Derivation of a clonal HEK293 suspension cell line for high yield AAV production

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P301

Background

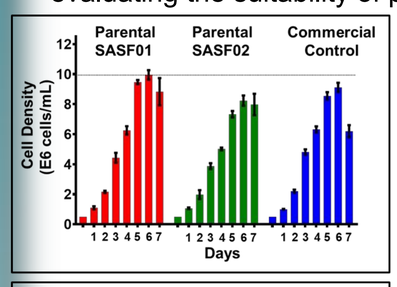
Andelyn Biosciences has developed a high yielding, robust suspension platform for AAV manufacturing which demonstrates scalability from 1–500L. A design-of-experiments approach was implemented to formulate a model for optimized AAV production and purification **(1)**.

Additional support for this platform has been provided by the development of an all-column chromatography process for downstream purification of AAV **(2; Poster 280)** and a HPLC-based method for evaluating the empty/full ratios of AAV productions **(3; Poster 331)**. In addition, we have also derived a suspension-adapted HEK293 clonal cell line to complement our suspension platform for AAV manufacturing. The above improvements augment our established in-house capabilities and are being integrated with our suspension platform as a result of continual iterative improvements to the manufacturing process.

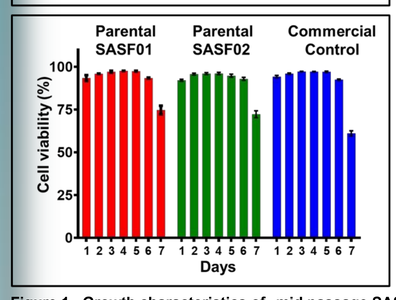
This presentation highlights some of the early development work on the clonal suspension cell line **(Figures 1–5)**. The Andelyn Biosciences adherent HEK293 line, a critical component of the in-house adherent AAV manufacturing platform, was used as the starting material to derive a series of clonal suspension-adapted HEK293 cell lines. The rationale was to simplify associated licensing requirements for the resulting clonal suspension cell line(s) and increase available client options.

Adaptation

- The adherent HEK293 cell line was adapted to growth in a series of decreasing serum concentrations.
- Cell lines were switched to growth in serum-free media, concurrent with growth under suspension conditions.
- At this point, they were designated as suspension-adapted, serum-free **(SASF)** cell lines.
- Growth, viability, and lack of clumping, were key criteria in evaluating the suitability of parental HEK293 cell lines.



Parental 01; P27; PDT ~24 hr
Parental 02; P26; PDT ~25 hr
Control; P26; PDT ~22 hr



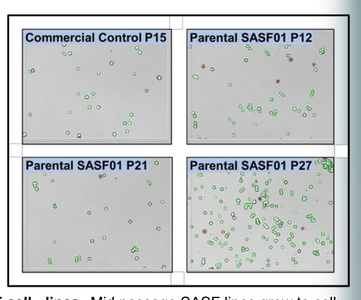


Figure 1. Growth characteristics of mid passage SASF cell lines. Mid passage SASF lines grow to cell densities above 10.0 E+6 cells/mL. Cell densities and viabilities are comparable or better than the commercial control cell line used by the Process Development group **(Top Left, Bottom Left Panels)**. SASF cell lines exhibit minimal clumping when grown in suspension. **(Bottom Right Panel)**. (**PDT** = Population Doubling Time)

Abstract

Cells are critical components of biotherapeutic productions and implementation of clonal cell lines is highly recommended in manufacturing processes. Cell line clonality mitigates the genetic heterogeneity inherent to most biological cell lines. Andelyn Biosciences utilizes a robust suspension platform for AAV production using a clonal HEK293 suspension cell line developed from an adherent HEK293 parental line. We subjected an existing adherent line of HEK293 to a selection series with decreasing serum concentrations before switching it to growth in serum-free media. These processes resulted in a family of suspension-adapted, serum-free HEK293 cell lines that were the parental lines for subsequent isolation of single-cell clones. Clonal selection was performed with the Solentim Verified In-Situ Plate Seeding system which delivers Day 0 assurance of clonality. Clonal cell lines were initially assessed for AAV production in 125mL shake flasks. Promising candidates were further assessed with the Ambr® 250 modular and then subjected to 2L yield assessments. The final candidate was selected from a panel of six comparable clones. It was scaled to a 50L production with AAV Serotype A using an Allegro STR50 bioreactor and purified to final fill. At all stages of production, AAV yields exceeded those produced from the commercially available control cell line. We will discuss further optimization of this newly developed cell line, including viral titers of AAV serotypes with our production platform.

Clonal Derivation

- SASF01 **(non-clonal)** was selected as the parental cell line for the derivation of clonal SASF HEK293 lines.
- Series of clonal cell lines were seeded in 96 well plates using Verified *in situ* Plate Seeding (VIPS).

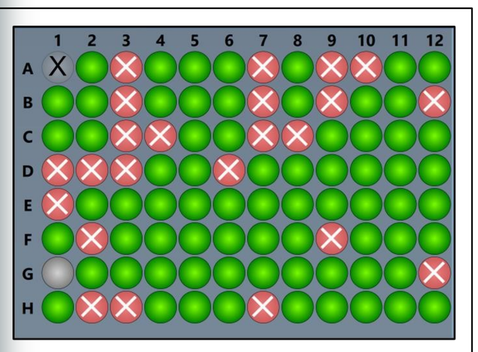


Figure 2. Top Panel: Verified *in situ* Plate Seeding generates single cell clones. The plate map represents single cell seeding events as they occurred in real time. A series of plates were seeded with cells from the parental HEK293 cell line SASF01.

Green circles: single cell seeding event, **CLONAL**; **red circle+X**: two or more cells seeded in a single well, **non-clonal**

Well **A1** is used as a dump well in the seeding process.

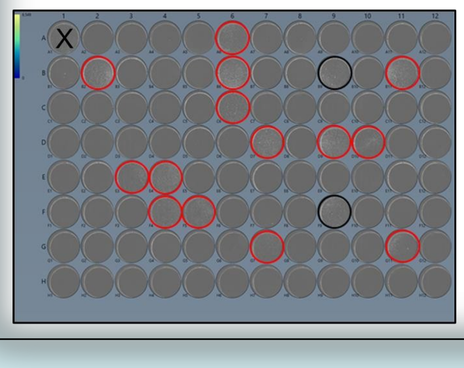


Figure 2. Bottom Panel: Clonal outgrowths arising from Verified *in situ* Plate Seeding events. Cell Metric was used to image plates at different days post-seeding. Some single cell clones have survived **(red circles)** and have given rise to clonal populations. This state is described as outgrowth and comprised ~14% of the total cells seeded. These clones were selected for expansion. Wells which have been seeded with two or more cells may also grow **(black circles)** but the resulting cell lines will not be clonal. Non-clonal lines were discarded.

Screening Clones

- Outgrowing clonal cell lines were expanded to 125mL shake flasks for small scale assessment of AAV yields.
- Clonal lines that were comparable, or better, than the in-house control HEK293 cell line for AAV production, were selected for further development.

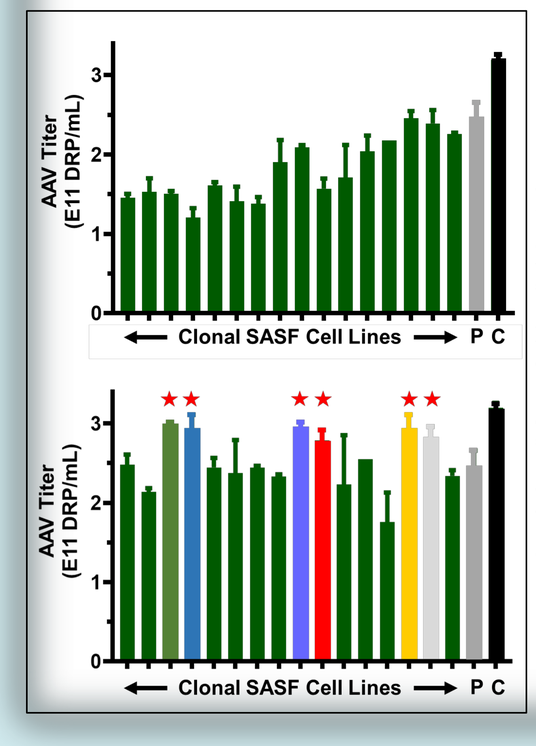


Figure 3. Small scale production of AAV in SASF clonal cell lines. 32 different cell lines were seeded in 125mL non-baffled shake flasks. Cells were transfected by a transient triple plasmid transfection method, standard in the Andelyn Biosystems PD group. FectoVIR-AAV was used as the transfection reagent. AAV titers at harvest (DRP/mL) were determined for all samples by qPCR. Data was plotted using GraphPad Prism. The mean and standard deviation are shown for each sample.

Red stars signify clonal cell lines chosen for yield evaluations at the Ambr®250 and 2L scales.

P=Parental SASF01 cell line;
C=Control cell line (benchmarking)

AMBR

- Six candidate cell lines were selected for further evaluation with the Ambr®250 mini-bioreactor.
- The Ambr®250 was used to model several growth parameters, such as rpm and DO, prior to scaling to 50L.
- All cell lines produce higher yields of AAV in baffled versus non-baffled Ambr®250 vessels.

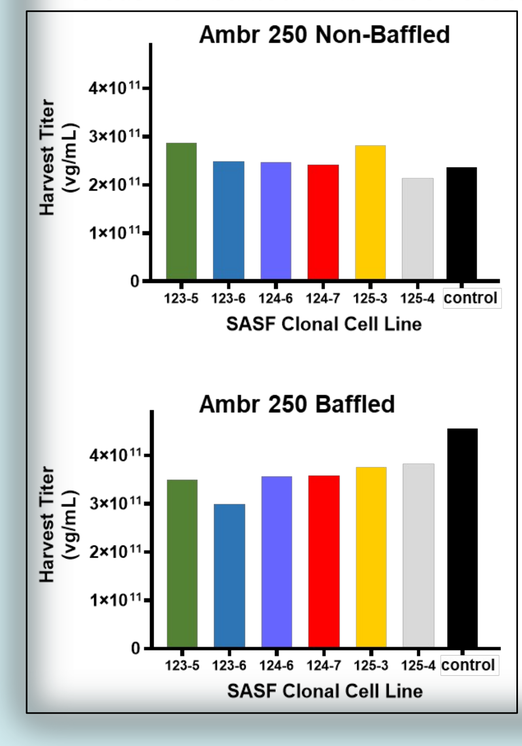


Figure 4. Top Panel: AAV harvest titers from candidate clonal cell lines grown in Ambr®250 non-baffled vessels.

Titers were estimated by ddPCR.

Control: cell line for benchmarking

The top two producing clones were SASF123-5 and SASF125-3.

Figure 4: Bottom Panel: Harvest titers of AAV from candidate clonal cell lines grown in Ambr®250 baffled vessels

Titers were estimated by ddPCR.

The top two producing clones were SASF125-4 and SASF125-3.

Yield Assessment

- In parallel, the six candidate cell lines were assessed for AAV production at the 2L shake flask scale.
- The titers of harvested supernatants ranged between **1.0 E+11 DRP/mL – 2.3 E+11 DRP/mL**. Again, these are comparable or better than the control cell line.
- Recoveries from harvest to final were in the range of **41% – 53%,** notably higher than the control line 20% – 30%.

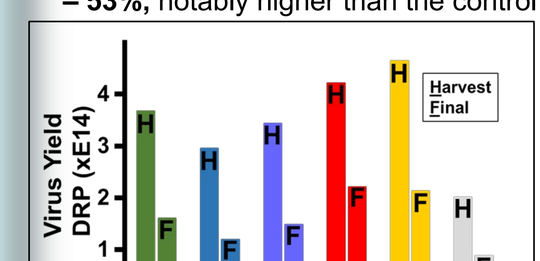


Figure 5. Top Panel: Harvest and final yields of 2L productions for candidate clonal cell lines. AAV titers were determined by qPCR on various plates. Total yields are presented.

The top two producing clones were SASF125-3 and SASF124-7.

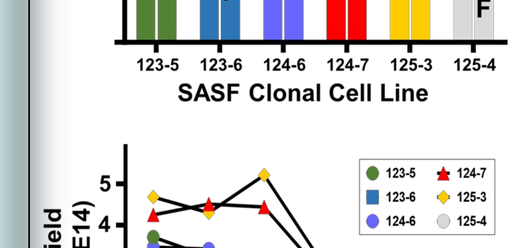


Figure 5. Bottom Panel: AAV yields from harvest through final steps for 2L productions. Titers were determined by qPCR on various plates – all steps from one preparation were analyzed on the same plate; different cell lines were analyzed on different plates.

STR50 Bioreactor

- HEK293 **SASF125-3** was selected as the final candidate for Andelyn Bioscience's clonal suspension cell line.
- Production was scaled up to a Pall Allegro STR50 bioreactor. AAV serotype A was purified to the TFF process stage. **(Table 1)**. FectoVIR-AAV was used for the transfection reagent.
- The AAV titer at harvest was **4.02 E+11 vg/mL**.
- Average AAV titers at harvest at the same scale with the PD control cell line are ~1.15 E+11 vg/mL (n=4). Final recoveries range from ~28% – 35%.
- **Caveats:** The data set for the 50L runs with AAV serotype A are small. There are some differences in process parameters between runs.

Table 1: STR50 AAV Process Yields

Sample/Stage	Yield (vg)
Harvest (1)	1.958 E+16
Harvest (2)	1.763 E+16
Clarification	1.619 E+16
TFF concentrate	1.527 E+16
TFF permeate	< LOQ

Observations

- A suspension-adapted, serum-free HEK293 cell line was derived from Andelyn Bioscience's adherent HEK293 line.
- The cell line is **clonal**. The VIPS platform provides Day 0 assurance of clonality; it is **21 CFR Part 11** compliant.
- All data presented in this poster is based on production parameters optimized for the PD control HEK293 cell line.
- AAV production parameters are being further optimized for SASF125-3. Upstream conditions are currently being optimized, downstream will follow.
- Available process data for small scale indicates over a 2-fold increase in AAV production, relative to our current standard. This is, of course, serotype dependent.
- The cell line is available now at research grade.
- The GMP MCB has been made and tested.

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References

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(2). A robust downstream strategy for high quality AAV using an all-column purification method. Poster 280. Byron Carper, Hannah Roodhouse, John Ketz, Matthew Janson & Samir Acharya. ESGCT 29th Congress, Edinburgh, Scotland, Oct. 2022.

(3). Novel HPLC applications for rapid in-process characterization during AAV platform development. Poster 331. Matthew Janson, Byron Carper, Hannah Roodhouse, John Ketz & Samir Acharya. ESGCT 29th Congress, Edinburgh, Scotland, Oct. 2022.