Automation for dual clonality assurance and reduced timelines in CHO Cell Line Development

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INTRODUCTION

Single-cell cloning during Cell Line Development (CLD) is a notable challenge and bottleneck in the generation of stable cell lines for the production of therapeutics. Using manual methods such as limiting dilution, the cloning process can take many months, is prone to error, and assurance of monoclonality is measured statistically rather than by an evidence-based approach. The incorporation of automation into the CLD workflow provides long-term cost savings, reduces timelines, and by incorporating evidence-based dual monoclonality assurance to aid regulatory approval, ultimately enables a more attractive and accelerated offering to clients. Menarini Biotech has developed a workflow which incorporates CYTENA automation devices – the UP.SIGHT single-cell printer and C.BIRD plate-based miniature bioreactor, and we have experimentally proven the extensive time-savings and improvements in cloning efficiency. Additionally, incorporating high-throughput titer screening enables titer ranking within 18 days post-cloning. This enables Menarini Biotech as a CDMO to offer a next generation agile CLD workflow for our clients.

SINGLE-CELL CLONING VIA UP.SIGHT

Single-cell cloning can pose a challenge for CLD workflows in two key areas: poor cell recovery post-cloning, and the aforementioned absence of clonality assurance. These are the result of shear stress on cells, the loss of cell-cell communication, as well as the statistical approach to clonality taken by techniques such as limiting dilution and cloning by FACS. By incorporating built-for-purpose cell cloning automation, such as the UP.SIGHT, into an optimised workflow, it has been possible to mitigate challenges both in clonal outgrowth and assurance of clonality (figure 1). Data suggests clonal outgrowth has been increased 2.5-fold, ultimately increasing the probability of selecting top-producing clones and therefore maximising productivity potential (figure 2).

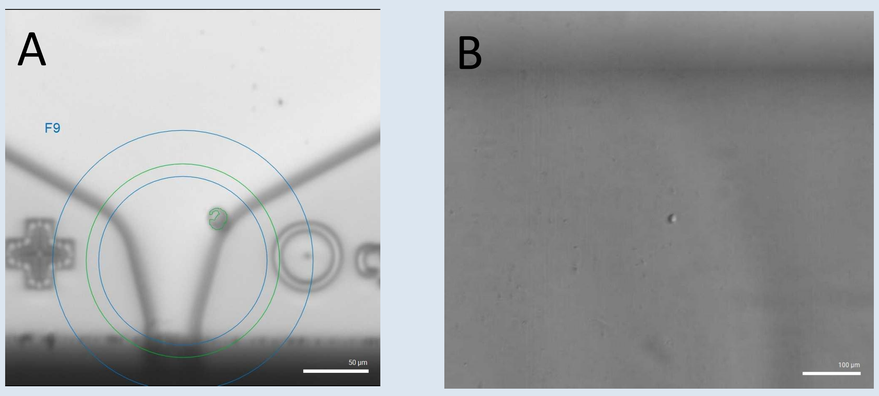

Figure 1: Clonality assurance of the top clone. (A) provides evidence of single-cell at the nozzle before dispensing, and (B) provides evidence of monoclonality within the well.

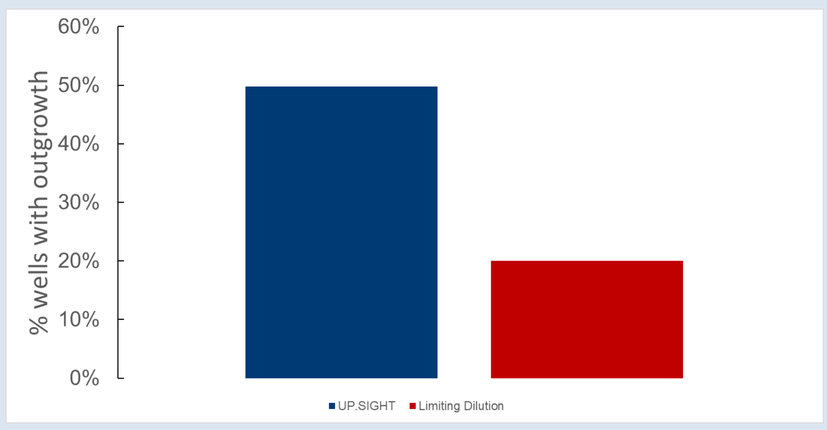

Figure 2: Percentage of wells with visible colony outgrowth 10 days post-cloning in 96 well-plates. UP.SIGHT seeded at 1 cell/well, limiting dilution plates seeded at 0.5 cells/well. Plates imaged using UP.SIGHT imager function. Data not adjusted for cell density at seeding. For each condition, 3 x 96-well plates were seeded, n = 285

In addition to UP.SIGHT integration, workflow efficiency has been improved through the optimisation of cloning supplement strategy, 96-well culture volume, re-feeding strategy, as well as length of time recovering in static plates before rapidly increasing the outgrowth rate using dynamic C.BIRD mixing (figure 3).

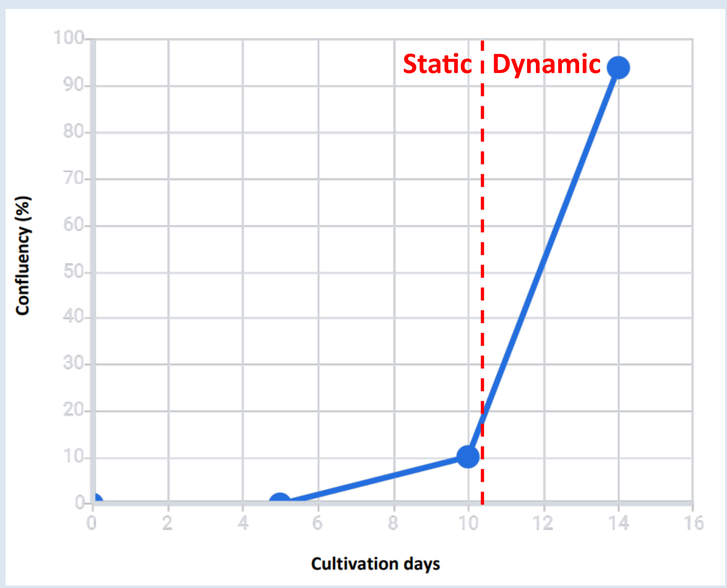

Figure 3: Confluency as measured on days 0, 5, 10 and 14 post-seeding for the top clone. On day 10, the plates were transferred to the C.BIRD miniature bioreactor for 4 days. Cells were allowed to settle before imaging for confluency on day 14. Graph produced and confluency calculated in C.STUDIO software (CYTENA)

HIGH-THROUGHPUT DYNAMIC UPSCALING USING C.BIRD

The utilisation of dynamic upscaling has increased cell-nutrient exposure by removal of the nutrient gradient present in static culture, and reduces competition through cell layers at higher confluency, therefore increasing cell proliferation and reducing upscaling time. By incorporating the C.BIRD miniature bioreactor into the cloning workflow alone, it has been possible to reduce time to shake flask from 33 days to 18 days (- 45%). Through the optimisation of C.BIRD and UP.SIGHT into the CLD workflow, Menarini Biotech is able to deliver clonally-derived cell lines in a reduced time and with greater assurance of clonality for regulator approval.

Additionally, by incorporating UP.SIGHT dual clonality assurance (Figure 4), it is possible to further reduce timelines, by removing the second round of limiting dilution. Therefore, when taking two rounds of limiting dilution into account, the total time to shake flask is reduced from 70 days using limiting dilution and static upscaling to 18 days using UP.SIGHT and C.BIRD automation (- 74%).

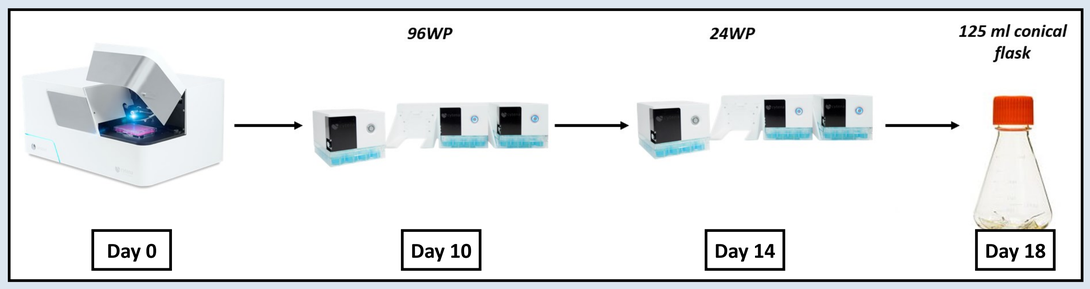

Figure 4: Cloning and upscaling workflow utilising CYTENA instruments to reduce time to clonally-derived cell lines in shake flask

TRACEABILITY AND CLONALITY ASSURANCE

Traceability of a clone is crucial from both a research and regulatory standpoint. Using the C.STUDIO software (CYTENA) it has been possible to track the progress from pre-dispense nozzle images, 3D-well images and confluency (figure 5).

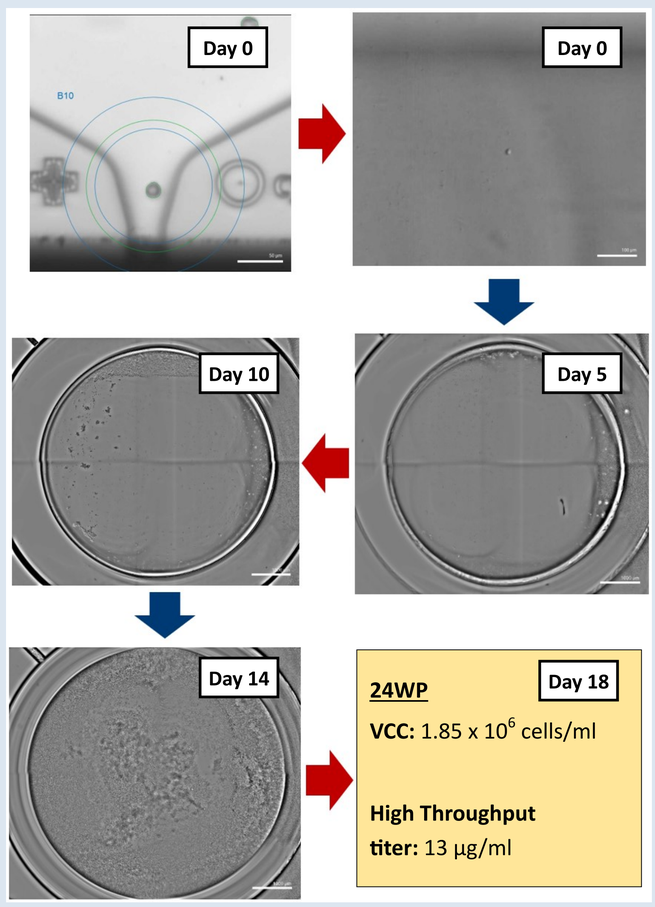

Figure 5: Traceability of the CLD cloning workflow from single-cell dispensing to outgrowth, tracing monoclonality, confluency and ultimately VCC (Vi-CELL XR) and titer (IgG titer ELISA) in 24-well plate. Top clones are therefore picked based on titer and q_p at an early stage. For comparison, the scale is presented on the bottom right of each microscope image

CONCLUSIONS AND ACKNOWLEDGEMENTS

- **A platform cloning process incorporating both single-cell printing technology and a plate-based miniature bioreactor (UP.SIGHT and C.BIRD, CYTENA) pushes boundaries for reduced timelines, increased monoclonality assurance and clone traceability**
- **A competitive timeline of 18 days to shake flask from single cell is achieved, a 74% time-saving compared to the limiting dilution process**
- **By augmenting cloning efficiency, the probability of finding top performing clones is greatly increased**
- **This ultimately ensures Menarini Biotech UK can support clients with a next generation workflow for CLD activities in the CDMO space**

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