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Novel Dual-Plasmid Transfection System Improves Titer And Product Quality In AAV Manufacturing

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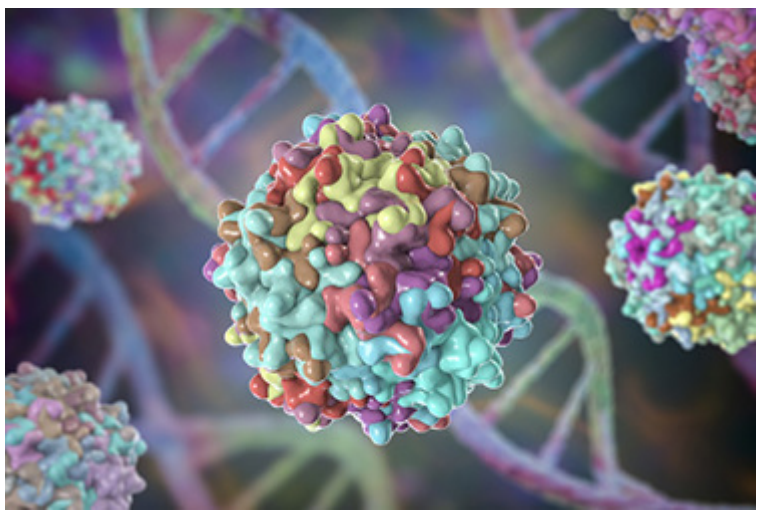

Adeno-associated virus (AAV) vectors are a widely-used, versatile, and appealing gene therapy delivery platform because of their high safety profile and ability to target many different cell types and transport healthy gene copies into a patient's cells. The recent clinical successes of AAV-mediated gene therapies[1] have ignited much interest in solutions for the scalable, good manufacturing practice (GMP) production of AAVs, and high demand for outsourced development and manufacturing capabilities. Yet, significant challenges remain, particularly around product quality, productivity, and consistency. Achieving high quality is incredibly important as it directly affects patients' health and safety. Productivity and consistency are also critical from a supplier and economics perspective.

Additionally, there's a well-recognized shortage of skilled workers in the manufacturing space. This is creating a massive technical challenge with inadequate access to expertise, especially as the US Food and Drug Administration (FDA) and other regulatory agencies are raising the bar for analytical expectations. On-time delivery can also be a struggle for some manufacturers when the process is complex, when there is a quality issue, or a supply chain bottleneck.

Oxford Biomedica (OXB) Solutions has developed a novel, proprietary dual-plasmid system as a means of addressing some of the issues with the traditional transient transfection process used to produce AAVs. This system has consistently demonstrated improved productivity and product quality across multiple serotypes, and it can be leveraged to obtain significant process gains in the manufacturing pathways of advanced therapies while maintaining the flexibility of transient transfection.

Challenging the Challenges: The Path to a Higher, Better-Quality Titer

Overcoming the challenges associated with AAV manufacturing can seem daunting, but it is possible with the right approach. The key to success is two-fold: 1) understanding all the technical needs early on and addressing them with innovative thinking, and 2) focusing on developing an optimal process from the start. This may sound easier said than done, but as the field is continuously evolving, manufacturers must also learn, adapt, and improve to be able to expertly support developers and provide a full breadth of capabilities spanning process development and GMP operations.

In search of a method to get better performance out of the transient transfection process typically used to manufacture AAVs, the team at OXB Solutions started with a simple question: Is it possible to improve upon the decades-old, two- and three-plasmid systems used to encode the necessary components for AAV vector production?

The standard three-plasmid method employs an AAV genome plasmid containing the gene of interest (GOI), a RepCap plasmid that provides the AAV replicase and capsid gene, and a plasmid with helper virus functions. Some groups have tried (and some still use) a dual plasmid system combining the RepCap and helper plasmids. This system, termed pDG, has resulted in comparable, but not higher, productivity to triple-plasmid transfection.

The novel dual-plasmid configuration developed by OXB Solutions was driven by the need to obtain more vector right out of the bioreactor. The team tested the three possible variations for a dual-plasmid system and ran a head-to-head comparison for vector genome (VG) productivity, capsid productivity, and percentage of calculated full vectors. From the results, one particular arrangement of sequences (GOI + RepCap and a separate helper plasmid) clearly stood out in every aspect (Figure 1).

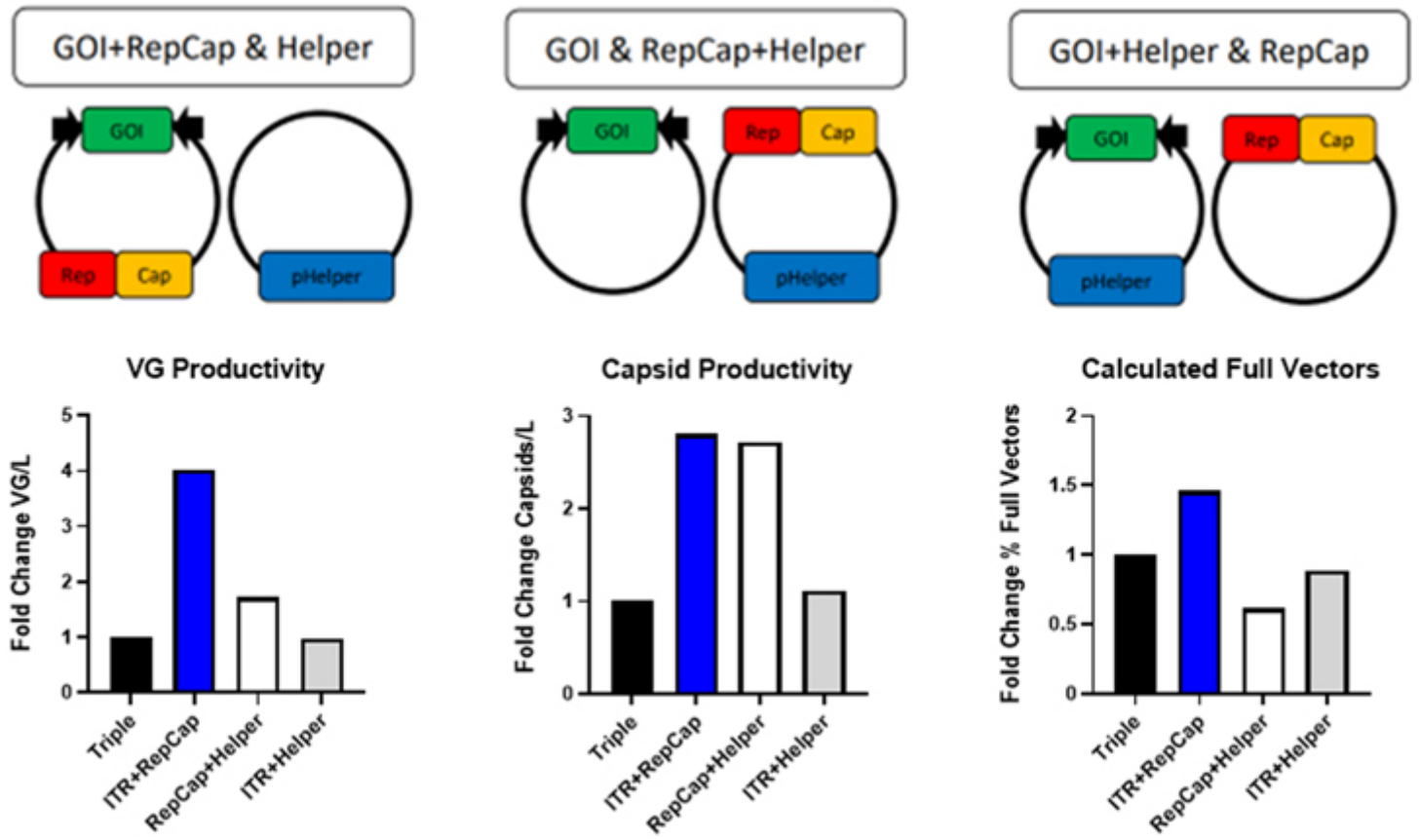

Figure 1: *Productivity assessment of three possible dual plasmid configurations (GOI+RepCap & Helper, GOI & RepCap+Helper, GOI+Helper & RepCap) and triple transfection. Crude lysate samples were quantified for VG productivity, capsid productivity, and the percentage of calculated full vectors.*

Repeated testing on seven distinct GOI constructs and seven different AAV capsid serotypes consistently and reproducibly demonstrated better performance, up to 3-fold in some cases (Figures 2 & 3). Importantly, this system yielded a higher percent of full packaged vectors (complete, intact) coming out of the bioreactor.

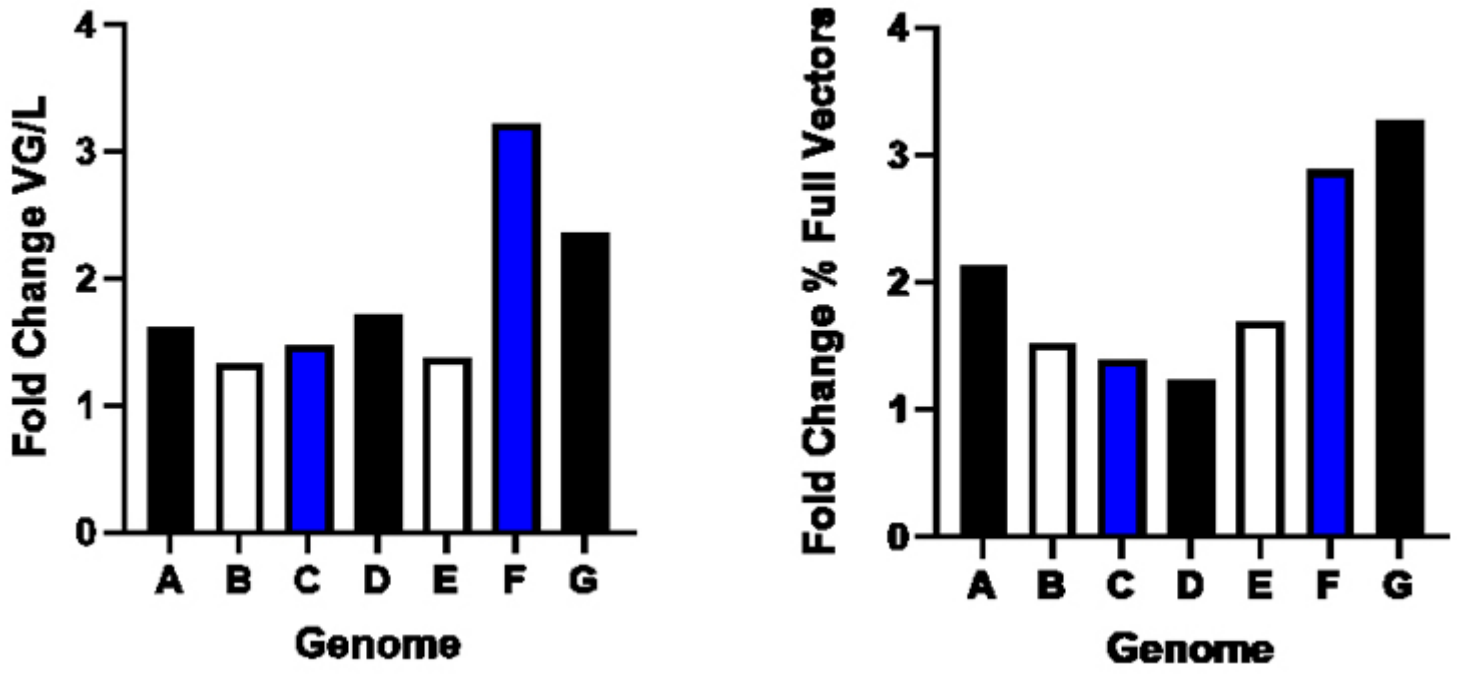

Figure 2. *Productivity assessment of seven AAV genomes comparing dual to triple transfection. Transfections were performed in 2 L bioreactors. All conditions were completed in duplicate.*

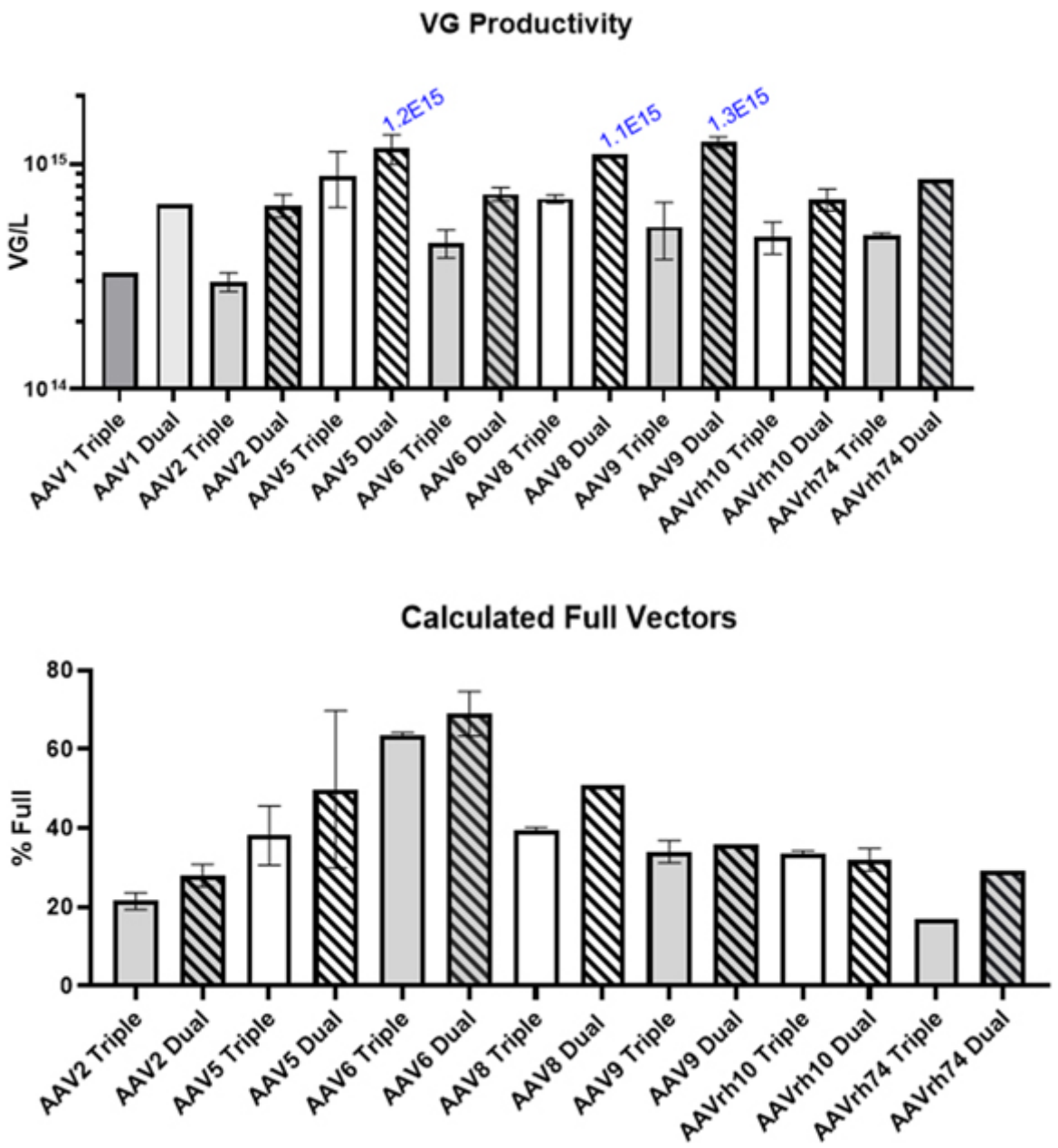

Figure 3. *Productivity assessment of seven AAV capsid serotypes comparing dual to triple transfection. Transfections were performed with dual or triple plasmids for each AAV capsid serotype in 2 L bioreactors. Crude lysate samples were quantified for VG production and calculated full vectors.*

Performance and Purity

This novel two-plasmid system has enabled researchers to achieve suspension bioreactor titers of 10^{15} vg/L, almost two orders of magnitude above the industry standard of 10^{13} -10^{14} vg/L. This is a crucial advance, especially to mass-produce drug product for non-rare indications and democratize therapeutic access in these large patient populations.

Obtaining high titers is key to high process performance, but equally important is the level of purity, which impacts product quality and ultimately patient safety. What manufacturers intend to produce are the fully intact vectors containing the GOI "cargo" – empty capsids are of no use; they are not the product. As such, the better the removal of empty/incomplete capsids, the higher the purity and product quality. Being able to consistently achieve high purity at scale is one of the biggest struggles in the AAV manufacturing business. Lot-to-lot variability is difficult to overcome without a highly efficient purification system.

OXB Solutions addresses purification with a two-column chromatography step. The resin is purchased off-the-shelf, but the operation of the columns is unique, patented, and incredibly adept at separating empty capsids from full ones. This is not a simple task as both empty and full capsids look the same and have similar weight. Being able to detect very subtle differences between the two and effectively purify the full capsids is a result of complex science and some ingenious creativity.

Scalability and Speed

When considering scale, the magic formula relies on two key words: capacity and capability. Pure capacity simply looks at meeting the demand for making enough vector. Capability, on the other hand, is all about the efficiency in achieving an optimal scale. This means having the ability to produce vector at the necessary capacity with optimal quality levels and a minimal number of batches. Capacity alone is akin to a one-winged bird. For large-scale GMP manufacturing to really take flight, capacity must go hand in hand with sufficient capability for late-phase products.

One of the greatest accomplishments of developing the novel dual-plasmid system is reaching this magic formula of scalability. Even at the 2000 L scale, it was possible to obtain high quality product with 90% fully intact vector. At the initial R&D stage, a small-scale shake flask (125 mL) was used for comparative analyses to tease out which configuration performed better. Then, the process was upgraded to a 2 L scale-down model qualified on the OXB Solutions manufacturing platform to ensure that results scaled linearly and representatively. From there, the process moved up to the 50 L scale typically used for toxicology work, and finally, the 500L or 2000 L scale for mass production. Compared to the triple-plasmid system, side by side, the new system exhibited the same improved performance and the same increase in quality at 2000 liters (Figure 4). In terms of how much vector can be made from a single 2000 L batch, it's in the quintillion (10^{18}) range – an enormous step up for scalability.

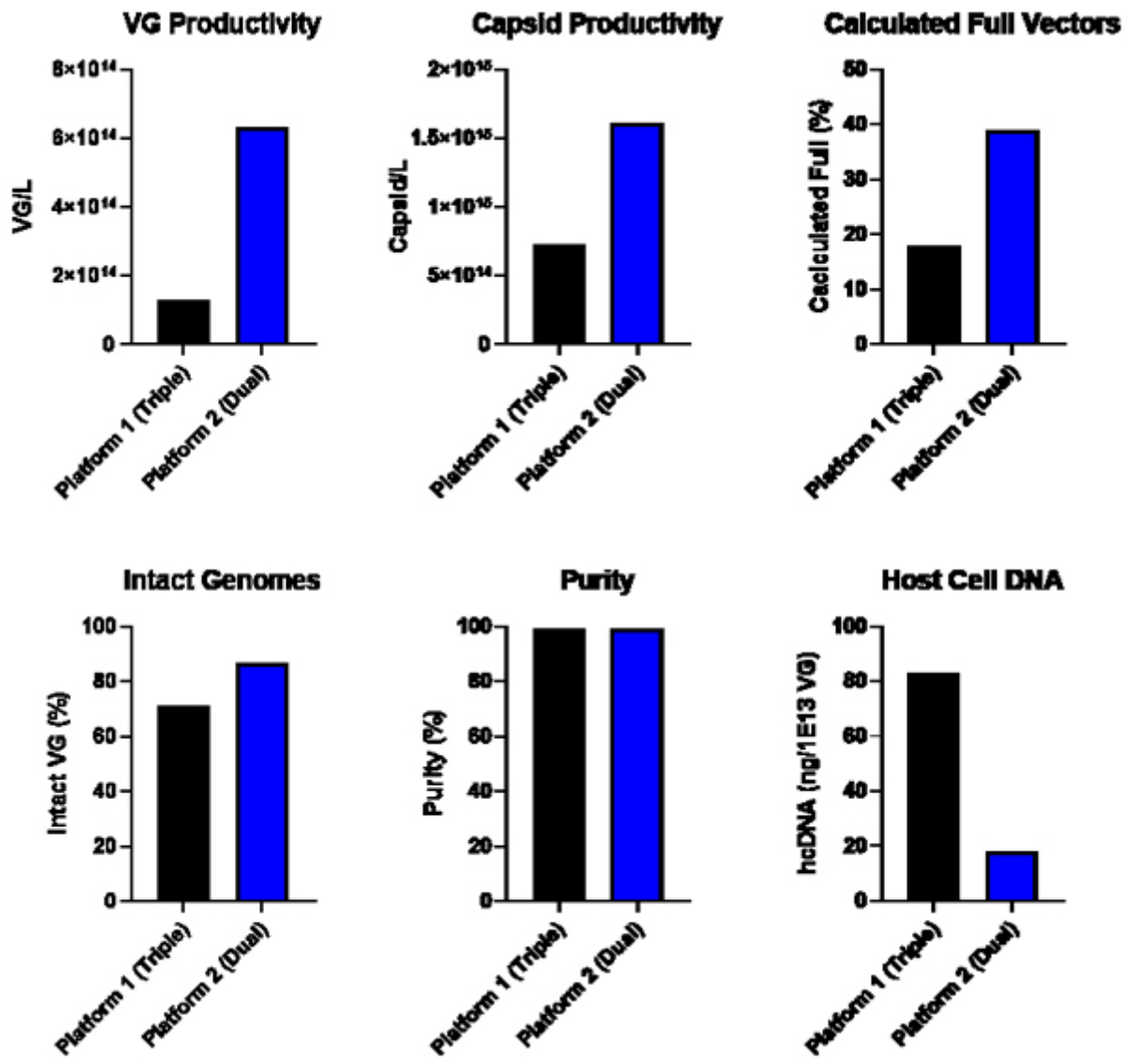

Figure 4. *Bioreactor productivity and final drug substance product quality comparison of Platform 1 (Triple plasmids) and Platform 2 (Dual plasmids) at the 2000 L scale.*

Another prime advantage coming from OXB Solutions' two-plasmid system is the boost in speed of the manufacturing process. With a new drug development partner, there is no need to go back to the drawing board and start designing the process from scratch to be able to run it on the platform. The partner's construct can be integrated immediately to create plasmids, plug those plasmids into the platform, and obtain the vectors. In this way, the OXB Solutions is able to move quickly through the entire development process by leveraging its platform, which now includes the dual transfection system. Thus, there's a time advantage – but importantly, it affects the overall picture, or in other words, effective speed to the client's end goal, not just their next activity on the development and manufacturing path. For instance, an accelerated drug development process that hits quality/safety 'speedbumps' at the clinical stage resulting in an FDA hold, does not translate to an overall fast timeline. OXB Solutions views the end game as success in the clinic, and the sooner that goal is reached, the faster the access to life-saving therapies.

Plug and Play

From the beginning, the intent of this plasmid innovation was to enable the support of a wide range of drug products. The OXB Solutions Platform has been tested for multiple genomes and AAV serotypes at different scales, from shake flasks to 2000L bioreactors, more than 800 times. Furthermore, over 500 different constructs (mixing and matching different GOIs and different serotypes) have been tested to reveal consistent performance across all of them. Note that there are slight titer variations between serotypes, but all serotypes have demonstrated an increase in VG productivity using dual rather than triple transfection. Additionally, equivalent or increased calculated full vectors were observed for all serotypes. This is what OXB Solutions refers to as the "plug and play" capability – virtually any GOI sequence can be plugged in to the system to obtain high-quality vectors quickly and efficiently.

Rigorous Analytical Validation and Comparability Testing

Comparability testing is a critical piece of validation if manufacturing changes occur in the process of producing a drug substance. For example, switching to a new technology or method such as the dual-plasmid system would require manufacturers to prove to the FDA that the change is not going to have a negative (or different) effect in the clinic. Therefore, to address this, OXB Solutions did a full analytical study comparing its novel dual-plasmid system to the traditional triple-plasmid system and providing analytics side by side to show that results are either similar or better (higher fully intact vector). This was done at the 2 L, 50 L, and 2000 L scale to confirm linearity.

The ultimate test of the system is to see how it performs in an animal model and whether the drug product works in vivo in the same, intended way. In vivo comparability testing indicated that efficacy was identical with no new/different side effects, confirming that the dual-plasmid system produced carbon-copy results in vivo.

To date, OXB Solutions has successfully cleared five investigational new drug (IND) applications as a company, and a sixth one will include the dual-plasmid system currently in the execution phase. As these plans have been discussed with the FDA, it's clear that there is a strong path forward for this innovation in AAV manufacturing, which is now being offered externally by the company.

Conclusion

The OXB Solutions team has developed a proprietary ‘plug and play’ dual-plasmid system for transient transfection that meets regulatory and compliance expectations. The system is capable of achieving remarkable titers and high-quality vectors necessary for effective AAV-based gene therapies. High titer, high product quality, proven scalability, and speed are the foundations of the system, resolving some of the key manufacturing challenges faced by AAV developers.

To learn more, email Oxford Biomedica Solutions.

References:

[1] Wang, D., Tai, P.W.L. & Gao, G. *Adeno-associated virus vector as a platform for gene therapy delivery*. Nat Rev Drug Discov 18, 358–378 (2019)