QIAGEN Supplementary Protocol:

Automated purification of DNA from fresh or frozen buffy coat on the Autopure LS®

This protocol is designed for purification of DNA from fresh or frozen buffy coat prepared from 1–5 ml or 5–10 ml fresh whole blood 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 RBC Lysis Solution (9500 ml), cat. no. 949004
- 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.**

   Important: When running protocols for 1–5 ml buffy coat, the sample volume must not exceed 5 ml. When running protocols for 5–10 ml buffy coat, the sample volume must not exceed 10 ml.

   Protocols for processing up to 10 ml fresh or frozen buffy coat

Protocol name	Sample size	Sample storage
Fresh Buffy Coats	1–5 ml or 5–10 ml	Samples stored for <24 h at room temperature (15–25°C) or <5 days at 4°C. Samples have not been frozen.
Frozen Buffy Coats	1–5 ml or 5–10 ml	Samples frozen at –80°C directly after collection and stored for less than 2 years at –80°C. Samples have not been thawed and refrozen. Make sure to thaw samples quickly at 37°C before placing on the Autopure LS.

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 1–5 ml buffy coat are denoted by ■ and reagent volumes for processing 5–10 ml buffy coat are denoted by ◆.

RBC lysis

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 ■ 15–20 ml or ◆ 25–35 ml Autopure RBC Lysis Solution (Reagent 1) into each input tube.**

   Note: The system uses Reagent 1 to balance the tubes before centrifugation. The amount dispensed into each tube varies depending on the initial sample volume. The total volume of sample and Reagent 1 is ■ 20 ml or ◆ 35 ml.
3. **Incubates the sample in Autopure RBC Lysis Solution for 5 min to lyse the red blood cells. The samples are rotated gently to mix during the incubation.**
4. **Centrifuges the samples at 3000 x *g* for 2 min to pellet the white blood cells.**
5. **During centrifugation, the Autopure LS dispenses ■ 5 ml or ◆ 10 ml Autopure 100% Isopropanol (Reagent 4) into output tubes in Row C (if running 16 samples).**
6. **After centrifugation, the supernatant from step 4 is poured into the waste tray.**

Cell lysis and protein precipitation

1. **Dispenses ■ 1.67 ml or ◆ 3.34 ml Autopure Precipitation Soln. (Reagent 3) vigorously into the center of the input tubes to disperse the white blood cell pellet and to precipitate the proteins.**
2. **Dispenses ■ 5 ml or ◆ 10 ml Autopure Cell Lysis Solution (Reagent 2) into each input tube to lyse the white blood cells.**
3. **Mixes the samples vigorously to precipitate the proteins.**
4. **Centrifuges the samples at 3000 x *g* for 2 min. The precipitated proteins will form a tight pellet at the bottom of the input tube.**
5. **During the centrifugation in step 4, the Autopure LS dispenses ■ 5 ml or ◆ 10 ml Autopure 100% Isopropanol (Reagent 4) into output tubes in Row C (if running 8 samples) or 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 2 min to pellet the DNA.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 ■ 5 ml or ◆ 10 ml Autopure 70% Ethanol (Reagent 5) into the output tubes.**
2. **Centrifuges the samples at 3000 x *g* for 1 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.