Conclusions
- The Beacon platform can isolate and culture thousands of single cells and provide verifiable evidence of single cell status in an automated process.
- Monoclonal populations cultured on OptoSelect Chips can be characterized with a quantitative secretion assay to rank clones while maintaining monoclonality enabling cloning and selection of 24 top clones in just 5 days.
- Clones recovered from the Beacon platform remain highly clonal during the export process.
- Recovered colonies retain high viability and are ready for scale up and banking.

References

Introduction
Berkeley Lights, Inc. (BLI) has developed the Beacon® platform, a flexible instrument that combines structured visible light technology and microfluidic design to automate isolation, growth, screening, and manipulation of thousands of monoclonal cell populations in parallel. These unique capabilities of the Beacon platform automate major segments of the Cell Line Development (CLD) process, saving both time and money. FDA guidelines require assurance of monoclonality for all cell lines used in commercial therapeutic production and typical CLD campaigns require multiple rounds of cloning to achieve this standard. In comparison, the CLD workflows executed on the Beacon platform generate human verifiable evidence of single-cell status for each clone and NanoPens isolation maintains monoclonality during expansion and antibody productivity assay. However, recovery of top-producing clones during the export process lacks similar human-verifiable images to prove monoclonality, therefore an extensive verification study was conducted to provide statistical evidence that clones recovered from the Beacon platform have a high probability of maintaining >99% monoclonality.

Typical well plate based workflow

Beacon™ based workflow

Beacon Platform
The Beacon platform blends novel microfluidic design with semiconductor technology to enable thousands of single cell experiments in parallel. BLI’s OptoSelect™ chip overlays a microfluidic system on a light-activated phototransistor array. This enables rapid and precise cloning through the movement and positioning of single cells using controlled patterns of light.

The OptoSelect chip contains thousands of flow-isolated single nanoliter chambers, known as NanoPens™, which physically isolate clones from flow but enable diffusion-based exchange.

With the Beacon, thousands of single cells can be isolated, cultured, assayed, and exported for analysis across multiple OptoSelect chips.
**Materials and Methods**

Monoclonality of recovered clones was demonstrated by executing 8 CLD workflows over a period of 4 weeks using 2 Beacon instruments. A transfected pool derived from the CHOZN® Chinese hamster ovary (CHO) line was used as a representative model. Single cells are imported and isolated on the Beacon platform. Single cell status was confirmed with multiple rounds of automated cell detection, and saved quality control images. Once isolated in NanoPens, clones were cultured in CD CHO Fusion media supplemented with 20% batch culture supernatant for a period of 4-5 days. Quantitative secretion assays are typically performed during culture, but are not relevant to the study of monoclonality and thus were omitted. Frequent, visual monitoring of growth ensured that the workflow was completed before fast-growing clones could overgrow their NanoPen, thereby potentially impacting the clone monoclonality. Clones were recovered using an automated export process, where clones were exported in a 5μL volume into a 96-well plate, after which four (4) 5μL blanks were dispensed into a 384-well plate for monoclonality assessment.

**Results**

During a 4 week period, a total of 30 OptoSelect chips were loaded, from which 650 clonal populations were selectively exported. 92.5% of the exported clones expanded successfully after recovery. An additional 2599 “blank” (negative control) exports were performed and these wells were stained, imaged, and manually scored for failures. Three (3) contaminating cells 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%.