A comparison of traditional cloning methods vs. CloneSelect Single-Cell Printer f.sight using CHO cell lines commonly used for mAb production

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Introduction

As regulations for cell line development become increasingly more stringent, researchers will be required to perform single-cell cloning and provide evidence that a cell line is derived from a single cell (proof of clonality). One accepted method for establishing clonality is limiting dilution (LD), a probability-based process whereby only a small subset of the wells (10–30%) in a plate is likely to contain a single cell. Cell sorting by flow cytometry (FC), another traditional cloning method, can dispense a single cell in each well of a plate with higher efficiency, but high fluidics pressure during sorting may have non-negligible effects on post-sort cell viability. Furthermore, the high costs associated with instrument maintenance and trainings creates barriers to users who have limited access to resources. Cost-effective cell line development workflows and highly efficient cloning methods are needed.

The CloneSelect™ Single-Cell Printer™ f.sight™ (f.sight) is designed to meet this need, gently depositing a single cell into a well with the entire cell deposition process imaged and time-stamped, providing pictorial evidence of monoclonality and excellent post-deposit cell growth for cell line development. In this study, we conducted six sets of experiments, cloning two CHO cell lines with animal-component-free (ACF) cell culture media and supplements, and compared the performance of f.sight versus two other accepted cloning methods (FC and LD).

Materials & methods

Two suspension CHO cell lines, a parental FreeStyle™ CHO-S cell line from Thermo Fisher Scientific (R80007) and a recombinant human IgG-secreting CHO DG44 cell line (Molecular Devices) both maintained in XP CHO Growth A medium (K8860, Molecular Devices) with 4mM L-glutamine were used for this study. For each cell line tested, the components of cloning medium and workflow for sample preparation were identical (Figure 1).

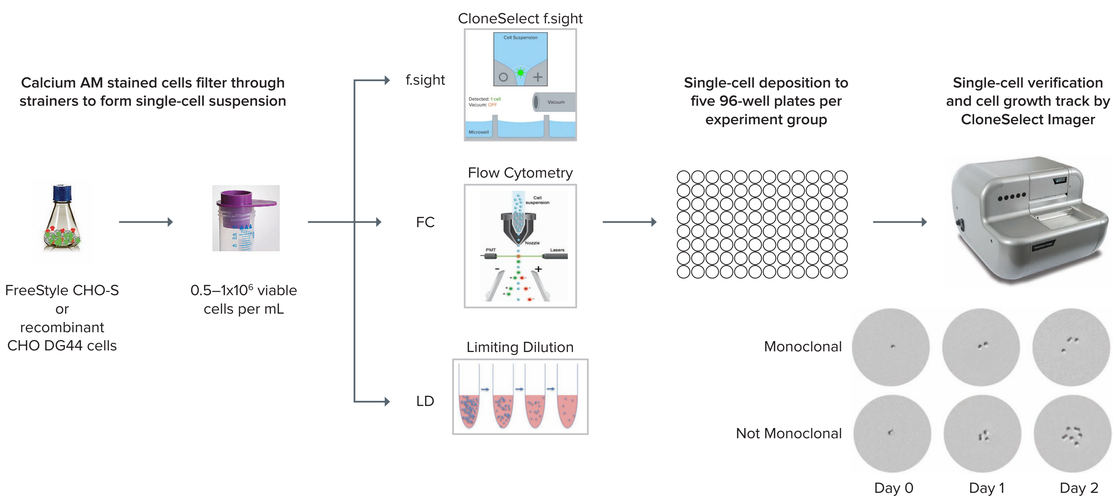

Figure 1. The cloning medium was freshly made from EX-CELL® CHO cloning medium (C6366, Sigma SAFC) supplemented with 4 mM L-glutamine and 2.5% ClonaCell™-CHO ACF supplement (3820, STEMCELL). On the day of cloning, 10 million cells at log phase were harvested and stained with 1 µM CAM (R8343A, Molecular Devices) at 37oC for 15 minutes. After staining, cells were washed twice and suspended in plain cloning medium, filtered through a 30 µm cell strainer, adjusted to the density of one million viable cells/mL, then split into three separate vials for cloning: (1) F.sight Method Plates were prefilled with complete cloning medium (200 µL/well). 60 µL of single-cell suspensions with the density of one million viable cells/mL was loaded into a disposable sterile cell cartridge. The cell cartridge was loaded onto the CloneSelect Single-Cell Printer f.sight and cells were deposited at a density of one cell per well into five standard 96-well plates (Corning 3300) based on user-defined parameters including cell size, roundness, and fluorescent intensity (2) FC Method Stained cells ready to be dispensed were transferred along with the prefilled plates (200 µL/well) to a BD FACS Aria™ Fusion sorter. Unstained cells with no/low fluorescence were used as a baseline reference for fluorescence cell sorting. Cells were passed through a nozzle (85 µm, sheath pressure 45 psi), sorted based on cell fluorescence intensity, and dispensed at a density of one cell per well into five 96-well plates (3) LD Method Stained cells were diluted 1:1000 (1000 viable cells/mL) to target a seeding density of 0.33 viable cells/well. A total of 165 viable cells (165 µL) were taken from the 1:1000 dilution and added into a media bottle containing 100 mL of complete cloning medium. Cells were mixed by gentle swirling the bottle and transferred into five standard 96-well plates with 200 µL per well. After cells settled down to the bottom of the plate, all plates were imaged on the Clone Select Imager (CSI) system (with transmitted light only) for the following days: 0, 1, 2, 7, 14 to track cell growth and confirm clonality from seeding to cell expansion. Single-cell counts and colony counts were assessed based on manual review of cell images. Cell confluency was automatically calculated on a per well basis using CSI software. The criteria used to determine counts was consistently applied across all six sets of experiments. All single-cell events were further verified by examining cell growth on the following days (day1, day2, or day7) post-deposition. Wells containing single-cell like particles or non-viable cells were counted as void wells. If a single-cell was deposited on the edge of the well, making it difficult to confirm clonality, then the well was excluded from the study.

Results & discussion

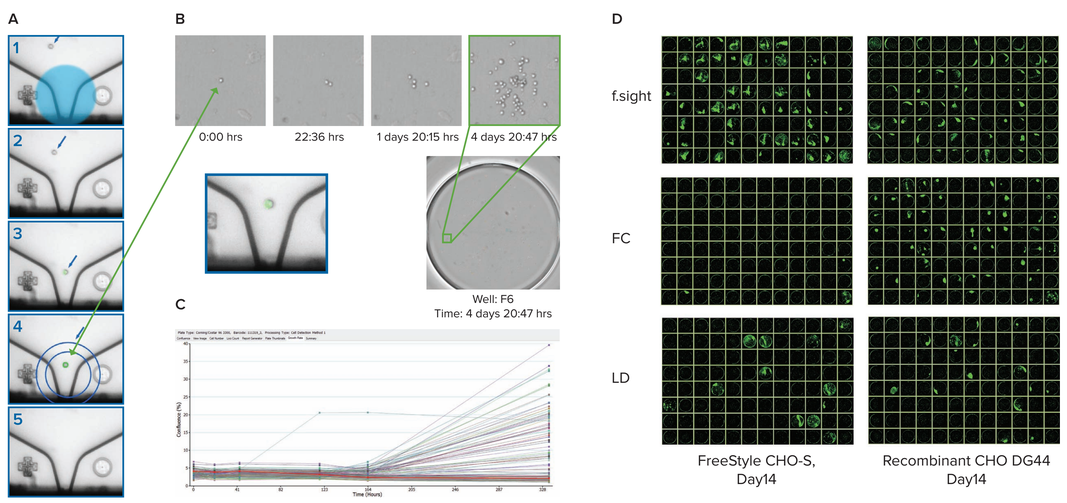

Figure 2. Combined f.sight and CSI workflow provides high assurance of monoclonality in a single round of cloning. There are five overlaid images (total 10 images) captured by f.sight from white light channel and green fluorescence channel at the nozzle of the cell cartridge (2A). Picture 1, 2, 3, 4 were taken before and at the event of single-cell dispensing which shows in (2B) the image of seeded single-cell captured by CSI on Day0. Figure 2C shows the cell growth tracking for each well from Day0 to Day14. Figure 2D shows cell growth images from different microplates on Day 14 after single-cell deposition. The green color indicates the cells.

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Cell Type	Cloning Method	Eligible Wells	Wells with Single Cell	Wells with Multiple Cells	Wells with Single-cell Derived Colony	Single Cell Efficiency (%)	Outgrowth Efficiency (%)
FreeStyle CHO-S	f.sight (SUM)	467	385	11	262	385/467	262/467
	Mean± SD (n=5)	93.4 ± 1.8	77.0 ± 3.1	2.2 ± 0.8	52.4 ± 6.0	82.4% ± 3.0%	56.1% ± 5.8%
	FC (SUM)	473	304	12	16	304/473	16/473
	Mean± SD (n=5)	94.6 ± 0.5	60.8 ± 2.4	2.4 ± 1.1	3.2 ± 1.3	64.3% ± 2.8%	3.4% ± 1.4%
	LD (SUM)	461	61	17	45	61/461	45/461
	Mean± SD (n=5)	92.2 ± 2.8	12.2 ± 2.4	3.4 ± 1.5	9.0 ± 2.3	13.2% ± 2.2%	9.7% ± 2.3%
Recombinant CHO DG44	f.sight (SUM)	466	402	14	247	402/466	247/466
	Mean± SD (n=5)	93.2 ± 1.3	80.4 ± 1.8	2.8 ± 0.8	49.4 ± 7.8	86.3% ± 3.0%	53.1% ± 8.8%
	FC (SUM)	462	307	11	228	307/462	228/462
	Mean± SD (n=5)	92.4 ± 1.8	61.4 ± 3.6	2.2 ± 1.8	45.6 ± 5.9	66.5% ± 4.1%	49.4% ± 6.9%
	LD (SUM)	466	61	32	51	61/466	51/466
	Mean± SD (n=5)	93.2 ± 1.9	12.2 ± 1.3	6.4 ± 2.7	10.2 ± 1.3	13.1% ± 1.3%	10.9% ± 1.3%

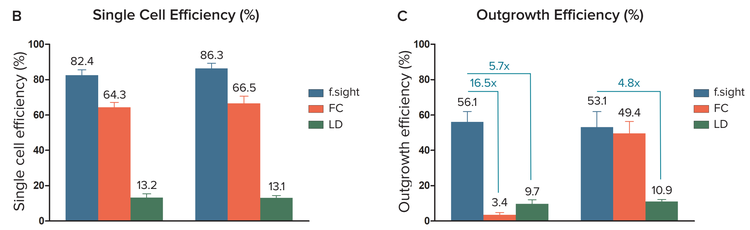

Figure 3. The single cell deposition efficiency was calculated as the counts of wells containing single-cell on day0 divided by total eligible wells (i.e. remaining wells not excluded using criteria described above). Outgrowth efficiency was calculated by dividing the number of wells containing a single-cell derived colony (with more than twenty cells clustered together) on day14 with the total number of eligible wells. As shown in Figure 3A, the single cell and outgrowth efficiency varies greatly among cell lines and the cloning method employed. In general, the average single-cell (deposition) efficiency via FC is about 10-20% lower than that of f.sight for both cell lines (64.3% ±2.8% vs. 82.4% ±3.0%, respectively for FreeStyle CHO-S; 66.5% ±4.1% vs. 86.3 ±3.0%, respectively for recombinant CHO DG44) (Figure 3B). It is unlikely that this decreased efficiency is an artifact of the lower viability observed post-deposit using FC, which could lead to a possible underestimation of single-cell counts, since CHO DG44 cells showed similar viability in all experiments. As expected, LD method yielded the lowest single cell efficiency among three cloning methods tested (13.2% ±2.2% for FreeStyle CHO-S, 13.1% ±1.3% for recombinant CHO DG44). The outgrowth efficiency via f.sight for FreeStyle CHO-S cell is up to 16.5 fold higher than that of FC and 5.7 fold higher than that of LD (f.sight vs. FC vs. LD: 56.1% ±5.8% vs.3.4% ±1.4% vs. 9.7% ±2.3%). However, for recombinant CHO DG44 cells, the average outgrowth efficiency via FC is comparable to that of f.sight (49.4% ±6.9% vs. 53.1% ±8.8%), which suggests that the efficiency of cloning via FC is cell line dependent (Figure 3C). The outgrowth efficiency via f.sight for recombinant CHO DG44 cells is about 4.8 fold higher than that of LD (53.1% ±8.8% vs. 10.9% ±1.3%).

Conclusion

The f.sight outperformed both limiting dilution and cell sorting by flow cytometry with respect to clonal outgrowth efficiency for each cell type tested.

As illustrated in this study, the f.sight improves upon traditional methods of cloning by providing a gentle and efficient cell sorting method for cell line development. This approach is particularly valuable for single-cell isolation of sensitive cell lines with inherent low tolerance to high fluidic pressure, which often leads to poor cell growth after fluorescence cell sorting.

MOLECULAR DEVICES