Research Progress on Plasmid DNA Production Process and Quality Control

Jiaming Chen *

College of Life Science, Hunan Normal University, Changsha, China

* Corresponding Author Email: 18930272967@163.com

Abstract. Plasmid DNA, as the core material of the new generation of nucleic acid vaccines, has broad market potential, especially playing a critical role in gene therapy and mRNA vaccine development. With the rapid development of biotechnology, the production process and quality control techniques of plasmid DNA are continuously innovated and optimized. This paper reviews key technological advances in plasmid DNA production, including strategies for increasing plasmid yield, post-fermentation processing, impurity removal methods, and the purification process for high-purity supercoiled plasmid DNA. In addition, the quality control system of plasmid DNA production is discussed in detail. The plasmid DNA production process encompasses multiple steps, including fermentation, extraction, purification, and concentration, where optimization and innovation at each stage significantly contribute to enhancing production efficiency and product quality. Regarding quality control, key indicators such as purity, endotoxin absence, sequence integrity, and concentration are strictly monitored to ensure the safety and effectiveness of plasmid DNA. Simultaneously, the application of automated production and antibiotic-free selection marker systems has brought new technological breakthroughs to plasmid production. In the future, as global quality control standards are gradually unified, the application prospects of plasmid DNA in vaccine development, gene therapy, and personalized medicine will become more promising. By continuously optimizing production processes, reducing costs, and improving quality control standards, plasmid DNA will continue to play an essential role in the biopharmaceutical field, driving further advancement of related technologies.

Keywords: Plasmid DNA, Production process, Gene therapy, mRNA vaccine.

1. Introduction

Plasmid DNA (pDNA) is a unique circular double-stranded DNA capable of independent replication in host cells, thus playing a crucial role in genetic engineering, gene therapy, vaccine development, and other biotechnology and pharmaceutical fields [1]. Plasmid DNA is not only easy to manipulate and amplify but can also serve as a vector for foreign genes, widely applied in gene editing, gene expression regulation, and drug delivery [2]. In gene therapy, plasmid DNA is used as a vector to deliver therapeutic genes into target cells to correct genetic disorders. In vaccine development, plasmid DNA serves as the basis for DNA vaccines, with applications extending from traditional vaccine platforms to new infectious disease control, such as the development of COVID-19 vaccines [3]. Therefore, the large-scale production process and quality control of plasmid DNA are critical to its successful application.

In recent years, the rapid development of the biopharmaceutical field has significantly increased the demand for plasmid DNA, driving continuous progress in plasmid DNA production processes and quality control technologies. Plasmid DNA production involves multiple steps, such as fermentation, extraction, purification, and concentration, where technical optimization and process innovation at each step have a major impact on production efficiency, product purity, and cost-effectiveness [4]. Moreover, the quality of plasmid DNA is directly related to its safety and efficacy in applications, requiring strict quality control measures to ensure its purity, endotoxin-free status, sequence integrity, and high expression efficiency [5].

The main objective of this review is to comprehensively examine the production process and quality control methods of plasmid DNA, analyze important technological advancements in this field in recent years, and explore possible future development directions.

2. Plasmid DNA Production Process

2.1. Overview of Plasmid DNA Production Steps

The preparation of plasmid DNA consists of several relatively independent process units. In GMP production practice, these process units are optimized and combined to form a production workflow with good performance. The production steps of plasmid DNA typically include establishing plasmid sequences and seed banks, fermenting engineered bacteria, lysing bacterial cells, preliminary and fine purification of plasmid, concentration, sterile filtration, and storage.

2.2. Fermentation

Plasmid DNA is produced through bacterial fermentation to reduce costs and achieve scalability. Currently, new technologies for enzymatic synthesis of plasmid DNA can avoid the uncontrollable risks associated with biological fermentation, but these methods remain to be perfected. In the fermentation process of genetically engineered bacteria, plasmid replication occurs during bacterial proliferation and is passed on to subsequent generations. However, plasmids are not essential for bacterial survival and can easily be lost in the absence of selection pressure [6]. The fermentation process must be optimized according to the strain and plasmid type by controlling parameters such as culture temperature, pH, rotation speed, and aeration. DOE (Design of Experiments) optimization experiments can be used to optimize process parameters, and fed-batch fermentation can promote high-density fermentation, improving plasmid yield. During fermentation, it is also necessary to monitor the growth status of the bacterial strain and microbial contamination, as well as key plasmid indicators such as yield, supercoiled ratio, and sequence consistency.

2.3. Extraction and Purification

After fermentation, cells are typically collected using centrifugation or membrane filtration. In large-scale production, a 0.22 μm tangential flow hollow fiber microfilter membrane can be used to save costs and significantly shorten processing time.

Because ultrasonic disruption and high-pressure homogenization can damage plasmid conformation and break genomic DNA, increasing the difficulty of purification, and enzymatic lysis introduces foreign proteins during production, so alkaline lysis is generally used to lyse bacterial cells. In large-scale plasmid DNA alkaline lysis processes, factors such as reaction system, lysis time, lysis pH, and stirring conditions are key factors affecting plasmid yield and purity.

After plasmid DNA is released from the cells, centrifugation is used to separate the solid and liquid phases, recovering the plasmid from the solution. Appropriate centrifugation conditions should minimize damage to the supercoiled conformation of the plasmid while effectively achieving solid-liquid separation. In addition to centrifugation, low-shear filtration can be used to separate flocculated cell debris and genomic DNA from the plasmid DNA solution [7].

Before chromatographic separation of plasmid DNA, calcium chloride precipitation is typically used, followed by tangential flow filtration (TFF) to remove high-molecular-weight RNA and low-molecular-weight RNA [8, 9].

The separation of plasmid DNA uses three chromatographic steps based on different principles: size exclusion chromatography, ion exchange chromatography, and affinity chromatography [10-14], which achieve high-purity plasmid free of host DNA, RNA, proteins, and endotoxins. Size exclusion chromatography effectively removes RNA impurities based on molecular size, collecting the target plasmid DNA sample. Affinity chromatography purifies the plasmid by using ligands that specifically bind to supercoiled plasmid DNA, effectively removing linear and open-circular plasmid forms. Anion exchange chromatography efficiently removes proteins and endotoxins based on charge differences [14, 15].

2.4. Storage

The storage conditions of plasmid DNA significantly impact its stability and the reliability of experimental results. By controlling storage temperature, using appropriate buffer solutions, avoiding freeze-thaw cycles and light exposure, and maintaining neutral pH, the long-term stability of plasmid DNA can be effectively enhanced. Plasmid DNA can be stored in a refrigerator at 4°C for short-term use, suitable for a few weeks [16]. For long-term storage, it is recommended to store plasmid DNA at -20°C or -80°C to reduce the risk of degradation [17]. -80°C is the optimal choice, significantly extending the shelf life of plasmid DNA.

2.5. Emerging Production Technologies

2.5.1. High-Efficiency Fermentation Systems

As gene therapy technology advances, the demand for plasmid DNA, a core raw material, continues to increase. From the perspectives of both capacity expansion and cost control, optimizing fermentation processes and achieving high volumetric yields have become focal points. The current mainstream process is high-density fermentation using fed-batch strategies. This technology can control the substrate concentration in the reactor at a low level, reducing the production of harmful metabolites like acetic acid. Selecting an appropriate feeding strategy to regulate the flow rate of nutrient components controls bacterial growth rates, ensuring stable cell growth while enhancing the expression level of the target product [18-20].

2.5.2. Continuous Purification Technology

With the continuous development of biotechnology, there is an increasing demand for efficient and economical plasmid production methods. Continuous purification technology, due to its high efficiency and cost-effectiveness, is gradually becoming an important means of plasmid production. Continuous purification technology is a method for uninterrupted separation and purification of target molecules throughout the production process. This technology typically combines multiple separation techniques, such as membrane separation, chromatographic separation, and precipitation, to achieve efficient separation and purification through automation and continuous flow. Continuous purification technology has demonstrated promising applications in plasmid production, increasing yield, reducing costs, and improving purity to effectively meet the needs of modern biotechnology.

3. Quality Control of Plasmid DNA

3.1. Quality Requirements for Plasmid DNA

Key quality control indicators for plasmid DNA include purity, endotoxin content, sequence integrity, and concentration. The purity of plasmid DNA primarily reflects the levels of contaminating proteins and organic solvents (such as phenol, alcohol) and salts. When plasmid DNA is used for cell transfection or gene therapy, the presence of endotoxins may lead to cell reactions and inaccurate experimental results. The sequence integrity of plasmid DNA reflects whether the DNA sequence remains unchanged, which can be verified by amplifying specific regions of the plasmid using PCR and sequencing. The concentration of plasmid DNA directly affects transfection efficiency and cloning success rates. Monitoring these indicators effectively evaluates the quality of plasmid DNA, providing a reliable basis for subsequent work.

The quality requirements for plasmid DNA also vary depending on the application scenario. For gene expression, plasmids must have efficient gene expression capability and remain stable within host cells, preventing loss or mutation while ensuring high purity and appropriate concentration. For vaccine development, plasmids must be non-pathogenic and capable of inducing an effective immune response, while maintaining stability for large-scale production. In gene therapy, plasmid safety is of utmost importance, with plasmids needing to avoid mutagenic or insertion mutation risks while ensuring efficient delivery into target cells. Simultaneously, plasmids must maintain stable long-term expression within cells to ensure genetic stability is unaffected.

3.2. Common Quality Control Methods

3.2.1. Purity

Agarose gel electrophoresis can be used to separate DNA fragments and visually observe their integrity and purity. High-performance liquid chromatography (HPLC) is suitable for higher separation efficiency and quantitative analysis, helping evaluate the purity and concentration of plasmid DNA.

3.2.2. Sequence Integrity

Sanger sequencing is the gold standard for detecting plasmid DNA sequences, confirming whether mutations or deletions are present by sequencing and comparing with reference sequences. PCR amplification can also detect specific regions of plasmid DNA, with gel electrophoresis analyzing the size and specificity of the amplification product to verify the sequence integrity of the plasmid.

3.2.3. Endotoxin

The Limulus Amebocyte Lysate (LAL) test is the standard method for detecting endotoxins. When endotoxins are present, LAL activates enzymatic reactions that lead to gelation, color change, or luminescence, which can be quantitatively analyzed using appropriate instruments.

3.2.4. Host Impurity Residue

Enzyme-linked immunosorbent assay (ELISA), DNA probe hybridization, fluorescence staining, and quantitative PCR are the main methods for detecting host cell protein (HCP) residues. The United States Pharmacopeia (USP) only recommends quantitative PCR for detecting residual host DNA in biological products.

3.3. Quality Control Standards and Regulations

Multiple international standards and guidelines have been established for the production and quality control of plasmid DNA, particularly by the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA). The FDA requires that plasmid DNA production must follow Good Manufacturing Practices (GMP), covering facility design, equipment maintenance, and process control. All raw materials must undergo strict quality control to ensure they are sourced reliably and meet standards. Additionally, molecular biology techniques (such as PCR and sequencing) must be used to identify plasmids, assess plasmid purity, including the removal of host DNA, RNA, and proteins, and ensure that plasmids exhibit the expected biological activity, such as transfection capability.

The EMA places particular emphasis on risk assessment and management throughout the production process to ensure product safety, efficacy, and quality control. Process validation is crucial to ensure stability and reproducibility. The EMA also requires microbiological contamination testing, including bacterial and fungal limit tests, and functional testing of plasmids in cell lines or animal models to verify their biological efficacy. The World Health Organization (WHO) has also issued guidelines on gene therapy products, emphasizing that the production and quality control of plasmid DNA should comply with international standards to safeguard global public health.

4. Process Optimization and Innovation

4.1. Process Optimization in Plasmid Production

Selecting the appropriate strain is a key factor in improving plasmid yield in plasmid DNA preparation. High-copy plasmid strains should be prioritized, and recombinase-deficient (recA) and endonuclease-deficient (endA) strains such as DH5α, TOP10, and JM109 should be used whenever possible. In these strains, recombinase mutations reduce the probability of homologous recombination in the plasmid, ensuring the stability of inserted DNA, while endonuclease mutations inhibit the synthesis of functional active nuclease I, further improving plasmid DNA integrity.

The improvement of plasmid DNA yield is closely related to the feeding strategy. It is crucial to select an appropriate nutrient feed strategy based on plasmid expression characteristics. Carbon sources and nitrogen sources are common limiting substrates, with glucose and glycerol often chosen as the preferred carbon sources due to their fast absorption and low cost, while yeast extract and peptone are commonly used as organic nitrogen sources in Escherichia coli high-density fermentation. Properly controlling the feed rate of these limiting substrates is critical to the success of fermentation [20, 21].

Traditional plasmid purification methods, such as alkaline lysis and phenol/chloroform extraction, have been widely used over the past decades, but their efficiency and safety are often limited. In recent years, emerging technologies such as ultrafiltration, affinity chromatography, and magnetic bead methods have provided new options for improving plasmid DNA purification efficiency and quality. Therefore, when developing plasmid purification processes, it is important to optimize lysis parameters, chromatography parameters, and ultrafiltration processes to identify the most suitable process for maximizing plasmid production.

4.2. Application of Emerging Technologies in Plasmid Production

Traditional plasmid production often relies on antibiotic selection marker systems (e.g., ampicillin) to select transformed cells. However, this method carries the risk of spreading antibiotic resistance and may negatively impact downstream applications, such as gene therapy. Therefore, the development of antibiotic-free selection marker systems provides a new direction for plasmid production. These systems include suicide gene systems, metabolic selection markers, CRISPR/Cas9 systems, and auxotrophic complementation systems [22-25]. Antibiotic-free systems can reduce the safety risks associated with antibiotics, particularly in high-safety-requirement fields such as gene therapy and vaccine production.

Moreover, DNA synthesis technology, as an important support for life sciences and synthetic biology, is driving the rapid development of industrial biotechnology. As the advantages of industrial DNA synthesis technology in terms of throughput, cost, and speed become increasingly apparent, it shows great potential in meeting the growing demand for large-scale DNA. This technology will help improve research and development efficiency, reduce R&D costs, and promote the industrialization of plasmid DNA production.

4.3. Challenges in Large-Scale Production of Plasmid DNA

The transition of plasmid DNA from laboratory-scale to industrial-scale production faces multiple challenges, mainly in terms of production efficiency, cost control, quality assurance, and scalability. In laboratory-scale production, shaking flask cultures are typically used, while industrial-scale production requires large-scale fermentation. This transition necessitates optimizing culture medium, gas exchange, temperature, and pH control. Inappropriate culture conditions may result in reduced plasmid yield and DNA degradation. Therefore, industrial fermentation processes need to be closely monitored to ensure stable cell growth and plasmid expression. However, current monitoring technologies are not yet fully mature, making it difficult to evaluate key parameters in real-time during production.

Laboratory media are often unsuitable for large-scale production, especially those containing expensive animal-derived components, which are difficult to justify in industrial production due to high costs. Developing more economical media suitable for large-scale production is a key challenge. Additionally, the purification process of plasmid DNA often constitutes the most expensive step in industrial production. Traditional purification methods (e.g., phenol/chloroform extraction and centrifugation) are inefficient and costly on a large scale. Therefore, new purification methods (e.g., affinity chromatography and ultrafiltration technology) need further optimization to reduce industrial production costs.

Ensuring the high purity and integrity of plasmid DNA is critical in large-scale production. Contaminants such as proteins, RNA, and endotoxins can affect the efficacy of plasmids in downstream applications. However, unified quality standards and testing methods are still lacking. Furthermore, quality consistency issues between different production batches remain a challenge, potentially leading to poor product reproducibility. Therefore, establishing standardized quality control systems and enhancing process stability will be key challenges in large-scale plasmid production.

4.4. Application and Prospects of Automated Production Lines in Industrial Plasmid DNA Production

The application of automated production lines in industrial plasmid DNA production is gaining increasing attention. By introducing automated systems, plasmid production efficiency has been significantly improved, and product quality consistency has been better ensured. For example, some companies have developed small-scale automated platforms suitable for medium to small-scale plasmid DNA production, enabling efficient production under laboratory conditions. Large biopharmaceutical companies have established fully automated plasmid production lines, where the entire process from cell culture to purification is controlled by automated systems, greatly enhancing production efficiency and product quality.

Automated production lines have broad application prospects in industrial plasmid DNA production. Automation technology not only improves production efficiency and reduces costs but also ensures the stability and consistency of product quality. Therefore, automation will be an important direction for the future development of plasmid DNA production. Throughout this process, technological innovation and interdisciplinary collaboration will continue to drive the advancement of plasmid production technology, further expanding its application potential in industrial-scale production.

5. Prospects and Challenges

5.1. Future Trends in Plasmid DNA Production Technology

Since the discovery of the DNA double helix structure in the 1950s, the chemical structure of DNA and the genetic information it carries have gradually been deciphered. The plasmid DNA extraction and preparation process has undergone several iterations, with production equipment becoming increasingly automated and intelligent. Some companies have already introduced semi-automated production equipment.

Currently, plasmid biosynthesis technology still requires improvement, and economic costs need to be further reduced. Once its advantages become evident, the future production of plasmid DNA will gradually shift from traditional fermentation and purification routes to biosynthetic routes, eventually achieving automation. In a toxin-free and RNA-free environment, plasmid DNA can be produced, further enhancing its safety.

5.2. Global Standardization of Quality Control

The widespread application of plasmid DNA in biopharmaceuticals, gene therapy, and synthetic biology demands standardized global quality control standards. However, differing quality control standards across countries may affect plasmid production processes, product quality, and market access strategies. Understanding these differences is crucial for companies' production strategies and international market expansion.

Strict standards in the United States and Europe often require more verification steps and compliance efforts, increasing the complexity of production processes. More time and resources are needed to optimize and adjust workflows. These differing quality standards may affect raw material selection and, in turn, production costs and efficiency. Furthermore, differences in regulatory approval processes between countries may result in significant variations in product registration times across different markets, affecting market promotion strategies and business decisions. Additionally, differing regulations across countries may make it challenging to ensure product quality consistency, thereby impacting the safety and efficacy of clinical applications.

Unified global quality control standards will help reduce trade barriers between countries and regions, increase international market interoperability, and enhance companies' global competitiveness. Standardization can also improve internal management efficiency and product quality within companies, increasing consumer trust in products. Furthermore, unified global quality standards can promote technology sharing and innovation, particularly in the biotechnology and pharmaceutical industries, where standardized regulations can drive technological progress across the entire industry.

However, achieving global standardization of quality control also faces many challenges. Cultural and institutional differences between countries complicate standardization in quality control practices and legal regulations. Economic disparities may place developing countries under significant economic pressure to implement high-standard quality control, affecting the universal acceptance of these standards. Additionally, rapid technological advancements may outpace the development and updating of quality control standards. Varying expectations and needs among companies, governments, and consumers further complicate the establishment of unified standards.

5.3. Future Demand in Plasmid DNA Applications

As fields like gene therapy and vaccine development continue to progress, plasmid DNA, as a key tool, is gaining widespread market attention. Plasmid DNA boasts advantages such as simple preparation processes and fast replication, making it widely used in basic experiments like gene cloning, sequencing, and site-directed mutagenesis, as well as in high-end applications such as gene therapy and immunotherapy. In recent years, plasmid DNA manufacturing services have experienced unprecedented growth, with more and more companies offering specialized, customized plasmid preparation services to meet the demand for high-quality plasmid DNA from research institutions and the biopharmaceutical industry.

According to market research institutions' forecasts, the plasmid DNA manufacturing services market will continue to grow rapidly. As the biopharmaceutical industry develops and market competition intensifies, the demand for high-quality plasmid DNA will continue to rise. Plasmid DNA manufacturers must continuously improve their technological capabilities and service levels to meet the market's demand for high efficiency, low cost, and high-quality plasmid DNA.

Traditional drugs primarily target common diseases and have broad applicability. However, many rare disease patients are few in number, and existing drugs often cannot meet their treatment needs. Personalized medicine offers new hope for these patients, enabling treatment plans tailored to their genetic information and significantly improving treatment precision. However, the successful implementation of personalized medicine depends on the seamless integration of innovative technologies and faces significant sustainability challenges [26]. The high cost and long development cycle of traditional drugs make it difficult to meet the needs of personalized medical interventions. In contrast, plasmid DNA offers a rapid and cost-effective solution for developing personalized medicines due to its short preparation cycle and low cost. The combination of plasmid DNA and mRNA drugs has demonstrated significant potential in advancing personalized medicine for cancer and tumor treatment [27].

Nevertheless, drug approval still requires substantial safety and efficacy data, which often increases the development time and costs for personalized medical products. Therefore, accelerating drug development, improving drug safety and efficacy, and meeting the needs of personalized medicine will be key research areas for future plasmid DNA applications.

6. Conclusion

Plasmid DNA plays a crucial role in gene therapy, vaccine development, and recombinant protein production in the biopharmaceutical field. With the rapid advancement of biotechnology, the production process and quality control technologies of plasmid DNA are continuously being improved and researched in greater depth. To meet the growing market demand, optimizing the construction, amplification, and purification processes of plasmids has become key to enhancing production efficiency and product quality.

The construction process of plasmids typically involves the cloning and amplification of DNA fragments, using methods such as restriction enzyme digestion, ligation, and PCR amplification. Choosing the right vector and promoter is essential for the stable expression of plasmids and subsequent purification. During plasmid production, transfection or transformation is used to introduce plasmids into host cells (such as Escherichia coli, yeast, etc.), followed by batch, continuous, or fermentation culture for cell amplification. To further increase plasmid yield, it is necessary to optimize the culture medium composition and culture conditions. Common plasmid extraction methods, such as alkaline lysis, affinity chromatography, and precipitation methods, aim to minimize cell impurities and DNA degradation during extraction to ensure the purity and quality of the product meet expected standards.

Plasmid DNA purity is an important aspect of quality control, typically evaluated through methods like gel electrophoresis, spectrophotometry, and high-performance liquid chromatography (HPLC). High-purity plasmids can significantly improve the success rate and effectiveness of downstream experiments. The concentration of plasmid DNA is another key indicator, with common measurement methods including UV spectrophotometry and fluorescence assays. These methods must consider the optical properties of the sample and the interference from impurities. Additionally, the integrity and correctness of plasmid construction are typically evaluated using restriction enzyme analysis and real-time PCR, ensuring their effectiveness in gene expression. For plasmid DNA used in drugs or vaccines, it is also essential to assess its biological activity and safety, with transfection efficiency experiments used to test the expression capacity and functional performance of plasmid DNA in cells.

As a key raw material, plasmid DNA demonstrates significant advantages in the development of innovative vaccines, such as DNA vaccines and mRNA vaccines. These advantages include short development cycles, low dosage, high safety, simple production, and good cost-effectiveness. In the future, plasmid DNA can be used to introduce foreign genes encoding antigenic proteins into host cells to induce a robust immune response or to introduce therapeutic genes into patients to repair, replace, or regulate defective genes, achieving the goal of disease treatment.

In the field of cell therapy, plasmid DNA also plays an important role. By modifying gene expression within cells, plasmid DNA can enhance cell function or impart new properties to cells, allowing it to be used in the treatment of various diseases. For example, in CAR-T cell therapy, plasmid DNA is used to construct CAR gene expression vectors, enabling T cells to recognize and kill tumor cells. This innovative technology offers new hope for cancer treatment.

In conclusion, the production process and quality control of plasmid DNA will continue to play an important role in the future development of biopharmaceuticals. As technology continues to innovate, its application prospects are broad, particularly in the fields of vaccine development, gene therapy, and cell therapy. By continuously optimizing production processes and improving quality standards, plasmid DNA production and applications will provide a stronger foundation for the development of the biotechnology field.

References

[1] Prather, K., Sagar, S., Murphy, J., & Chartrain, M. (2003). Industrial scale production of plasmid DNA for vaccine and gene therapy: Plasmid design, production, and purification. Enzyme and Microbial Technology, 33 (7), 865-883. https://doi.org/10.1016/S0141-0229 (03)00205-9.

[2] Sousa, F., Passarinha, L., & Queiroz, J. (2009). Biomedical application of plasmid DNA in gene therapy: A new challenge for chromatography. Biotechnology and Genetic Engineering Reviews, 26(1), 116-183. https://doi.org/10.5661/bger-26-83.

[3] Schmeer, M., Buchholz, T., & Schleef, M. (2017). Plasmid DNA manufacturing for indirect and direct clinical applications. Human Gene Therapy, 28(10), 856-861. https://doi.org/10.1089/hum.2017.159.

[4] Przybylowski, M., Bartido, S., Borquez-Ojeda, O., Sadelain, M., & Rivière, I. (2007). Production of clinical-grade plasmid DNA for human Phase I clinical trials and large animal clinical studies. Vaccine, 25 (27), 5013-5024. https://doi.org/10.1016/j.vaccine.2007.04.077.

[5] Carnes, A., & Williams, J. (2014). Plasmid fermentation process for DNA immunization applications. In J. A. Williams (Ed.), Methods in Molecular Biology (Vol. 1143, pp. 197-217). Springer. https://doi.org/10.1007/978-1-4939-0410-5_13.

[6] Chen, X. (2022). Exploration of the adaptation and stability evolution mechanism of tet (X4)-positive plasmid in bla_(NDM-5) and mcr-1 multiple drug-resistant Escherichia coli [Doctoral dissertation, Yangzhou University]. https://doi.org/10.27441/d.cnki.gyzdu.2022.001127.

[7] Progress in Microbiology and Immunology. (2006). Prog in Microbiol Immunol, 34 (2).

[8] Eon-Duval, A., Gumbs, K., & Ellett, C. (2003). Precipitation of RNA impurities with high salt in a plasmid DNA purification process: Use of experimental design to determine reaction conditions. Biotechnology and Bioengineering, 83 (5), 544-553.

[9] Eon-Duval, A., MacDuff, R. H., Fisher, C. A., & Harris, M. J. (2003). Removal of RNA impurities by tangential flow filtration in an RNase-free plasmid DNA purification process. Analytical Biochemistry, 316 (1), 66-73.

[10] Sun, B., Yu, X., Yin, Y., Liu, X., Wu, Y., Chen, Y., Zhang, X., Jiang, C., & Kong, W. (2013). Large-scale purification of pharmaceutical-grade plasmid DNA using tangential flow filtration and multi-step chromatography. Journal of Bioscience and Bioengineering, 116 (3), 281-286.

[11] Latulippe, D. R., & Zydney, A. L. (2009). Size exclusion chromatography of plasmid DNA isoforms. Journal of Chromatography A, 1216 (30), 6295-6302.

[12] Eon-Duval, A., & Burke, G. (2004). Purification of pharmaceutical-grade plasmid DNA by anion-exchange chromatography in an RNase-free process. Journal of Chromatography B, 804 (2), 327-335.

[13] Bo, H., Wang, J., Chen, Q., Shen, H., Wu, F., Shao, H., & Huang, S. (2013). Using a single hydrophobic-interaction chromatography to purify pharmaceutical-grade supercoiled plasmid DNA from other isoforms. Pharmaceutical Biology, 51 (1), 42-48.

[14] Cernigoj, U., & Štrancar, A. (2021). Scale-up of plasmid DNA downstream process based on chromatographic monoliths. In N. Luza (Ed.), DNA Vaccines: Methods and Protocols (pp. 167-192). Springer.

[15] Parker, T.; Cherradi, Y.; Mishra, N. Scalable Purification of Plasmid DNA: Strategies and Considerations for Vaccine and Gene Therapy Manufacturing; Application Note MS-WP7159EN Ver; Millipore Sigma: Burlington, MA, USA, 2020; Volume 1.

[16] Sambrook, J., & Russell, D. W. (2001). Molecular cloning: A laboratory manual (3rd ed.). Cold Spring Harbor Laboratory Press.

[17] Wilfinger, W. W., Mackey, K., & Chomczynski, P. (1997). Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Biotechniques, 22(3), 474-478.

[18] Schmidt, T., Schleef, M., Friehs, K., & Flaschel, E. (2006). Hochzelldichtefermentation zur Gewinnung von Plasmid-DNA für Gentherapie und genetische Impfung. BIOforum.

[19] Chen, W., Graham, C., & Ciccarelli, R. (1997). Automated fed-batch fermentation with feedback controls based on dissolved oxygen (DO) and pH for production of DNA vaccines. Journal of Industrial Microbiology and Biotechnology, 18(1), 43-48.

[20] Carnes, A. E., Hodgson, C. P., & Williams, J. A. (2006). Inducible Escherichia coli fermentation for increased plasmid DNA production. Biotechnology and Applied Biochemistry, 45 (3), 155-166.

[21] Liu, J., & Zhang, Q. (2004). Advances in large-scale production of plasmid DNA. Biotechnology Communication, 4, 409-413.

[22] Chen, C.-C., Nien, C.-J., Chen, L.-G., Huang, K.-Y., Chang, W.-J., & Huang, H.-M. (2019). Effects of Sapindus mukorossi seed oil on skin wound healing: In vivo and in vitro testing. International Journal of Molecular Sciences, 20 (7), 4178. https://doi.org/10.3390/ijms20174178.

[23] Haenseler, W., Sansom, S. N., Buchrieser, J., Newey, S. E., Moore, C. S., Nicholls, F. J., Chintawar, S., Schnell, C., Antel, J. P., Allen, N. D., Cader, M. Z., Wade-Martins, R., James, W. S., & Cowley, S. A. (2017). A highly efficient human pluripotent stem cell microglia model displays a neuronal-co-culture-specific expression profile and inflammatory response. Stem Cell Reports, 8 (6), 1727-1742. https://doi.org/10.1016/j.stemcr.2017.05.017.

[24] Khan, S., Mahmood, M. S., Rahman, S. U., Zafar, H., Habibullah, S., Khan, Z., & Ahmad, A. (2018). CRISPR/Cas9: The Jedi against the dark empire of diseases. Journal of Biomedical Science, 25 (1), 29. https://doi.org/10.1186/s12929-018-0425-5.

[25] Dong, W. R., Xiang, L. X., & Shao, J. Z. (2010). Novel antibiotic-free plasmid selection system based on complementation of host auxotrophy in the NAD de novo synthesis pathway. Applied and environmental microbiology, 76 (7), 2295-2303.

[26] Beccia, F., Hoxhaj, I., Castagna, C., Strohäker, T., Cadeddu, C., Ricciardi, W., & Boccia, S. (2022). An overview of personalized medicine landscape and policies in the European Union. European Journal of Public Health, 32 (6), 844-851.

[27] Goetz, L. H., & Schork, N. J. (2018). Personalized medicine: Motivation, challenges, and progress. Fertility and Sterility, 109 (6), 952-963.