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 (MCIR)), 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™•LIVESTOCK (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 from livestock.

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 microsatellite, SNP genotyping and microarray. For these reasons, we show the suitability and reliability of nasal samples collected from cattle into Performagene-LIVESTOCK on the ABI PRISM® 3100 Genetic Analyzer (Taqman), Sequenom® MassARRAY and Illumina BovineSNP50™ BeadChip.

Nasal samples were collected into the Performagene-LIVESTOCK 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-LIVESTOCK protocol. Using fluorescent quantification, we show that the Performagene-LIVESTOCK 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-LIVESTOCK

- 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 ul aliquot of the nasal sample was processed according to the DNA Genotek Quick to PCR protocol PD-PR-098 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.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Collection Method</th>
<th>Hands on time per sample</th>
<th>Processing time per sample</th>
<th>Hands on time per 24 samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Non-invasive</td>
<td>1h 40 min</td>
<td>28 min</td>
<td>1h 31 min</td>
</tr>
<tr>
<td>Blood</td>
<td>Non-invasive</td>
<td>30 min</td>
<td>41 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Hair (50 follicles)</td>
<td>Non-invasive</td>
<td>30 min</td>
<td>41 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Nasal (1 mL of sample)</td>
<td>Quick to PCR</td>
<td>45 min</td>
<td>28 min</td>
<td>45 min</td>
</tr>
<tr>
<td>Nasal (1 mL of sample)</td>
<td>Quick to PCR</td>
<td>20 min</td>
<td>27 min</td>
<td>20 min</td>
</tr>
<tr>
<td>Nasal (1 mL of sample)</td>
<td>Quick to PCR</td>
<td>1h 30 min</td>
<td>1h 30 min</td>
<td>1h 30 min</td>
</tr>
</tbody>
</table>

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

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 run at 80 volts for 45 minutes.

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

<table>
<thead>
<tr>
<th>Assay</th>
<th>Microsatellite ABI PRISM® Genetic Analyzer - Taqman assay</th>
<th>BovineSNP50™ BeadChip</th>
<th>Illumina BovineSNP50™ BeadChip</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequenom® MassARRAY Analysis of Nasal sample</td>
<td>Quick to PCR 98.74% 99.49% 95.96%</td>
<td>Quick to PCR 94.73% 100.00%* 100.00%*</td>
<td>Full Purification 99.63% 100.00% 100.00%</td>
</tr>
</tbody>
</table>

Discussion

- Performagene-LIVESTOCK provides a quick and easy method for collecting large amounts of high quality DNA from cattle using non-invasive methods.
- Performagene-LIVESTOCK 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 SRC GenServe Laboratories™ and DNA Landmarks for providing DNA analysis services.