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mRNA as novel active ingredient Ã¢â?¬â?? PlasmidFactory providing the template for tomorrow's vaccines

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PlasmidFactory GmbH was founded in 2000 in Bielefeld/Germany with 4 employees. In the meantime, under the founder and managing director Dr. Martin Schleef, the company has become a well-known contract manufacturer (CDMO) for plasmid and minicircle DNA. Today, PlasmidFactory has ~50 highly qualified employees, and has established a GMP manufacturing facility.



Q: The usage of messenger RNA (mRNA) as novel active ingredient has attracted public attention in recent years, and it has become clear that it has profoundly changed biotechnology. How did this come about?

A: RNA, especially mRNA, has shown its capability to catalyze the development of advanced therapeutics, including engineered proteins, next-generation vaccines, and novel pharmaceuticals, whereby its versatility is unmatched, with its potential applications spanning infectious diseases, oncology, and rare genetic disorders. Besides the platform's inherent adaptability, its scalability facilitates rapid production and deployment, particularly critical during pandemic responses or

personalized pharmaceuticals.

These properties streamline the preclinical and clinical development phases, accelerate the path from laboratory to bedside and make mRNA an extremely valuable drug with great potential that has not yet been exhausted.

Q: Usage of RNA as new active ingredient represents a paradigm shift in modern medicine. Especially the deployment of mRNA vaccines against SARS-CoV-2 has underscored their transformative potential in vaccinology. What are the main components and what are the advantages?

A: RNA-based vaccines are genetic vaccines, often based on mRNA molecules that have been encapsulated in a lipid shell for both stability and efficiency of membrane transport. Though initially thought to be limited by its instability, RNA vaccines have improved over the years due to important innovations such as nucleoside modifications, better purification strategies, modifications to the untranslated regions, and better delivery through lipid nanoparticles. Even though RNA vaccine manufacturing is a cost-intensive in vitro process, it has been possible to optimize it to be more productive. The processivity of RNA polymerases makes it possible to yield mRNA products several orders higher than the input of template plasmid DNA.

Generally, the DNA from the template plasmid is transcribed *in vitro* into mRNA. mRNA contains cis-acting structures, namely, 7-methylguanosine cap and polyadenylate tail along with other sequence motifs. These structures are crucial for translation initiation and elongation, and they can be targeted to enhance RNA expression efficiency. The novel mRNA vaccines developed during the last pandemic showed excellent effectiveness in combating COVID-19, thus definitely ushering in the next generation in vaccine technology.

The manufacture of mRNA is a multistep in vitro process that depends on multiple enzymes and intermittent purifications. Finally, the mRNA molecules must be modified for stability and enhanced expression (cap and tail) and then formulated within its special lipid capsule which acts as an efficient vehicle for delivery into the cells. Such mRNA-based vaccines offer the advantage that they never enter the nucleus in the cells of the recipient. They do not integrate into the chromosome and are degraded in time after expression has taken place. This offers transient expression of the antigenic protein without any undesirable consequences of long-term continued expression.

Q: The demands on mRNA are high, and the same applies to the starting material. Are there hurdles in the production process? Which are the most significant, and what are their consequences?

A: Crucial to the efficiency and scalability of this process is the availability of sufficient amounts of the starting material, plasmid DNA, in the necessary quality. Any errors in the plasmid sequence, especially in the promoter region, the coding sequence or in the polyA homopolymer sequence element, will have consequences for the quality of the produced RNA. Single strand breaks in the plasmid DNA sequence due to degradation will result in reduced efficiency of *in vitro* transcription. This is e.g. manifested in the generation of mRNAs of shortened length. Recombination events during plasmid replication leading to a loss or shortening of the polyA homopolymer sequence stretch results in downstream production of shortened unstable mRNAs. All these basically lead to a possibility of inhomogeneous mRNA population and therefore represent a quality risk in biopharmaceutical production.

Q: So, depending on the application, the starting material must be of very high quality and fulfill all requirements. How can this be guaranteed?

At PlasmidFactory, we offer different quality grades, tailored to the customer's various applications. Thus, we ensure that individual requirements can be fully met.

Our Research grade quality is a kit alternative for basic research based on a fermentation workflow. This cost-efficient option is recommended for early-stage research.

The CCC grade quality provides optimized, highly efficient DNA for basic research, pre-clinical and toxicology studies. The DNA provided in this quality grade consists of ? 95% ccc-form, the only intact and most efficient plasmid topology present in E. coli bacteria used for high yield production.

High Quality (HQ) grade DNA is produced based on a cell bank (RCB) created at PlasmidFactory and the uniquely effective proprietary ccc Grade DNA technology. For both the cell bank and the DNA product, PlasmidFactory offers a wide range of in-process and quality controls, so a product is ultimately created that is tailor-made for the respective application. HQ grade DNA meets the requirements for use as a starting material for GMP production of RNA for clinical applications.

The production process is supported by PlasmidFactory's proprietary POLYARESCUE® technology, which can amplify plasmid DNA containing long (> 120A) polyA stretches, which is of highest value when regarding mRNA applications. Moreover, our Next Generation Sequencing technology complements the optimized fermentation process, allowing sequencing and quantification of polyA sequences while maintaining intact plasmids, thus leading to homogenous products for efficient mRNA production.

For product safety reasons, only raw materials with TSE/BSE certificates are used throughout the entire production chain. Moreover, the highest possible product purity is guaranteed through reliable separation of impurities, e.g., bacterial chromosomal DNA or damaged plasmids. To prevent further contamination, only one plasmid is produced at a time in the facility used exclusively for High Quality (HQ) Grade plasmids; no parallel plasmid productions occur in the same facility.

Moreover, the HQ fermentation is physically separated from the purification (chromatography) to ensure that downstream processing of the sensitive DNA is not affected by live contaminants.

PlasmidFactory's HQ grade DNA is already used as a starting material in clinical trials studies, being also ready to support our clients with starting material for their mRNA market supply. To also be able to provide plasmid and minicircle DNA as API for direct human application, PlasmidFactory has established a GMP facility in which plasmids and minicircles can be produced. In this facility, we exploit single-use equipment. The qualification process is ongoing, enabling us to begin the next year with a brand-new GMP facility ready for operation. DNA produced in this facility will be GMP-certified and meet the stringent requirements for therapeutic products for human application.

Q: Has this GMP facility exclusively been built for the production of plasmids?

No, it was not. Actually, this facility was primarily built for the production of minicircle DNA, making it the worldwide first minicircle GMP production facility.

Q: What exactly is the difference between a plasmid and a minicircle them?

A: Basically, the two products are similar, but the minicircle is much smaller, as the name implies. PlasmidFactory uses a proprietary technology to produce non-synthetic minicircle DNA.

A plasmid containing the gene of interest (GOI) serves as the starting material, which is why the facility can be used for production of both plasmid and minicircle DNA.

The GOI is inserted into the so-called parental plasmid, followed by intramolecular recombination. The resulting minicircle DNA contains almost exclusively the customer's sequence and its regulatory sequence motifs as well as a short residual sequence region (~150 bp). Superfluous bacterial backbone sequences such as the origin of replication or the antibiotic resistance genes are completely removed.

Especially in the field of cell and gene therapy, the smaller size of minicircles compared to standard plasmid vectors is an advantage. It makes for increased cargo capacity as well as greater transfection and expression efficiency, while a reduction in the number of CpG motifs reduces the risk of immunogenicity.

To sum it up, minicircles are structurally identical to plasmids as they are supercoiled covalently closed circular (ccc), but without a classical bacterial backbone, resulting in an extremely small but at the same time highly efficient molecule. In contrast to other small minimalistic vectors, no selection process is used in their production and, of course, no sequence element as such is included.

Q: Where are these plasmids and minicircles produced? Is your new GMP facility based in close proximity to your other facilities?

A: All manufacturing, research and development is concentrated at the Bielefeld site in Germany, for almost 25 years now. In 2000 the company was founded, and it has grown ever since, including dedicated facilities for each quality grade. In parallel to the constructional expansion, various products and proprietary technologies have been developed.

Over the years a great deal of fruitful cooperations and partnerships have been forged with academic institutions, industrial companies as well as strategic partners. The partnership with ARCHIMED has enabled us to invest in further expansion and created a broad network of healthcare partners. Another strategic relationship was recently entered into to expand our network to the Asian market.

Q: Speaking of the Asian market, what is your connection to Asia and what are your plans for the future?

A: For almost 25 years, PlasmidFactory has been supplying customers around the world and has delivered its innovative products to researchers in over 40 countries around the globe, including Asia. Since the Asian continent is vast and the biotechnology and pharmaceutical market is growing exhilaratingly fast, PlasmidFactory has started implementing a network of agents all over Asia, beginning with Dr. Nagaraj Rao, RRR Labs, who supports our customers in India.

Besides this new network, the team at Bielefeld is always available to all our global customers, with a quick response time and highly competent customer support. Just feel free to reach out and find out for yourself.



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