ORAgene®·ONE

Quality, yield and bacterial content of DNA from human saliva collected and purified using Oragene®·ONE

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Oragene•ONE is a non-invasive DNA collection kit from the Oragene family of products. In this document, we demonstrate that the average DNA yield recovered from 0.5 mL of saliva is 11.6 ug. The DNA has a corrected $A_{260}/_{A280}$ ratio of 1.6 - 1.8, and a molecular weight > 23 kb. The median bacterial DNA content in the Oragene•ONE/saliva sample is 7.4%.

Introduction

Non-invasive and easy-to-use sample collection methods are becoming increasingly important criteria to improve patient care within clinical trial screening and molecular genetic testing. As a result, saliva samples are frequently used as a convenient source of DNA. The Oragene•ONE kit is designed for self-collection of human genomic DNA from saliva. The kit is completely non-invasive, convenient, and easy-to-use. Once the saliva sample is delivered, the kit is capped and the Oragene•ONE stabilizing solution is released. This solution minimizes the bacterial content of the sample and preserves the DNA at ambient temperature. Studies have shown that DNA from oral samples gives equivalent results to DNA from blood for applications like PCR and SNP genotyping[1, 2]. Oragene•ONE is an ideal collection method for applications where single testing is required.

Materials and methods

Saliva collection/purification

Saliva samples were collected from 40 healthy adult donors. Within 72 hours of collection the samples were heated at 50°C for 1 hour in a water bath. Following the heating step a 250 µL aliquot was purified according to the Oragene•ONE manual purification protocol[3].

DNA analysis

DNA was quantified by fluorescence following the *DNA quantification using SYBR® Green I dye and a micro-plate reader*[4] protocol. The A_{260}/A_{280} ratios were determined using a microplate reader and UV transparent plates. The A_{260} and A_{280} values were corrected for minor amounts of turbid material by subtracting the A_{320} value. The molecular weight of the DNA was determined by electrophoresis on a 0.8% agarose gel using a Lambda/Hind III DNA marker.

Bacterial DNA analysis

Bacterial DNA was quantified using a real-time PCR assay. PCR primers were chosen from a region of the 16S rRNA gene which is known to be conserved across a wide variety of microorganisms and is not found in humans[5]. The 40 Oragene•ONE samples were tested for the 16S rRNA gene by quantitative PCR using a Rotor-Gene™ 6200 real-time thermal cycler (Corbett Research). Each reaction used 15 ng of total DNA as the template. To check the efficiency of each reaction, a second 15 ng aliquot from each sample was spiked with 5 ng of bacterial control DNA and run alongside the first sample. If the reaction were perfectly efficient, the amount of bacterial DNA should be 5 ng plus the amount of the unknown bacterial DNA. A standard curve was used to quantify the samples. Purified bacterial control DNA used to construct the standard curve was obtained from Sigma (E.coli, strain B, Cat. #D4889).

Results

DNA yield and spectral quality

The average amount of DNA recovered from a 250 µL aliquot of the sample was 1.45 µg, ranging from 0.25 to 4.46 µg.

The average corrected A_{260}/A_{280} ratio was 1.7, with a range of 1.6 to 1.8. A representative absorbance scan (Figure 1) demonstrates absorbance of a raw Oragene•ONE/saliva sample and a purified DNA sample from which all Oragene•ONE components and most protein has been removed.

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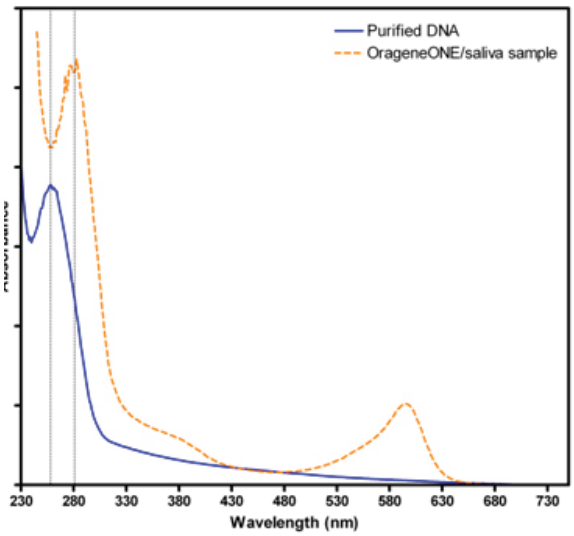

***Figure 1**: Absorbance spectrum scan of a non-purified Oragene•ONE/saliva sample (dashed orange) and the corresponding purified DNA (solid blue), with an A_{260}/A_{280} =1.7.*

The quality of the samples was further assessed by running 12 randomly selected purified samples on an agarose gel (Figure 2). The purified DNA had a molecular weight >23 kb.

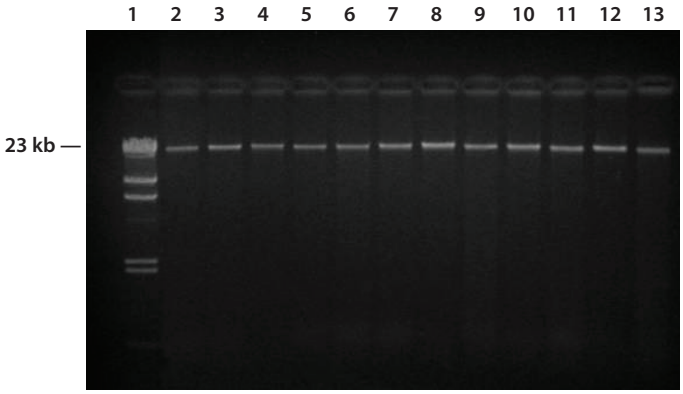

***Figure 2**: Agarose gel electrophoresis (0.8% agarose, 90 V, 60 minutes) of DNA from 12 donors purified within 72 hours of sample collection. A Lambda-Hind III digest was used as a marker in Lane 1.*

Bacterial content

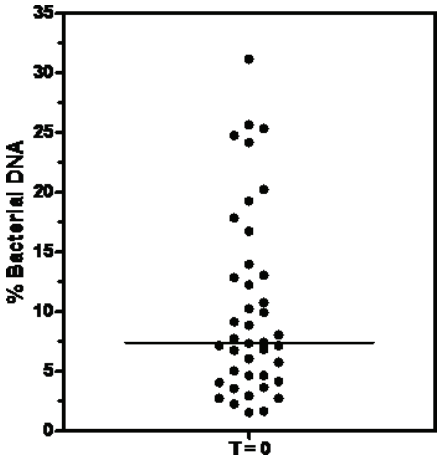

***Figure 3**: Bacterial DNA as a percentage of total DNA in 40 Oragene•ONE samples. The horizontal line represents the median value (7.4%).*

Conclusion

Oragene•ONE is a self-collection kit that stabilizes DNA in saliva at ambient temperature. The resulting purified DNA is of high quality as assessed by the A_{260}/A_{280} ratio (1.7) and high-molecular-weight (>23 kb) as assessed by agarose gel electrophoresis. For the 40 collected samples, we observed an average DNA yield of 11.6 µg per 0.5 mL of saliva, and 1.5 µg per 0.25 mL of Oragene•ONE/saliva. The purification protocol efficiently removes Oragene•ONE components as seen by the disappearance of the peak around 600 nm in Figure 1 and also efficiently removes salivary proteins as demonstrated by the disappearance of the 280 nm peak.

Previous studies have reported that the amount of human genomic DNA in oral samples may only be a small percentage of the total DNA yield because of the significant amount of contaminating bacterial DNA. Feigelson et al. (2001) found that the median percentage of human DNA in mouthwash samples was 34% of the total DNA yield[6]. Similarly, Garcia-Closas et al. (2001) found that the median percentage of human DNA in mouthwash samples was 49.5% and 11.5% in cytobrush samples[7].

In contrast, the majority of DNA from Oragene•ONE/saliva samples is human genomic DNA, with a median bacterial DNA content of only 7.4%. Oragene•ONE contains potent antibacterial agents which prevent the growth of bacteria between the time of collection and the time of DNA purification.

In summary, the Oragene•ONE kit is an easy and non-invasive method for collecting high quality human genomic DNA.

References

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6 Feigelson, H., Rodriguez, C., Robertson, A., Jacobs, E., Calle, E., Reid, Y. et al. (2001) Determinants of DNA yield and quality from buccal cell samples collected with mouthwash. *Cancer Epidemiology, Biomarkers & Prevention*. 10, 1005-1008.

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Oragene®·ONE is not available for sale in the United States.

All DNA Genotek protocols, white papers and application notes, are available in the support section of our website at www.dnagenotek.com.

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