APPLICATION NOTE

FDA-Accepted Monoclonality Assurance on the Beacon® Optofluidic System for Cell Line Development

In this Application Note we outline:

- Industry leading technology for confident assurance of clonality in regulatory submissions
- Rapid, automated workflow provides complete end-to-end image record of every clone
- Independently validated >99% probability of monoclonality with a single round of cloning

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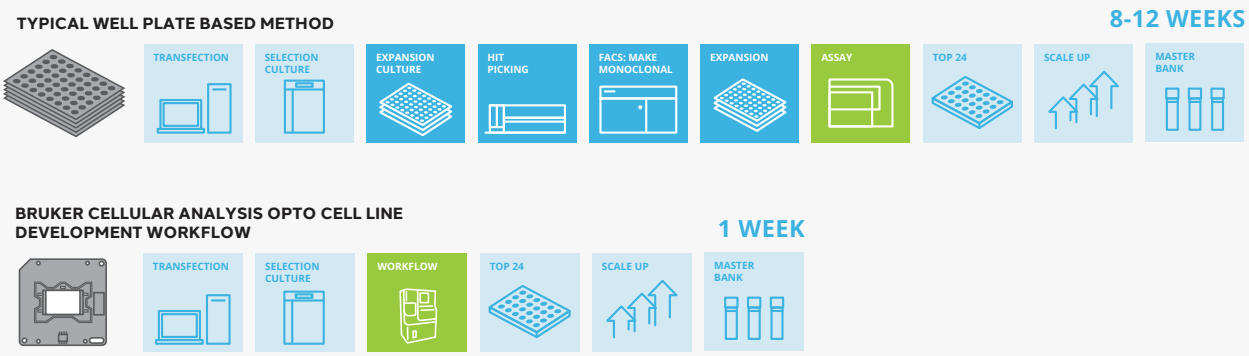

Figure 1 | Opto Cell Line Development with the Beacon system.

Introduction

In recent years the FDA and other regulatory authorities have increased their emphasis on proof of monoclonality of mammalian cell banks, as referenced by FDA guidelines, ICH Q5D, and EMA/CHMP. While there is no explicit regulation stating that a production cell line should be monoclonal, a clonally derived Master Cell Bank is seen as a critical component of a robust, consistent process that ensures drug product quality and safety. Insufficient evidence of clonality in a regulatory submission may necessitate additional manufacturing controls and lead to costly delays of clinical trials and/or drug commercialization.

The Beacon optofluidic system from Bruker Cellular Analysis offers an integrated end-to-end automated workflow for rapid cloning and selection in mammalian cell line development (Figure 1). Clones can be selectively enriched for unique phenotypes and multiple assays can be used to measure productivity of different types of antibody molecules. Used globally by many leading pharmaceutical companies and CDMOs, the Beacon system's industry leading technology delivers superior assurance of monoclonality for regulatory submissions.

Its Opto® Cell Line Development workflow has been independently demonstrated to provide >99% probability of clonality from a single automated round of cloning[1], while in-process imaging provides direct visual evidence of clonal derivation. Recently a customer IND application incorporating monoclonality assurance data from the Beacon system was granted approval by the FDA.

Superior Cloning Technology

Traditional methods of single cell cloning have significant drawbacks for monoclonality assurance. Limiting dilution and flow cytometry must rely on indirect measurements as evidence of single cell isolation. Colony picking from semi-solid media (ClonePix) has potential for crosscontamination of colonies, so that "a single round of cloning is likely not sufficient without extremely good supporting data"[2]. Cell imaging systems can provide direct assurance, yet are confounded by artifacts and debris, especially along well edges, and contaminating "ghost cells".

OptoSelect® chips for the Beacon system's cell cloning and selection workflows contain thousands of tiny NanoPen® chambers connected to a continuous fluidic path (Figure 2). Each chamber is less than 2 nanoliters in volume with an area over 700X smaller than a microtiter plate well. Their small dimensions and narrow focal plane allow for integrated pen imaging with minimal artifacts, clear edges, and virtually nowhere for cells to be obscured from view (Figure 3).

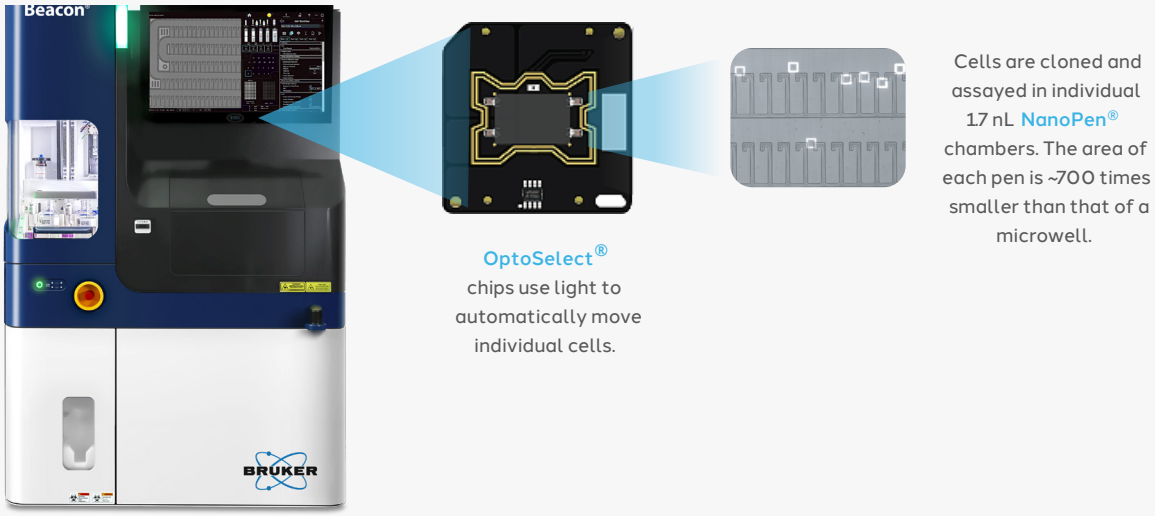

Figure 2 | The Beacon Platform. The Beacon system is a combination of optics and nanofluidics called optofluidics. Single cells in large numbers are moved with light so they can be isolated, cultured, assayed, and exported.

Single cells are sorted into individual NanoPen chambers by opto-electropositioning (OEP®) technology. OEP uses light to capture and manipulate hundreds of single cells in parallel from the fluidic channel into the chambers, where they are perfusion cultured and assayed over several days. Expanded clones with desired phenotypes can then be recovered with OEP and deposited into wells of a 96-well plate for scale-up and further characterization.

Monoclonality Assurance for a Successful IND

Automated on-chip imaging of every NanoPen chamber is performed throughout the Opto CLD workflow. The software provides a complete visual record of every clone through several days of culture, productivity assays, and recovery of top producers. In addition, multiple measures and in-process controls ensure that clonality is preserved from single cell deposit to recovery of top clones.

1. Assuring cells are clonally derived

In the first stage of the Opto CLD workflow, cells from a transfected pool are loaded into the OptoSelect chip fluidic channel. Once cells are distributed throughout the channel, imaging software identifies and targets single cells. OEP is applied to the targeted cells, and each cell is moved individually into a nearby NanoPen chamber ("penning") (Figure 4).

Measures to ensure clonal derivation:

- Laminar flow ensures that cells are retained in the channel until OEP is applied and do not passively enter NanoPen chambers.
- Images of all NanoPen chambers are captured just before loading of cells onto the chip, and again after penning of single cells. A software processing algorithm uses these images to verify that only one cell is penned in each NanoPen chamber.

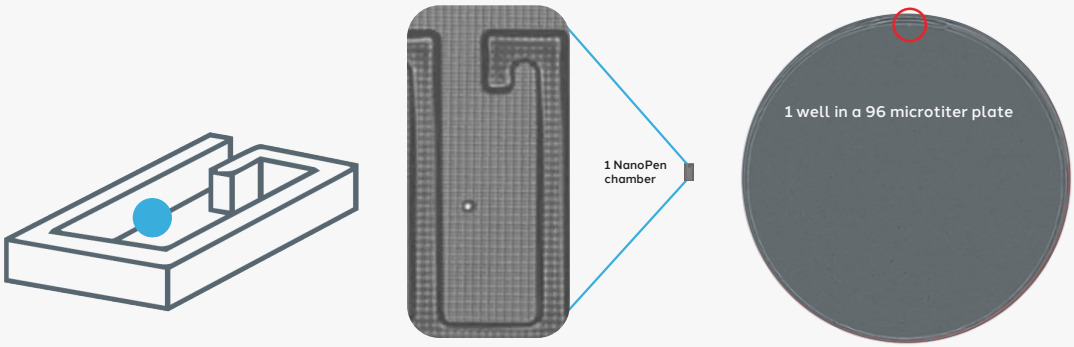

Figure 3 | **Definitive visual confirmation.** The small size of the NanoPen chamber offers superior imaging resolution of isolated single cells as evidence of clonality.

- Automated cell counting enables the software to detect chambers loaded with more than one cell during initial penning. These cells can then be unloaded back into the channel with OEP and flushed out of the chip.

2. Assuring isolation of cells during culture

After penning, the chip is perfused with media to maintain cell viability and enable on-chip culture over several days. As the cells expand in the chamber microenvironment, fluorescence-based assays can be performed at multiple time points to identify clones with desired attributes, such as growth rate, secreted antibody titers, and relative per cell productivity (rQp) (Figure 5).

Measures to ensure clone isolation:

- Laminar flow along the channel ensures that no unwanted cells or debris can move into the NanoPen chambers after cells inside have been clonally deposited.
- The unique design of the chambers ensures that even rapid flow rates in the channel do not disturb the cells inside. Though each NanoPen chamber is connected to the channel by a small opening to enable diffusion of nutrients, fluorescent reagents, and waste products, the chamber volume remains free of any turbulence.
- If a clone expands too quickly in the NanoPen chamber there is some increased risk that cells could escape into the channel. This risk is mitigated by strict monitoring of cell growth throughout the workflow. Automated export or "pruning" of chambers is performed with OEP and channel flush before cells can expand beyond the recommended occupancy level in the NanoPen chamber.

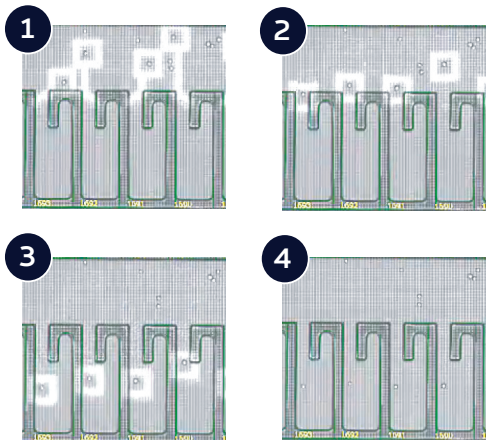

Figure 4 | Penning sequence on an OptoSelect chip.

Figure 5 | Visual record of a single NanoPen chamber with a cloned CHO cell over five days, with fluorescent secretion assay images from days 3, 4, and 5.

- Imaging and automated counting occur at multiple time points throughout the workflow, for complete record of morphology, growth rate, and behavior over several days for every clone (Figure 5).

3. Assuring clonality is maintained when recovering clones

After 4-6 days of on-chip culture, clones with desired characteristics are selected for recovery from the chip. OEP is used to direct a single clone — typically consisting of 8-20 cells — out of its NanoPen chamber and into the fluidic channel (Figure 6). Once the cells are out of the chamber and suspended in the channel, media is flushed through the channel to remove them from the chip and deposit them into a single well of a 96-well plate for scale up ("Export"). This process is repeated for each selected clone, alternating with media flushes to prevent crosscontamination.

Measures to ensure purity during export:

- Prior to each clone export, the chip channel is flushed with media. This media is collected in a separate well of a 96-well plate ("Blank").
- After a clone is exported, an additional media flush is performed and collected before unpenning the next clone.
- The blank wells can be stained and imaged by microscopy to confirm that no contaminating cells were present in the channel before or after exporting of a clone (Figure 7).
- Blank wells can also be incubated for a period of time to check for growth as further verification.

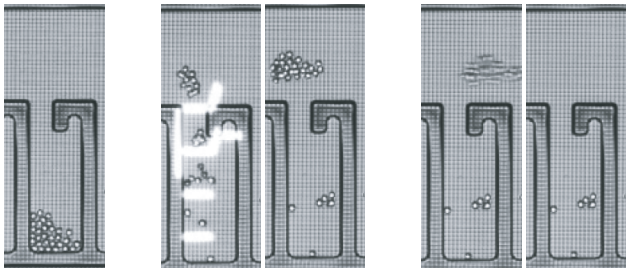

Figure 6 | **Unpenning and export of a clone from NanoPen chamber with OEP.** Light is used to gently move cells of selected clone out of the chamber and into the fluidic channel. Flushing the channel with media removes the clone from the chip and deposits into a well of a 96-well plate.

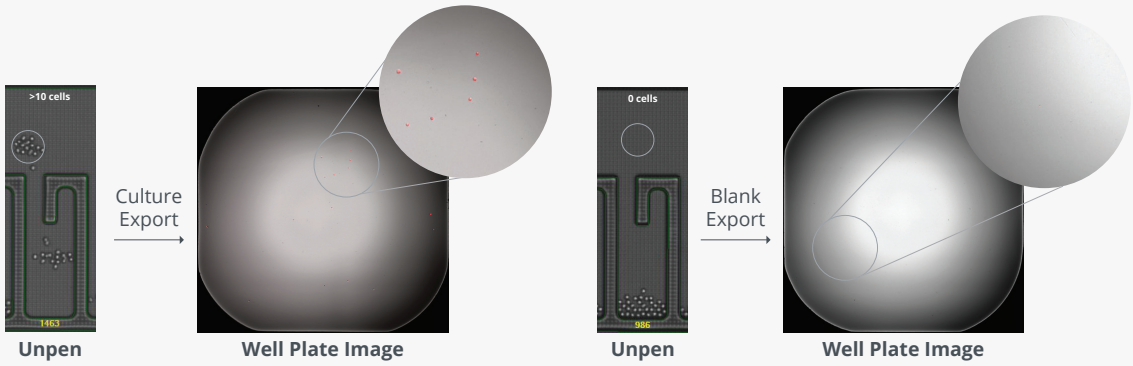

Figure 7 | Verifying clonality during clone recovery with alternating blank wells.

Monoclonality Validation Study

During a four-week period, a total of 30 OptoSelect chips were loaded with CHO cells on two different Beacon systems, from which 650 clonal populations were selectively unloaded. An additional 2,599 blank exports were performed, and these blank wells were stained, imaged, and manually scored for growth.

Table 1 breaks down the results by Beacon instrument, experiment, and chip. In total, only three contaminating colonies were identified across all blanks, yielding an average monoclonality of 99.88%. Calculating the 95% binomial confidence interval around this data using the Clopper-Pearson method results in a lower bound of 99.66%. This is equivalent to four rounds of limiting dilution.

Conclusion

The Beacon system has been demonstrated to have a high probability of maintaining >99% monoclonality when operating the workflow according to recommended procedures, as shown by Bruker Cellular Analysis and independently by Le K, *et al*. Integrated imaging capability in combination with in-process quality controls provide superior end to end monoclonality assurance compared to microtiter plate based cloning approaches. These data have been and can confidently be included in IND and BLA applications to the FDA in support of a biologics drug approval.

	Beacon System 1				Beacon System 2				
	Workflow 1	Workflow 2	Workflow 3	Workflow 4	Workflow 5	Workflow 6	Workflow 7	Workflow 8	Total
# of Clones Exported	74	57	95	94	91	49	95	95	650
# of Viable Clones	57	52	94	92	79	47	90	90	601
Export Yield	76.6%	92%	99%	98%	87%	94%	95%	95%	92.5%
# of Blanks	296	224	380	380	364	195	380	380	2,599
# of Failures	0	0	0	0	1	1	0	1	3
% Monoclonality	100%	100%	100%	100%	99.7%	99.5%	100%	99.8%	99.88%

Table 1 | Monoclonality with Opto CLD workflow on the Beacon system.

References

1. Le, K et al. Assuring Clonality on the Beacon Digital Cell Line Development Platform. *Biotechnology Journal*. 2019. 15(1): e1900247.

2. From presentation by Sarah Kennett, 2014 Symposium on the Interface of Regulatory and Analytical Sciences for Biotechnology Health Products. https://cdn.ymaws. com/www.casss.org/resource/resmgr/WCBP_Speaker_Slides/2014_WCBP_Sarah_Kennett.pdf