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Simplifying Progress

See the wood for the trees: A holistic route to an optimized AAV production process

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Introduction

The promise to provide long lasting therapeutic effects, especially for monogenetic diseases, has resulted in over 2000 viral-based therapeutics projects in the pre-clinical and clinical pipelines. The vast majority of viral vectors is presented by adeno associated virus (AAV) expressed by HEK293 as preferred host. Viral vector manufacturers are facing unique challenges to reduce time-to-market and cost of goods sold (COGS), as viral expression processes are still highly variable. Optimization must consider diverse factors like cell line, serotype, plasmids, cell culture media, transfection conditions and the cultivation process as well as complex analytics to characterize the final product. In this work, we present a "Design Space" approach, addressing these key process variables. Our holistic approach facilitates, accelerates, and increases the chance of selecting the best media and processes for AAV production.

AAV production process and its bottlenecks

AAV production processes consist of many steps. A pre-selected media panel, transfection reagents and platforms for purification and analytics facilitate and accelerate the screening phase to identify optimal procedures. Transfection protocols are optimized using design of experiments (DoE). Enhancer and feeds can be added to increase titer. In addition, instruments and software are key to generate and analyze data from multi parameter screenings and to characterize cell lines.

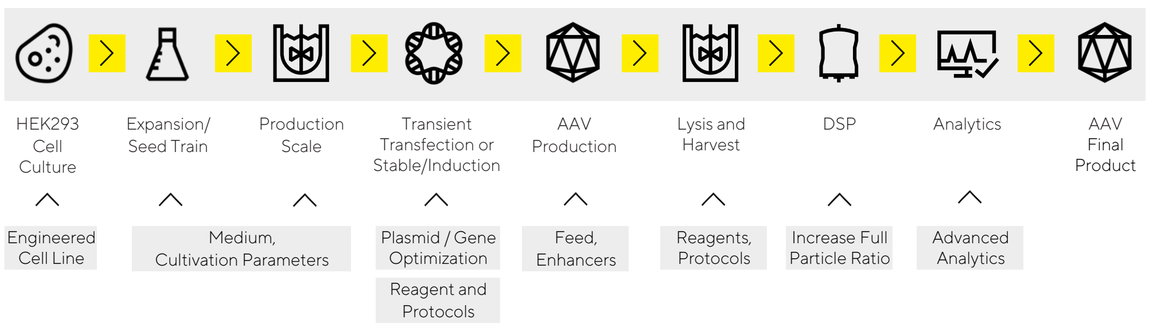
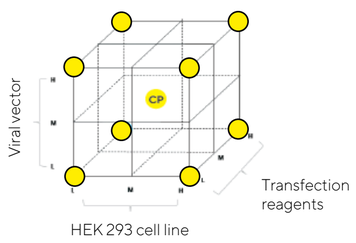


Figure 1: AAV production process steps. A design space approach is used to screen, optimize and characterize parameters in each step to increase AAV titer and quality.

Design space: Cell lines, vectors and transfection reagents

A design space of cell lines, respective media, viral vectors and transfection reagents allow identification of optimal process parameters for AAV production. The combination of DoE, high-throughput automation, and a panel of cells, media, vectors and reagents increase the efficiency and reduce the cost and time required for AAV production optimization. In addition, the systematic identification and controlling of critical process parameters is key for the implementation of a quality by design (QbD) approach.



1. Identification of optimal media for cell cultivation

The optimal medium is highly cell line and clone dependent. Therefore, a panel of media must be screened to identify a medium providing fast suspension growth rate, high cell density and viability.

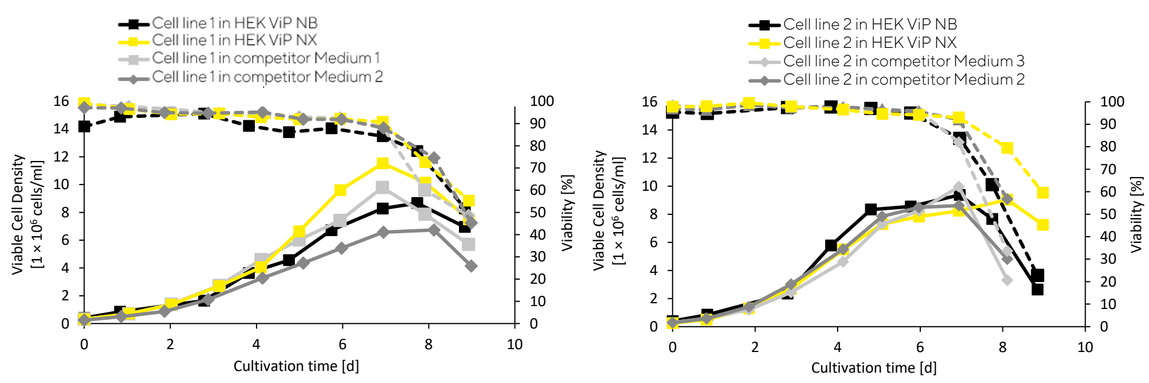
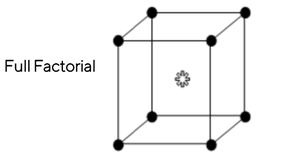


Figure 2: Batch cultivation of two HEK293 cell lines in different media. Cells were inoculated at 3 × 10^5 cells/ml and cultivated for 9 days.

2. Optimize cultivation process

Transfection and production induce stress in cells, making it crucial to cultivate them under optimal conditions. Cultivation in controlled systems is preferred as it reduces the risk of varying results between screening phase and up-scaled production. The Ambr®15 system enables down-scaling of experiments in a controlled environment, resulting in optimized cultivation processes with minimal effort using DoE and MODDE®.

Parameter	Range
Stirring speed	400 – 800 rpm
pH	6.9 – 7.3
DO	30 – 70 %
Responses	VCC, viability



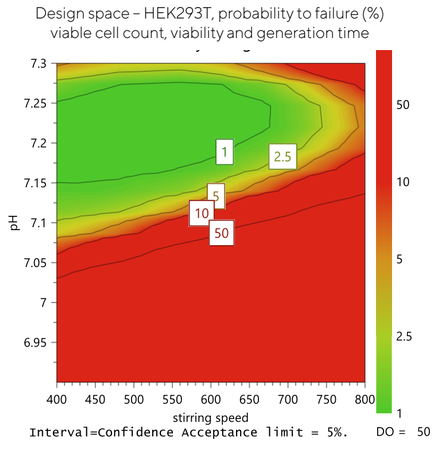


Figure 3: Optimization of three cultivation parameters in an Ambr®15 choosing a 2-level full factorial design with center points. The figure depicts the chosen parameter and levels (left), a schematic diagram of a full factorial design with center point (middle) and the resulting probability to failure depending on pH and stirring speed at a DO of 50 %. No significant effect of DO was measured. HEK293T cells were cultivated for four days.

3. Optimizing production: A comprehensive approach

The choice of cell lines, media, and transfection reagents significantly impacts AAV titer. Therefore, a panel of these parameters should be tested for different serotypes, as it is challenging to predict in advance which combination provides the most efficient production process. The example illustrates that the optimal process resulting in high AAV titer varies for different serotypes. Once the set up with the highest productivity is identified, transfection process can be further optimized by DoE and powerful software, e.g. to identify optimal plasmid : reagent ratio and ideal cell density.

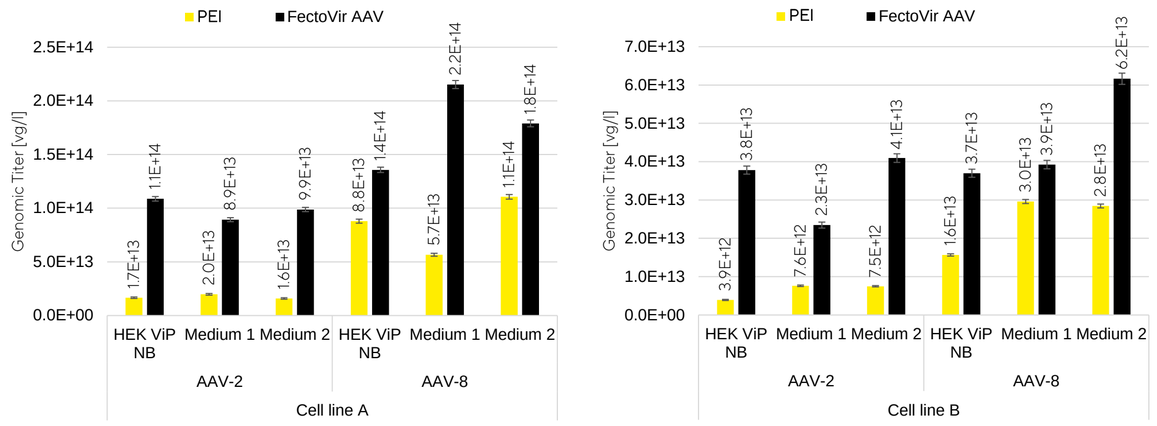


Figure 4: Production of AAV-2 and AAV-8 in two different cell lines transfected either with PEI or FectoViR®-AAV. Cells were cultivated in shake flasks in commercially available HEK ViP NB (Sartorius Xell) or two media formulations under development (Medium 1 or Medium 2; SartoriusXell). One day before transfection, cells were seeded at 1.9 × 10^6 cells/ml. On the day of transfection, 30 % fresh medium (v/v) was added to the cultures and 3 × 10^5 cells/ml were transfected with 3 µg/ml DNA (2 plasmid system, Plasmid Factory, Bielefeld) and 8.1 µg/ml PEI or 3 µl/ml FectoViR®-AAV. Cells were lysed 72 hours post transfection and genomic titer was measured in crude cell lysates by ddPCR.

4. Enhancer and feeds increase AAV-titer

AAV-titer can be further increased by addition of enhancer substances and feeds. Sodium butyrate increased the relative titer 3.5-fold. In a controlled experiment using an Ambr®15 system, the addition of feed resulted in a higher titer. The effect of feed was strongly cell line dependent but increased the AAV titer in all three cell lines.

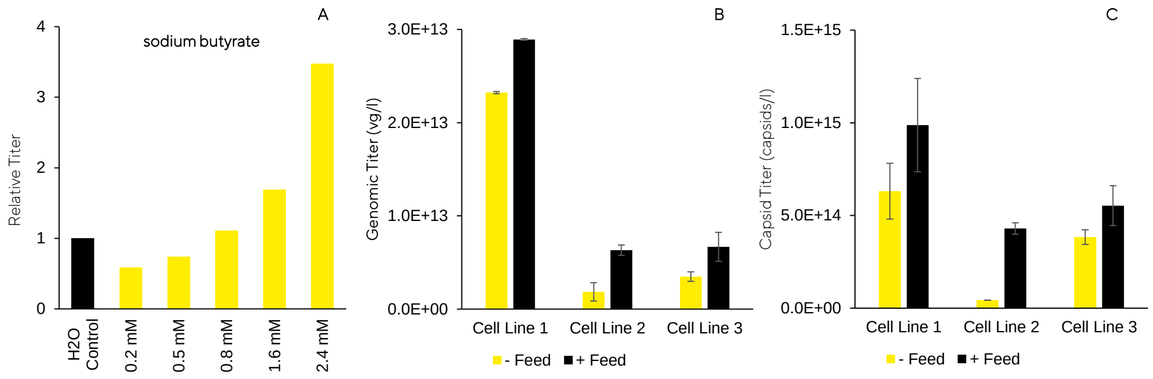
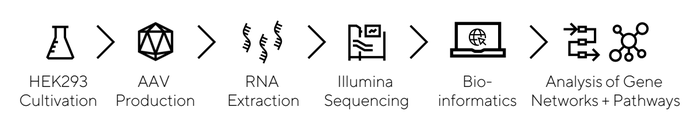


Figure 5: Screening of sodium butyrate concentrations to increase AAV-2 titer. HEK293 cells were transfected in 24-well plates with PEI and sodium butyrate was added 5 hours post transfection (A). Effect of 10 % HEK FS (Sartorius Xell) feed addition (v/v) 24 hours post transfection was evaluated in three different HEK293 cell lines cultivated in an Ambr®15 at pH 7.2, 630 rpm and 37°C. Cells were PEI-transfected as described in Figure 4. Resulting genomic and capsid titer were measured by ddPCR (B) and ELISA (C) after harvest and lysis 72 hours post transfection.

5. Learn for success: Characterization of cells by omics

New initiatives for knowledge generation are ongoing. RNA sequencing approaches comparing different cell lines and the effect of transfection and AAV production reveal new options for future development and improvement. The differences observed underline the demand for cell line-specific processes and media.



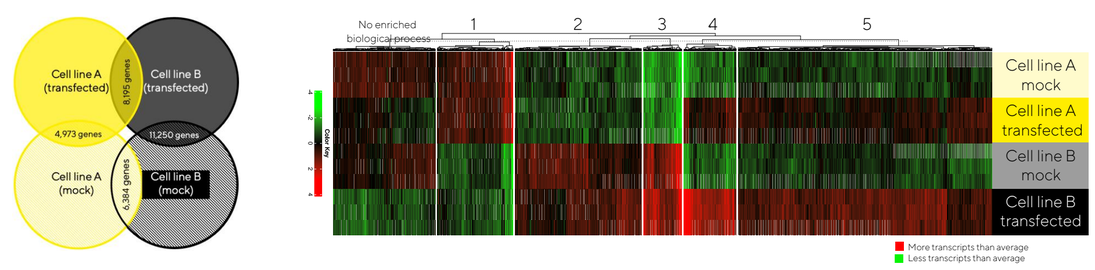


Figure 6: Transcript analysis of high and low producing HEK293 cell lines. Cell lines were PEI-transfected with AAV-2 coding plasmids (transfected) or with PEI only (mock) in shake flasks (left). Culture for RNA sequencing was sampled 18 hours post transfection. The analysis identified significant difference between the two mock transfected cell lines and the effect of transfection and AAV production (right).

Conclusion

- AAV production is impacted by a multitude of factors, including serotype, vector, cell line, medium, transfection, and cultivation.
- Optimizing these factors can be challenging, resulting in high costs and long timelines.
- Special consideration should be given to media and cell line selection early on.
- High-throughput screening and DOE methodology is well suited to improve complex steps, such as transfection.
- Automated systems like Ambr®15 allow optimization in a controlled environment simplifying up-scaling.
- Addition of suitable enhancers and feeds increase AAV titer significantly.
- Outsourcing the process or parts of it accelerates development timelines.
- A holistic design space approach increases the speed of development by reducing the total effort and leads to an efficient AAV production platform.