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Assuring Clonality on the Beacon Digital Cell Line Development Platform

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During biomanufacturing cell lines development, the generation and screening for single-cell derived subclones using methods that enable assurance of clonal derivation can be resource- and time-intensive. High-throughput miniaturization, automation, and analytic strategies are often employed to reduce such bottlenecks. The Beacon platform from Berkeley Lights offers a strategy to eliminate these limitations through culturing, manipulating, and characterizing cells on custom nanofluidic chips via software-controlled operations. However, explicit demonstration of this technology to provide high assurance of a single cell progenitor has not been reported. Here, a methodology that utilizes the Beacon instrument to ensure high levels of clonality is described. It is demonstrated that the Beacon platform can efficiently generate production cell lines with a superior clonality data package, detailed tracking, and minimal resources. A stringent in-process quality control strategy is established to enable rapid verification of clonal origin, and the workflow is validated using representative Chinese hamster ovary-derived cell lines stably expressing either green or red fluorescence protein. Under these conditions, a >99% assurance of clonal origin is achieved, which is comparable to existing imaging-coupled fluorescence-activated cell sorting seeding methods.

1. Introduction

The development of biotherapeutics relies on manipulating mammalian cells to secrete desired proteins. To ensure maximal productivity and as part of the overall control strategy, regulatory agencies require that all biomanufacturing cell lines be "cloned from a single cell progenitor" to ensure safety and consistent product quality.[1–3] Additionally, guidelines from the FDA released over the past few years also established an expectation for industrial sponsors to provide high assurance of clonality.[4–6] Historically, subcloning was performed through limiting dilution plating, and clonality assurance was assessed based on statistical arguments.[7,8] To improve assurance, multiple rounds of limiting dilution were often performed to obtain a desired probability of clonal derivation.[9,10] Modern cloning approaches instead employ specialized instruments to ensure that a single cell is seeded into microtiter plates through interrogation of cells at the point of deposit. These include fluorescence-activated cell sorting (FACS), Cytena single-cell printer (SCP),[11] and Solentim verified in-situ plate seeding (VIPS).[12–16] In the above examples, cell deposition is usually coupled with the use of microscopic imaging of well plates before deposition, after deposition (within 24 h), and during subsequent colony expansion. Additionally, a manual verification step of these images by one or more trained scientists is performed to confirm single cell origin and select candidate clones for subsequent colony picking.

The emerging, fully integrated Berkeley Lights Beacon nanofluidic technology holds the potential to transform traditional manual cell culture to digital analysis and manipulation. The Beacon platform is equipped with opto-electropositioning (OEP), microfluidics, and microscopy to enable cells to be manipulated, cultured, and assayed on nanofluidic chips.[17–20] We have previously demonstrated that the Beacon instrument can be employed to generate high quality cell lines with reduced resource requirements compared to traditional FACS-based methods. However, no reported clonality assessment has been published for the Berkeley Lights Beacon platform.[21]

Each nanofluidic chip contains 1758 NanoPen chambers arrayed along four continuous channels. The NanoPens have a narrow opening to the channel for nutrients and cellular waste diffusion and are not completely separated by physical barriers, leading to the possibility that on-chip mixing of cells from one NanoPen to another can occur. The Beacon platform prevents on-chip mixing of cells from one NanoPen to another in two ways. First, the nanofluidic chips are tilted at a small angle from horizontal so that cells settle to the bottom of NanoPens and

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away from the narrow opening to the channel. Second, the chips are engineered so that there is no net flow into or out of the NanoPens. The fluidic regime, whether laminar or turbulent, can be assessed by the non-dimensional Reynolds number which is a ratio of inertial forces to viscous forces. For a typical Beacon flow rate of 1 μL s^{-1} through the chip's channels, the Reynolds number is ≈1.2, whereas the accepted transition to turbulent flow begins to occur at a Reynolds number of 2300. Thus, we can assume that all flows in these channels are laminar and that cells can only be moved into and out of the NanoPens using light-based manipulation. However, whether these methods are sufficient to prevent on-chip mixing and to ensure clonality when culturing and exporting highly optimized production cell lines has not been previously published. In this manuscript, we describe how the Beacon's integrated imaging capability, in combination with in-process controls, is employed to generate a reliable clonality data package. Using a representative Chinese hamster ovary (CHO) model system that constitutively expresses either green fluorescent protein (GFP) or red fluorescence protein (RFP), we have validated this workflow and present unrivaled clonality assurance compared to FACS and limiting dilution-based procedures.

2. Experimental Section

2.1. Cells and Cell Culture

Model cell lines were generated by transfecting a clonal CHO host with plasmid DNA encoding either a GFP or an RFP and a selection marker. Following transfection, stably expressing pool populations were generated through repeated passaging under selection pressure until the cells reached above 90% viability. Selection pressure was then removed, and the cells were monitored for stable expression of GFP or RFP through fluorescence microscopy. Pools were then re-transfected with plasmid DNA encoding heavy and light chains from a human monoclonal antibody with a metabolic selection marker through a process typical of a standard cell line development campaign. Antibody secreting pools were selected through passaging in a selective seed-train growth medium until the cells reached above 90% viability and maintained consistent doubling times. Subclones were isolated from pools through three rounds of sequential limiting dilution, fluorescence-activated cell sorting (FACS), and Berkeley Lights Instrument (BLI) subcloning methods. Isolated clones were expanded and screened for stable fluorescent protein expression. Throughout the process, the 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 spin tubes (TPP, Trasadingen, Switzerland) in growth media at 36°C, 5% CO_2 and 85% humidity. Cells were maintained by passaging multiple times a week at a target seed density.

2.2. Single Cell Cloning by Limiting Dilution

Limiting dilution cloning was performed through measurement of viable cell density of a stable culture using a trypan blue dye exclusion cell analyzer (Vi-Cell XR, Beckman Coulter, Brea, CA), dilution in custom cloning media at a target density of approximately 0.7 cells per 180 μL per well and transferring into a sterile 96-well microtiter plate (Corning, Corning NY).

2.3. Single Cell Cloning by Flow Cytometry

The FACS AriaIIu (Becton Dickinson, Franklin Lakes, NJ) cell sorter was used to isolate and deposit single cells from stable transfected pools directly into 96-well microtiter plates (Corning, Corning, NY) prefilled with a custom cloning medium. Sorting parameters were set for three doublet discrimination gates, the highest stringency conflict resolution setting, and flow rates that conferred optimal purity. Manual verification of single cells sorted on a glass slide by microscopy was performed to increase sorting stringency (if doublets or aggregates were detected) until zero doublets were observed. Following optimized instrument set-up, single cell sorting into the wells of a 96-well microtiter plate commenced. Internal plate control wells include wells bulk sorted with single color cells.

2.4. Imaging and Image Verification after FACS or Limiting Dilution Seeding

After depositing, plates were centrifuged and immediately imaged on a high-throughput microscopic imager (Cell Metric, Solentim, Dorset, UK or CloneSelect Imager, Molecular Devices, San Jose, CA). Imaging was performed periodically post seeding to track the formation of a single colony. Clonally derived cell lines were confirmed using several criteria: 1) clear proof of a single cell on day 0; 2) absence of significant artifacts in the well; 3) formation of single round colony; and 4) independent verification by two different scientists.

2.5. Single Cell Cloning by Berkeley Lights Beacon Instrument

Stable pools were single cell loaded on OptoSelect chips (Design 1750, Berkeley Lights, Emeryville, CA) using the Beacon instrument (Berkeley Lights, Emeryville, CA). OEP settings and scripts for loading and exporting cells were provided by BLI. Cells were cultured on the chips for 4 days using seed train growth medium containing additional growth supplements and manufacturer-recommended settings. Repeated imaging and cell counting were performed using the integrated 4× microscope and camera on the Beacon instrument. Cell counting algorithms were provided by BLI. Evidence of clonal derivation was achieved by an image of a single cell in a NanoPen. Selected pens were exported using OEP to guide between 2–20 cells out of NanoPens into the main channel, followed by flushing off the chip into a 96-well microtiter plate prefilled with a custom cloning medium. Blank exports were performed before and after each export where an equal volume line sample is deposited into even-column wells. Exports and blank samples are incubated for at least 14 days to monitor growth and contamination events in the system lines. Fluorescence in plates was monitored using the Cell Metric FL imaging system (Solentim, Dorset, UK) using the manufacturer supplied "monitor clonality scripts." Detection of RFP and GFP expressing were optimized through single color controls and validation using the EVOS FL Cell Imaging Microscope (Thermo Fisher, Waltham, MA)

Table 1. Validation of the Beacon cloning process steps and the overall clonality assurance rates.

Process step	True positive	False positive	Negative	Assurance rate
Beacon loading	1205	3	550	99.7%
Beacon NanoPen Isolation	1758	0	n.a.	100%
Beacon export purity	112	0	n.a.	100%

Scores of true positive, false positive, and negative events for individual workflow steps described in Figure 2. For Beacon Loading, true positive event is defined as single cell of known phenotype, false positive is defined as single cell of unknown phenotypic identity, negatives defined as blank pen (280), pen loaded with multiple cells (182), or incorrectly categorized by BLI algorithm as blank (88). For Beacon NanoPen Isolation, true positive defines pens that maintained homogenous phenotypic identity on day 4 when compared to day 0. For Beacon Export Purity, true positive is defined as clone of homogenous phenotypic identity that is consistent with phenotypic identity of clone selected for export.

2.6. Calculation of Clonality Probability

To evaluate clonality assurance, the probability of clonality (PoC) was calculated by estimating the percentage of true positives among all clone candidates.[22] Among clone candidates that have passed all bright field imaging and verification criteria, true positive and false positive are determined by using independent fluorescent imaging at least 14 days of outgrowth to check whether the culture has a single-color phenotype. Clones that have passed verification criteria and later determined to have a dual-color phenotype are false positives. To compensate for the inability to see wells that contain cells of the same color, the number of false positive wells were doubled (2×). To account for the effect of sample size, the Wilson method was used as previously described.[23] Briefly, a one-sided upper 95% confidence interval for the probability was calculated as a conservative estimate. The data in **Table 1** was used to calculate the confidence interval using the Wilson method:

95% upper confidence limit probability

$$= \frac{\hat{p} + \frac{z_{1-\alpha}^2}{2n} + z_{1-\alpha}\sqrt{\frac{\hat{p}(1-\hat{p})}{n} + \frac{z_{1-\alpha}^2}{4n^2}}}{1 + \frac{z_{1-\alpha}^2}{n}},$$

where n is the total number of observed wells, $z_{1-\alpha}$ is the $(1-\alpha)$th percentile of the standard normal distribution; $1-\alpha$ is the target confidence level, and $\hat{p}$ is the observed proportion of false positives among all wells.

3. Results and Discussion

3.1. The Beacon Platform Provides a Complete Image Data Package for Clonal Assurance

A common paradigm used to assure a single cell progenitor is to establish assurance of single cell deposition into a culture vessel, assurance of culture isolation through the duration of the culture, and assurance of purity when transferred out (**Figure 1**A). Typically, cloning processes occur in a single well of a microtiter plate. Assurance of single cell deposition (Figure 1A, blue box, i) is determined through a statistical distribution assumption for limiting dilution, imaging during deposition (SCP), or experimentally measured and calculated (FACS).[23] To improve assurance, microscopic imaging at the bottom of the well (Figure 1A, green boxes) within 24 h of plating provides evidence of single cell isolation and deposition. Images are often taken before (iii) and after cell seeding (iv), followed by repeated imaging to monitor growth of a localized colony (v). The probability of cells that may reside outside of the image area (Figure 1A, red box, ii) is experimentally determined and calculated as "ghost" wells.[24] Together, the plurality of data is manually verified and accepted as clonally derived.

The Berkeley Lights Beacon platform follows a similar clonality assurance paradigm through microscopic imaging and manual verification (Figure 1A, Bottom). The platform provides improved integrated imaging capability in combination with in-process quality controls compared to microtiter plate based cloning approaches. An OptoSelect NanoPen is over 100 000× smaller in volume (≈1.7 nL) than a well in a 96-well microtiter plate (≈200 µL). This size reduction results in improved image quality due to minimal z-depth (≈40 µm), clear edges, and reduced debris and artifacts. Automated focus and calibration capability combined with artificial intelligence (AI) cell identification simplifies the imaging workflow, enabling near real-time image analysis.

The initial cell deposition step utilizes an AI algorithm for cell detection followed by OEP to guide single cells into NanoPens (vii). The entire chip imaged before (viii) and immediately after cell loading is completed to provide pictorial proof of a single cell progenitor (ix). In the second stage of on-chip cell culturing and characterization, the fluidic flow in channels outside NanoPens is optimized to maintain completely in laminar regime with no occurrence of turbulence within the NanoPens where the cells are growing or in the chip channels. Time-course imaging and an AI cell count algorithm are used as in-process monitoring tools to ensure the empty NanoPens remain empty (xi) and cells do not expand beyond the maximum recommended height inside the NanoPen (x). In case when overgrowth occurs, there is a risk that the growing clones will expand into the channels and contaminate the export flow path. This risk is mitigated through strict monitoring of growth and ensuring that exports are performed before the cells expand beyond the recommended height (roughly ¾ of pen height filled). In the last stage of export for each clone, the complete export flow path is thoroughly flushed. Pen images are taken before (xii), during (xiii), and immediately after (xiv) export, as well as the export well plate (xv) to account for all cells on the chip. Throughout the export procedure, in-process fluidic samples are taken before and after each export. These in-process samples are deposited into a medium containing well on the same well plate to assess for clonal contamination or cross contamination in system lines. The well plate is further incubated for 2 weeks and re-imaged to ensure growth only in the intended wells and not in the in-process fluid control samples (xvi-red boxes). Manual verification of images is performed as a last step for data verification together with a complete image data set tracking cells

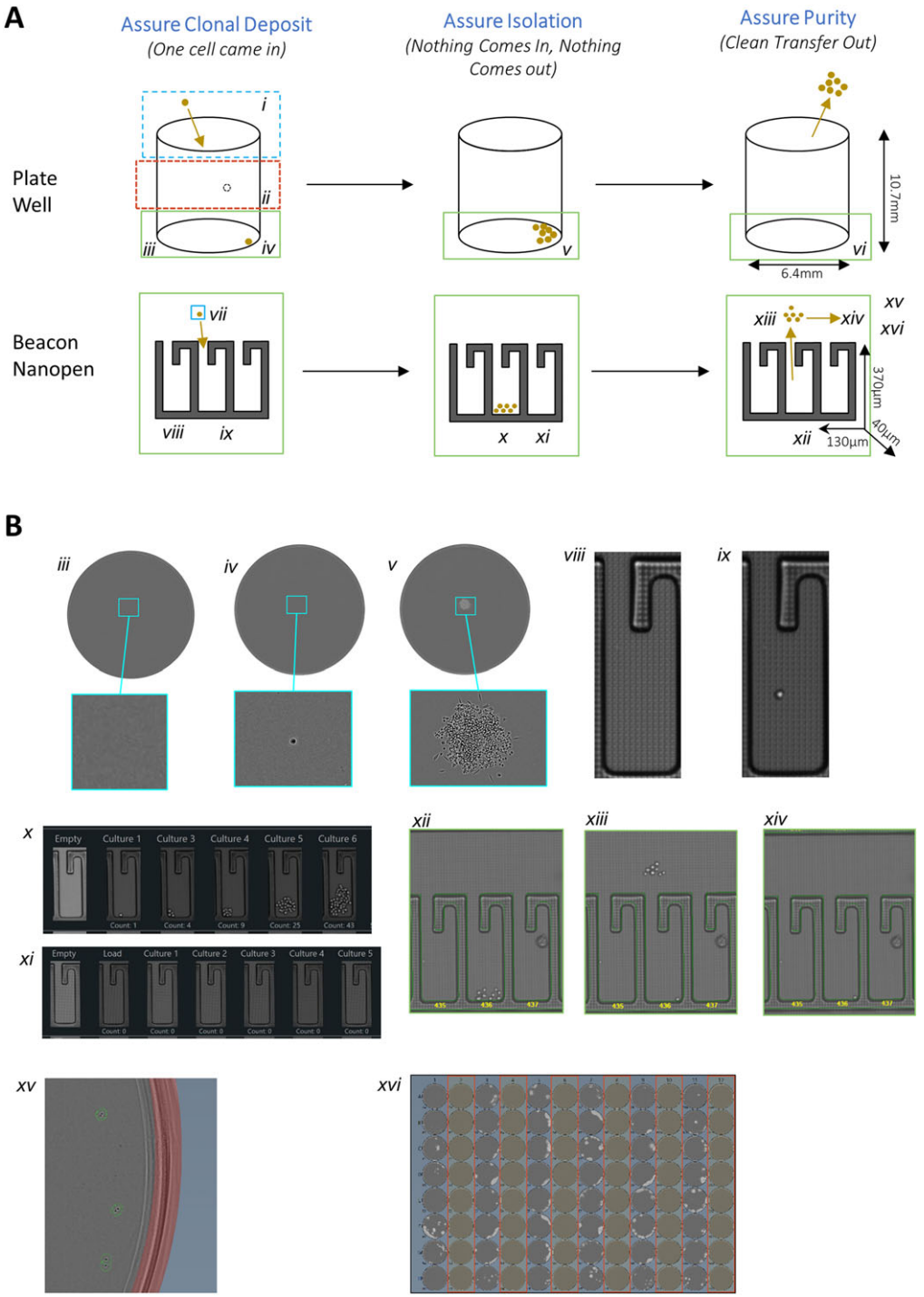

Figure 1. Subcloning workflow and single cell progenitor controls. A) Schematic of comparing single cell cloning workflows associated with microtiter plate-based cloning (top) and Beacon platform (bottom). Blue squares designate primary cell isolation and deposition methods steps. Green squares designate microscopic imaging image evidence and respective imaging areas captured. Red dotted square represent areas unable to capture using imaging and assurance is provided through measurement of "ghost-well" rates. Roman numerals indicate specific image data points or statistical measurements. B) Examples of image evidence used to support single cell progenitor assurance. Evidence from plate sorting methods include image prior to seeding (iii), image directly after seeding (iv), and image of a single localized colony (v). Evidence from Beacon cloning involve image of empty NanoPen prior to seeding (viii), image of single cell after OEP seeding (ix), time series of NanoPen growth (x), evidence of zero cross-contamination on chip through time series of adjacent empty NanoPens (xi), image record of OEP export including before (xii), after unpenning (xiii) and after flushing into well plates (xiv), image evidence of cells deposited into well plates (xv), evidence of growing culture after incubation (xvi), evidence of lack of contamination from cultured in process fluidic samples (xvi wells in red boxes)

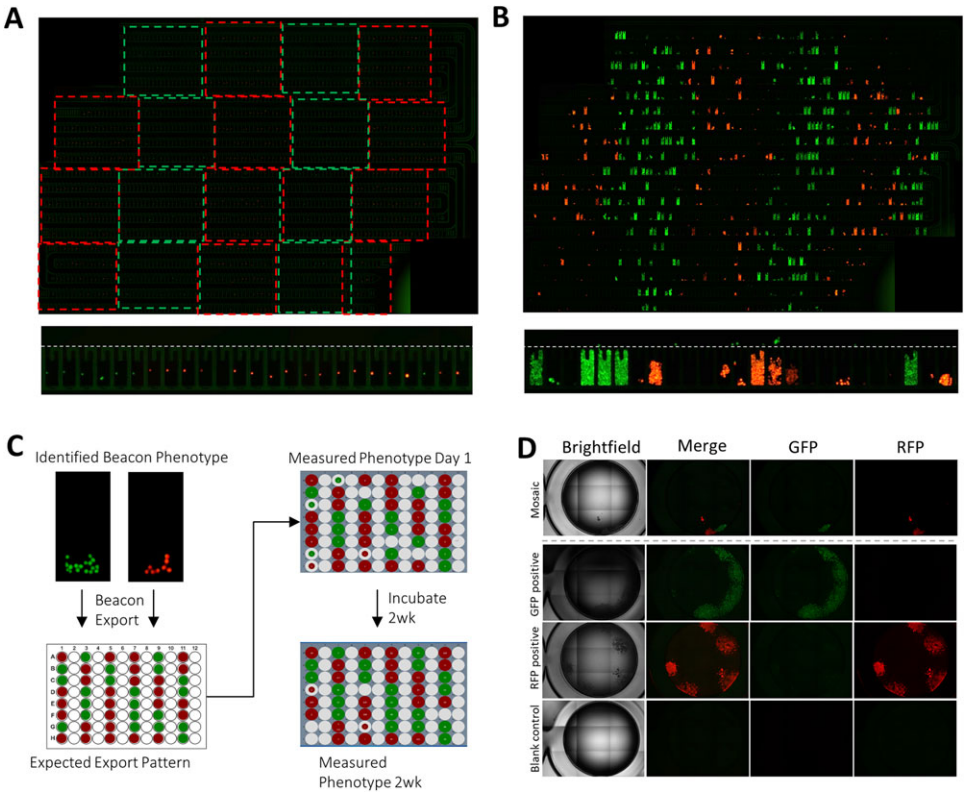

Figure 2. Validation of workflow steps using representative model CHO system. A) Single cells expressing expected fluorescent protein (GFP or RFP) were loaded onto the desired zones (dotted boxes) on the chip. Red and green fluorescence are measured and merged in example image of entire chip (top) and zoomed section (bottom). B) Cells are cultured over extended time to monitor and confirm that there is no mixing between pens during laminar flow of culture media. White dotted line depicts the border between flowing channel (above line) and inside NanoPens (below line). C) Cells with the expected phenotype were exported as shown by imaging of well plates post export using a fluorescence enable plate imager. D) Example images of clones expressing fluorescent proteins acquired after export into well plates. Images corresponding to mixed colony were acquired and scaled independently.

on and off the Beacon that enables rapid data verification and documentation.

4. Validation of Beacon Cloning Process Using Representative Model CHO System

To validate that the process described earlier does assure for clonal derivation, we used two representative CHO model cell lines that constitutively express either GFP or RFP. They were developed following a standard cell line development workflow, subcloned multiple times with multiple techniques, and the final clones were selected to have similar growth phenotypes and a stable fluorescent phenotype. In **Figure 2**A, the Beacon instrument's ability to correctly deposit single cells of known fluorescent phenotypes into desired pen locations was assessed. As shown in Table 1, single red and single green cells were placed into the desired zones on the chip. Immediately after load, pens were interrogated with the integrated fluorescent imaging capabilities to verify correct loading and tracking of cell origins. The Beacon correctly loaded, identified and documented a single clone with a single color 99.7% of the time.

Figure 2B illustrates the monitoring of cross-contamination events during cell culturing. Here the chip was cultured for an extended duration (10 days), which is two times longer than typical process (5 days) and imaged daily. Zero instances of multi-fluorescence pens or altered fluorescence pens were observed. Finally, the export purity rate off the instrument was determined (Figure 2C). Fluorescence identity of pens was determined and selected for export at days 4–6. Fluorescence imaging was performed on the exported well plates after export was complete and 2 weeks after (Figure 2D). All export colors and expected blank wells out of 112 attempts were as expected. Results are summarized in Table 1.

To measure the overall clonality assurance on the Beacon platform and compare to industry standard microtiter plate-based cloning approaches (limiting dilution and FACS), an equal volume mixture of the GFP- and RFP-expressing cells was subcloned using one of the three methods: limiting dilution seeding combined with plate image verification, FACS assisted cell deposition combined with plate image verification, and Beacon with integrated imaging and process controls (**Table 2**). After each process output, clones were manually assessed for positive evidence of clonality using the described process in Figure 1. Verified clones were then reassessed using a second independent instrument (Solentim CellMetric FL) to determine the number of single-color output cultures and multi-color output cultures. The number of multi-color output cultures provides a surrogate

Table 2. Overall clonality assurance rates.

Cloning process	True positive	False positive	Negative	No cell	Attempts	Assurance rate	Wilson assurance
Beacon cloning process	419	0	26	349	794	100%	99.36%
FACS cloning process	179	0	219	1226	1624	100%	98.52%
Limiting dilution cloning process	66	0	311	775	1152	100%	96.08%

An equal ratio of cells stably expressing either GFP or RFP proteins were cloned using one of three approaches: limiting dilution, FACS, and Beacon. True positives are defined as clones that have passed clonal verification acceptance criteria and then measured to have a single color phenotype. False positives have passed clonal verification acceptance criteria and measured to have a multi-color phenotype. Negatives are clones that have failed acceptance criteria defined by each method. "No cell" refers to the number of wells that show no growth after seeding or exporting.

to estimate the false positives, which is needed to calculate the probability of clonality (PoC).[22,24] To calculate the probability that the process ensures a single cell origin, we calculate the measured probability of clonality (number of true positives/(number of true positives + false positives)).[22] The probability is also calculated using one-sided upper 95% confidence interval by Wilson method to take in account for sample size.[7,23] The Beacon platform was shown to provide equivalent clonality assurance to reported methods (100% and 99.33%, respectively). This coincides with a similar monoclonal probability assessment performed by the instrument manufacturer. When comparing between growing colonies, the Beacon cloning and confirmation process calls 94% of exported cultures as positive that they were clonally derived. This is compared to 45% for a FACS and 17% for a limiting dilution process. These lower rates are due to a number of reasons including multiple cells seeded in a single well, strict imaging acceptance criteria (e.g., away from well edge, focus, high contrast, round cell shape), difficulty ensuring a high-quality day zero image, and maintaining a single localized colony over the imaging time frame. Additionally, Beacon clones that have been selected for export into 96-well microtiter plates have a higher recovery rate (56%) (positive + negative / attempts) compared to FACS (24%) and limiting dilution (33%) in the same plate format. The improved recovery can be attributed to preselection of healthy, growing clones on the chip, and the fact that multiple cells are seeded into plates from the Beacon process. In comparison, the direct well plate seeding methods require a single cell clonality image inside a well, and therefore must recover from a lower effective density, that is, a single cell. Together, the overall cloning efficiency (true positive, clonal verified, growing colonies/attempted seeded wells) is 52% for Beacon, 11% for FACS, and 5.7% for limiting dilution.

5. Conclusions

It is well established that manufacturing cell line clonality derivation can be assured using microscopic imaging and verification. Clonality assurance on the Berkeley Lights Beacon platform follows the same imaging and validation paradigm. However, through improved integrated imaging capability, cell detection algorithms, and in-process quality controls, the Beacon provides an overall stronger data package for single cell progenitor assurance. Using a representative CHO model system, we have demonstrated the Beacon platform provides improved efficiency over FACS and limiting dilution approaches, while offering comparable assurances of single cell derivation.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

Berkeley Lights, cell line development, Chinese hamster ovary cells, clonality assurance, digital cell culture

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[1] M. Plavsic, *ICH Q5D Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products*, European Medicines Agency, Amsterdam **2017**.
[2] *Quality, Non-Clinical and Clinical Aspects of Medicinal Products Containing Genetically Modified Cells*, European Medicines Agency, Amsterdam **2012**.
[3] J. Y. Baik, K. H. Lee, *Biotechnol. J.* **2018**, *13*, 1700230.
[4] S. Kennett, presented at WCBP 2014, Washington, DC, January **2014**.
[5] R. Novak, *CDER/OPQ/OBP/DBRRI*, **2017**. https://cdn.ymaws.com/www.casss.org/resource/resmgr/cmc_no_am_jan_spkr_slds/2017_CMCJ_Novak_Rachel.pdf (accessed: May 2019).
[6] J. Welch, *CDER/OPQ/OBP/DBRRIV*, **2017**. https://cdn.ymaws.com/www.casss.org/resource/resmgr/ce_pharm_speaker_slides/2017_CE_Pharm_-_Welch_Slides.pdf (accessed: May 2019).
[7] H. A. Coller, B. S. Coller, *Hybridoma* **1983**, *2*, 91.
[8] J. Quiroz, Y.-S. Tsao, *Biotechnol. Prog.* **2016**, *32*, 1061.
[9] Y. Zhou, D. Shaw, C. Lam, J. Tsukuda, M. Yim, D. Tang, S. Louie, M. W. Laird, B. Snedecor, S. Misaghi, *Biotechnol. Prog.* **2018**, *34*, 559.
[10] T. Lai, Y. Yang, S. K. Ng, *Pharmaceuticals* **2013**, *6*, 579.
[11] M. Yim, D. Shaw, *Biotechnol. Prog.* **2018**, *34*, 1454.
[12] T. Kelly, A. M. Tuckowski, K. D. Smith, presented at the 26th ESACT Meeting, Copenhagen, Denmark, May **2019**, P-154.
[13] F. Boldog, S. Rajendran, S. Balasubramanian, L. Webster, M. Lee, A. Gough, C. Richards, T. Purcell, E. Hart, M. Fox, D. Vavilala, N. Kulikov, J. Minshull, O. Beske, presented at the 26th ESACT Meeting, Copenhagen, Denmark, May **2019**, P-173.

[14] A. Gough, C. Richards, I. Taylor, presented at the 26th ESACT Meeting, Copenhagen, Denmark, May **2019**, P-595.
[15] A. Gough, C. Richards, I. Taylor, presented at the 26th ESACT Meeting, Copenhagen, Denmark, May **2019**, P-449.
[16] J. Fieder, P. Schulz, I. Gorr, H. Bradl, T. Wenger, *Biotechnol. J.* **2017**, *12*, 1700002.
[17] H. Y. Hsu, A. T. Ohta, P. Y. Chiou, A. Jamshidi, S. L. Neale, M. C. Wu, *Lab Chip* **2010**, *10*, 165.
[18] A. Mocciaro, T. L. Roth, H. M. Bennett, M. Soumillon, A. Shah, J. Hiatt, K. Chapman, A. Marson, G. Lavieu, *Commun. Biol.* **2018**, *1*, 41.
[19] M. Jorgolli, T. Nevill, A. Winters, I. Chen, S. Chong, F. F. Lin, M. Mock, C. Chen, K. Le, C. Tan, P. Jess, H. Xu, A. Hamburger, J. Stevens, T. Munro, M. Wu, P. Tagari, L. P. Miranda, *Biotechnol. Bioeng.* **2019**, *116*, 2393.
[20] A. Winters, K. McFadden, J. Bergen, J. Landas, K. A. Berry, A. Gonzalez, H. Salimi-Moosavi, C. M. Murawsky, P. Tagari, C. T. King, *mAbs* **2019**, *11*, 1025.
[21] K. Le, C. Tan, S. Gupta, T. Guhan, H. Barkhordarian, J. Lull, J. Stevens, T. Munro, *Biotechnol. Prog.* **2018**, *34*, 1438.
[22] C. Chen, K. Le, H. Le, K. Daris, N. Soice, J. Stevens, C. T. Goudar, *Biotechnol. J.* **2019**, 1900289.
[23] K. Evans, T. Albanetti, R. Venkat, R. Schoner, J. Savery, G. Miro-Quesada, B. Rajan, C. Groves, *Biotechnol. Prog.* **2015**, *31*, 1172.
[24] D. Shaw, M. Yim, J. Tsukuda, J. C. Joly, A. Lin, B. Snedecor, M. W. Laird, S. E. Lang, *Biotechnol. Prog.* **2018**, *34*, 584.

