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Application Note

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Integrated Two-Step Membrane Chromatography Platform for Scalable Plasmid DNA Purification

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Abstract

Plasmid DNA (pDNA) is a critical raw material for gene therapies, mRNA production, and DNA vaccines, requiring robust and scalable purification solutions. Membrane chromatography enables efficient processing through convective mass transfer, supporting high capacity at short residence times and straightforward scale-up.

We developed a two-step membrane chromatography platform for pDNA purification. Capture using a Sartobind® Q membrane achieved high recovery (~ 97%) with effective removal of RNA and process-related impurities while maintaining plasmid integrity. Subsequent polishing with a Sartobind® Phenyl membrane further improved purity and enabled enrichment of the supercoiled pDNA fraction, with high recovery (~ 94%).

The integrated process delivered up to 98% total pDNA purity and demonstrated robustness across different plasmid sizes, confirming platform versatility. Overall, this approach provides a scalable, high-throughput solution for producing high-quality pDNA for advanced therapeutic applications.

Introduction

Plasmid DNA (pDNA) is a well-established non-viral platform for gene-based therapeutics, enabling in vivo expression of therapeutic proteins, antigens, and genome editing components. Its favorable safety profile and compatibility with scalable manufacturing — demonstrated by applications such as DNA vaccines — are driving increased demand for robust and cost-effective production processes.

Despite advances in upstream fermentation, downstream purification remains a key bottleneck. Efficient removal of host cell impurities combined with preservation of the biologically relevant supercoiled pDNA isoform is essential for ensuring product quality and regulatory compliance.

Conventional resin-based chromatography is often limited by diffusional mass transfer and scale-up constraints when processing large biomolecules such as pDNA. In contrast, membrane chromatography enables convective flow, supporting high throughput, short residence times, and straightforward linear scale-up.

In this work, a two-step membrane chromatography platform was developed to streamline pDNA purification. Capture using a Sartobind® Q membrane enabled efficient binding of negatively charged pDNA and robust removal of RNA and process-related impurities. Subsequent polishing using a Sartobind® Phenyl membrane supported further impurity removal and improved isoform separation.

Materials and Methods

pDNA production and pretreatment

Two model plasmids (pcDNA3 FLAG p53 and HPV-16 E6/E7) were produced in E. coli DH5α and harvested after overnight cultivation in Terrific Broth medium. pDNA was isolated using a modified alkaline lysis process followed by clarification, isopropanol precipitation, and ammonium sulfate treatment to reduce protein and RNA impurities. The resulting clarified pDNA feedstock was stored at –80 °C and used for subsequent chromatographic studies.

Chromatography

Sartobind® Q chromatography (capture step)
pDNA capture was performed using Sartobind® Q strong anion-exchange membrane adsorbers. Initial screening and dynamic binding capacity (DBC) studies were conducted using 1 mL membrane devices, while process optimization, robustness evaluation, and integration into a two-step membrane chromatography workflow were performed using 3 mL devices.

Chromatographic experiments were carried out on an ÄKTA™ pure system at 5 membrane volumes per minute (MV/min) with UV detection at 260 nm and in-line conductivity monitoring. Membranes were equilibrated with 20 mM Tris, 10 mM EDTA buffer (pH 8.0), and sample conductivity was adjusted prior to loading. Elution conditions were optimized to maximize pDNA recovery and purity, and fractions from the loading, washing, and elution steps were collected for analysis. The final optimized operating conditions were evaluated in triplicate to confirm process reproducibility.

Sartobind® Phenyl chromatography (polishing step)
Polishing was performed using 3 mL Sartobind® Phenyl membrane adsorbers operated in hydrophobic interaction chromatography (HIC) mode. Experiments were conducted on an ÄKTA™ pure system (Cytiva) at 1.67 MV/min, with UV detection at 260 nm and conductivity monitoring. The reduced flow rate was selected to minimize pressure increases associated with the high salt concentrations required for HIC operation.

Prior to loading, samples were conditioned with 3 M ammonium sulfate in 20 mM Tris, 10 mM EDTA buffer (pH 8.0) to promote pDNA binding. Following loading and washing, pDNA was eluted using a decreasing ammonium sulfate gradient. Elution conditions were optimized to maximize impurity removal while maintaining pDNA recovery, and collected fractions were analyzed throughout process development. The final optimized method was performed in triplicate to demonstrate robustness and reproducibility.

Analytical methods

The following analytical methods were used in the study:

- pDNA quantification using CIMac pDNA Analytical Columns (UV detection at 260 nm).
- Isoform analysis by agarose gel electrophoresis.
- Impurity analysis using bicinchoninic acid (BCA), Limulus amebocyte lysate (LAL), and qPCR assays to measure proteins, endotoxins, and genomic DNA (gDNA), respectively.

Results and Discussion

Capture step optimization

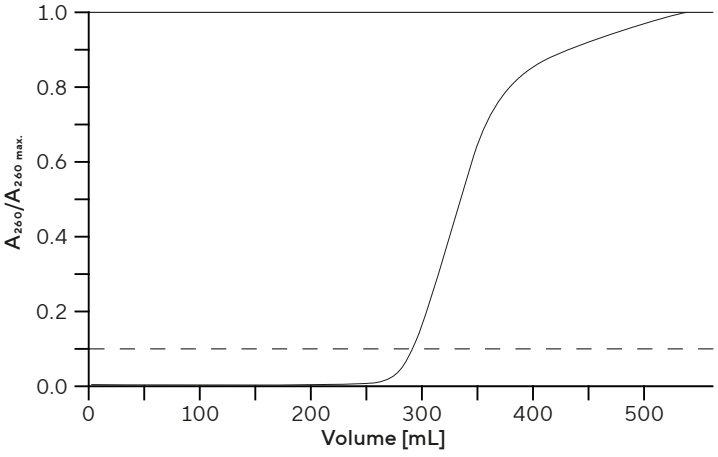
DBC and feasibility of the capture step
pDNA capture was performed using Sartobind® Q strong anion-exchange membrane adsorbers. DBC was evaluated on a 1 mL membrane at 5 MV/min using both purified pDNA and clarified lysate feedstocks. The purified pDNA sample (21.5 µg/mL, 97% supercoiled pcDNA3 FLAG p53, 6.7 kb) was adjusted to 20 mS/cm conductivity prior to loading to support binding under low-ionic-strength conditions.

The breakthrough curve showed efficient pDNA binding, with a 10% breakthrough capacity of 6.3 mg pDNA/mL membrane and a saturation capacity of 11.2 mg pDNA/mL membrane (Figure 1).

To establish representative capture conditions, DBC was also determined using a clarified lysate feedstock containing 19.5 µg/mL pDNA and approximately 14% pDNA purity. A 10% breakthrough point of 3.0 mg pDNA/mL membrane was obtained. Subsequent optimization studies were performed at 70% of the breakthrough capacity, corresponding to a loading of approximately 1.8 mg pDNA/mL membrane.

A preliminary bind-and-elute experiment was then conducted using 450 mL of clarified lysate loaded onto a 1 mL Sartobind® Q membrane. Elution was performed using a 40 MV linear gradient to 2 M NaCl in 20 mM Tris, 10 mM EDTA (pH 8.0). The results demonstrated effective separation of pDNA from RNA impurities, with RNA eluting at lower salt concentrations and pDNA eluting later in the gradient. Complete pDNA recovery was achieved at NaCl concentrations below 1 M, indicating that the final 2 M salt concentration was unnecessary. Consequently, the maximum NaCl concentration used in subsequent experiments was reduced, lowering buffer consumption while maintaining efficient pDNA recovery.

Figure 1: Dynamic breakthrough profile of a 97% supercoiled pcDNA3 FLAG p53 (6.7 kb) sample loaded onto a 1 mL Sartobind® Q membrane (5 MV/min) for DBC determination. The dotted line represents 10% breakthrough.



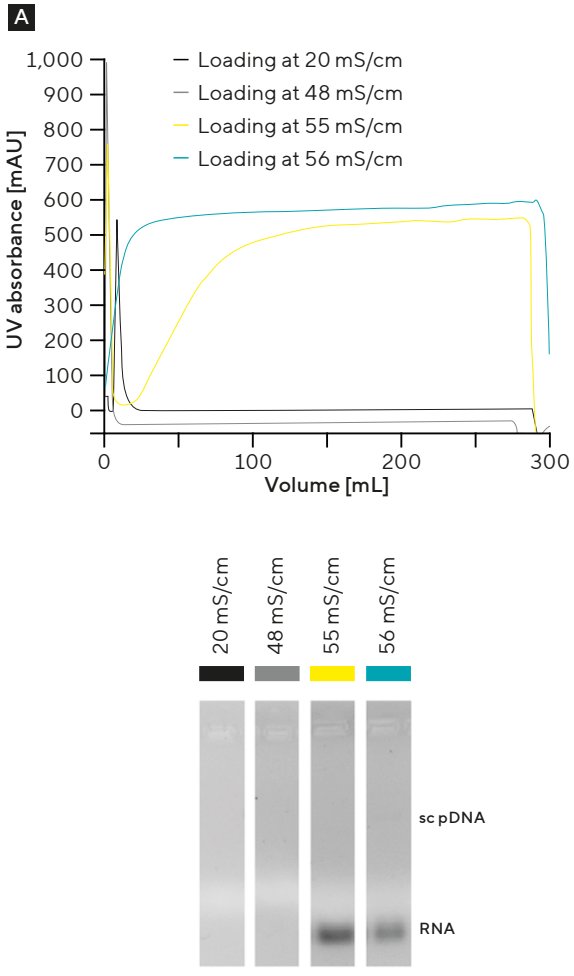
Evaluation of loading conductivity
Loading conductivity is a critical parameter in anion-exchange chromatography, as it influences the binding of both pDNA and process-related impurities. To identify optimal capture conditions, clarified lysate samples were loaded onto 3 mL Sartobind® Q membranes at 5 MV/min using four conductivities (20, 48, 55, and 56 mS/cm).

UV profiles and agarose gel analysis showed that pDNA and RNA were efficiently retained at 20 and 48 mS/cm (Figure 2A). At 55 and 56 mS/cm, increased signal in the flowthrough indicated reduced retention of RNA and other weakly bound species. While higher conductivity improved RNA clearance and increased pDNA purity, excessive conductivity began to compromise pDNA binding. At 56 mS/cm, pDNA breakthrough was observed, resulting in reduced recovery.

Bound material was eluted using a linear gradient to 1 M NaCl in 20 mM Tris and 10 mM EDTA (pH 8.0) over 80 membrane volumes. A clear trade-off between purity and recovery was observed: increasing conductivity improved pDNA purity by reducing RNA co-retention but decreased recovery at the highest conductivity tested due to pDNA losses during loading (Figure 2B).

Based on the combined purity and recovery results, 55 mS/cm was selected as the optimal loading conductivity, providing the best balance between efficient impurity removal and robust pDNA capture performance.

Figure 2: (A) Loading chromatograms at different conductivities with the respective agarose gel analyses (20, 48, 55, and 56 mS/cm). **(B)** Corresponding pDNA purity and recovery after capture.



Evaluation of elution salt

Following optimization of the loading conditions, the elution strategy was evaluated to further improve pDNA purity. Elution was performed on 3 mL Sartobind® Q membranes using either NaCl or guanidine hydrochloride as the elution salt. A linear gradient to 1 M salt in 20 mM Tris, 10 mM EDTA (pH 8.0) was applied over 80 membrane volumes at 5 MV/min.

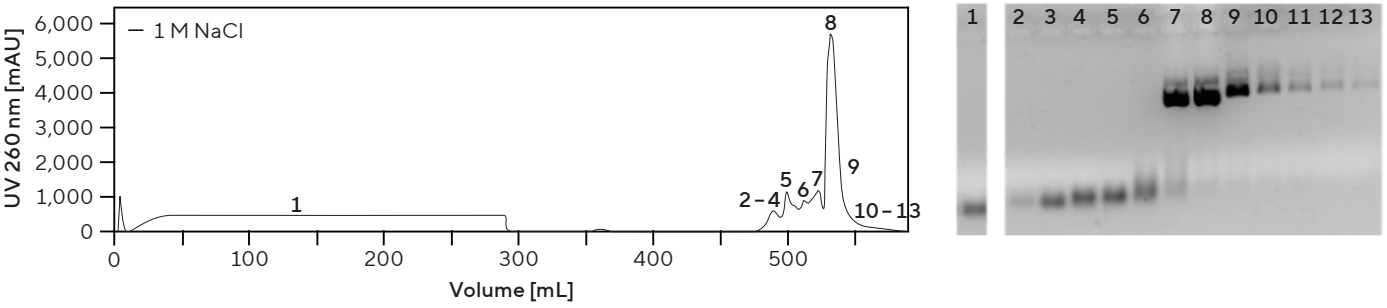
The chromatographic and gel electrophoresis results showed clear differences in impurity separation (Figure 3A and B). When NaCl was used, RNA was distributed across the early gradient fractions with some co-elution observed in the pDNA-containing fractions. In contrast, guanidine hydrochloride concentrated RNA in the initial elution fractions and produced sharper pDNA elution peaks with improved separation between RNA and pDNA.

This improved resolution translated into higher product quality and yield (Figure 3C). Elution with guanidine hydrochloride achieved 96.1% pDNA purity and 96.8% recovery, compared with 90.4% purity and 89.9% recovery when using NaCl. These results demonstrate that guanidine hydrochloride provides more effective separation of pDNA from RNA contaminants while maintaining excellent recovery.

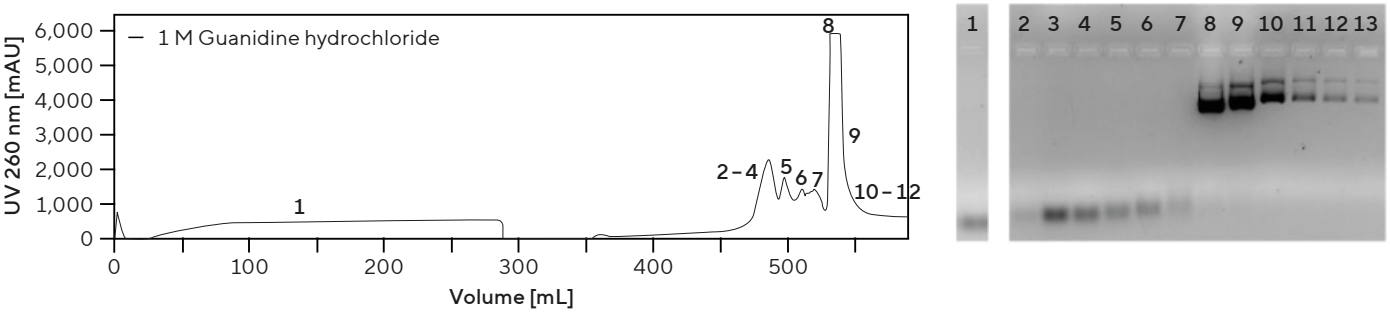
Additionally, after loading approximately 300 mL of clarified lysate, the majority of the pDNA was recovered in only three concentrated 10 mL fractions, enabling significant product concentration during the capture step. Based on these findings, guanidine hydrochloride was selected as the preferred elution salt for subsequent Sartobind® Q capture chromatography experiments.

Figure 3: Capture elution profiles and corresponding agarose gel analyses using **(A)** NaCl and **(B)** guanidine hydrochloride. **(C)** pDNA purity and recovery.

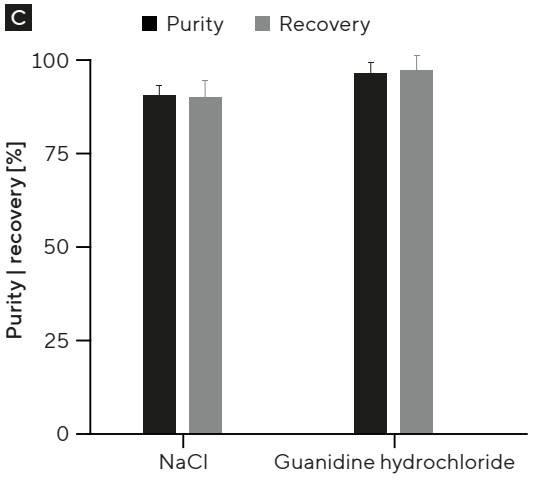
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Reproducibility analysis

Following identification of the optimal capture conditions, reproducibility studies were performed using a loading conductivity of 55 mS/cm and guanidine hydrochloride as the elution salt. Experiments were conducted on 3 mL Sartobind® Q membranes, and the chromatographic profiles obtained across three independent runs showed highly consistent pDNA binding and elution behavior (Figure 4).

The optimized process combined selective RNA removal during loading with enhanced pDNA–RNA separation during elution, resulting in 96.1 ± 2.6% pDNA purity and 96.8 ± 3.9% recovery (Table 1). Run-to-run variability was low, with a relative standard deviation below 4%, demonstrating excellent process robustness and reproducibility.

The performance achieved with Sartobind® Q compares favorably with reported state-of-the-art convective media for direct lysate purification, where pDNA recoveries are typically in the range of 85 – 93%. The combination of high recovery, high purity, and reproducible operation highlights the suitability of Sartobind® Q membranes for efficient pDNA capture in a scalable purification workflow.

Figure 4: Reproducibility of Sartobind® Q capture under optimized conditions (55 mS/cm, guanidine hydrochloride elution).

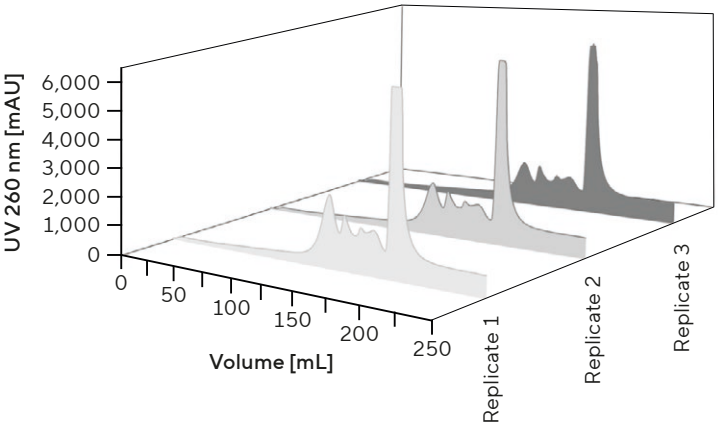


Table 1: Reproducibility of capture chromatography on Sartobind® Q (3 mL) under optimized conditions (purity and recovery).

	Replicate 1	Replicate 2	Replicate 3	Mean ± standard deviation
Purity [%]	98.7	96.3	93.4	96.1 ± 2.6
Recovery [%]	97.9	92.5	100.0	96.8 ± 3.9

Polishing step optimization

DBC

To evaluate the suitability of Sartobind® Phenyl for pDNA polishing, DBC studies were performed using 3 mL membrane adsorbers operated in hydrophobic interaction chromatography (HIC) mode. A purified pcDNA3 FLAG p53 sample (31.6 µg/mL, 96% supercoiled) was conditioned to 3 M ammonium sulfate, filtered through a 0.45 µm filter, and continuously loaded at 5 mL/min (1.67 MV/min). The reduced flow rate was selected to limit pressure increases and minimize precipitation under high-salt conditions.

The breakthrough experiment showed that 4.5 mg of pDNA was required to reach 10% breakthrough, corresponding to a DBC of 1.5 mg pDNA/mL membrane.

For subsequent optimization studies, the loading amount was set to 70% of the breakthrough capacity, corresponding to approximately 2.6 mg of pDNA per run. These results demonstrate that Sartobind® Phenyl provides sufficient binding capacity to support its implementation as an effective polishing step within the two-step membrane chromatography workflow.

Effect of capture elution conditions on polishing performance

High-purity pDNA production typically requires the integration of a capture and a polishing chromatography step. While these operations can be optimized individually, capture elution conditions may significantly influence polishing performance by affecting the composition and physicochemical properties of the capture eluate.

To evaluate this effect, the impact of different Sartobind® Q elution conditions on subsequent Sartobind® Phenyl HIC polishing was investigated. Attention was given to impurity carryover, salt composition, and their influence on pDNA binding and separation during polishing.

The results showed that capture conditions directly affected polishing efficiency. Improved separation of RNA during the capture step reduced impurity burden in the polishing feed and enhanced downstream purification performance. In addition, the choice of elution chemistry influenced the conditioning requirements prior to HIC and the overall effectiveness of impurity removal.

These findings highlight the importance of considering capture and polishing steps as an integrated process rather than as isolated unit operations. Optimizing capture elution conditions can simplify downstream processing, improve polishing performance, and contribute to the development of robust, high-throughput pDNA purification workflows with enhanced product quality.

NaCl-eluted samples

The performance of Sartobind® Phenyl polishing was first evaluated using capture eluates generated with NaCl during the Sartobind® Q step. Because NaCl-based capture resulted in partial RNA co-elution, residual RNA was carried into the polishing feed and influenced subsequent HIC separation performance.

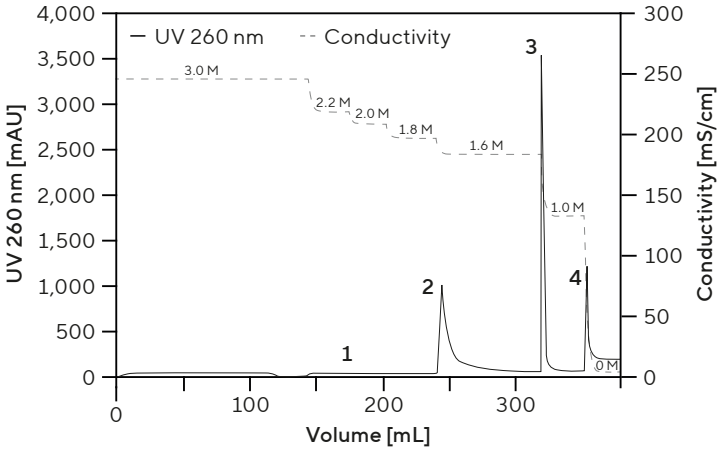
To establish the elution behavior of plasmid isoforms and residual RNA, the 3 mL Sartobind® Phenyl membrane was equilibrated with 3 M ammonium sulfate in 20 mM Tris and 10 mM EDTA (pH 8.0). One-third of the capture eluate was loaded at 5 mL/min, and stepwise decreases in ammonium sulfate concentration were applied to achieve sufficient resolution between nucleic acid species. The results showed distinct elution of open circular (oc) and supercoiled (sc) pDNA, with the more compact sc isoform exhibiting stronger retention and eluting at lower salt concentrations.

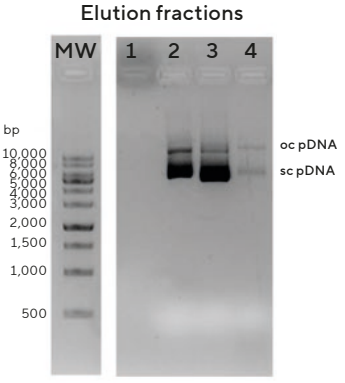
Several elution strategies were evaluated to maximize sc pDNA enrichment (Figure 5). Introduction of an intermediate 2 M ammonium sulfate step improved removal of oc pDNA and yielded 95% sc pDNA purity with 70% recovery. Further optimization using a simplified step-elution strategy at 1.65 M and 0.8 M ammonium sulfate provided the best overall performance, producing pDNA containing 97.7% sc isoform and 2.3% oc isoform, with a recovery of 79%.

Although efficient isoform enrichment was achieved, trace RNA was still detected in the later polishing fractions, indicating residual RNA carryover from the Sartobind® Q capture step. These results demonstrate that the impurity profile generated during capture directly influences polishing performance and underline the importance of optimizing capture conditions to maximize downstream separation efficiency and process robustness.

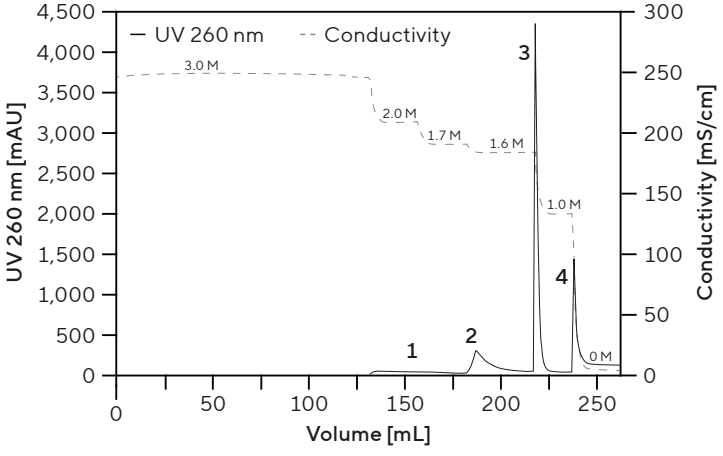
Figure 5: Optimization of HIC polishing on Sartobind® Phenyl membranes. Stepwise ammonium sulfate gradients show isoform separation across **(A)** initial screening, **(B)** intermediate conditions, and **(C)** optimized conditions.

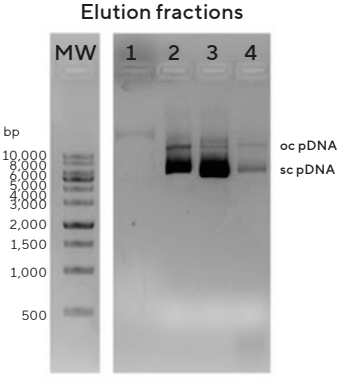
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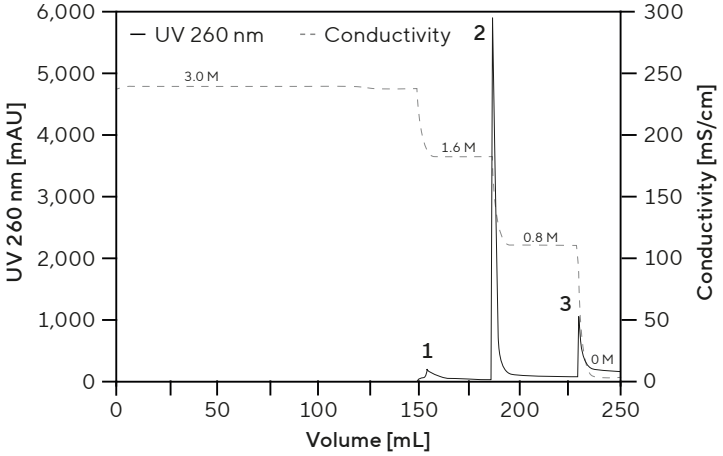


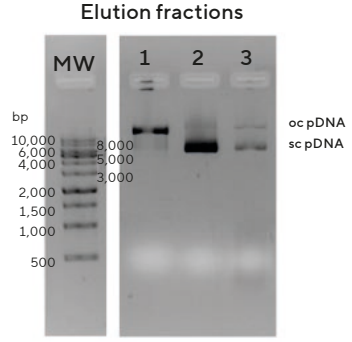
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Guanidine hydrochloride-eluted samples

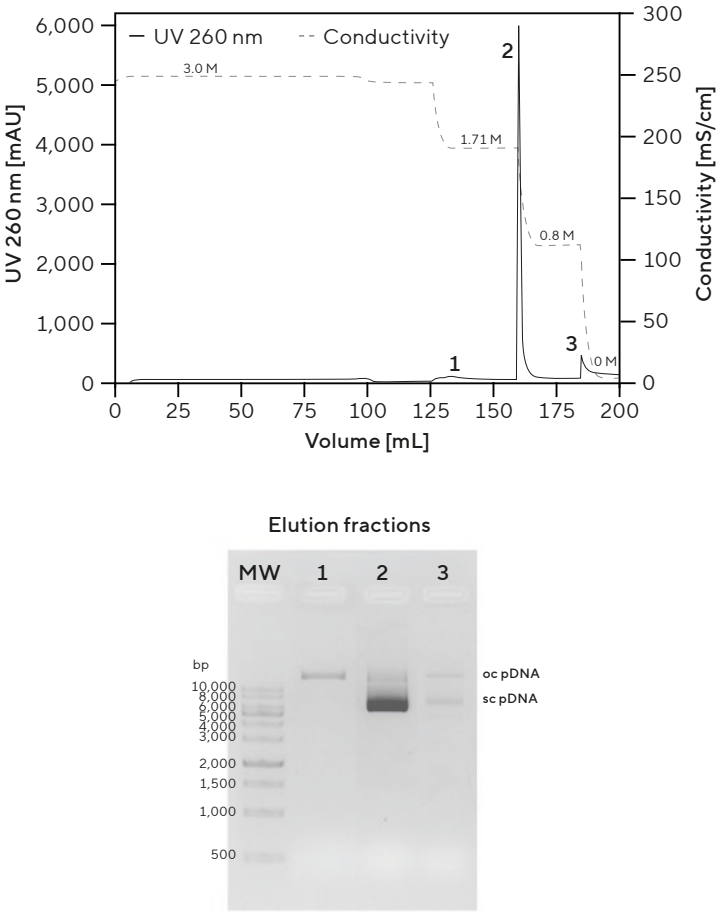
The optimized HIC polishing process was subsequently evaluated using capture eluates generated with guanidine hydrochloride. When the step-elution strategy developed for NaCl-eluted material was applied directly, the chromatographic profile changed and the sc and oc pDNA isoforms co-eluted, indicating that the reduced RNA burden and altered eluate composition affected separation behavior on Sartobind® Phenyl.

To restore isoform resolution, the intermediate elution step was adjusted from 1.65 M to 1.71 M ammonium sulfate (Figure 6). This minor modification improved separation between plasmid isoforms, enabling preferential recovery of the supercoiled (sc) pDNA fraction while reducing contamination by the open circular (oc) form. Under these optimized conditions, the recovered product contained 97.4% sc pDNA.

In contrast to NaCl-eluted material, no residual RNA was detected during downstream polishing of guanidine hydrochloride capture eluates. The improved impurity clearance achieved during the Sartobind® Q capture step simplified the subsequent HIC operation and enabled isolation of highly pure sc pDNA using a streamlined polishing strategy.

Overall, these results demonstrate that polishing conditions should be optimized according to the chemistry of the capture eluate. Capture elution with guanidine hydrochloride provided superior impurity removal, reduced polishing complexity, and supported robust production of highly pure sc pDNA while maintaining high process recovery.

Figure 6: Optimized polishing profile for guanidine hydrochloride-eluted samples using a stepwise ammonium sulfate gradient.



Reproducibility analysis

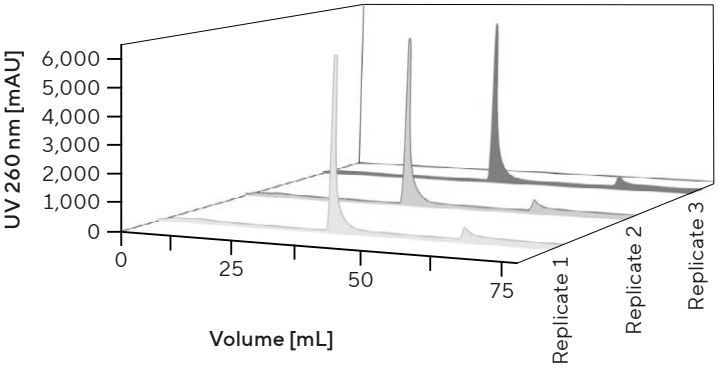
To assess process robustness, reproducibility studies were performed using the optimized Sartobind® Phenyl polishing method developed for guanidine hydrochloride-eluted Sartobind® Q capture fractions. The selected step-elution strategy consisted of 3.0, 1.71, 0.8, and 0 M ammonium sulfate (Figure 7).

Highly consistent chromatographic profiles were obtained across replicate runs, demonstrating reproducible binding and elution behavior. The low variability in peak shape, elution volume, and overall chromatographic pattern confirms the robustness and reliability of the polishing process when applied to guanidine hydrochloride capture eluates.

Analytical characterization showed efficient separation of the open circular (oc) and supercoiled (sc) plasmid isoforms, yielding approximately 99% sc pDNA purity with a recovery of ~93% (Table 2).

Overall, the optimized polishing step delivered highly reproducible performance and enabled robust production of highly enriched sc pDNA, providing a strong complement to the Sartobind® Q capture step within the integrated two-step membrane chromatography workflow.

Figure 7: Reproducibility of Sartobind® Phenyl polishing under optimized conditions.



Process robustness across plasmid sizes

To assess process robustness, the optimized two-step membrane chromatography workflow was evaluated using a larger plasmid, HPV-16 E6/E7 (8.7 kb). For the capture step, the conditions optimized with pcDNA3 FLAG p53 were applied: clarified lysate was loaded onto Sartobind® Q at approximately 55 mS/cm and eluted with 1 M guanidine hydrochloride (Figure 8A).

The E6/E7 clarified lysate contained a higher relative RNA burden than the p53 feed, resulting in increased UV absorbance during loading and early elution fractions. Despite this more challenging impurity profile, no pDNA was detected in the flowthrough, confirming effective binding under the selected capture conditions. However, the higher RNA content reduced peak resolution during elution and affected capture performance, yielding 96.8% pDNA purity and 85.5% recovery.

Because plasmid size and feed composition can influence HIC behavior, both optimized Sartobind® Phenyl polishing gradients were evaluated. The gradient consisting of 3.0, 1.65, 0.8, and 0 M ammonium sulfate provided the best isoform separation for the E6/E7 plasmid, producing a final sc pDNA fraction with 99.1% sc purity and 92.4% recovery (Figure 8B). In comparison, the alternative gradient of 3.0, 1.71, 0.8, and 0 M ammonium sulfate resulted in lower isoform resolution and reduced sc pDNA purity (92.1%, Figure 8C).

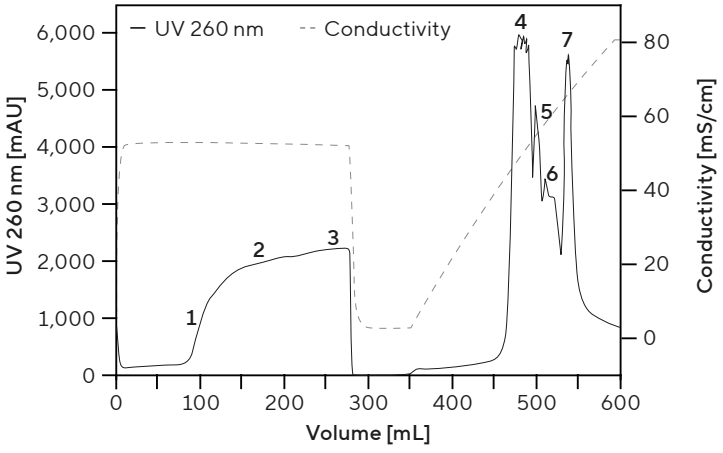
Overall, the 3.0 – 1.65 – 0.8 – 0 M ammonium sulfate elution scheme was selected as the most suitable polishing strategy for the larger E6/E7 plasmid. The final performance was comparable to that obtained with pcDNA3 FLAG p53, with differences below 1% in both purity and recovery, demonstrating good process transferability across plasmid constructs.

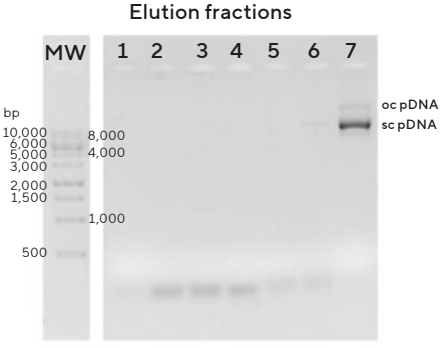
Table 2: Reproducibility of polishing chromatography on Sartobind® Phenyl membranes (3 mL).

	Replicate 1	Replicate 2	Replicate 3	Mean ± standard deviation
Supercoiled pDNA purity [%]	98.8	97.9	99.7	98.8 ± 0.9
Supercoiled pDNA recovery [%]	92.4	98.8	89.4	93.5 ± 4.8

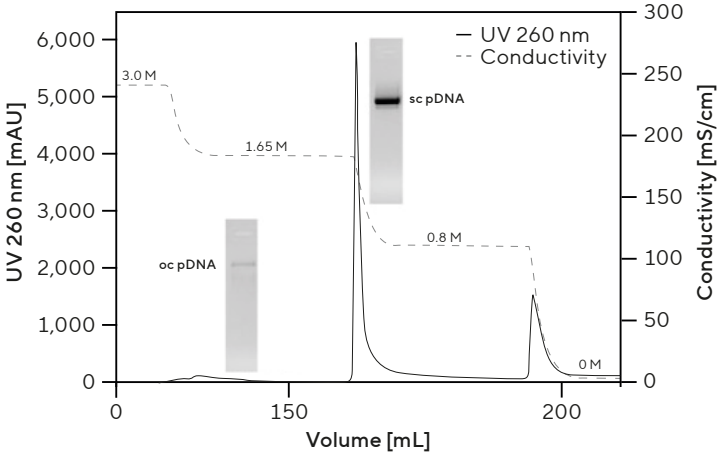
Figure 8: Capture and polishing of HPV-16 E6/E7 plasmid. **(A)** Capture profile (guanidine hydrochloride elution). **(B – C)** Polishing under different ammonium sulfate step gradients.

A

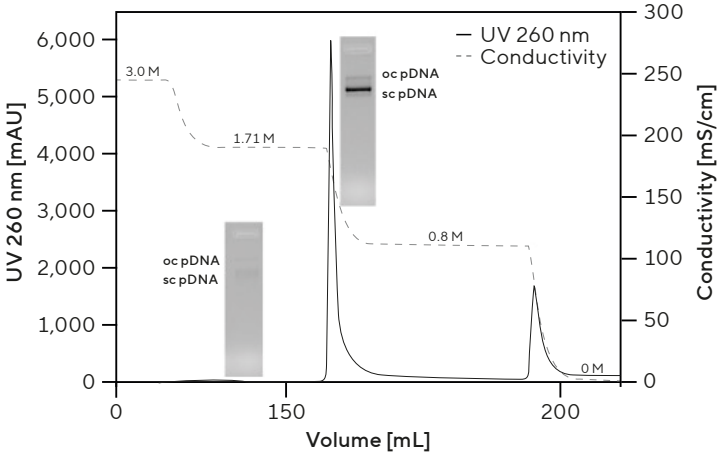




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C



Final product quality assessment against regulatory expectations

Final product characterization confirmed efficient impurity clearance and favorable product quality (Table 3). Across the tested plasmids, polishing resulted in sc pDNA levels above 97%, while host cell impurities were strongly reduced. Up to 99.4% of gDNA was removed, no protein contamination was detected in the final sc pDNA fractions, and endotoxin levels remained below the accepted threshold for the reported assay output unit. These results demonstrate that the integrated Sartobind® Q capture + Sartobind® Phenyl polishing workflow is robust toward differences in plasmid size and lysate composition, supporting its use for high-purity sc pDNA purification.

Table 3: Summary of process performance across plasmids (pcDNA3 FLAG p53 and HPV-16 E6/E7), including isoform distribution, and impurity clearance (gDNA, host cell protein, and endotoxins).

Plasmid	Step	pDNA integrity (% sc)	% oc pDNA	E. coli gDNA (µg gDNA/mg pDNA)	Host cell protein (µg protein/mg pDNA)	Endotoxin (EU/mg pDNA)
p53 (6.7 kb)	Lysate	–	–	64.7	58.3	0.5
	Capture	96.2	3.8	15.3	Undetectable	0.1
	Polishing	97.6	2.4	0.4	Undetectable	0.1
HPV-16 E6/E7 (8.7 kb)	Lysate	–	–	140.2	69.5	0.4
	Capture	91.6	8.4	58.2	Undetectable	0.3
	Polishing	97.9	2.1	1.4	Undetectable	0.2
Quality criteria	FDA	>80%	–	< 10 µg gDNA/mg pDNA	< 1.0 µg HCP/mg pDNA	<40

Conclusion

This study demonstrates the development of an integrated two-step membrane chromatography workflow for high-purity plasmid DNA (pDNA) purification, combining Sartobind® Q membrane adsorbers for capture with Sartobind® Phenyl membrane adsorbers for polishing. By leveraging complementary charge-based and hydrophobic interaction mechanisms, the process enables efficient impurity removal, preservation of plasmid integrity, and enrichment of the sc pDNA isoform.

During the capture step, optimization of loading conductivity and elution conditions proved critical for balancing pDNA recovery and impurity clearance. Operation at 55 mS/cm minimized RNA co-retention while maintaining strong pDNA binding, and the use of guanidine hydrochloride as the elution salt improved separation of pDNA from RNA compared with conventional NaCl-based elution. Under optimized conditions, Sartobind® Q achieved approximately 96% pDNA purity and 97% recovery with excellent run-to-run reproducibility.

Subsequent polishing on Sartobind® Phenyl enabled efficient separation of supercoiled and open circular plasmid isoforms through optimized ammonium sulfate step-elution strategies. The polishing step routinely delivered up to 99% sc pDNA purity with recoveries of approximately 93 – 94%, while demonstrating robust and reproducible performance.

Importantly, the study highlights the value of process integration. Capture elution chemistry directly influenced polishing performance, with guanidine hydrochloride reducing RNA carryover and simplifying downstream HIC purification. The workflow also proved robust across different plasmid constructs, maintaining high purity and recovery for both 6.7 kb and 8.7 kb plasmids despite differences in feed composition and impurity burden.

Overall, the combination of Sartobind® Q capture and Sartobind® Phenyl polishing provides a scalable and efficient membrane chromatography platform capable of delivering regulatory-grade sc pDNA, representing an attractive alternative to conventional multistep resin-based purification processes.

Acknowledgments

The authors gratefully acknowledge Jens Meyer and Alexander Tappe (Application & Testing, Separation Technology Consumables) for their support and for enabling this collaboration.

Additional Resources

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