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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.

Methods

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

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

Linearisation of pDNA was performed using buffer without BSA, 25°C, 4 h. Purity of linear pDNA was confirmed using AGE (shown in poster), HPLC and CGE (data not shown in poster).

Purification of linearized pDNA was done on CIMmultus® C4 HLD (Conditions: MPA: 50mM Tris 10mM, EDTA 2.5M AS pH 7.2, MPB: 50mM Tris 10mM EDTA pH 7.2, Method: 10 min MPA, 20 min step 100% MPB)

Linearisation of pDNA
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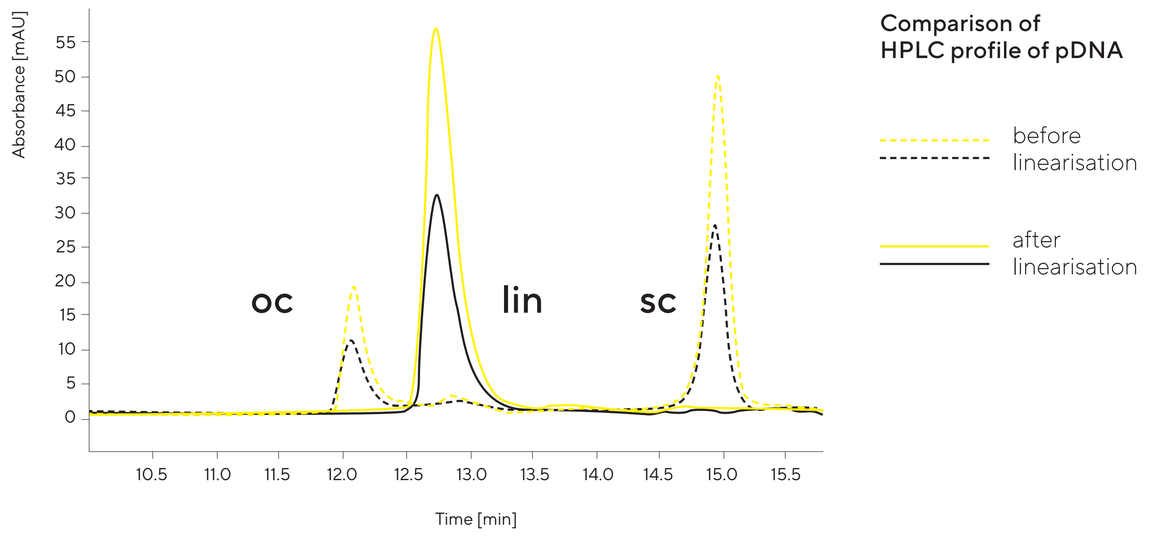

Figure 3: Linearisation of pDNA.
Analytics: CIMac™ pDNA 0.3 mL analytical column, MPA: 50 mM HEPES, 1% Tween, pH 7.5, MPB: 50 mM HEPES, 1 M guanidine-HCl, 1% Tween, pH 7.5, Method: 25% - 100% of MPB in 25 min.

Results

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

Capture with CIMmultus® DEAE
Conditions: for protocol, see HiP² Plasmid processing pack 1 mL, product number 100.0011-2

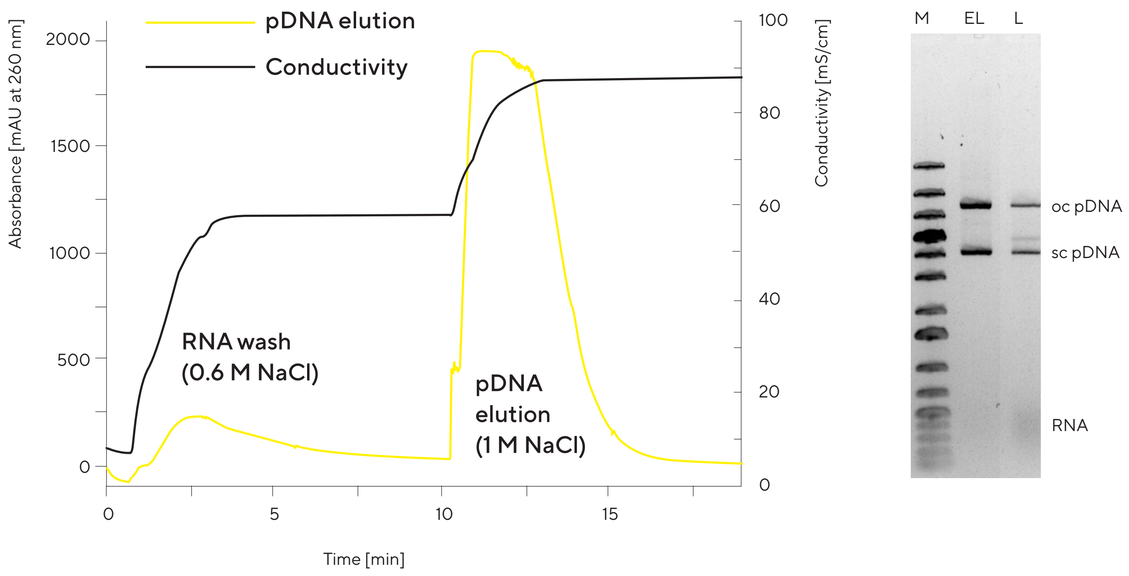

Figure 1: Capture with CIMmultus® DEAE

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

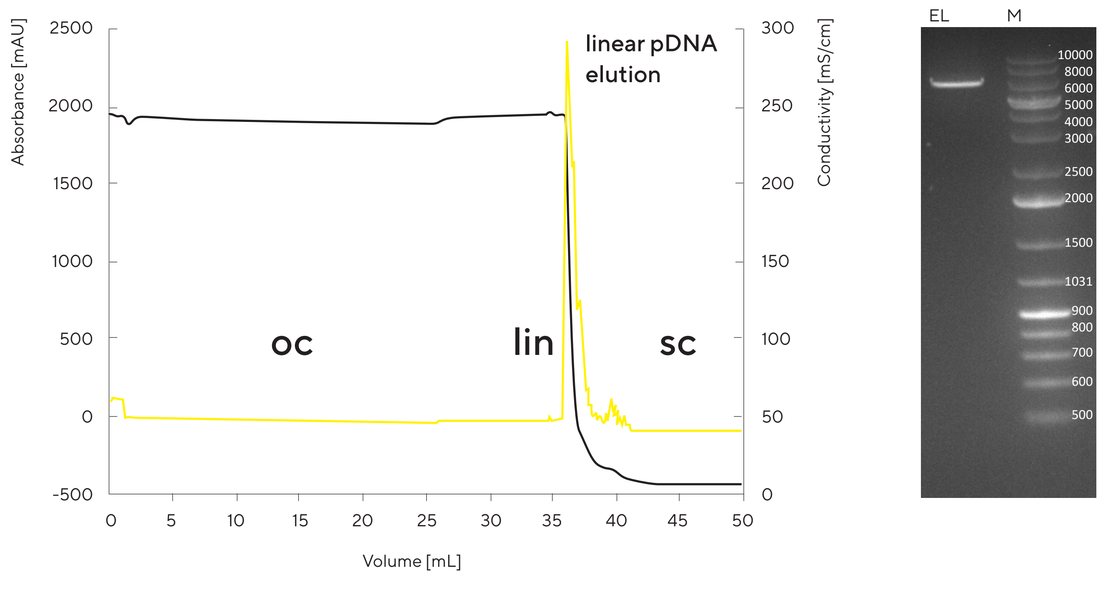

Figure 2: Purification with CIMmultus® C4 HLD.
Purity of linear pDNA was confirmed using AGE (shown), HPLC and CGE (data not shown). C4 HLD binds protein contaminants with strong interaction (elution of protein using 1 M NaOH).

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