ORAgene•ANIMAL
For DNA Collection

Quality, Yield, Performance and Bacterial Content of DNA from Canine Saliva Collected and Purified using Oragene®•ANIMAL

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The Oragene•ANIMAL for DNA sample collection product is designed for non-invasive sample collection from animals. In this document, we demonstrate that the average DNA yield recovered when used with canines is 11.6 µg. The DNA has an average corrected A_{260}/A_{280} ratio of 1.6, and a molecular weight > 23kb. The average bacterial DNA content in the Oragene•ANIMAL/saliva sample is 16.1%.

Introduction

Non-invasive and easy-to-use sample collection methods facilitate and accelerate molecular genetic experiments.

The Oragene•ANIMAL kit is designed for non-invasive collection of genomic DNA from mid-sized animals. The kit is non-invasive, convenient, and easy-to-use. Once the saliva sample is collected into the Oragene•ANIMAL solution, large amounts of genomic DNA is released and the DNA is stabilized at room temperature. The solution is bacteriostatic and minimizes the bacterial content in the sample after collection. Studies have shown that DNA from oral samples provides equivalent results to DNA from blood for applications like PCR and SNP genotyping (ref. 1, 2, 3).

Buccal swabs generate insufficient yield of genomic DNA (ref. 1) and often result in failed samples. Blood samples are difficult to obtain due to ethical restrictions, logistical challenges and compliance. Oragene•ANIMAL is the ideal collection method for the collection of DNA from saliva as it is painless and non-invasive for the animal and provides a high quantity and quality of genomic DNA for downstream applications.

Materials and Methods

Canine Saliva collection/purification

Saliva samples were collected from 33 canines by their owners in a non-supervised setting. After collection, samples were transported to the lab and stored at room temperature until they were processed. Due to the ease of collection, samples were typically returned to the lab within 72 hours. Once at the lab, 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•ANIMAL manual purification protocol (PD-PR-095).

DNA Analysis

DNA was quantified by fluorescence following the DNA Quantification Using Sybr Green I dye and a Micro-Plate Reader protocol (PD-PR-075). The absorbance spectrum between 320nm and 230nm was determined using a Tecan Infinite M200. 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/HindIII DNA marker.

Bacterial DNA Analysis

Bacterial DNA was quantified using a real-time PCR assay (PD-PR-065). PCR primers were chosen from a region of the 16S rRNA gene which is known to be conserved across a wide variety of microorganisms (ref. 4). The 33 Oragene•ANIMAL 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 & Spectral Quality

Saliva collected from 33 canines was used for these experiments. Following the purification protocol supplied with the Oragene•ANIMAL kit (protocol PD-PR-095) the DNA was measured using fluorescence with Sybr Green I dye according to protocol PD-PR-075. The average amount of collected canine DNA was 11.6 µg, ranging from 2.1 to 81.4 µg. The purity of the recovered DNA was assessed by the corrected A_{260}/A_{280} ratio. Absorbance at wavelengths 260 nm, 280 nm, and 320 nm was measured. The absorbance at wavelength of 320 nm which corresponds to presence of turbid (insoluble) material was subtracted from the A_{260} and A_{280}

values before calculating the A_{260}/A_{280} ratio. The average A_{260}/A_{280} ratio for the 33 canine samples was 1.6. A representative absorbance scan (Figure 1) demonstrates absorbance of a purified Oragene•ANIMAL/saliva sample. The quality of the samples was further assessed by running 10 randomly selected purified samples on an agarose gel (Figure 2). Using a Lamda-HindIII ladder we were able to demonstrate that the purified DNA had a molecular weight >23kb.

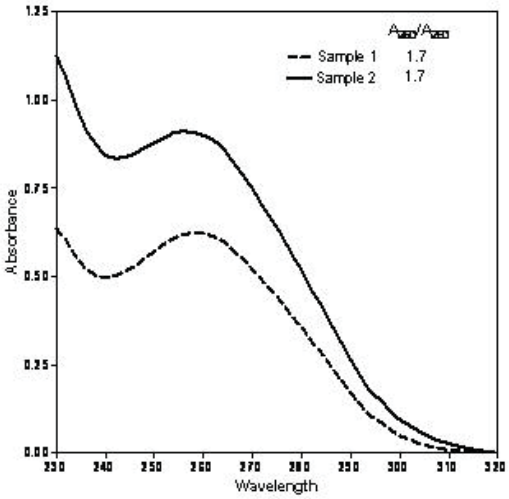

Figure 1: Absorbance spectrum scan of a purified Oragene•ANIMAL /saliva sample.

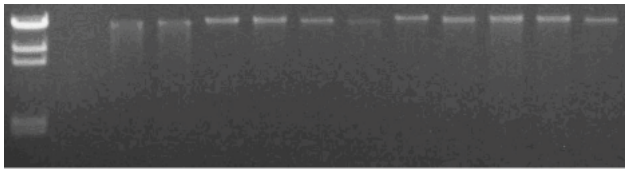

Figure 2: Agarose gel electrophoresis (0.8% agarose, 90 V, 60 min) of DNA from 10 canine samples purified within 72 hours of sample collection. A Lambda-HindIII digest was used as a marker in Lane 1.

Bacterial DNA Content

Bacterial DNA content was assessed using a real-time PCR assay with universal bacterial primers. The universal primers flank a highly conserved region of the 16S rRNA gene allow for an accurate assessment of total bacterial DNA content in canine samples. Using this method bacterial DNA has been identified as the minor component of total DNA in canine saliva samples with an average of 16.1%, ranging between 1.8% and 44.1%.

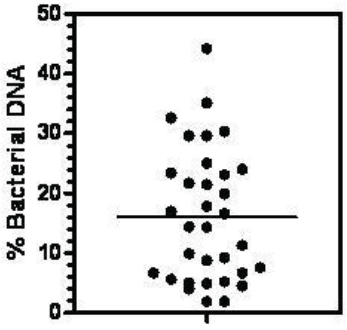

Figure 3: Bacterial DNA as a percentage of total DNA in 33 Oragene•ANIMAL samples. The horizontal line represents the median value (16.1%).

Conclusion

Oragene•ANIMAL is a non-invasive collection kit that stabilizes DNA from animal saliva at room temperature. The resulting purified DNA is of high quality as assessed by the A_{260}/A_{280} ratio (1.6) and high molecular weight (>23 kb) as assessed by agarose gel electrophoresis. For the 33 collected samples, we observed an average DNA yield of 11.6 µg per collected sample, and 1.5 µg per 0.25 mL of Oragene•ANIMAL/saliva. The majority of DNA from Oragene•ANIMAL/saliva samples is canine genomic DNA, with an average bacterial DNA content of only 16.1%. Oragene•ANIMAL 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•ANIMAL kit is an easy and non-invasive method for collecting high quality genomic DNA from animals.

References

1. Chang, M.L. et. al. (2007) Large-Scale SNP Genotyping with Canine Buccal Swab DNA. *Journal of Heredity*. 98(5), 428-437.
2. Terasaki, P., Chia, D., and Sugich, L. (1998) Saliva as DNA source for HLA typing. *Human Immunology*. 59, 597-598.
3. Todesco, L., Torok, M., Krahenbuhl, S., and Wenk, M. (2003) Determination of 3858G –› A and 164C –› A genetic polymorphisms of CYP1A2 in blood and saliva by rapid allelic discrimination: large diff erence in the prevalence of the 3858G-›A mutation between Caucasians and Asians. *European Journal of Clinical Pharmacology*. 59, 343-346.
4. Muyzer, G., de Waal, E. and Uitterlinden, A. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reactionamplified genes coding for 16S rRNA. *Applied Environmental Microbiology*. 59, 695-700.

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