Compatibility of the AUTOPURE® with Oragene™ DNA Collection

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DNA samples collected with Oragene and purified with the Autopure LS instrument have an average DNA yield of 82.3 µg and A_{260}/A_{280} ratio of 1.87. The DNA is high molecular weight and suitable for downstream applications like PCR.

Introduction

To maximize the participation rate in population genetic studies, alternatives to invasive whole blood collection are increasing. One such alternative is the Oragene DNA Self-Collection Kit (DNA Genotek, Canada). After saliva is collected, samples can be stored for years at room temperature prior to DNA purification. Along with the benefit of a non-invasive collection method, samples can be shipped at room temperature which saves expensive delivery costs associated with blood collection. The purpose of this study was to assess the yield and quality of DNA purified on Gentra Systems' AUTOPURE LS Instrument from the entire 4-ml sample contained in the Oragene vial.

Materials and Methods

Sample Collection and Handling

Saliva (2 ml) was collected from 16 individual donors following the instructions for collection provided with the Oragene Kit. As the Oragene vial was capped, saliva automatically mixed with 2 ml of Oragene DNA-preserving solution. Samples were then incubated at 50°C overnight in an air incubator and stored at room temperature. Prior to DNA purification, the 4-ml lysate samples were transferred to AUTOPURE input QUBES and 1 ml of PUREGENE Cell Lysis Solution containing RNase A was added. Samples were mixed well by vortexing vigorously.

Protocol Overview

The lysate samples were placed on the AUTOPURE LS and the DNA was purified in a fully automated format using PUREGENE chemistries (Gentra Systems, Inc., Mpls., MN) and the protocol for 1-5 ml cell lysate samples. After samples were loaded on the instrument, Protein Precipitation Solution was added and the samples were vigorously mixed to precipitate the proteins. The precipitated proteins and other contaminants were then collected by centrifugation and the cleared lysates were transferred to the output QUBES containing 100% Isopropanol. The samples were gently mixed and centrifuged to pellet the precipitated DNA. Residual salt was removed by washing the samples with 70% Ethanol followed by centrifugation to collect the DNA pellet. The samples were then hydrated by adding 300 µl of DNA Hydration Solution and incubating at 65°C for 1 hour followed by an overnight incubation at room temperature.

UV Absorbance Determination

DNA yields were determined by UV spectrophotometry using a Beckman DU-64 spectrophotometer (Beckman-Coulter, Fullerton, CA). A 10 µl volume of each DNA sample was diluted with 190 µl ultrapure deionized water and vortexed at high speed for 5 seconds. Absorbencies were measured at 260 nm, 280 nm, and 320 nm (background). To calculate DNA concentration, the 320 nm background reading was subtracted from the 260 nm reading, and the resulting value was multiplied by the DNA extinction coefficient of 50 µg/ml and the dilution factor (200 µl/10 µl). The DNA yield was calculated by multiplying each concentration by the respective DNA volume. The A_{260}/A_{280} ratio was determined by subtracting the background reading from each of the two readings before they were divided.

Genomic DNA Analysis

To assess DNA integrity, 100 ng from each sample was separated by 0.7% agarose gel electrophoresis. The DNA was electrophoresed for 1 hour at 75 volts using 0.125 µg/ml ethidium bromide in the gel. The gel was photographed on a UV transilluminator using a Kodak Digital Imaging System EDAS 120 LE (Rochester, NY). Lambda DNA digested with *Hind*III was used as a size reference.

DNA Amplification

Purified DNA was evaluated for amplification performance using primers specific to a 1.5 kb target in one of the cytochrome P450 genes (CYP2D6 locus). For each reaction, 100 ng DNA was amplified in a 50 µl volume containing: 1×Epicentre Failsafe™ Buffer E (Madison, WI), 1.0 µM of Primer MP1 and MP2 (Genosys, The Woodlands, TX), 2.5 U of Promega Taq polymerase (Madison, WI). The amplification conditions for the CYP2D6 target were: 30 cycles: 94°C 30 seconds, 63°C 30 seconds, and 72°C 2 minutes; 72°C hold 6 minutes, 4°C hold. A volume of 10 µl from each reaction was analyzed by 1% agarose gel electrophoresis. The DNA was electrophoresed for 60 minutes at 75 V using 0.125 µg/ml ethidium bromide in the gel and running buffer. A 1 kb ladder was used as a size reference. The gel was photographed on a UV transilluminator using a Kodak Digital Imaging System EDAS 120 LE (Rochester, NY).

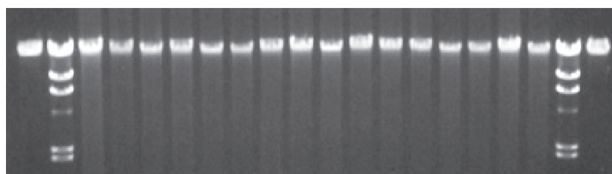
Results

DNA yields and A_{260}/A_{280} readings from the samples purified on the AUTOPURE LS Instrument are shown in Table 1. The average DNA yield from the Oragene-collected saliva samples was 82.3 µg of DNA. The average A_{260}/A_{280} was 1.87, which suggests pure DNA free of

contaminants. Electrophoretic analysis of the purified DNA samples (Figure 1) shows high quality DNA was purified for all samples, as indicated by the high molecular weight band of at least 50 kb and the lack of low molecular weight DNA. The purified DNA amplified well using the primer set for the CYP2D6 locus (Figure 2).

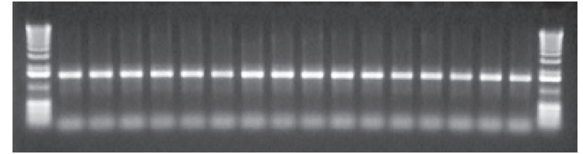
Sample	DNA Yield (µg)	A_{260}/A_{280}
1	112	1.83
2	45.8	1.85
3	267	1.81
4	36.7	1.91
5	153	1.84
6	101	1.87
7	62.4	1.79
8	45.2	1.89
9	18.4	1.99
10	60.6	1.84
11	32.3	1.90
12	21.0	1.99
13	102	1.81
14	29.1	1.93
15	119	1.84
16	111	1.84
Average	**82.3**	**1.87**

Table 1. DNA Yield and quality of saliva collected with Oragene and purified on the AUTOPURE LS Instrument with PUREGENE chemistries. DNA was purified from 16 individual samples following the 1-5 ml Cell Lysate Protocol on the instrument. The DNA was analyzed by UV spectrophotometry to determine DNA yield and A_{260}/A_{280} ratios.



Lane	Description
1, 20	Uncut Lambda
2, 19	Lambda x *Hind*III Ladder
3-18	Samples 1-16

Figure 1. Integrity of DNA purified from Oragene collected saliva samples on the AUTOPURE LS Instrument. DNA was purified from 16 individual samples following the 1-5 ml Cell Lysate Protocol on the instrument and analyzed by agarose gel electrophoresis to determine the quality of the DNA.



Lane	Description
1, 18	1 kb ladder
2-17	Samples 1-16

Figure 2. Amplification of DNA purified from Oragene-collected saliva samples on the AUTOPURE LS Instrument. DNA was purified from 16 individual samples following the 1-5 ml Cell Lysate Protocol on the instrument and subsequently amplified using primers for the CYP2D6 locus. Amplified DNA was analyzed by agarose gel electrophoresis.

Discussion and Conclusions

The AUTOPURE LS Instrument purifies DNA in a fully automated format from the entire saliva sample collected in Oragene, producing high quality DNA that is free from detectable protein contamination. Agarose gel electrophoresis analysis of the purified samples showed that the integrity and quality of the DNA was excellent. In addition, a 1.5 Kb target was amplified successfully from the purified DNA samples, indicating the DNA is free of contaminants or inhibitors and suitable for PCR analysis. The AUTOPURE Instrument purifies high yields of high quality DNA in a fully automated high-throughput format, processing up to 128 samples of 4-ml Oragene samples during an 8 hour shift.

Recommendations

To purify from the entire 4-ml saliva sample collected with Oragene, add 1 ml of Cell Lysis Solution to the input QUBES along with the 4-ml lysate sample and follow the AUTOPURE LS 1.0-5.0 Cell Lysate Protocol.

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