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# Compatibility of the PUREGENE® DNA Purification Kit with Oragene™ DNA Collection

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*DNA samples collected with Oragene and purified with Puregene have an average DNA yield of 85.5 µg and $A_{260}/A_{280}$ ratio of 1.79. The DNA is high molecular weight and is 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 manually with Gentra Systems' PUREGENE chemistries from the entire 4-ml sample contained in the Oragene vial.

## Materials and Methods

### *Sample Set-up*

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 50 ml tubes and 1 ml of PUREGENE Cell Lysis Solution containing 25 µl RNase A was added. Samples were mixed well by vortexing vigorously. The DNA was purified following protocol 400244 for 4-ml saliva samples collected in Oragene vials. The samples were 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 manually with PUREGENE are shown in Table 1. The average DNA yield from the Oragene collected saliva samples was 85.5 µg of DNA. The average $A_{260}/A_{280}$ was 1.79, 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).

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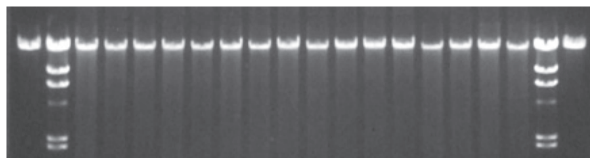
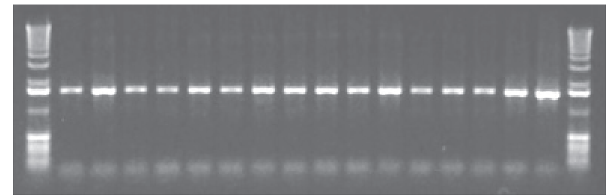
| Sample | DNA Yield (µg) | $A_{260}/A_{280}$ |
|---|---|---|
| 1 | 107 | 1.77 |
| 2 | 61.1 | 1.78 |
| 3 | 200 | 1.79 |
| 4 | 63.8 | 1.78 |
| 5 | 147 | 1.78 |
| 6 | 112 | 1.83 |
| 7 | 76.6 | 1.73 |
| 8 | 60.1 | 1.79 |
| 9 | 31.5 | 1.80 |
| 10 | 49.7 | 1.80 |
| 11 | 39.1 | 1.76 |
| 12 | 37.5 | 1.81 |
| 13 | 130 | 1.78 |
| 14 | 39.2 | 1.84 |
| 15 | 111 | 1.79 |
| 16 | 103 | 1.82 |
| **Average** | **85.5** | **1.79** |

***Table 1.*** DNA yield and quality of saliva collected with Oragene and purified manually using PUREGENE. DNA was purified from 16 individual samples following the PUREGENE 4 ml Saliva Oragene Lysate protocol. 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 manually purified using PUREGENE from Oragene collected saliva samples. DNA was purified from 16 individual samples following the PUREGENE 4 ml Saliva Oragene Protocol 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.*** PCR amplification of DNA manually purified using PUREGENE from Oragene-collected saliva samples. DNA was purified from 16 individual samples following the PUREGENE 4 ml Saliva Oragene Protocol and subsequently amplified using primers for the CYP2D6 locus. Amplified DNA product was analyzed using agarose gel electrophoresis.

## Discussion and Conclusions

High yields of high quality DNA were purified from Oragene-collected saliva samples using the PUREGENE DNA Purification Kit. 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.

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