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

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 training 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™ 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 Medium (KB860, Molecular Devices) with 4 mM 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).

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 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.

Figure 3. The single cell deposition efficiency was calculated as the counts of wells containing single-cell on day0/day14 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 (4.3% ± 2.8% vs. 82.4% ± 3.0%, respectively for FreeStyle CHO-S; 66.5% ± 14% vs. 86.3% ± 3.0%, respectively for recombinant CHO DG44 (Figure 3B). It’s unlikely that this decreased efficiency is an artifact of the lower visibility 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. 5.7x vs. 49.4% ± 6.9%). 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% ± 3.3%).

Conclusion

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.