Continuously improving Quality and Efficiency of AAV Manufacturing

Dr. Daniel Last, Luba Kisselova, Maike Werdin, Jule Greschok, Niklas Buck, Mathias Kahl
Process Development Department, IDT Biologika, Magdeburg, Germany

Introduction

The field of AAV-based gene therapies is rapidly evolving. This applies to aspects such as manufacturing, analytics and regulations which are core competencies of IDT Biologika, a full-service CDMO specialized in virus-based therapies and vaccines.

In the following, we present insights into our cross-serotype, robust high-yield AAV manufacturing platform. The platform was established with the objective of providing short timelines and a cost-effective scalable process to produce clinical trial and commercial material in up to 2000-liter bioreactors. Current and future areas of process and analytical development are outlined in this presentation.

USP Platform

Status

Key parameters and materials of the AAV upstream process were optimized in order to provide a high-titer, scalable, and robust USP platform. This includes, but is not limited to, selection of the cell line, the culture medium, a feeding strategy (Fig. 1) as well as fine-tuning of the transient transfection protocol.

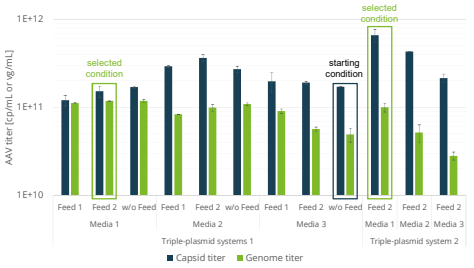

Fig. 1: Optimization of media and feeding strategy.

IDT Biologika's proprietary HEK293 cell line facilitates an economic production of AAV by showing better performance in terms of yield and full virus particles when compared to a widely-used commercial cell line (Fig. 2).

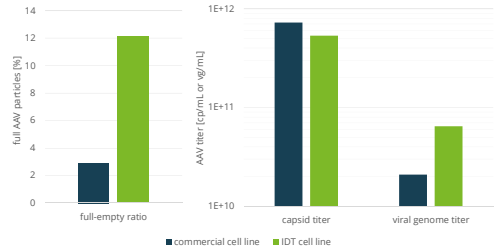

Fig. 2: Comparison of IDT Biologika's proprietary HEK293 cell line and a widely-used commercial cell line.

With all critical parameters identified, IDT Biologika's USP platform can be easily adapted to clients plasmids and other serotypes (Fig. 3).

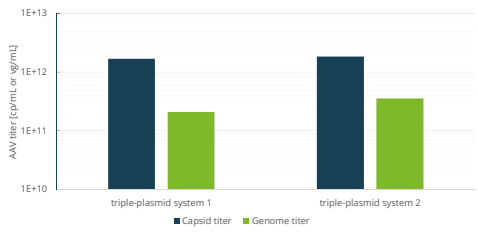

Fig. 3: Comparison of two triple-plasmid systems at 10-liter scale demonstrates the high yield and robustness of the USP platform.

Outlook

While triple-plasmid systems are still the industry standard, alternatives are emerging that hold promise of improved economies and/or mitigated risks. In this regard we evaluate optimization strategies such as viral sensitizers, which downregulate the host cell's antiviral defense.

DSP Platform

Status

Extensive in-house studies using multi-factorial decision matrix enabled us to establish a generic and robust DSP platform for AAV purification. The platform is well characterized and can be easily adjusted to purifiy different AAV capsid serotypes.

Key to success is the combination of an affinity and an ion exchange chromatography. The latter step mediates the removal of empty particles (Fig. 4), which are therapeutically non-functional but might induce adverse side effects.

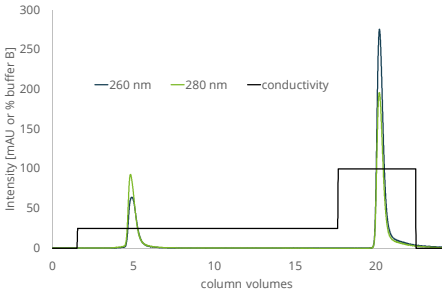

Fig. 4: Separation of full and empty AAV particles by IEX indicated by the switch in 280 nm / 260 nm UV absorption ratio.

The AAV platform reliably yields more than 5E12 vg/mL and about 80 % full particles in bulk drug substance. The overall recovery of viral genomes is about 30 % (Fig. 5). For highly engineered capsid variants that do not interact with the affinity chromatography ligand, alternative purification schemes are established.

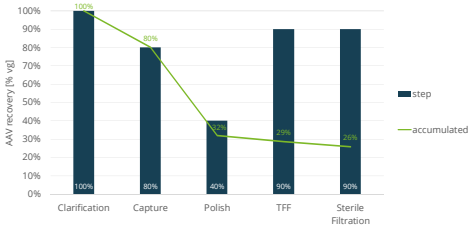

Fig. 5: Performance of the individual DSP steps and the combined overall recovery leading to highly pure and active AAV preparations.

Outlook

In order to provide advanced and economic DSP capabilities in the AAV field, we are constantly assessing alternative technologies and materials, including prototypes from various suppliers that are not yet available on the market.

Contact our Business Development Team

BD@idt-biologika.com

www.idt-biologika.com

Analytical Platform

Status

At IDT Biologika, AAV-specific analytical methods are well-established, including the viral genome titration, cross-serotype capsid titration, and the full-empty ratio determination (Fig. 6). In addition, our next-generation sequencing platform as well as our targeted mass spectrometry further enables in-depth characterization of the AAV particles. The potency assay, which is specific to the therapy's target, can be provided by the client or will be developed in-house.

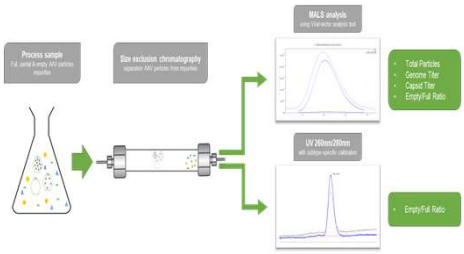

Fig. 6: Full-empty ratio determination of AAV particles by SEC-MALS.

Outlook

The expanding repertoire of analytical methods, such as charge detection mass spectrometry, enables profound identification and monitoring of well-known and novel AAV-related impurities and heterogeneities. Consequently, the impact of these molecular determinants, such as post-translational modifications on the safety and efficacy of AAV-based therapies is becoming increasingly evident. In the light of this evolving knowledge, IDT is continuously refine its entire AAV platform, to help paving the way for the next generation of AAV gene therapeutics.

Challenge our AAV Platform!

We are eager to produce your specific AAV construct! In this regard, we offer the following short-term and cost-effective feasibility studies:

- USP only: Please, provide us with your plasmids or alternative systems.
- DSP only: Please, provide us with four liters of your harvest solution.
- USP & DSP: Please, provide us with your plasmids or alternative systems.

Samples may be sent to you in order to facilitate the comparison with your internally gained data.