## QIAGEN Supplementary Protocol:

# Automated purification of DNA from up to 10 ml cell lysate from compromised samples on the Autopure LS®

This protocol is designed for purification of DNA from 5 ml or 10 ml of cell lysate from compromised samples 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 Precipitation Soln. (3800 ml), cat. no. 949008
- Autopure DNA Hydration Soln. (3800 ml) or DNA Hydration Solution (500 ml), cat. nos. 949010 and 158916
- Autopure Glycogen Solution (5 ml), cat. no. 949002
- 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 points before starting

- Ensure that you are familiar with operating the Autopure LS. Refer to the *Autopure LS User Manual* for operating instructions.
- Reagent volumes for processing 1–5 ml samples of cell lysate are denoted by ■ and reagent volumes for processing 5–10 ml samples of cell lysate are denoted by ◆.

### 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. **Add ■ 66.5 µl or ◆ 133 µl of Autopure Glycogen Solution to output tubes.**
3. **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*.**
4. **Select the protocol "Comp Lysate Finish" and the sample volume "1–5 ml" or "5–10 ml".**

   **Important**: When running the protocol for 1–5 ml cell lysate, the sample volume must be 5 ml. When running the protocol for 5–10 ml cell lysate, the sample volume must be 10 ml. The Autopure LS does not equalize sample volumes at the start of the protocol. Samples must be either 5 ml or 10 ml to avoid initial weight failures and a possible centrifuge imbalance error.
5. **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.**
6. **When instructed to do so by the software, remove the purified DNA from the Autopure LS.**
7. **After removing the purified DNA from the instrument, incubate at 65°C for 1–2 h to dissolve the DNA.**
8. **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 1–5 ml samples of cell lysate are denoted by ■ and reagent volumes for processing 5–10 ml samples of cell lysate are denoted by ◆.

### 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 ■ 2.2 ml or ◆ 4 ml Autopure Precipitation Soln. (Reagent 3) vigorously into the center of the input tubes.**
3. **Mixes the samples vigorously for 2 min to precipitate the proteins.**
4. **Centrifuges the samples at 3000 x *g* for 5 min. The precipitated proteins will form a tight pellet at the bottom of the input tube.**
5. **During the centrifugation in step 4, the instrument dispenses ■ 6.5 ml or ◆ 12 ml Autopure 100% Isopropanol (Reagent 4) into output tubes in Row C (if running 8 samples) or Row C and Row D (if running 16 samples).**
6. **Pours the DNA-containing supernatant from step 4 into the output tubes that contain Autopure 100% Isopropanol.**

### 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 tray, and inverts the output tubes for 1 min to evaporate any remaining alcohol.**

### DNA wash

1. **Dispenses ■ 6.5 ml or ◆ 12 ml Autopure 70% Ethanol (Reagent 5) into the output tubes.**
2. **Centrifuges the samples at 3000 x *g* for 5 min to pellet the DNA.**
3. **Pours the ethanol supernatant into the waste container, 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.**

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