Simplifying Progress

Using PATfix® Analytical HPLC Platform to Optimize Lysis Conditions in pDNA Downstream Processing

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1. Introduction

Optimizing processing steps in sc pDNA isolation is critical for obtaining good process yields as well as high product purity. HPLC with convective chromatography media (e. g. monolith) offers a rapid analytical method to characterize complex biomolecular mixtures and gives immediate feedback during process development. *E. coli* lysis represents such a challenging step, where multiple critical quality attributes need to be identified and critical processing parameters optimized. This approach leads to better yields and product purity, allowing for simplified downstream steps. A new PATfix® analytical HPLC platform presented here uses CIMac™ pDNA column, to separate and characterize plasmid from impurities, allowing for easy optimization of key parameters such as RNA removal.

2. Length of Lysis Optimization Using PATfix®

Alkaline lysis (0.1 M NaOH, 0.5% SDS)

1, 2, 3, 4, 5, 15, 30 min incubation

Neutralization (CH_3COOK)

Analysis (CIMac™ pDNA, PATfix®)

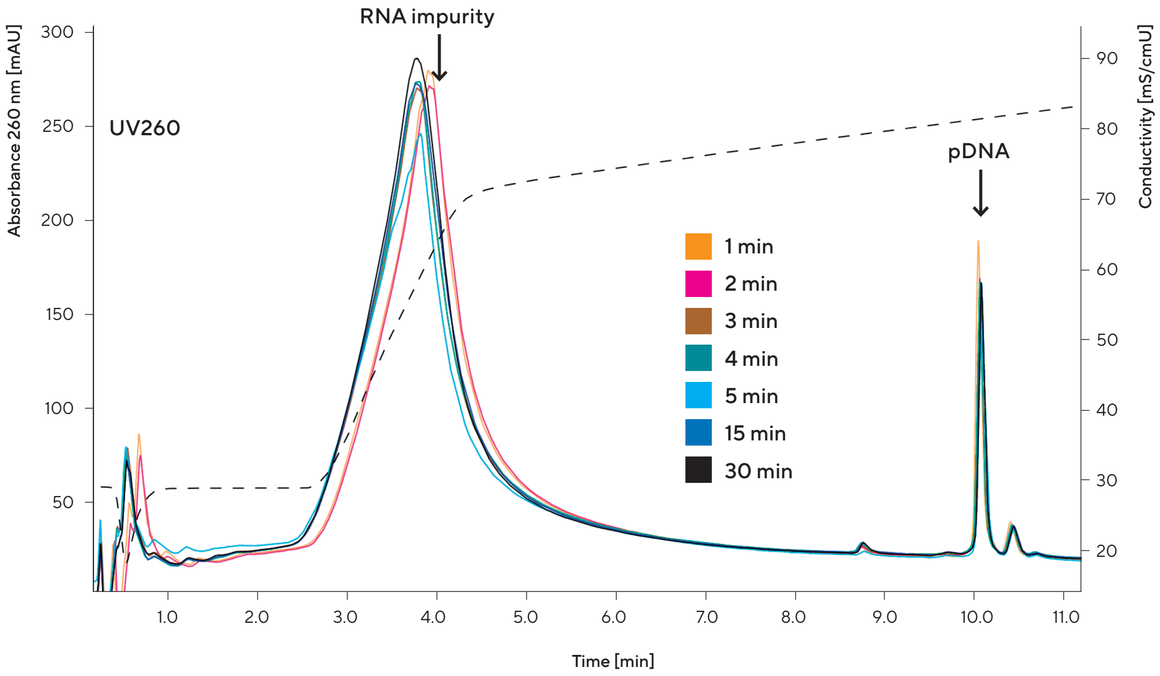
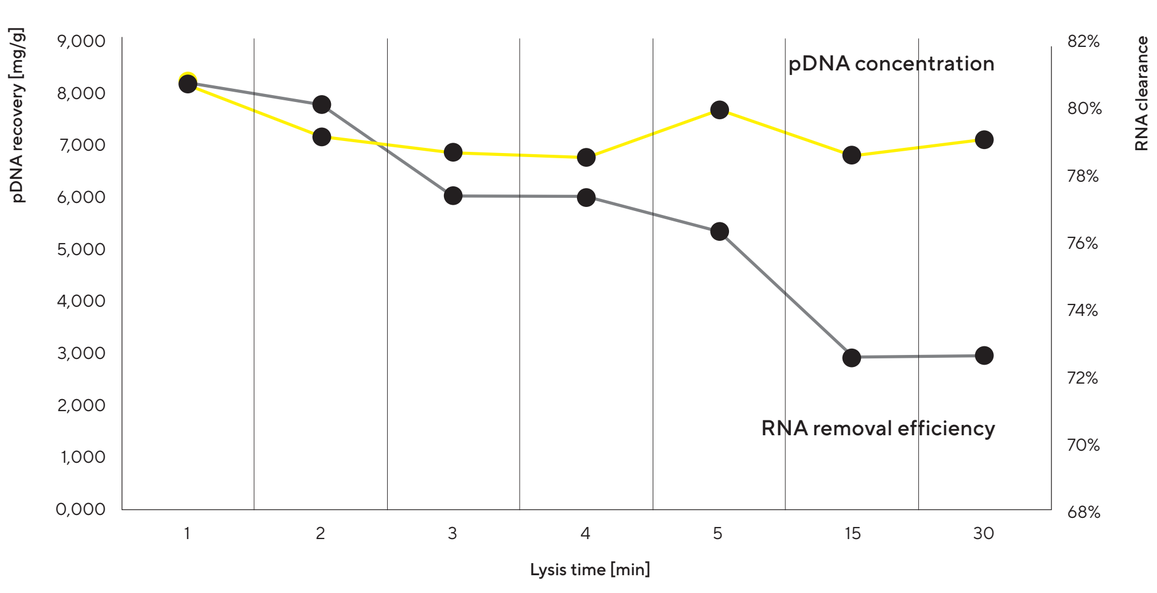


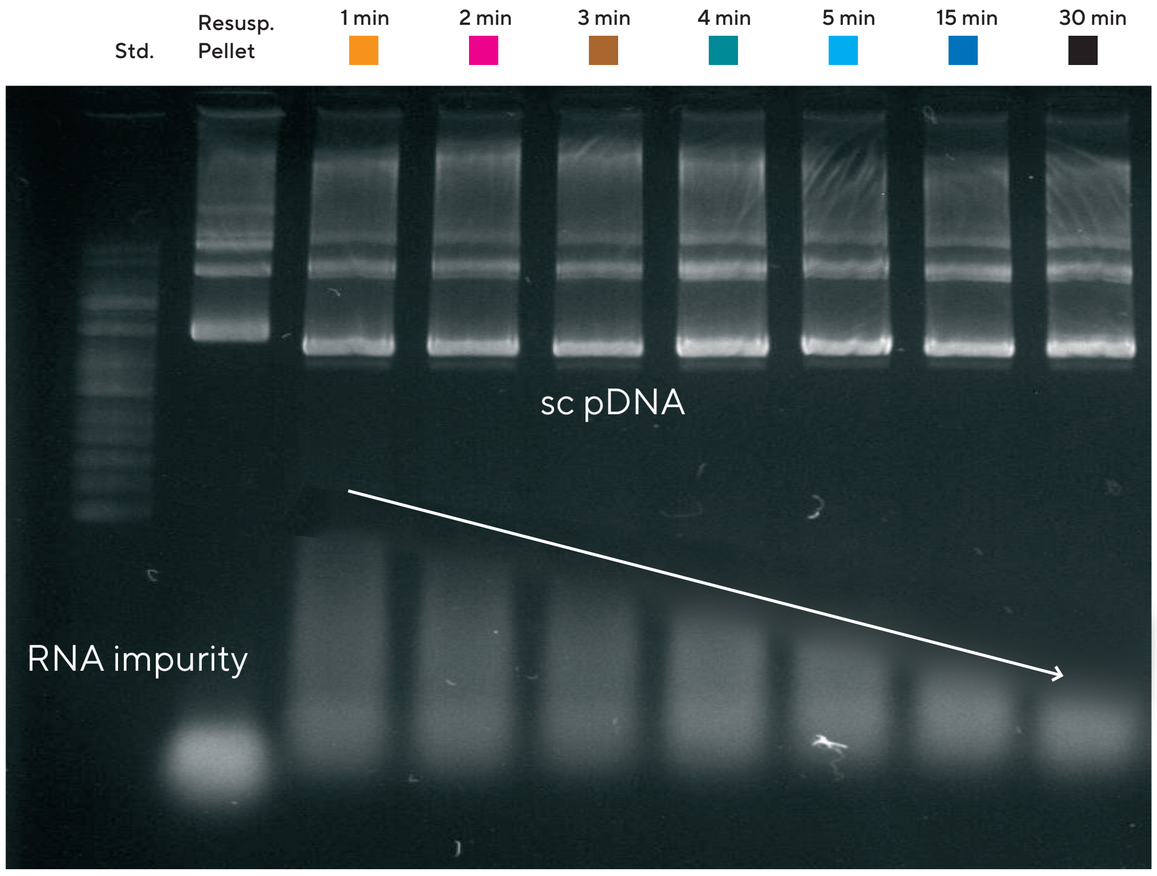
Figure 1: CIMac™ pDNA 0.3 mL on PATfix® HPLC system. Samples: Lysis solution containing 6.8 kbp plasmid, neutralized and analysed after variable increasing time of lysis. Sample were diluted 50x in MPA before injection. MPA: 50 mM HEPES pH 7.5, 1% Tween pH 7.5, MPB: 50 mM HEPES pH 7.5, 1 M guanidine HCl, 1% Tween pH 7.5, Flow rate: 1 mL/min. Method: 100 µL injection, 1.5 min 74% MPA, 1.5 min gradient from 74% to 23% MPA, 8 min gradient from 23% to 8% MPA, 1.5 min 0% MP A, 4.5 min 74% MPA. Detection: absorbance (260 nm). Conductivity shown in black dashed line.

Removing RNA released during *E. coli* lysis is a critical step in pDNA purification process. At the same time pDNA losses due to processing must be minimized and thus increasing yields. Optimization of alkaline lysis steps, such as lysis time, neutralization time, quantity of $CaCl_2$ addition and time of precipitation with $CaCl_2$ can be performed using the CIMac™ pDNA column on the PATfix® analytical HPLC system. By tracking the area of 260 nm absorbance peaks of RNA and pDNA at different time points quantitative analysis can be achieved, helping to optimize pDNA processing parameters.



3. Gel Electrophoresis Gives the Wrong Impression

Using agarose gel electrophoresis, shorter alkaline lysis times before neutralization can seem sub optimal at first glance. A smear of larger RNAs can be observed, which disappears as the time of alkaline lysis increases. However, the RNA is only being fragmented into shorter pieces by the caustics conditions, while the absolute amount is actually increasing. This gives an example how the optimization of alkaline lysis by agarose gel electrophoresis alone could be misleading without the use of analytical CIMac™ pDNA column.



4. Conclusion

- CIMac™ pDNA column can separate pDNA from contaminants, such as proteins, RNA, gDNA etc.
- PATfix® analytical HPLC allows for easy sample processing and quantification of impurities as well as pDNA in complex mixtures
- With appropriate methods, critical quality attributes (CQA) can be identified and critical process parameters (CPP) optimized
- RNA clearance is an example of important CQA in pDNA production
- Agarose gel electrophoresis alone cannot be used for pDNA quantification or assessing RNA removal in alkaline lysis steps usage of CIMac™ pDNA columns is thus essential