Early Plasmid Optimization For Long-Term Commercial Success

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The proliferation of targeted cell and gene therapies as first-line treatments for many rare and complex disease states has spurred demand for materials that meet the downstream needs for these applications. Plasmid DNA, purified from bacteria, serves several important roles in the biologics and cell and gene therapy spaces – from transfection to sequencing, cloning to PCR, plasmids have a ranging utility that has helped drive personalized medicine innovations.

Securing the right plasmid for a given application can be a complex endeavor. Between the wide variance that can occur in their development to the shifting regulatory standards that define their production, plasmids require the right expertise and experience to optimize their development for varying manufacturing paradigms.

Choosing the right plasmid can help prevent costly delays in the development life cycle. Core to this is selecting a supplier with the quality assurance protocols and good manufacturing practices (GMPs) in place to facilitate optimized plasmid production.

Finding the Right Plasmid: Considerations

Plasmid DNA – small, double-stranded DNA molecules independent from a cell's chromosomal DNA – is often a highly varied material, developed with unique considerations for bespoke pharmaceutical applications. In response to increased demand for pDNA to keep pace with personalized medicine innovations, contract development and manufacturing organizations (CDMOs) have begun pioneering a greater range of plasmids with more utility than ever before.

As with many aspects of the space, companies have trended toward securing the highest-quality plasmids available for a given application. This has largely been in response to evolving regulatory standards – many companies, keen on staying ahead of the curve, have sought out materials well above what is necessary for their process. As a result, many have begun to incur the cost constraints inherent to that approach. Right-sizing with a supplier able to meet evolving needs for a project along its development can help companies mitigate those costs and optimize productivity.

There are essentially three grades of plasmid DNA: research-, clinical-, and commercial-grade. But within these categories exist levels of nuance; commercial-grade plasmids that are intended to serve as raw materials, for example, possess regulatory standards much less stringent than for plasmids selected to serve as active pharmaceutical ingredients (APIs). That stringency is even greater for plasmids that function as a finished product. These varying degrees of regulatory rigor, dependent on a plasmid's intended use, result in compounding cost and time constraints for companies working toward return on investment.

In response to progress across the cell and gene therapy field, many biopharmas are working now to identify efficiencies in their manufacturing processes and create more sustainable, scalable production. As plasmids have become a more common component to these therapies, the FDA and other regulatory bodies have continued to iterate on their quality standards. Under existing regulations, there are no allowable contaminants for plasmids, which makes the purification processes fundamental to their manufacture a critical focal point for innovation. This consideration goes hand in hand with a plasmid's initial design – its functionality alone is insufficient to guarantee its scalability as part of a manufacturing paradigm. Companies must also consider other factors inherent to its design, including yield, transient transfection rates, and its capacity to stably transduce cell lines in downstream applications.

For companies at the proof-of-concept stage, these and other considerations, such as the antibiotic resistances of different plasmid backbones or how many generations a backbone is removed from its genesis, all serve to impact the yield and contamination profile of the pDNA. Decisions related to these considerations, made as early as possible, can help companies avoid the delays associated with reworking a plasmid that demonstrates suboptimal yield or a tendency toward contamination at later stages of development.

Optimizing Plasmid Design And Production

Poor plasmid design can lead to a range of issues, from issues regarding the integrity of inverted terminal repeats (ITRs) to partial transgene packaging and everything in between. There are a wide variety of factors, energetical-

ly, stoichiometrically, and chemically, that can impact this design. Ideally, companies can access the necessary data, either internally or in concert with a partner, to address each in turn – data that illustrate the issues that can influence backbone packaging, such as transgene size, or that support the selection of helper plasmids capable of reducing or virtually eliminating replication-competent adenovirus.

Finding a plasmid supplier able to support a project's vertical integration from early research grade through toxicology, clinical manufacturing, and commercialization is paramount to ensuring its success. Capacity is nearly as important a consideration as competency in this respect – plasmid suppliers that have invested in their capacity to meet market demand, as well as those able to demonstrate their ability to control lead times in the face of potential supply chain constraints, are invaluable to companies looking to scale a therapy commercially. Access to pDNA is one of the biggest bottlenecks in downstream biopharmaceutical manufacturing; instability in manufacturing or the identification of issues post-sequencing are perhaps bigger factors in this problem than capacity, but both are important considerations for biopharmas seeking to streamline their development efforts.

One of the best ways for companies to ensure this streamlining is by partnering with a plasmid supplier capable of transitioning alongside them through every phase of development. This lack of third-party intercession, coupled with a continuity in data collection, serves to close gaps, both in the time needed to scale up and the communication required to scale up the right way. Squaring the scale of manufacturing a plasmid against the yield profile of the commensurate vector manufacturing to determine how to produce enough material for each phase of development is a complex endeavor. Being able to communicate with a provider that can help companies understand their yield goals and the variables that can impact achieving them is key to avoiding the pitfalls that insufficient pDNA can have on the overarching manufacturing process.

Selecting The Right Partner For The Future

Traditional plasmid manufacturing has been around for a long time, and most plasmid manufacturers are working with a similar and well-codified process. But as gene therapies continue to proliferate, innovations to the status quo surrounding plasmid manufacturing are becoming more common. Things such as new resins introduced to the purification process, as well as single-use disposable fermenters in place of traditional stainless-steel ones, have helped minimize the potential for contamination. Similarly, the traditional approach to bacteria growth was to centrifuge bacteria in order to pellet it down; today, new technologies are starting to emerge that allow users to filter out media and replenish it with new media or solution that aids the downstream purification processing, as well.

There are even more promising developments on the horizon that portend even greater advancement for the space: innovations like the "doggy bone" DNA technology, a synthetic, rapid, cost-effective alternative to backbone plasmids that eliminates antibiotic resistance genes, which can represent a "contaminant" for plasmids due to their potential to introduce antibiotic resistance in patients. This and other developments that represent the next frontier of plasmid development are important milestones for the

space, but equally important is a commitment to the state-of-the-art: by streamlining and optimizing plasmid DNA in the short term, companies are well-positioned to reap compounding benefits as they scale their therapies.

Ultimately, partnering with a plasmid supplier that can scale alongside a therapy's development can save biopharmas time and money through standardized, connected protocols, comprehensive data aggregation, and in-house expertise. Companies should consider their plasmid and plasmid supplier as early as possible in the development pipeline; doing so in a way that accounts for the factors that can impact yield and stability, as well as the changing regulatory considerations that surround plasmids, can help biopharmas ensure their long-term commercial success in a rapidly evolving landscape.

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