WACKER

CREATING TOMORROW'S SOLUTIONS

PLASMITEC®

WHITE PAPER I WACKER BIOTECH I pDNA

ADVANCING GENE THERAPIES – KEY CHALLENGES AND CONSIDERATIONS

Insights and a case study

ADVANCING GENE THERAPIES – KEY CHALLENGES AND CONSIDERATIONS:
INSIGHTS AND A CASE STUDY

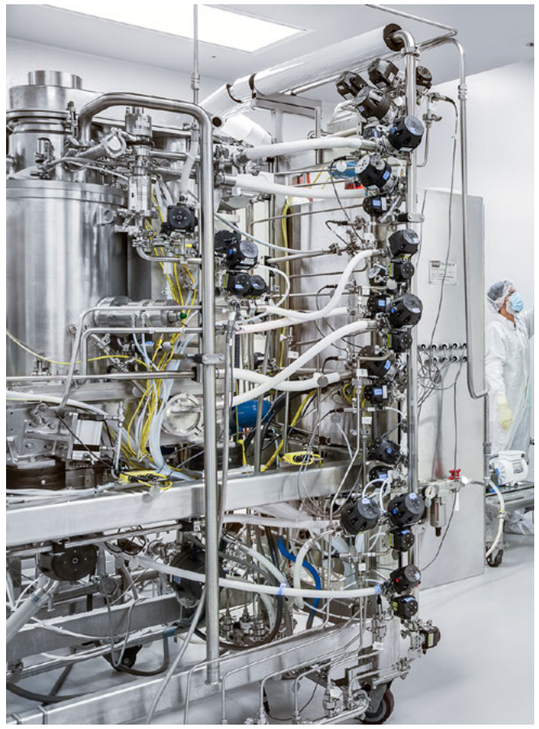
In 2024, Wacker Biotech in San Diego received a critical request from a client: two plasmids were urgently required in GMP grade. These plasmids were the key for the client's mRNA gene therapy product, specifically for an IND-enabling toxicology study prior to entering phase I clinical trials. The product was designed to encode for a bioengineered enzyme to execute highly precise gene editing once in the target cell – in this case retina cells in the eye – to treat Stargardt's disease, a genetic disease causing blindness for which no approved therapies exist.

At the San Diego site of Wacker Biotech, process development (PD) experiments went exceptionally well. Titer and critical quality attributes such as percentage of supercoiled plasmid were meeting expectations even though the plasmid was coding for a longer-than-usual poly-A tail (104 As). The poly-A tail is a critical feature for downstream mRNA stability and translation efficiency, with greater tail length correlating to increased stability and translational potential. Indeed, six development runs and 12 weeks after the initial feasibility study, the PD team was transferring to GMP production via an engineering run, with the goal of a percentage of supercoiled pDNA > 70% and a concentration of 1mg/mL at larger scale (30 L scale).

While scalability is always at the forefront of process development at Wacker Biotech, cell lysis, the least controllable part of a pDNA manufacturing process (Figure 1), can always present inherent challenges. The following aspects always need to be considered carefully when moving from process development to larger-scale GMP production:

- Thawing time of cell paste
- Mixing and resuspension of the cell paste
- Consistent lysis contact time

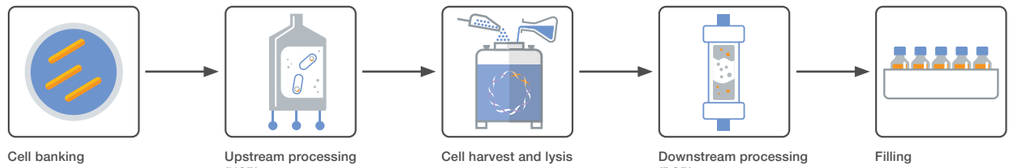
The team particularly focused on ensuring consistency by introducing appropriate combinations of pumps and static mixers to keep the lysis contact time consistent prior to neutralization.



As a CDMO, Wacker Biotech operates a pDNA center of excellence in San Diego, California, USA.

An engineering run approach (where material was produced at GMP scale but without every GMP quality assurance step) allowed the Wacker Biotech team to finetune parameters in the USP part of the process as well as ensure that thawing times and lysis contact times were appropriate for the scale prior to moving to final GMP production. The final GMP batch yielded 1 kg of critical starting material for the drug product. The GMP-grade starting material met specifications (Table 1) and was released on time for further processing at the next step of the supply chain.

Figure 1: Typical process for pDNA production from master cell bank (MCB) to bottled starting material or API



PLASMITEC® is a registered trademark of Wacker Chemie AG.

Table 1: Specifications of GMP-grade critical starting material of the drug substance produced at 30 L scale

Type	Test	Specification	GMP batch
General	Appearance	Clear and colorless liquid free from visible particles	Clear and colorless liquid
	pH	8.0 ± 0.5	8.0
	Nucleic acid concentration	1.0 ± 0.2 mg/mL	1.1
	260/280 purity	1.8 – 2.0	1.9
Purity	DNA homogeneity by AGE	≥ 70% supercoiled pDNA	79%
	Residual host RNA	≤ 5% w/w (RNA/pDNA)	< 0.5%
	Residual host cell DNA	≤ 5% w/w (gDNA/pDNA)	0.02%
	Residual protein	≤ 2% w/w (protein/pDNA)	1%
	Residual kanamycin	≤ 5 ppm	< 0.5 ppm
ID	Identification by restriction digest	Conforms to expected band pattern	Conforms to expected band pattern
		BspQI: Approximates 4010bp	BspQI: Approximates 4010bp
		HindIII/PvuI: Approximates 1463bp & 2547bp	HindIII/PvuI: Approximates 1463bp & 2547bp
	Sequencing (full plasmid DNA sequence)	Comparable to reference sequence (excluding poly-A tail)	Pass
Safety	Endotoxin	≤ 5 EU/mg	< 0.227
	Bioburden	TAMC: < 2 CFU/10 mL	0 CFU/0 mL
		TYMC: < 2 CFU/10 mL	0 CFU/10 mL
	Mycoplasma	Not detected	Pass
Overall			**Pass**

Figure 2: pDNA using Wacker Biotech's PLASMITEC® platform can be produced at significantly higher yields compared to standard and competitor strains.

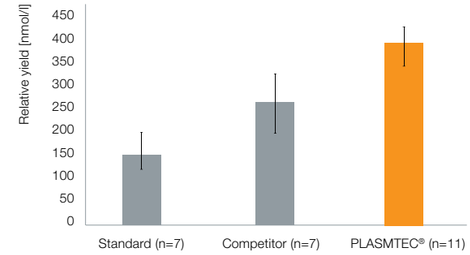
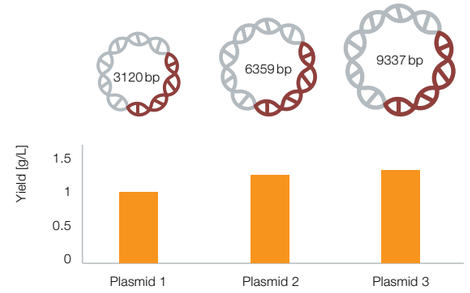


Figure 3: The PLASMITEC® platform yields consistently superior concentrations of pDNA across plasmid sizes. Yields are plotted below for three different plasmids ranging in size from 3120 base pairs to 9337 base pairs.



Setting the stage and informing future pDNA and mRNA projects

While the project was successful, meeting the client's specifications and positioning Wacker Biotech as a partner who delivers, the achieved yields showed that relying on the DH5α *E. coli* strain for pDNA production has limitations. In the past few years, Wacker Biotech has also developed PLASMITEC®, a technology platform for pDNA production utilizing a superior, proprietary *E. coli* strain. Developed in collaboration between the central R&D team at Wacker Biotech's parent company in Munich and the bioprocess development team at Wacker Biotech, PLASMITEC® can offer a 2.4 times faster pDNA production rate and increased yields across a variety of different plasmid sizes (Figure 2 and Figure 3). It is also capable of generating plasmids that are greater than 90% supercoiled.

The collaboration described in this paper exemplifies the power of partnership in advancing genetic medicines. By combining the client's innovative gene coding technology with Wacker Biotech's expertise in plasmid DNA manufacturing, partnerships like this are paving the way for transformative gene therapies to treat previously undruggable targets and benefit patients worldwide.

WACKER

Wacker Biotech GmbH
Jena and Halle, Germany
Phone: + 49 3641 5348-0

Wacker Biotech B.V.
Amsterdam, The Netherlands
Phone: +31 20 750 3600

Wacker Biotech US Inc.
San Diego, CA, USA
Phone: +1 858 875 4700

info.biologics@wacker.com
www.wacker.com/biologics

Follow us on:

1180en/12.25