A Novel Mammalian Cell Line Development Platform Utilizing Nanofluidics and OptoElectro Positioning Technology

Kim Le
Drug Substance Technologies, Process Development, Amgen Inc., Thousand Oaks, CA, 91320

Christopher Tan
Drug Substance Technologies, Process Development, Amgen Inc., Thousand Oaks, CA, 91320

Shivani Gupta
Drug Substance Technologies, Process Development, Amgen Inc., Thousand Oaks, CA, 91320

Trupti Guhan
Drug Substance Technologies, Process Development, Amgen Inc., Thousand Oaks, CA, 91320

Hedieh Barkhordarian
Drug Substance Technologies, Process Development, Amgen Inc., Thousand Oaks, CA, 91320

Jonathan Lull
Drug Substance Technologies, Process Development, Amgen Inc., Thousand Oaks, CA, 91320

Jennitte Stevens
Drug Substance Technologies, Process Development, Amgen Inc., Thousand Oaks, CA, 91320

Trent Munro
Attribute Sciences, Process Development, Amgen Inc.Thousand Oaks, CA, 91320

DOI 10.1002/btpr.2690
Published online September 19, 2018 in Wiley Online Library (wileyonlinelibrary.com)

Generating a highly productive cell line is resource intensive and typically involves long timelines because of the need to screen large numbers of candidates in protein production studies. This has led to miniaturization and automation strategies to allow for reductions in resources and higher throughput. Current approaches rely on the use of standard cell culture vessels and bulky liquid handling equipment. New nanofludic technologies offer novel solutions to surpass these limits, further miniaturizing cell culture volumes (10^5 times smaller) by growing cells on custom nanofluidic chips. Berkeley Lights' OptoElectro Positioning technology projects light patterns to activate photoconductors that gently repel cells to manipulate single cells on nanofluidic culturing chips. Using a fully integrated technology platform (Beacon), common cell culture tasks can be programmed through software, allowing maintenance and analysis of thousands of cell lines in parallel on a single chip. Here, we describe the ability to perform key cell line development work on the Beacon platform. We demonstrate that commercial production Chinese hamster ovary cell lines can be isolated, cultured, screened, and exported at high efficiency. We compare this process head to head with a FACS-enabled microtiter plate-based workflow and demonstrate generation of comparable clonal cell lines with reduced resources. © 2018 American Institute of Chemical Engineers *Biotechnol. Prog.*, 34:1438–1446, 2018
Keywords: Cell line development, automation, nanofluidics

Introduction

The development of a successful therapeutic biologic is a lengthy, multiple step process which begins with isolation of a highly productive mammalian cell line to generate a cell line bank for future clinical and commercial production. Typical mammalian cell line development processes are resource intensive and require lengthy timelines because of the slow recovery of cells during selection, single cell subcloning, and the need to perform multiple screening assays to identify suitable production hosts.[1] Additionally, regulatory agencies require that clinical trials be initiated with material from clonally derived cell lines and banks.[2]

A typical cloning and clone selection process is among the most labor-intensive steps during cell line development as a result of the several technical challenges. First, single-cell isolation is inefficient. Single cells are deposited in individual wells of a cell culture plate by limiting dilution,[3] single cell printing,[4] or

Kim Le and Christopher Tan contributed equally to this work.

Correspondence concerning this article should be addressed to Kim Le at khle@amgen.com

fluorescence-activated cell sorting (FACS).[5,6] Each of these methods have degrees of error in isolating individual cells and clonality must be verified by manual imaging of each individual well, followed by visual confirmation of the presence of a single cell in the well.[7] Second, individual cells grow poorly in microtiter plates likely as a result of the loss of cell-to-cell signaling, or the dilution of autocrine and paracrine factors secreted into the media and require time to recapitulate a healthy culture.[8] Finally, the lack of good high-throughput methods for analyzing protein secretion at the single-cell level that can predict large-scale production titers requires scaling of clones to large scale to allow for protein analytics and screening of product titer and quality.[9] As increasing the number of clones analyzed increases the likelihood of identifying rare clones with high titer and product quality attributes, hundreds if not thousands of clones must be carried from single cell to large scale cell cultures.

To address these challenges, significant efforts have been employed integrating miniaturized cell culture systems with high-throughput liquid handling automation.[10] These systems are usually coupled with microliter scale high throughput analytics to quickly identify potential candidate cell lines for manufacturing.[11] For example, a common miniaturization platform is to use shaken microtiter plates coupled with automated liquid handling.[12,13] However, when increasing throughput (number of wells per plate), technical limitations such as evaporative loss, increased aeration and agitation requirements, low density recovery efficiency, pipetting accuracy at submicroliter volumes, parallel processing liquid handling, and low limits of protein detection (ng) required limit the improvement of these approaches.[14]

Nanofluidic technologies offer a promising solution to further miniaturize cell culture processes.[15] One such technology platform has been developed by Berkeley Lights.[16] Their Beacon platform combines the use of nanofluidics on a temperature-controlled chip arrayed with 1758 pens (OptoSelect™ Chips), single cell manipulation though OptoElectro Positioning™ (OEP),[17–19] and high-resolution fluorescence imaging for downstream analytics. OEP can manipulate hundreds of cells in parallel on OptoSelect™ Chips, allowing multiplexed depositing of single cells into an array of individual pens with nanoliter volumes (nanopen). The entire chip can be imaged in 8 min, and coupled with artificial intelligence-based cell counting, allows for efficient monitoring of clonality and growth of all clones on the chip.[3] Additionally, bead and diffusion-based fluorescent assays can be adapted for scoring secreted antibody on chip. Thus, relative protein productivity can be established on the instrument and only clones with acceptable productivity can be selected for export to microtiter plates and scaled-up for further studies. Captured data can then combined to document cell growth measurements, proof of clonal origin, single-cell secretion, and overall population compositions.

To assess the potential of the Beacon platform in production cell line development, we compared a Beacon-based subcloning workflow with a traditional FACS-based process. We aimed to compare the throughput, timelines, resource requirement, data quality, and overall performance of cell lines generated.

Materials and Methods

Cell lines

Cell lines were generated by transfection of an Amgen proprietary clonal CHO host cell lines with plasmid DNA encoding for a heavy chain and light chain from a human monoclonal antibody. Following transfection, stably expressing pool populations were created through the repeated passaging in selective growth media until cells reach percent viabilities greater 90% and maintain consistent doubling times. Further selection using increasing levels methotrexate is also applied to improve productivity. Cells were cultured in either 96-well, 24-well, or 24-deepwell microtiter plates (Corning, Corning, NY), 125 mL shake flasks (Corning, Corning, NY), T-175 flasks (Corning, Corning, NY), or 50 mL TubeSpin (TPP, Trasadingen, Switzerland) in Amgen proprietary media at 37°C, 5% CO_2, and 85% humidity. Cells were maintained by passaging multiple times a week at a targeted seed density.

Single cell cloning by flow cytometry and imaging

A FACSAriaIIu (Becton Dickinson, Franklin Lakes, NJ) cell sorter was used to isolate and deposit single cells from a stable transfected cell line directly into 96-well microtiter plates prefilled with proprietary cloning medium. Sorting conditions were set to internally determined parameters that encourage high assurance of single cell isolation. After depositing, plates were centrifuged and immediately imaged on a high-throughput microscopic imager (Cell Metric, Solentim, Dorset, UK). Imaging was performed periodically to track the formation of a single colony. Clonally derived cell lines are confirmed using a criterion of (1) clear proof of a single cell, (2) absence of other significant artifacts in the well, (3) formation of single round colony, and (4) verification by two scientists.

Berkeley lights beacon instrument

Cell lines were single cell loaded on OptoSelect™ Chips (Design 1750, Berkeley Lights, Emeryville, CA) using the Beacon Instrument (Berkeley Lights, Emeryville, CA). OptoElectro positioning settings and scripts for loading and exporting cells were provided by Berkeley Lights Inc. Cells were cultured on the OptoSelect™ chips for up to 4 days using proprietary growth media and manufacturer recommended settings. Repeated imaging and cell counting were performed using the integrated 4X microscope and camera on the Beacon instrument. Evidence of clonal derivation is achieved by image of a single cell in a nanopen (0.05 mm^2 area, and 0.002 mm^3). Secretion assays were performed using the Spotlight HuIg2 Assay (Berkeley Lights, Emeryville, CA) with supplied method scripts and assay analyzer software (Cell Analysis Suite, Build 30552). Selected pens were exported using OEP to move cells out of pens followed by flushing off the chip into a 96-well microtiter plate prefilled with proprietary cloning media.

Scale up of exported subclones

Exported cells were scaled up through dilution into proprietary passaging medium into increasing sized microtiter plates and cultured in static incubators at 37°C, 5% CO_2. Transfer steps were assisted using a Biomek Fx^P liquid handling robot (Beckman Coulter, Brea, CA). Cells were transitioned to suspension culturing using shaken 24-deepwell plates (Corning, Corning, NY) and 50 mL TubeSpin bioreactors (TPP, Trasadingen, Switzerland). Growth in microtiter plates was monitored using the Cell Metric imaging system (Solentim, Dorset, UK).

Small-scale production cultivation

Fed-batch evaluations were performed in TubeSpin bioreactors (TPP, Trasadingen, Switzerland). Individual tubes were set up with a working volume of 30 mL of production media, incubated at 36°C, 5% CO_2, 85% relative humidity, and shaken at 225 rpm with at 50 mm orbital diameter in a large-capacity ISF4-X incubator (Kuhner AG, Basel, Switzerland). Cultures were inoculated at a target cell density ranging from 8×10^5 to 1×10^6 cells/mL and were fed a single bolus feed at Days 3, 6, and 8. In-process samples were taken from cultures on Days 0, 3, and 6–10 for analysis. Cell concentrations and viability of cultures were determined using a Vi-Cell XR cell counter (Beckman Coulter, Brea, CA). Antibody titers were measured by affinity Protein A high-performance liquid chromatography.

Bioreactor production

Seven-liter Bioreactors (Applikon, Foster City, CA) were used for a standard production culture with a 4.4 L working volume. Temperature set-point and agitation were controlled by digital control units. pH set-point was controlled automatically by CO_2 or 1 M Na_2CO_3 addition. Dissolved oxygen set-point was controlled by sparging oxygen through a drilled-tube and a sintered-steel spargers. Proprietary production medium were used. Daily samples were analyzed for viable cell density (VCD) and viability (%) using a Bioprofile CDV (Nova Biomedical, Waltham, MA), glucose, lactate, NH4+ and osmolality using BioProfile Flex Analyzer (Nova Biomedical, Waltham, MA), and pH, pCO_2, and pO_2 using Corning 248 blood gas analyzer (Medfield, MA). Supernatant samples were also analyzed for titer.

Results

The Beacon can efficiently clone and recover production CHO cell lines

To determine how the nanofluidic platform would best fit into an industrial cell line development platform, we closely examined each cell line development step. We identified subcloning operations as the most impactful application of the technology because of the (1) high-throughput cell culture needs, (2) requirement to proving clonal derivation and tracking, and (3) the greatest potential for resource and timeline savings through the early decision of a final clone.

To explore the efficiency of performing the cloning workflow on the Beacon instrument, we evaluated the ability to efficiently isolate single cells and document clonality, growth of the cells on the chip under perfusion culture, export of the cells into microtiter plates, and outgrowth and performance of the resulting cell lines. To ensure that there were no product or cell line specific influences on growth and export, 17 unique CHO cell lines were tested from host different lineages, selective pressures, expressing three modalities (monoclonal antibody, a-glycosylated antibody, and bispecific antibody), with a range of titers. We examined efficiency for the four major steps of the workflow (Figure 1A), such as isolation of single cells (Figure 1B), growing clonally derived cells in nanopens (Figure 1C), exporting grown populations out of nanopens into microtiter plates (Figure 1D), and expanding cultures in microtiter plates (Figure 1E). A total of 10 chips with 1,758 pens each were OEP-loaded with the described cell lines to achieve an average of 1,038 single cells per chip (59%), with a range 820–1,258 clones (46%–71%) (Figure 1B). Out of 9,365 single clones, 65% show at least one doubling after 3 days (Figure 1C). Figure 1D describes the range of cell numbers that can moved out of the pen using OEP. Out of 221 unpen attempts (move cultures outside of pen), OEP was able to remove 209 of pens (94%) with at least 3 cells. Following unpenning, cell populations were deposited into 96-well microtiter plates. Of the attempted depositions, 81% form viable colonies in plates (Figure 1E). In total, a single chip can generate from 406–623 viable clones from a single load (or 23%–35% to available pens).

The Beacon workflow can isolate and screen clones for productivity

We next compared subcloning procedures on a cell line expressing a secreted biologic with traditional microtiter plate-based methods vs. a nanofluidic workflow on the Beacon Instrument. Figure 2 describes a standard subcloning operation where a starting heterogenous population is isolated and deposited into microtiter plates using FACS-based cell sorting. Immediately after deposition, high-quality, high-throughput whole well imaging is used to verify a single cell in a well as described above. After growth and repeated imaging, colonies are picked and consolidated using automation liquid handlers. As secretion and growth rates are difficult to measure early on, dozens of clones are scaled up to perform a high-throughput small scale production assay. In contrast, single cell isolation, growth assessment, and high-throughput screen are performed while on the chip (dotted box) in the nanofluidic workflow, and only those clones that meet the desired criteria are exported and expanded for further evaluation.

High expressing CHO stable pools secreting an antibody fusion modality were subcloned using a FACS-based cloning method, and the same pools were cloned and analyzed on the Beacon platform. A total of 1,920 wells (20 plates) were seeded using the standard process as compared to 3,518 pens (2 Chips) on the Beacon process. The FACS process resulted in 360 wells with single cell-derived colonies that passed a stringent image analysis screening that consists of repeated image tracking of colony outgrowth originating from a verified single cell. Alternatively, for the Beacon process, 1,603 clones were derived from single cells as determined by high quality imaging of each pen after loading on the OptoSelect™ chips. Cells were then assessed for growth for up to 4 days though repeated bright-field imaging of pens and the use of a cell counting algorithm. (Figure 3A). To measure secreted antibody, a diffusion-based assay was employed (Spotlight Assay). In this assay, nanopens are first saturated with a fluorophore-tagged small molecule targeting human IgG Fc. Following equilibration of fluorescent signal between the pen and the channel, the chip is flushed to clear signal from the channel and diffusion of the fluorescent molecule out of the pens is observed. Pens containing secreted antibody have the fluorescent small molecule bound to the antibody forming a complex that diffuses slower as a result of its high molecular weight as compared with pens with little or no antibody where most of the fluorescent signal diffuses quickly. This difference in diffusion rates is measured and quantified on the Beacon platform (Figure 2). A total of 51 clones were exported for scale up (highlighted) which exhibited a wide range of growth (Figure 3B) and productivity characteristics (Figure 3A). Following export, populations were scaled to suspension culture for small-scale fed-batch screening.

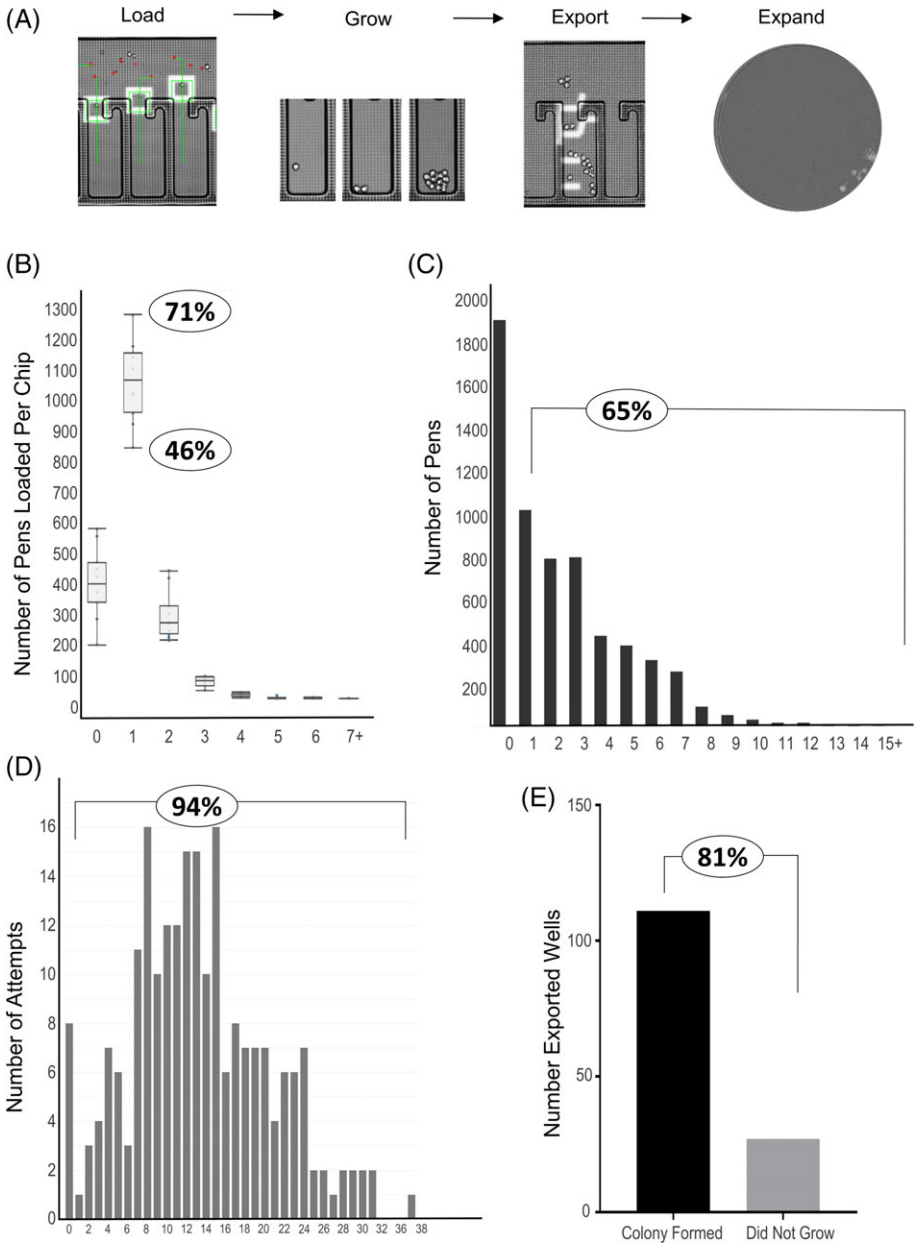

Figure 1. Assessment of workflow efficiency of the Beacon platform. (A) Representation of workflow with images of cells and colonies. (B) Number of pens that contain empty, single, or multiple cells per chip after loading operation. Percentage denotes highest and lowest percent singles loaded. (C) Cell count of pens after 3 days from single cell loads. Percentage denotes number of single clones with positive cell growth. (D) Counts of unpenned cells after export workflow. Percentage denotes numbers of pens with exports greater than three cells. (E) Count of wells with growth after export.

To determine whether the Spotlight assay is predictive of larger scale fed-batch productivity, the Spotlight titer measurements for each clone were compared to resulting 10-day fed-batch specific productivity following approximately 50 days of scaleup. Plotted in Figure 3C is the comparison of early Spotlight assay score (*x*-axis) vs. the specific productivity of each clone during fed-batch (*y*-axis). Overall, we see decent correlation of titer scores with the final productivity of cells (Pearson product correlation coefficient = 0.7395).

The Beacon cell line development workflow generates comparable cell lines as traditional methods

Beacon generated cell lines were compared with equal numbers of clones expanded from FACS-based workflow. The overall total time from seeding to suspension screening start was comparable between processes. Clones from both processes were run side by side in a small-scale fed-batch screening experiment. On average, the plate-based process yielded clones with an average normalized titer of 1.0 and range from 0.1 to 2.2. The Beacon clones on average had a normalized titer of 1.25 with a range of 0.3 to 2.9 (Figure 4A). Following small scale fed-batch screening, the top three clones from each process were run on bench scale bioreactors. VCD, viability, cumulative titer, and specific productivity vs. process duration (respectively clockwise, left to right) are plotted in Figure 3C. The top Beacon clones (black) perform similarly to top standard clones (white) in terms of titer and viability. Two Beacon isolated clones show higher specific productivity by achieving comparable titer with lower total cell mass. Overall, the top beacon clone

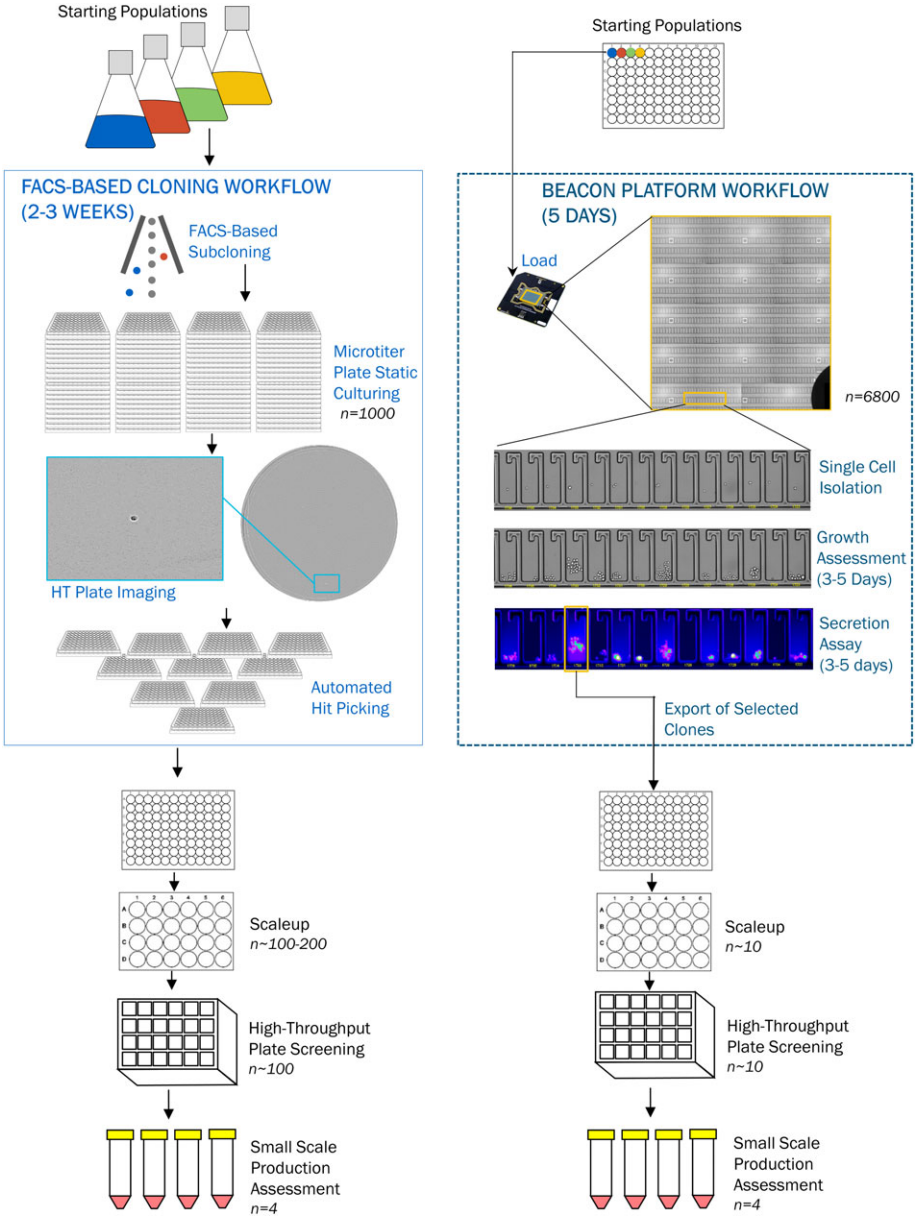

Figure 2. Comparison of a microtiter plate-based cloning workflow vs. a nanofluidic chip subcloning workflow. A depiction of the steps involved in performing a clonal isolation and expansion workflow using two approaches. Differences are highlighted in boxes for FACS-based workflow(solid) and Beacon workflow (dotted).

(Beacon-A) performed comparable to the top plate-derived clone (FACS-B).

The Beacon platform can generate high expressing production cell lines with reduced resources

As the Spotlight secretion measurements have been shown to correlate with later clone productivity, it can be employed to preselect top clones and therefore reduce the numbers of clones needed to scale up and analyze. To demonstrate this, the Beacon was again compared side by side to a standard FACS process for a new model monoclonal antibody. In this case, a standard FACS-based process yielded a total of 157 mature clonally-derived candidate cell lines. For the Beacon process, only 10 clones were scaled and evaluated. These 10 clones were preselected by the Spotlight assay and growth on the chip and represented the top 25% of secretors and top 50% of doubling times. The fed-batch screening was performed resulting in the FACS-based process analyzed 92 clones with an average normalized titer of 1.0 and range from 0.01 to 2.0. The 10 Beacon clones had an average normalized titer of 1.19 with a range of 0.5–1.6. The top 4 clones of each process were then run in an alternative intensified process that favors higher cell mass accumulation and higher viability. Top Beacon clones performed similarly to top standard clones in terms of titer and viability. Similar to the previous study, the two Beacon clones were identified having higher specific productivity. Overall, the Beacon process was able to achieve comparable clones as the FACS-based process despite scaling up a fraction of the number of clones.

The Beacon platform allows for rich analysis of data

In addition to execution of existing cell line workflows at higher efficiency, the Beacon platform generates a richer data

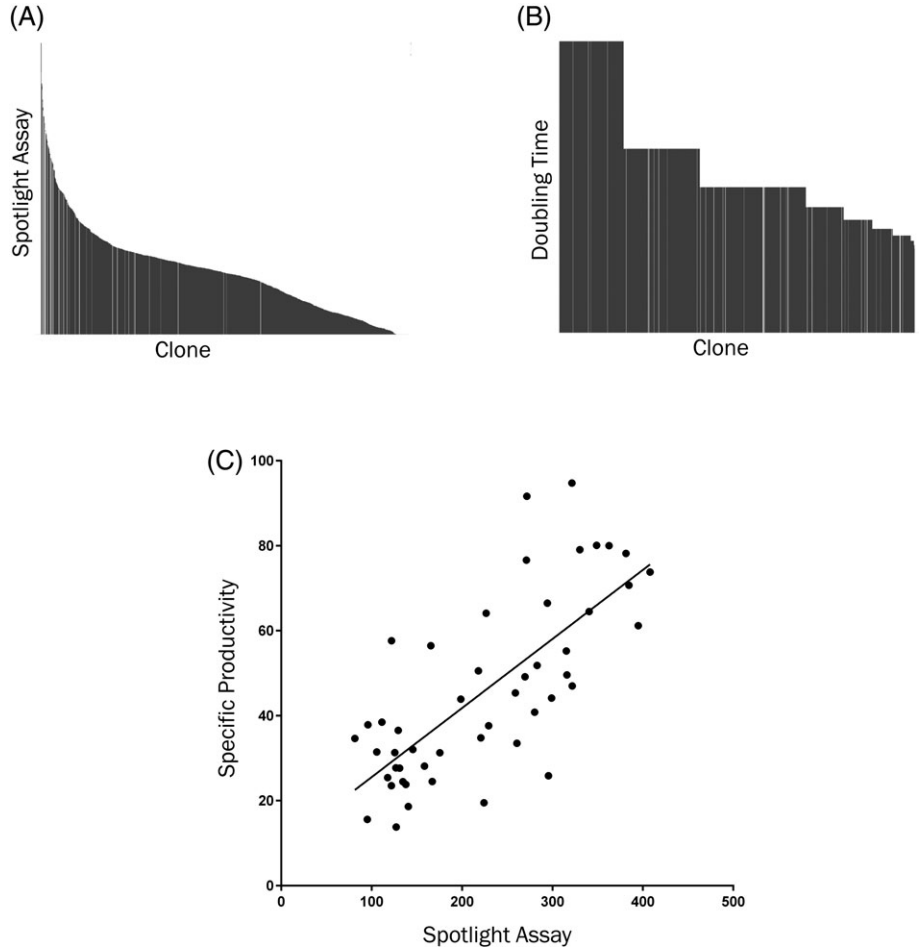

Figure 3. Correlation of Spotlight Titer analysis of early clones with fed-batch specific productivity of mature clones. (A) Secretion and growth measurements measured on OptoSelect™ chips. The Spotlight secretion assay generates a ranked assay score. (B) Growth assessment on chip is performed through performing cell counts and calculation of doubling time. Selected clones with a wide range of growth and secretion highlighted in white. (C) Scatter plot of individual clones analyzed early on the Beacon using Spotlight Assay (X-axis-Titer score) and analyzed 50 days later in a suspension fed-batch production experiment (Y-axis-Relative specific productivity).

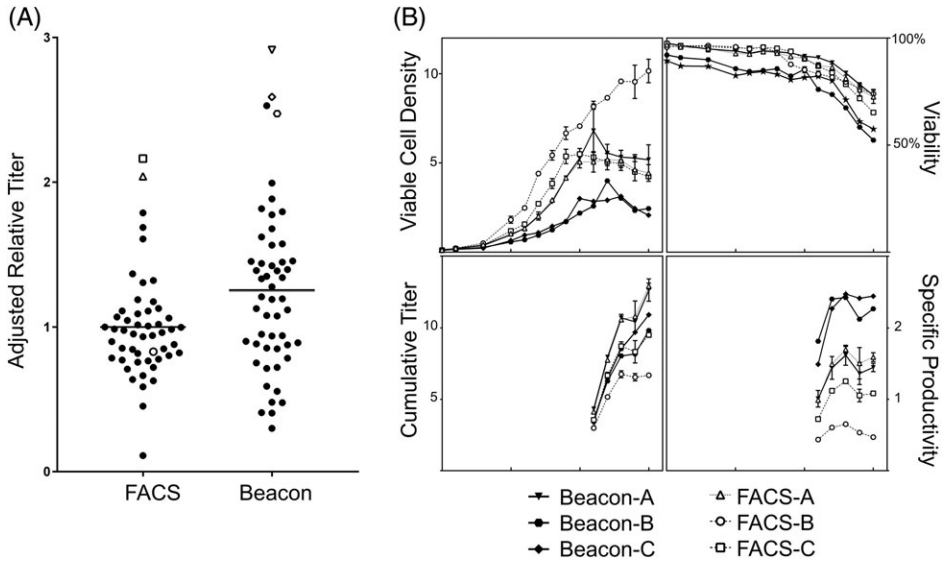

Figure 4. Comparison of clonally derived cell lines from FACS-based and Beacon Workflows. (A) Fed-batch screening of mature clones. Clones from both methods screened using a fed-batch production screen and accumulated titer measured (Normalized grams/liter). Top clones from each method highlighted with open circles. (B) Bioreactor screening of top clones. Top 3 Beacon clones (Black) screened with top 3 FACS-based clones (white). Plot of VCD (Top Left), viability (Top Right), accumulated titer (Bottom Left), and specific productivity (Bottom Right) over process duration.

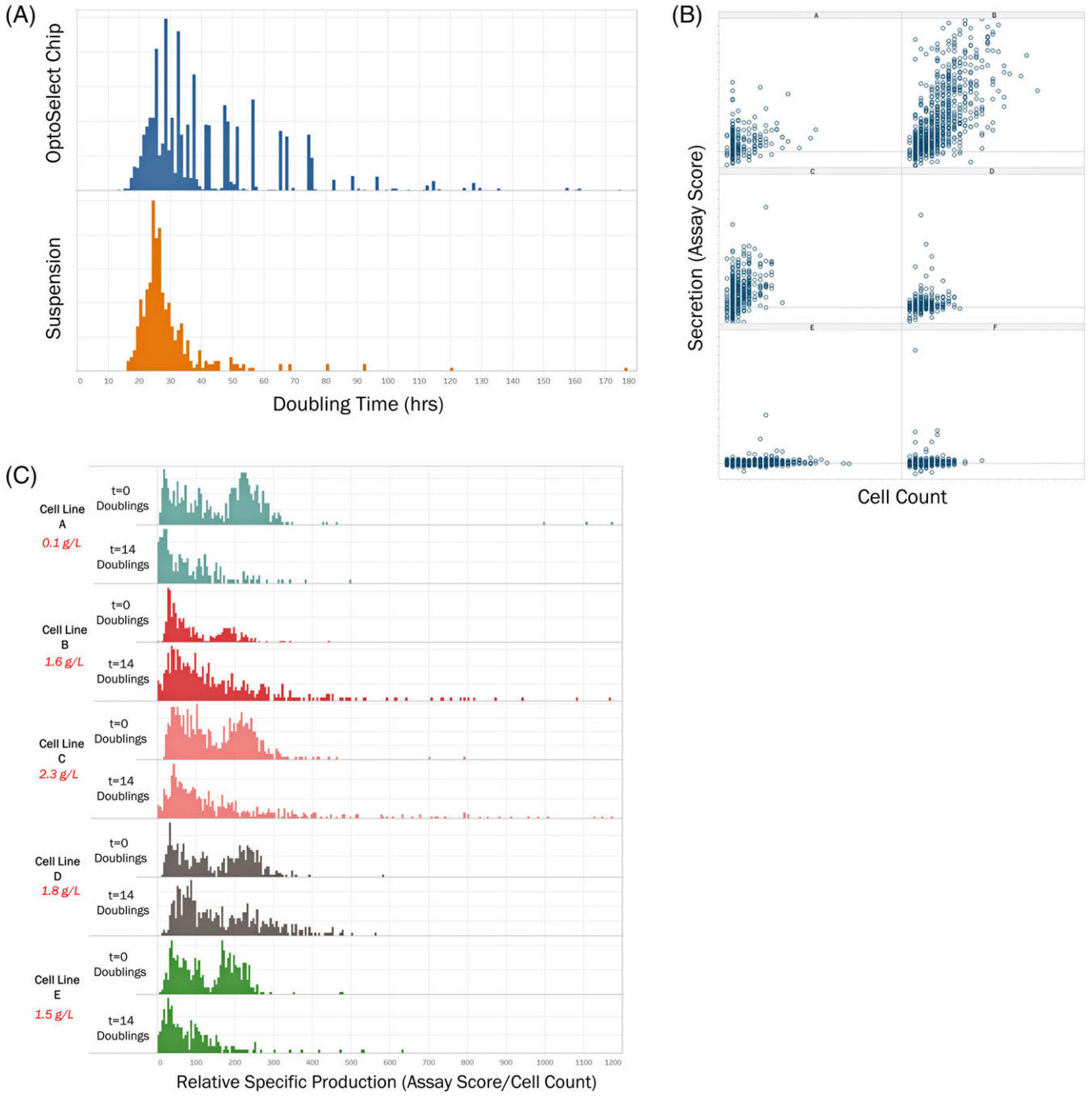

Figure 5. Assessment of stable cell lines on the Beacon platform. (A) Interrogating cell growth from single cells (blue) vs. shaken suspension pools (orange). (B) Single clone analysis of stable populations for growth and secretion. Plot of cell numbers after 3-day growth vs. spotlight secretion assay score. (C) Specific productivity profiles of five stable pools after culturing.

package as compared to FACS or batch screening data. Unlike typical FACS analysis, the Beacon platform allows for the assessment of secretion and growth at a single cell level. As a result, a better understanding of the dynamics of the overall populations and subpopulations is visualized. Figure 5A compares the doubling time of a selected antibody-expressing pool population. The cell line was either passaged in shaken suspension (shake flasks) vs. clonally isolated and grown in individual nanopens. While the median doubling time on the Berkeley Lights instrument was marginally higher (28 vs. 26 h) than shake flask doubling time, the overall distributions were similar, indicating that production CHO lines are capable of comparable growth on the instrument. The lower mean growth rate on the instrument is not surprising as shake flasks represent a large population of cells where faster growing cells will outgrow slower growing cells and be over-represented in the culture. Indeed, rare clones with doubling times of >100 hours can be seen on the instrument and represent an interesting population of cells we have not previously been able to isolate because of the masking by the faster growing cells in microtiter plates.

The Beacon data set also allows single cell population comparisons across multiple pools. Figure 5B plots secretion vs. 3-day growth of individual pens for an unamplified vs. methotrexate (MTX) amplified pool expressing a monoclonal antibody. This analysis provides a full view of a population

heterogeneity of not only relative expression but also doubling times. For example, the unamplified pool A is primarily constituted by a fast-growing population with relatively low expression and a few rare slower growing outliers, while some have high productivity. Upon amplification to 150 nM MTX, population B profile changes dramatically, with a shift to a higher producing, faster growing population. Importantly one can then potentially isolate cells with a unique growth and productivity profile. Interestingly, a 300 nM MTX amplified pool (C) shows a population centered around lower growth population relative to the 150 nM pool, suggesting increasing levels of MTX lead to diminishing productivity returns. In panels D–F, a separate cell line profile shows that one of the populations has a low productivity and rapidly dividing subpopulation (E), population (F) exhibits a slow-growing high-productive phenotype, and population (D) has a combination of growth and secretion.

Figure 5C further visualizes the distribution of specific productivity in a pooled population. Here, four stable pool cell populations are secreting the same protein and only differ by transfection event. The productivity profiles generated on the Beacon correlate well with the measured batch titers at the later time point. Interestingly one can observe how these pools change over time in culture as pool B and C appears to have higher production populations that are emerging during the culture period. These figures exemplify how richer data obtained from the Beacon gives insight into the interrogated population, allowing for stronger assessments and decision making.

Discussion

We have demonstrated the feasibility of performing a cell line development workflow on a novel nanofluidic platform utilizing software driven OEP and fluorescent detection. As a result, a simplified workflow to maintain and screen over 3,000 subclones simultaneously, document clonality, and efficiently select highly productive clones and export clonal cell lines is achievable.

The case study described demonstrates the ability to reduce overall resources by dramatically decreasing the total number of subclones that need to be individual maintained, scaled, and analyzed. Through screening early on chip, we show that it is only necessary to isolate a fraction of Beacon-selected clones as compared to FACS based methods to achieve higher average fed-batch titers though the elimination of poor producing clones (less than 0.5 relative titer). Screening significantly fewer clones in plate-based formats results in a corresponding decrease in cell culture load that translates to reduced labor, required incubator space, and laboratory footprint.

Another critical resource advantage of utilizing an integrated nanofluidic device for commercial cell line development is the ability to generate high quality images throughout the entire subcloning process for generating complete proof of clonality reports. Time lapse imaging of growth from the single cell stage to export provides confidence of clonal origin, a complete record of cell growth, and secretion phenotypes. Simultaneous imaging of empty pens allows for the in process monitoring of cross-contamination throughout the duration of the experiment. Exports are tracked by on-instrument imaging at each step of the OEP export process and complemented by subsequent off-instrument imaging of export plates. Together, a complete image documentation package can be created to provide to regulatory agencies to assure clonal derivation.

In addition to streamlining the cloning process and reducing resources, this study also potentially highlights the power of the Beacon to select cells with rare phenotypes or desirable traits. For example, in both case studies, the Beacon workflow derived clones had the highest specific productivity. While plate subcloning and production screening selection can be biased towards faster growing clones, slower growing clones with higher specific productivity can be interrogated in the nanofluidic environment for use in long-term perfusion-based manufacturing platforms where steady growth is optimal for example. Further, the single cell data obtained from cell lines provides insights into defining population characteristics of the production cell lines that are not currently possible with the traditional FACS-based methods. Data generated in this study provide additional understanding of rapidly shifting populations dynamics that occur during prolonged culturing of cells in vitro (Figure 5). Ultimately, this data can be combined holistically with manufacturing data to develop predictive models for cell line selection.

Top clone selection is arguably one of the most important decisions made over the lifecycle of bringing a novel biotherapeutic to patients. The resulting master and working cell banks are used over the entire life-cycle of the marketed product that can last multiple decades. Currently, a great deal of effort and scrutiny is involved in the selection of the top clone through assessment of performance and product quality in small scale production runs followed by protein analytics. The Beacon platform offers an opportunity to obtain large amounts of similar information from thousands of clones at the point of cloning. As described in the Figure 1, we can obtain detailed growth information, secreted antibody titers, and specific productivity. Additional information can be obtained through modulating the culturing conditions on chip such as media, temperature, gasses, and treatments. Bead-based capture and diffusion assays can also be developed to interrogate product quality immediately after secretion. Together, this package may potentially be used as surrogate data for the selection of the final master cell bank and therefore significantly reduce resources.

Conclusions

Developing a commercial production cell line usually involves three general activities, the culturing of CHO cells, the sterile manipulation of cell culture, and the cellular analytics. The nanofluidic platform offered by Berkeley Lights provides the ability to do all three activities efficiently within an integrated workflow. For the first time, we have shown this type of technology can be directly applied to generate clonal CHO cells suitable for commercial development. We show this is particularly effective when applied to the subcloning workflow. When compared to a traditional plate-based workflow, the Beacon platform is capable of efficiently producing comparable manufacturing cell lines. Additionally, a rich data set is generated to support clonality, tracking, and population understandings and enable early decisions and identify high performing cell lines.

Acknowledgments

The authors thank Tanner Neville, Troy Lionberger, Gang Wang, and contributors from Berkeley Lights Inc. for insights and technical support. The authors also thank their colleagues

in Amgen Drug Substance Technologies and Attribute Sciences for project support.

Literature Cited

1. Wurm FM. Production of recombinant protein therapeutics in cultivated mammalian cells. *Nat Biotechnol.* 2004;22(11):1393–1398.
2. Frye C, Deshpande R, Estes S, Francissen K, Joly J, Lubiniecki A, Munro T, Russell R, Wang T, Anderson K. Industry view on the relative importance of "clonality" of biopharmaceutical-producing cell lines. *Biologicals*. 2016;44(2):117–122.
3. Zhou Y, Shaw D, Lam C, Tsukuda J, Yim M, Tang D, Louie S, Laird MW, Snedecor B, Misaghi S. Beating the odds: the poisson distribution of all input cells during limiting dilution grossly underestimates whether a cell line is clonally-derived or not. *Biotechnol Progress*. 2017.
4. Gross A, Schoendube J, Zimmermann S, Steeb M, Zengerle R, Koltay P. Technologies for single-cell isolation. *Int J Mol Sci.* 2015;16(8):16897–16919.
5. DeMaria CT, Cairns V, Schwarz C, Zhang J, Guerin M, Zuena E, Estes S, Karey KP. Accelerated clone selection for recombinant CHO CELLS using a FACS-based high-throughput screen. *Biotechnol Progress*. 2007;23(2):465–472.
6. Misaghi S, Shaw D, Louie S, Nava A, Simmons L, Snedecor B, Poon C, Paw JS, Gilmour-Appling L, Cupp JE. Slashing the timelines: opting to generate high-titer clonal lines faster via viability-based single cell sorting. *Biotechnol Progress*. 2016; 32(1):198–207.
7. Evans K, Albanetti T, Venkat R, Schoner R, Savery J, Miro-Quesada G, Rajan B, Groves C. Assurance of monoclonality in one round of cloning through cell sorting for single cell deposition coupled with high resolution cell imaging. *Biotechnol Progress*. 2015;31(5):1172–1178.
8. Lim UM, Yap MG, Lim YP, Goh LT, Ng SK. Identification of autocrine growth factors secreted by CHO cells for applications in single-cell cloning media. *J Proteome Res.* 2013;12(7): 3496–3510.
9. Arkin MR, Glicksman MA, Fu H, Havel JJ, Du Y. Inhibition of protein-protein interactions: non-cellular assay formats. In: Sittampalam GS, Coussens NP, Brimacombe K, Grossman A, Arkin M, Auld D, Austin C, Baell J, Bejcek B, TDY C, Dahlin JL, Devanaryan V, Foley TL, Glicksman M, Hall MD, Hass JV, Inglese J, Iversen PW, Kahl SD, Kales SC, Lal-Nag M, Li Z, McGee J, McManus O, Riss T, Trask OJ Jr, Weidner JR, Xia M, Xu X, editors. *Assay Guidance Manual*. Rockville, MD: Bethesda; 2004.
10. Woodruff K, Maerkl SJ. A high-throughput microfluidic platform for mammalian cell transfection and culturing. *Sci Rep.* 2016;6: 23937.
11. Priola JJ, Calzadilla N, Baumann M, Borth N, Tate CG, Betenbaugh MJ. High-throughput screening and selection of mammalian cells for enhanced protein production. *Biotechnol J.* 2016;11(7):853–865.
12. Chaturvedi K, Sun SY, O'Brien T, Liu YJ, Brooks JW. Comparison of the behavior of CHO cells during cultivation in 24-square deep well microplates and conventional shake flask systems. *Biotechnol Rep.* 2014;1-2:22–26.
13. Markert S, Joeris K. Establishment of a fully automated microtiter plate-based system for suspension cell culture and its application for enhanced process optimization. *Biotechnol Bioeng.* 2017; 114(1):113–121.
14. Halldorsson S, Lucumi E, Gomez-Sjoberg R, Fleming RM. Advantages and challenges of microfluidic cell culture in polydimethylsiloxane devices. *Biosens Bioelectron.* 2015;63: 218–231.
15. Sackmann EK, Fulton AL, Beebe DJ. The present and future role of microfluidics in biomedical research. *Nature*. 2014;507(7491): 181–189.
16. Chiou PY, Ohta AT, Wu MC. Massively parallel manipulation of single cells and microparticles using optical images. *Nature*. 2005;436(7049):370–372.
17. Valley JK, Neale S, Hsu HY, Ohta AT, Jamshidi A, Wu MC. Parallel single-cell light-induced electroporation and dielectrophoretic manipulation. *Lab Chip*. 2009;9(12):1714–1720.
18. Hsu HY, Ohta AT, Chiou PY, Jamshidi A, Neale SL, Wu MC. Phototransistor-based optoelectronic tweezers for dynamic cell manipulation in cell culture media. *Lab Chip*. 2010;10(2): 165–172.
19. Mocciaro A, Roth TL, Bennett HM, Soumillon M, Shah A, Hiatt J, Chapman K, Marson A, Lavieu G. Light-activated cell identification and sorting (LACIS) for selection of edited clones on a nanofluidic device. *Commun Biol*. 2018;1(1):41.

Manuscript received Jan. 26, 2018, revision received Jun. 26, 2018, accepted Jun. 27, 2018.