A Novel Dual Plasmid Platform Provides a Scalable Transfection Process Yielding Improved Productivity and Packaging Compared to Triple and pDG Dual Plasmids

Laura van Lieshout[1], Miranda Rubin[2], Stacy Ota[1], Dicky Gilmore[1], Shannon Wallace[1], Diane Golebiowski[1], Marissa Stanvick[1], Ify Iwuchukwu[1], Jin Yin[1], Tim Kelly[1]

[1]Oxford Biomedica Solutions Bedford, MA, [2]Homology Medicines Inc, Bedford, MA

Introduction

Transient transfection of mammalian cells using plasmid DNA is a standard method to produce adeno-associated virus (AAV) vectors allowing for flexible and scalable manufacture. Typically, three plasmids are used to encode the necessary components to facilitate rAAV vector production; (1) an AAV genome plasmid containing the gene of interest (GOI) expression cassette with appropriate regulatory elements flanked by inverted terminal repeats (ITR), (2) a RepCap plasmid that provides the AAV replicase and capsid gene expression *in trans*, and (3) a helper plasmid providing helper virus functions. However, a dual plasmid system, termed pDG, was introduced over two decades ago demonstrating sequences could be combined resulting in comparable productivity to triple transfection. We have developed a novel dual plasmid system, with an alternative arrangement of sequences that results in significantly increased AAV vector productivity and percentage of full capsids packaged in comparison to pDG and triple transfection. Here, we demonstrate the reproducibility of these findings across seven recombinant AAV genomes and multiple capsid serotypes as well as the scalability of this novel dual plasmid transfection at 50L bioreactor scale. Purified drug substance showed a consistent product quality profile in line with triple transfected vectors, except for a substantial improvement in intact genomes packaged using our GOI+RepCap dual transfection system. This dual plasmid design represents an innovation in AAV manufacturing resulting in significant process gains while maintaining the flexibility of a transient transfection platform.

Contact Information

Oxford Biomedica Solutions
solutions.partnering@oxb.com

A Novel Dual Plasmid System Outperforms pDG Dual & Triple Transfection

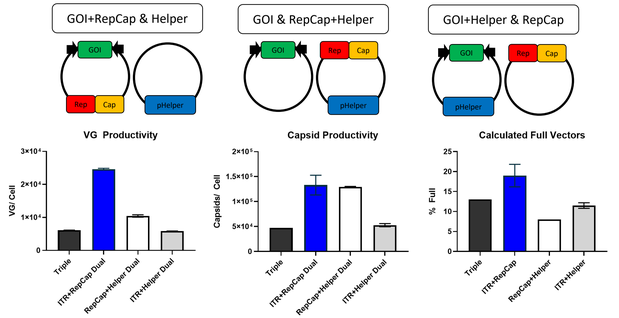


Figure 1: Productivity Assessment of Three Possible Dual Plasmid Configurations and Triple Transfection. 125mL shake flasks were transfected with GOI+RepCap & Helper, GOI & RepCap+Helper, GOI+Helper & RepCap or standard triple transfection at a 1:1 or 1:1:1 molar ratio. Crude lysate samples were quantified for VG productivity, capsid productivity and the percentage of calculated full vectors. All conditions were completed in duplicate, and the data is displayed as mean ± SD.

We evaluated the three combinations that the components of triple transfection could be arranged onto two plasmids. The RepCap+Helper design is also known as the "pDG" dual plasmid, however to the best of our knowledge the GOI+RepCap and GOI+Helper dual plasmids have not yet been published. The combination of GOI+RepCap along with a separate pHelper resulted in substantial improvement in VG titers as well as an increase in the percentage of calculated full vectors. Therefore, the GOI+RepCap dual plasmid design was selected for further development and is shown in the remainder of this poster.

Results

Increased VG Productivity Across Seven Distinct GOI Constructs at 2L Scale

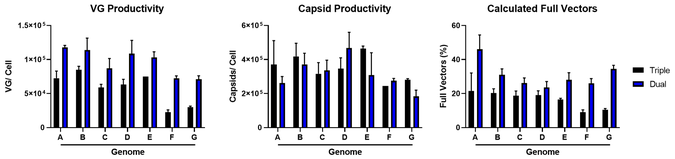


Figure 2: Productivity Assessment of Seven AAV Genomes Comparing Dual to Triple Transfection. Transfections were performed with dual or triple plasmids for each GOI-ITR genome sequence in 2L bioreactors. Crude lysate samples were quantified for VG production, capsid production and calculated full vectors. All vectors were packaged with AAVHSC15 except for genome F which was packaged with AAVHSC17. All conditions were completed at a minimum of n=2 and the data are displayed as mean ± SD.

Consistent Product Quality Profile at 50L Scale

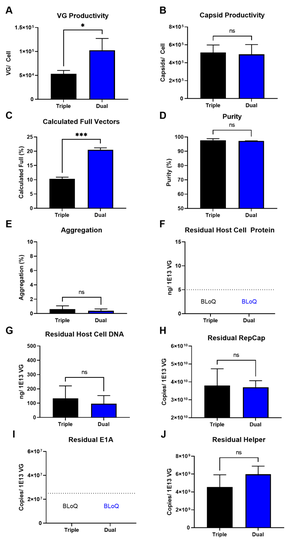


Figure 3: Productivity and Product Quality Assessment of Dual and Triple Plasmid Produced Drug Substance. Genome D was produced at 50L bioreactor scale using dual (n=2) and triple plasmid (n=3) transfection. Crude lysate samples were analyzed for (A) VG productivity, (B) capsid productivity and (C) calculated full vectors while purified vectors from these lots were analyzed for (D) purity, (E) aggregation, (F) residual host cell protein, (G) residual packaged host cell DNA, (H) residual packaged RepCap DNA, (I) residual packaged E1A DNA and (J) residual packaged helper DNA. Data are displayed as mean ± SD. The dashed lines indicate the limit of detection for the assays where samples were determined to be below the limit of quantification (BLoQ). Statistical significance was determined using a student's t-test, * p<0.05, ***p<0.001 and ns- not significant.

In addition to producing higher VG yields, our dual plasmid transfection platform produces a comparable product quality profile to triple transfection. The only product quality attribute that differed was improved AAV genome packaging with our dual plasmid transfection (see below).

Improved Vector Packaging Profile with up to 90% Full Capsids With Fewer Partial Genomes & Empty Capsids

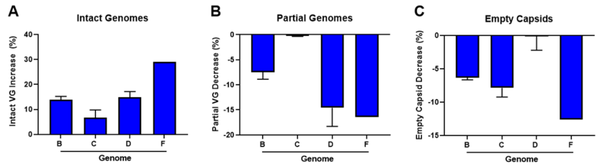
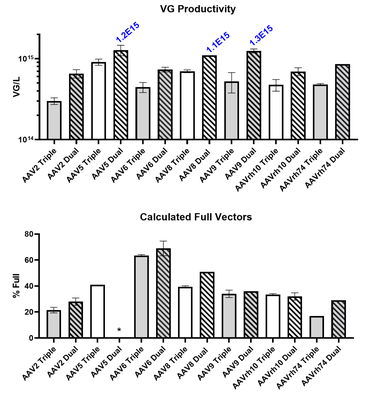


Figure 4: Increase in Packaged Intact Vector Genomes and Reduction in Partial Packaged Vectors and Empty Capsids Observed for Dual Compared to Triple Transfection. AUC was used to analyze purified vectors produced with dual and triple plasmid transfection to determine the proportion of capsids containing full length intact genomes, partial packaged genomes, or empty capsids. All data were generated at the 2L bioreactor scale except for genome D which was produced in 50L bioreactors. Data is displayed as the mean ± SD of (A) the absolute increase in intact packaged genomes, (B) the absolute decrease in partial packaged genomes and (C) the absolute decrease in empty capsids for dual produced vectors as compared to the averaged historical data for triple transfection for each genome. Dual produced vectors were analyzed in the following replicates: genome B (n=3), genome C (n=3), genome D (n=2) and genome F (n=1).

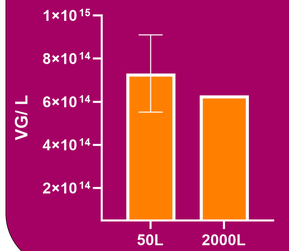
Platform Application of Dual Plasmid Transfection Across Seven AAV Serotypes



Our dual plasmid transfection platform allows for "plug-and-play" use across seven diverse AAV capsids (clades A, B, E & F). All serotypes demonstrated an increase in VG productivity using dual rather than triple transfection. Additionally, equivalent or increased calculated full vectors was observed for all serotypes tested.

Figure 4: 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 2L bioreactors. Crude lysate samples were quantified for VG production and calculated full vectors. All vectors utilized genome A and were completed in duplicate with the exception of AAV8 dual and AAVrh74 dual transfections which were in singlicate. *AAV5 % full data is pending.

Scalability of Dual Plasmid Transfection From 50L to 2000L



Our dual plasmid transfection platform has resulted in consistent VG productivity at 50L and 2000L scale. With VG titers >6E14 VG/L at 2000L scale, transient transfection is a viable approach for commercial supply of indications with larger patient populations.

Figure 5: Consistent Vector Genome Productivity Across 50L to 2000L Bioreactor Scale Up. Genome D was produced using a platform upstream process with dual plasmids at 50L and 2000L scale. Average crude lysate VG productivity is displayed as mean ± SD, 50L (n=4) and 2000L (n=1).

Conclusions

- **Our novel dual plasmid transfection platform provides improved productivity across multiple GOIs and capsid serotypes demonstrating the breadth of our platform**
- **Up to 90% of vectors in drug substance contain genomes using our dual plasmid transfection demonstrating an improvement in product quality**
- **OXB Solutions is now offering access to this platform capability externally**