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Accelerated High Secretion Cell Line Development

CellXpress™ - automated cloning, clone validation, growth and stability tracking

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Cloning stable cell lines with high specific protein secretion (e.g., antibody) is challenging, time consuming, and a major bottleneck in biopharmaceutical process development. Optimal production cell lines have high levels of secretion of the target recombinant protein, stable expression of such secreted protein and growth characteristics indicative of successful transferability to bioreactors.

Traditionally, clone-selection using limiting-dilution cloning or colony picking is labor and materials intensive, time consuming, and not amenable to significant automation. Large numbers of presumptive clones must be distributed over many multi-well plates, manually identified and verified as clones, and grown to significant cell numbers for assay before ranking and selection. Random isolation of clones followed by large scale assay inherently leads to significant amounts of effort wasted on non-secreting clones. Once the ranking process is completed, a large number of clones must be maintained over time in order to select clones with stable secretion rates. Inherent limits of scale and lack of automation restrict the size of selection pools to 1,000 – 2,000 cells and precludes multiple parallel projects.

CellXpress™

CellXpress is a fundamentally new process that integrates an in situ live-cell fluorescence secretion assay, brightfield imaging and in situ laser purification to clone selection, clone validation, growth tracking, and secretion stability tracking. CellXpress provides direct, automated, in-plate clone selection using standard growth media within a constantly sterile environment. Cells are not, for example, transferred to a semi-solid growth medium as required for semi-automated colony pickers. CellXpress reduces the time from stable pool to a set of clones ranked on implied specific productivity to less than 24 hours. This substantially reduces workload up-front focusing only on clones that have high potential value downstream. Subsequently, clones are assessed and ranked based upon in situ measurement of growth rates and secretion stability permitting early attrition of many clones. CellXpress has been effectively implemented on a number of cell types, including CHO and NS0, used in biopharmaceutical manufacturing. CellXpress is powered by LEAP™, a novel, high-throughput, in situ laser processing platform, that (1) images the full well of a multi-well plates, (2) identifies each cell via fluorescence or brightfield imaging, (3) quantifies fluorescence intensity from each cell, (4) gates cell populations on combinations of cell-level features, and (5) rapidly (>1,000 pulses per second) targets undesired cells for elimination from a well via precisely targeted laser irradiation.

Cell Secretion Assay, Selection and Cloning

The CellXpress process is initiated with an in situ live-cell secretion assay. This unique assay involves mixing cells in standard culture medium with C-lect™ reagents; plating cells into wells of a multi-well plate and incubating for a period of time, typically 10-15 hours (e.g. overnight). Cell-secreted protein is captured in close proximity to the cell producing the protein. After incubation, cells are washed and incubated with a live cell stain and each entire well imaged in fluorescence using LEAP. The fluorescence of the viable cell channel and the secreted protein channel are recorded (Fig. 1) providing a cell location and quantified cell secretion level for each cell. Additional cell-level features, such as size and shape, are also available within the real-time gating interface for use in identifying the best cell. LEAP targets all undesired cells leaving the desired clone, which is not physically exposed to the laser beam. Imaging and laser processing requires less than one hour per 384-well plate. The clone remains in the sterile cloning plate. The in situ secretion assay

has been demonstrated to provide excellent correlation (>0.9) to conventional specific productivity measures, such as HPLC, providing selection and cloning based directly on specific productivity.

Clone Validation and Growth Tracking

The second phase of the CellXpress process is clone validation and growth tracking. Within the original cloning plate, live cells are either identified using a live cell stain followed by fluorescence imaging or by label-free conditions with brightfield imaging using LEAP (Fig. 2). Label-free brightfield imaging provides a key benefit in that no dyes are added that may impact the health of fragile clones. Cell growth can be non-invasively monitored at regular intervals using brightfield imaging during clonal expansion allowing determination of growth rates of each clone early in the process. Clones with growth rates outside the desired range can be eliminated, reducing the workload.

Secretion Stability Tracking

The third phase of CellXpress provides a unique capability to routinely assess secretion stability of an expanding clone. The in situ live-cell secretion assay enables the unique ability to assess the distribution of secretion values within a population requiring few cells. This capability provides an easy and rapid method to repeatedly monitor the stability of secretion of a clonal population during culture expansion. Samples of cells (50 – 100 cells) at each stage can be used to assess the distribution of secretion. Clones that have reduced or stopped secreting can be removed. It is also possible to identify clonal populations beginning to “drift” showing an increased number of low secretors (Fig. 3) while the average secretion values indicate the clone is still good. This allows early identification of secretion instability in a clone enabling early termination often 2-3 weeks before conventional assays can be performed. Since the assay requires a limited sample size and can be accomplished in high throughput, this assessment can be performed throughout the expansion phase as well as into downstream processes.

Summary

Generation of high-producing clones using CellXpress™ on LEAP™ enables highly parallel direct selection of clones based on criteria important in the final selection of clones for biomanufacturing, including high specific productivity, appropriate growth rates and stable secretion rates over time. Clones can be produced using standard culture medium, maintained in a sterile environment, and clonality can be visually documented automatically. Experience has shown that over 10 fold higher numbers of highly-secreting clones are generated per plate with CellXpress versus standard cloning. Further, CellXpress enables larger selection pools to be screened. A single 384-well plate allows selection of the best 0.1 – 0.5% of secretors from >75,000 cells. As many as 8-10 plates can be processed in a day enabling more expansive assessment of challenging cloning projects or opening bandwidth to process multiple cloning projects in parallel increasing productivity. In summary, CellXpress provides significant savings by reducing time to high-value clones, reduced labor and materials costs, and a scale not currently accessible by conventional approaches, offering the potential to reduce risks of project failure due to limited numbers of high-quality clones.

FIGURE 1

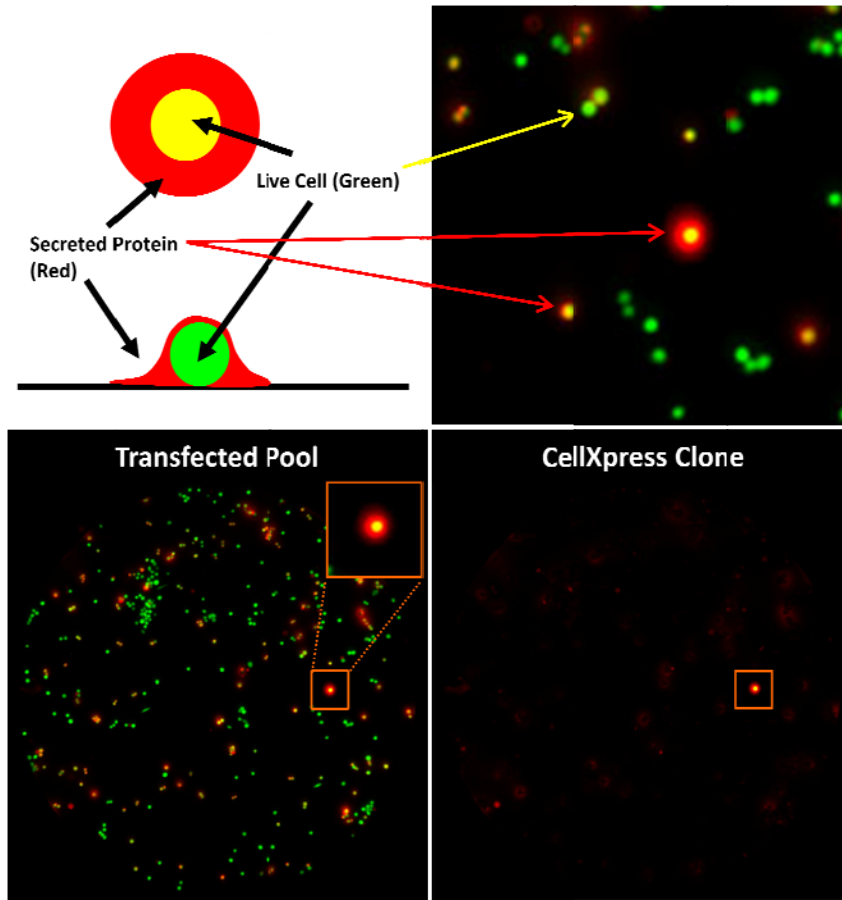


Figure 1. **Diagram and real image demonstrating detection of living cell and secreted protein.** (Top Panel) Live cells are identified in green, and associated secreted protein is visible as a red halo around each cell. Image on right was captured on the LEAP platform. (Transfected Pool) Heterogeneously secreting cell population in transfected pool prior to cloning. (CellXpress Clone) Single highest secreting cell remaining after laser processing.

FIGURE 2

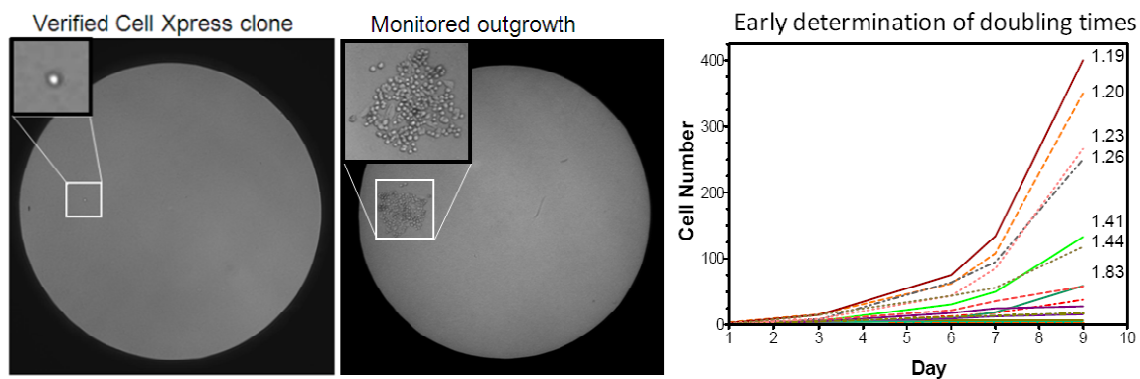


Figure 2. **Single clone verification and growth tracking.** The left panels are bright field images captured on LEAP verifying a single remaining cell after laser processing and outgrowth of this single cell to a colony one week later. The right panel shows growth rates of individual clones that were non-invasively determined *in situ* during the first ~1 week of growth.

FIGURE 3

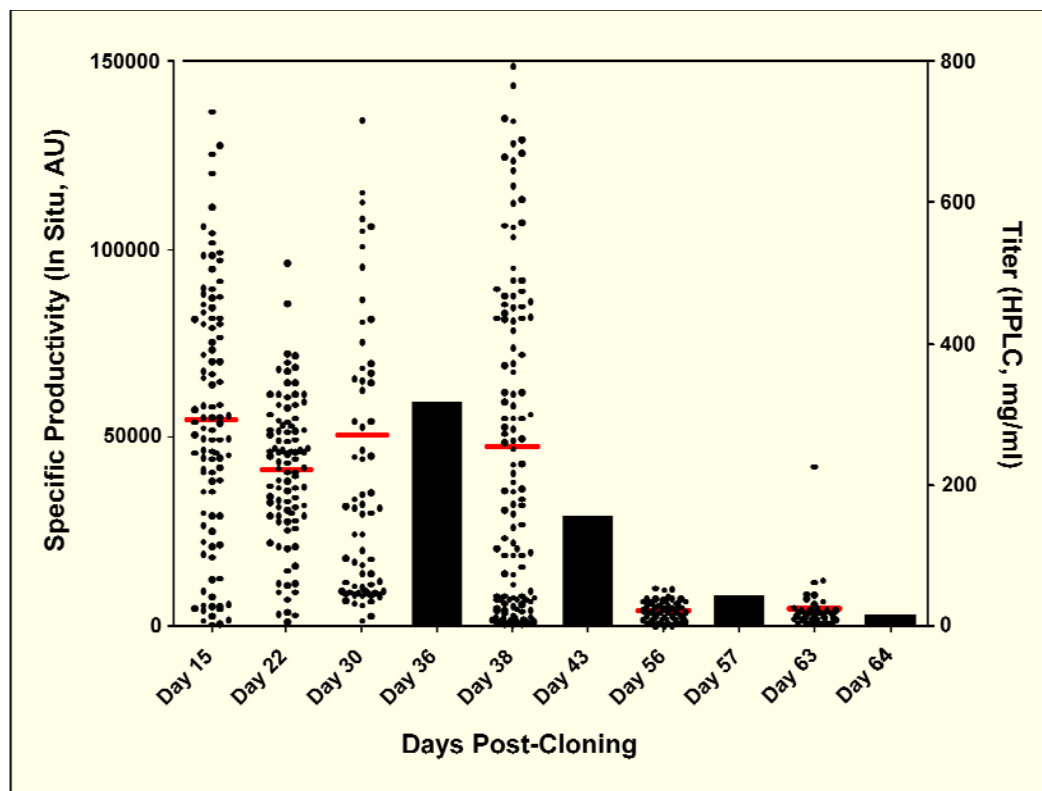


Figure 3. **Secretion stability tracking of expanding clonal population.** Scatter plots show specific secretion activity of each cell within the same clonal population at different days post-cloning. Within 30 days of cloning, secretion instability can be identified as increased number of individual cells with lower secretion levels, even though the average population secretion (red lines) remains high. Solid bars represent HPLC results. Instability can be observed 2-3 weeks before HPLC results can be acquired.