Overcoming the Challenges of Large-Scale Plasmid Production

Gene therapy involves inserting a new section of DNA into a patient's cells, often encoding a healthy gene to replace a disease-causing copy. This DNA is delivered by viral vectors which are usually built from plasmids. Plasmids are small sections of genetic material which can be combined to contain all the genes necessary to deliver the gene therapy. Adeno-associated virus (AAV) and lentiviral plasmids are commonly used for gene therapy delivery.

Many challenges associated with manufacturing therapies on a large scale are caused by difficulties in working with plasmids. When manufacturing a plasmid, the final product must be consistent across batches and must not have altered genetic material or other variations that affect quality. Often, small-scale manufacturing processes using adherent cells require significant expertise and process optimisation to adapt to suspension culture for clinical or commercial use.[1] There are also regulatory and timescale challenges associated with plasmid production which delay drug development.

In this article, we will examine how processes for the design and manufacturing of AAV and lentiviral plasmids can be constructed to meet the quality, regulatory and timescale challenges of bringing a new drug to market.

Optimising Plasmid Design

To meet the challenge of large-scale production, a plasmid must be able to reliably produce a high yield of viral product. Technologies which make plasmid design easier and faster are beneficial for achieving this goal, facilitating rapid creation of custom constructs and streamlined optimisation of plasmid design for high viral yields. Modular approaches to plasmid design, for example, utilise huge catalogues of DNA elements and can make it simple to swap elements in and out of a plasmid. These approaches can be used for optimised design of AAV and lentiviral plasmids by allowing rapid generation of custom constructs.[2]

Improved AAV Packaging Plasmids

The adeno-associated virus (AAV) genome is a single strand of DNA comprised of a *rep* gene, which encodes four regulatory proteins involved in genome replication, and a *cap* gene, which is responsible for the production of three capsid proteins. AAV is not infectious by itself; it requires a co-infecting helper virus, like adenovirus or herpes simplex virus to infect a host cell, and then relies on the cell's own DNA replication machinery to replicate its genome, since AAV does not encode its own polymerase. AAV plasmids can be designed with large-scale manufacturing and regulatory preferences in mind.

Most rAAV production systems retain the configuration of *rep* and *cap* genes found in the viral genome. However, since the cytotoxic and cytostatic effects of *rep* are well documented,[3,4] it can be beneficial to minimise *rep* expression by placing it under the control of a different promoter to *cap*.

Alongside the RepCap and gene of interest plasmid, AAV production also requires adenoviral help. This can be provided by co-transfection of the core adenoviral helper genes in a third plasmid or by adenoviral infection. The latter generally results in much higher AAV yield, but also significant adenoviral contamination, which then requires costly downstream processing to remove. Simplifying the Ad helper plasmid can improve helper-free AAV production. A reduced plasmid size can save costs as smaller plasmids often grow better than larger ones, giving higher yields and requiring fewer DNA preparations. The removal of additional adenoviral hexon gene sequences is also regarded favourably by regulators, a benefit when bringing a new drug to market.

Improved Lentiviral Packaging Plasmids

Lentiviral plasmids co-opt the ability of viruses to deliver their own genetic material into cells, utilising this function for the delivery of gene therapies. Originally derived from the human immunodeficiency virus, lentiviral plasmids have been optimised throughout the last 20 years. Multiple "third-generation" lentiviral plasmids have entered clinical trials.[5] Like AAV plasmids, they can be designed with manufacturing and regulatory requirements in mind.

To deliver a gene therapy, a lentiviral system needs a combination of DNA elements with complementary functions. These include genes which encode Gag, a polyprotein that forms the viral core structure; Pol, the reverse transcriptase; Rev, which is essential for HIV-1 protein expression; and VSV-g, responsible for fusing the viral envelope to the host cell membrane. However, the system must be prevented from producing a virus capable of its own replication.

Third generation plasmids are safer than second generation systems because the genes required for a replication competent lentiviral, such as *gag-pol* and *rev*, are kept in different plasmids. This means that an extra recombination event would be required to bring these genes together. Third generation systems also eliminate *tat*, a gene that activates viral transcription. This means that when the viral DNA integrates into the host genome, it doesn't activate transcription, so even if a recombination event were to occur, the provirus would still not be transcribed.

However, the enhanced safety features of a third-generation lentiviral packaging plasmid system often come at the cost of viral titre. Plasmids can be further optimised to counteract this problem and therefore to meet the demands of large-scale manufacturing.

GMP Grade AAV and Lentiviral Plasmid Manufacture and Supply

Manufacturing plasmid DNA according to good manufacturing

process (GMP) grade is required for clinical applications. As such, a number of companies manufacture and supply AAV and lentiviral plasmids at clinical and commercial GMP grades.

This requires careful planning from the beginning. For example, cell banks should be set up and downstream processes and analytical techniques assays should be confirmed before GMP manufacture. Antibiotic-free manufacturing processes are often advantageous; they are preferred by regulators for reducing the spread of antibiotic resistance and the risk of adverse reactions.[6] Developers may find it beneficial to work with a contract development and manufacturing organisation (CDMO) with extensive process development and testing expertise, established quality management systems, flexibility in manufacturing scale, and antibiotic-free processes, and the ability to provide the required documentation for investigational new drug (IND) filing.

Plasmid supply is a recognised bottleneck within the cell and gene therapy industry, with long turnaround times causing substantial delays for the delivery of GMP products. To meet the industry need for rapid turnaround, CDMOs can keep reliable stocks of raw materials and combine their molecular biology expertise with investments in processes and facility development for shorter turnaround timescales.

Through a combination of novel plasmid design technologies and robust GMP manufacturing processes, the industry is working to address the challenges of large-scale plasmid production and helping to accelerate time to market for new therapeutics.

REFERENCES

1. Manceur AP et al. (2017). Scalable Lentiviral Vector Production Using Stable HEK293SF Producer Cell Lines. Hum Gene Ther Methods 28, 330–339.
2. Patent GB2496849.
3. Yang Q et al. (1994). Characterization of cell lines that inducibly express the adeno-associated virus Rep proteins. J Virol 68, 4847–4856.
4. Schmidt M et al. (2000). Adeno-associated virus type 2 Rep78 induces apoptosis through caspase activation independently of p53. J Virol 74, 9441–9450.
5. Milone MC and O'Doherty U (2018). Clinical use of lentiviral vectors. Leukemia 32, 1529–1541.
6. Hardee CL et al. (2017). Advances in Non-Viral DNA Vectors for Gene Therapy. Genes (Basel) 8, 65.

Dr. Katie Roberts

Dr. Katie Roberts is Content Manager at OXGENE, a WuXi Advanced Therapies company. WuXi Advanced Therapies, a global Contract Testing Development and Manufacturing Organization (CTDMO), is the advanced therapies business unit of WuXi AppTec and offers integrated platforms to transform the discovery, development, testing, manufacturing, and commercialization of cell and gene therapies for customers worldwide.

Web: www.oxgene.com
www.advancedtherapies.com