High yield, high quality genomic DNA from cattle nasal samples using Performagene™

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Introduction

Genetics has been utilized for decades to improve livestock breeding, as a result of its increased industry adoption, genetic research and DNA testing is accelerating worldwide. Common forms of genetic testing being utilized by the industry include: parentage verification, trait characteristics (e.g., coat colour determination of red vs. black [MC1R]), and various genetic diseases. These applications require a reliable, high quality DNA sample; previous sample sources such as semen, blood, hair follicle or ear tissue have been inconvenient for the producer to collect or difficult, unreliable and costly to process in the lab. Performagene™ (DNA Genotek, Ottawa) is a DNA collection kit which uses nasal samples resulting in an easy-to-use, quick and reliable sampling method that provides high quantity, high quality genomic DNA.

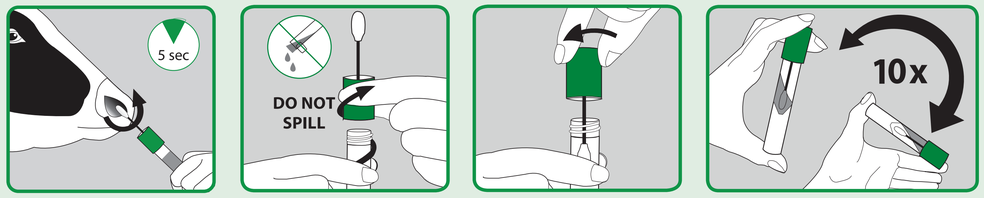
An unreliable sample or difficult sampling method impacts performance in downstream applications such as parentage verification and genotyping for trait characteristics. Commonly used assays for parentage and genotyping include microsattelite, SNP genotyping and microarray. For these reasons, we show the suitability and reliability of nasal samples collected from cattle into Performagene on the ABI PRISM® 3100 Genetic Analyzer (Taqman), Sequenom® MassARRAY and Illumina BovineSNP50™ BeadChip.

Nasal samples were collected into the Performagene kit from 38 beef and dairy cattle with minimum restraint. To compare sample types from the same animal, additional blood and tail hair samples were collected from 5 cattle. All samples were purified using the Performagene protocol. Using fluorescent quantification, we show that the Performagene DNA collection method yields a high quantity and high quality of genomic DNA. This study reports that purified DNA is suitable for any downstream application.

Materials and methods

Nasal samples

Figure 1: Nasal sample collection using Performagene



- 38 nasal samples were collected from cattle according to the DNA Genotek protocol PD-PR-099 and stored at room temperature until processed.
- Samples were collected by 7 different people and were collected from the following breeds of cattle: Holstein, Jersey, Limousin and Charolais.
- 0.5 mL aliquot of the nasal samples was purified according to the DNA Genotek purification protocol PD-PR-083 and the purified DNA was quantified by fluorescence, evaluated by agarose gel electrophoresis, and analyzed on the Illumina BovineSNP50 BeadChip.
- 100 µL aliquot of the nasal sample was processed according the DNA Genotek Quick to PCR protocol PD-PR-00220 and analyzed by Sequenom and Taqman assays.

Blood samples

- 5 blood samples were collected from Holstein cattle for which nasal samples were also collected (see Table 2).
- Blood was collected from the tail vein using EDTA tubes and kept at 4°C until processed.
- 200 µL of blood sample was purified according to the Qiagen® QIAamp Blood Mini Kit.

Tail hair samples

- 5 hair samples were collected from Holstein cattle for which blood and nasal samples were also collected (see Table 2).
- The hairs were pulled from the tail, counted and inspected for clean, intact root bulbs then aligned in a plastic bag until processed.
- For each animal, DNA from 50 hair follicles was purified using an SDS and proteinase K protocol.
- Briefly, hair follicles were placed in 0.1% SDS with 0.2 mg proteinase K and incubated at 50°C for 1 hour. After incubation, 20 µL of 2.5 M of KCl was added and incubated on ice for 10 minutes and then centrifuged at maximum speed for 5 minutes. The supernatant was then ethanol precipitated and gDNA was recovered.

Results

Table 1: Comparison of sample workflows

Workflow impact	DNA source			
	Nasal (Full purification)	Nasal (Quick to PCR)	Blood	Hair (50 follicles)
Collection time	2 min	2 min	5 min	7 min
Ease of collection	Non-invasive	Non-invasive	Invasive	Non-invasive
Processing time per sample	1h 40 min	1h 30 min	28 min	1h 31 min
Hands on time per sample	20 min	5 min	17 min	31 min
Processing time per 24 samples	2h 05 min	1h 36 min	41 min	4h 38 min
Hands on time per 24 samples	45 min	11 min	30 min	3h 38 min

Table 2: Quantification results for A) nasal and B) paired samples

A)

Sample ID	DNA yield per 1 mL of sample (µg)	Sample ID	DNA yield per 1 mL of sample (µg)	Sample ID	DNA yield per 1 mL of sample (µg)
1	71.64	15	11.90	29	19.72
2	15.03	16	2.70	30	16.57
3	7.29	17	4.83	31	22.24
4	6.79	18	4.51	32	10.79
5	4.01	19	16.85	33	14.33
6	11.61	20	3.58	34	5.96
7	2.75	21	3.40	35	38.22
8	2.71	22	10.58	36	9.94
9	3.05	23	1.24	37	1.68
10	8.73	24	9.71	38	12.92
11	2.29	25	22.02	**Average**	**13.07**
12	21.18	26	3.27	**Median**	**10.03**
13	10.12	27	37.59	**Max**	**71.64**
14	24.75	28	19.97	**Min**	**1.24**

B)

Sample ID	DNA yield per aliquot purified (µg)		
	Nasal (1 mL of sample)	Blood (200 µL of sample)	Hair (50 follicles)
175	10.12	15.66	0.85
134	4.51	14.00	1.12
94	4.83	18.09	1.20
353	2.70	9.21	0.05
304	21.18	9.14	0.57
Average	**8.67**	**13.22**	**0.76**
Median	**4.83**	**14.00**	**0.85**
Max	**21.18**	**18.09**	**1.20**
Min	**2.70**	**9.14**	**0.05**

Figure 2: A) Sample comparison of nasal (N), blood (B) and hair (H) high molecular weight DNA. B) Representative gel of 16 nasal samples. 50 ng of total DNA was loaded into a 0.8% agarose gel and run at 80 volts for 45 minutes.

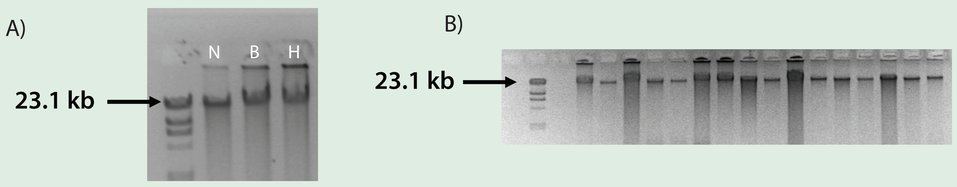
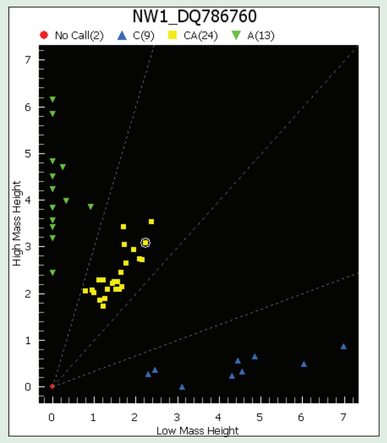


Table 3: Summary of results from downstream applications: results from Sequenom, Microsatellite and Illumina arrays

Assay	Processing method (Nasal)	Average call rate (Nasal)	Average concordance (nasal with blood)	Average concordance (nasal with hair)
Sequenom® MassARRAY analysis of nasal	Quick to PCR	98.74%	99.49%	95.96%
Microsatellite analysis ABI PRISM® genetic analyzer - Taqman assay	Quick to PCR	91.73%	100.00%*	100.00%*
Illumina BovineSNP50™ BeadChip	Full purification	99.63%	100.00%	100.00%

*** no calls not included in concordance calculation**

Figure 3: Representative scatter plot from Sequenom data



Discussion

- Performagene provides a quick and easy method for collecting large amounts of high quality DNA from cattle using non-invasive methods.
- Performagene offers two DNA preparation methods which offer decreased hands on time and result in DNA of high quality suitable for downstream applications.
- DNA collected from nasal samples is the same as DNA from blood as shown through the high concordance rates on all three platforms tested.

DNA Genotek would like to extend their appreciation to all parties who assisted in the collection of this data, including but not limited to the Cattlemen who collected the samples, as well as GenServe Labs and DNA Landmarks for providing DNA analysis services.

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