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Rapid Desalting of *E. Coli* Cell Lysate for CGE Analysis Using Monolithic Spin Columns

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Introduction

Neutralised *E. coli* cell lysate is a complex intermediate sample in the production process of plasmid DNA (pDNA), which is a workhorse for use in modern vaccination as well as in gene and cell therapy. Lysate composition analysis is important in-process characterization which enables optimal planning of the pDNA upstream and downstream processing. pDNA isoform composition is often analysed using capillary gel electrophoresis with laser induced fluorescence detection (CGE-LIF), a very sensitive and high-resolution method.

1. Experimental problem

The complexity of the *E. coli* cell lysate matrix together with typically low pDNA concentration is a challenge for the implementation of a fast and robust analytical method. Low sample matrix conductivity is particularly essential for robust injection in CGE (Figure 1); therefore, sample preparation (such as desalting) is a crucial step before lysate analysis. The main risks and challenges with lysate sample preparation are:

- retaining the same nucleic acid profile as in the original sample;
- speed and robustness of the sample preparation method;
- high analyte recovery;
- not diluting a sample with an already low concentration.

2. Rapid and innovative sample desalting approach

We have developed an innovative and rapid method for desalting nucleic acids solutions using Convective Interaction Media (CIM®) monolithic spin columns (CIMspin Swiper) and tested it for desalting of neutralized *E. coli* lysate. CIMspin Swiper columns utilize the bind-elute mechanism, which can efficiently remove unwanted salts, as the column binds only various nucleic acids. Elution can be then performed directly with CGE sample buffer (Figure 2). The novel sample desalting approach is presented and compared to ultrafiltration-based desalting, which is standardly applied with centrifugal filters with membranes of different cut-off pore sizes.

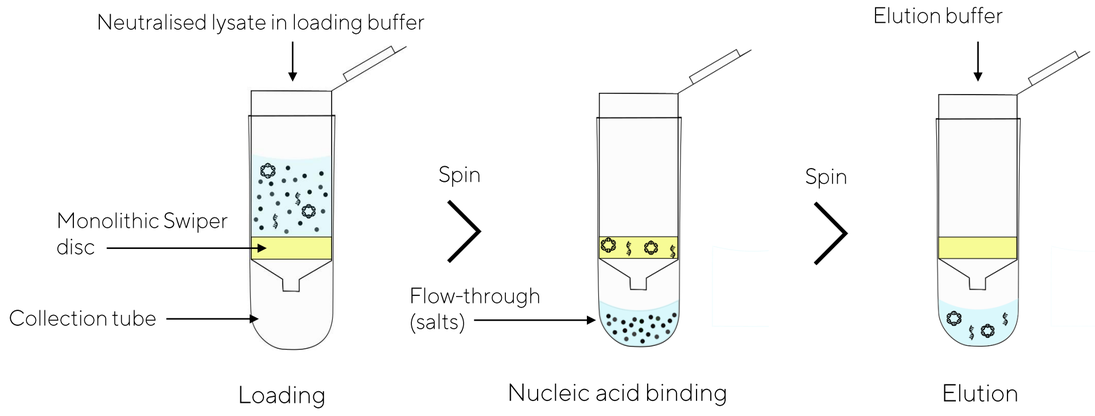

Figure 2: Schematic illustration of desalting of neutralized lysate with CIMspin Swiper column.

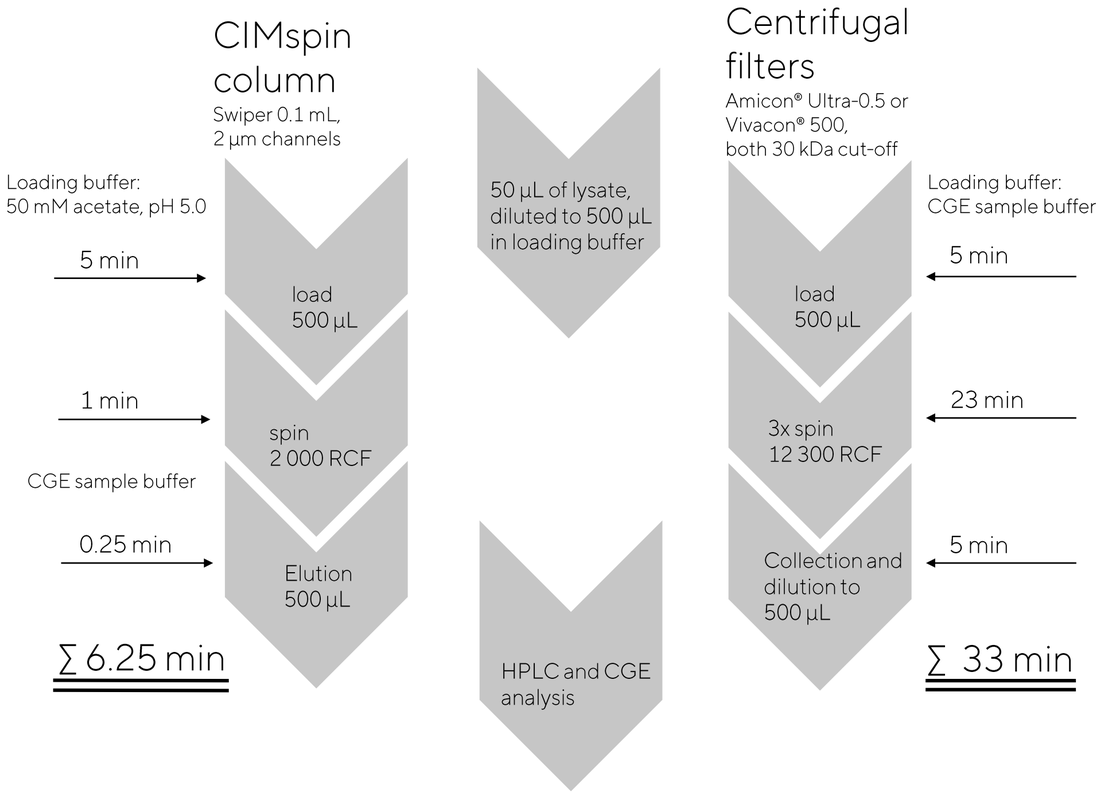

Figure 3: Workflow of desalting using two different procedures used in our study.

Firstly, we desalted the same neutralized *E. coli* lysate with CIMspin column or centrifugal filters, following the procedures in Figure 3. Next, we analyzed the control and processed samples by CGE-LIF, using a method for quantitative pDNA isoform composition analysis with electrokinetic injection. Analysis was performed on a PA800 Plus system (SCIEX) [1].

Processed samples were also analyzed on PATfix® HPLC system with CIMac pDNA-0.3 analytical column (6 µm), which provides not only quantitative results of pDNA isoform composition, but also concentration of pDNA [2,3].

	% RNA relative to total area of all detected nucleic acids*		% SC pDNA isoform relative to total pDNA*	
	HPLC	CGE	HPLC	CGE
Neutralised lysate	84	ND	88	ND
CIMspin	88	77	89	89
Amicon 30 kDa	74	48	87	89
Vivacon 30 kDa	86	72	88	89

Table 1: Analyses results as average, *n = 3, RSD < 5%.

	Relative SC pDNA TCA [%]*
	CGE
Neutralised lysate	0
CIMspin	100
Amicon 30 kDa	77
Vivacon 30 kDa	68

Table 2: Impact of sample pretreatment on efficiency of electrokinetic injection on CGE-LIF, expressed as relative time-corrected areas (TCA) compared to the highest detected area (CIMspin desalting).

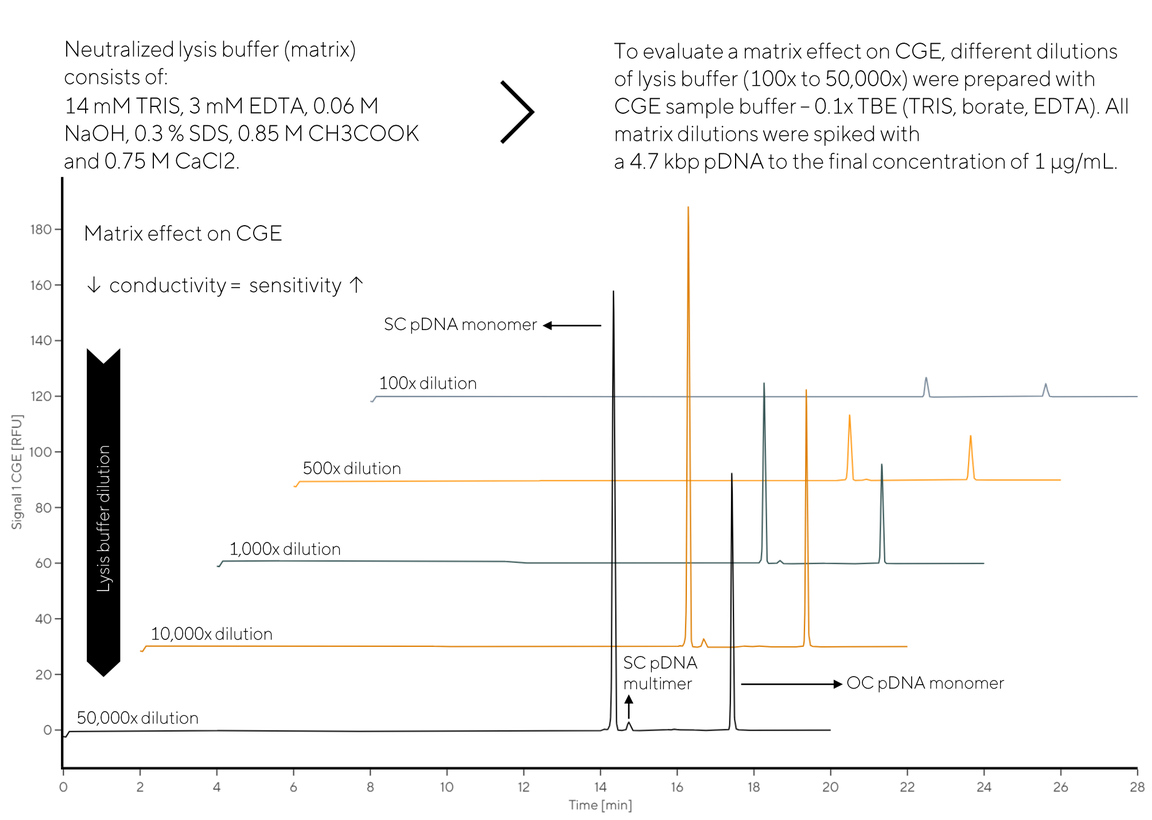

Figure 1: Impact of sample matrix on electrokinetic injection on CGE-LIF. Each sample contains 1 µg/mL pDNA (4.7 kbp) in serial dilutions of lysis buffer (100x – 50,000x). The response (at constant analyte concentration) enormously depends on sample conductivity/salt content, demonstrating the need for efficient sample desalting prior to CGE-LIF analysis.

3. Analysis of desalted lysate samples

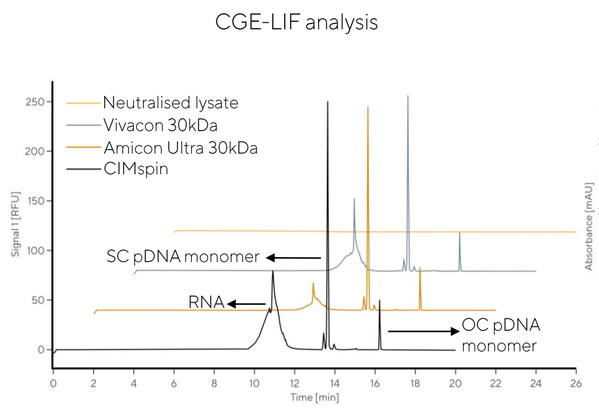

Figure 4: Electropherograms of *E. coli* neutralised lysate, processed by different procedures.

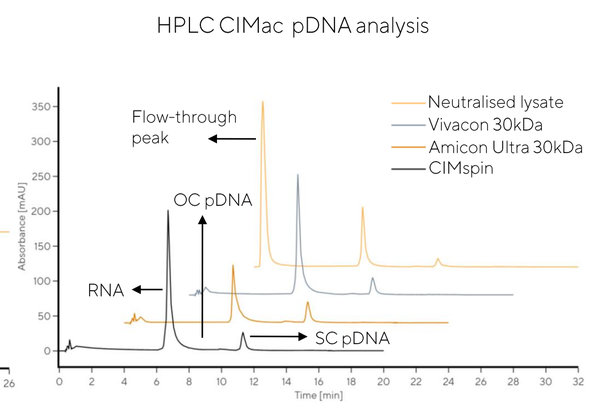

Figure 5: Analytical chromatograms of *E. coli* neutralised lysate, processed by different procedures.

- Lysate processed with CIMspin Swiper column resulted in the highest CGE signal sensitivity for nucleic acids (Figure 4 and Table 2).
- Vivacon® (30kDa) and CIMspin Swiper column based-processing retained the nucleic acid profile of the lysate, whereas Amicon® Ultra (30kDa) lost some of the bacterial RNA through the pores into the permeate (Figures 4 and 5, Table 1).
- CGE analysis has a higher resolution between pDNA isoforms (SC pDNA multimer is well resolved from its monomer), however, the HPLC-based method is more robust (Figures 4 and 5).
- Unprocessed (without desalting) neutralized lysate can only be analyzed with HPLC CIMac pDNA column (Figure 5), as the salt content is too high for efficient electrokinetic injection on CGE (Figure 4).
- Determined RNA:pDNA ratio in the lysate is lower for CGE compared to HPLC processed samples (Table 1), probably because staining sensitivity for intercalating fluorophores (used in CGE analysis) is highly dependent on the number of nucleic acid strands. Reagents optimized for double-stranded species have low sensitivity for single stranded species.

4. Conclusion

	CIMspin Swiper columns	Centrifugal filters
+	▪ Rapid processing (each spin step ~15 s) ▪ Applicable for various nucleic acid types and sizes ▪ Lower centrifugal forces ▪ Reusable ▪ Procedure can be transferred to CIMmultus column line for preparative desalting (not shown here)	▪ No need for specific loading buffer ▪ Analyte specific cut-offs ▪ Possibility to concentrate the sample
–	▪ Use of specific elution buffer ▪ Not possible to concentrate nucleic acids above 500 µg/mL ▪ Adjusting the sample buffer to enable efficient nucleic acids binding	▪ Longer processing time ▪ Higher centrifugal forces ▪ Smaller cut-offs must be used not to lose RNA (size-specific method) ▪ Non-specific adsorption of analytes to membrane

5. References

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