# Achieving better AAV vector productivity and product quality

Diane Golebiowski and Marissa Stanvick

**In a recent episode of The BioInsights Podcast, we spoke to two experts from Oxford Biomedica Solutions about their experiences in optimizing transient transfection of plasmids into suspension HEK293 cells for the production of recombinant adeno-associated virus (rAAV) gene therapies. Here, we sum up some of their key thoughts.**

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### HOW IMPORTANT IS PLASMID DESIGN TO VECTOR PRODUCTIVITY AND QUALITY?

"Our team has worked very hard in the last few years to come up with an ideal arrangement of sequences that is critical to AAV production, and we have generated a novel dual plasmid design which results in a significant increase in AAV productivity and, more importantly, increases the percentage of full capsids upstream. This has been a huge innovation for our AAV manufacturing platform process, leading to significant process gain while still maintaining the same flexibility as triple plasmid transfection."

– **Diane Golebiowski,** Head of Vector Engineering Group

### "... AND WHAT ABOUT PAYLOAD SEQUENCE?

"This is just as critical, if not more. We advocate for biology being the main driver for the decision-making process when nominating a lead for development, but you have to consider the whole package. If you have two candidates that are comparable expression-wise and efficacy-wise, it is crucial to look at how it manufactures, so that doesn't lead to problems later. We offer end-to-end services to support construct design, and look for red flags early that could cause problems with drug quality."

– **Diane Golebiowski,** Head of Vector Engineering Group

### WHAT WERE THE CRITICAL ASPECTS YOU FOCUSED ON FOR SUCCESSFUL SCALE-UP?

"First, we designed a cell expansion strategy. Considering how unpredictable supply chain is we also needed to design a flexible process. The next area we focused on was ensuring the volume and process timing would be appropriate and consistent among scales. We came up with strategies to evaluate in a scaled down bioreactor model, before ultimately deciding on our two thousand litre scale recommendation.

Transient transfection traditionally has a reputation for poor productivity and scalability. We set out to prove that wrong and create a high-performing system that offers the flexibility of transient transfection with the productivity you might see with other systems. We've shown that we can scale our process from two to two thousand litres for three programs."

– **Marissa Stanvick**, Director of Upstream Process Development

### WERE YOU ABLE TO INCREASE BIOREACTOR PRODUCTIVITY?

"Ultimately, we were able to optimise our platform conditions around the novel dual-plasmid design, and find a set of parameters that were ideal for multiple constructs and AAV serotypes. Once we combined these parameters, we increased our productivity by over a log to over 1E15 vg/L. We also found nearly 50% full capsids in the affinity product as a result of the dual plasmid design. Our purification sciences team designed a downstream platform where the final products achieved over 90% fully intact vectors in the drug substance, with high product quality."

– **Marissa Stanvick**, Director of Upstream Process Development

CELL & GENE THERAPY INSIGHTS

*Cell & Gene Therapy Insights* 2022; 8(7), 793 • DOI: 10.18609/cgti.2022.120



In collaboration with: Oxford Biomedica Solutions