PERFORMAgene™

Quality, yield, performance and bacterial content of gDNA from cattle nasal samples collected and purified using Performagene™

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Performagene™ sample collection product is designed for non-invasive collection of genomic DNA from animals. In this document, we demonstrate that the average DNA yield recovered from cattle nasal samples is 24.2 ug. The DNA has an average corrected A_{260}/A_{280} ratio of 1.7, and a molecular weight > 23 kb. The average bacterial DNA content from the Performagene samples is 3.3%.

Introduction

Quick, reliable and easy-to-use sample collection methods facilitate and accelerate molecular genetic experiments.

The Performagene kit is designed for reliable collection of genomic DNA from animals. The product is an all-in-one system for collection, stabilization, transportation and extraction of DNA from samples. Once the sample is collected into the Performagene solution a large amount of DNA is released and stabilized at room temperature. The solution is bacteriostatic and minimizes the bacterial content in the sample after collection. Performagene is ideal for the collection of large amounts of high quality DNA from animals where blood is difficult to obtain and other sampling methods are inefficient to process and can provide a low quality and quantity of DNA.

Materials and methods

Cattle nasal sample collection/purification

Nasal samples were collected from 225 unrestrained cattle ranging in age from 4 days to 14 years. During the field collection, no regard was taken to the last time the cattle ate or drank. After collection using Performagene samples were transported at room temperature to the lab. Once at the lab the samples were heated at 50°C for 1 hour in a water bath. Following the heating step a 500 µL aliquot was purified according to the Performagene purification protocol[1].

DNA analysis

DNA was quantified by fluorescence following the protocol[2]. The absorbance spectrum between 320 nm and 230 nm 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. Purified DNA was shown to be of sufficient quality for real-time PCR by amplifying a portion of the bovine thymidylate synthetase gene.

PCR assay

Performagene samples can also be quickly prepared for use in downstream applications without the need for full purification. Raw (unpurified) samples were prepared for real-time PCR according to protocol[3]. Up to 2.5 µL of the prepared sample was used in each PCR reaction.

Bacterial DNA analysis

Bacterial DNA was quantified using a real-time PCR assay[4]. PCR primers were chosen from a region of the 16S rRNA gene which is known to be conserved across a wide variety of microorganisms. Randomly selected, 68 Performagene 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. Assuming the reactions 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).

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Results

DNA yield and spectral quality

A subset of nasal samples collected from 225 unrestrained cattle were used for the experiments. Following the purification protocol[1] supplied with the Performagene kit the DNA was measured using fluorescence with SYBR Green I dye according to protocol[2]. The average amount of collected bovine DNA was 24.2 µg. A similar amount of DNA was collected from different breeds (Table 1). A yield scatter plot (Figure 1) demonstrates the yield distribution based on animal age. 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 68 randomly selected bovine samples was 1.7. 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 >23 kb.

Breed of cattle	Average amount of total DNA (µg)
Holstein	24.0
Hereford	31.2
Brown Swiss	15.9
Simmental	21.9

***Table 1**: Average amount of DNA collected from 4 different bovine breeds.*

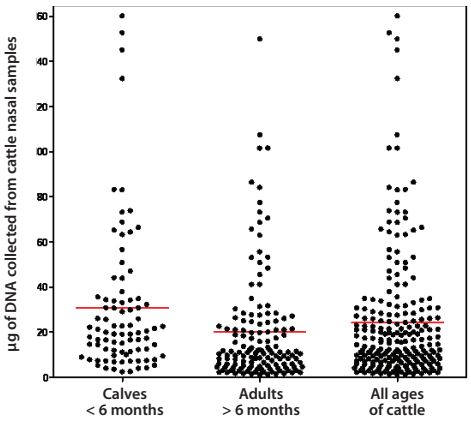

***Figure 1**: Scatter plot of DNA yield from 225 cattle (85 calves and 140 adults). The average amount of DNA is shown by the red line, calves= 31 µg , adults= 20.1 µg , total= 24.2 µg.*

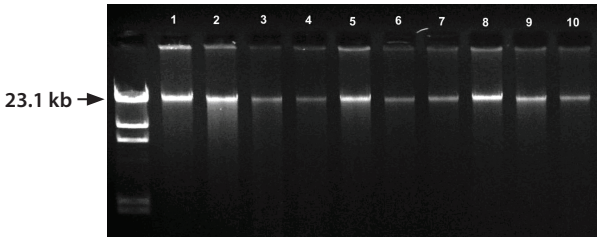

***Figure 2**: Agarose gel electrophoresis (0.8% agarose, 90 V, 60 minutes) of DNA from 10 bovine samples purified within 72 hours of sample collection. A Lambda-HindIII digest was used as a marker in Lane 1.*

The purified DNA was shown to be of sufficient quality for use in real-time PCR assays by amplifying a section of the bovine thymidylate synthetase gene. Both calf and cow samples successfully amplified 100% of the time (Figure 3).

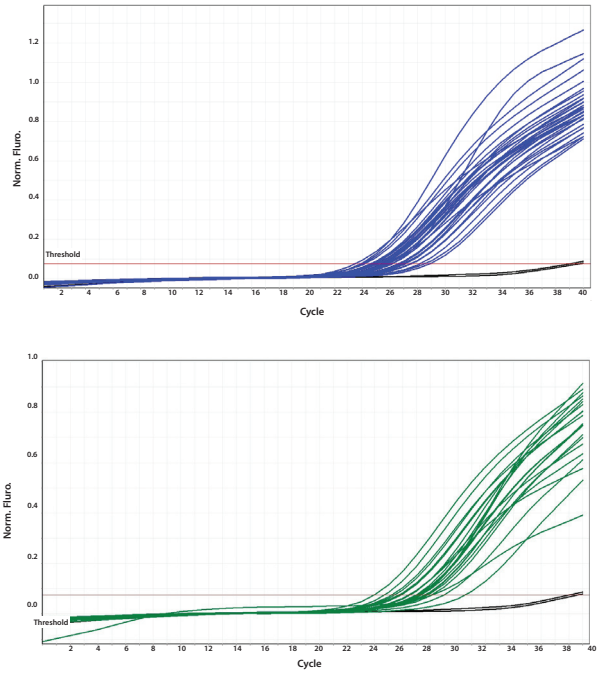

***Figure 3**: Real-time PCR analysis of cattle samples. Curves represent successful amplification of the bovine thymidylate synthetase gene. Blue lines represent DNA collected from calves < 6 month old. Green lines represent DNA collected from cattle > 6 month old. Black lines are no template controls.*

PCR assay

In order to streamline sample processing, Performagene samples can be quickly prepared for downstream applications such as real-time PCR according to protocol[3]. An aliquot of the same samples that were purified in the above experiments were also processed according to protocol[3].

The prepared samples were used in a real-time PCR assay which amplified a portion of the bovine thymidylate synthetase gene. All of the tested samples amplified successfully (Figure 4).

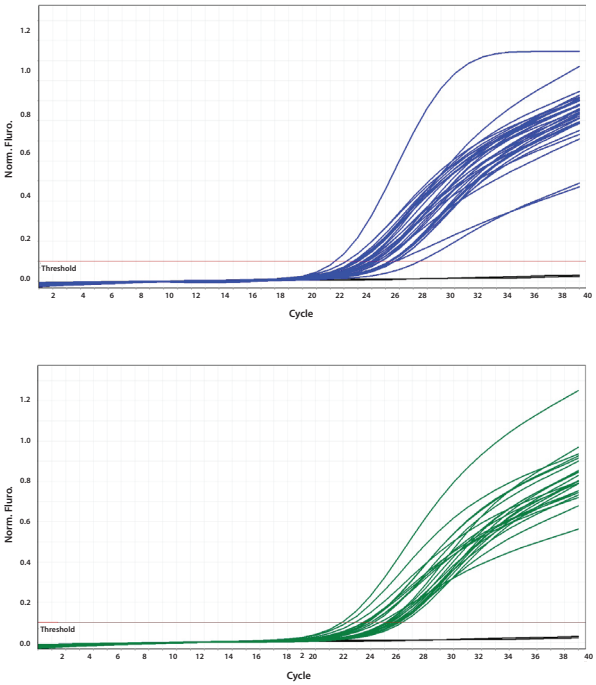

***Figure 4**: Real-time PCR analysis of bovine samples after protocol[3]. Curves represent successful amplification of the bovine thymidylate synthetase gene. Blue lines represent DNA collected from calves < 6 month old. Green lines represent DNA collected from cattle > 6 month old. Black lines are no template controls.*

Bacterial DNA content

Bacterial DNA content was assessed using a real-time PCR assay with universal bacterial primers. The universal primers against a highly conserved region of the 16S rRNA gene allow for an accurate assessment of total bacterial DNA content in bovine samples. Using this method bacterial DNA has been identified as the minor component of total DNA in bovine nasal samples with an average of 3.3%, ranging between 0.4% and 14.2% (Figure 5).

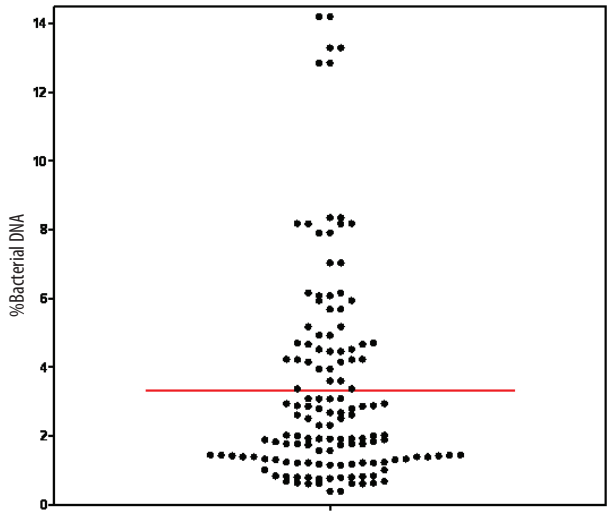

***Figure 5**: Bacterial DNA as a percentage of total DNA in 68 bovine Performagene samples. The horizontal red line represents the median value (3.3%).*

Conclusion

Performagene is a non-invasive collection kit that stabilizes DNA from bovine nasal samples at room 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 225 collected samples, we observed an average DNA yield of 24.2 µg. Bovine nasal samples contain very little bacterial DNA, with an average bacterial DNA content of only 3.3%. Performagene contains potent antibacterial agents which prevent the growth of bacteria between the time of collection and the time of DNA purification. In summary, the Performagene kit is an easy and reliable method for collecting high quality bovine genomic DNA.

References

1 Laboratory protocol for manual purification of DNA from 0.5 mL of Performagene sample. DNA Genotek. PD-PR-083.
2 DNA quantification of Oragene/saliva samples using SYBR Green I Dye and a micro-plate reader. DNA Genotek. PD-PR-075.
3 Laboratory protocol for processing a 50 µL aliquot of Performagene sample using PG-AC2/3 reagent package. DNA Genotek. PD-PR-00220.
4 Bacterial DNA assay. DNA Genotek. PD-PR-065.