WHITE PAPER

Improving scalability and productivity of transient transfection AAV production

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VIRALGEN

All-In AAV™

Employing a transient transfection system using HEK293 cells to produce recombinant adeno-associated viral vectors (rAAVs) has a number of advantages, particularly for discovery, early development, and product optimization. Early speed and flexibility are the central drivers that lead most to employ transient transfection — iterating on a product upstream is made simpler with transient transfection than with many other methods, such as the use of helper viruses, and can serve to streamline candidate selection leading into preclinical studies.

As these programs transition from concept to clinic, the advantages of transient transfection become more in line with those of other platforms, and often, developers are faced with unique considerations around scaling these systems. Ultimately, demonstrating that a manufacturing process has the controls in place to ensure purity for these products is paramount. Many contract development and manufacturing organizations (CDMOs) have demonstrated process control in leveraging transient transfection for rAAV production; despite this, there are still significant avenues for optimization that companies can explore when making the leap to larger scales of production.

When it comes to suspension-based systems, operators can achieve a high level of accuracy, performing varying degrees of optimization as they develop individual constructs. By refining variables like starting DNA and reagents and optimizing them against scale-independent parameters such as cell density, media composition, or reaction kinetics, as well as with scale-dependent ones that affect hydrodynamics and mixing like rotational speed, working volume, and delivery rates, companies and their manufacturing partners can arrive at a process that has been linearly scaled with minimal losses in productivity.

Achieving linear scalability from the start with AAV

The variability of the transgenes and serotypes, both natural and modified, that are leveraged for rAAV applications, as well as the diversity of therapeutic indications these products target, can create hurdles for bulk downstream recovery. These losses can become especially evident at vial presentation with the concentration of the final drug product; for smaller indications where operators have less material to work with, employing creative pooling strategies that minimize the losses incurred by retains and analytics can be useful. But these types of strategies fall outside of process control and optimization. In the end, being able to maintain consistent productivity and product quality as a product scales is the most integral means of preserving a drug's efficacy.

This linear scalability is crucial to the future of rAAV-based drug products which, as they proliferate within the greater biopharmaceutical pipeline, experience increasing pressure to lower the costs passed on to the patient. This evaluation often starts in early development, with some organizations closely examining the manufacturability of candidates in terms of COGS and beginning to model a possible future for a drug's manufacturing within the larger healthcare economy. There tends to be a wide distribution of COGS across existing rAAV production platforms — the result of siloed, one-off development across the space. Bringing these costs in line uniformly through concerted standardization efforts will be an industrywide push in the coming years, with many CDMOs leading the charge as their expertise in rAAV deepens.

At Viralgen, this has manifested itself in a focus on a chemistry, manufacturing, and controls (CMC) strategy that can balance early speed with continuous refinement. By working in conjunction with clients to demonstrate a product's commercial potential as early as

possible in development, Viralgen has started to zero in on optimizing for COGS on top of process optimization, productivity and quality considerations. With an established platform that can meet the needs of most products in order to generate high-quality, clinical-grade material, Viralgen has worked to become more targeted with a product's critical quality attributes (CQAs) and target product profile (TPP), setting process goals with an eye toward cost savings and manufacturing efficiencies as well as safety and quality.

Leveraging analytics to characterize and standardize transient transfection

Performing analytics for transient transfection rAAV production has likewise seen a degree of normalization across the space: to measure productivity, most employ transgene-specific droplet digital PCR (ddPCR) or quantitative PCR (qPCR). Employing ddPCR early in development to measure product-specific titer in combination with size exclusion chromatography (SEC) or enzyme-linked immunosorbent assay (ELISA) — or in Viralgen's case, both — to perform these fundamental analytics is key to supporting a transfection control strategy, measuring transfection efficiency and affording operators a better understanding of the effects of rAAV-delivered genes. Viralgen has likewise correlated its SEC to a 260/280 ratio to extrapolate full and empty capsids for a robust response model in platform fit and early process optimization.

In the early days of rAAV development, time was often the most prioritized variable in evaluating transfection, with less emphasis placed on the physical attributes of transfection composition, the reaction itself, or mixing. This negatively impacted linearity, with product losses that compounded as a program scaled into larger bioreactors. To circumvent this, most developers and operators have increasingly emphasized mixing, focusing on characterization of mixing time and transfer time. Yet there are other, less common optimizations that can be pursued to further refine transfection — assays such as dynamic light scattering (DLS) can be used to correspond reaction time with complexation size. By analyzing the rate at which polyethyleneimine (PEI) condenses with DNA over time to form complexes, for example, organizations can define the threshold at which cell uptake efficiency is lost, tightly defining optimal productivity and driving deeper process characterization.

Establishing a right-sized analytical strategy for transient transfection of AAV means balancing approaches that have been demonstrated to work with more novel analytics aimed at driving deeper process understanding. Scale-down models are likewise key to establishing this process understanding — demonstrating consistency across scales and over time is both critical and challenging, and transfection remains a major source of the lack of process understanding and potential variability for rAAV processes. Even with the best controls for transfection, transfer time, mixing, complex formation, and other key considerations, most processes rely on the consistency of their instrumentation, their starting materials, and that their reaction is occurring the same way every time. As the industry continues to standardize this more and more at every scale of production, other related optimizations around cell lines and other process steps, such as having at-line analytics, are likely to result in compounding gains for these applications.

Balancing early advantages with long-term efficiency

An emphasis on deep characterization and better process understanding is ultimately the path forward for perfecting rAAV transient transfection. While transient transfection offers undeniable advantages in speed and flexibility for rAAV production during discovery and early development, scaling this system effectively requires a focus on process control and optimization. Linear scalability, minimizing product loss, and optimizing COGS are crucial, particularly as rAAV therapies proliferate and cost pressures mount. Industrywide standardization efforts, coupled with CDMO expertise in rAAV manufacturing, will be key to driving down costs and ensuring the long-term viability of this promising therapeutic platform. Viralgen exemplifies this approach, balancing early speed with continuous refinement, optimizing for COGS alongside process efficiencies, and employing a robust analytical strategy to ensure consistent, high-quality rAAV production for its clients.

About Viralgen

Viralgen was founded in 2017 as a joint venture between AskBio and Columbus Venture Partners (a Spanish venture capital firm). We are one of the world's leading manufacturers of cGMP-certified AAV. We use the Pro10TM cell based platform, a technology licensed by AskBio, which enables us to ensure greater scalability, performance and precision of AAV therapies. Located in Spain, in the Gipuzkoa Science and Technology Park, Viralgen is a CDMO (contract development and manufacturing organization) that produces AAV gene therapy treatments for pharmaceuticals and biotech companies with the aim of accelerating the delivery of new treatments that can improve patients' lives.

One of our technology's key benefits is the manufacturing of high-quality cGMP-certified rAAV in high yields thanks to our unique and robust manufacturing platform. Viralgen's clinical facilities have four GMP manufacturing suites, with 250L and 500L bioreactors. In 2020, Viralgen expanded within the Scientific Park with the construction of a new module that received the authorization from the Spanish Agency for Medicines and Medical Devices (AEMPS), as part of the EMA network, for the commercial manufacturing facility to support 500 – 2,000L AAV GMP manufacturing. This enables our clients to take their products to market after successfully passing the regulatory process.

In October 2020, Viralgen was acquired by the German multinational Bayer. Since then, we continue to work as an independent manufacturing unit. Our team has grown to more than 400 highly skilled employees, known for their scientific, manufacturing and managerial expertise.

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