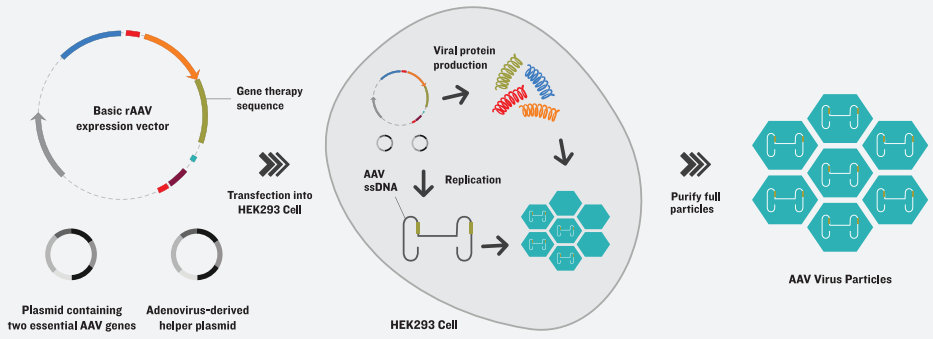
TRIPLING DOWN ON EFFICIENT GENE THERAPY PRODUCTION

Three is the magic number when it comes to manufacturing vectors for NOVEL THERAPIES

FIGURE 1. OVERVIEW OF THE TRIPLE-TRANSFECTION STRATEGY FOR AAV PRODUCTION

This approach eliminates the need for a 'helper' adenovirus by introducing key genes in a DNA plasmid (top left), accompanied by two other plasmids (bottom left). The three plasmids are transfected into the production cell line (middle), which produces mature AAV capsids containing the transgene of interest. Once purified (right), these viral particles can infect patient cells to deliver their DNA payload, but cannot replicate further.



Given the considerable time and effort companies have invested in gene therapy, most prefer to play it safe when it comes to manufacturing. Accordingly, the large majority of recombinant adeno-associated virus (rAAV)-based therapies—which account for most gene therapies—are still produced with a method developed more than two decades ago, known as the 'triple transfection' technique.

One of the central challenges of rAAV production is the need for a 'helper virus' to facilitate replication within a host mammalian cell. In the early days of rAAV, cultured cells would first be infected with adenovirus, after which they would be transfected with two DNA plasmids: one containing a pair of essential rAAV genes, and the other a sequence to be employed for the gene therapy in question. This approach yields a reasonable output of fully-assembled, gene therapy-ready rAAV particles. However, these must then be separated from the also-abundant adenovirus particles that contaminate the resulting virus preparation, and can put patients at risk.

In 1998, two different research groups—one led by R. Jude Samulski at the University of North Carolina and the other by Peter Colosi at Avigen—determined that they could avoid these hassles by shifting key functions of the adenovirus on to a third, 'helper' plasmid. In this approach, researchers can simply transfect their preferred host cell—in most cases, human embryonic kidney (HEK) 293 cells—with all three plasmids in a stepwise procedure (see Figure 1). The combined activity of the adenovirus-derived genes on the helper plasmid with the rAAV genes on the second plasmid proved sufficient to generate functional rAAV particles containing the transgene from the third plasmid.

MODULAR CONSTRUCTION

Besides eliminating unwanted adenoviruses, this procedure has several advantages. There are numerous different serotypes of rAAV with distinct properties and capacity to infect different human organs, and researchers can readily swap different genetic elements into this system in a modular fashion to determine which virus is generated. Furthermore, the transfection procedures used to deliver the three plasmids to cells are robust, well-established, and can be performed at manufacturing scale.

Today, at least 11 companies are producing experimental or clinically-approved rAAV-based therapies using this approach, including Spark Therapeutics' Luxturna and a pair of other therapies now in phase 3 testing from Avexis and Gensight. Many contract manufacturers, such as Vigene Biosciences, also adopt this approach, which can be routinely scaled up to generate HEK293 cultures in volumes of up to 500L per bioreactor, generating rAAV at an output of 10^5 viral genomes per cell.

The biggest limitation to this approach is scalability. HEK293 cells are typically maintained in adherent cultures, but far greater culture volumes are possible with cells floating free in suspension. To address this, many teams have developed various strategies for producing suspension cultures of triple-transfected HEK293 cells. Published results initially demonstrated equivalent productivity to adherent culture in a 20L volume, but in principle could scale up to culture volumes of 200L or more to achieve far greater rAAV productivity.

Triple transfection technology has propelled commercial launch of rAAV-based gene therapy for eye-disease patients across the US. Despite newer and more efficient systems emerging, this approach will almost certainly remain instrumental for the manufacture of rare disease gene therapies as well as for proof-of-concept clinical trial materials. ■

source: www.vigenebio.com. By Permission.

Vigene Biosciences
Excellence in Gene Delivery