An optimized and validated workflow for developing stable producer cell lines with >99.99% assurance of clonality and high clone recovery

Julia Scherzinger, Daniel Türk, Fernando Aprile-Garcia
CYTENA GmbH, Freiburg, Germany
Correspondence to: fernando.aprile.garcia@cytena.com

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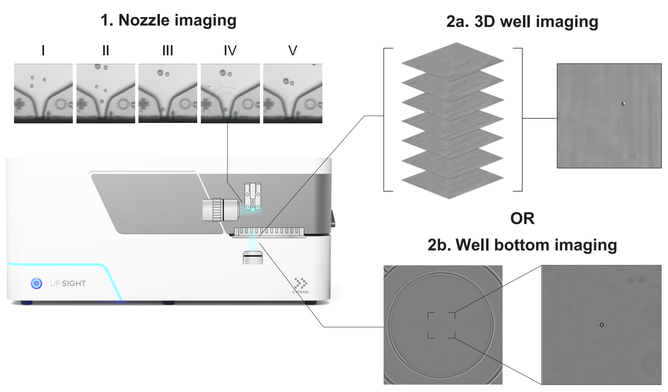
There is a constant pressure to reduce timelines in mammalian cell line development (CLD) for biotherapeutic protein production and gene and cell therapy. Demonstration of clonal derivation of the generated cell lines is key for health authorities' approval. To meet these regulatory and process-oriented demands, single-cell dispensers and plate imagers have become vital in any CLD laboratory.

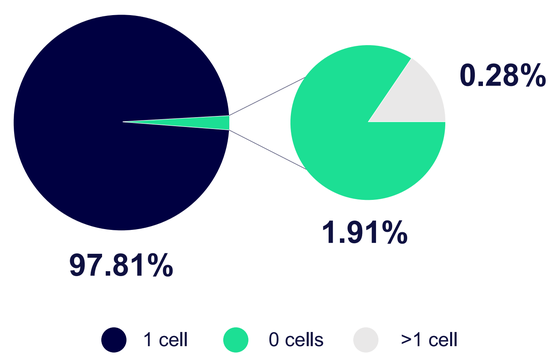
Here we present the 2nd gen UP.SIGHT (CYTENA GmbH), the all-in-one solution for the generation of monoclonal cell lines. The full workflow from single cell cloning to selection of high producing clones with comprehensive documentation for IND/BLA submissions can be performed, while assuring >97% single-cell dispensing efficiency (SCDE) and >99.99% probability of clonal derivation (p(clonal)). Our results show that the 2nd gen UP.SIGHT enables fast and efficient CLD workflows, with full documentation of clonal derivation.

1 The 2nd gen UP.SIGHT gently dispenses single cells in 384- and 96-well plates and provides double assurance of clonal derivation



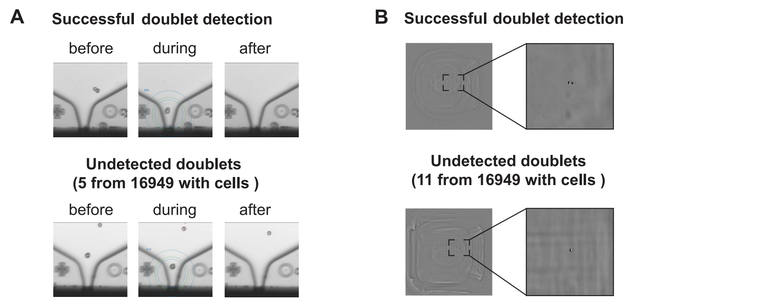
1. Nozzle imaging: Droplets containing one cell are dispensed. I, before single-cell dispensing; II, cell enters region of interest; III, cell is at the nozzle; IV, same image with cell detection overlay; V, after single-cell dispensing to verify that cell left nozzle.
2a. Day 0 3D well imaging: Generation of image stack to confirm single cell deposition.
2b. Day 0 well bottom imaging: Acquisition of single image once cell has settled to the bottom of the well to confirm single cell deposition.

2 Single cell dispensing efficiency on the 2nd gen UP.SIGHT is higher than 97%



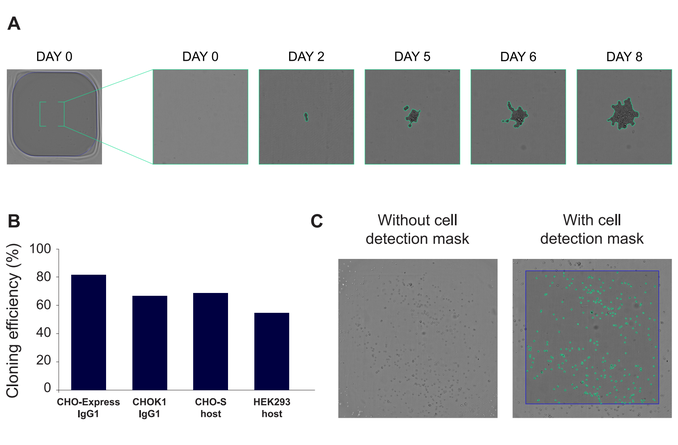
CHO-GFP cells were dispensed in 3 independent experiments with 3 2nd gen UP.SIGHTs each. In total, 45 384-well plates (17280 wells) were analyzed. The number of cells deposited in each well was determined by well bottom imaging with a NYONE imager. Results were curated manually to correct algorithm-caused mistakes.

3 Probability of clonal derivation using 3D well imaging on the 2nd gen UP.SIGHT is higher than 99.99%



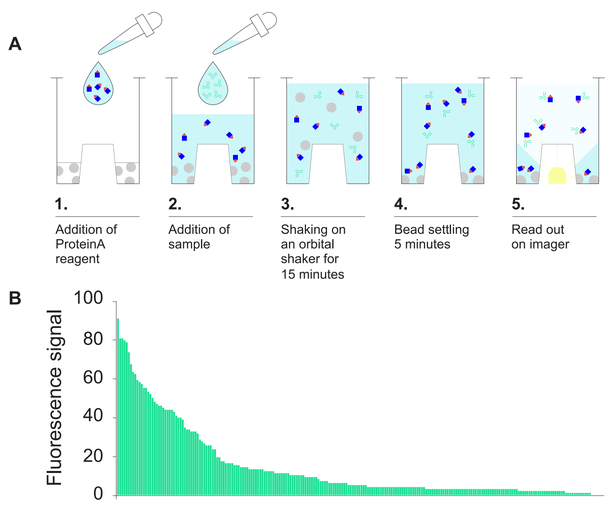
A) Nozzle images. Top: doublet detection. Bottom: no doublet detection; nozzle imaging system fails at detecting the doublet. This accounts for the nozzle system detection error. B) Individual stack images from 3D well imaging. Top: doublet detection. Bottom: no doublet detection; 3D well imaging system fails at detecting the doublet. This accounts for the 3D well imaging system detection error. C) A clone is of non-clonal origin if both imaging systems failed to detect a doublet. The error rate of each imaging system was statistically derived with the Wilson Method after image inspection and the resulting probability of clonal derivation was calculated as shown.

4 Well bottom imaging enables growth monitoring of suspension and adherent cell lines by confluency measurement and cell counting



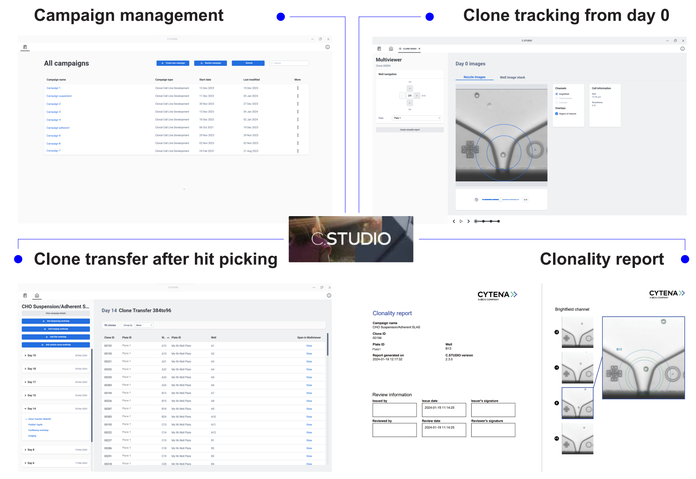
A) 2nd gen UP.SIGHT well bottom images at different timepoints after CHO single cell dispensing. B) Typical cloning efficiencies after medium optimization for several suspension cell lines. Cloning efficiency values expressed as percentage of wells occupied by colonies on 384-well plates after 14 days of cloning. N=3-5, error bars: s.e.m. C) Representative cell count image with and without cell detection mask ($2.5x10^4$ cells/ml). All image analysis performed with C.STUDIO.

5 F.QUANT-based mAb titer measurement for clone selection



A) Schematic protocol for F.QUANT Fc Titer Assay. B) A minipool of IgG1-expressing CHO cells was dispensed into 384-well plates using the 2nd gen UP.SIGHT. On day 10, plates were imaged and confluency determined with C.STUDIO. The top 25% clones (227 clones) were picked into 96-well plates. 3 days later, 5 µl supernatant was used for F.QUANT-based titer measurement. Ranked relative titer values are shown.

6 C.STUDIO: Analysis, clone tracking & reporting



- Select your best clones based on all 2nd gen UP.SIGHT-derived data and even import custom data.
- Generation of clonality reports to prove clonal derivation of producer cell lines.
- Confluency measurement to track cell growth over time.
- Determination of cell counts.
- Comprehensive mAb monitoring for high-producing clone identification.
- Software-guided clone transfer: C.STUDIO creates clone transfer lists to aid in manual pipetting.

Conclusions

- The 2nd gen UP.SIGHT is a gentle single cell dispenser with extremely high single cell dispensing efficiency and assurance of clonal derivation.
- The 2nd gen UP.SIGHT's plate imaging capabilities combined with C.STUDIO image analysis enables colony tracking and clone characterization over time.
- C.STUDIO allows the selection of high-producing clones based on 2nd gen UP.SIGHT-derived titer measurements.
- All the steps from single-cell dispensing with assurance of clonality and colony tracking are covered by the same instrument. This should not only result in a faster and more efficient cloning workflow, but also in better documentation for improved quality of the final biological product.

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Scherzinger, J., Türk, D., and Aprile-Garcia, F. (2022). An optimized and validated workflow for developing stable producer cell lines with >99.99% assurance of clonality and high clone recovery. bioRxiv, 2022.12.16.520697. 10.1101/2022.12.16.520697.