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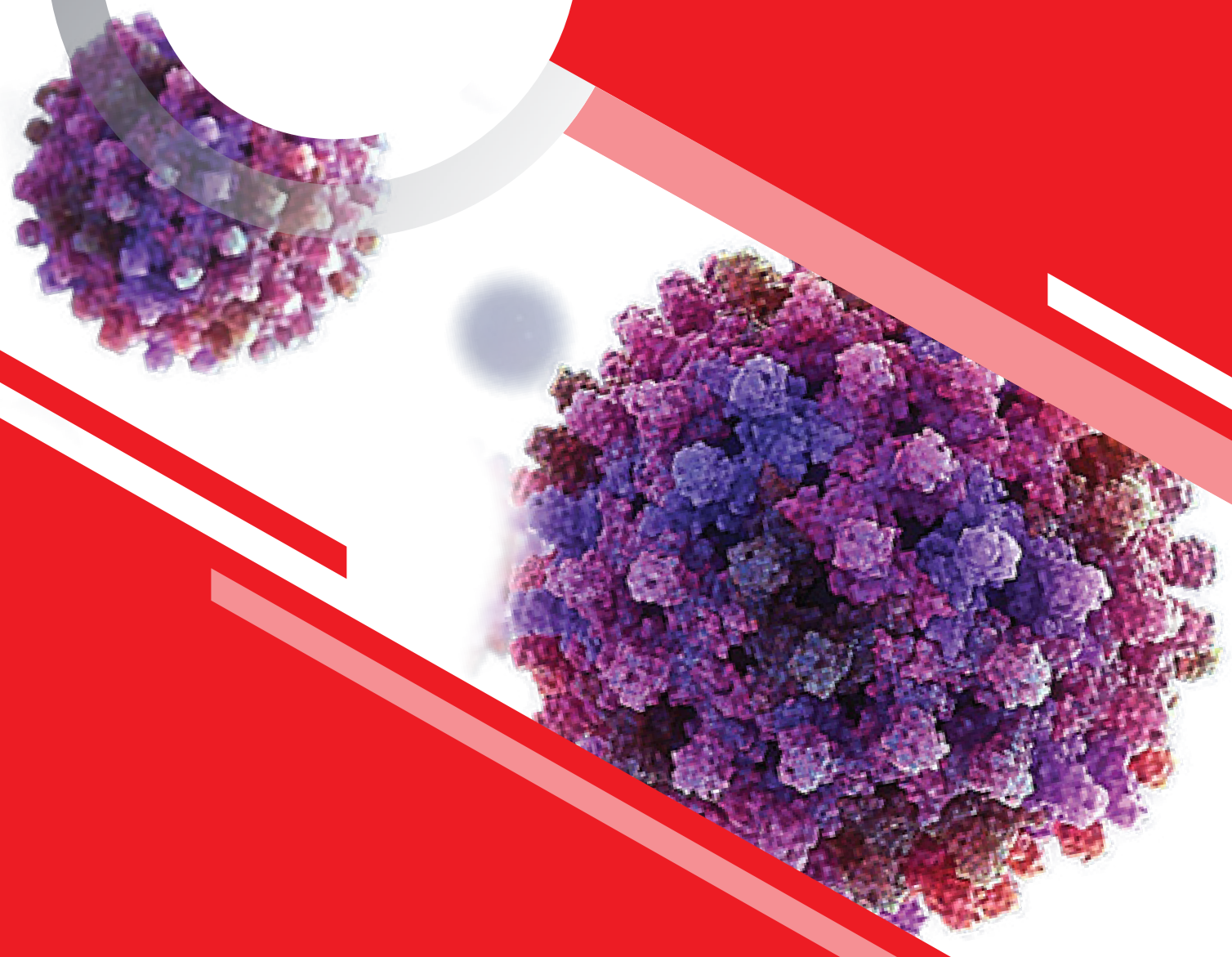

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GENE THERAPY WORKFLOW

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DEVELOPING A FINE-TUNED MACHINE

Gene therapy—modifying a person's genetic code for the benefit of human health[1]—is not a new idea, but it has recently advanced by leaps and bounds.[2] Current gene therapy approaches involve integrating corrective genetic sequences into cellular genomes for transcription and translation. This can be done in one of two ways: Genetic material is introduced into harvested or cultured cells *ex vivo*, which are subsequently re-introduced into the body. Alternatively, genetic material is directly introduced into the host, where it enters cells and integrates with the genome.[1] In either approach, exogenous genetic material must be able to cross cellular membranes.

Taking Advantage of Viral Vectors

Gene therapy hinges on the ability to transfer genetic material into cells. Viruses have an innate ability to accomplish this very task, so they are prominent cell modification candidates.[2] Scientists previously developed lentivirus and adenovirus vectors, but adeno-associated viruses (AAVs) have emerged as the most popular vector candidate in recent years. AAVs are generally accepted as the least immunogenic and least toxic viral vectors. They are also replication deficient, with no human pathology currently associated with their infection. Finally, they are highly versatile and have been demonstrated to be efficacious for the treatment of diseases across a wide range of tissues, including the heart, eyes, liver, and central nervous system.[1,2]

The Nuts and Bolts of AAV Production

The ability of AAVs to be used for clinical gene therapy applications relies on achieving sufficient viral vector yields to meet clinical demand. Viral vector generation is closely tied to gene therapy manufacturing capacity; generating viral vectors involves multiple stages and components, and optimizing production workflows is a meticulous process. An example of the complexity associated with viral vector production is the optimization of the plasmid transfection step. The genetic material that encodes viral proteins must be introduced into production cells—something commonly accomplished via multi-plasmid transfection.[3] Transfection efficiency is affected by plasmid design, transfection method, reagent composition, enhancers, and media. Low transfection efficiency typically translates to a decreased viral production yield.

These are all tied closely to the choice of production cell. Not only must it synergize with transfection strategy, but certain cells are—independent of other factors—simply better at producing certain viruses. Furthermore, inherent cellular properties such as metabolism, division rate, and lifespan can affect long-term production efficiency, and cell culture conditions such as environmental properties and media composition must be tailored to boost cellular productivity. HEK293 cells are currently the most popular within the scientific community for AAV production, largely due to their compatibility with suspension cell culture facilitating easy scale-up, but manufacturers use other cell types as well.[4]

Finally, viral vector production quality is just as important as quantity. In order to ensure the viral vector preparation is free from impurities that can be introduced from the manufacturing process itself, a robust quantitative analytical assessment workflow is needed that includes the detection of residual byproducts and reagents as well as an analysis of the final vector product.

Where to Seek Assistance

One of the main challenges of AAV production is the transition from early development to process development and clinical phases where a manufacturing process that can scale is needed.

Increasing production ten-fold is rarely as easy as simply multiplying everything in a workflow by ten. Scaling typically requires considerable optimization to identify how one might need to adapt an existing workflow to meet commercially relevant production targets. Fortunately, scientists have a wealth of support available to them. To address gaps and streamline the transition to clinical and commercial manufacturing, commercial entities such as Thermo Fisher Scientific have solutions specifically designed to improve production efficiency at scale. These solutions are supported by an extensive design optimization process that is validated for robustness and repeatability, taking the trial-and-error burden largely out of the scientists' hands.

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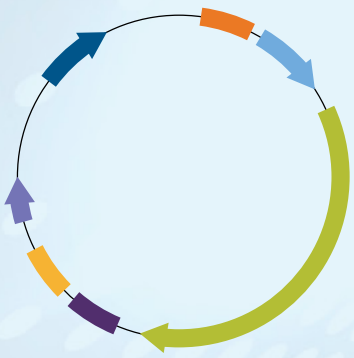
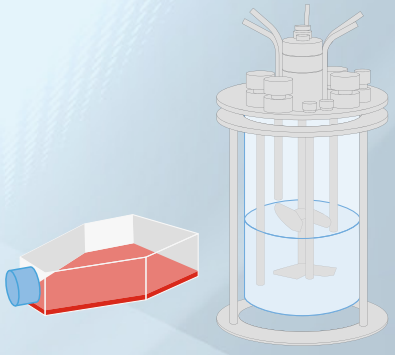
Producing an Adeno-Associated Virus (AAV) for Gene Therapy

AAV production for gene therapy is an involved process. Factors both upstream and downstream of cell-driven production can affect efficiency, consistency, and purity.



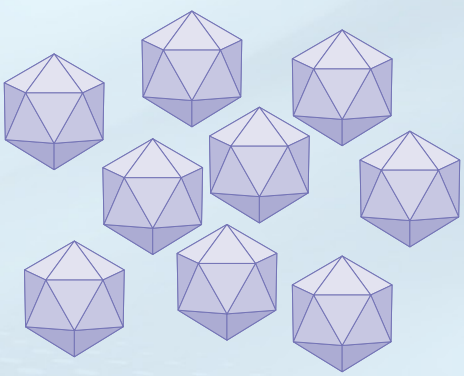
Step 1: cGMP-Grade Plasmids

- Plasmid DNA production is the first step in viral vector production.
- Consideration of plasmid design and quality attributes that are compliant with current regulatory requirements can help reduce costly adjustments and re-optimizations downstream.



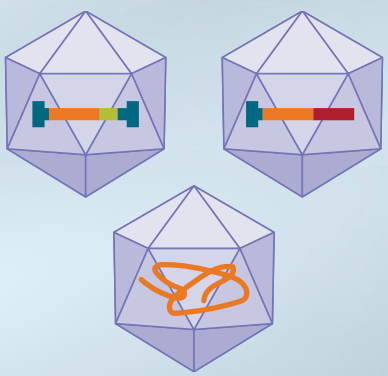
Step 2: Viral Vector Production

- Optimizing viral vector production can involve many variables including viral production cells, media, transfection reagents, supplements, and culture methods.
- Production cells adapted to suspension culture are critical when considering clinically and commercially relevant production yields, while adherent culture platforms can be suitable for research-scale applications.



Step 3: Viral Purification

- Affinity-based chromatography is a single-step technique for separating viral vectors from process- and product-related impurities.[1]
- Ion exchange chromatography using a high-resolution resin can separate empty and full capsids.[2]



Step 4: Viral Characterization and Analytics

- Partially-filled, empty, or aggregate viral capsids can decrease efficacy, necessitating higher clinical dosages and promoting immunogenicity.
- Researchers must examine each viral vector batch for contaminants, such as residual DNA and proteins.

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BUILD IT OR BUY IT?

Turning the discovery of a potential gene therapy target into a viable therapeutic agent is a long process—one that inevitably requires a regulatory-compliant dedicated manufacturing facility. To develop such facilities requires considerable time, energy, and capital, which is why scientists often look towards third parties to supply manufacturing capabilities—a dichotomy coined as the "build or buy" decision. To make this decision, researchers consider a number of factors concerning their gene therapy product, assess the resources available to them, and perform due diligence on what third party options are available.

Building It

Most gene therapy programs are born from discoveries made in small-scale research environments, whether academic or industry. To move from the bench to the clinic, scientists must shift their gene therapy products from these humble beginnings into large-scale manufacturing facilities. This journey comes with many hurdles: sourcing specialized equipment, hiring and training scientists and administrators, learning about manufacturing regulations, building out or locating an appropriate facility to house it all, and raising enough capital to afford all of the costs. However, a benefit for undertaking this journey is a layer of control. Researchers and entrepreneurs who construct their own gene therapy product manufacturing facility are able to oversee every detail of this process, such as protocols, instruments, or personnel training, giving them the ability to make immediate decisions and adjustments when necessary. "Building it" can also provide advantages when it comes to protecting intellectual property (IP).

Buying It

The alternative to "building it" is to seek out commercial third parties (contract development and manufacturing organizations; CDMOs) with established gene therapy product manufacturing capacity and expertise such as Thermo Fisher Scientific's Patheon brand. Leveraging the expertise of a third party and transferring your manufacturing needs to a CDMO removes the property, logistic, and personnel expenditures that come with outfitting a manufacturing facility. Third parties also offer knowledge in terms of meeting rigorous regulatory standards and meeting the varying needs dependent on whether you are in clinical or commercial stages. Finally, CDMOs are more flexible when it comes to absorbing market forces: they may already possess the necessary infrastructure for scaling up, while scaling down no longer results in sunk costs from idle equipment for the client scientist.

The Hybrid Approach

The "build it or buy it" question can be reassessed over time and may not be such a clearly delineated point. Capital and infrastructure availability, expertise and knowledge requirements, and product supply demands all change over time—as observed by the COVID-19 pandemic. Ultimately, scientists must make decisions based on a company's short and long-term needs and capabilities at a specific point in time.

A hybrid approach may offer the most flexibility. For a start-up, this may take the form of a partnership with academic centers for Phase I and II clinical trials, followed by a transition to CDMO manufacturing for Phase III trials and beyond. For an established biopharma company, a hybrid approach could encompass securing a CDMO partner as a back-up strategy to complement in-house manufacturing for existing products. This CDMO partnership could then be extended for additional products coming through the pipeline.

The ideal CDMO provides an end-to-end solution for all of a company's product-related needs, from plasmid and viral vector production, to product manufacturing, clinical supply, and distribution, with an option of flexibility to partner and effectively help you achieve your goals.

References

Article 1: Developing a Fine-Tuned Machine

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2. H.J. Wagner et al., "Synthetic biology: emerging concepts to design and advance adeno-associated viral vectors for gene therapy," *Adv Sci (Weinh)*, 8(9):2004018, 2021.
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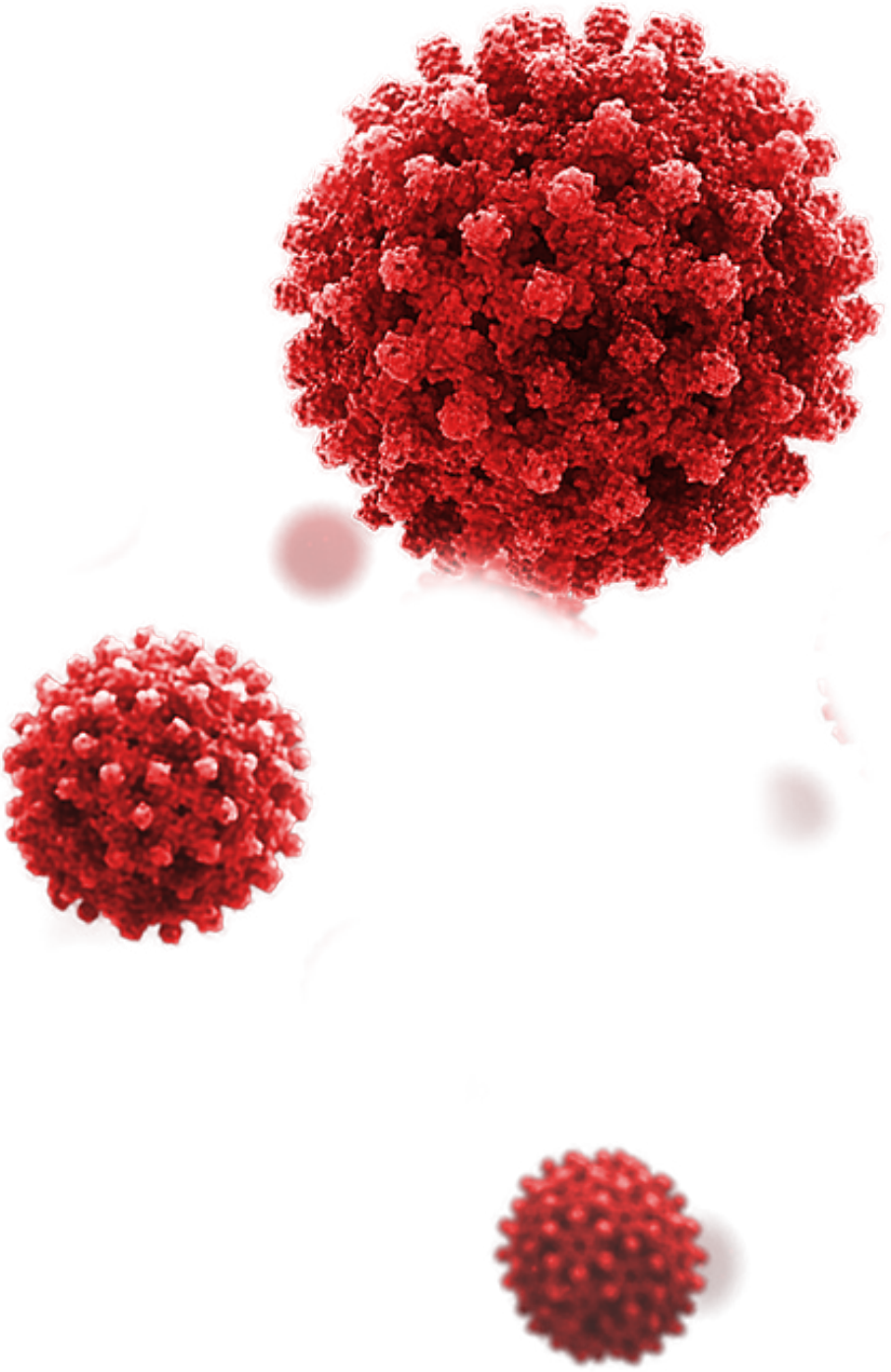

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