NorthX Biologics

PLASMID DNA – THE RIGHT QUALITY FOR EACH APPLICATION

Background

With the tremendous advances in cell and gene therapies, and the unprecedented success of vaccines based on nucleic acid seen during the Covid pandemic, the need for high quality plasmid DNA have increased dramatically. For cell and gene therapy irrespective if the therapy is an ex-vivo gene therapy like CAR-T cells, or an in-vivo direct gene therapy mediated by viral vectors, the plasmids are transfected into cells to generate the protein that possess the immunogenic or therapeutic effect. Depending on the application, the plasmid DNA is considered a critical starting material or a drug substance with the accompanied differences in regulatory and quality expectations. Considering the importance of the plasmid DNA in providing the blueprint for the therapeutic protein, the need for a well-designed, phase appropriate control and testing strategy cannot be underestimated.

Choosing the right quality level for the different steps in the therapy development process is crucial to balance the costs for bringing the treatment to market and ensuring an effective and safe treatment.

Ever since the first plasmid manufacturing process the cost per gram of plasmid has been decreasing but still remains a major part of the cost of goods in the manufacturing of the drug product. For an AAV treatment, the most common approach is to utilize the proven three plasmid transfection system where one plasmid carries the transgene and the other two provide the sequences needed for the viral structural proteins and replication mechanism. This directly implies that to manufacture one AAV therapy you need to source at least three different plasmids of the right quality.

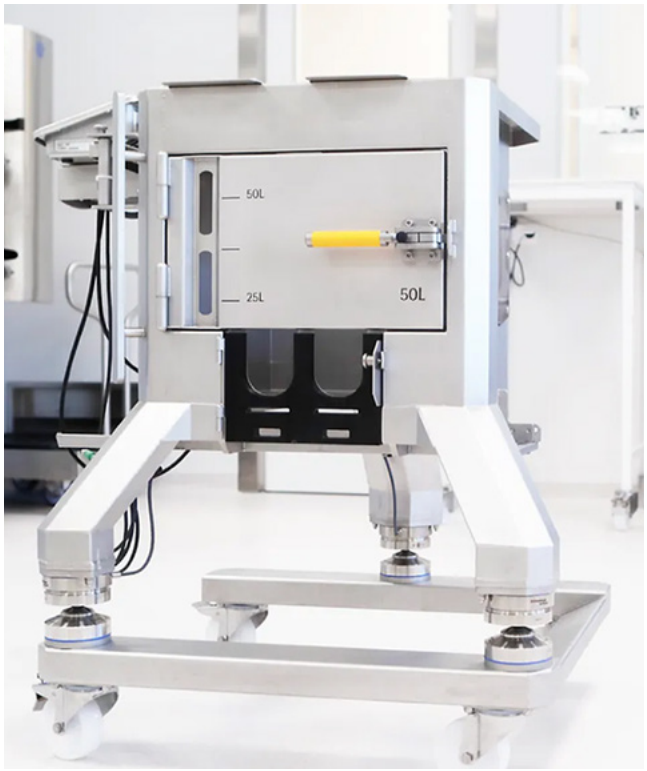
Several quality grades are available and, as expected, increased quality levels come with an increased price tag. Several factors contribute to the cost of plasmid DNA manufacturing, including the cost of raw materials, the cost of equipment, and the cost of labor. Choosing the right quality level, at the right time, will therefore be key to making the therapy more accessible to patients by not adding unnecessary costs too early in the program but still generating enough study data to support the progression into the next phase.

Plasmid Quality grades

For plasmid DNA three main quality grades are available, R&D grade, High Quality (HQ) grade and GMP grade. Some suppliers market additional versions of the less regulated quality classes making it crucial for the user to understand the requirement and needs for their specific application as well as understanding what is included in the service in terms of quality, testing and documentation.

R&D grade plasmid are commonly used for discovery and research applications and are not to be used in clinical applications. R&D grade is not regulated in any way and hence the variation between different suppliers in what is included can be vast. There are no guidelines for recommended specification or quality requirements, so R&D grade encompasses all plasmids not manufactured or tested under GMP or under the guidelines available for HQ plasmids. The R&D plasmids can be manufactured according to simple processes using "ready to use" kits or using full processes including multistep chromatographic purifications like the higher quality grades. Typically, the level of control and documentation is lower and normally several plasmids can be manufactured in parallel in the same lab to keep the costs within reason.

HQ plasmid is a "GMP-like" quality grade relevant for plasmids used as critical starting materials eg. for the manufacturing of viral vectors or as templates for mRNA drugs. The plasmids are normally fit for use in toxicology studies or for viral vector manufacturing in clinical phase I-II. There is no naming consensus of this quality class so different suppliers have decided on their own brand name which they in some cases trademark. The important attribute for these plasmids is that they offer a step up in quality from R&D grade but without adding the full cost of GMP manufactured products. Some of the quality elements typically added are the use of fully traceable raw materials, manufacturing according to predefined batch records, and segregated manufacturing suites where only one plasmid is processed at a time. Cleaning, product change-over and gowning procedures are described in SOP's and often single use processing equipment is adopted to minimize the risk for contamination and product carry over between batches. The establishment of the middle way between the non-regulated R&D grade and fully regulated GMP quality grades is the industry's answer to the guidelines available. Plasmid DNA for critical starting material is not required to be manufactured under a GMP license but current regulatory guidelines tell us that the plasmids shall be manufactured complying with the principles of GMP. The meaning of "principles of GMP" was received by the industry as a rather vague formulation opening up for individual interpretations. To answer this EMA in 2021 published a Q&A document explaining the regulators view explaining the expectations. →



GMP grade plasmids are plasmids manufactured to the highest quality grade in compliance with the guidelines stipulated by the relevant authorities. For the US, GMP is enforced by the FDA under the 21 Code of Federal Regulations and in Europe by EMA according to Eudralex Volume 4. A plasmid manufactured under GMP is produced in facilities approved by the authorities, and by organizations that have a QMS in full compliance with regulatory expectations. Key parts in this are the use of segregated manufacturing suites designed with logical material and personnel flows, environmental monitoring, qualified equipment, and phase appropriate validation of analytical methods. The personnel must have well-documented training and follow SOPs for all interventions and procedures. A Quality Assurance organization, independent from operations, oversees the manufacturing and testing, reviews all critical steps and in Europe, certifies the compliance to GMP for the release of the plasmid product via a Qualified Person (QP). Plasmid manufactured and tested under GMP is fit for all applications including lates stage and commercial viral vector manufacturing, direct administrated gene therapies and plasmid DNA vaccines.

Discussion

Choosing the right quality for the current stage and application of the program is crucial for not adding unnecessary cost or causing delays in making the important treatments available to the patients. R&D grade plasmids provide a cost-effective alternative normally with short turnaround times that are well suited for the early development work. The undefined character of R&D plasmids does however open the possibility for quality variation if the supplier is not chosen wisely. The use of R&D grade plasmids also implies that the developer is educated on what the critical quality attributes are. It is important to be aware and thoroughly check that the needed specifications are met to draw robust conclusions from the data generated. Even if the consensus in offering between suppliers is higher and there are regulations in place, a lot of this also holds true when it comes to the “GMP like” HQ grade plasmids. A variety of processes and analytical methods used are present and choosing too low a standard at this point may affect the possibility to use data in later phases without extensive comparability studies. It is therefore recommended that a developer looks for a partner that can offer high quality services within all three grades to avoid costly and time-consuming sourcing of the next quality grade plasmid. Identifying a supplier with extensive GMP experience within biological manufacturing normally paves the way for a successful long-term relationship even if the immediate need is for lower quality grades.

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NorthX Biologics is offering a comprehensive pDNA service ranging from R&D grade to GMP plasmids manufactured in our top modern facilities approved by the EMA. To ensure smooth transfer between the phases the processes and testing, although flexible, are aligned between the quality grades.

Table 1. Overview of NorthX Biologics plasmid DNA quality elements

Quality element	R&D	High Quality	GMP
Facility	Development Lab	Segregated CNC Production facilities with restricted access	Qualified GMP Clean Room Suites
Environmental monitoring	No	No	Yes
Raw materials	Research Grade	Raw materials aligned with GMP process Sourced from GMP approved suppliers	GMP grade raw materials, QC tested and released for manufacturing
Personnel	Trained	Documented process specific training	QA controlled documented process and GMP training
Equipment	Documented maintenance and calibration	Documented maintenance and calibration	Qualified equipment with documented maintenance/ calibration with QA oversight
Product change over	Standard lab cleaning	Procedural SOP controlled cleaning and product change over routines. Fully document-ed and traceable	Full GMP line clearance/ control and qualified cleaning and disinfection with QA oversight
Analytical testing	Flexible panel	Aligned with GMP and verified as needed QC issued CoA	GMP phase appropriate qualification of methods QC issued CoA with QA review and approval
Documentation	Raw data capture in laboratory notebooks Study report	Client approved Manufacturing Specification Batch Manufacturing Specification Operations review and approval	Client approved Manufacturing Specification Batch Manufacturing Specification QA review and approval
Quality Agreement	Not applicable	At client request (recommended for clinical use)	Mandatory
Product storage (cell bank, plasmid product)	Development lab	Qualified, monitored and alarmed	Qualified, monitored and alarmed
TSE/BSE certification	Possible but not standard	TSE/BSE certification	TSE/BSE certification

References

Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/chemistry-manufacturing-and-control-cmc-information-human-gene-therapy-investigational-new-drug

EudraLex The Rules Governing Medicinal Products in the European Union Volume 4 Good Manufacturing Practice Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products

https://health.ec.europa.eu/system/files/2017-11/2017_11_22_guidelines_gmp_for_atmps_0.pdf

Questions and answers on the principles of GMP for the manufacturing of starting materials of biological origin used to transfer genetic material for the manufacturing of ATMPs

https://www.ema.europa.eu/en/documents/other/questions-answers-principles-gmp-manufacturing-starting-materials-biological-origin-used-transfer_en.pdf

NorthXBiologics

A leader in new technologies

NorthX Biologics provides process development and manufacturing services with expertise in plasmids, proteins and other advanced biologics. We sit in the heart of Sweden, and our team has been manufacturing biologics to GMP since 1988. In 2021 we were recognized as a national innovation hub for advanced therapeutics and vaccines.

We take pride in adapting new technologies and different innovative platforms to our GMP processes. This enables development, upscaling and finally GMP manufacturing of innovative clinical material and supports our collaboration partners to help patients. We work closely with our staff and clients to provide solutions for biologics and ATMP's.

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