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Application Note

June, 2021

Keywords or phrases
pDNA, PATfix®, pDNA analytics, in-process control, plasmid purification, plasmid linearization

PATfix® pDNA Analytics for In-Process Control of Linear pDNA Production

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Abstract

We describe the use of the PATfix® pDNA HPLC Platform for in-process control of linear plasmid preparation. Analytical chromatography is performed using a PATfix® pDNA optimized method on the CIMac™ pDNA analytical column. pDNA purification, monitoring of pDNA linearization progression and accurate pDNA quantification are enabled by the PATfix® pDNA analytical package.

Introduction

A key step in mRNA production is linearization of plasmid DNA, which is utilized as a template in IVT reactions. For efficient transcription in the IVT reaction, it is crucial to remove contaminants e.g. RNA and host cell proteins, before plasmid DNA linearization. Unwanted contaminants can create additional impurities, complicating downstream process (DSP) purification, and lead to poor yields of the mRNA product.

The PATfix® pDNA HPLC Platform, designed for in-process control of linear pDNA production, enables monitoring of pDNA linearization progression. Fully optimized and validated analytical methods, as well as guidelines for buffer and sample preparation come as part of the HPLC system, allowing users to focus on their specific application. In addition, the PATfix® pDNA analytical package includes a pDNA calibration standard, which enables accurate quantification of the pDNA species of interest.

Applying PATfix® pDNA analytical methods at different stages of linear plasmid production, allows fast and precise monitoring of contaminants removal (RNA, host cell proteins) as well as degradation products (oc or multimer pDNA). This ensures that each production step in plasmid preparation is correctly optimized during the process development (PD) stage as well as performing optimally during production. PATfix® pDNA analytics can be used to determine the initial plasmid concentration in the cell harvest providing production yields and losses at every step.

Materials

- PATfix® pDNA HPLC Platform
- CIMac™ pDNA column
- Buffer components: Tris, guanidine hydrochloride, Tween-20, NaCl
- Sample: pFix5 (plasmid pDNA standard; size 4.7 kb)

Methods

Usage of CIMac™ pDNA Analytical Column for in-process control of linear pDNA production
The optimized method provided with the PATfix® pDNA platform was used to generate the data in both Figures 1 and 2. The sample in Figure 2 is the provided pDNA standard, pFix5 (plasmid size 4.7 kb).

Column	CIMac™ pDNA-0.3 analytical column, 1.4 µm (5.2 mm id × 15.0 mm)
Injection volume	50 µL
Buffer A (loading | running buffer)	100 mM TRIS, 300 mM Guanidine-HCl, 1% Tween-20, pH 8.00
Buffer B (elution buffer)	100 mM TRIS, 300 mM Guanidine-HCl, 700 mM NaCl, 1% Tween-20, pH 8.00
Detection	UV: wavelengths 260 nm and 280 nm (10 mm UV cell) Conductivity and pH Fluorescence: Trp λ_{EX} = 280 nm; λ_{EM} = 348 nm (optional)
Flow rate	1 mL/min
Run time	17 min
Column temperature	30 °C
Auto-sampler temperature	4 °C
Method	pDNA_analytics_17min_UV detection pDNA_analytics_17min_UV & FLD detection

Gradient

Time [min]	Buffer A [%]	Buffer B [%]	Flow [mL/min]
0	86	14	1.0
1	86	14	1.0
3	42	58	1.0
11	27	73	1.0
11.02	0	100	1.0
12.5	0	100	1.0
12.52	86	14	1.0
17	86	14	1.0

Results

Figure 1 shows the in-process monitoring of plasmid purification and linearization for the purpose of mRNA production. Fully optimized and validated analytical methods allow tracking of pDNA isoforms and impurities from clarified lysate through capture, linearization and polishing.
Removal of contaminants (RNA and HCPs) and degradation products (oc or multimer pDNA) can be tracked. PATfix® pDNA analytics can be used to determine the initial plasmid concentration in the cell harvest providing production yields and losses at every step. Figure 2 shows the pDNA calibration standard for quantification of oc and sc isoforms (left) and the separation of key pDNA species (right).

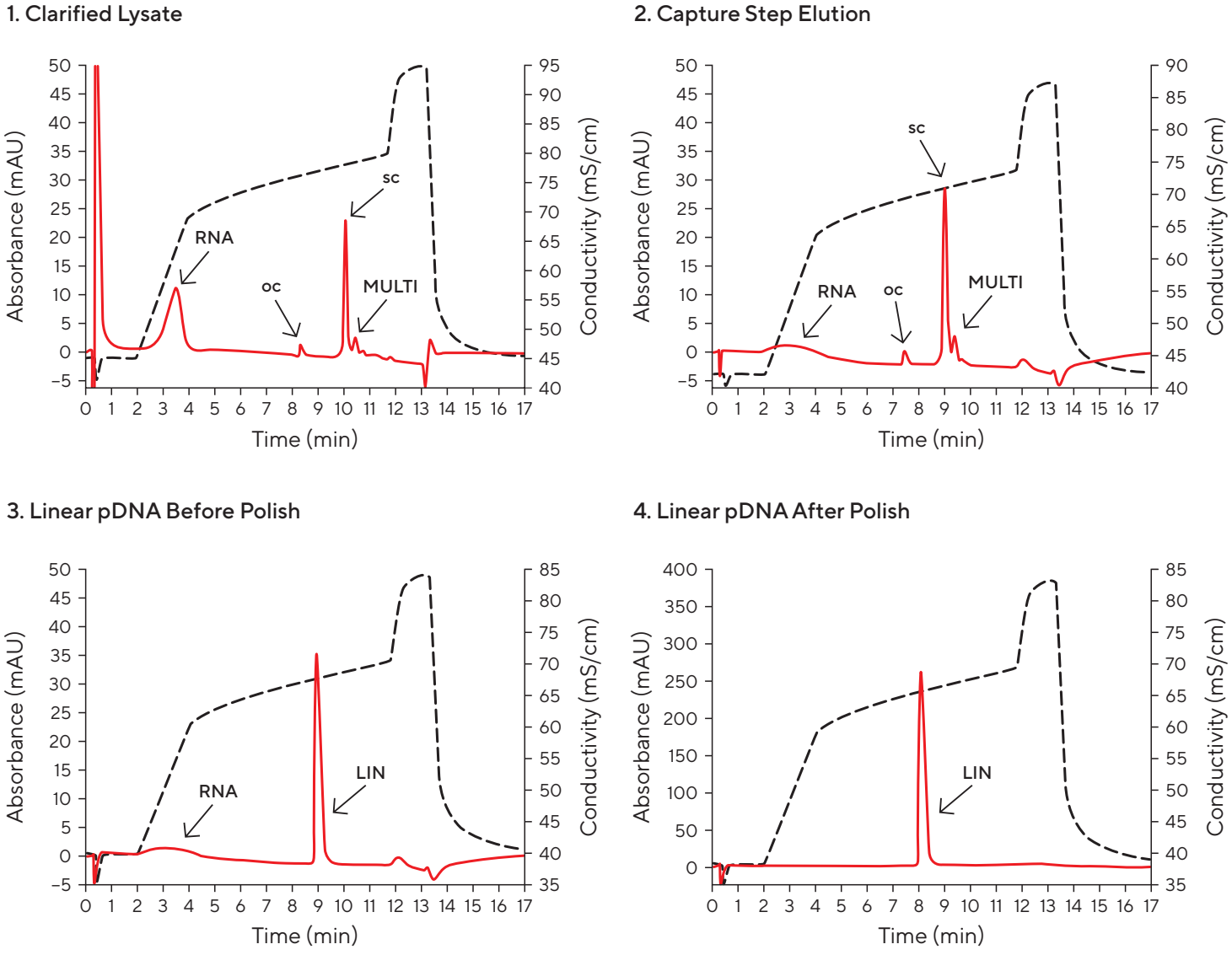

Figure 1: *PATfix® pDNA Application for In-Process Control of Linear Plasmid Preparation.*
Analytical chromatography was performed using a PATfix® pDNA optimized method on the CIMac™ pDNA analytical column.
The red line shows the 260 nm UV signal, while the black line shows the conductivity. Chromatograms from the most complex (graph 1) to the simplest sample (graph 4) are shown.

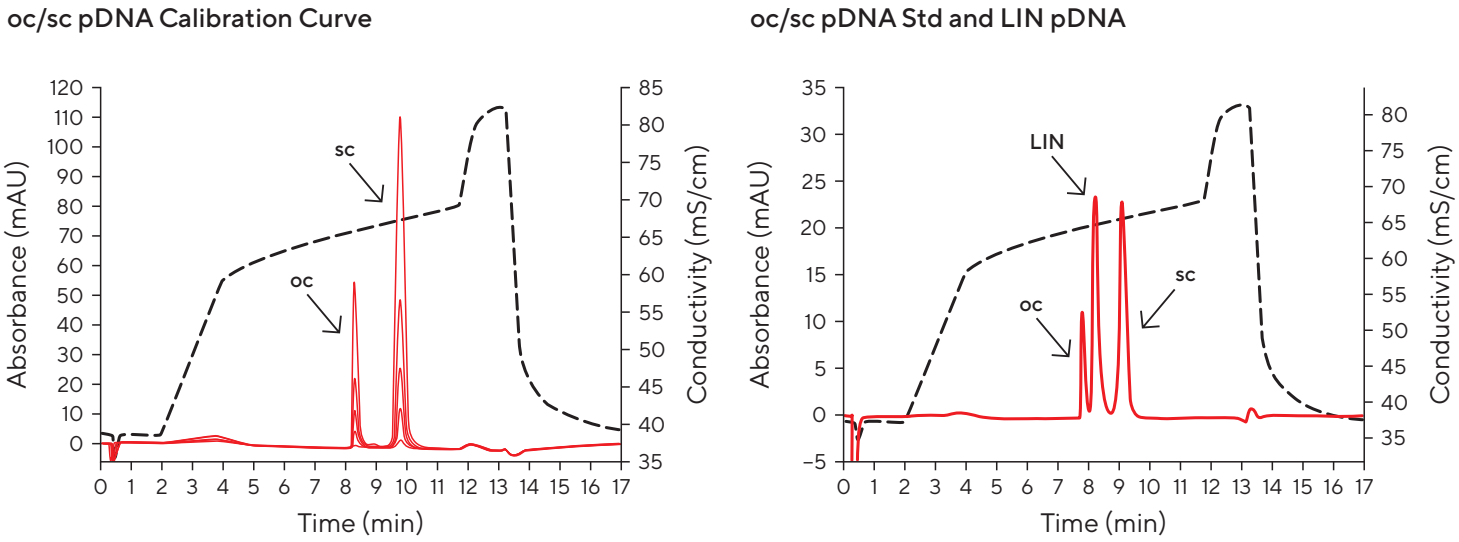

Figure 2: *pDNA Calibration Standard for Quantification of oc and sc Isoforms (Left) And Separation of Key Species (Right).* The red line shows the 260 nm UV signal, while the black line shows the conductivity.

Conclusion

The PATfix® pDNA analytical platform allows for monitoring of key process parameters during process development and production of linear pDNA. With the included calibration standard, absolute quantification of pDNA isoforms can be determined.

When used as part of a complete production process, each in-process control check is completed in less than 20 minutes (Figure 1), speeding up process development and cutting down on lengthy lag times during pDNA downstream processing.

PATfix® pDNA application enables the separation of pDNA isoforms (oc/sc/LIN/MULTI), allowing for product quality control that leads to more predictable production runs.

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Status 11 | 25 | 2021