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An Efficient, End-to-End Process for Plasmid DNA Purification

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Abstract

Plasmids are an essential element in many advanced therapy medicinal products, which are becoming increasingly important in treating various diseases. The growing demand for plasmid DNA (pDNA) requires efficient, robust, and cost-effective production processes in the biopharmaceutical industry. This application note outlines a downstream purification case study of pDNA using Sartorius technologies and solutions, from cell lysis to final sterile filtration. Comprehensive analytics demonstrate high yield, purity, and integrity of pDNA, achieving >98% supercoiled pDNA and meeting regulatory requirements for key critical quality attributes.

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Introduction

Advanced therapy medicinal products (ATMPs) offer innovative treatment options for a range of diseases. Their enormous potential is evident in clinical development and in the increasing availability of already approved therapies based on AAV, lentivirus, or mRNA-LNP products.

Plasmids are critical raw materials in advanced therapies, particularly for vector construction and genetic information delivery. With the increase in clinical trials and the approval of new products, the demand for plasmid DNA (pDNA) is expected to rise significantly, necessitating efficient, reliable, and cost-effective production processes at an industrial scale.

However, industrial-scale downstream pDNA processing faces several challenges:

1. Separating pDNA from impurities
2. Avoiding shear force damage, preserving the integrity of supercoiled isoforms
3. Achieving high recovery rates

Addressing these challenges requires optimized, robust downstream processes to ensure cost efficiency, safety, and high-quality pDNA with high yield.

This application note outlines the steps involved in the downstream processing of a representative 4.7 kb pDNA, from cell lysis of the plasmid-expressing *E. coli* to final bulk sterile filtration. Table 1 provides an overview of the downstream pDNA processing steps and technologies utilized.

The entire end-to-end pDNA downstream process is monitored by at-line analytical chromatography using the PATfix® pDNA Platform, which delivers insights into pDNA yield, integrity of supercoiled pDNA, and purity of pDNA (presence of *E. coli* RNA and residual DNA impurities) at each process step—from crude lysate to purified supercoiled pDNA. The PATfix® pDNA Platform supports in-process control during pDNA development and production by utilizing the unique analytical capabilities of the monolithic CIMac pDNA column in conjunction with user-friendly software for efficient data management.

Table 1: *Steps of the pDNA Purification Case Study Using Sartorius Downstream Processing Units*

Process Steps	Description	Sartorius Solution
1. Cell lysis	Alkaline cell lysis of harvested *E. coli* cells	Automated in-line lysis system: ▪ Alkalizator 1000-4FC-SU-Tower ▪ Lysis Reactor LR 1000-SU-EPOXI
2. Clarification and bioburden reduction	Removal of cell debris and other impurities by initial clarification followed by a 0.2 µm filtration for bioburden reduction.	▪ Sartopure® PP3 | 20 µm ▪ Sartopore® 2 XLG 0.8 | 0.2 µm
3. pDNA capture For capture of the pDNA, the clarified lysate was processed using two alternative approaches: 1. Concentration and buffer exchange followed by anion exchange (AEX) chromatography (3.1, 3.1.1) 2. In-line dilution followed by AEX chromatography (3.2)		
3.1 Concentration and buffer exchange by ultrafiltration | diafiltration	Volume reduction and removal of impurities | salts using tangential flow filtration (TFF). ▪ An alternative to in-line dilution for sample preparation before AEX when volume reduction is required.	Sartoflow® Smart benchtop system: ▪ Trial 1: Sartocon® Slice 200 Cassette | Hydrosart® | 300 kDa | 0.02 m² ▪ Trial 2: Sartocon® Slice 200 Cassette | PESU | 100 kDa | 0.02 m²
3.1.1 pDNA capture	Capture of pDNA and removal of host cell RNA, proteins, endotoxins, and smaller impurities by AEX.	▪ CIMmultus® DEAE 40 mL (2 µm channels) monolithic column
3.2 pDNA capture after in-line dilution	In-line dilution of the clarified lysate with purified water to reduce conductivity to 35 mS/cm to enable processing on the AEX column. ▪ An alternative approach to 3.1 for sample preparation before AEX when fast processing is preferred, and volume reduction is not required. Capture of pDNA and removal of host cell RNA, proteins, endotoxins, and smaller impurities by AEX.	▪ Resolute® RCC MU ▪ CIMmultus® DEAE 800 mL (6 µm channels) monolithic column
4. pDNA polishing	Removal of remaining contaminants (unwanted pDNA isoforms, *E. coli* DNA, *E. coli* RNA, *E. coli* proteins, and endotoxins) by hydrophobic interaction chromatography (HIC).	▪ Resolute® RCC MU ▪ CIMmultus® C4 HLD 400 mL (2 µm channels) monolithic column
5. Concentration and buffer exchange (ultrafiltration | diafiltration)	Buffer exchange and final concentration of the pDNA by TFF.	Sartoflow® Smart benchtop system: ▪ Trial 1: Sartocon® Slice Cassette | Hydrosart® | 100 kDa | 0.02 m² ▪ Trial 2: Sartocon® Slice 200 Cassette | PESU | 100 kDa | 0.02 m²
6. Sterile Filtration	Final sterile filtration of the bulk	Trial 1: Sartopore EVO® 0.8 | 0.2 µm | 4.5 cm² EFA

Additionally, the yield at each process step was assessed against various critical quality attributes (CQAs), including *E. coli* DNA (dPCR), *E. coli* RNA (PATfix® pDNA Platform), protein content (Qubit® assay), and bacterial endotoxins (LAL test) to ensure compliance with regulatory standards (Table 2).

Table 2: *Overview of the CQA Analysis Carried Out After Each Process Step*

Analytics	Method	Sartorius Solution
pDNA yield, purity, and integrity of supercoiled pDNA	Analytical chromatography	PATfix® pDNA Platform
Genomic DNA	dPCR | analytical chromatography	PATfix® pDNA Platform
Host cell RNA	Analytical chromatography	PATfix® pDNA Platform
Host cell protein	Qubit® assay	-
Bacterial endotoxins	LAL test	-

Materials, Methods, and Results

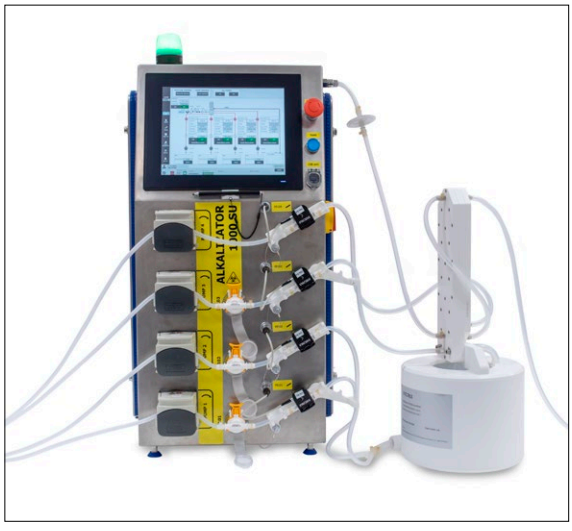
1. Cell Lysis

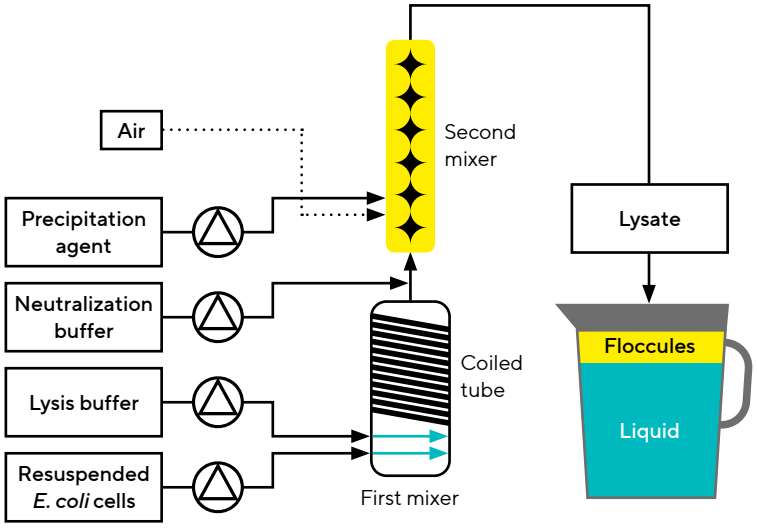
Alkaline lysis of the harvested *E. coli* cells was carried out with the automated in-line lysis system Alkalizator, which consists of the multi-use Alkalizator Tower (Alkalizator 1000-4FC-SU-Tower) and a single-use lysis reactor (Lysis Reactor LR 1000-SU-EPOXI). Alkalizator and a schematic diagram of the system are shown in Figure 1.

Alkalizator incorporates four peristaltic pumps, which are precisely controlled via ultrasonic flow sensors, ensuring tight control over the cell lysis process conditions.

The first mixer ensures efficient mixing of resuspended cells and lysis buffer. This mixture passes through the coil, defining the duration of lysis. It then passes through the first part of the second mixer, enabling efficient neutralization of lysed cells. The neutralized lysate passes through the second part of the second mixer for $CaCl_2$ treatment, which enables a precise separation of liquid and flocks during precipitation, increasing the lifespan of filters in subsequent clarification.

Figure 1: *System Setup of the Alkalizator and a Schematic Diagram of the System*





Alkalizator mixed the resuspended cells with lysis buffer (0.2 M NaOH, 1% SDS) to a final concentration of 0.1 M NaOH and 0.5% SDS. After 5 minutes of alkaline lysis, the lysate was neutralized by adding 3 M potassium acetate (CH_3COOK), pH 5.5, to a final concentration of 1 M. The precipitation of *E. coli* DNA, RNA, and proteins was induced by adding 5 M calcium chloride ($CaCl_2$) to a final concentration of 0.75 M.

The exact quantity and quality of the pDNA material in the lysate were determined using the PATfix® pDNA Platform, which can analyze pDNA in complex matrices without special sample preparation; only dilution into mobile loading phase is required. The analysis revealed a total of 1.62 g of pDNA, with 95.5% in the supercoiled form, highlighting the effective recovery of supercoiled pDNA achieved through automated in-line cell lysis with the Alkalizator. An analytical chromatogram of the lysate sample is shown in Figure 7.

2. Clarification and Bioburden Reduction

Following alkaline cell lysis, cell debris and other impurities were removed by sequentially filtering the lysate. Initial clarification was achieved using the Sartopure® PP3 filter with a pore size of 20 µm. Sartopure® PP3 filters offer exceptional robustness for prefiltration applications. The polypropylene fleece-based membrane retains particles with high efficiency, ensuring secure and reliable operation even under varying process conditions. Its all-polypropylene design minimizes unspecific binding, maximizing product yield. For the subsequent bioburden reduction filtration step, Sartopore® 2 XLG 0.8 | 0.2 µm filters were used. The Sartopore® 2 XLG includes a coarser pre-filter layer with pore sizes of 0.8 µm before the sterilizing-grade final membrane (0.2 µm), which helps prevent early blockage when filtering biological fluids.

Filtration was carried out up to a maximum differential pressure of 2 bar. Sartopure® PP3 and Sartopore® 2XLG achieved a filter capacity of 80 kg of lysate/m² filtration area. As both filters can operate up to 5 bar, the filter capacity could also be increased to support an individual filtration process.

Subsequent PATfix® analysis of the filtrate showed a total pDNA recovery exceeding 93% from the clarified lysate, with 91% in the supercoiled form (Figure 7). Fast filtration processes are preferred to maintain a high integrity of supercoiled pDNA, preventing the pDNA from long contact times in lysate conditions.

3. Capture

Preparation of the clarified lysate for anion exchange chromatography (AEX) utilized two alternative methods: the first method involved an ultrafiltration | diafiltration (UF | DF) step using tangential flow filtration (TFF) technology, while the second employed in-line dilution with purified water to achieve the desired conductivity for effective processing by AEX.

3.1 Tangential Flow Filtration

TFF was used to concentrate the pDNA sample (clarified lysate) in a first step, followed by a diafiltration step to remove impurities (e.g., *E. coli* RNA and salts) and to perform a buffer exchange into a Tris-EDTA buffer to stabilize the pDNA. TFF is a potential approach to prepare a pDNA sample to be loaded onto an AEX column, particularly when volume reduction is required. For this process step, two TFF modules made of different membrane materials with different molecular weight cutoffs (MWCO) were used. Trial one was performed with a Sartocon® Hydrosart® E-Screen Cassette, with a MWCO of 300 kDa (Figure 2). In trial two, a Sartocon® PESU E-Screen Cassette featuring a MWCO of 100 kDa was used (Figure 3). The appropriate feed volume was determined in previous screening trials (4 mL of clarified lysate/cm²).

Figure 2: *Detailed Process Parameters of pDNA UF | DF Using a Hydrosart® 300 kDa Cassette*

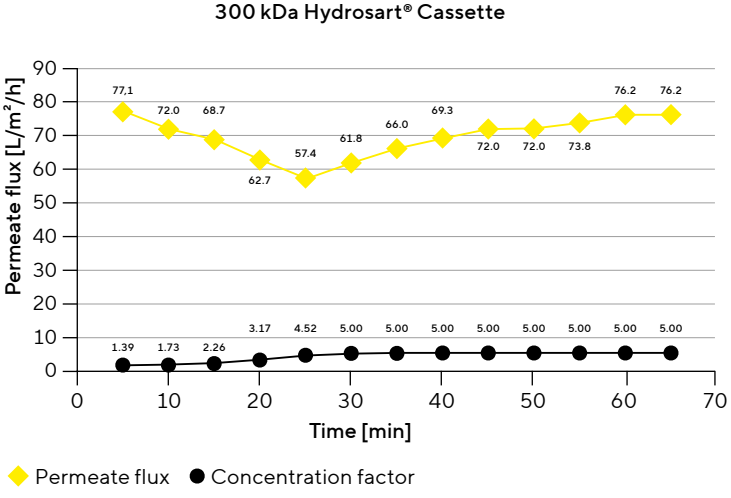


Figure 3: *Detailed Process Parameters of pDNA UF | DF Using a PESU 100 kDa Cassette*

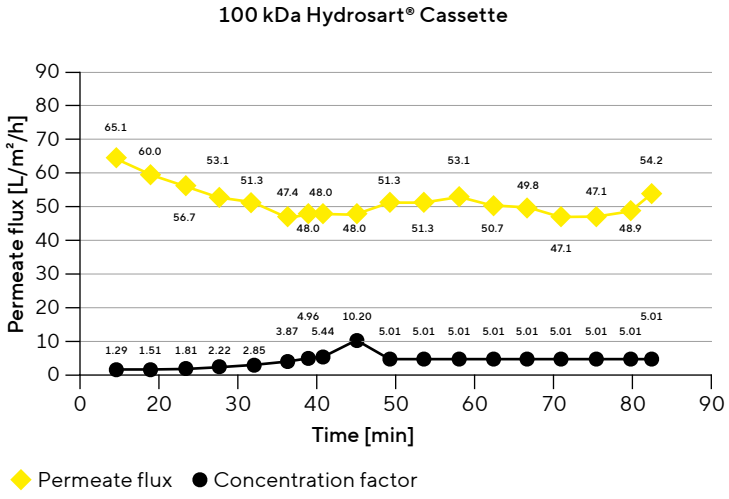


Table 3: *Process Conditions and Results of the UF | DF Process*

Process Conditions	Results	
	Hydrosart® 300 kDa	PESU 100 kDa
Membrane area [m^2]	0.02	0.02
Load volume [mL/cm^3]	4	4
Pressure (in) initial 5 min | after 5 min [bar]	0.5 | 1.0	0.5 | 1.0
Pressure (perm) initial 5 min | after 5 min [bar]	0.0 | 0.0	0.0 | 0.0
TMP initial 5 min | after 5 min [bar]	0.25 | 0.5	0.25 | 0.5
UF average flux [$L/m^2/h$]	67.6	53
DF average flux [$L/m^2/h$]	71	50
Processing time [min]	65	85
Concentration factor	5	5

Both trials were performed using a Sartoflow® Smart modular TFF system with automatic process control (Figure 4).

Figure 4: *Sartoflow® Smart Benchtop TFF System*

During UF, the pDNA-containing feed was five-fold concentrated. The 300 kDa MWCO of the Hydrosart® cassette allowed a faster permeate flux compared to the PESU membrane with a 100 kDa MWCO, with average permeate fluxes of 67.6 $L/m^2/h$ and 53 $L/m^2/h$, respectively. During DF, the feed was processed with five diafiltration volumes. The average permeate flux during diafiltration was 71 $L/m^2/h$ with the Hydrosart® and 50 $L/m^2/h$ with the PESU cassette, resulting in overall process times of 65 minutes for the Hydrosart® cassette and 85 minutes for the PESU cassette.

During TFF, both pore size and transmembrane pressure (TMP) can significantly impact product loss and the formation of open circular pDNA. PATfix® analysis of the retentates demonstrated pDNA recovery rates of 81% and 85% from the filtrate with the Hydrosart® (300 kDa MWCO) and the PESU cassette (100 kDa MWCO), respectively. The proportion of supercoiled pDNA was 90% with the Hydrosart® and 86% with the PESU cassette. These findings indicate that both membranes are suitable for effectively processing medium-sized pDNA of 4.7 kb. However, since the time from cell lysis to buffer exchange is a critical parameter for preserving pDNA integrity and thus achieving a high recovery rate of supercoiled pDNA, the 300 kDa Hydrosart® cassette is preferable for faster processing, particularly for larger pDNA molecules.

3.1.1 pDNA Capture by AEX After TFF

pDNA obtained from UF | DF (3.1) was purified by AEX chromatography utilizing a CIMmultus® DEAE 40 mL monolithic column (2 µm channel diameter).

CIMmultus® DEAE is a cGMP-compliant high-performance AEX column designed for fast and efficient purification of biomolecules, especially pDNA. Column capacity is 7 mg of pDNA per mL of monolith.

The AEX chromatogram is shown in Figure 5. Throughout the entire process, backpressure remained low, increasing only slightly from 0.6 bar to 0.7 bar during the sample loading phase at a flow rate of 2 CV/min (FT1). After the sample was loaded onto the column, it was washed with 0.6 M NaCl to effectively remove bound RNA impurities (RNA wash). The elution of pDNA was accomplished using 0.9 M NaCl (E), with the majority of the pDNA eluting within 3 CV. Any remaining material was subsequently eluted using 1 M NaCl (Strip) before cleaning in place (CIP) was carried out with 1 M NaOH and 2 M NaCl solution.

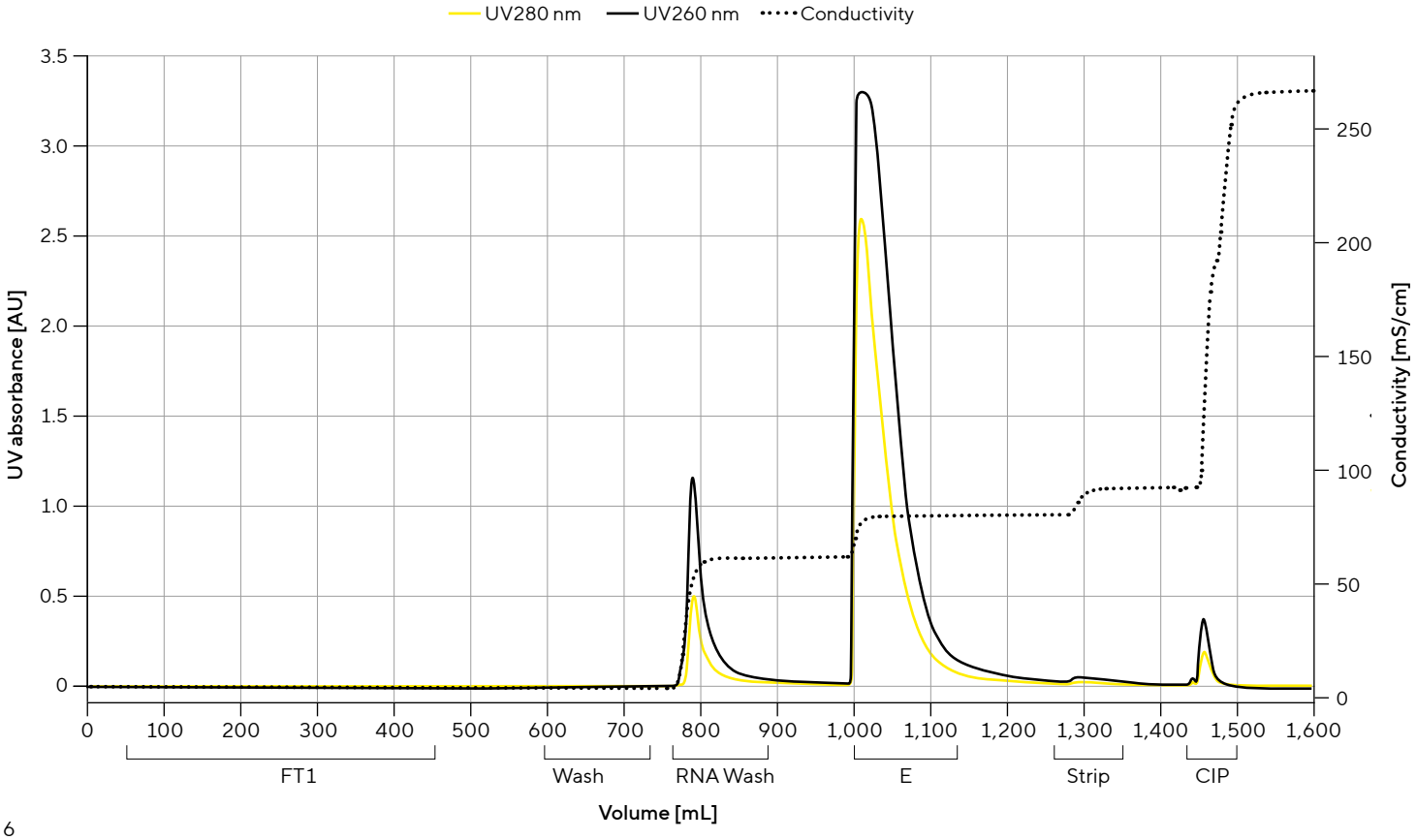
The PATfix® analysis demonstrated highly efficient pDNA capture, achieving a recovery rate of 97%, with 87% super-coiled pDNA (Figure 7).

3.2 In-Line Dilution Followed by pDNA Capture Using AEX

In this trial, in-line dilution of the clarified lysate with purified water using the Sartorius Resolute® RCC MU system was applied as an alternative to TFF for conductivity adjustment.

The diluted pDNA sample was directly loaded onto a CIMmultus® DEAE 800 mL with 6 µm channels (column capacity is 2.5 mg of pDNA per mL of monolith), allowing for higher flow rates (>2.5 CV/min) due to the low pressure-drop: ~27 L of the filtrate was six-fold in-line diluted and loaded onto the CIMmultus® DEAE column within 70 minutes. During the loading phase, back pressure increased only slightly from 0.9 to 1.1 bar. Under these conditions, the majority of *E. coli* RNA went into the flow-through (FT). After loading was completed, the column was washed with 0.6 M NaCl to effectively remove bound RNA impurities (RNA wash). The majority of pDNA was then eluted in 3 CV of 0.9 M NaCl (E), while the remaining material was eluted using 1 M NaCl (Strip). Thorough CIP of the column was ensured by using 1 M NaOH and 2 M NaCl solution (Figure 6).

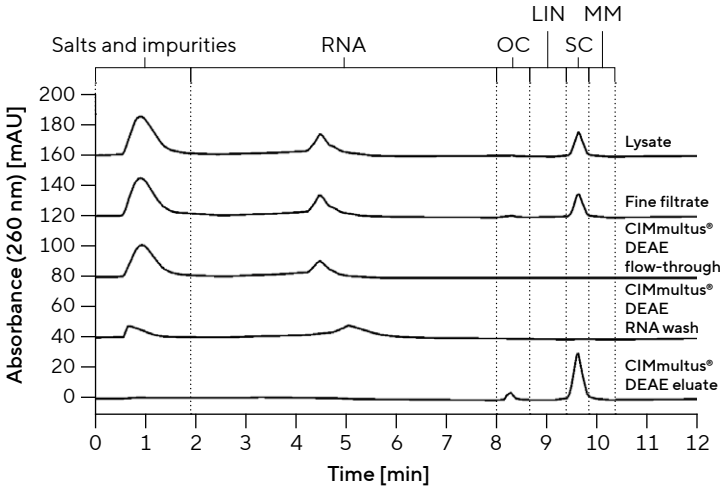
Figure 5: *Chromatogram of pDNA Capture by AEX Using a CIMmultus® DEAE 40 mL Monolithic Column With 2 µm Channels*



Changing buffer conditions (salt concentration, pH) of different chromatography fractions can affect the accuracy of simple UV-spectrophotometric readouts; chromatographic PATfix® analysis is more reliable. It showed a total pDNA recovery of 95% from the filtrate, with 89% of this being supercoiled pDNA (1.19 g), highlighting the effectiveness of in-line dilution followed by AEX as a suitable alternative to UF | DF via TFF.

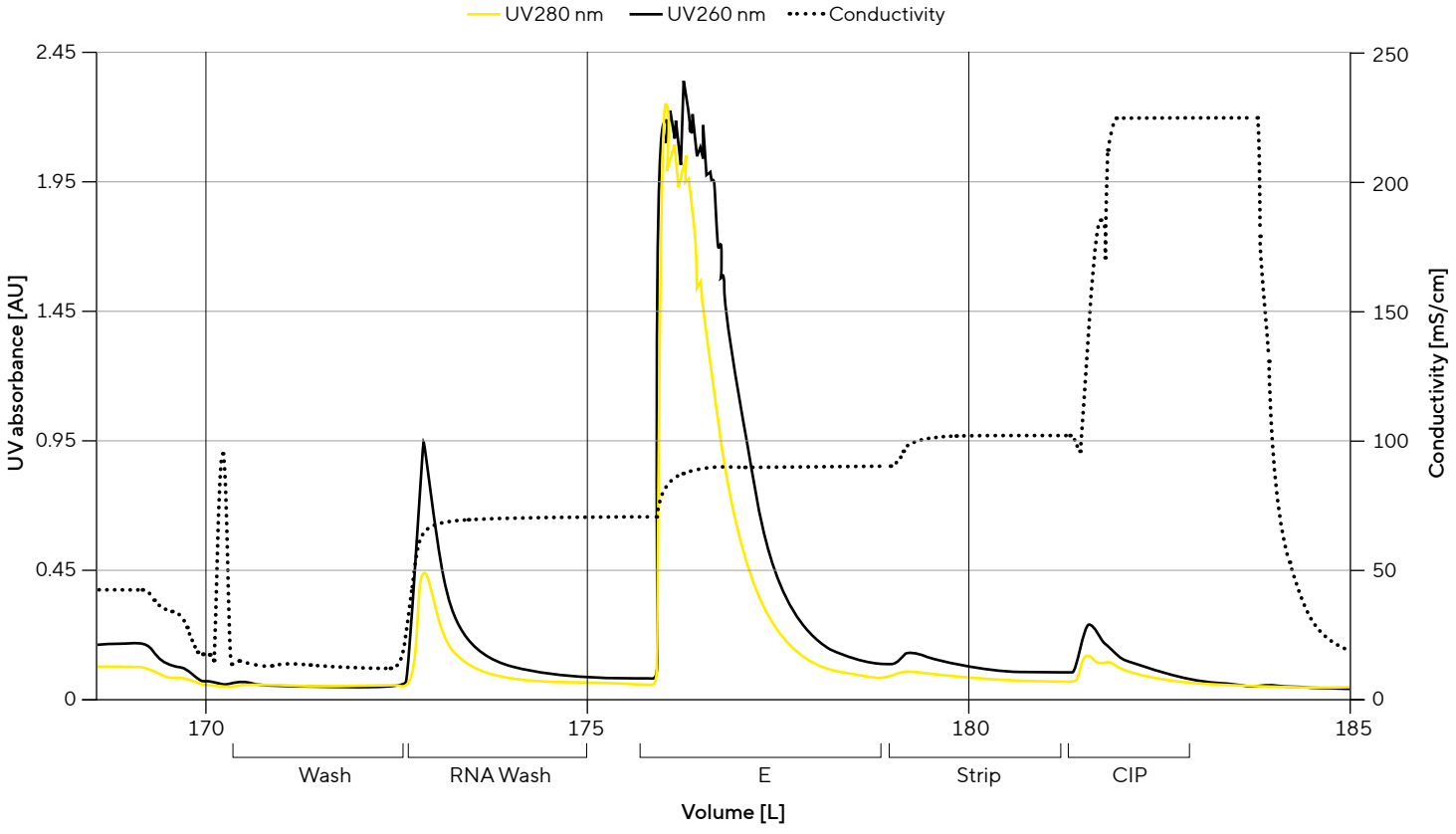
Processing the clarified lysate by in-line dilution followed by AEX shortens the process time, which can be critical at this stage due to the harsh conditions after cell lysis—characterized by high salt and enzyme activity—which can contribute to pDNA degradation.

Figure 7: *Overlay of Analytical Chromatograms for Process Samples From Lysis to the AEX Capture Step*



Note. SC = supercoiled, OC = open circular, LIN = linear, MM = multimeric

Figure 6: *Chromatogram of pDNA Capture by AEX Using a CIMmultus® DEAE 800 mL Column With 6 µm Channels (Focused on the Elution Phase)*



4. Selective Hydrophobic Interaction Chromatography
Polishing of the pDNA was achieved by processing the CIMmultus® DEAE eluates from both capture trials via hydrophobic interaction chromatography (HIC) on a CIMmultus® C4 HLD 400 mL column (2 µm channels). Column capacity under the conditions used is 2 mg of pDNA per mL of monolith. The CIMmultus® DEAE eluates (3.1.1 and 3.2) were pooled and adjusted by the addition of a stock solution of ammonium sulfate (AS) to increase the AS concentration to 1.5 M, followed by the addition of loading buffer in the same volume as the CIMmultus® DEAE eluate.

The prepared feed was divided into two parts and loaded onto the column in two consecutive runs at a flow rate of 1 CV/min, since the amount of pDNA after AEX was too high to be processed in a single run. This way, we also demonstrated that CIMmultus® columns are suitable for cycling, allowing the use of a smaller bed volume for processing a large amount of material. Under selective HIC (SHIC) conditions, open circular pDNA, linear pDNA, and *E. coli* DNA flowed through the column, while the more hydrophobic supercoiled pDNA remained bound. The supercoiled pDNA was eluted with 0.9 M AS, with the major proportion eluted within 3 CVs (E). Remaining pDNA and RNA impurities were eluted with salt-free buffer (Strip), followed by CIP with 1 M NaOH (Figure 8).

PATfix® analysis of samples from the flow-through, eluate, and Strip fractions showed that open circular pDNA, linear pDNA, and *E. coli* DNA were efficiently removed in the flow-through, while host cell RNA was removed in the Strip fraction. The analysis showed an impressive supercoiled pDNA recovery of >90% in the eluate and a high quality of the pDNA with >98% in the supercoiled form (1.04 g).[1]

5. Ultrafiltration | Diafiltration (TFF2)
After polishing, the purified pDNA was concentrated and rebuffered to 10 mM Tris, 1 mM EDTA, pH 8, using UF | DF conducted via TFF. Two types of TFF modules were evaluated for their suitability. One trial utilized a Sartocon® PESU E-Screen Cassette with a MWCO of 100 kDa (Figure 9), while the other employed a Sartocon® Hydrosart® E-Screen Cassette with an identical MWCO (Figure 10).
Process parameters were controlled and monitored using the Sartoflow® Smart benchtop crossflow system. UF | DF process conditions and results are summarized in Table 4.

Figure 8: *Chromatogram of pDNA Polishing by HIC Using a CIMmultus® C4 HLD 400 mL Column With 2 µm Channels*

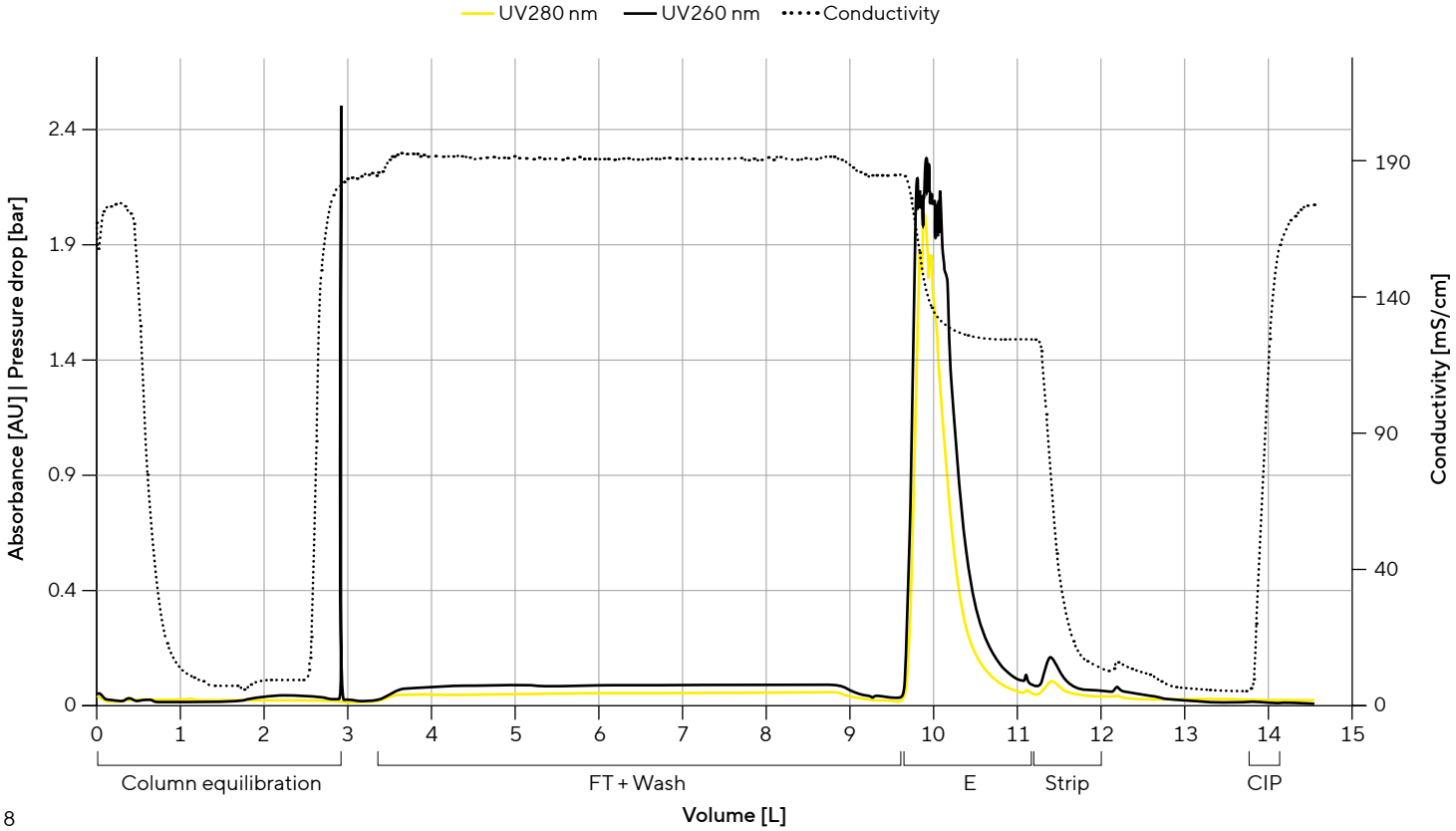


Figure 9: *Detailed Process Parameters of the pDNA UF | DF Using a 100 kDa PESU Cassette*

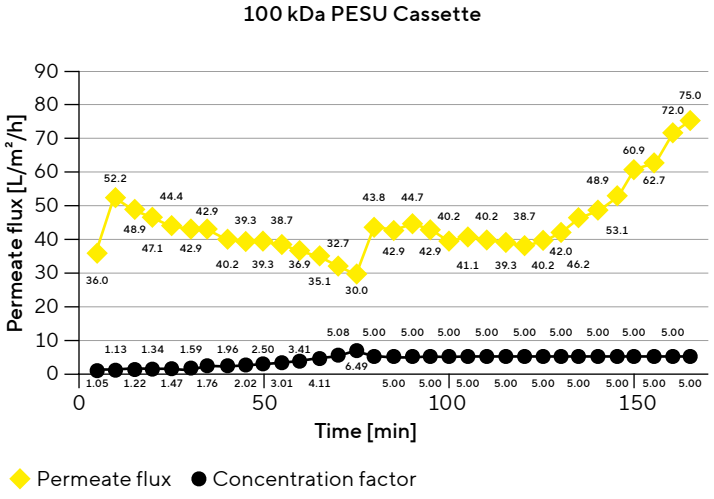
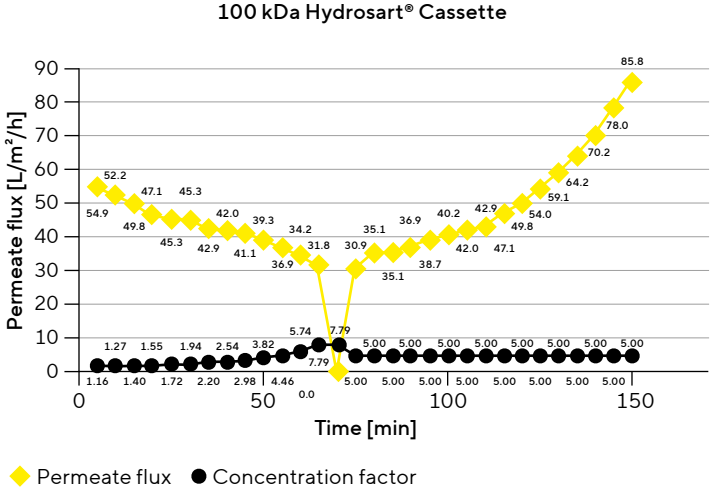


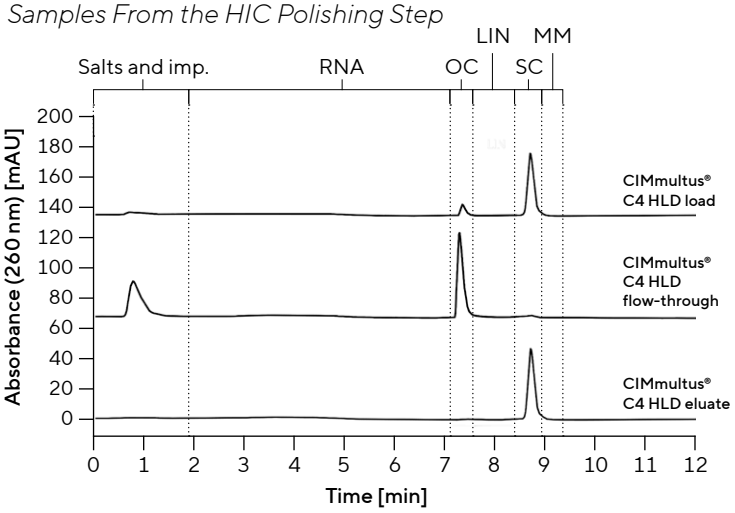
Figure 10: *Detailed Process Parameters of pDNA UF | DF Using a 100 kDa Hydrosart® Cassette*



PATfix® analysis of the retentates revealed an outstanding pDNA recovery of over 99% and 96% for Hydrosart® and PESU cassettes, respectively, with 98% of the pDNA retained in the supercoiled form (0.99 g).

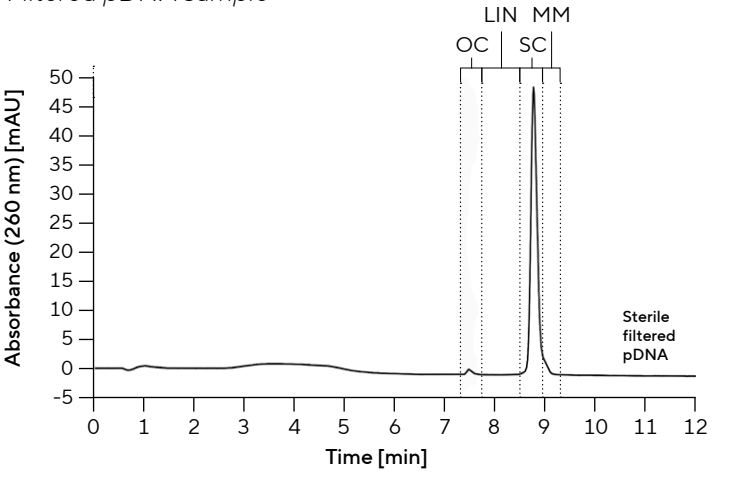
These results indicate that both membrane types with 100 kDa MWCO are suitable for diafiltration and concentration of medium sized pDNA after polishing. For slightly faster processing and consistency of materials used in downstream pDNA processing (compared with the TFF1 step), the use of a Hydrosart® membrane is recommended.

Figure 11: *Overlay of Analytical Chromatograms for Process Samples From the HIC Polishing Step*



Note. SC = supercoiled, OC = open circular, LIN = linear, MM = multimeric

Figure 12: *Analytical Chromatogram for the Final Sterile Filtered pDNA Sample*



Note. SC = supercoiled, OC = open circular, LIN = linear, MM = multimeric

Table 4: *Process Conditions and Results of the UF | DF Process*

Process Conditions	Results	
	Hydrosart® 100 kDa	PESU 100 kDa
Membrane area [m²]	0.02	0.02
Load volume [mL/cm³]	6	6
Pressure (in) initial 5 min | after 5 min [bar]	0.5 | 1.0	0.5 | 1.0
Pressure (perm) initial 5 min | after 5 min [bar]	0.0 | 0.0	0.0 | 0.0
TMP initial 5 min | after 5 min [bar]	0.25 | 0.5	0.25 | 0.5
UF average flux [L/m²/h]	36.4	40.4
DF average flux [L/m²/h]	50.6	48.6
Processing time [min]	150	170
Concentration factor	5	5

7. Final Filtration

Final sterile filtration of the bulk was performed using the Sartopore Evo® 0.8 | 0.2 µm sterilizing-grade filter, which incorporates a coarser pre-filter layer with a pore size of 0.8 µm followed by a final membrane with a pore size of 0.2 µm. The trial was carried out using a Sartoscale 25 flat filter disc device with an effective filtration area of 4.5 cm^2.

The total filter capacity was 1,300 L/m^2, which shows that the heterogeneous double-layer membrane construction of Sartopore Evo® provides outstanding filtration capacity for optimal process economy. With its newly developed, low-binding membrane surface modification, a pDNA recovery of 100% with 98% of the pDNA retained in the supercoiled form (0.99 g) was determined by PATfix® pDNA Platform.

8. Quality Control Analytics

The effectiveness of each pDNA purification step in removing unwanted contaminants throughout the end-to-end process was verified by analyzing the CQAs. All investigated CQAs — host cell DNA, RNA, proteins, and endotoxins — met FDA requirements. Table 5 gives an overview of the analysis results.

9. Overall pDNA Process Recovery

Table 6 summarizes the individual process step recoveries for total pDNA and supercoiled pDNA. Overall, the total pDNA recovery for the complete purification process of the pDNA was at 63% and at 64% for the supercoiled pDNA. The recovery data is influenced by the experimental setup, evaluating several different options for certain process steps and prolonged holding times for processing the material. Under optimized process conditions, especially faster processing in a defined commercial manufacturing process, even higher supercoiled pDNA recoveries can be expected.

Table 5: *Summary of the CQAs of the pDNA Purification Process*

Fraction	pDNA Integrity (% SC)	*E. coli* DNA (µg DNA/mg pDNA)	*E. coli* RNA (PATfix® pDNA area %)	*E. coli* Host Cell Protein (µg HCP/mg pDNA)	Endotoxin (EU/mg pDNA)
Lysate	96	14.4	67	353	16585
Filtrate after clarification and bioburden reduction	91	6.3	67	293	773
UF | DF	88	4	10	458	1224
AEX after UF | DF	87	4.2	< LOD	117	35.8
AEX after in-line dilution	89	4.4	< LOD	19	< 4.6
HIC	99	0.7	< LOD	< 1*	< 0.57
Final UF | DF	>98	0.6	< LOD	< 1*	< 0.33
Final bulk filtration	>98	0.6	< LOD	< 1*	< 0.33
US FDA Acceptance criteria	>80	< 10	n.a.	< 1.0	< 40
USP	> 97	< 2	< 0.2 µg RNA/mg pDNA	< 3	< 10

*Results were below limit of quantification (LOQ) — to get a numeric result, values were set to LOQ

Table 6: *Recovery of Total pDNA and Supercoiled pDNA Within the Individual Steps of the Downstream Process in Relation to the Amount of pDNA | Supercoiled pDNA of the Feed Processed in the Individual Step (Step Recovery)*

Downstream Processing Step	pDNA Step Recovery (%)*	Supercoiled pDNA Step Recovery (%)*
1. Cell lysis	N/A	96
2. Clarification and bioburden reduction	>93	88
Filtered material was divided into two equal small-scale TFF trials and in-line dilution followed by AEX as follows:		
3.1 UF | DF trial 1 Hydrosart® Cassette	81	76
3.1 UF | DF trial 2 PESU Cassette	85	77
3.2 In-line dilution AEX CIMmultus® DEAE-800 (6 µm)	95	93
Retentates after TFF were pooled together and processed on lab-scale AEX:		
4. Capture AEX CIMmultus® DEAE-40 (2 µm)	97	97
Materials after two different AEX capture runs were pooled together and polished on a HIC column as follows:		
5. Polishing (HIC) CIMmultus® C4 HLD-400 (2 µm)	79	91
Eluate from HIC was divided into two equal parts and processed in two different TFF trials:		
6.1 UF | DF trial 1 Hydrosart® Cassette	99	99
6.1 UF | DF trial 2 PESU Cassette	96	96
After TFF, fractions were pooled together, divided into two equal parts, and sterile filtered on two sterile filters		
7.1 Sterile filtration trial 1 Sartopore Evo® 0.8 | 0.2 µm double-layer filter	100	100
Overall recovery	63	64

Discussion and Conclusion

The increasing use of ATMPs across various medical indications necessitates robust and high-yield downstream processing of high-quality pDNA. Maintaining the supercoiled conformation during pDNA purification is crucial, as this stable form is less prone to mutations. Additionally, its compact nature significantly enhances cellular uptake, ensuring higher transfection efficiency in cell engineering.

The end-to-end downstream purification process utilizing Sartorius technologies, from the lysis of the pDNA-containing cell paste to final bulk filtration, demonstrated highly efficient pDNA purification with high recovery and quality.
The final filtrate contained 98% pDNA in the supercoiled form, significantly exceeding the FDA requirement of >80% supercoiled pDNA.

In addition to the pDNA composition, important CQAs were analyzed throughout the purification process.
Table 5 provides an overview of the results of the individual CQA analyses, all of which met the FDA requirements.

Lysis of the pDNA-expressing *E. coli* cells was carried out with the cell lysis system Alkalizator. The automated system ensures precise process control and employs a specialized mixing strategy that supports efficient $CaCl_2$ precipitation. This is crucial as it facilitates the formation of stable flocs during alkaline cell lysis, enabling efficient separation of lysate and flocs and resulting in a lysate with high pDNA content.

Clarification and bioburden reduction were accomplished by filtering the lysate sequentially through a Sartopure® PP3 filter (20 µm pores) and a Sartopore® 2 XLG 0.8 | 0.2 µm filter. The Sartopure® PP3 filter effectively captures particles, while its all-polypropylene design minimizes nonspecific binding and enhances product yield. This efficient prefiltration protects the Sartopore® 2 XLG sterile filter, thereby ensuring excellent filtration capacity for bioburden reduction.

The clarified lysate was prepared for pDNA capture by AEX using two approaches: one utilized UF | DF via TFF on the Sartoflow® Smart benchtop system for concentration and removal of impurities | salts, while the other employed in-line dilution to reduce conductivity and enable sample processing by AEX. Both methods proved to be effective preparatory techniques for achieving high recoveries of pDNA and efficient removal of host cell impurities with AEX (Tables 5 and 6).

The in-line dilution process facilitates the rapid transfer of pDNA from harsh lysate conditions, which can compromise pDNA stability, to plasmid-stabilizing buffer conditions. In-line dilution can be applied to CIMmultus® DEAE columns with 2 µm or 6 µm channels. The latter enables faster processing at higher flow rates but requires a larger column due to its lower pDNA binding capacity. In contrast, sample preparation by TFF significantly reduces the sample volume before AEX capture, decreasing the need for high flow rates and allowing the use of CIMmultus® DEAE columns with 2 µm channels, which offer higher capacity.

Final polishing of the pDNA by removing remaining contaminants was achieved by HIC employing the CIMmultus® C4 HLD 400 mL (2 µm) monolithic column in SHIC conditions. CIMmultus® C4 HLD enables fast and gentle polishing of the pDNA. Impurities were effectively removed while the supercoiled pDNA was recovered at high yield.

Before final sterile filtration, the polished pDNA underwent a further UF | DF cycle for concentration and buffer exchange to a long-term stabilizing Tris-EDTA buffer. There were small losses of pDNA regardless of which TFF cassette, Sartocon® Hydrosart® or PESU, was used (Table 6).

Sartopore Evo® single- and double-layer filters were used for the final sterile filtration of the bulk material. The filter capacity calculation showed a 120% higher capacity of the double-layer filter, while the pDNA yield and quality were essentially unaffected by the filtration, regardless of the filter used.

Overall, Sartorius technologies ensure robust, end-to-end pDNA downstream processing with efficient removal of impurities, high product yield, and purity, along with a significant proportion of supercoiled pDNA maintained across all purification steps. Furthermore, the process meets the FDA requirements for supercoiled pDNA content and successfully fulfills the tested CQAs.

Reference

1. Božič, K., Sedlar, A., Kralj, Š., Černigoj, U., Štrancar, A., & Sekirnik, R. (2024). Selective hydrophobic interaction chromatography for high purity of supercoiled DNA plasmids. *Biotechnology and Bioengineering, 121*(5), 1739–1749. https://doi.org/10.1002/bit.28667

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