Continuous Improvement of Suspension Cell Lines for AAV Manufacturing

Michael Mcilhatton, John Ketz, Brittany Hurley, Esheksha Gundre, Samir Acharya

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AAV CURATOR® PLATFORM

Introduction

HEK293 cells are extensively used for production of viral gene therapy vectors in both adherent and suspension platforms. Suspension cell culture has emerged as a critical tool in biomanufacturing due to its scalability and better control and monitoring of growth parameters throughout production. One of the challenges during derivation of suspension cell lines is the propensity of cells to aggregate or form clusters specially at higher cell densities. This issue has the potential to exacerbate as one transitions to larger bioreactors when using high cell densities (e.g., 500L, 2000L) and necessitates continuous improvement of established cell lines. While suitable culture conditions, growth parameters and anti- clumping agents can be used to circumvent these issues during development of optimized conditions using small bioreactors, the robustness of scale up remains at risk. We have addressed this by developing a proprietary method of selection for deriving cell lines with minimized aggregation potential even at high cell densities.

Methods

Cells were grown in different media and continuously selected for a low aggregation phenotype, as determined by number of cell clusters, over >30 passages. Following this, the selection process was stopped and cells grown for >20 more passages. Cells were monitored for growth, cluster formation and viability. They were evaluated for AAV production by transient triple transfection in baffled shake flasks. One principal cell line was designated and used to manufacture AAV at scale in a STR50 bioreactor.

Observations & Conclusions

- Media type can be used to effect desirable phenotypic changes in cell line characteristics.
- Derived lines varied in their growth characteristics, but viable cell density, viability and reduced cell clumping were all improved.
- Observed changes in phenotypes persisted after the selection regimen was terminated, suggesting that these changes were heritable and genotypically stable.
- The new cell lines offer a robust scale up opportunity with larger bioreactors.

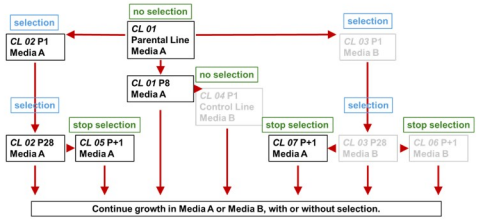

Figure 1. Outline of selection process and growth strategy for cell line generation.
CL 01, an Andelyn HEK293 line was used as the parental source to derive a series of cell lines that demonstrate reduced clumping phenotypes during cell growth, especially at higher cell densities.

Cell Line	Selection	Media
CL 01 (parental)	No	A
CL 02	Yes	A
CL 03	Yes	B
CL 04 (control)	No	B
CL 05	Stop	A
CL 06	Stop	B
CL 07	Stop	Shift B to A

Table 1: Summary of cell line selection strategy.

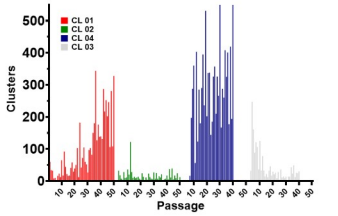

Figure 2. The selection process generated several cell lines with stable low-clump phenotypes that persist over many generations.

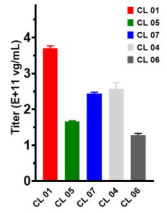

Figure 3. The selection regimen modifies AAV production in CL 01–derived cell lines.

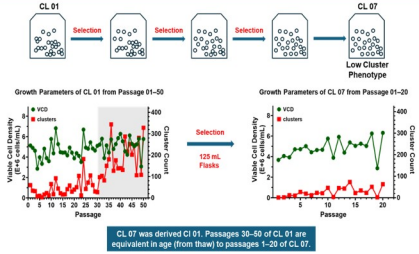

Figure 4. Continuous selection in combination with media choice results in cell lines with stable low-clump phenotypes.

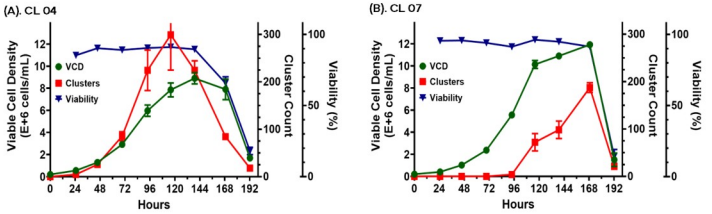

Figure 5. Growth Curves Reveal Phenotypic Differences Between Cell Lines.
CL 07 grows to a higher cell density than CL 04, 11.9 E+6 cells/mL versus 8.9 E+6 cells/mL, a 33% increase, demonstrates less clumping at higher cell densities, and maintains higher viability over a longer period than CL 04.

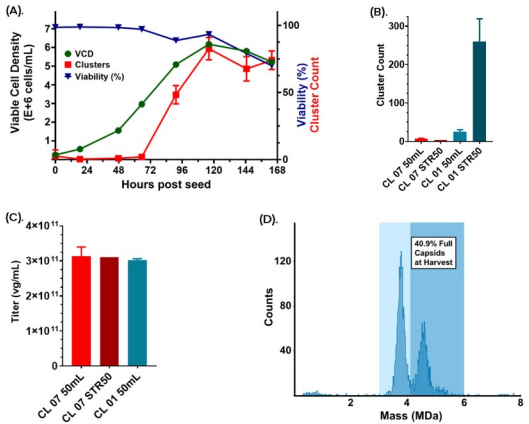

Figure 6. At scale production in a STR50 bioreactor with CL 07.
(A). Growth parameters of CL 07 over the course of the 50 L run. (B). CL 07 displays minimal cell clumping at the time of transfection. (C). AAV production with CL 07 scales from a 50 mL shake flask to the 50 L bioreactor. (D). AAV manufactured with CL 07 is ~41% full (by Samux) at harvest.

For inquires, please contact: andelynbusiness@andelynbiosciences.com

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