



## QIAGEN Supplementary Protocol:

### Automated high-throughput purification of plasmid DNA using the BioRobot® Universal System

This protocol is for high-throughput purification of molecular biology grade DNA from up to 4 x 96 bacterial cultures in parallel using the QIAprep® 96 Turbo BioRobot Kit in combination with the BioRobot Universal System.

#### Introduction

The BioRobot Universal System enables fully automated purification of high-copy plasmid DNA from 1–5 ml overnight cultures of *E. coli* grown in LB (Luria-Bertani) medium. The purification procedure, in 96-well format, uses proven silica-membrane technology and provides molecular biology grade DNA that is ready for immediate use in a wide range of downstream applications, such as sequencing, restriction enzyme digestion, cloning, transfection of robust cells, and PCR. Up to 4 x 96 samples can be processed in parallel and the automated, standardized procedure provides yields of up to 20 µg DNA per well.

**IMPORTANT:** Please read the *QIAprep Miniprep Handbook*, paying careful attention to the “Safety Information” and “Important Notes” sections, before beginning this procedure.

**IMPORTANT:** Please read the *BioRobot 8000 User Manual*, paying careful attention to the safety information, before beginning this procedure.

#### Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- BioRobot Universal System, cat. no. 9001094
- Application Pack, Sequencing, cat. no. 9016757
- QIAprep 96 Turbo BioRobot Kit (4), cat. no. 962141
- QIAGEN conducting disposable filter-tips (1100 µl) for use with BioRobot systems, cat. no. 9012598
- S-Blocks (required if processing fewer than 96 samples in a single run), cat. no. 19585
- Ethanol (96–100%)\*
- Disposable troughs (80 ml), cat. no. 9013653

\* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

- Hard-Shell® Thin-Wall skirted 96-Well PCR Plates (50) (Bio-Rad, cat. no. HSP 9631; see [www.bio-rad.com](http://www.bio-rad.com) )
- Centrifuge with rotor suitable for 96-well plates, capable of 2100 x g (e.g., QIAGEN 4K15C, see ordering information, page 7)
- Optional: AirPore Tape Sheets (50), cat. no. 19571 for covering flat-bottom blocks

## Important notes

### Reagents

Sufficient reagents are supplied to purify DNA from 4 x 96 samples. If fewer than 96 samples are processed in each run, additional reagents may need to be purchased to process the same number of samples in total (see ordering information, page 7).

### Buffer P1

Before use, add the provided RNase A solution to Buffer P1, mix, and store at 2–8°C.

### Buffer PE

Before use, add 4 volumes ethanol (96–100%) to 1 volume Buffer PE.

## Worktable accessories

### Plasticware

For a single run of 96 samples, one flat-bottom block, one TurboFilter 96 plate, one QIAprep 96 plate, and two PCR plates are required. When placing the items of plasticware on the BioRobot worktable, make sure that position A1 is located at the upper left corner. The flat-bottom block, TurboFilter 96 plate, and QIAprep 96 plate can be discarded after use.

If fewer than 96 samples are to be processed in a run, an S-Block is required. When placing the S-Block on the BioRobot worktable, make sure that position A1 is located at the upper left corner.

### Tape pad

Tape sheets from the tape pad are used to seal unused wells of the QIAprep 96 plate and TurboFilter 96 plate if fewer than 96 samples are to be processed in a run.

### Partially using a QIAprep 96 plate or TurboFilter 96 plate

The QIAprep 96 plate and TurboFilter 96 plate can be used for runs of 32–96 samples (sample number must be a multiple of 32).

If only part of a plate is used (e.g., the first 32 wells), seal the unused wells with a sheet from the tape pad and leave them sealed throughout the purification procedure. Ensure that complete columns of 8 samples are processed. After use, keep the unused wells sealed and store the plates at 4°C in the blister pack in which they were supplied.

When reusing partially used plates, label used wells with a waterproof marker pen and remove the adhesive tape covering the unused wells. Cover the previously used wells with adhesive tape before beginning the purification procedure.

### Setting up the BioRobot Universal System

The QIAsoft 5 software guides you through worktable setup. For a summary of worktable setup for a single run of 96 samples, see Tables 1 and 2 and the figure on the next page.

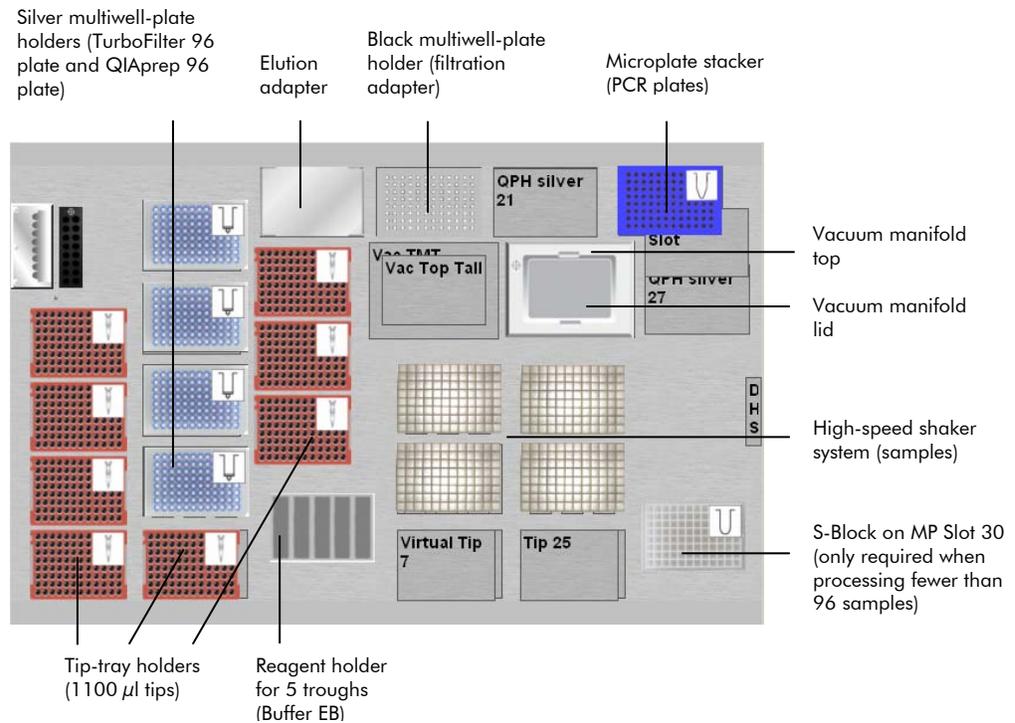
**Table 1. Positions of Reagents and Buffers**

Item	Position	Minimum volume (ml) for 96 samples
Buffer EB	Disposable 80 ml trough in the 5-trough reagent holder (Subslot A of the cooling and heating system)	12
Buffer P1 (with RNase added)	Rotor Slot 1 of the reagent carousel	97
Buffer PB	Rotor Slot 2 of the reagent carousel	203
Buffer P2	Rotor Slot 3 of the reagent carousel	97
Buffer PE	Rotor Slot 4 of the reagent carousel	287
Buffer N3	Rotor Slot 5 of the reagent carousel	108
System liquid	Rotor Slot 8 of the reagent carousel	500
Samples	High-speed shaker system	–

**Table 2. Positions of Accessories for a 4-Plate Run**

Item	Position	Holder/adapter
Disposable trough (80 ml)	Subslot A of cooling and heating system (VariTherm Slot)	Trough holder in reagent-holder tray
TurboFilter 96 plate	MP Slots 6, 7, 8, and 9	Silver multiwell-plate holder
QIAprep 96 plate	On top of TurboFilter 96 plate in MP Slots 6, 7, 8, and 9	–
Filtration adapter	QIAplate Holder black 16	Black multiwell-plate holder
Elution adapter	MP Slot 11	–
Five PCR plates	Stacker Slot 26	Microplate stacker placed on MP Slot 26
Disposable filter-tips	Tip racks 2–5, 10, 12–14, and 20	Red tip-tray holder
Samples (flat-bottom block)	High-speed shaker system	–

**Worktable Overview (4-Plate Run)**



## Protocol: High-throughput purification of plasmid DNA using the BioRobot Universal System

This protocol is for purification of molecular biology grade DNA from up to 4 x 96 overnight cultures of *E. coli* using the BioRobot Universal System.

### Important points before starting

- Before beginning the procedure, read “Important Notes” on pages 2–4 of this protocol.
- Bacterial cultures can be 1–5 ml. To grow cells in a 96-well flat-bottom block, culture volumes of 1.3 ml should be used (see step 1 of the procedure).
- Ensure that you are familiar with operating the BioRobot Universal System.

### Things to do before starting

- Check that Buffers P1 and PE have been prepared according to the instructions on page 2 of this protocol.
- Check that Buffers P2 and N3 do not contain a salt precipitate. If necessary, warm Buffers P2 and N3 to 37°C to dissolve the precipitate. Do not shake Buffer P2 vigorously.

## Procedure

### Cell cultivation in a 96-well block

- 1. Fill each well of a 96-well flat-bottom block with 1.3 ml of growth medium containing the appropriate selective agent. Inoculate each well from a single bacterial colony. Incubate the cultures for 20–24 h at 37°C with vigorous shaking.**

The wells in the block may be protected against spill-over by covering the block with a plastic lid or adhesive tape. AirPore microporous tape sheets promote gas exchange during culturing (see ordering information, page 8). If non-porous tape is used, pierce 2–3 holes in the tape with a needle above each well for aeration.

- 2. Harvest the bacterial cells in the flat-bottom block by centrifugation for 5 min at 2100 x g in a centrifuge with a suitable rotor for 96-well plates, preferably at 4–10°C. The block should be covered with adhesive tape during centrifugation. Remove media by inverting the block.**

To remove the media, peel off the tape and quickly invert the block over a waste container. Tap the inverted block firmly on a paper towel to remove any remaining droplets of medium.

**WARNING:** Ensure that the buckets on the rotor have sufficient clearance to accommodate 2 ml flat-bottom blocks before starting the centrifuge.

### Automated high-throughput purification of plasmid DNA

**3. Make sure that the BioRobot Universal System is switched on.**

The power switch is located on the lower right of the front BioRobot panel.

**4. Switch on the computer and monitor.**

**5. Launch the QIAsoft 5 Operating System.**

The QIAsoft 5 software can be started from the Microsoft® Windows® “Start” menu, where it is located under Programs/QIAsoft 5/QIAsoft 5.

**6. Enter your user name and password in the “Login” dialog box, and click “OK” to access the QIAsoft 5 software.**

**7. Select the “QIAprep Turbo UNIV Protocol” from the protocol selection box in the “Execute” environment toolbar.**

**8. Click  to start the protocol.**

The QIAsoft 5 software will now take you through the remaining steps required to set up the BioRobot Universal System for the “QIAprep Turbo UNIV Protocol”. Follow the steps detailed in each protocol message before continuing.

You will be prompted to enter information about the following:

- Number and positions of samples to be processed.
- The elution volume; must be in the range 70–100  $\mu$ l.
- Whether a load check should be performed; choice of complete or partial.
- If processing one or two 96-well plates, an optional wash with Buffer PB can be performed.

**9. A software message on the screen will indicate when the purification procedure is finished, and protocol messages will guide you through the steps for worktable cleanup.**

**10. The purified plasmid DNA is ready to use in downstream applications or can be stored at 2–8°C for 24 h or at –20°C or –80°C for longer periods.**

**11. Follow the maintenance instructions described in the “Maintenance” environment of the QIAsoft 5 software and in the *BioRobot 8000 User Manual*.**

## Ordering Information

Product	Contents	Cat. no.
QIAprep 96 Turbo BioRobot Kit (4)	For 4 x 96 high-purity plasmid minipreps, 4 each: TurboFilter 96 and QIAprep 96 Plates; Flat-Bottom Blocks and Lids, Reagents, Buffers, Collection Microtubes (1.2 ml) and Caps, 96-Well Microplates RB and Lids, Tape Pads	962141
BioRobot Universal System	Robotic workstation, computer-controlled vacuum pump, computer, QIAsoft 5 Operating System, installation, 1-year warranty on parts and labor*	9001094
Application Pack, Sequencing	Protocols and application-specific accessories for plasmid DNA purification, PCR cleanup, and sequencing reaction setup on the BioRobot Universal System	9016757
<b>96-Well Centrifugation System</b>		
Centrifuge 4K15C	Universal refrigerated laboratory centrifuge with brushless motor (100 V, 50/60 Hz)	81200 <sup>†</sup>
Centrifuge 4K15C	Universal refrigerated laboratory centrifuge with brushless motor (120 V, 60 Hz)	81210 <sup>‡</sup>
Centrifuge 4K15C	Universal refrigerated laboratory centrifuge with brushless motor (220 V, 50 Hz)	81220 <sup>¶</sup>
Plate Rotor 2 x 96	Rotor for 2 QIAGEN 96-well plates, for use with QIAGEN Centrifuges <sup>§</sup>	81031
<b>Accessories</b>		
Disposable Filter-Tips, 1100 µl (960)	Conducting disposable filter-tips; pack of 960	9012598
Buffer P1 (500 ml)	500 ml Resuspension Buffer (RNase A not included)	19051
Buffer P2 (500 ml)	500 ml Lysis Buffer	19052
Buffer N3 (500 ml)	500 ml Buffer N3	19064
Buffer PB (500 ml)	500 ml Binding Buffer	19066

\* Warranty PLUS 2 (cat. no. 9239573) recommended: 3-year warranty, 1 preventive maintenance visit per year, 48-hour priority response, all labor, travel, and parts.

<sup>†</sup> For Japan; <sup>‡</sup> For US; <sup>¶</sup> For rest of world.

<sup>§</sup> The Plate Rotor 2 x 96 is available exclusively from QIAGEN and its distributors. Under the current liability and warranty conditions, the rotor may only be used in Centrifuges 4-15, 4-15C, and 4K15C from QIAGEN and freely programmable models of centrifuges 4-15, 4K15, 6-10, 6K10, 6-15, and 6K15 from Sigma Laborzentrifugen GmbH.

<b>Product</b>	<b>Contents</b>	<b>Cat. no.</b>
Buffer PE (concentrate, 100 ml)	100 ml Wash Buffer (5x concentrate for 500 ml buffer)	19065
Buffer EB (250ml)	250 ml Elution Buffer	19086
RNase A (17,500 U)	2.5 ml (100 mg/ml; 7000 units/ml, solution)	19101
Flat-Bottom Blocks (24)	96-well blocks with 2 ml wells: 24 per case, lids included	19579
AirPore Tape Sheets (50)	Microporous tape sheets for covering 96-well blocks during bacterial cultivation: 50 sheets per pack	19571
Tape Pads (5)	Adhesive tape sheets for sealing multiwell plates and blocks: 25 sheets per pad, 5 pads per pack	19570
Disposable Troughs, 80 ml (10)	Troughs for holding up to 80 ml of liquid; pack of 10	9013653
S-Blocks (24)	96-well blocks with 2.2 ml wells, 24 per case	19585

The BioRobot Universal System is intended for life science research applications. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations. The PCR process is covered by the foreign counterparts of U.S. Patents Nos. 4,683,202 and 4,683,195 owned by F. Hoffmann-La Roche Ltd.

Selected handbooks can be downloaded from [www.qiagen.com/literature/handbooks/default.aspx](http://www.qiagen.com/literature/handbooks/default.aspx).  
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from [www.qiagen.com/ts/msds.asp](http://www.qiagen.com/ts/msds.asp).

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