Application Note:

Pairing automated cloning and monoclonality verification for rapid and efficient cell line development

Abstract

Proof of monoclonality is a regulatory requirement for cell lines used in immunotherapy development. In this study, cellenONE®, an automated cloning system and Celigo, an image cytometer, were combined to evaluate how single cell dispensing and monoclonality verification technologies could significantly accelerate cell line development.

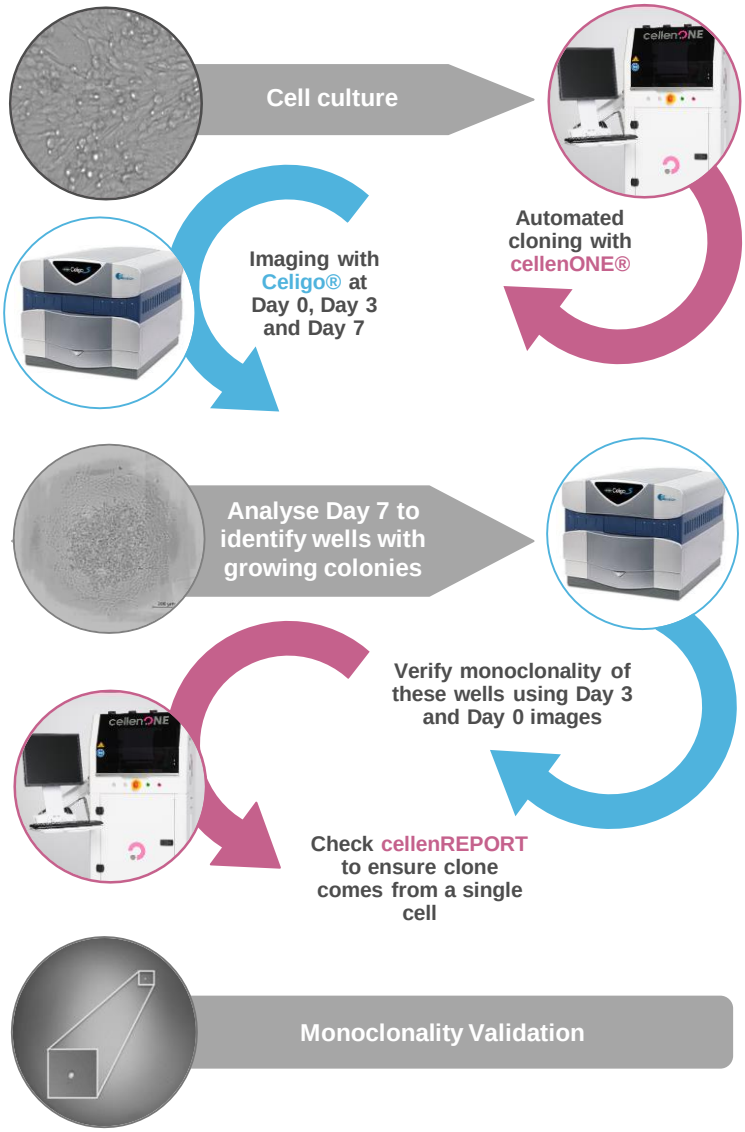
Materials and Methods

CHO and A549 cell lines were cultured using standard methods (DMEM/F12 media supplemented with 10% FBS, Penicillin, Streptomycin and Amphotericin B) in 6-well plates (Nunc). Prior to cloning, cells were detached using trypsin, dissociated, washed and resuspended in PBS (200 cells/µL).

cellenONE® F1.4, an automated single cell isolation and dispensing device, was used for seeding cells into 96-microwell plates (Nunc Edge 2.0; 3 plates per cell line) prefilled with culture media and maintained at 4°C throughout the cloning experiment. cellenONE selects single cells using diameter, circularity and elongation criteria. It was set to dispense one single cell per well with A549 detected cell Diameter 19-28 µm, Circularity < 1.1, and Elongation < 1.85; CHO detected cell Diameter 17-25 µm, Circularity < 1.1, and Elongation < 1.85. For each microplate a positive control well (A1) was prepared by pipetting about 4000 cells. After automated cloning, plates were stored in a cell culture incubator until imaging with Celigo.

Celigo®, a microwell plate based image cytometer, was used to automatically image every well of the microplates to visualise single cells and colonies at different time points: 30 minutes after cloning (Day 0) and after 3 and 7 days (Day 3 and Day 7) of culture. Positive control wells (A1) were used to set the focal plane and adjust imaging settings before the automated imaging of every well of each plate.

Monoclonality Verification began with identifying all wells containing single colonies from Celigo Day 7 images. Subsequently, images of these wells at Day 3 and Day 0 were inspected together with cellenREPORT pre-isolation images to confirm monoclonality. Only wells containing a single clone at Day 0 (Figure 1) and single colony at Day 7 were considered monoclonal and subsequently used to calculate clonal outgrowth (Figure 3).



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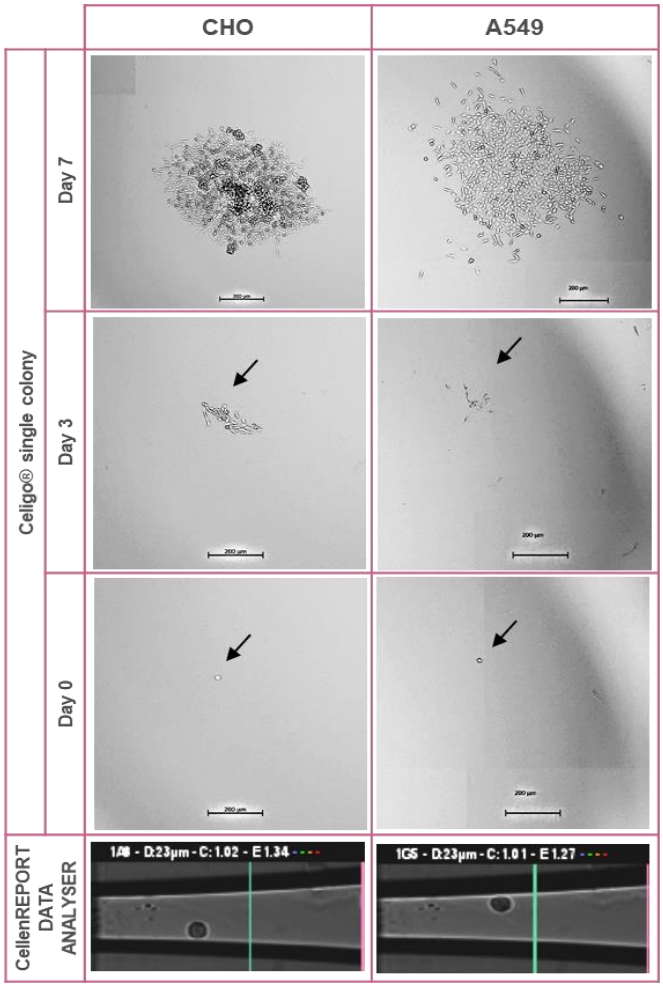

Figure 1. Celigo images of colonies at d7, d3 and d0 (top) and cellenREPORT images (bottom) of the corresponding single cell during automated cloning. The green line denotes the ejection region.

Results and Discussions

Automated cloning with cellenONE F1.4 took on average 3 min 57 s and 3 min 35 s per 96-microwell plate, for A549 and CHO cells respectively. Imaging each microwell plate using Celigo averaged 3 min 30 s per plate.

Overall, out of just three 96-microwell plates for each cell line, 246 CHO and 183 A549 validated monoclonal colonies were prepared in a single round of cloning, corresponding to an average monoclonal outgrowth of 86.3% and 64.2% respectively.

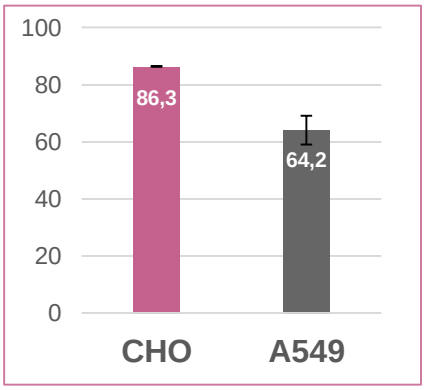

Figure 2. Average % of wells containing monoclonal colonies at Day 7 (n=3).

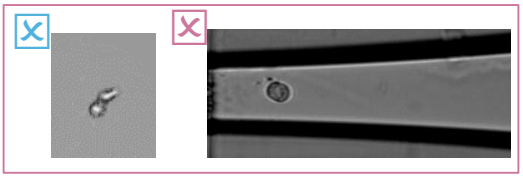

Figure 3. Celigo image showing a well containing 2 clones on Day 0 (left) and confirmation from cellenREPORT images showing the corresponding cell aggregate (right).

Additional features *(not used in this study)*

Both cellenONE and Celigo are compatible with a wide range of microwell plates (96, 384 and 1536), and can utilize fluorescence for cell sorting and imaging making these platforms ideal partners for efficient cell line development.

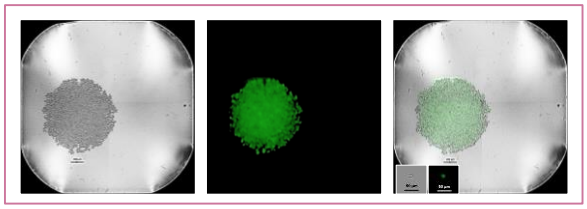

Figure 4. Celigo brightfield (left), fluorescence (centre), and composite (right) images from a HEK-GFP colony grown in a 384-microwell plate after 7 days of culture.

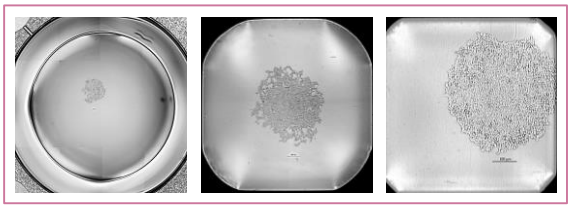

Figure 5. Celigo images of 96, 384 and 1536 microwell plates after 7 days of culture.

Conclusions

In this short study, the complementarity of cellenONE and Celigo platforms was demonstrated for cell line development. Combining these systems enables drastic accelerations in cell line development as efficient cloning and demonstrable monoclonality will inevitably provide more clones of interest from fewer rounds of cloning.

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