Application Note

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Plasmid DNA, pDNA purification, isoform separation, supercoiled pDNA, plasmid process pack, polishing step, sample displacement, CIMmultus® C4 HLD

# Increasing Productivity of pDNA DSP Using Sample Displacement Chromatography

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## Abstract

Plasmid DNA (pDNA) as a pharmaceutical product has stringent purity and efficacy requirements, and often one or more chromatographic steps are used in the downstream process. High ligand density butyl-modified chromatographic monolith (CIMmultus® C4 HLD, part of CIMmultus® HiP² Plasmid Process Pack™ ) is currently employed in the polishing step of a pDNA purification process. It is mainly used for separation of supercoiled (SC) pDNA separation from open circular (OC) and linear pDNA isoforms and the removal of remaining gDNA and RNA.

This application note presents a comparison of two different polishing processes employing monoliths, namely bind-elute (BE) and the more recently described sample displacement purification (SDP).

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# Introduction

Cell lysate containing 9.1 kbp pKLAC (Generi Biotech, Czech Republic) was captured by 8 mL CIMmultus® DEAE Advanced Composite Column, part of CIMmultus® HiP² Plasmid Process Pack™. Following the capture step on DEAE, the collected sample was divided into two parts subjected to the before-mentioned polishing methods on a 1 mL CIMmultus® C4 HLD Advanced Composite Column.

BE purification requires a high concentration of ammonium sulphate (AS) during loading. Elution is then achieved by descending AS gradient. After choosing optimal wash and elution mobile phases from screening experiments, optimal run conditions were selected (Figure 1).

SDP utilizes different relative binding affinities of components in a sample mixture and separates pDNA isoforms under overloading conditions, where sc pDNA isoform acts as a displacer of oc or linear pDNA. Optimal AS concentration range was determined from screening runs. Plasmid DNA was loaded in 1.8 M AS, and the main elution was collected in 1.2 M AS (Figure 2).

Two different analytical techniques analysed elution fractions: HPLC analytics using CIMac™ pyridine-0.1 Analytical Column (gradient elution from 2.5 M to 0 M AS) and agarose gel electrophoresis (AGE) (Figure 1 and Figure 2).

Supercoiled pDNA production yield and homogeneity of the sc isoform in the main elution fraction were estimated (Figure 3) for both methods. The homogeneity of sc pDNA isoform was determined as the ratio between the area of sc pDNA isoform and the area of both oc pDNA and sc pDNA isoforms in the main elution fraction. Both methods were compared regarding the loaded amount of sc pDNA, the mass of reagents, i.e., AS and deionized water, load volume, and process time needed for the purification of 1 mg of sc pDNA using 1 mL C4 HLD column (Table 2).

# Materials

- CIMmultus® DEAE-8 Advanced Composite Column
- CIMmultus® C4 HLD -1 Advanced Composite Column
- CIMac™ pyridine-0.1 Analytical Column

We gratefully acknowledge Generi Biotech (Machkova, Czech Republic) for providing us biomass containing pKLAC plasmid DNA.

# Methods

### Conditions for capture step

| | |
|---|---|
| Column | CIMmultus® DEAE-8 Advanced Composite Column |
| Conditions | Buffer A: 50 mM TRIS 10 mM EDTA pH 7.2;<br>Buffer B: 50 mM TRIS 10 mM EDTA 1 M NaCl pH 7.2 |
| Detection | UV at 260 nm |
| Flow rate | 80 mL/min, elution with 10 mL/min |
| Sample | Elution of pDNA was performed with 1.0 M NaCl |

### Conditions for polishing step in bind-elute method

| | |
|---|---|
| Column | CIMmultus® C4 HLD-1 Advanced Composite Column |
| Conditions | Binding buffer: 50 mM TRIS 10 mM EDTA 3.0 M AS pH 7.2;<br>Washing buffer: 50 mM TRIS 10 mM EDTA 1.95 M AS pH 7.2;<br>Elution buffer: 50 mM TRIS 10 mM EDTA 1.2 M AS pH 7.2;<br>Stripping buffer: 50 mM TRIS 10 mM EDTA pH 7.2 |
| Detection | UV at 260 nm |
| Flow rate | Loading: 4.0 mL/min<br>Wash and elution: 2.0 mL/min |
| Sample | Elution fraction from DEAE capture step containing 2.0 mg pKLAC plasmid was diluted with 50 mM TRIS 10 mM EDTA 4.0 M AS pH 7.2 in volumetric ratio 1:3 |

## Conditions for polishing step in sample-displacement method

| | |
|---|---|
| Column | CIMmultus® C4 HLD-1 Advanced Composite Column |
| Conditions | Binding buffer: 50 mM TRIS 10 mM EDTA 1.8 M AS pH 7.2;<br>Washing buffer: 50 mM TRIS 10 mM EDTA 2.0 M AS pH 7.2;<br>Elution buffer: 50 mM TRIS 10 mM EDTA 1.2 M AS pH 7.2;<br>Stripping buffer: 50 mM TRIS 10 mM EDTA pH 7.2 |
| Detection | UV at 260 nm |
| Flow rate | 4.0 mL/min |
| Sample | Elution fraction from DEAE capture step containing 1.9 mg pKLAC plasmid was diluted with 50 mM TRIS 10 mM EDTA 4.0 M AS pH 7.2 in volumetric ratio 1:0.82 |

## Chromatographic conditions for pDNA analytics

| | |
|---|---|
| Column | CIMac™ pyridine-0.1 Analytical Column |
| Conditions | Buffer A: 50 mM TRIS 10 mM EDTA 2.5 M AS pH 7.2;<br>Buffer B: 50 mM TRIS 10 mM EDTA pH 7.2 |
| Detection | UV at 260 nm |
| Flow rate | 1.0 mL/min |
| Sample | Elution fractions from preparative run |
| Injection volume | 200 µL |
| Method | Linear gradient from 2.5 M AS to 0 M AS in 4.0 min |

# Results

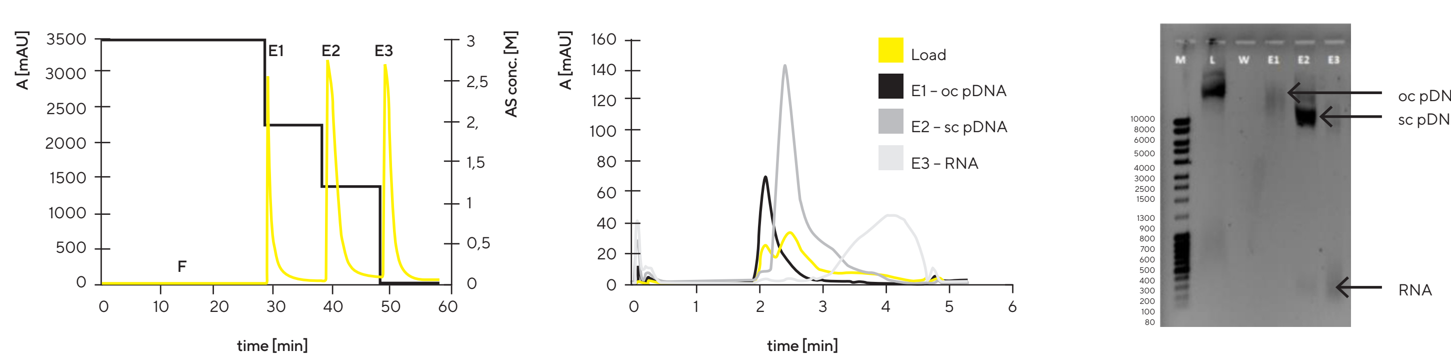


Figure 1: Bind-elute polishing step – left: preparative chromatographic run (load in 3.0 M AS, E1: 1.95 M AS (mainly oc pDNA isoform), E2: 1.2 M AS (mainly sc pDNA isoform), E3: 0 M AS (mainly RNA); center: HPLC analytics with CIMac™ pyridine-0.1 Analytical Column; right: Agarose electrophoresis (AGE) analysis of elution fractions.

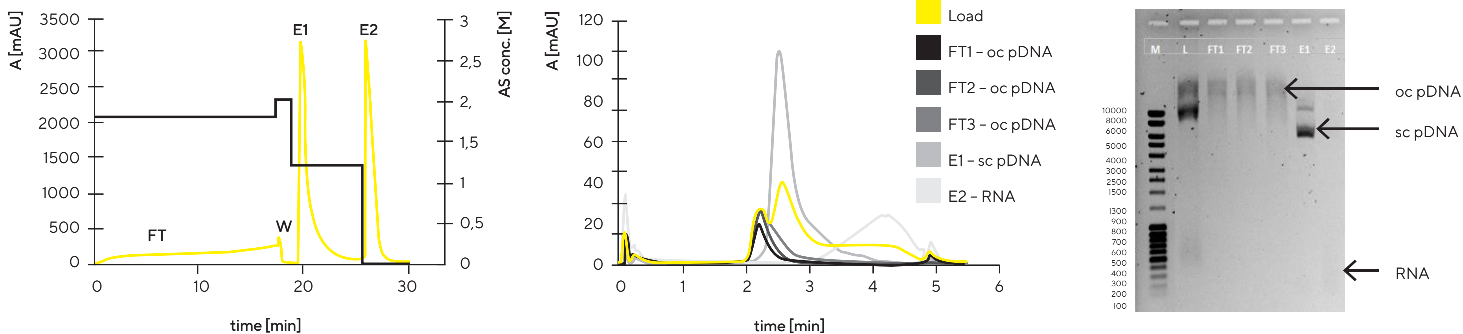


Figure 2: SDP polishing step – left: preparative chromatographic run (load in 1.8 M AS; FT (mainly oc pDNA isoform), W: 2.0 M AS, E1: 1.2 M AS (mainly sc pDNA isoform), E2: 0 M AS (mainly RNA); middle: HPLC analytics with CIMac™ pyridine-0.1 Analytical Column; right: AGE analysis of elution fractions.

| | pDNA isoform ratio | | RNA presence |
|---|---|---|---|
| Method | sc pDNA | oc pDNA | RNA [%] |
| Load | 59.8 | 21.1 | Observed on AGE and in analytical chromatographic run (estimated between 20% and 30% of the total nucleic acids amount in loading sample) |
| Main elution BE | 98.4 | 1.6 | Not detected on AGE; estimated below 5% of the total nucleic acids amount in the main elution sample (from analytical chromatographic run) |
| Main elution SDP | 98.7 | 1.3 | |

Table 1: The composition of loading sample and main elution fraction for both downstream protocols (BE and SDP).

# Discussion

High homogeneity (98%) of sc pDNA in the main elution fraction was achieved in both methods, while SDP resulted in 5% higher yield compared to BE. This 5% increase in pure product yield (sc pDNA isoform) using SDP was achieved alongside a 60% reduction in chemicals consumption and a 20% reduction in processing time per gram of product. These figures represent a significant improvement over bind-elute and could translate into higher profit in an industrial pDNA downstream process.

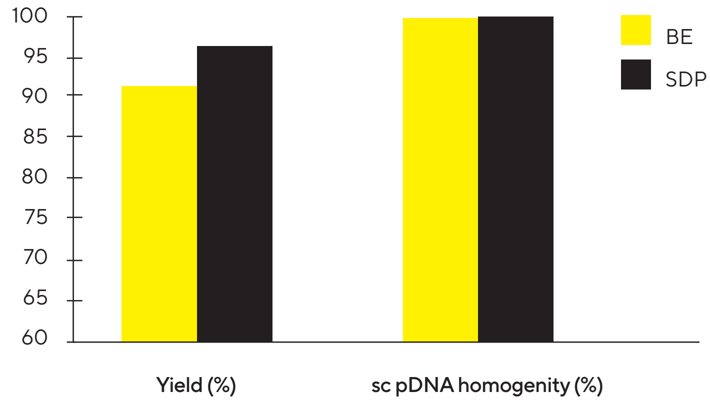


Figure 3: Yield of purified sc pDNA isoform and homogeneity of sc pDNA isoform for BE and SDP

| Method – single run | Loaded amount of sc pDNA [mg] | sc pDNA in final elution fraction | m [g] AS / mg sc pDNA | m [g] $H_2O$ / mg sc pDNA | V [ml] load / mg sc pDNA | t [min] method / mg sc pDNA |
|---|---|---|---|---|---|---|
| BE | 1.4 | 1.24 | 69.1 | 155.3 | 58.8 | 106.2 |
| SDP | 1.1 | 1.06 | 27.3 | 84.3 | 32.3 | 84.0 |

Table 2: Comparison of two polishing chromatographic processes – classical bind–elute (BE) versus sample displacement purification (SDP).

# Conclusion

Both chromatographic methods – classical bind-elute purification (BE) as well as sample displacement purification (SDP), are suitable for polishing purification step of plasmid DNA.

# References

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