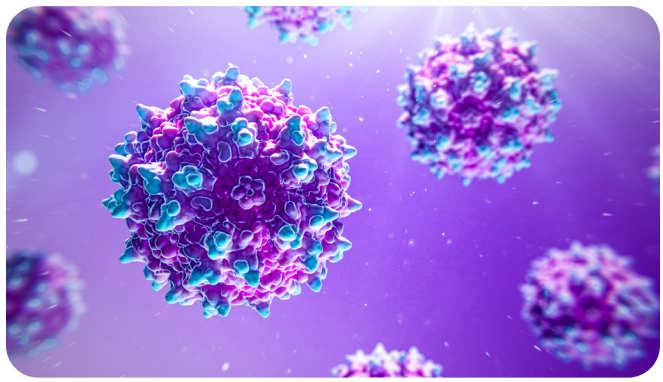
WHITE PAPER

Advancing gene therapy with custom AAV engineering

Adeno-associated virus (AAV)-based gene therapies have demonstrated significant success in pre-clinical and clinical applications, with seven approved therapies on the market and over 200 ongoing clinical trials.[1] As gene delivery vehicles, AAVs are a popular choice due to their nonpathogenic nature, ability to establish long-term gene expression across various tissues, and capacity to transduce both dividing and non-dividing cells. Additionally, their broad tropism has driven their widespread adoption in clinical trials targeting a variety of diseases, including ocular, hematological, neurological, and neuromuscular conditions.

Most of the AAV vectors in therapeutic development originate from naturally occurring AAV serotypes, with AAV9, AAV8, AAV2, and AAV5 being the most common.[1] However, natural serotypes face several limitations. First, a significant proportion of the population (more than 50%)[2] has pre-existing immunity due to natural exposure to wild-type AAV, which can reduce treatment efficacy. Second, AAV serotypes exhibit natural tropism for certain tissues,[3] potentially preventing efficient transduction of target tissues. AAV vectors also face challenges relating to large-scale manufacturing and often require high therapeutic doses, which can increase the risk of immune responses and production costs, further complicating their widespread clinical adoption.

To overcome these challenges, there is growing interest in AAV vector engineering to improve performance in target tissues. This involves either optimizing the DNA cargo—such as incorporating tissue-specific promoters or regulatory elements—or modifying the viral capsid.



Randomized approaches for AAV capsid engineering, including evolutionary approaches such as peptide display and DNA family shuffling,[4] are driving the development of next-generation vectors with improved transduction efficiency and specificity of transgene expression. These engineered vectors show promise in overcoming the limitations of natural serotypes, combined with a potentially favorable immunological profile, and expanding the therapeutic potential of AAV-based gene therapies.

This white paper examines progress in AAV capsid engineering and its implications for the future of gene therapy.

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Tailoring the AAV capsid

The AAV capsid plays an essential role in cell binding, internalization, and intracellular trafficking. Modifications to the capsid can significantly enhance vector performance in several ways, including:

- **Improved tissue tropism:** Engineered capsids can more precisely target specific cell types or tissues, potentially lowering the required therapeutic dose and improving efficacy.
- **Access to new targets:** Optimized vectors can target previously inaccessible or inefficiently transduced cell types, expanding the range of treatable conditions.
- **Reduced off-target effects:** By improving the vector specificity, engineered capsids can minimize unintended transduction of non-target tissues, lowering toxicity risks.
- **Altered immune response:** Capsid modifications can improve the vector's immunological profile by reducing recognition by pre-existing antibodies.

There are various approaches to capsid engineering, but two widely used evolutionary techniques are peptide display and DNA family shuffling (Table 1).

Peptide display

Peptide display involves inserting small peptide sequences—typically 7 to 12 amino acids—into surface-exposed variable regions of the capsid. These modifications can introduce new or altered interactions with cellular receptors, improving tissue targeting or evading neutralizing antibodies. Although the changes are subtle, they enable the creation of highly diverse libraries containing billions of different sequences. This modular approach allows for the generation of peptide libraries across different AAV serotypes, depending on the specific requirements of the vector.[5]

A key advantage of peptide display is its compatibility with amplicon next-generation sequencing (NGS), which enables the generation of large amounts of quantitative data in a cost-efficient manner. Furthermore, short peptide sequences can be tracked across different tissues and screening parameters, simplifying the identification of proprietary vectors with optimized properties.

DNA family shuffling

An alternative method for AAV evolution is DNA family shuffling. This 'mix and match' technique involves fragmenting AAV capsid genes from different serotypes into various sized pieces, typically through enzymatic digestion with DNAse. Because these genes share partial sequence homology, the fragmented pieces can be reassembled into new chimeric capsid variants and amplified by PCR. This process generates a customized shuffled AAV library containing new capsid variants with long, randomized regions.[4]

The composition of the shuffled library can be customized by selecting specific serotypes based on desirable properties and specific needs to achieve improved targeting or enhanced gene delivery. The resulting library can then be screened for capsids with novel or improved properties. This screening can reveal capsids capable of transducing challenging cell types or that exhibit other advantageous traits, enabling the development of proprietary vectors with enhanced functionality.[6]

Table 1: **Comparison of AAV peptide display and DNA family shuffling approaches for capsid engineering.** This table highlights key similarities and differences in modification strategies, library diversity, screening methods, and applications of each technique.

Feature	Peptide display	DNA family shuffling
Modification type	Small peptide insertions (7-12 amino acids)	Chimeric capsids from fragmented and reassembled genes
Targeted regions	Surface-exposed variable regions	Long randomized regions across capsid genes
Diversity potential	High library diversities achievable	Combination of multiple diverse serotypes possible
Screening capability	Easy read-out by amplicon next-generation sequencing (NGS)	Technological improvements in long-read sequencing enable in-depth analysis
Customization	Modular approach across different AAV serotypes	Tailored serotype selection based on specific needs
Limitations	Flanking regions and serotype context may prove crucial for performance	Potential impact on assembly-activating protein (AAP) encoded within the cap gene resulting in impaired capsid assembly
Applications	Tissue targeting with improved efficiency and specificity, potential novel receptor interactions, reduced off-target transduction, lower therapeutic dose	Transduction of challenging cell types feasible, potentially improved immunological profile, reduced off-target transduction, lower therapeutic dose
Outcome	Proprietary novel vectors with optimized properties	Proprietary novel vectors with optimized properties

AAV evolution workflow

A typical AAV evolution project consists of two main phases: an iterative screening campaign followed by a biodistribution study using a subset of vector candidates (Figure 1). Successful screening requires careful planning, as researchers need to define certain criteria in advance. These include the library design (or combination of designs), selection of AAV serotype, route of administration, and choice of screening model—all factors that shape the outcome of the campaign. Once the project is initiated, two to three selection rounds are typically performed in translational large animal models—such as non-human primates—to identify enriched AAV variants. These selected variants are recovered from the tissue of interest and used to generate a focused sub-library for further analysis.

In the second phase, a set of 50 to 100 promising candidates is selected and produced with individually barcoded expression cassettes for a biodistribution study. This approach enables a quantitative assessment of candidate vector performance at both the genomic and transcriptional levels while minimizing the use of large animal models by allowing evaluation of an AAV pool.[7] In particular, the use of single nucleus RNA sequencing enables the analysis of gene expression patterns across different tissues, providing detailed insight into on- and off-target activity. By leveraging this high-throughput analysis, the most promising AAV candidates with optimized properties for therapeutic applications can be identified.

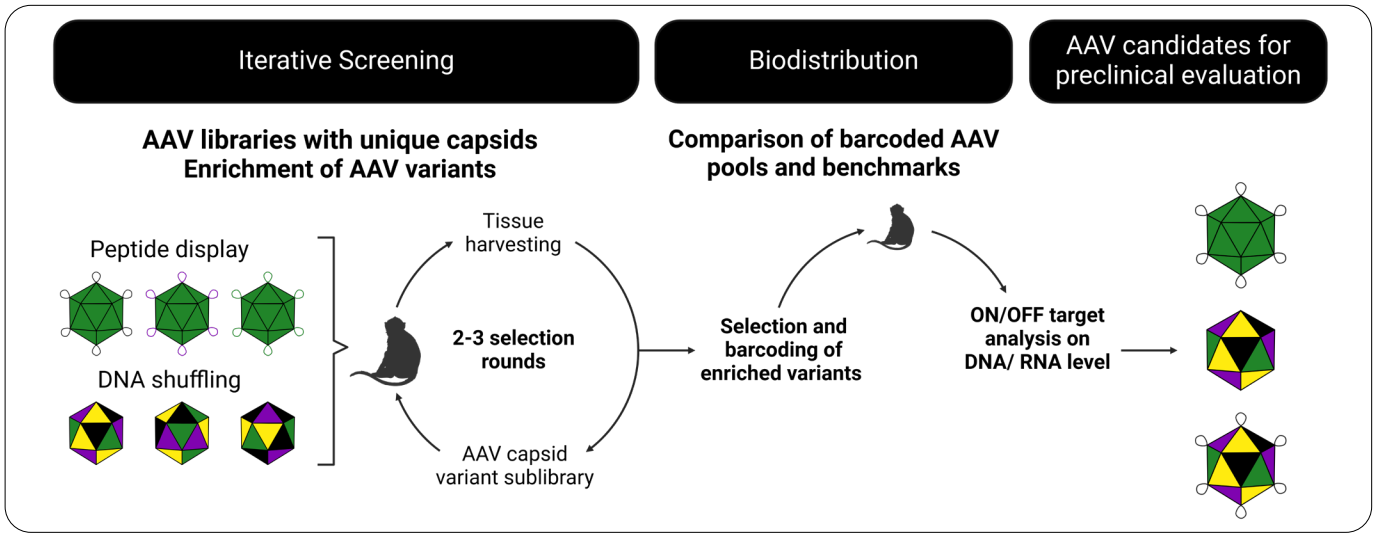

Figure 1: Screening of AAV libraries to identify novel vector candidates. Created with BioRender.com.

Conclusion

AAV vector engineering using evolutionary approaches, such as peptide display and DNA family shuffling, is transforming gene therapy by overcoming the limitations of natural AAV serotypes. These advanced techniques enable the development of tailored vectors with improved tissue specificity, altered immune interactions, and enhanced transduction efficiency. By leveraging iterative screening and high-throughput biodistribution studies, researchers can identify and refine the most promising candidates for their specific therapeutic applications.

As AAV-based gene therapies continue to evolve, scalable and reproducible vector manufacturing processes are essential to support clinical translation. Optimized production workflows using suspension cell systems, combined with advanced downstream purification techniques, help to ensure the delivery of high-quality AAV batches. Further optimization approaches can enhance yields, supporting more efficient and cost-effective vector development.

At Revvity, we provide comprehensive support across the entire AAV development pipeline—from vector engineering and high-throughput screening to optimized large-scale manufacturing. Drawing on our track record of innovation, deep expertise, and partnership network, we can partner with you to develop novel AAV vectors that can help to accelerate your gene therapy's journey to the clinic.

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