



QIAGEN Supplementary Protocol:

Automated purification of DNA from fresh or frozen cultured-cell pellets on the Autopure LS®

This protocol is designed for purification of DNA from up to 1×10^7 , $1-2 \times 10^7$, $2-5 \times 10^7$, or $0.5-1.5 \times 10^8$ cells using Autopure reagents on the Autopure LS.

The Autopure LS provides automated purification of archival-quality DNA from a variety of large samples. Proven Gentra® Puregene® chemistries and optimized protocols provide high yields of pure DNA ready for use in sensitive downstream applications or for DNA archiving. Purified DNA typically has an A_{260}/A_{280} ratio between 1.7 and 1.9. Either 8 or 16 samples can be processed per run.

IMPORTANT: Please read the *Autopure LS User Manual*, paying careful attention to the safety information, before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, consult the appropriate material safety data sheets (MSDSs), available from the product supplier. The Autopure LS instrument is intended to be used only in combination with Autopure reagents for applications described in the *Autopure LS User Manual*.

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.

- Autopure LS, cat no. 9001340
- Autopure Cell Lysis Solution (3800 ml), cat. no. 949006
- Autopure Precipitation Soln. (3800 ml), cat. no. 949008
- Autopure DNA Hydration Soln. (3800 ml), cat. no. 949010 or DNA Hydration Solution (500 ml), cat. no. 158916
- Autopure 100% Isopropanol (3800 ml), cat. no. 949016
- Autopure 70% Ethanol (3800 ml), cat. no. 949018
- Autopure Qubes® E (192), cat. no. 949020 or Autopure Qubes D (192), cat. no. 949022
- Autopure Waste Container, cat. no. 9017686
- Water bath heated to 65°C

Important point before starting

- Ensure that you are familiar with operating the Autopure LS. Refer to the *Autopure LS User Manual* for operating instructions.

Things to do before starting

- Heat the water bath to 65°C for use in step 6 of the procedure.

Procedure

1. Make sure that the Autopure LS is switched on. The power switch is located at the back left side of the instrument.
2. Log in to the instrument software. Prepare the samples and the rack, and follow the steps for starting sample processing described in the *Autopure LS User Manual*.
3. Select the appropriate protocol for the sample type and size. See the table below for more information.

Protocols for processing up to 1.5×10^8 cells

Protocol name	Sample size	Important note
Cultured Cells (Pellet)	0–10 million cells	Cells must be pelleted and supernatant removed before loading onto the Autopure LS.
Cultured Cells (Pellet)	10–20 million cells	
Cultured Cells (Pellet)	20–50 million cells	
Cultured Cells (Pellet)	50–150 million cells	

4. Select "Run Rack" to start the run. The Autopure LS will then perform the automated purification procedure. For more detailed information about the procedure, see "Steps performed by the Autopure LS", page 3.
5. When instructed to do so by the software, remove the purified DNA from the Autopure LS.
6. After removing the purified DNA from the instrument, incubate at 65°C for 1–2 h to dissolve the DNA.
7. Incubate at room temperature (15–25°C) overnight with gentle shaking. Ensure tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

Steps performed by the Autopure LS

The amount of reagent used depends on the protocol being run. Reagent volumes for processing up to 1×10^7 cells are denoted by ■, reagent volumes for processing $1-2 \times 10^7$ cells are denoted by ◆, reagent volumes for processing $2-5 \times 10^7$ cells are denoted by ●, and reagent volumes for processing $0.5-1.5 \times 10^8$ cells are denoted by ▲.

Cell lysis and protein precipitation

1. Scans and verifies the input and output cap bar codes and weighs the tubes to check that input tubes contain samples and that output tubes are empty.
2. Dispenses ■ 1 ml, ◆ 3 ml, ● 6 ml, or ▲ 12 ml Autopure Cell Lysis Solution (Reagent 2) into each input tube.
3. Mixes the samples vigorously for 2 min to lyse the cells.
4. Incubates the sample for 15 min.
5. Centrifuges the samples at 3000 x g for 2 min to collect all liquid at the bottom of the tube.
6. Dispenses ■ 0.34 ml, ◆ 1 ml, ● 2 ml, or ▲ 4 ml Autopure Precipitation Soln. (Reagent 3) into each input tube.
7. Mixes the samples vigorously for 2 min to precipitate the proteins.
8. Centrifuges the samples at 3000 x g for 15 min. The precipitated proteins will form a tight pellet at the bottom of the input tube.
9. During the centrifugation in step 8, the Autopure LS dispenses ■ 1 ml, ◆ 3 ml, ● 6 ml, or ▲ 12 ml Autopure 100% Isopropanol (Reagent 4) into the output tubes.
10. After the centrifugation, the supernatant from step 8 is poured into the output tubes.

DNA precipitation

1. Rotates the output tubes gently 50 times to precipitate the DNA.
2. Centrifuges the samples at 3000 x g for 5 min to pellet the DNA.
3. Pours the isopropanol supernatant into the waste container, and inverts the output tubes for 1 min to evaporate any remaining alcohol.

DNA wash

1. Dispenses ■ 1 ml, ◆ 3 ml, ● 6 ml, or ▲ 12 ml Autopure 70% Ethanol (Reagent 5) into the output tubes.
2. Centrifuges the samples at 3000 x g for 2 min to pellet the DNA.
3. Pours the ethanol supernatant into the waste tray, and inverts the output tubes for 1 min to evaporate any remaining alcohol.

DNA hydration

1. Dispenses the volume of Autopure DNA Hydration Soln. (Reagent 6) selected by the user into the output tubes to rehydrate the DNA.
2. Displays message to inform user that the protocol run has finished.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from 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.

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