Application Note

Positive-pressure-based plasmid preparation using QIAprep® chemistries

Semi-automated plasmid DNA extraction from *E. coli*, using the QIAprep 96 Turbo Miniprep Kit and the QIAprep 96 *Plus* Kit on the Resolvex® A200 from Tecan®

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Introduction

Extraction of plasmid DNA is a standard procedure in molecular biology laboratories, and plasmids are still a go-to resource for cloning and modifying genes of interest.

QIAprep chemistries offer fast and cost-effective plasmid DNA preparation based on cell lysis and clarification, following a simple bind–wash–elute procedure. The resulting DNA is ready for immediate use in subsequent applications. While this plasmid purification can be performed manually, high-throughput screening or production labs – e.g., in the pharma sector – require purification of several hundred samples per day and can benefit from workflow automation.

In this application note, we describe a semi-automated protocol for the extraction of plasmid DNA (*E. coli* cells) using the QIAprep 96 Turbo Miniprep Kit (cat. no. 27191 or 27193) and the QIAprep 96 *Plus* Miniprep Kit (cat. no. 27291) on a Resolvex A200 positive-pressure workstation. The workflow provides highly efficient extraction of plasmid DNA for life science applications, using low-pressure profiles for filtration, washing and elution steps, plus fast buffer exchange.

Materials and Methods

Resolvex A200 instrument configuration

A Resolvex A200 positive-pressure workstation (Figure 1) was used to process different QIAprep 96-well plates, using spacers designed specifically for this application (available from Tecan on request). The six buffers required for QIAprep protocols were prepared and transferred to the reagent bottles attached to the Resolvex A200, and all lines were primed using standard flushing volumes (2 x 4 ml). An optimal working pressure of 5.5 bar (80 PSI) dry air or nitrogen was chosen.

Figure 1. Resolvex A200 positive-pressure workstation.

Sample growth

E. coli cells containing a pCMVbeta plasmid were grown overnight in Luria-Bertani (LB) medium. One-and-a-half milliliter aliquots were transferred to QIAGEN flat-bottom blocks and centrifuged for 10 minutes at 5000 x *g*. The supernatants were discarded, and the pellets were frozen and stored in flat-bottom blocks at –80°C until purification.

Sample resuspension, transfer and extraction into 96-well plates

QIAprep 96 Turbo and QIAprep 96 *Plus* Miniprep protocols were used for the purification of plasmid DNA, comparing the results of semi-automated processing on the Resolvex A200 workstation with the BioRobot® Universal System (reference method). Adaptation to the Resolvex A200 included adjustment of buffer volumes and optimization of solvent-dispensing speeds and pressure gradients. Introduction of a double-elution step improved DNA yields.

A. QIAprep 96 Turbo Miniprep protocol

Bacteria were resuspended and lysed under alkaline conditions using Buffers P1 and P2, respectively, then shaken with Buffer N3 to neutralize the samples. All buffer dispensing steps were performed according to kit protocol using the Resolvex A200 (Figure 2).

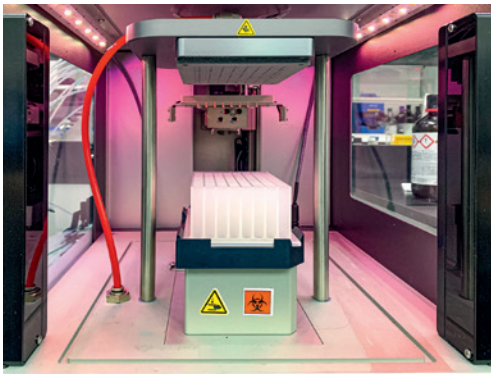

Figure 2. Buffer dispensing to resuspend, lyse and neutralize cells using Resolvex A200 (shaking performed offline).

Samples were then manually transferred to a TurboFilter® 96 Plate (cat. no. 120025) and placed on top of a QIAprep 96 Plate (located on top of a deep-well plate to collect the flow-through, Figure 3).

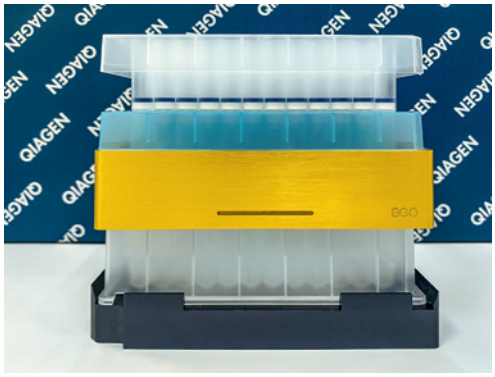

Figure 3. TurboFilter 96 Plate placed on top of a QIAprep 96 Plate and deep-well plate with adapters.

The plate combination was positioned on the Resolvex A200 instrument using the required adapters, and the samples were pushed through the TurboFilter 96 Plate via a pressure gradient, loading them directly onto the QIAprep 96 Plate. After removing the TurboFilter 96 Plate and the deep-well plate, only the QIAprep 96 Plate stayed in the Resolvex A200 (Figure 4), and interferences were removed using several washing steps. In the final step, Buffer EB was dispensed twice to elute plasmid DNA into a collection plate via a pressure gradient.

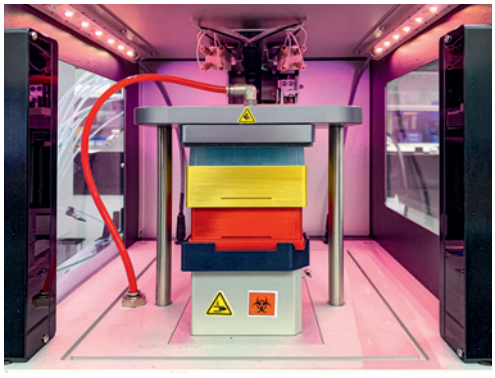

Figure 4. QIAprep 96 Plate placed for DNA binding and washing steps, using adapters to fit under the pressure head.

Table 1. Schematic overview of the QIAprep 96 Turbo protocol using the Resolvex A200

Step	Processing method	Procedure
1	Automated	Dispense buffers P1, P2 and N3 (Figure 2)
2	Manual	Shake sample plate
3	Manual	Transfer samples to TurboFilter 96 Plate
4	Manual	Place TurboFilter 96 Plate on top of a QIAprep 96 Plate + deep-well plate with adapters (Figure 3)
5	Automated	Push samples through the TurboFilter 96 Plate using pressure gradient
6	Manual	Remove TurboFilter 96 Plate and deep-well plate (QIAprep 96 Plate stays in Resolvex A200, Figure 4)
7	Automated	Dispense Buffer BB and wash buffer, and process using pressure gradients
8	Automated	Dry plate under constant pressure
9	Manual	Put the collection plate under the binding plate
10	Automated	Dispense Buffer EB and collect eluates in elution plate using pressure gradient

B. QIAprep 96 *Plus* Miniprep protocol

Bacteria were resuspended and lysed under alkaline conditions using Buffers P1 and P2, then shaken with Buffer S3 to neutralize the samples. All buffer dispensing steps were performed using the Resolvex A200 according to kit protocol. Samples were then manually transferred to a TurboFilter 96 Plate, which was placed on top of an S-Block (Figure 5), and both plates were loaded onto the Resolvex A200 (Figure 6).

Lysates were clarified using positive pressure, then the TurboFilter 96 Plate was removed, leaving the collection plate in place for dispensing of Buffer BB. This was followed by offline shaking and manual transfer of the samples to the Plasmid *Plus* 96 Plate, which was positioned over a drip tray (Figure 7).

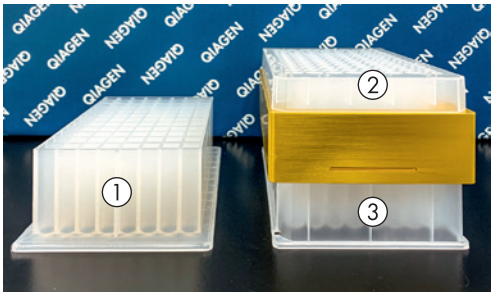

Figure 5. Lysis and neutralization were performed in 2 ml plates, then lysed cells were transferred to a TurboFilter 96 Plate located on top of a collection plate with an adapter. 1: Buffer S3 added to lysed cells (Buffer P1 and P2 added earlier). **2**: TurboFilter 96 Plate. **3**: S-Block.

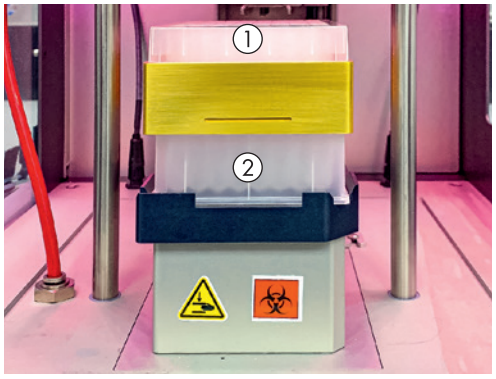

Figure 6. Both plates were then placed onto the Resolvex A200 to clarify the lysates using positive pressure. 1: TurboFilter 96 Plate. **2**: S-Block.

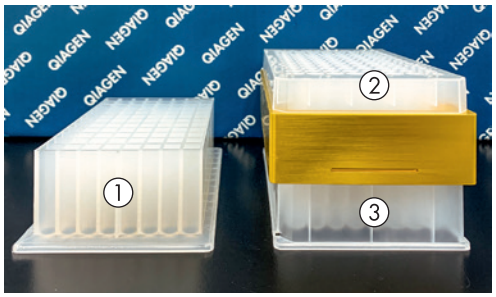

Figure 7. Filtered lysates were collected in a 2 ml plate, then filtered cells were transferred to a Plasmid *Plus* 96 Plate on top of a drip tray using an adapter. 1: Filtered lysate with Buffer BB added. **2**: Plasmid *Plus* 96 Plate. **3**: Drip tray.

Both plates were placed on the Resolvex A200 to load samples onto the sorbent via positive-pressure gradient (Figure 8).

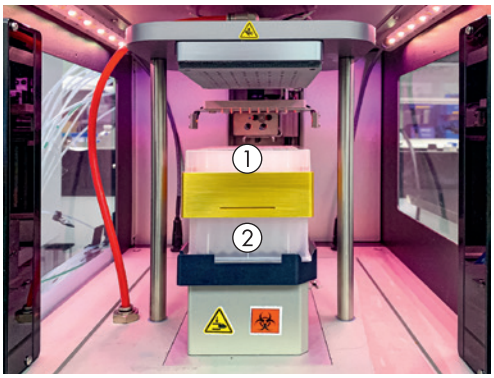

Figure 8. Both plates were then placed onto the Resolvex A200 to load samples onto the sorbent via positive pressure. 1: Plasmid *Plus* 96 Plate. **2**: Drip tray for discarding flow-through.

The drip tray was removed, and the Plasmid *Plus* 96 Plate was washed twice by automated buffer dispensing (Buffer PE). Finally, Buffer EB was dispensed twice, and the plasmid DNA was eluted into the collection plate via a slow pressure gradient (Figure 9).

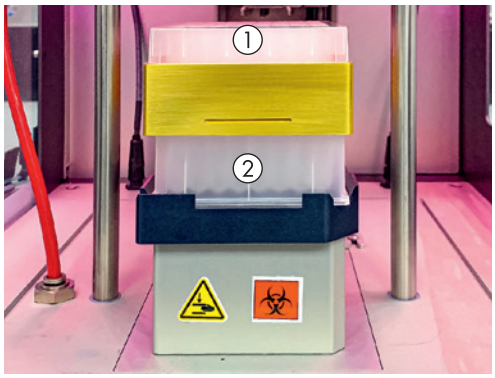

Figure 9. Plasmid *Plus* 96 Plate and elution plates were placed on the Resolvex A200 for dispensing of Buffer EB. A slow pressure gradient was applied to elute the samples. To maximize recovery, the elution step was performed twice. **1**: Plasmid *Plus* 96 Plate. **2**: Elution plate.

Table 2. Schematic overview of the QIAprep 96 *Plus* Miniprep protocol using the Resolvex A200

Step	Processing method	Procedure
1	Automated	Dispense Buffers P1, P2 and S3
2	Manual	Shake sample plate
3	Manual	Transfer samples to TurboFilter 96 Plate
4	Manual	Place TurboFilter 96 Plate on top of the S-Block (Figure 5) and load onto the Resolvex A200 (Figure 6)
5	Automated	Push samples through plate using pressure gradient
6	Automated	Dispense Buffer BB
7	Manual	Shake the S-Block
8	Manual	Transfer samples to the Plasmid *Plus* 96 Plate (Figure 7)
9	Automated	Load samples onto the sorbent using a positive pressure gradient (Figure 8)
10	Manual	Remove drip tray and place the Plasmid *Plus* 96 Plate on the Resolvex A200
11	Automated	Dispense Buffer PE, and process using pressure gradient
12	Automated	Dry the plate under constant pressure
13	Automated	Put the collection plate under the binding plate
14	Automated	Dispense Buffer EB and collect eluates in elution plate using pressure gradient (Figure 9)

Results and Data Analysis

Plasmid isolation on the Resolvex A200

Tables 3 and 4 show plasmid DNA yields across a 96-well plate using the QIAprep 96 Turbo and QIAprep 96 *Plus* Miniprep protocols, respectively, with the Resolvex A200.

Table 3. Plasmid DNA yields (µg) across a 96-well plate isolated using the QIAprep 96 Turbo Miniprep protocol on the Resolvex A200 (double elution, resulting in ~100 µl eluates)

	1	2	3	4	5	6	7	8	9	10	11	12
A	8.66	8.97	8.80	7.08	7.91	8.99	8.10	7.22	8.25	7.74	7.76	7.01
B	10.27	8.31	8.00	8.78	7.52	8.11	7.93	6.93	9.04	8.62	7.85	7.61
C	7.91	9.06	7.97	8.43	8.96	11.95	9.40	8.55	9.70	10.08	9.65	7.92
D	8.34	7.62	8.91	7.85	8.04	8.71	8.04	8.13	11.14	8.14	8.39	8.43
E	7.42	9.71	7.45	7.35	7.39	8.56	9.50	7.73	9.24	8.65	9.42	7.47
F	7.93	9.47	8.15	8.22	8.71	9.32	9.14	7.78	11.24	9.09	10.34	8.72
G	10.75	8.37	9.05	7.82	10.93	9.18	8.96	8.20	12.10	9.82	9.68	7.03
H	10.75	8.48	9.78	7.41	7.59	8.76	11.02	9.86	9.92	8.84	10.07	12.96

Table 4. Plasmid DNA yields (µg) across a 96-well plate isolated using the QIAprep 96 *Plus* Miniprep protocol on the Resolvex A200 (no ETR wash, double elution, resulting in ~115 µl eluates)

	1	2	3	4	5	6	7	8	9	10	11	12
A	10.36	10.84	10.50	11.26	11.10	11.05	10.67	10.16	10.69	10.88	11.16	10.22
B	10.70	12.72	11.39	11.28	11.09	10.85	10.43	11.26	11.21	10.85	11.12	11.53
C	11.39	12.22	11.28	11.60	11.81	11.01	10.59	11.25	11.67	11.40	11.75	11.25
D	10.99	12.53	10.74	10.76	11.45	11.25	10.73	11.16	11.07	10.76	10.41	11.66
E	10.38	11.93	11.61	11.16	10.86	11.58	10.80	10.79	11.45	11.06	10.94	10.98
F	10.42	11.80	11.72	10.50	10.20	10.82	10.70	10.80	10.30	11.16	11.85	10.34
G	11.69	11.14	10.89	10.72	9.97	10.32	10.59	11.03	11.59	11.47	11.49	11.32
H	11.70	12.18	11.28	10.43	10.14	11.13	10.75	11.42	10.50	11.29	11.59	11.52

Comparison between the Resolvex A200 workflow and the BioRobot Universal workflow

Comparison of the results obtained using the Resolvex A200 method with the BioRobot method is based on absorbance measurements at 230, 260 and 280 nm, delivering information on DNA yield and purity (using $A_{260/280}$ and $A_{260/230}$ ratios). Figure 10 shows the purity of the extracted DNA, demonstrating the same high-quality results for the Resolvex A200 workflow and the BioRobot method.

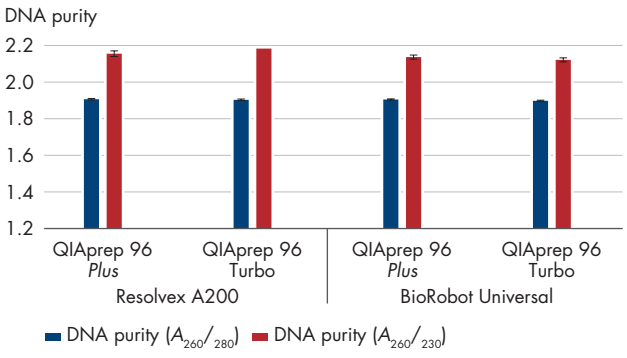

Figure 10. Comparison of DNA purity using different protocols and workstations.

Introduction of a double elution step to the QIAprep 96 *Plus* Miniprep protocol ensures excellent reproducibility, with CVs consistently below 5% and an average yield of 11.09 µg/ml (Figure 11). This workflow also employs extra sample transfer steps – both into the filtration plate and for loading the filtered lysates into a plasmid binding plate – which resulted in higher yields and lower %CVs compared to the QIAprep 96 Turbo Miniprep protocol.

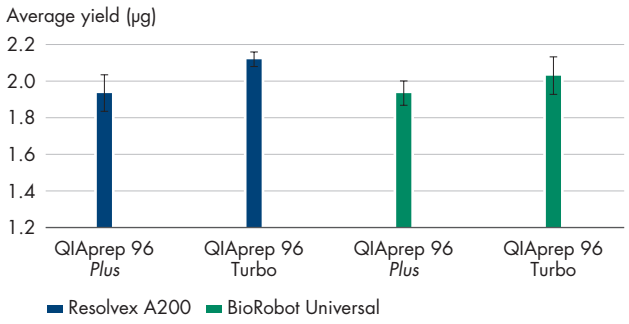

Figure 11. Comparison of yields and CVs across a 96-well plate for plasmid DNA extracted using different protocols on the Resolvex A200 and the BioRobot Universal workstations.

The advantage of the combined plate set-up (TurboFilter 96 Plate + QIAprep 96 Plate + deep-well plate) used in the QIAprep 96 Turbo Miniprep workflow is reduced hands-on time, enabling a fast, efficient DNA extraction protocol using the Resolvex A200. Although this results in a lower average yield (8.77 µg/well) and slightly reduced reproducibility (>13 %CV) compared to the QIAprep 96 *Plus* Miniprep protocol on the Resolvex A200, this is most likely due to less efficient sample transfer using the combined plate set-up. Despite this, both protocols deliver results equivalent to the reference method with lower variation using the QIAprep 96 *Plus* Miniprep protocol on the Resolvex A200.

Summary

Extraction of plasmid DNA using the QIAprep 96 Turbo and QIAprep 96 *Plus* Miniprep protocols on the Resolvex A200 positive-pressure workstation results in high purities and comparable yields to BioRobot methods, demonstrating the suitability of this system for routine use in high-throughput labs.

The faster QIAprep 96 Turbo Miniprep protocol reduces sample transfer steps and hands-on time but results in lower yields compared to the QIAprep 96 *Plus* Miniprep protocol. The QIAprep 96 *Plus* Miniprep protocol delivers higher plasmid DNA yields with lower variation, but it requires additional sample handling steps.

The Resolvex A200 workflow reduces handling times and errors compared to the BioRobot method by providing direct dispensing of all buffers and consistent pressure gradients.

Ordering Information

Product	Contents	Cat. no.
QIAprep 96 *Plus* Miniprep Kit (4)	For 4 x 96 plasmid minipreps: TurboFilter 96 Plates, Plasmid *Plus* 96 Plates, buffers, reagents, S-blocks, and elution microtubes; requires use of QIAvac 96 and Elution Microtube Adapter (available from QIAGEN Technical Service), or a centrifugation system suitable for 96-well blocks	27291
QIAprep 96 Turbo Miniprep Kit (4)	For 4 x 96 high-purity plasmid minipreps, 4 each: TurboFilter 96 and QIAprep 96 Plates; S-Blocks, reagents, buffers, collection microtubes (1.2 ml), caps	27191
QIAprep 96 Turbo Miniprep Kit (24)	For 24 x 96 high-purity plasmid minipreps, 24 each: TurboFilter 96 and QIAprep 96 Plates; S-Blocks, reagents, buffers, collection microtubes (1.2 ml), caps	27193
Related products		
TurboFilter 96 Plates (96)	TurboFilter 96 Plates (96)	120025

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