

# Microsatellite and SNP analysis for parentage verification using bovine nasal samples with Performagene™

E.J. Maclean, J.O. Niles, C.M. James and R.M. Iwasiow  
DNA Genotek Inc., Ottawa, Canada

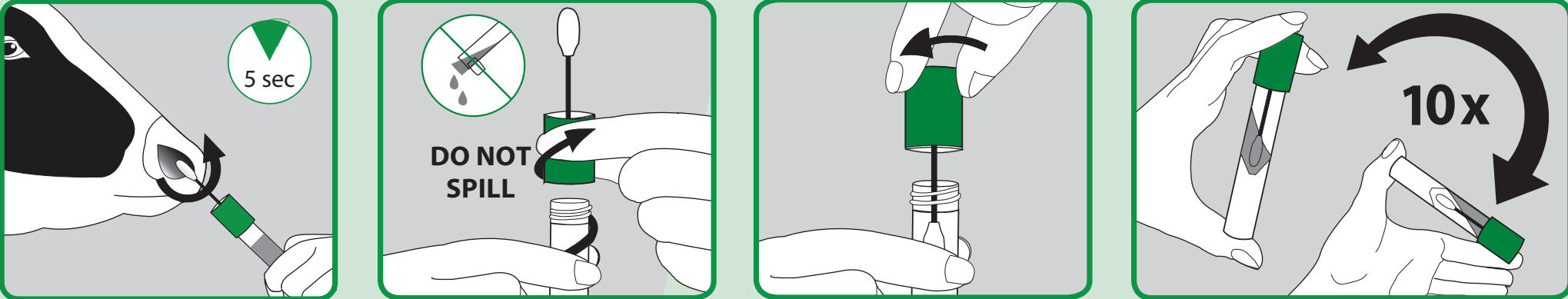
## Introduction

Parentage analysis of livestock animals is becoming increasingly utilized in the agriculture industry to improve the accuracy of herd books and to select breed crosses that increase production traits and herd efficiency. To date, the international standard of parentage analysis is done using microsatellites (ISAG Conference, 2008), however, parentage can also be determined using SNP genotyping (Heaton et al, 2002). Although both the microsatellite and SNP genotyping method have been shown to be effective for parentage analysis, the benefit of analyzing parentage using SNP genotyping is that additional SNP's for production traits can be analyzed in parallel. Obtaining both parentage and trait information through a single sample and test offers producers more information and decreases the cost of genetic based breeding decisions.

As the use of parentage analysis transitions from microsatellite testing to more cost effective and informative SNP genotyping, the total use of genomics is increasing. With growing adoption of genetic testing, the demand for efficient and reliable solutions for DNA sample collection and processing is increasing. Performagene is an all-in-one solution for the collection, stabilization and extraction of DNA from nasal samples. This method enables more efficient parentage analysis by streamlining sample collection, transport and processing. Using the Performagene DNA collection kit developed by DNA Genotek, Inc., we demonstrate the reliable collection and processing of high yield, high quality genomic DNA from cattle nasal samples. Nasal samples were collected from a total of 349 purebred Holstein, Jersey, Limousin and Charolais cattle during routine parentage analysis. Microsatellite analysis was conducted on the nasal samples using the ABI PRISM® 3100 Genetic Analyzer. The results demonstrate the suitability and reliability of Performagene nasal samples for use in parentage analysis in the existing ISAG standard. SNP genotyping was performed using the Illumina BovineSNP50™ BeadChip and Bovine3K™ assay shows the reliability of Performagene nasal samples for use in a combined parentage and production trait analysis.

## Material and methods

Figure 1: Nasal sample collection using Performagene



The following data is based on a total of 349 nasal samples collected according to the DNA Genotek protocol PD-PR-099 and stored at room temperature until processed.

### SNP analysis with Illumina Bovine3K

- 21 nasal samples were collected by 2 individuals from Holstein cattle.
- 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 analysed on the Illumina Bovine3K assay.

### SNP analysis with Illumina BovineSNP50 BeadChip:

- 38 nasal samples were collected by 7 individuals 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 analysed on the Illumina BovineSNP50 BeadChip.

### Microsatellite analysis with ABI PRISM Genetic Analyzer:

- 290 nasal samples were collected by Holstein UK members from Holstein cattle.
- 100 µL aliquot of the nasal sample was processed according the DNA Genotek purification protocol PD-PR-00220 and using Bovine Genotype™ Panel 1.2 and 2.2 (Finnzyme) with ABI PRISM 3100 Genetic Analyzer.

## Results

Table 1: Quantification results for 59 nasal samples collected for the Illumina Bovine3K analysis and the Illumina BovineSNP50 BeadChip analysis

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	28.67	22	71.64	43	10.58
2	97.61	23	15.03	44	1.24
3	87.59	24	7.29	45	9.71
4	101.72	25	6.79	46	22.02
5	17.97	26	4.01	47	3.27
6	39.24	27	11.61	48	37.59
7	58.99	28	2.75	49	19.97
8	31.56	29	2.71	50	19.72
9	59.40	30	3.05	51	16.57
10	30.82	31	8.73	52	22.24
11	144.82	32	2.29	53	10.79
12	69.53	33	21.18	54	14.33
13	47.52	34	10.12	55	5.96
14	45.39	35	24.75	56	38.22
15	17.49	36	11.90	57	9.94
16	83.44	37	2.70	58	1.68
17	37.63	38	4.83	59	12.92
18	155.40	39	4.51	Average	30.79
19	35.63	40	16.85	Median	17.49
20	46.81	41	3.58	Max	155.40
21	83.14	42	3.40	Min	1.24

Figure 2: 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

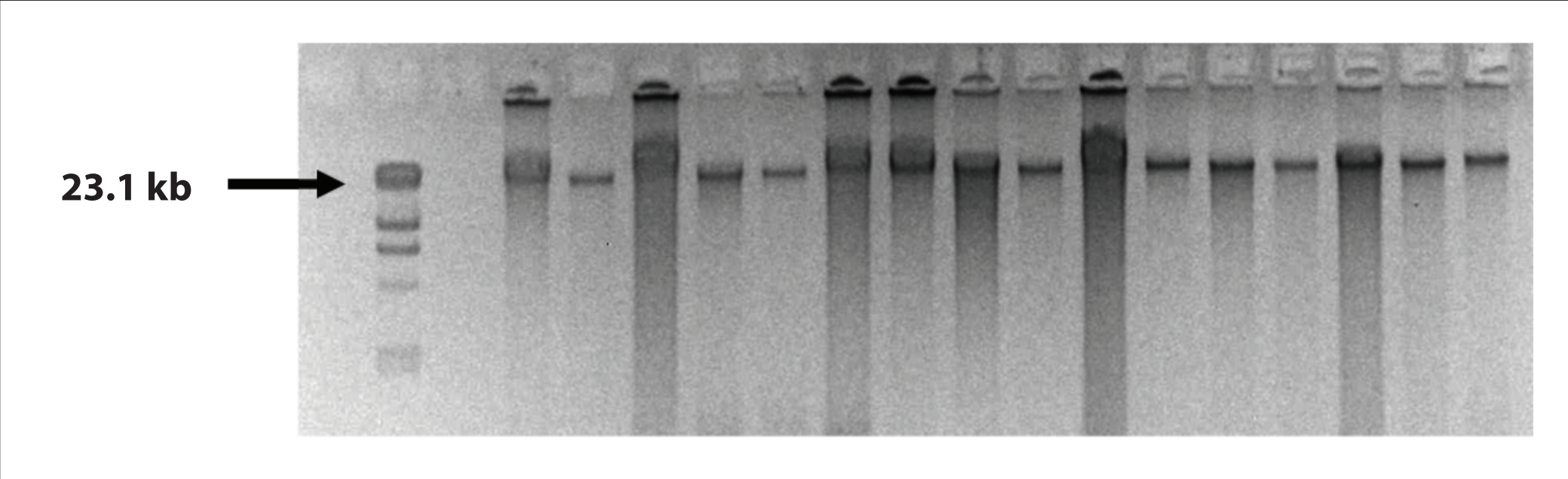
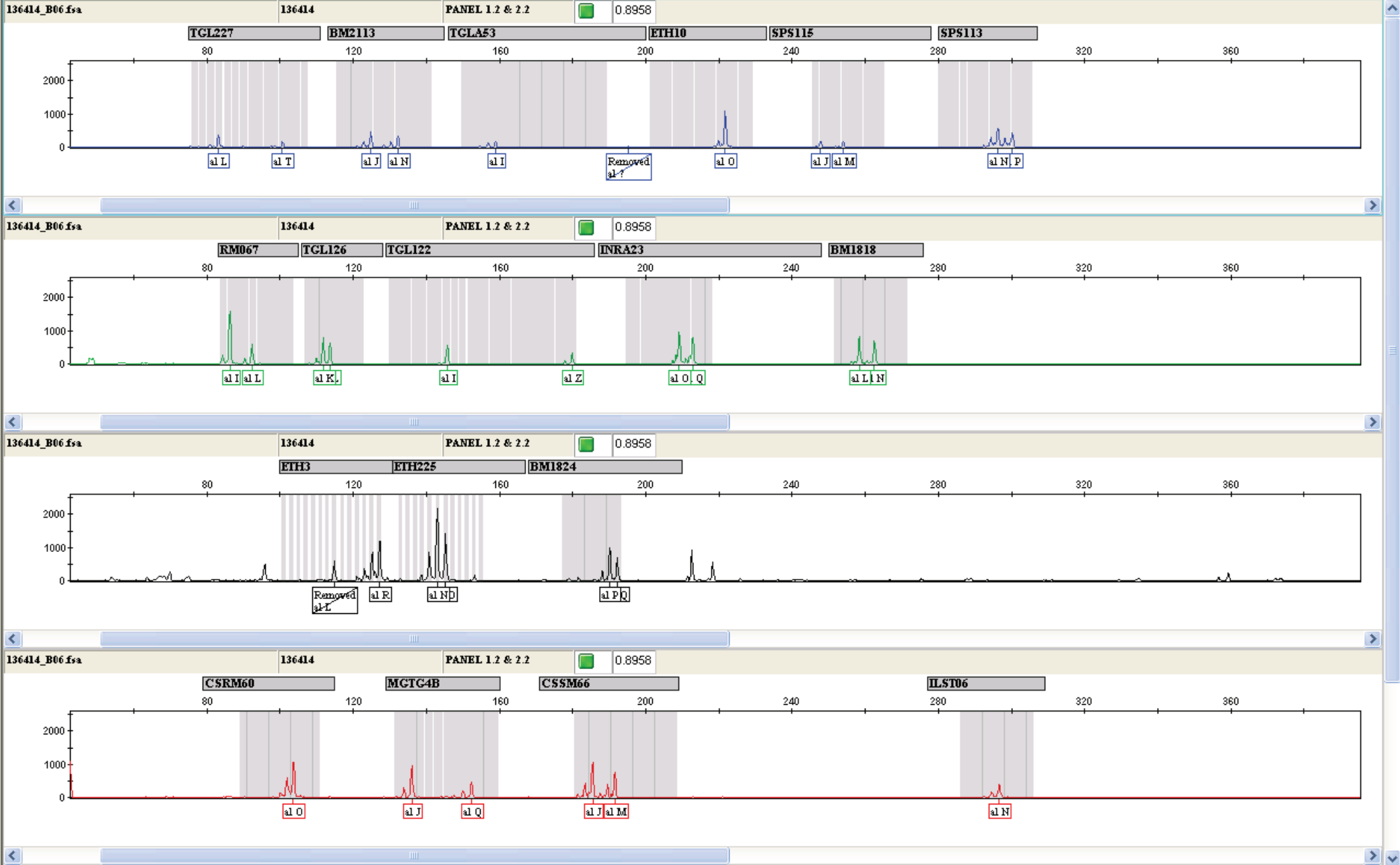


Table 2: Summary of call rate results from SNP Genotyping and Microsatellite analysis

Assay	Processing method (nasal)	Average call rate (nasal)
Illumina Bovine3K	Full purification	98.19% <sup>1</sup>
Illumina Bovine SNP50™ BeadChip	Full purification	99.63%
Microsatellite Analysis ABI PRISM Genetic Analyzer	Quick to PCR	97.15%

<sup>1</sup> No calls were not included in the calculation of call rates (6 samples)

Figure 3: Representative electropherogram from ABI PRISM® Genetic Analyzer



## Discussion

- Performagene provides a quick and easy-to-use method for collecting large amounts of high quality DNA from cattle.
- Performagene facilitates the transition of parentage analysis from microsatellite to SNP genotyping by performing reliably on both methods as illustrated by the high call rates.
- Samples collected using Performagene can be used with either microsatellite or SNP analysis for accurate parentage determination.

DNA Genotek would like to extend their appreciation to Holstein UK and Weatherby's for assisting in the collection and analysis of the Microsatellite data presented in this poster. DNA Genotek would also like to thank Illumina for conducting and providing the Bovine3K data analysis.



Superior samples  
Proven performance

