

Development of an In Situ Secretion Assay that Enables Accelerated Cloning and Tracking of Highly-Secreting Cell Lines

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Abstract

Cloning stable cell lines with high specific protein secretion is challenging and time consuming. A fundamentally new process, CellXpress™, has been developed that purifies initial clones based on specific cell secretion, reliably produces a larger pool of better clones, and greatly reduces the time and effort required compared to other approaches. CellXpress is a streamlined process which combines a novel cell secretion assay with LEAP™, an innovative platform for imaging and laser processing of cells. The first step (1) in the process is in situ quantitative measurement of secretion on individual living cells within a multi-well plate followed by (2) laser-mediated elimination of all non- and poorly-secreting cells. Subsequent steps in the process consist of (3) validation of clonality and colony growth tracking, and (4) secretion stability tracking of individual expanding clones. This new enabling process provides direct, automated, in-plate clone selection using standard growth media within a constantly sterile environment. CellXpress reduces the time from stable transfected pool to clones ranked on specific productivity to less than 24 hours. The CellXpress automated 4 stage process has been shown to generate >10 fold more high secreting clones than standard limiting dilution. Growth characteristics are tracked using label-free brightfield imaging, permitting early attrition of many clones. A major issue in secreted protein cell line development is secretion instability. CellXpress provides a direct means of measuring secretion instability. Unstable clones can be identified 2-3 weeks earlier than using conventional methods. CellXpress is powered by LEAP, a high-throughput, in situ laser-processing platform, that images the full well of multiwell plates, identifies each cell via fluorescence and/or brightfield imaging, quantifies fluorescence intensity from each cell, gates cell populations on combinations of cell level features, and rapidly targets undesired cells for elimination from a well via precisely targeted laser irradiation, while protecting the desired cell to be retained. The CellXpress process significantly reduces the time and resources needed to generate highly-secreting cell lines providing the opportunity to process multiple projects in parallel.

COMPARISON:

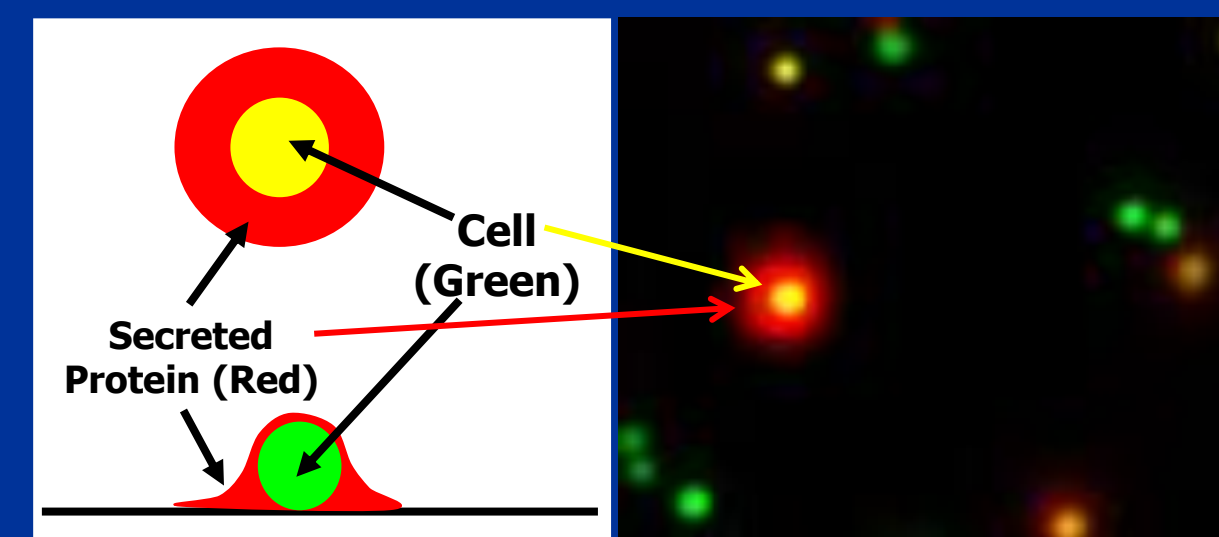
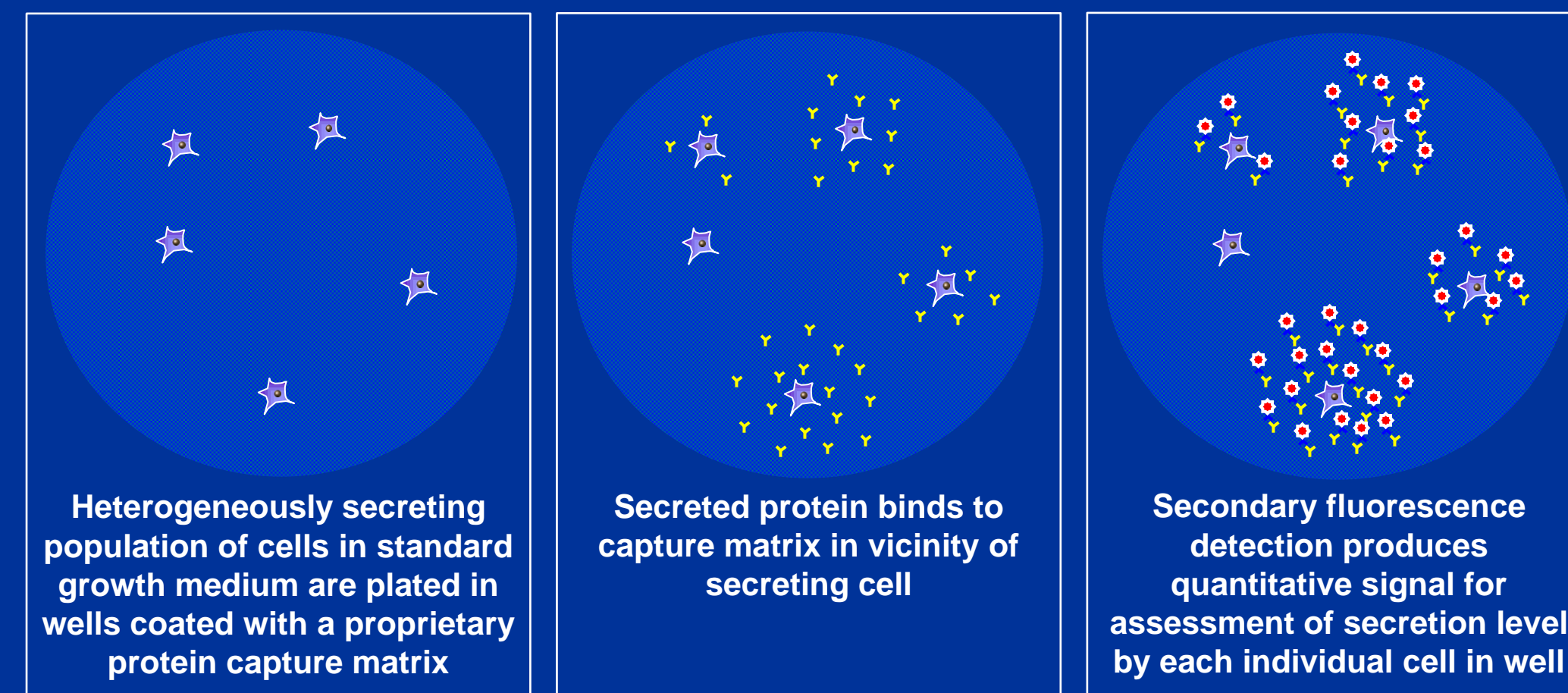
Limiting Dilution Cloning

vs.

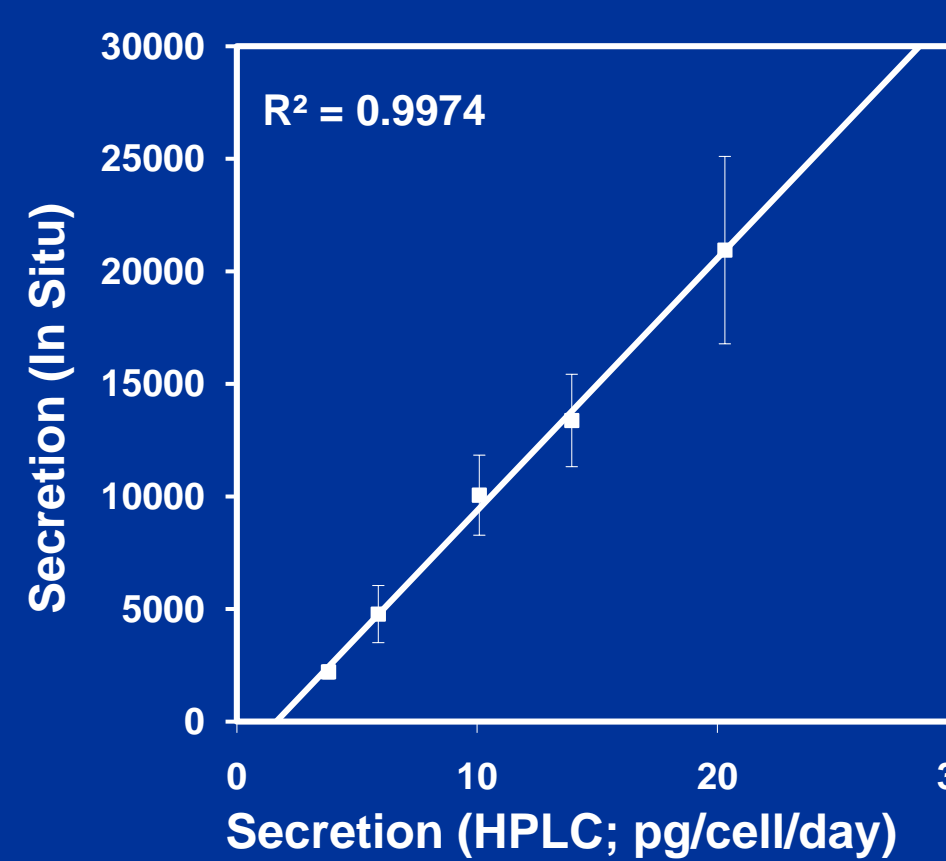
CellXpress

	CellXpress	Limiting Dilution	CellXpress Improvement vs. Limiting Dilution
Pool size for selection	600,000	<1000	600x
Time to clone isolation	1 – 2 Days	10 – 16 Days	>90%
Total time to 24-well plate	15 – 22 Days	45 – 106 Days	50 – 85%
Time to first HPLC results	4 – 6 Weeks	9 – 18 Weeks	30 – 78%
Number of potential parallel cloning projects	3 – 4	1	3 – 4x

STEP 1: In Situ Quantitative Measurement of Cell Secretion



Cells in 384-well plate imaged on LEAP. Live CHO cells are labeled with green Calcein-AM dye. Halos of captured secreted antibody are labeled with red fluorescent secondary detection.



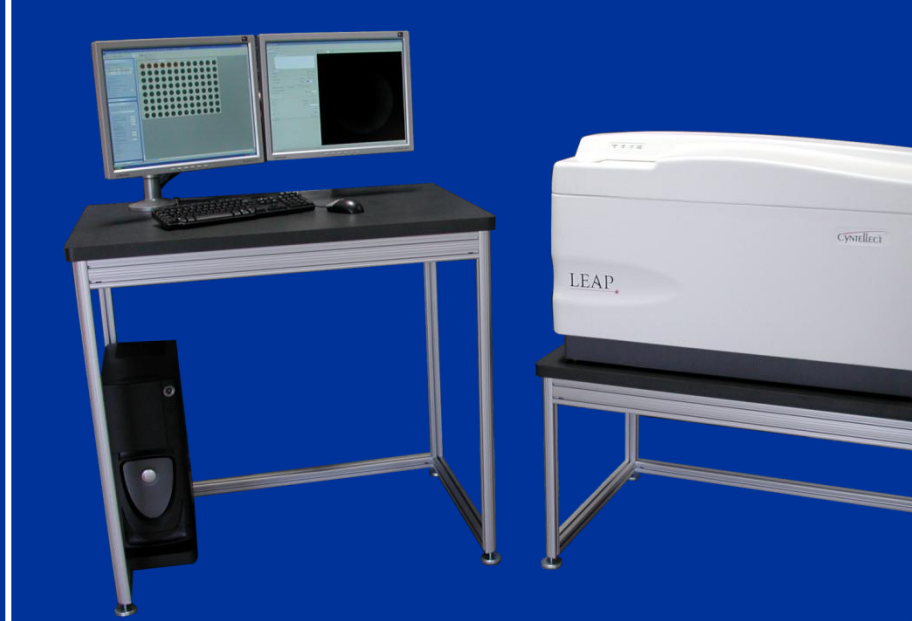
Quantitative comparison of CellXpress image based secretion measurement and HPLC. Several clonal cell lines secreting differing levels of IgG were analyzed by both CellXpress secretion measurement and conventional HPLC. In situ secretion measured by CellXpress is highly correlated with HPLC measurement from supernatant.

STEP 2: Laser Purification of Best Secreting Clone



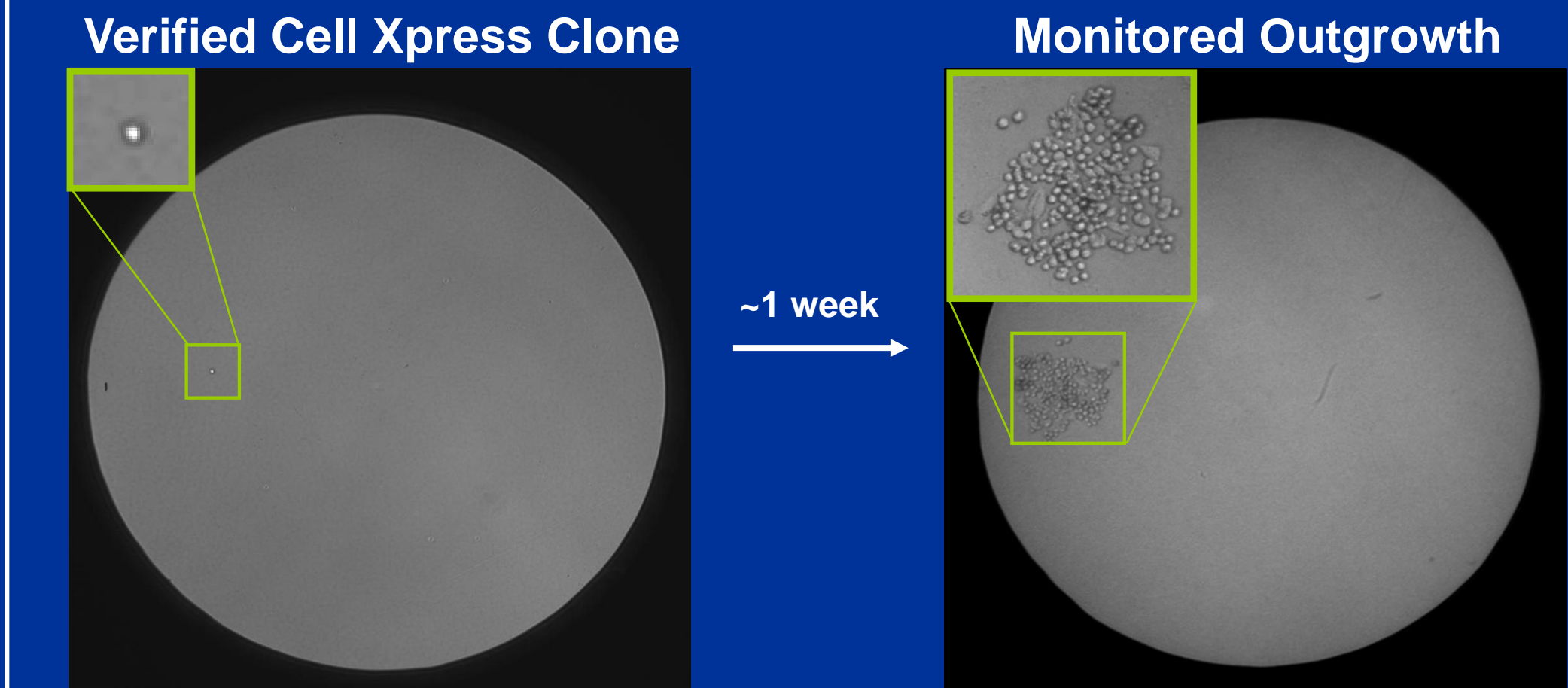
(Transfected Pool) Pool of heterogeneous IgG secreting suspension-adapted CHO cells in a 384-well plate. Cells are identified by green fluorescence. Cell-associated IgG secretion halos are identified by red fluorescence. (CellXpress Clone) The single remaining best secreting cell after CellXpress-mediated clonal purification. (Expanded Clone) Homogeneous cell line population after outgrowth of CellXpress clone for ~50 doublings.

LEAP (Laser Enabled Analysis and Processing)



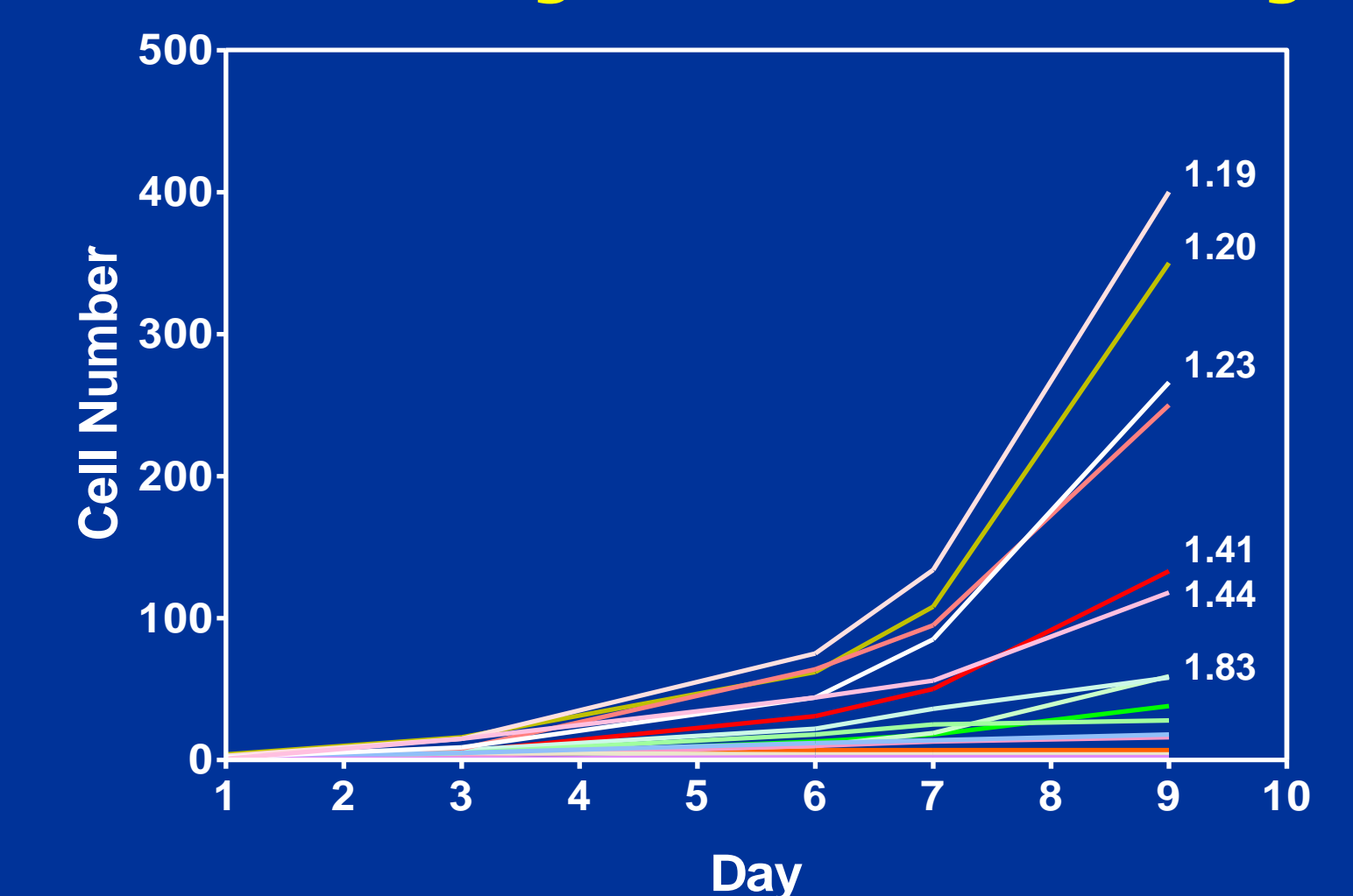
- Fluorescence and bright-field illumination
- Mega-pixel CCD camera
 - (365-700 nm; 8 position wheel)
- Adjustable magnification (3, 5, 10, 20X)
- Image whole wells of multi-well plates
- Rapid auto-focus
- Laser-based cell manipulation
 - Two wavelengths for various applications
 - Cell targeting at >1,000 per second
- Computer and software automation
 - Automated control of all hardware features

STEP 3: Validation of Clonality and Colony Growth Tracking



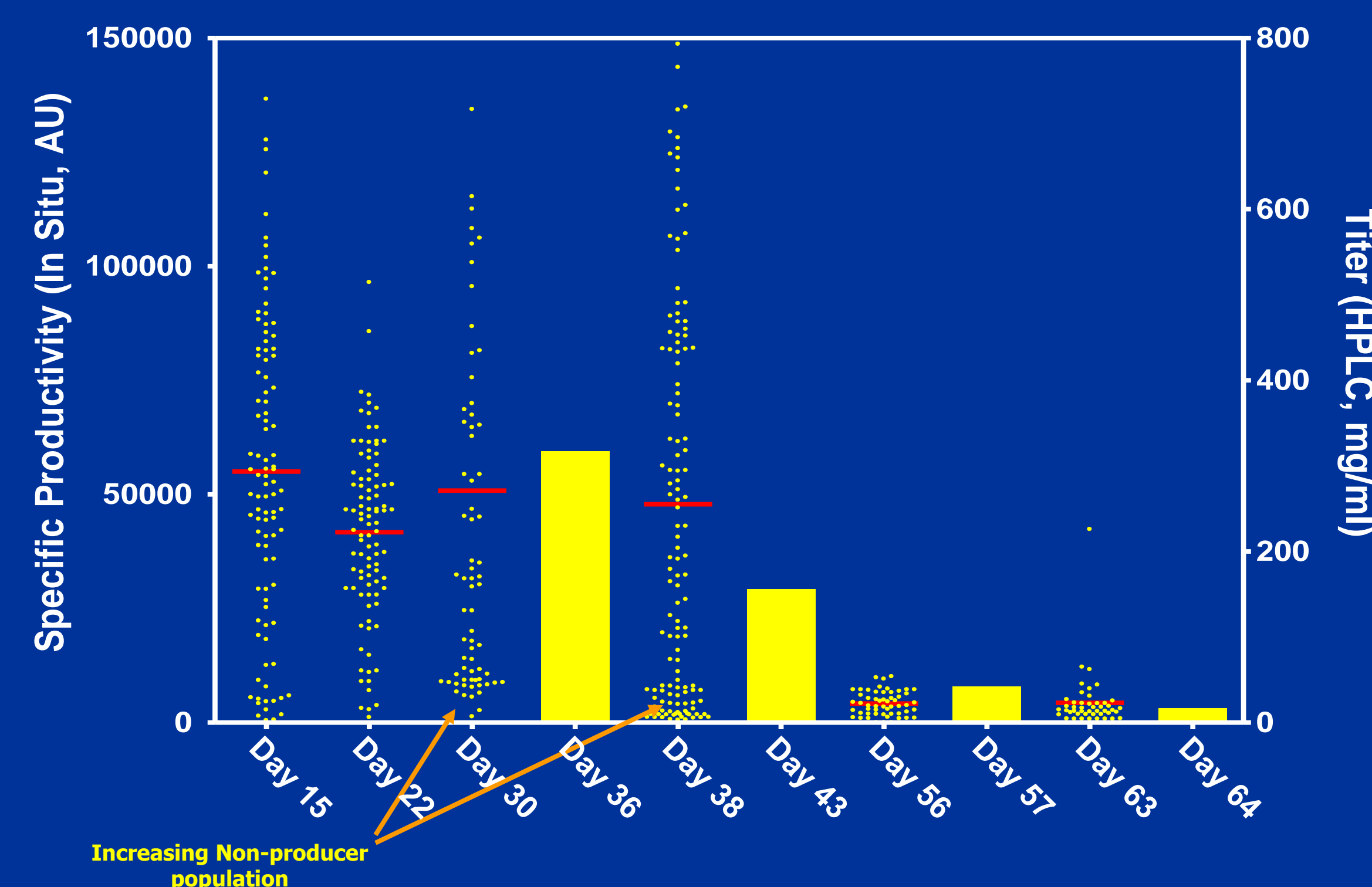
Single clone verification and growth tracking: Brightfield images captured on LEAP verifying a single remaining cell after laser processing and outgrowth of this single cell to a colony one week later.

Real-time growth rate monitoring

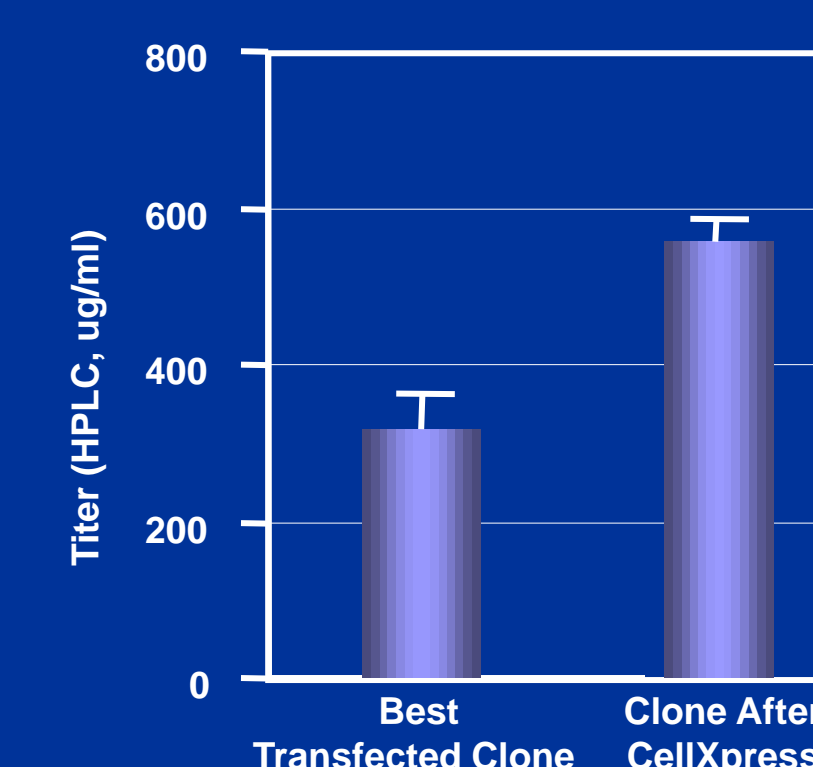


Growth rates (doubling times) of individual clones that were noninvasively determined in situ using brightfield imaging and cell counting on LEAP during the first week of growth.

STEP 4: In Situ Tracking of Colony Secretion Stability During Expansion



Assessment of the distribution of secretion levels within populations of cells provides early insight into the quality of the culture and enables reduction in overall workload. CellXpress data are shown as scatter plots obtained much earlier than currently possible with HPLC (solid bars). As early as Day 30, this clone exhibited an increase in non-producers within the population (lower population), even while the mean production level was maintained (red bar), eventually leading to complete loss of secretion.



Comparison of antibody secretion for the best parental line derived by limiting dilutions, and a new clonal line derived by CellXpress.

Conclusions

Development of the CellXpress process has created a powerful application for rapid and robust generation of highly-secreting cell lines. The unique nature of the application guarantees isolation and verification of a single cell clone and enables detailed tracking of colony expansion and secretion. The quantitative image analysis associated with CellXpress provides accurate assessment of clone secretion in the earliest stages, thereby eliminating effort wasted on non-productive clones. The in situ nature of the process also allows serial enrichment steps prior to cloning, further maximizing robustness of resulting clones. With a throughput capability of examining over 600,000 individual potential candidates in a day, CellXpress makes finding that one in a million clone feasible. CellXpress may also be used for generating optimized cell lines for non-secreted protein expression.