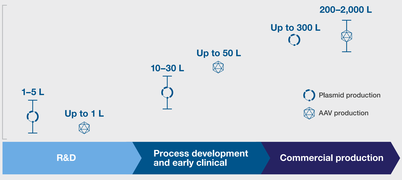
Raw material demands for gene therapy development

To facilitate a smooth transition from research and development (R&D) to commercial production, AAV gene therapy developers should establish manufacturing processes that can be reproduced across different scales. It will be important to understand the volumes of plasmid and vector needed at each stage of development, as well as the required quality of any incoming raw materials.

Required volumes of plasmids and AAV



Product quality attributes: the mark of a successful process

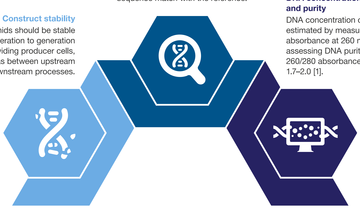
There are certain quality attributes that gene therapy developers should be looking for in their plasmids and AAV, and these attributes should be carefully monitored throughout scale-up.

Plasmids

DNA identity and homogeneity
Restriction mapping and DNA sequencing is used to confirm successful incorporation of the gene of interest and a complete sequence match with the reference.

Construct stability
Plasmids should be stable from generation to generation of dividing producer cells, as well as between upstream and downstream processes.

DNA concentration and purity
DNA concentration can be estimated by measuring the absorbance at 260 nm. When assessing DNA purity, an ideal 260/280 absorbance ratio is 1.7–2.0 [1].

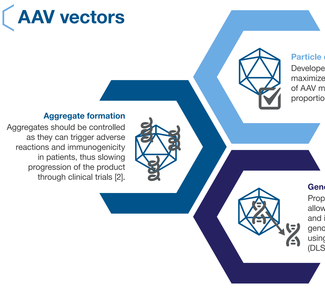


AAV vectors

Particle quality
Developers should also aim to maximize the yield and quality of AAV molecules, as well as the proportion of full capsids.

Aggregate formation
Aggregates should be controlled as they can trigger adverse reactions and immunogenicity in patients, thus slowing progression of the product through clinical trials [2].

Genome release
Proper capsid uncoating will allow the DNA to be released and integrated into the host genome, and can be measured using dynamic light scattering (DLS) [3].



Choosing a raw materials supplier

To maximize the chances of success and delivery of the desired plasmid and AAV quality attributes, developers should consider these three things when choosing a raw materials supplier:

Supply assurance

Expedited clinical and regulatory pathways have compressed timelines. Choosing a vendor with strong supply chains helps prevent potentially costly stoppages, which could result in disrupted clinical trials, lost CDMO slots, or downstream knock-on effects.

GMP validation

AAV vectors are highly complex molecules. Be sure to choose a supplier that can deliver raw materials that have been developed and documented via validated processes demonstrated by an ISO certification—these can help provide consistency and process control, thereby reducing variability and risk.

Raw material quality

Impurities from incoming raw materials can disrupt performance and lead to additional qualification steps. Opt for a supplier with a raw materials qualification program that carefully monitors variability and can deliver high-quality products.

GMP misconceptions

It is important to understand the differences between GMP-manufactured raw materials and GMP-manufactured end products. Raw materials are manufactured and tested under a quality management system that aims to maintain consistency between batches. However, for drug products, a stricter quality management system is expected and includes more extensive testing and control due to the proximity of products to patients (i.e., direct administration to patients).

References:

1. Thermo Fisher Scientific (2012) Interpretation of nucleic acid 260/280 ratios. https://tools.thermofisher.com/content/sfs/brochures/T123-nanodrop-lite-interpretation-of-nucleic-acid-260-280-ratios.pdf
2. Penaud-Budloo M, François A, Clément N et al. (2018) Pharmacology of recombinant adeno-associated virus production. *Mol Ther Methods Clin Dev* 8:166–180. https://doi.org/10.1016/j.omtm.2018.01.002
3. Cole L, Fernandes D, Hussain MT et al. (2021) Characterization of recombinant adeno-associated viruses (AAVs) for gene therapy using orthogonal techniques. *Pharmaceutics* 13(4):586. https://doi.org/10.3390/pharmaceutics13040586

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