RegMedNet Spotlight

Addressing quality standards of plasmid DNA

Ensuring the quality of plasmid-based cell and gene therapy products

Plasmids are composed of DNA sequences that encode key proteins required for the production of cell and gene therapy products. As a critical starting component, plasmids must meet quality standards to ensure product safety and efficacy. There is, however, a lack of commercially available plasmid standards, both in physical reference materials and in documentary guidelines, leading to challenges in standardization such as:

- Limited guidance on control strategies
- Unharmonized production procedures
- Unstandardized analytical testing methods
- Variations of plasmid DNA specification among suppliers

Establishing clear guidelines is crucial for:

- Improving plasmid-based gene therapy products
- Assisting manufacturers in aligning quality standards of starting materials
- Ensuring the safety of gene therapy products

Ensuring quality: plasmid purification and testing

The quality of plasmid DNA has been shown to have a direct impact on product quality.

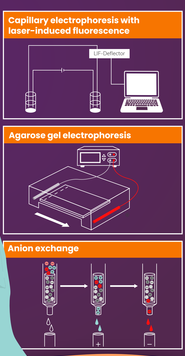
The conformation of the plasmid DNA is one of the most important quality attributes, with supercoiled plasmid being the most desired form.

Nicked **Supercoiled** **Linear**

Prior to production, manufacturers are required to assess the topology of their plasmid starting material to ensure it meets their quality standards.

The three most common methods for assessing plasmid topology are:

- Capillary electrophoresis with laser-induced fluorescence (CE-LIF)
- Agarose gel electrophoresis
- Anion exchange chromatography



Residual plasmid DNA as a process-related impurity

While plasmids are essential in early manufacturing, residual plasmid DNA is an unwanted impurity in the final therapeutic product. Residual plasmid DNA is known and shown to:

- Pose a safety risk
- Trigger unintended immune responses
- Transfer antibiotic resistance genes
- Integrate into a patient's genome

To minimize plasmid-related impurities, downstream purification processes, such as endonuclease treatment and chromatography, are commonly used to remove unwanted DNA fragments. However, to ensure the safety of the product, tests are required to analyze the amount of residual DNA in drug products. Typically, either quantitative PCR (qPCR) or digital droplet PCR (d(d)PCR) are used for this assessment.

Enhancing quality standards for gene therapy

To aid gene therapy manufacturers, USP has developed physical reference materials and a documentary reference standard. The physical reference materials will serve as standards for residual plasmid DNA quantification, qualification and plasmid topology assessment.

USP Standards for plasmid DNA

USP has developed the *General Chapter <1040> Quality Considerations of Plasmid DNA as a Starting Material for Cell and Gene Therapies*. This chapter was collectively drafted by group of experts to provide best practices on sourcing, qualifying and testing plasmid DNA.

Plasmid for Qualification (topology assessment)

- Six plasmids ranging in size from 4.2 kb to 12.3 kb, ensuring all customers have a plasmid that matches the size of the plasmids they use
- These reference materials can serve as controls to support manufacturers in the qualification and validation of different DNA topology methods, as each plasmid will be assessed by CE-LIF, Agarose Gel Electrophoresis and Anion Exchange Chromatography

Plasmid for Residual DNA Quantification

- Consists of linearized plasmid DNA with common target regions for residual plasmid PCR methods
- Serves as a positive control for dPCR and qPCR methods when quantifying residual plasmid DNA
- Functions as a standard curve calibrant in qPCR assays
- Well-characterized using multiple analytical methods, including dPCR, to ensure accuracy and reliability

Learn more about USP gene therapy solutions:

Gene therapy analytical guide
USP Biologics

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