

High-throughput DNA purification with the Qiagen BioRobot™ EZ1

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The BioRobot EZ1 is an automated high-throughput DNA purification system that uses silica-magnetic-particle technology. The EZ1 can purify up to six Oragene®•DNA/saliva samples in less than 20 minutes, with excellent DNA recovery.

Introduction

Large-scale population studies may involve the collection of thousands of donor samples. Manual purification of DNA from these samples can be time- and labor-intensive. A number of manufacturers offer automated high-throughput DNA purification systems such as the BioRobot line of machines from Qiagen. The purpose of this study was to determine the DNA purification performance of the BioRobot EZ1 System with saliva samples collected with Oragene•DNA.

Materials and Methods

DNA collection

Oragene•DNA Self-Collection Kits contain 2 mL of DNA-preserving solution. The kits were used to collect 2 mL of saliva from 18 donors (4 mL total volume of Oragene•DNA/saliva sample). Prior to purification with the EZ1 System, the Oragene•DNA/saliva samples were incubated overnight at 50°C, following the Oragene•DNA kit instructions.

DNA purification

DNA was extracted from 200 µL of Oragene•DNA/saliva sample using the BioRobot EZ1 System with the EZ1 DNA Tissue Kit and the EZ1 DNA Blood Kit (Qiagen). For both kits, purified DNA was eluted in the recommended volume of 200 µL (ref. 1). DNA was also extracted from 200 µL of Oragene•DNA/saliva sample using the manual Oragene•DNA protocol.

Different elution volumes

Five Oragene•DNA/saliva samples were processed using the EZ1 DNA Tissue Kit and eluted in volumes of 50, 100, and 200 µL.

DNA analysis

DNA yield was determined by the highly specific Fluorescence/DNase method (ref. 2). The F/D method quantitates DNA using SYBR Green I™ dye (Molecular Probes) and is more accurate than quantification by absorbance. DNA purified with each of the three methods was analyzed by agarose gel electrophoresis with ethidium bromide staining. The molecular weight of the DNA was determined by comparison with a Lambda-Hind III digest ladder.

Results

DNA yield

From 18 Oragene•DNA/saliva samples, the median DNA yield with the EZ1 Tissue Kit was 3.2 µg per 200 µL of starting sample (Figure 1). This was slightly higher than 2.9 µg obtained with the manual Oragene•DNA purification protocol. The EZ1 Blood Kit produced a significantly lower yield of 1.2 µg.

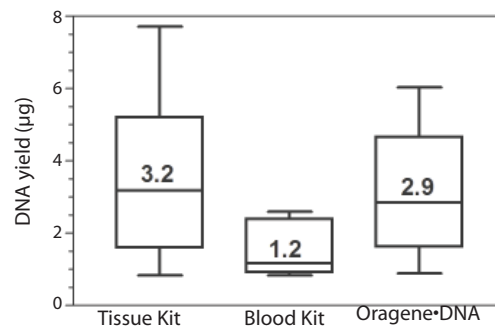


Figure 1. Median DNA yield from 200 µL of Oragene•DNA/saliva sample with the EZ1 Tissue Kit, Blood Kit, and Oragene•DNA purification protocol.

Elution volume

From five Oragene•DNA/saliva samples, the average DNA yield from 200 µL of starting sample was 2.9 µg with an elution volume of 200 µL. Elution volumes of 100 and 50 µL gave lower DNA yields of 2.4 and 2.2 µg, respectively (Figure 2A). Conversely, the average DNA concentration increased with decreasing elution volume: 14.6, 23.7, and 44.6 ng/µL for elution volumes of 200, 100, and 50 µL, respectively (Figure 2B).

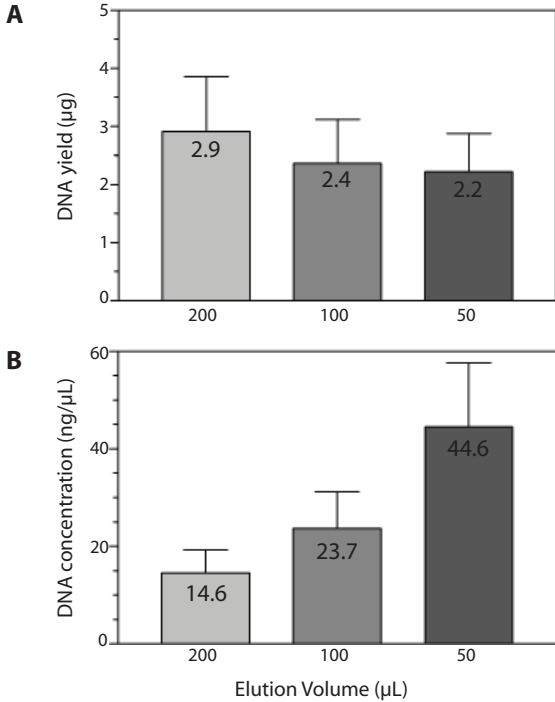


Figure 2. Average DNA yields (A) and concentrations (B) with different elution volumes.

Molecular weight of DNA

All three purification procedures produced DNA with a molecular weight of 23 kb or higher (Figure 3).

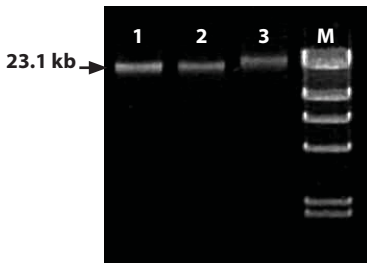


Figure 3. Agarose gel electrophoresis of DNA purified with the Blood Kit, Tissue Kit, and Oragene•DNA protocol in Lanes 1, 2 and 3, respectively. A Lambda-Hind III digest was used as the marker.

Discussion and Conclusions

Up to six Oragene•DNA/saliva samples may be purified in less than 20 minutes using the BioRobot EZ1 System. The eluted DNA is of high molecular weight. For maximum DNA yield, the EZ1 Tissue Kit is preferred over the Blood Kit, and the DNA should be eluted in a volume of 200 µL. Higher throughputs may be obtained using larger BioRobots such as the M48 or M96, which use the same MagAttract™ silica-magnetic-particle technology as the EZ1.

References

1. EZ1 DNA Handbook. Second Edition. Qiagen. February, 2004.
2. DNA Quantification Using the Fluorescence/DNase (F/D) Assay. DNA Genotek.