# GOOD HELP IS HARD TO FIND

Infecting mammalian cells with helper viruses greatly boosts AAV vector production for gene therapy, but creates EXTRA WORK IN PURIFICATION

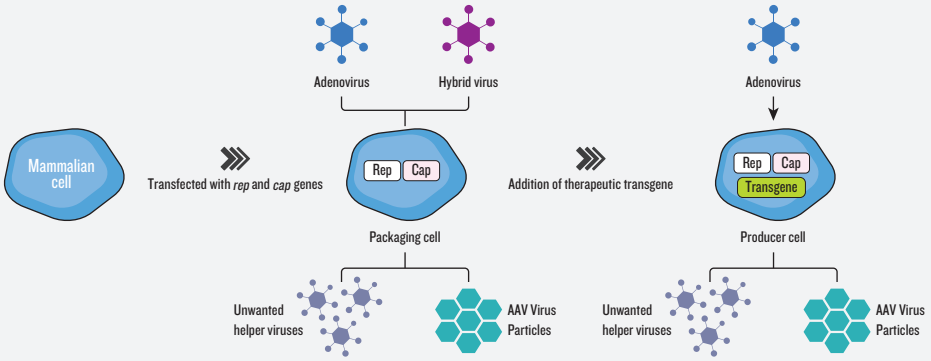
**When Robert Atchison and his colleagues** at the University of Pittsburgh first stumbled across adeno-associated viruses (AAV) in 1965, they initially supposed they were merely defective byproducts of the well-studied adenovirus. These AAV particles could not replicate within host cells on their own, but could when the cells were also infected with adenovirus. It has since become clear that AAV is actually a 'dependovirus' that can only be produced with assistance from other helper viruses.

AAV offers a number of advantages for gene therapy: it can efficiently deliver genetic material into patients' cells without causing cell damage or illness, and its inability to independently reproduce prevents uncontrolled generation of new viruses within a host. A 1984 paper by Paul Hermonat and Nicholas Muzyczka offered the first demonstration that AAV might provide a safe and effective vehicle for transgene delivery, with helper adenovirus infection enabling vector production in mammalian cells. This method offered good proof-of-concept, but was too inefficient for large-scale manufacturing. What's more, it needed extensive clean-up to eliminate adenovirus particles that might otherwise trigger a pathological or immune response in patients.

Newer iterations of the helper-virus-assisted approach improve performance. One strategy converts mammalian cells into 'packaging cells' by genetically modifying them to express the AAV genes *rep* and *cap*, which help copy and package viral genetic material. For gene therapy manufacturing, one can convert packaging cells to 'producer cells' by further modifying their genomes to include the therapeutic transgene along with sequences that signal for the transgene to be bundled into the AAV particle (see Figure 1).

**FIGURE 1: PACKAGER AND PRODUCER CELLS**

Mammalian cells (either HEK293, HeLa or A549) can be modified to express the adeno-associated virus (AAV) genes *rep* and *cap*, and become 'packaging cells' to help create recombinant AAV particles. By adding a therapeutic transgene, packaging cells can become 'producer cells', pumping out rAAV without the need for an additional hybrid helper virus.




Producer cell lines serve as durable rAAV factories: upon infection with adenovirus, they will churn out fully functional gene therapy particles. This technique is compatible with cells grown in free-floating suspension cultures under serum-free conditions, achieving an output of 5,000–50,000 viral genomes per cell. However, it is also very labor-intensive, as each new gene-therapy product requires a new producer-cell line.

Alternatively, some groups stay with the packaging cells, which they co-infect with two different adenoviruses: one wild-type, and one containing the AAV gene-therapy elements. In this scenario, a single packaging-cell line can be used to generate many different gene therapy constructs. However, it has not yet been used in large-scale manufacturing. Furthermore, both the packaging- and producer-cell approaches also create adenovirus particles, requiring an extra purification step to eliminate the unwanted helpers. This problem can be partially remedied by using adenoviruses that have been genetically modified to be temperature-sensitive or replication-defective.

AAV is not exclusively reliant on adenovirus; Friedrich Weindler and Regine Heilbronn demonstrated in 1991 that herpes simplex virus (HSV) can also play the role of helper. This method, in development by Applied Genetic Technologies Corporation (AGTC), requires no packaging-cell line; instead, suspension-cultured mammalian cells are infected with two modified HSV particles. One contains the transgene of interest, and the other holds the essential AAV genes. This approach is faster and simpler than the adenovirus-based methods, and can achieve AAV output levels that rival or exceed producer-cell systems. However, contamination is a recurrent problem of helper-mediated production systems and, despite using replication-defective HSV constructs, purification remains essential.

In spite of these potential risks, the high titer production by HSV is attractive to the developers of the therapies that require high-dose AAVs, such as those for Duchenne Muscular Dystrophy. In addition to AGTC, Solid Biosciences has adopted an HSV production system for its AAV production. Good help appears to be on the way. ■