Automating Cell Line Development for Biologics

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ABSTRACT

Cell line development is a highly-involved, multi-week workflow that involves the plating, screening, and expansion of single cell-derived clones to establish a protein-expression cell line. This complex process required significant throughput of sterile cell manipulations, long-term incubations, centrifugation, cell counting, monoclonality determination and growth tracking, and protein production assessment. Automating the cell line development process on the Biomek i7 Automated Workstation enabled us to meet the throughput needed for the workflow while minimizing the manual errors that lead to costly rework. Assay data from multiple instruments and time points were used as toll gates to identify the wells that met the criteria to continue through the lengthy process and these data ultimately drove automated hit-picking and cell expansion. Further improvements on workflow efficiency and sample and data integrity can be gained by integrating the various analyzers to the liquid handler and scheduling overlapping campaigns to maximize system utilization.

AUTOMATED CELL LINE DEVELOPMENT

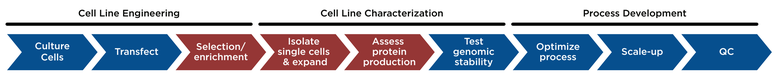

Figure 1. Cell Line Development Workflow. The cell line development process begins with transfecting cells (e.g. CHO) with a construct expressing your protein or antibody of interest, followed by removal of untransfected cells through growth in selective media and/or enriching for high expressing cells through cell sorting or colony picking. Here we focus on automating the enrichment of expressing cells and the generation and characterization of single cell-derived populations for maximizing protein production.

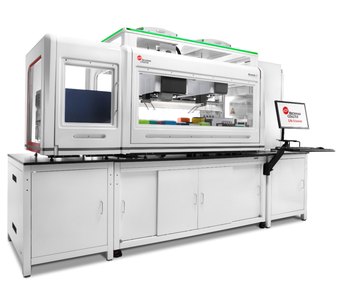

Figure 2. Biomek i7 Workstation. All cell manipulations and sample preparations were performed on a Biomek i7 Workstation with Span-8 pipettors and a 1200µL capacity 96-channel head. A HEPA-filtered enclosure and sterile filtered tips were utilized to maintain cell sterility. The Biomek was integrated with a Cytomat 2C incubator (right), a microplate centrifuge (not shown), and a Vi-CELL XR Cell Viability analyzer (not shown). Cell imaging and protein quantification were performed offline.

ENRICHMENT OF EXPRESSING CELLS

Figure 3. Semi-Solid Media Automation. To enrich the population of cells secreting IgG prior to clonal expansion, CHO cells were counted on an integrated Vi-CELL XR and 1,800 cells were dispensed to 22mL semi-solid CloneMedia CHO Growth A medium containing a 1:100 dilution of CloneDetect reagent (Molecular Devices). Using fine pipetting control, cells were slowly resuspended in the media and 3mL/well (250 cells) were dispensed to 6-well plates without introducing bubbles (A). Plates were incubated for 12 days to form colonies, then analyzed and picked using the ClonePix 2 (Molecular Devices, B).

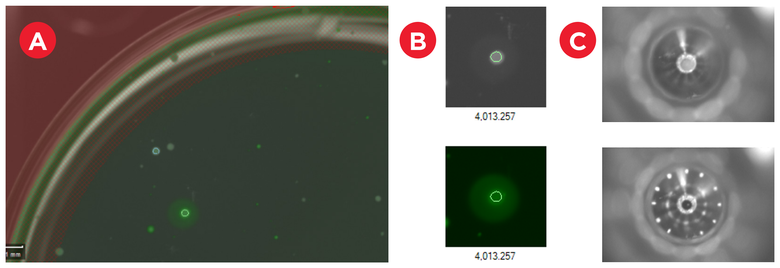

Figure 4. Picking IgG-Secreting Colonies. A) Images of the 6-well plates were taken in brightfield and FITC channels with the ClonePix 2. B) Colonies that were secreting IgG showed a halo of fluorescence as IgG molecules aggregated near the colony within the semi-solid media and were stained by the CloneDetect reagent. C) Based on characteristics such as colony size, shape, and external FITC intensity, a subset of colonies were picked into 96-well plates containing 200µL of XP CHO Growth A medium for further culture, analysis, and monoclonal colony formation.

ISOLATE SINGLE CELLS & EXPAND

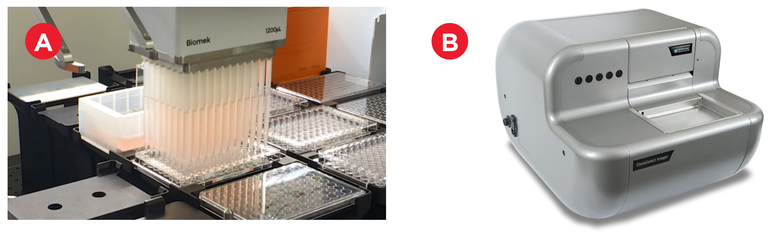

Figure 5. Automated Limiting Dilution. To create single-cell colonies, suspension CHO cells were mixed and dispensed to an integrated Vi-CELL XR Cell Viability Analyzer for counting. 100,000 cells were then stained with 0.25µM calcein AM at 37°C for 30 min. Following an initial 25-fold dilution, ~467 cells were added to 140mL XP CHO Growth A medium (Molecular Devices) such that there was one cell per 300µL on average. Robust mixing and rapid plate filling were achieved through the ability to aspirate and dispense 96mL at one time with the 1200µL-capacity multichannel head. Plates were spun in the integrated microplate centrifuge at 200×g for 4 min to ensure all cells were at the plate bottom prior to imaging on the CloneSelect Imager (B).

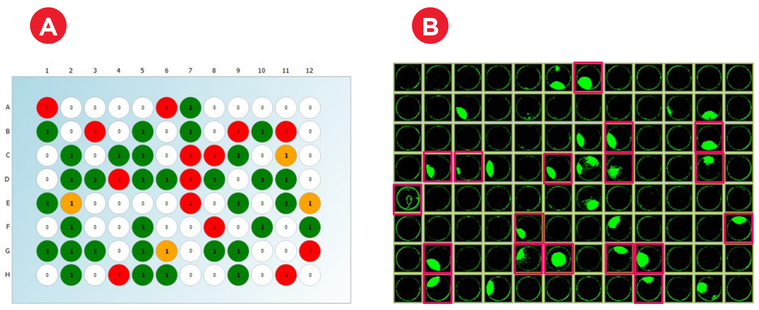

Figure 6. Monoclonality and Colony Growth. A) Immediately after plating, wells were imaged in brightfield and fluorescent channels to count calcein-stained cells. As expected with a limiting dilution approach, an average of 33% of wells had a single cell across six experiments (representative plate shown). B) Cell confluence was assessed at multiple time points for three weeks. Cell-conditioned media was able to achieve growth in roughly 50% of the monoclonal wells (pink highlights), resulting in ~16% of the plated wells being eligible for IgG production assessment.

ASSESS PROTEIN PRODUCTION

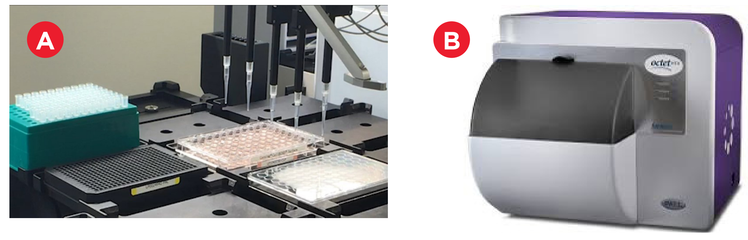

Figure 7. Hit Picking for IgG Analysis. Media from the ~16% of wells with colony growth from a single cell were sampled using the Span-8 pipettors and transferred to a 384-well tilted-bottom plate for analysis on the Octet HTX (Pall ForteBio, B). Protein A Dip and Read Biosensors were used to bind IgG for quantification.

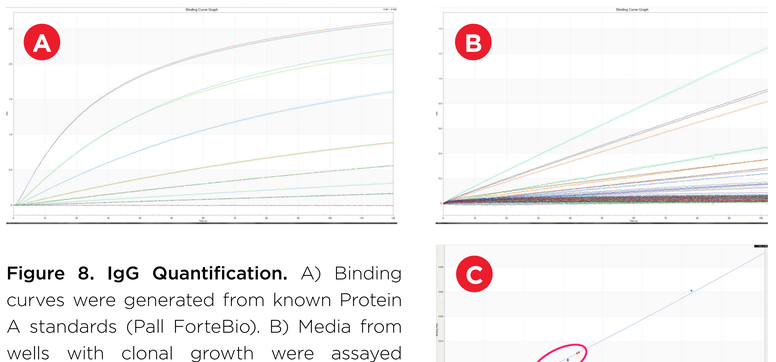

Figure 8. IgG Quantification. A) Binding curves were generated from known Protein A standards (Pall ForteBio). B) Media from wells with clonal growth were assayed for IgG binding to Protein A biosensors. C) IgG concentration was determined for the clonal wells using the standard curve. Cells in high-expression wells (circled) were resuspended and transferred to larger-area 24-well plates for further expansion and analysis (e.g. genomic stability).

INTEGRATED WORKFLOW

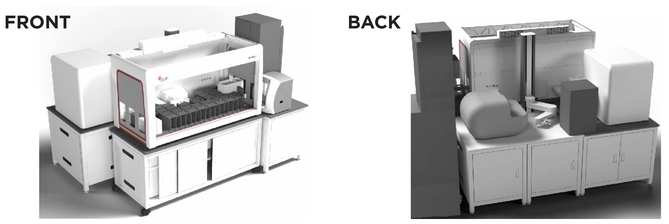

Figure 9. Illustrative Integrated System. The devices described here can be integrated into a single workstation that reduces human intervention and thereby saves time and lowers the opportunity for errors. In addition, data from the various analyzers can be directly utilized to drive downstream steps such as hit picking, without the need for manual data transfers or manipulations. By integrating the devices, both sample and data integrity are improved throughout the lengthy workflow. Finally, software tools that track the labware locations and device capacities can maximize the utilization of the system by enabling the overlap of campaigns as plate throughput allows.

CONCLUSIONS

- Automation can overcome many cell line development challenges including:
  - Sample sterility
  - Consistent pipetting of semi-solid media
  - High-throughput limiting dilution
  - Hit picking for IgG analysis
- Integrating devices can enable:
  - Cell counting for in-process calculations of transfer volumes
  - Centrifugation of cells for immediate monoclonality determination
  - Sample and data integrity across multiple analyzers over multiple weeks

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