Advantages of Standardization and Large-scale Manufacturing of Helper Plasmids for Viral Vector Production

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Abstract

Success in gene and cell therapy has increased demand for plasmid DNA used to produce viral vectors, both in quantities for commercial products as well as the breadth of different vectors for the expanding number of programs in development. The data and analysis indicate that standardization and large-scale production of helper plasmids, those that are the same regardless of the specific viral vector produced, represent an opportunity to significantly reduce timeline, cost, and risk. Aldevron has developed processes to quickly produce a set of helper plasmids that consistently generate functional viral vectors, are immediately available for research and clinical production and are free of any royalties or future payments. To meet production scale requirements, our technical operations team has developed and deployed a platform based around a single-use, 300 L fermentation device and process train capable of purifying up to 100 grams of a plasmid in a single processing event. The manufacture of the output from an individual fermentation process can take as little as seven days. Scaling work done in the early engineering phase for this train was integral to the design of a new 70,000 square foot manufacturing plant. The data show that fermentation scalability can support large processing events and the production of optimized helper plasmids can enable high-titer lentiviral vector production. Standardization of lentiviral plasmids supports consistency and efficiency at large scale and across multiple programs. Our data show the scalability of fermentation for a typical plasmid at 30 L and 300 L, with specific yield maintained as scale is increased. The data show the performance of lentiviral vectors produced with optimized plasmids, indicating improved performance over other plasmid systems. The availability, cost, freedom to operate, and consistency of these plasmids will help address the growing demands of cell and gene therapy.

Introduction

Success in gene and cell therapy has increased demand for viral vectors, both in the quantities needed for commercial products as well as the breadth of different vectors required for the expanding number of programs in development. The most widely used viral vectors in modern gene therapy are recombinant adeno-associated virus (rAAV) and recombinant lentivirus (rLenti). Several methods have been developed for producing these vectors and significant resources are being devoted to increasing yields and decreasing costs. One of the most common production methods is transfection of plasmid DNA into HEK293 (or HEK293T) cells. This method requires between two and four separate plasmids depending on the vector and arrangement of genes in the plasmids. These plasmids are the most critical components in rAAV and rLenti production.

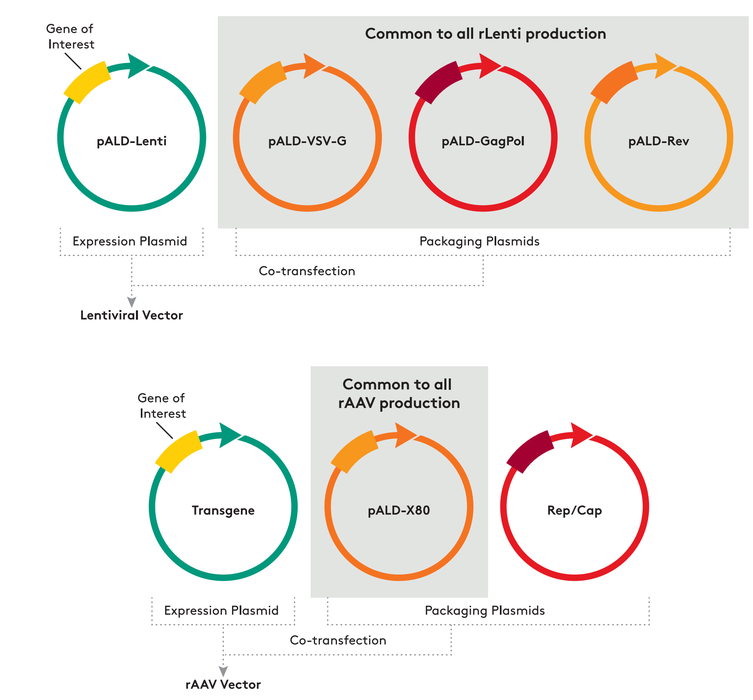

Figure 1 Plasmids used in viral vector production illustrating the plasmids that are common to all rLenti and rAAV production.

Triple transfection of three plasmids into HEK293 cells is a widely used method for rAAV production. Figure 1 illustrates the three plasmids required for this method. Other methods have been developed using a two-plasmid system, in which case the adenoviral helper genes and the rep/cap genes are combined into one plasmid. Although this method confers some benefits, triple transfection is still the most frequently used production method. Figure 1 shows that the helper plasmid in triple transfection is independent of the transgene or AAV serotype. Figure 1 also shows the similar production method for rLenti, which uses four plasmids, with the three packaging plasmids independent of the transgene. Since they are independent of the specific viral vector produced, standardization and large-scale production of the rAAV helper and rLenti packaging plasmids represent an opportunity to reduce cost, complexity, and variation in viral vector manufacturing.

Materials and Methods

Scalable plasmid DNA production

Fermentation was performed using the HyPerforma™ Single-Use Fermenter System (Thermo Fisher Scientific) at 30 L and 300L scales. This utilized the Aldevron proprietary fermentation protocol and media formulations as well as interim quality control analysis performed via OD600 and miniprep analysis at hourly intervals starting at elapsed fermentation time (EFT) 21:00 through harvesting. Fermentations were harvested at approximately EFT 28:00 via batch centrifugation using an Avanti JXN-26 centrifuge.

Recombinant AAV production

Plasmids encoding AAV2 rep and cap (pAAV-RC2, VPK-422) and the AAV2 genome expressing GFP (pAAV-GFP, AAV-400) were purchased from Cell Biolabs, Inc. The pAAV-GFP plasmid was modified to encode firefly luciferase, giving pAAV-Luc. Helper functions were provided by the pALD-x80 plasmid. The three plasmids (pAAV-RC2, pAAV-Luc, and pALD-x80) were combined in a 1:1:1 ratio (w/w/w), formulated with TransIT-X2 transfection reagent (Mirus), and added to HEK293 cells according to the manufacturer's protocols. After incubation for 48-72 hours, the media and cells were recovered and subjected to three freeze-thaw cycles. After clarification, the extracts were added to HEK293 cells at various dilutions. After incubation for 72 hours, the luciferase activity was measured using the Bright-Glo™ Luciferase Assay System (Promega).

Materials and Methods (cont.)

Recombinant lentivirus production

To produce rLenti in adherent HEK293T cell line cultured in DMEM + 10% FBS, cells are seeded in 6-well plates one day prior to transfection and transfected at 70-80% confluence. Transfection complex is prepared by diluting 2.75 µg/well of pALD-Lenti packaging plasmid mix, 1.25 µg/well of lentiviral transfer plasmid and 10 µg/well of 25 kD branched polyethylenimine (B-PEI) in 400 µL/well of Opti-MEMTM medium (Gibco/Thermo Fisher). The transfection complex is incubated at room temperature for 20 minutes before applied to the pre-seeded cells.

To produce rLenti in suspension HEK293 cell line cultured in BalanCDTM HEK293 medium (Irvine Scientific), cells are seeded in 125 mL Erlenmeyer flasks at 1x106 cells/mL, 22.5 mL/flask one day prior to transfection. Transfection complex is prepared by diluting 17.2 µg/flask of pALD-Lenti packaging plasmid mix, 7.8 µg/flask of lentiviral transfer plasmid and 50 µL/flask of PEIproTM (Polyplus) in 2.5 mL/flask of BalanCD HEK293 medium. The transfection complex is incubated at room temperature for 10 - 15 minutes before applied to the pre-seeded cells. 72 hours post transfection, the lentiviral-containing supernatant or cell suspension is collected and centrifuged at 1000 x g for 10 minutes to pellet any cells or debris before submitted to analytical assays. Infectious titre assay is performed in adherent HEK293T cell line.

Results and Discussion

The demand for plasmid DNA has grown significantly and this growth is predicted to continue, if not accelerate. To meet production scale requirements, we have developed and deployed a platform based around a single-use, 300 L fermentation device and process train capable of purifying up to 100 grams of a plasmid in a single processing event. The manufacture of the output from an individual fermentation process can take as little as seven days. Scaling work done in the early engineering phase for this train was integral to the design of a new 70,000 square foot manufacturing plant dedicated to plasmid DNA production. Figure 2 shows data from a 300 L and two 30 L production runs. This data shows the consistency of fermentation production at both scales. Although this data is not from rAAV helper or rLenti plasmids, the plasmid used in these runs is larger than any of those plasmids. Production of the rAAV and rLenti plasmids at 300L scale is underway and initial results show consistency of yield for these constructs.

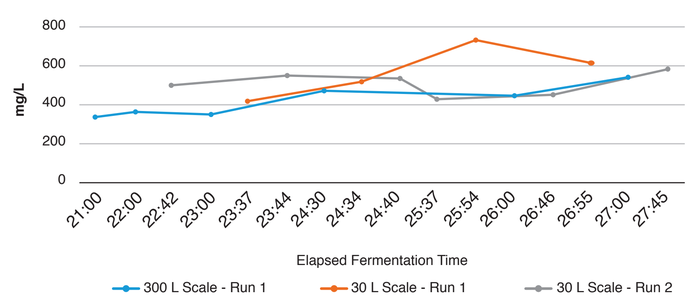

Figure 2 Fermentation data showing scalability of plasmid DNA production between 30 L and 300 L scales.

Realization of the benefits of large-scale production of common plasmids requires standardization of the plasmid used across programs. Standardization allows for maintaining an inventory of plasmids that, given the stability of plasmid DNA, is available "on demand" for clinical and commercial production of viral vectors. For rAAV production the pXX6-80 plasmid published in 1998 by Xaio, et al., is an attractive choice given its wide use to produce rAAV for discovery research and clinical trials. A version of this plasmid, named pALD-X80, is commercially available and includes kanamycin resistance, which is preferred by regulatory agencies over ampicillin. Figure 3 shows our data comparing transduction of rAAV produced with pALD-X80 with another commercially available rAAV helper using two different plasmid production methods. This data is generally consistent with the large body of published literature supporting the performance of pXX6-80 for rAAV production.

The four-plasmid system of rLenti production represents an even greater opportunity to impact costs and timelines with standardized plasmids. Figures 4 and 5 show the infectious titer of rLenti (encoding eGFP) produced using the pALD-Lenti standard plasmids compared to other commercially available packaging plasmids in adherent HEK293T cell line (Figure 4) and Oxford Genetics' proprietary clonal suspension HEK293 cell line (Figure 5), respectively. The pALD Lenti plasmids were developed by Oxford Genetics and optimized for rLenti production. The pALD-Rev and pALD-VSV-G plasmids have been codon optimized and extraneous sequences minimized. Homology with HIV/VSV and inter-cassette homology has also been minimized.

Results and Discussion (cont.)

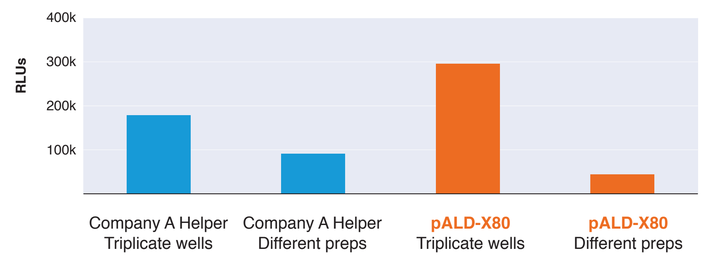

Figure 3 Transduction data from AAV2 viral vector with luciferase transgene indicating performance of pALD-X80 relative to commercially available helper plasmid.

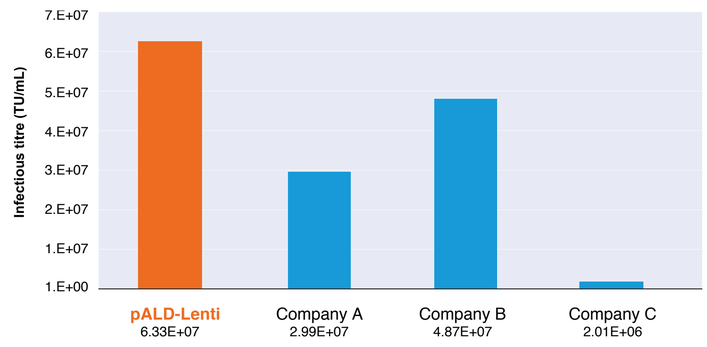

Figure 4 Infectious titer data for lentiviral vector production in adherant HEK293 cells relative to commercially available packaging plasmids.

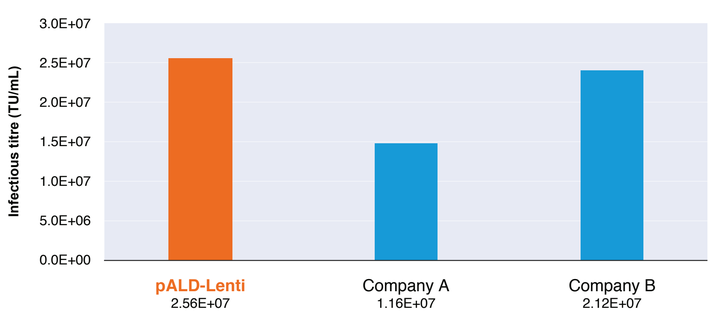

Figure 5 Infectious titer data for lentiviral vector production in suspension HEK293 cells relative to commercially available packaging plasmids.

Conclusions

Our analysis of the state of rAAV and rLenti viral vector manufacturing combined with our data allows us to identify plasmids common to each vector production method that can be used for any program or stage of development. We have also shown our ability to scale plasmid production up to 300 L. Large-scale batches of the rAAV helper plasmid, pALD-X80, are now available at research and clinical quality grades for immediate delivery. Large-scale batches of lentiviral plasmids, pALD-Rev, pALD-VSV-G and pALD-GagPol are now available at research grade with clinical grade quality available in the summer of 2019.

In addition to cost and supply chain complexity, innovators in genetic medicine must also be concerned with intellectual property if they wish to bring therapies to patients as quickly as possible. The pALD-X80 and pALD-Lenti plasmids are available with no royalties, milestones, or other reach-through costs. The features of these plasmids combine to make them excellent options for gene therapy development and stand to become the basis for future breakthrough medicines.

References

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