Supercoiled DNA matters – even for linear pDNA template for RNA drug substance manufacturing

And deliberate USP and DSP steps can maximize it

In their paper "Supercoiled DNA percentage: A key in-process control of linear DNA template for mRNA drug substance manufacturing", Piao and colleagues argue that "understanding the correlation between DNA template quality and product-related CQAs is crucial in mRNA process development and manufacturing" [1]. For five pDNA constructs, they investigated the correlations between supercoiled percentages ranging from 59% to 98% and purity, integrity, residual dsRNA content, Poly(A) tail integrity, per cent capping efficiency, and potency – six main Critical Quality Attributes (CQAs) of mRNA drug substance for vaccines and therapeutics. They used a COVID mRNA and a FLuc mRNA construct and applied lithium chloride (LiCl) precipitation and oligo-dT-based purification to both.

Critical quality attribute (CQA)	Correlation to percentage supercoiled observed
% capping efficiency	–
Poly(A) tail	–
Purity/integrity	+
Residual dsRNA content	+/– (depending on mRNA contruct)[1]
Potency	+

Table 1: Correlation between percentage supercoiled pDNA and mRNA CQAs (+ signifies that the paper (Pioa et al. 2024) reported a correlation; – signifies that no correlation was observed)

The correlations were more "significant" in the case of the LiCl-based purification process. However, since LiCl purification is not currently industry standard and definitively not applied in Wacker Biotech's plasmid manufacturing process, we will focus on the results of the study that are associated with the oligo-dT-based manufacturing strategy.

To summarize their study, capping efficiency and Poly(A) tail integrity are not linked to the percentage of supercoiled pDNA. However, according to the authors, the percentage of supercoiled pDNA does determine the homogeneity of linearized pDNA template, which in turn affects the percentage of full-length mRNA purity and integrity. For purity and integrity, the right purification strategy – in this case oligo-dT rather than LiCl precipitation – can also rescue a deficiency. Indeed, in the study, oligo-dT-purified mRNA constructs yielded consistently > 83% purity, even with percentage supercoiled pDNA as low as 59%. For LiCl-purified mRNA construct, purity dropped to 6% for those low-percentage supercoiled pDNA starting materials.

For residual dsRNA, we need to paint a more nuanced picture. At least in this study, the amount of residual dsRNA after purification seems to be more correlated with the mRNA construct (COVID versus FLuc) than with the percentage supercoiled pDNA or even with the mRNA purification strategy.

For potency on the other hand, the quality of the starting material in terms of the percentage of supercoiled pDNA matters more than the purification strategy. Additionally, even though not a CQA, yield was also correlated with percentage supercoiled pDNA. The authors did not differentiate between total percentage of supercoiled pDNA and percentage of monomers of supercoiled pDNA, nor did they investigate the effect of linearization and linearized template purification strategy on the different mRNA CQAs.

Regardless of those limitations, the paper supports the fact that, for linearized pDNA template, in the absence of better CQAs, percentage supercoiled pDNA can and should serve as an in-process control (IPC) standard.

Influencing the percentage of supercoiled pDNA

While it is considered industry standard and in line with "Guidance for Industry: Considerations for Plasmid DNA Vaccines for Infectious Disease Indications (FDA) [2] that the percentage supercoiled pDNA should be > 80% for pDNA as starting material for mRNA process, little attention is given to which (potential) critical process parameters (CPPs) and key performance parameters (KPPs) affect percentage supercoiled pDNA during manufacturing.

Downstream processing (DSP)

In our experience, downstream process (DSP) unit operations matter, certainly for plasmid purity and to some extent for influencing the percentage of supercoiled pDNA. Approaching DSP appropriately, for instance by using HIC (Hydrophobic Interaction Chromatography), an increase in percentage supercoiled pDNA

[1] The study revealed that for one RNA construct a correlation was observed, while for the other one it was not.
[2] For viral vector and direct pDNA application, the requirements are greater and the threshold higher.

of more than 10% can be achieved. The DSP part of a pDNA manufacturing process is critical not only to the capture of the molecule of interest – in the current case, the monomeric form of supercoiled pDNA – but also to the reduction of process-related impurities.

Upstream processing (USP) and primary recovery

However, what matters even more are the upstream process (USP) and the primary recovery, including lysis. For the USP and primary recovery steps, several aspects are important:

- The production/host cell strain
- Oxygenation during harvest
- Harvest and lysis conditions

First and foremost, the host cell strain matters. Some strains are better at plasmid production than others. Furthermore, some specific plasmids such as AAV plasmids are better produced in specific cell lines. For pDNA as a starting material for RNA, the most important fact in addition to topology is yield. An expression system such as Wacker Biotech's PLASMITEC® strain can be of advantage by providing high yield while maintaining high purity (Figure 1).

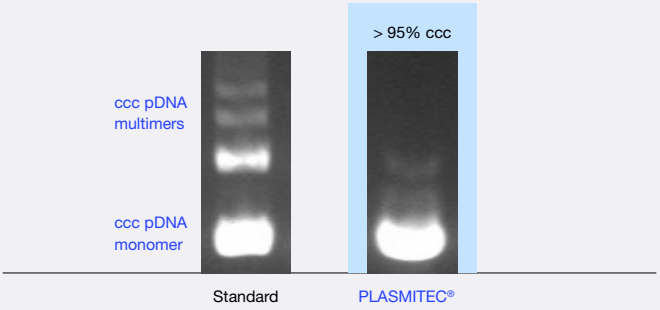
In addition to the strain and a suitable upstream media and feed composition, conditions during harvest need to be considered. For instance, oxygen concentration during harvest can impact enzymes responsible for topology (topoisomerase). If negatively impacted, the percentage of supercoiled forms of pDNA might be reduced during harvest.

Primary recovery, including harvest and lysis, matters. The two most important aspects are without doubt contact time and the pH of the lysis buffer. Indeed, if the contact time is extended, integrity of the plasmid can decrease. On the other hand, keeping that time too short can result in a suboptimal lysis. Finding the sweet spot, for instance via the design of experiment-based studies, can be beneficial. The composition, and hence also the pH, of the lysis buffer matters to ensure sufficient lysis without detrimental effects. Furthermore, flux during the separation process can affect the impurity profile of the intermediate while not necessarily affecting the topology.

Correlations between all these different process parameters (pH of lysis buffer, contact time, etc.) and critical quality attributes (in this case in particular topology or percentage of supercoiled pDNA) are typically investigated during process development and characterization, determining, on a product-specific basis, which parameters are critical process parameters (CPPs). An appropriate control strategy is then developed (Figure 2 on page 3).

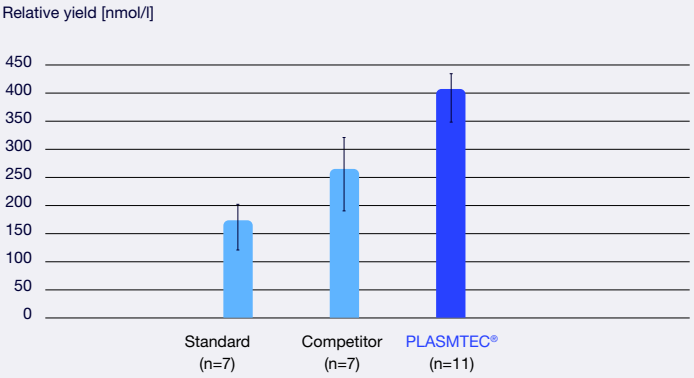
As pDNA is considered a critical starting material but not an active ingredient for an mRNA-based therapeutic or vaccine, a formal process performance qualification to be included in a dossier submitted for a BLA application is not required. However, it is essential to ensure that this critical raw material is consistently produced to the required quality, meeting all the required specifications. This is ensured by developing true process understanding, creating a meaningful control strategy, and verifying its effectiveness through continued process verification.

High pDNA purity



In a qualitative comparison (via AGE) of plasmid purity (covalently closed circular forms), Wacker Biotech's PLASMITEC® *E. coli* strain and PLASMITEC® production process yields almost exclusively monomers of covalently closed circular (ccc) forms compared to a standard *E. coli* cell line and production process (left) where both multimeric and monomeric forms are detected.

Class-leading plasmid yields



Wacker Biotech's PLASMITEC® platform, with proven track record at GMP scale, has demonstrated 40% greater relative plasmid yield compared to competitor plasmid (statistical significance p=.0001) and more than double the yield of the industry standard. USP pDNA titers of up to 1.5 g/L and overall DSP yields of up to 62% can be achieved.

Figure 1: Illustration, from left to right, of the purity and yield that can be achieved with Wacker Biotech's PLASMITEC® platform.

	1. Cell banking	2. Fementation	3. Cell harvest and lysis	4. DEAE chroma / Alternative: Monolithic chormatography	5. HIC chroma	6. TFF	7. pDNA product
CPPs	Identity starting material	1. Identity cell bank 2. Media feed comp. 3. Feed profile	1. Buffer pH 2. Contact time	Resin type		1. Diafiltration volumes 2. Formulation buffer	–
CQAs	Identity	Topology		Topology, endotoxin, residual RNA/protein/gDNA		Appearance, concentration, residuals	Microbial contaminants
Control strategy	Site-specific contamination control strategy	1. Released, characterized 2. Qualified, maintained scale 3. Predefined feed	1. Instructed, recorded max. duration/pump flow rates 2. Qualified, maintained scale	Typical KPPs for chromatographic step might include flow rate, column load, elution gradient, elution buffer composition		1. Flow rate, fixed volume, measured dia-filtration buffer consumed 2. Qualified, maintained scale	Site-specific contamination control strategy

Figure 2: Illustration of a typical quality control process strategy for manufacturing pDNA as a critical starting material for RNA production processes

References:

1. Xijun Piao, Yujie Tang, Xiuzhi Li, Weicheng Zhang, Wei Yang, Xining Xu, Wenjing Wang, Jiajia Jiang, Jun Xu, Kunkun Hu, Meiling Xu, Mengjie Liu, Mengfei Sun, Lin Jin. "Supercoiled DNA percentage: A key in-process control of linear DNA template for mRNA drug substance manufacturing." Molecular Therapy – Nucleic Acids (Cell Press), vol. 35, No. 2, 11 June 2024. https://www.sciencedirect.com/science/article/pii/S2162253124001100
2. Guidance for Industry Considerations for Plasmid DNA Vaccines for Infectious Disease Indications. Food and Drug Administration (2007). https://www.fda.gov/media/73667/download

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