

QIAGEN Supplementary Protocol

Automated purification of DNA from saliva samples collected with the Oragene[®] DNA Self-Collection Kit

Protocol menu name	Cell Lysate
Instrument	AP-0196 or AP-0098
Sample material	5 ml lysate
Batch size	8 or 16
Editable parameters	Volume of DNA Hydration Solution

This protocol is designed for purification of DNA from saliva samples collected with the Oragene DNA Self-Collection Kit. Cell lysates are prepared manually, and the purification procedure is performed by the Autopure[®] LS.

The Autopure LS provides automated purification of archival-quality DNA from a variety of large samples. Proven Autopure chemistries and optimized protocols provide high yields of pure DNA that is 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 and is up to 200 kb in size.

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

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.

- Oragene DNA Self-Collection Kit (see www.dnagenotek.com for more information)
- Autopure RNase A Solution (19 ml), cat. no. 949014
- Autopure Glycogen Solution (5 ml), cat. no. 949002
- Water bath or air incubator capable of attaining 65°C
- Vortexer
- Tube rocker

Things to do before starting

- Collect saliva samples with the Oragene DNA Self-Collection Kit according to the manufacturer's instructions.
- Heat water bath or air incubator to 50°C
- Incubate the Oragene DNA vial at a convenient time after sample collection at 50°C in a water bath for a minimum of 1 hour; alternatively, incubate in an air incubator for a minimum of 2 hours.

Note: This incubation step may be performed at any time after collection of the saliva sample before purification of the DNA. The sample may be incubated at 50°C overnight if this is more convenient. A longer incubation is necessary when using an air incubator since temperature equilibration is slower compared to a water incubator.

- Heat water bath to 65°C for use in step 26 of the procedure.

Procedure

Manual cell lysate preparation

1. **Transfer 2–4 ml saliva sample in the Oragene DNA vial into each Qube® input tube.**

Note: The volume may be varied to facilitate different Oragene DNA Self-Collection Kit formats and different user requirements. For highest DNA yields, we recommend using a sample volume of 4 ml.

2. **Add Cell Lysis Solution to each Qube input tube to bring the total volume to 5 ml.**

Note: The instrument will not equalize sample volumes at the start of this protocol; therefore all samples must contain 5 ml lysate to ensure that the centrifuge is balanced.

3. **Add 25 µl RNase A Solution (4 mg/ml) to each Qube input tube.**
4. **Vortex at high speed for 10 s to mix sample.**
5. **Incubate for 10 min at room temperature (15–25°C).**

Run preparation

6. Make sure that the Autopure LS is switched on. The power switch is located at the back left side of the instrument.
7. Log in to the instrument with your user ID and password.
8. Prepare the samples and the rack, and follow the steps for starting sample processing described in Section 6.2 of the *Autopure LS User Manual*.

Note: Select *Cell Lysate* from the "Protocol" dialog field and *1–5 ml* from the "Sample size" dialog field. The instrument will not equalize sample volumes at the start of this protocol; therefore all samples must contain 5 ml lysate to ensure that the centrifuge is balanced. Select the hydration volume from the "Hydration Vol. (μ l)" dialog field. We recommend a hydration volume between 300 μ l and 500 μ l.

9. Add 40 μ l of Glycogen Solution (20 mg/ml) into each output tube.
10. Select *Run Rack* to start the run.

Protein precipitation

11. Scans and verifies the input and output cap IDs, and weighs all of the tubes to verify presence and volume of sample (input tubes) or to ensure tubes are empty (output tubes).
12. Dispenses 1.67 ml Reagent 3 (Protein Precipitation Solution) into each input tube.
13. Mixes the samples vigorously for 2 min to precipitate the proteins.
14. Centrifuges the samples at 3,000 x g for 2 min to pellet the proteins.
15. Dispenses 5 ml Reagent 4 (100% isopropanol) into each output tube during the centrifugation.
16. Pours the DNA-containing supernatant into the output tubes containing isopropanol.

DNA precipitation

17. Mixes the tubes gently 50 times to precipitate the DNA.
18. Centrifuges the samples at 3,000 x g for 2 min to pellet the DNA.
19. Pours the isopropanol supernatant to waste.
20. Drains the output tubes for 1 min to evaporate the alcohol.

DNA wash

21. Dispenses 5 ml Reagent 5 (70% ethanol) into each output tube.
22. Centrifuges the samples at 3,000 x g for 1 min to pellet the DNA.
23. Pours the ethanol supernatant to waste.
24. Drains the output tubes for 1 min to evaporate the alcohol.
25. Dispenses the user-selected volume of Reagent 6 (DNA Hydration Solution) into each output tube to hydrate the DNA.

DNA hydration

26. When the instrument protocol is complete, remove the samples from the Autopure LS and incubate at 65°C for 1–2 h to dissolve the DNA.
27. Incubate at room temperature (15–25°C) overnight on a rocker. Ensure the tube caps are tightly closed to avoid leakage.
28. Store the purified DNA at 2–8°C for short-term storage or at –20°C to –80°C for long-term storage.

Note: The samples may be centrifuged briefly and transferred to smaller tubes for storage.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature. Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/Support/MSDS.aspx.

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