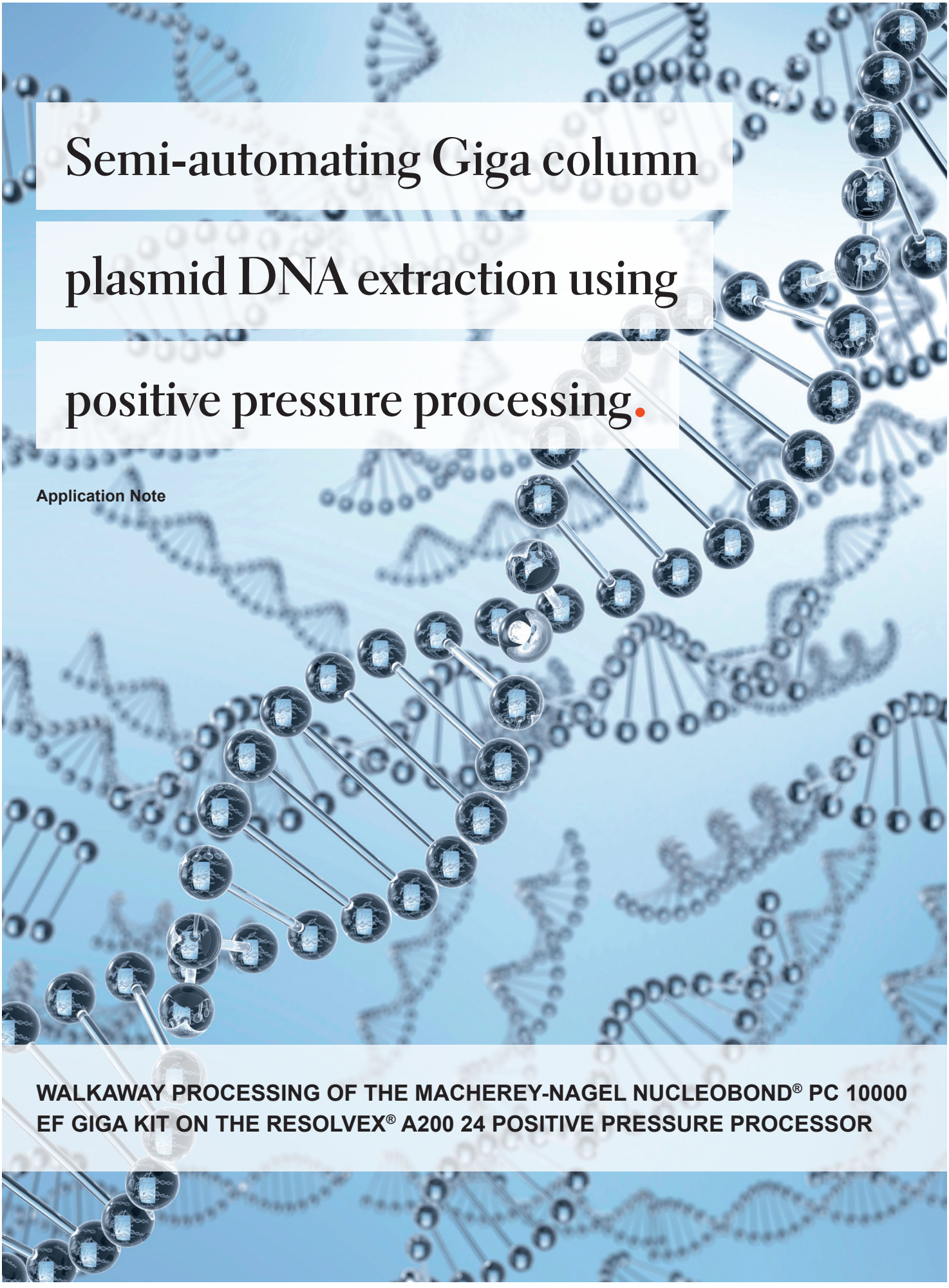

Semi-automating Giga column plasmid DNA extraction using positive pressure processing.

Application Note

WALKAWAY PROCESSING OF THE MACHEREY-NAGEL NUCLEOBOND® PC 10000 EF GIGA KIT ON THE RESOLVEX® A200 24 POSITIVE PRESSURE PROCESSOR

TECAN.

INTRODUCTION

Homogeneous, highly purified preparations of supercoiled plasmid DNA (pDNA) from bacterial cultures are used for various downstream molecular biology applications, such as cloning, transfection and protein production. Commercially available kits for plasmid purification have streamlined the procedure, which can yield pDNA concentrations from 150 ng/µl to several mg/ml. The NucleoBond PC 10000 EF Giga kit (MACHEREY-NAGEL) uses alkaline lysis of bacterial cell pellets with subsequent anion exchange chromatography on silica-based resin columns to generate yields between 2 and 10 mg of purified plasmid per 10 g wet weight of bacterial pellet.

The manual Giga prep process comprises gravity flow-based chromatography steps, which are rate limiting due to the difficulty of controlling the progress. Depending on the nature of the sample, these steps take three to four hours to complete, and the operator is unable to leave the process unattended, as constant monitoring is required. Furthermore, column clogging often makes the manual plasmid extraction process tedious.

In this application note, the suitability of the Resolvex A200 24 positive pressure processor was investigated for semi-automating the extraction of plasmid DNA using the NucleoBond PC 10000 EF Giga kit. The workflow uses automated pressure profiles for the filtration, washing and elution steps to yield high quality plasmid DNA. This semi-automated workflow provides the user with walkaway time, as well as a significant reduction in overall procedure time.

MATERIALS AND METHODS

Resolvex A200 24 configuration

The Resolvex A200 24 (Figure 1A, #30219820) is a next generation Resolvex A200 positive pressure processor with a revised pressure manifold, dispense head and waste design. To operate the Giga column and workflow (Figure 1B), the four channel dispense head is converted to a two channel set-up. This simple procedure takes less than 15 minutes to complete, and requires the same software as previous iterations of the instrument. A specially designed rack was used to accommodate the height and width of the Giga column, and an application-specific spacer is also recommended to prevent contamination through splashing.

Resolvex A200 24 **Resolvex A200 24 side view**

Figure 1A: Resolvex A200 24 Positive pressure processor

Resolvex A200 Giga column elution stack with 350 ml trough **Side view with rack and spacer set-up to prevent contamination** **Giga column process rack set-up**

Figure 1B: Resolvex A200 24 giga column accessories and set-up

Labware and reagents

- Bacteria was cultured overnight in 5 l flasks (Corning, #431684), and pelleted using 500 ml centrifuge bottles (Corning, #430282).
- DNA was eluted into sterile, nuclease-free reagent troughs (Corning, #RES-SW96-HP-SI).
- Alcohol precipitation steps were carried out in 250 ml centrifuge bottles (Corning, #430776).
- Reagents and consumables from the NucleoBond PC 10000 EF Giga kit (#740548) containing AX 10000 Giga columns were used for the Giga prep procedure.

Giga prep plasmid purification

The Giga prep purification procedure was performed according to the manufacturer's recommendations. Briefly, plasmids were transformed into DH5α competent E. coli (NEB) cells, and starter cultures were grown in Luria-Bertani (LB) broth with plasmid marker-specific antibiotics at 37 °C until an OD_{600} of 0.2 to 0.4 was reached, which usually took six hours. Subsequently, 300 µl was inoculated into 5 l flasks containing 1.5 l of fresh medium. This culture was incubated in a shaker (Jeio Tech, ISS-7200) at 150 rpm for 16h at 37 °C. The bacterial culture was pelleted by centrifugation, then processed according to the manufacturer's recommendations.

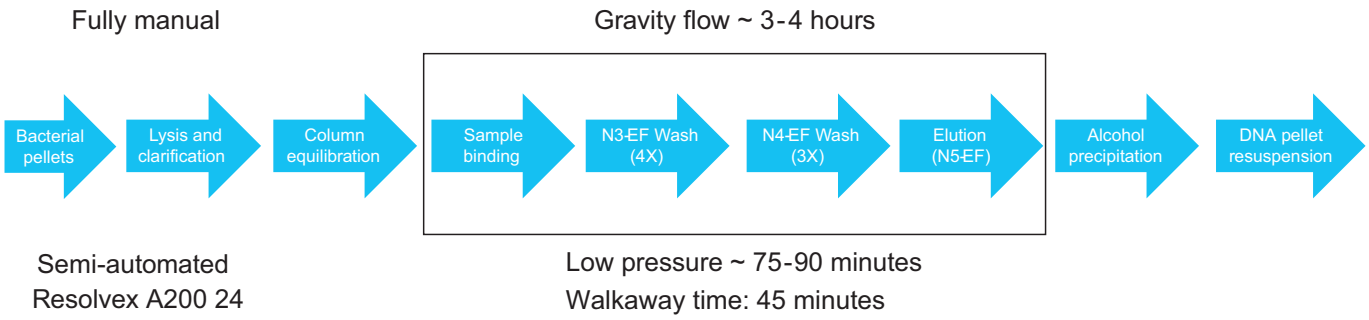

Figure 2: Comparison of manual and semi-automated workflows

The crude lysates were clarified using a vacuum filter that came with the kit, and the cleared lysate was applied to the Giga column, which was equilibrated using the Resolvex A200 24.

Step	Time/step	Description
Step 1: Manual	1 min	Assemble the Giga processing stack
Step 2: Resolvex A200 24	2 mins	Equilibrate the Giga column with buffer N2-EF
Step 3: Manual	1 min	Add ~80 ml of cleared bacterial lysate to Giga column
Step 4: Resolvex A200 24	3 mins	Apply low pressure to pass the cleared lysates through the equilibrated column
Repeat steps 3 and 4 until all the cleared lysate has passed through the Giga column (usually two repeats)		
Step 5: Resolvex A200 24	5 mins	Wash with 100-120 mL Buffer N3-EF
Repeat step 5 three more times (four washes in total)		
Step 6: Resolvex A200 24	4 mins	Wash with 100-120 ml of N4-EF buffer
Repeat step 6 two more times (three washes in total)		
Step 7: Manual	1 min	Assemble the Giga elution stack
Step 8: Resolvex A200 24	1 min	Add 100 ml of N5-EF elution buffer to the Giga column
Wait 5 minutes		
Step 9: Resolvex A200 24	1 min	Elute the pDNA into the collection trough
Proceed with alcohol precipitation of the eluted pDNA solution according to manufacturer's instructions.		

Table 1: Resolvex A200 24 workflow for NucleoBond PC 10000 EF Giga kit prep

The chromatography steps were semi-automated as described in Table 1. After column equilibration, the cleared lysates were poured onto the column in three batches. The subsequent washes – four times with N3-EF buffer and three times with N4-EF buffer – were completed using the Resolvex A200 24. Finally, the spacer was replaced with a nuclease-free trough, and the pDNA was eluted in N5-EF buffer.

The alcohol precipitation and DNA pellet resuspension steps were carried out according to the manufacturer's recommendations. The pelleted pDNA was reconstituted in an appropriate amount of TE-EF buffer, and the resulting plasmid preparation was assayed for DNA concentration, purity and endotoxin levels.

RESULTS

Side-by-side comparison of manual and semi-automated plasmid extraction workflows

The same plasmid was purified in duplicate either manually or using the semi-automated Resolvex A200 24 workflow. As indicated in Table 2, the yield and purity by both methods was comparable, and the purity ($A_{260/280}$) and endotoxin levels were within acceptable limits. However, purification with the Resolvex A200 24 resulted in almost a one-third reduction in chromatography time compared to the manual procedure.

Yield and purity of pDNA purified by the Resolvex A200 24

Eleven unique plasmids were purified using the Resolvex A200 24 on two different days. Table 3 describes the yield and purity of these samples. For samples 3-11, the method was further modified to reduce the run time from 1.5 to 1 hour. The yield of pDNA ranged from 1.5 mg to 6 mg, depending on the wet pellet weight and nature of the plasmids, and the average plasmid purity ($A_{260/280}$) obtained was 1.88. Endotoxin levels for two representative samples were within the acceptable limits of <0.1 EU/µg. Multiple samples that were purified sequentially on the Resolvex A200 24 were also tested, and no adverse impact to the yield and purity of plasmids was found (data not shown).

Sample ID	Pellet wet weight (g) per Giga prep	Purification process	Chroma-tography time (hr)	Yield (ng/µL)	Final Volume (mL)	Yield (mg)	Yield (mg/g pellet weight)	Purity A260/280	Endotoxin levels (EU/µg)*
Plasmid X-1-A	6.4	Manual	4	1119	5	5.6	0.875	1.89	0.045
Plasmid X-1-B	7.8	Manual	4	1859	5	9.3	1.19	1.87	0.00003
Plasmid X-1-C	6.8	Resolvex A200	1.5	1280	5	6.4	0.941	1.89	0.00059
Plasmid X-1-D	6.9	Resolvex A200	1.5	922	5	4.6	0.670	1.91	0.00016

*Endotoxin levets of <0.1 EU/µg acceptable for downstream applications

Table 2: Side-by-side comparison of manual and semi-automated workflows

Sample Number	Wet pellet weight (g)	Chromatography time (hr)	Yield (ng/µl)	Final volume (ml)	Yield (mg)	A260/280	Endotoxin levels
1	7	1.5	922	5	4.7	1.91	<0.1 EU/µg
2	7	1.5	1280	5	6.4	1.89	<0.1 EU/µg
3	7	1	921	5	4.6	1.90	Not tested
4	6	1	560	3	1.7	1.89	Not tested
5	7	1	490	3	1.5	1.89	Not tested
6	7	1	1063	5	5.3	1.89	Not tested
7	8	1	1003	3	3.0	1.88	Not tested
8	7	1	712	5	3.6	1.89	Not tested
9	7	1	950	5	4.8	1.88	Not tested
10	7	1	767	3	2.3	1.86	Not tested
11	6	1	704	3	2.1	1.88	Not tested

Table 3: Yield and purity of pDNA purified by the Resolvex A200 24

SUMMARY

The Resolvex A200 24 positive pressure processor could be easily adapted for Giga prep workflows using the NucleoBond PC 10000 EF Giga kit. Side-by side comparison of the manual and semi-automated methods revealed that both techniques produce equivalent yields and purities. Crucially, automating part of the workflow gives users up to 45 minutes of walkaway time, and cuts the overall chromatography time by half.

The yields and purity of 11 unique plasmids was compared, and it was observed that yields were lower than expected for two samples, presumably due to the nature of these plasmids. Yields from the semi-automated procedure can be improved by decreasing the pressure applied for the binding and wash steps, however, this will increase the overall procedure time. The Resolvex A200 24 workflow is limited to processing one sample at a time, making it less desirable if multiple samples need to be processed. In this study, multiple samples were sequentially processed with the Resolvex A200 24, with no adverse impact on plasmid yield or purity. Finally, only columns supplied by MACHEREY-NAGEL can currently be used, as other kits have incompatible column heights and widths. However, it is clear that the Resolvex A200 24 is a highly versatile instrument that can be used with other Giga prep kits if custom columns with precise specifications are obtained. Overall, the Resolvex A200 24 provides the user with significant benefits by reducing hands-on time and errors for Giga prep plasmid purification.

LITERATURE

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ACKNOWLEDGEMENTS

This protocol was developed in collaboration with the High Throughput Technologies and Operations (HTTO) group at Spark Therapeutics. Samples were provided by Research Vector Core, Therapeutic Area Research Groups, and Mustafa Yazicioglu, rAAV Vector Engineering Lead at Spark Therapeutics. Application-specific adaptations for the Resolvex A200 24 set-up were supported by Karsten Liegmann, Senior Application Specialist at Tecan. Dr Marc Wenger, Christopher Daniels, Matthew Miezeiewski (Spark Therapeutics), Qi Huang and Shang Tsai (Tecan) also provided critical feedback.

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402549 V1.0, 2023-03

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