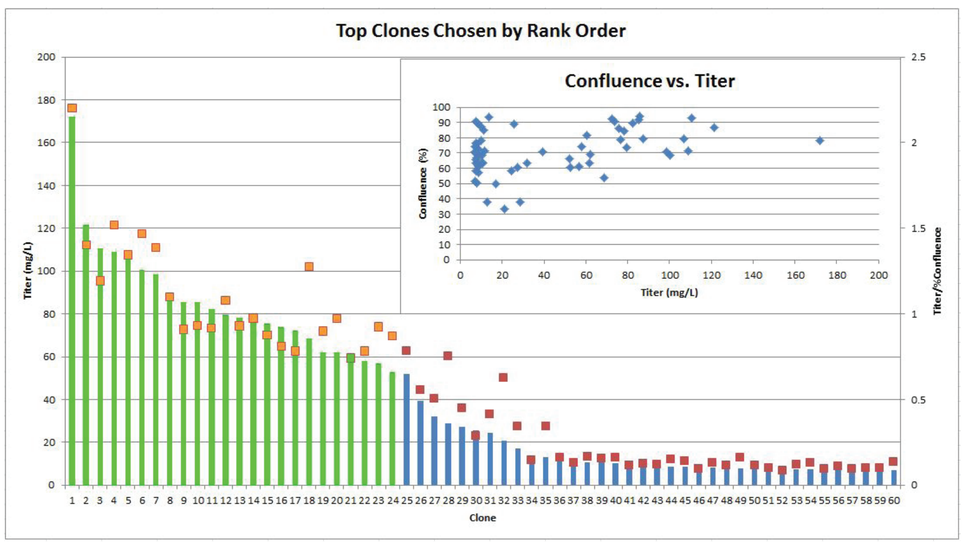

Figure 3. Top clones chosen via rank order.

Green bars show top clones that would have been scaled up via traditional means (titer only). Orange dots show top clones that would have been scaled up via new “normalized” method (Titer/%Confluence).

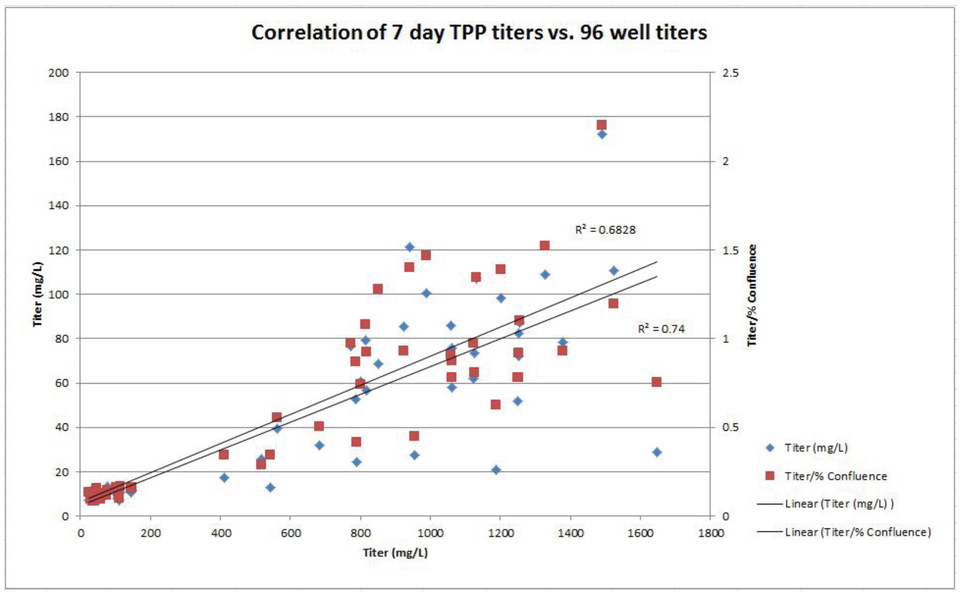

Figure 4. Correlation of 7 day TPP titers to both 96 well titer and 96 well normalized titer.

Both 96 well data sets correlate well with the 7 day TPP data, indicating that the 96 well static assay can be used to identify top performing clones for scale up and further characterization.

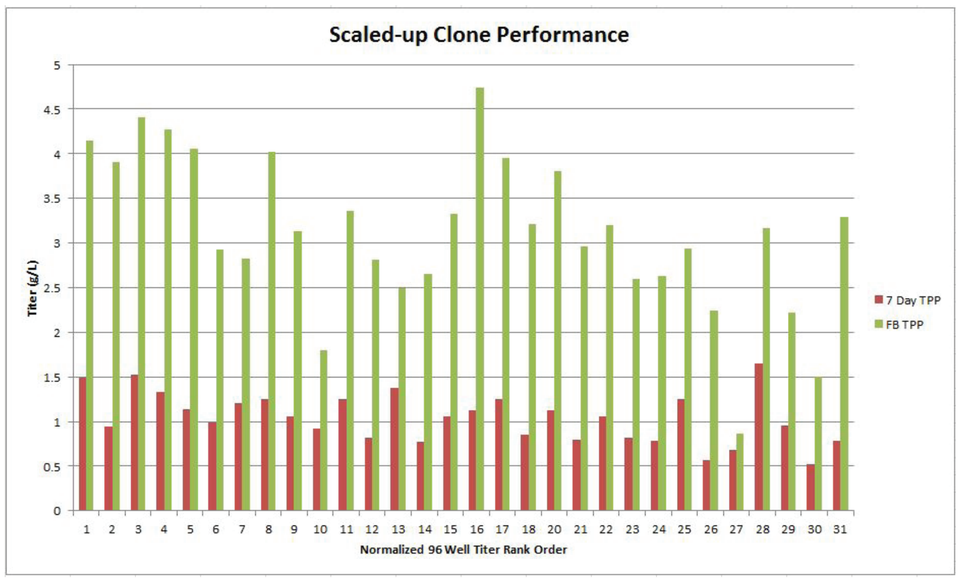

Figure 5. Peak productivity for the lead 30 clones.

The Top 30 clones shown by 96 well static titer rank order. 7 day TPP titer is compared to 14-day fed-batch titer, showing the robust increase between the two time points.

Conclusions/Future Directions

- With this new information, in the future we can eliminate the 7 day assay from our cell line development workflow, and simply use confluency and titer data from the consolidated 96 well plates for our early clone selection.
- The titer normalization method shows a good correlation, but not necessarily much better than our standard process which does not take into account confluency. However, for minipools normalized titers may be more predictive because of greater heterogeneity in cell recovery/growth across different pools. The single cell clones in this study were all generated from one minipool which is already a fairly homogeneous population.

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