



QIAGEN Supplementary Protocol:

Automated purification of DNA from 10 ml samples of mouthwash on the Autopure LS[®]

This protocol is designed for purification of DNA from 10 ml samples of mouthwash 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 Pro-K Pump, cat no. 9017687
- Autopure Cell Lysis Solution (3800 ml), cat. no. 949006
- Autopure Proteinase K Solution (3500 μ l), cat. no. 949012
- 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
- Recommended: Autopure Glycogen Solution (5 ml), cat. no. 949002 (if DNA yields are expected to be low i.e., <20 μ g)

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 7 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. **Recommended: Add 5 µl Autopure Glycogen Solution to each output tube.**
4. **Select the protocol "Mouthwash".**
Important: When running the mouthwash protocol, the sample volume must not exceed 10 ml. To avoid a centrifuge imbalance error, make sure that all samples have the same volume.
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

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. Centrifuges the samples at 3000 x g for 5 min to pellet the cells.
3. During the centrifugation in step 2, the instrument dispenses 1 ml Autopure 100% Isopropanol (Reagent 4) into output tubes in Row C (if running 16 samples).
4. Pours the mouthwash supernatant into the waste container.
5. Dispenses 1 ml Cell Lysis Solution (Reagent 2) into each input tube.
6. Mixes the samples gently and incubates for 15 min to lyse the cells.
7. Dispenses 10 μ l Autopure Proteinase K Solution into each input tube.
8. Mixes the samples vigorously for 2 min.
9. Incubates the samples for 10 min.
10. Dispenses 0.33 ml Autopure Precipitation Soln. (Reagent 3) into the input tubes to precipitate the proteins.
11. Mixes the samples vigorously for 2 min to precipitate the proteins.
12. Incubates the sample for 10 min at 4°C.
13. Centrifuges the samples at 3000 x g for 10 min at 4°C. The precipitated proteins will form a tight pellet at the bottom of the input tube.
14. During the centrifugation in step 13, the Autopure LS dispenses 1 ml Autopure 100% Isopropanol (Reagent 4) into output tubes in Row C (if running 8 samples) or Row D (if running 16 samples).
15. Pours the DNA-containing supernatant from step 13 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 1 ml Autopure 70% Ethanol (Reagent 5) into the output tubes.
2. Centrifuges the samples at 3,000 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.

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