Simplifying Progress

Preparation of Linear Plasmid DNA for in Vitro Transcription Reaction

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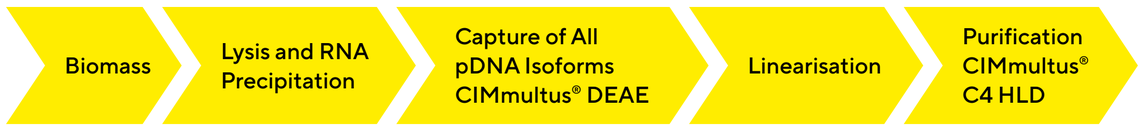
Introduction

Linearised pDNA is currently the starting point of In-Vitro-Transcription processes to synthesize mRNA.

Large scale purification protocols for manufacturing of pDNA used for Gene Therapy applications typically include two chromatography steps. The first step captures both linear, open circular and supercoiled pDNA species. The polishing step enriches supercoiled pDNA, while discarding other isoforms.

We describe a single-step-capture strategy to maximize the recovery of pDNA for further linearization.

Optimised Purification Workflow



Methods

Capture with CIMmultus® DEAE

After lysis and RNA precipitation capture of all pDNA isoforms was achieved with CIMmultus® DEAE (Conditions: see protocol that comes with HiP² Plasmid Pack).

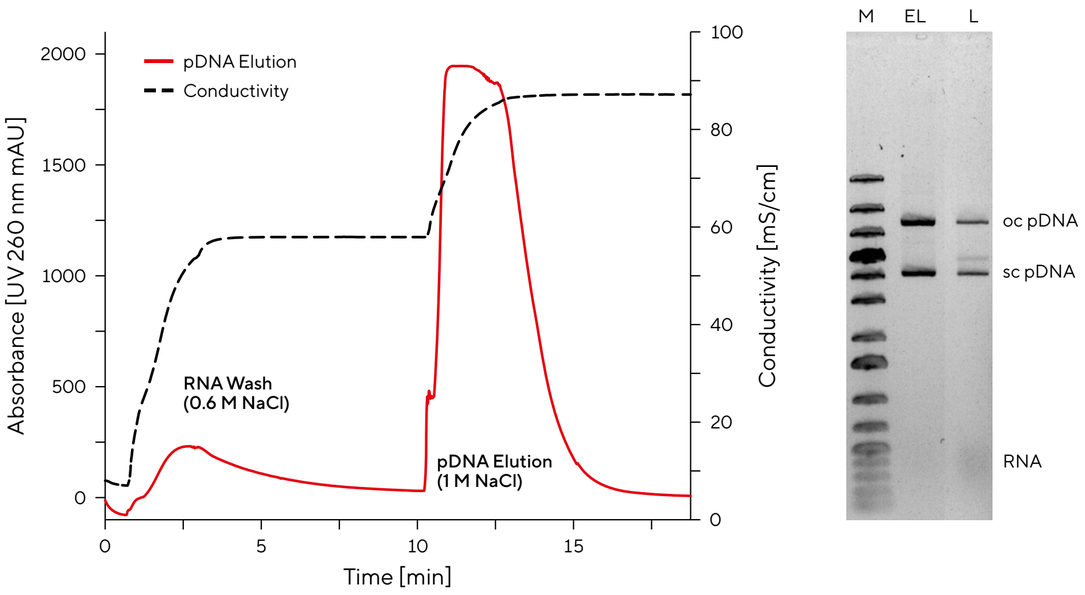


Figure 1: *Preparative Chromatogram of Capture Step on CIMmultus® DEAE*

Linearisation of pDNA

Linearisation of pDNA was performed using buffer without BSA, 25 °C, 4 h.

Sample: pDNA, 6409 bp, *E. coli* biomass lysed with 0.1 M NaOH, RNA precipitation with 0.75 M $CaCl_2$, two step filtration.

Analytics: CIMac pDNA 0.3 mL analytical column, MPA: 50 mM HEPES, 1% Tween, pH 7.5, MPB: 50 mM HEPES, 1 M guanidine-HCl, 1% Tween, pH 7.5, Method: 25% – 100% of MPB in 25 min.

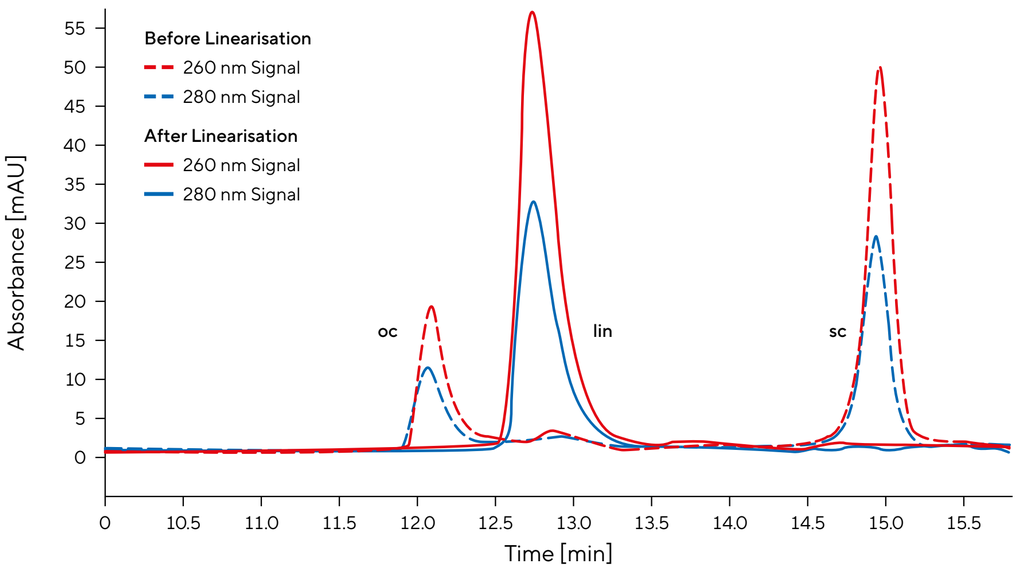


Figure 2: *Comparison of HPLC Profile of pDNA Before (Dashed Line) And After the Linearisation (Solid Line).*

Purification with CIMmultus® C4 HLD

Conditions: CIMmultus® C4 HLD 1mL, MPA: 50 mM Tris 10 mM EDTA 2.5M AS pH 7.2, MPB: 50 mM Tris 10 mM EDTA pH 7.2, Method: 10 min MPA, 20 min step 100% MPB

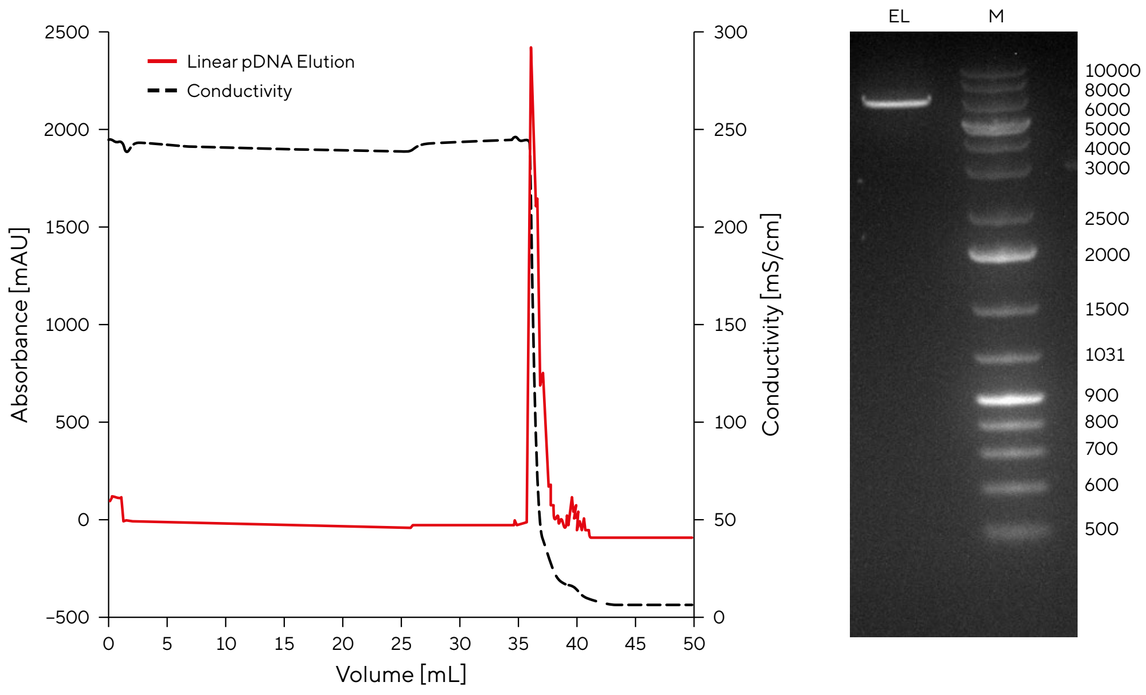


Figure 3: *Purification of Linearised pDNA Using CIMmultus® C4 HLD. Purity of Linear pDNA Was Confirmed Using AGE (Shown on the Right), HPLC and CGE (Data Not Shown).*

Results

	Recoveries
DEAE capture	83% recovery, 92% RNA removal, oc content 31%
Linearisation	100% yield
C4 HLD purification	86% recovery

Conclusion

- Employing a single capture step strategy provides about 40% increase in starting material (depending on percentage of non-supercoiled pDNA isoforms).
- Starting material with mixed isoforms is suitable for linearization procedure
- Single step capture strategy is fully scalable
- Linearisation without BSA is perfomed with 100% efficiency
- CIMmultus C4 HLD suited for purification of linearised pDNA
- CIMac pDNA Analytics supports fast pDNA isoform characterisation