DNA Collection with Performagene Nasal Swabs: Quantity, Quality, and Cost Effectiveness

A Senior Project

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by

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This project would not have been possible without the help of Dr. Bruce Golden. This project was offered to me 6 months prior to taking the class which allowed for the data to be processed. Dr. Bruce Golden aided me in handling the DNA samples post collection by sending them to Iowa State where they were processed for DNA extraction. Dr. Bruce Golden also arranged for the samples to be processed at Geneseek where DNA was observed through the use of the BovineSNP50 Bead Chip. Golden walked me through the statistical analysis and data analyzing to be able to compile a logical paper.

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ABSTRACT

The objective was to determine the quality and quantity of DNA collected via nasal swab in Holstein and Jersey cows compared to the time taken to collect the samples through a person inexperienced with the Performagene™ LIVESTOCK product. DNA was collected from 100 cows at the California Polytechnic State University San Luis Obispo Dairy. Holsteins (n=47) and Jerseys (n=53) were collected and samples were shipped to Iowa State University where DNA was extracted by Dr. Jim Reecy's lab. Results were compiled into a spreadsheet based on DNA quantity in ng/ul, and protein to DNA ratios (A260/A280). Extracted DNA was sent to GeneSeek to be genotyping where the BovineSNP50 Bead Chip was used to determine single nucleotide polymorphisms (SNPs). Data were analyzed by comparing mean, median, standard deviation, minimum, and maximum numbers within the data set. Numbers were observed based on "pass" or "fail" percentage for call rate (>80%=pass), and were compared by breed. Data showed significant signs of reliability compared to blood and hair sampling resulting in a 92% pass rate. Average DNA quantity was significantly higher than blood sampling (n=108.18ng/ul). There may have been some behavioral differences between Holsteins and Jerseys that cause a few animals to be more difficult to sample. However, this was not an important issue in this study. If used on a larger sample of animals a higher call rates could be better represented as well as a DNA average that better summarized results from a larger population . This would result in more accurate characterization of the new technology. Possible follow-up work should include testing animals in different conditions. Another suggestion for testing would be to sample a lot more animals and

breeds to make comparisons across breeds. Next research steps should test if longer nasal passage time results in greater quantity DNA.

Key words: DNA, nasal swab, call rate, SNP.

INTRODUCTION

The dairy industry has been changing in the area of genetic testing. Cows have been tested for production and type traits to produce higher producing offspring. The original method of calculating the predicted transmitting ability (PTA) was a long process. It involved breeding cows and then waiting until the offspring were producing milk. This would then show the PTA of specific traits that are desired, from animal size and udder features to milk production and components.

Before genomic PTAs became available, reliability of a young female's predicted merit was low until she began producing. However, with the introduction of genomic testing the reliability of PTAs for young animals can increase to much higher levels. This is because markers are being predicted from a large amount of individuals with high reliability. The markers are resolved with genomic tests (SNPs) and then applied to the test results of young animals. This method allowed for early detection of desired traits such as: milk production, stature, udder, and components.

Genomic tests require collection of tissue for DNA extraction.Typical tissue sources for DNA extraction have included blood, tail hair, milk, and semen. The most popular of these has been blood because of the reliability of quality and quantity DNA to extract and test. However, blood collection requires developing proficiency and proper handling to be able to process. This was also an invasive way of collecting DNA which may be a downside. In addition to the previous collection methods, nasal swabbing has become a popular method for DNA collection which is non-invasive and can be performed at any stage of life of the animal.

The ability for DNA to be collected at a young age allowed people to test their calves genomically to confirm how good of an animal it will become. Bull studs have used genomic testing to rank bulls that yield high producing daughters, or sons, that can be used for breeding or production in the future.

Genomic testing began to uncover trackable traits that were not typically observed previously. This has allowed for mastitis resistance in genetics to be tracked and also predicting energy balance of the animal. This ability to track less common traits provides bull studs with complex genetic evaluations of the animal. This allows dairyman to mate animals based on a larger variety of traits.

The objective of this study was to determine the quality and quantity of DNA collected via nasal swab for Holstein and Jersey cows compared to the time taken to collect the samples through a person inexperienced with the Performagene™ LIVESTOCK product.

LITERATURE REVIEW

History of Genetic Evaluation

For more than 40 y dairy breeders have used genetic evaluations to identify superior cows. Selective use of these superior animals improved phenotype measures for milk, and also components predominantly in the Holstein breed (Sonstegard and Van Tassell, 2001). In the 1970's Genetic selection was based on daughter and dam comparisons which observe differences and strengths of traits passed on to the next generation. An issue with this was selection for response in the next generation, not a response in the long run without observing diversity within the herd. Popular bulls typically were the most predominant bulls used to artificially inseminate (AI), and if selected incorrectly would result in a high inbreeding coefficient. The higher the coefficient, the more problems were experienced. Inbreeding had deleterious effect on milk production, udder health, calving performance, fertility, and survival (Mc Parland et al. 2007)

Another issue with breeding solely based on predicted breeding values was that it limited ability to improve lowly heritable traits without severely affecting production (Sonestegard and Van Tassell, 2001). Common low heritable traits include disease resistance, reproduction, duration of productive life, and some traits related to fitness.

Milk production traits have been greatly improved by the use of predicted genetic merit in selected bulls. Hypothetically, genetic improvement could be accelerated even further for milk yield and other economically important traits. Genomic testing can be useful to address these issues. Genomic science helped identify economic trait loci (ETL) which have been known to positively or negatively affect traits in cattle. Most ETL have

been identified through the granddaughter design, but because ETLs have not been resolved well enough for accurate selections in current populations. Economic trait loci analyses have been or are being extended to include ancestral animals that connect family pedigrees and current generations of nonprogeny-tested animals from within the founder animal pedigree (Sonstegard and Van Tassell, 2001).

The granddaughter design ranks bulls based on their offspring. This form of ranking took many years to do because the bull must reach puberty before semen can be collected and tested in cows. The cow would then have to carry out a full pregnancy and have the calf. If it was a daughter then the calf would need to be raised until she is then bred. Following calving, when the cow produced milk, the cow's traits could be observed. Observed traits with the cow were milk production, components, stature, and udder traits along with many others. The granddaughter design predicted transmitting ability (PTA) of type traits were analyzed and converted into a linear type scoring. Many traits were examined to better select a bull to mate with a suitable cow to produce offspring that benefit the rest of the herd.

Genomic Testing Overview

Collecting DNA has become much more common and affordable in the past years allowing for genetic advancement in dairy cattle. There are three popular sources of tissue used to extract DNA: blood sampling, hair samples, and nasal swabs. Of these, blood has been the most common. However, recently, nasal swabs are available through the company DNA Genotec, making specific swabs for cattle or livestock. This nasal swab was specifically named Performagene™ ·LIVESTOCK, which was the basis for the experiment that was performed.

Genomic PTAs have played a large part in the genetic advancement so far. Genomic testing allows for a wide spectrum of the cows genes to be displayed on microarrays to identify ETL. This method for assessing genotype for cattle was very successful and was continued on for more research. The genomic PTAs were becoming much more affordable because of the availability of supplies and technology to collect and analyze DNA.

Blood sampling has been the "go to" method for DNA extraction because of the simplicity of the materials used, and the availability of the blood. Blood sampling involved a skilled professional, typically a veterinarian, to draw the blood. Once drawn, blood coagulated unless stopped otherwise by Ethylenediaminetetraacetic acid (EDTA) which acted as an anticoagulant. This allowed for extended storage until processing. There was also a chemically treated paper card that the blood could be applied to where the DNA would be preserved and dried and not degrade because the chemical based paper destroyed any bacteria that had potential to degrade the sample. Once dried the FTA card has potential to be stored in room temperature without degrading. Sampling of DNA from this card only required a punch of 3mm that was then re-suspended in solution for analysis of the DNA genomically (McClure et al. 2009).

An alternative method that increased in popularity is hair sampling. Hair sampling involved plucking 15 to 30 tail hairs where the follicles were stuck to a small card. Excess hair that stuck out of the edge of the card was trimmed for cleanliness. Samples have potential to last forever because they have been stored and identified for re-sampling the DNA for further advancement of genomics. This method for extraction of DNA has shown to be effective and produced a high call rate and quantity of DNA.

Cattle have been gnomically tested to improve dairy cattle genetic evaluations. This has evolved from phenotypes and pedigrees that were the basis of selection for the previous 100 years (VanRaden et al.). Rapid developments in genotyping tools have lowered the cost of collecting genomic data to just over $200 per animal (VanRaden et al.). Samples collected can be analyzed using the Illumina BovineSNP50 BeadChip which identifies more than 50,000 single-nucleotide polymorphisms (SNP) which span the entire bovine genome. From these thousands of markers, genetic effects can be traced across families. In using genomic data in genetic evaluations, reliability of predicted merit is greatly increased when matched with phenotypes for a large number of animals (VanRaden et al.). Large gains of reliability require large families and large numbers of SNP because traits are affected by many genes of small effect. Recently adopted genomic technology has replaced the traditional model for animal evaluations (VanRaden et al.). In the past few years, tens of thousands of cattle in North America have been genotyped with the Illumina BovineSNP50 BeadChip. These SNP markers represent base changes in nucleotides (Adenine (A), Thymine (T), Guanine (G), or Cytosine (C)) within the DNA sequence of the animal tested whether it is a bull or cow (Weigel, Kent). A SNP was defined as: a DNA sequence variation occurring when a single nucleotide in the genome differs between members of a biological species or paired chromosomes in an individual (Single 2012). In high-density SNP arrays, hundreds of thousands of probes are arrayed on a small chip, allowing for many SNPs to be interrogated simultaneously (SNP 2012).

Performagene™ ·LIVESTOCK nasal swab was designed to be an efficient, non-invasive way to collect quality DNA. The product is an all-in-one system for collection,

stabilization, transportation and extraction of DNA from nasal samples (Iwasiow et al.) The swab has a twist off cap with a sponge attached to the cap for swabbing. The cap and swab can be inverted for collection to prevent loss of buffer solution. Post collection, the swab is submersed in the buffer solution and vigorously shaken 10x.Results from other tests have shown bacterial content averages about 3.3% which is relatively low meaning a quality amount of DNA is available for testing. The buffer solution was used to preserve the collected DNA for a year at room temperature.

Using Genomic Data to Improve Genetic Evaluations

Once there was enough genetic markers available for an animal a breeding value can be predicted based on genotypes for: milk yield, somatic cell score (SCC), productive life (PL), daughter pregnancy rate, fat, and protein. In Weigel's experiment he genotyped bulls and cows of Holsteins, Jerseys, and Brown Swiss from 1952-2009 with the Illumina BovineSNP BeadChip. The genotypes and phenotypes were used to estimate specific traits that were mentioned above. Results from this test showed that there was a range of increase between different traits from -1% to +50% meaning gains in reliability from genomic information was significant in all but one category, foot angle, which was not significant. This significant information then was useful with bull studs such as: ABS Global, Accelerated Genetics, Alta Genetics, Genex Cooperative, Select Sires, Semex, and Taurus Service, for detailed predicted transmitting ability (PTA). Genomically tested bulls better reveal the accuracy of the transmitting ability of specific traits for the offspring such as: lifetime merit, fat yield, protein yield, milk yield, and also physical attributes regarding the udder and the cow (Weigel, Kent).

A study was done between four different dairy countries: Australia, Netherlands, United States, and New Zealand, to determine if the reliability of genomic breeding value (GEBV) was much higher than breeding values from parental averages (Hayes et al., 2009). Results conclude that GEBV reliability is much higher from the four countries, although the United States and New Zealand had a lot more bulls to sample than did Australia.

Genomic Selection and its Effects on Fertility in High Producing Dairy Cows

In the past few decades cow fertility has been on a decline, and further genomic testing has been done to bring fertility rates back up. In these past two decades the number of days from calving to conception increased by 24 days in the United States (Veerkamp and Beerda, 2007). Genomic testing has allowed researchers to test the transmitability of fertility traits from bulls to offspring, with which appropriate bull selection seemed like a practical way to bring to solve fertility problems (Veerkamp and Beerda, 2007).

The decline in fertility was linked to a desire for higher production. Evidence showed that increased genetic merit for yield without considering genetic merit for fertility reduced fertility. The addition of 1000kg milk yield had the potential to increase calving interval by 5-10 days. But keep in mind this trend varied from herd to herd phenotypically and genetically (Veerkamp and Beerda, 2007). It has also been found that when animals are bred for production, the energy partitioning was altered and therefore had an effect on body condition which has a major effect on fertility and conception. Continued research is being done on heritability of fertility to better understand fertility issues and address them.

Genomic Effects on Resistance of Mastitis

Mastitis has been a large issue that was dealt with on every dairy. Genomic testing has been done to address the heritability of traits affecting mastitis recovery time and incidences. This trait is very complex, but is also related to physiological and environment factors (Rupp and Biochard, 2003). Although sanitary conditions are the best aid in mastitis prevention, perfect conditions are virtually impossible to achieve. Mastitis was a very frequent and costly disease for dairyman to deal with making this study something worthwhile for researching. If dairyman can breed for mastitis prevention, lots of money can be saved by not administering costly treatments to clinical cases. Also it has been noted that there is increasing number of clinical mastitis cases in several countries in the Holstein breed. This is a topic of concern because Holsteins were the largest breed of cows that are milked, so slowing down this progression would help with the issues of mastitis in this prominent breed.

Accumulated results have shown a moderate to low heritability for somatic cell count (SCC). Higher SCC counts were found also in cows that milk fast, also called milking ease, which was a heritable trait that is looked at when looking at mastitis as a whole. Research showed that these cows have higher SCC counts than normal cows, but with rapid flushing of the udder there is a better chance of avoiding clinical mastitis (Rupp and Biochard, 2003). The major issue with breeding for low mastitis was that worldwide there was not enough records kept to have a reliable number. The idea sounds acceptable but may take more years than anticipated to increase reliability of the numbers.

Genomic Effects on Predicting Energy Balance

Genomic testing was used for identifying many traits in cows. The idea of predicting energy balance (EB) was to investigate the genetic basis of EB and the potential use of genomic selection in selection programs (Verbyla et al. 2010). Due to decreased calving performance and conception rates at first service, fertility was a major trait that was included within national selection indices (Verbyla et al. 2010). A reason for the fertility decline was the difference between energy intake and energy usage also known as energy balance. The EB trait was an essential link between production and non-production traits because both depend on a common source of energy. Energy must be partitioned efficiently to keep a cow from negative energy balance. This typically is more common in the early stages of lactation when the cow was producing a lot of milk and using a lot of stored energy. This usage of stored energy decreases fertility and health in most lactating cows. The use of high density SNPs identified locations of the specified trait target and quantifies the desired trait. Genomic testing for many traits became much more popular because of the extent of analysis that can be done on DNA. The cost has also become much more affordable allowing testing to be done on not as popularly followed traits as mentioned above. Overall, genomic testing was rapidly advancing and becoming much more popular and affordable resulting in a complex analysis of the bovine genome that can be applied to selective breeding for specific desired traits.

MATERIALS AND METHODS

Animal Housing

The experiment was carried out at the California Polytechnic State University Dairy. One hundred milk cows, Holstein (n=47) and Jersey (n=53), were selected randomly along the line of locking stanchions. At the time there were about 225 total milking cows also roughly half Jersey and half Holstein. Cows were housed in free stalls bedded with compost. The animals were separated into different pens by breed on opposite mangers. Cows were fed a total mixed ration twice daily corresponding to the twice daily milking. The cows have been milked in a double-8 herringbone with no rapid exit. There has been a unique labor force which consisted of roughly 40 students all on a part-time schedule working around class schedule. The inconsistency of laborers has made management difficult. Each quarter student's classes changed and therefore their time availability changed. The dairy was run on a very timely schedule that was not always forgiving with class time. Therefore, alternate students filled spots that were not able to be covered by the student that was possibly more skilled in the job required.

Animal behavior was remarkably different from other facilities I had visited. Animals were not startled by human presence. The animals were so "friendly" because of their upbringing. The animals at a young age were halter broken for the annual Fit and Show contest. The whole herd has been halter broke and exhibited this throughout all stages of life.

Data Collection

We started from the North of the milk cow free stall barn and collected DNA samples from the Holsteins that were feeding in the locked stanchions on one half of the

barn. After 47 collections from the Holsteins then we collected starting from the South of the same barn and also collected 53 more samples from the locked up Jerseys. performagene™ ·LIVESTOCK nasal swabs were used on a portion of the milk cows at California Polytechnic State University's Dairy facility. One hundred milk cows were selected to be swabbed, 47 were Holstein, and the remaining 53 were Jersey. The nasal swab cost $6 per unit, which would have cost $600 of product to collect the DNA samples. The nasal swabs were donated for this study. Collection day was May 4, 2011 in the spring quarter, where I was assisted by Dr. Golden and Rich Silacci, herd manager. Performagene™ ·LIVESTOCK nasal swabs were provided to me without any further verbal instruction about how to use it. Written and picture directions were provided by the manufacturer (Figure 1).

Figure 1. Instructions on individual nasal swab package. © DNA Genotek Inc. All rights reserved. Used with permission.

Instructions were read and interpreted by me only for a few minutes prior to the beginning of the collection. I opened up one sample at a time and worked my way down the headlocks holding the cows head and swabbing the right nostril. The entire collection process was performed by me only to calculate what a producer would experience in a

production setting. Dr. Golden used a stopwatch to time each sample to collect an average time of collection for each sample. Sample time was recorded to determine if collection time was faster based on experience and understanding of the product. Upon collection the all inclusive swab is inserved into the tube with buffer and is closed off and shaken vigorously 10x. The swab sample was coded and identified to the cow's identification tag to avoid mixing of samples. Swab tubes were collected back into the cardboard box they were received in because sample solution is not degraded with temperature or handling.

Figure 2. Photograph taken of me by Dr. B. L. Golden during collection.

Data Processing

Performagene™ ·LIVESTOCK nasal swab tubes were boxed and shipped to a lab at Iowa State University (ISU). The lab was run by Dr. Jim Reecy and extracted and quantified. From ISU the DNA was sent to Geneseek for genotyping. This DNA was genomically tested using the BovineSNP50 BeadChip. The chips were analyzed for call rates to quantify and qualify the DNA that was processed. Also from the chips the specific genotype was displayed for each sample on individual BeadChips. Genotyping cost was $80 per sample, however price since then has dropped to about $70, but could be as high as $120 depending who you were, and purpose behind the project. Commercial applications usually have a higher cost of processing.

Data Analysis

Data collected were analyzed to determine the differences of quality and quantity of DNA between the Holstein and Jerseys of Cal Poly's Dairy. Differences that were observed were: collection time, breed, and call rates from DNA sample, DNA quantity in ng/ul, and A260/A280 ratios. The A260/A280 ratio has been used to compare DNA and RNA concentration to the concentration of protein. Ratios indicated the expected quality of the samples collected. Data was extracted from excel spreadsheets to observe DNA quantity and collection time based on breeds. Standard deviation, mean, and median were computed to analyze differences within each breed for time of collection, DNA yield, call rate, and A260/A280 ratio. Averages of each of these were compared to each other to observe differences by time and breed. Cost per swab is $6.00 per swab. This can be kept in consideration to be able to have a cost breakdown to accurately inform consumers how much the process cost per animal.

RESULTS AND DISCUSSION

Results

Collection proceeded at precisely 12:21p.m. on the Holsteins. Sample collection time for both breeds averaged 7.46s per cow and standard deviation was 2.28s. Holsteins average collection time was 6.99s and standard deviation was 1.73s. A total of 47 Holsteins were sampled and took 33 min. to collect. This does not include the time of post collection handling procedures and writing down sample number, time of day, cow identification and any other notes on animal behavior during collection. Then at precisely 12:55p.m. we began collection from the Jerseys. Sample collection time average was 7.93s per cow and standard deviation was 2.8s. A total of 51 Jerseys were sampled and took 43 min which was about 10 minutes more than the Holsteins. And included a 10min break before starting

Call Rates for the 100 samples ranged from 44.07% to 99.69% from both the Holsteins and the Jerseys (Table 1). Holsteins had the minimum call rate, while both breeds had the same maximum of virtually 100% (Table 1). These call rates averaged 94% for the Holsteins (Table 2), and 95% for the Jerseys (Table 3), but the minimum call rate for the Jerseys was 29% (Table 3) and the minimum for Holsteins was 44% (Table 2). The standard deviation for the breeds was 10% for the Jerseys (Table 3) and 12% for the Holsteins (Table 2).

Table 1. Analyses of DNA results by Holstein and Jersey breeds (n=100)

	ng/ul	A260	A280	260/280	Call Rate
Avg	108.18	2.16	1.26	1.75	94.39%
Median	45.33	0.91	0.55	1.73	98.64%
Stdev	154.40	3.09	1.75	0.37	11.01%
Min	-3.69	-0.07	-0.05	0.64	44.07%
Max	905.09	18.10	10.35	3.98	99.69%

Table 2. Analyses of DNA results by Holstein (n=47)

	ng/ul	A260	A280	260/280	Call Rate
Avg	102.84	2.06	1.19	1.74	0.94
Median	48.74	0.98	0.58	1.73	0.99
Stdev	141.88	2.84	1.60	0.23	0.12
Min	1.75	0.04	0.01	1.39	0.44
Max	752.82	15.06	8.27	3.06	1.00

Table 3. Analyses of DNA results by Jersey (n=53)

	ng/ul	A260	A80	260/280	Call Rate
avg	112.90	2.26	1.31	1.75	0.95
median	43.75	0.88	0.54	1.72	0.98
stdev	165.93	3.32	1.89	0.46	0.10
min	-3.69	-0.07	-0.05	0.64	0.49
max	905.09	18.10	10.35	3.98	1.00

The A260/A280 ratios were compared by breed to determine the difference of ratios and also composition of each DNA. The Jerseys had both the minimum and maximum protein to DNA ratios ranging from .64 to 3.98 (Table 3). The Holsteins had a range from 1.39 to 3.06, which is a much narrower range (Table 2). The averages were almost the same, Holsteins at 1.74 (Table 2) and Jerseys at 1.75 (Table 3). Another number was observed was the ng/ul. This is the measure of the amount of actual DNA that was extracted from the sample. Jerseys once again had the largest range of DNA which ranged from -3.69ng/ul to 905.09ng/ul (Table 3). Holsteins ranged from 1.75ng/ul

to 752.82ng/ul (Table 2). This was a smaller range, but average ng/ul was still very close with the Holsteins at 102.84ng/ul (Table 2) and the Jerseys at 112.90ng/ul (Table 3). These numbers consist of all samples tested including the 8 with a call rate less than 80%. Within these low call rate samples half were Holstein (n=4) and the other half were Jersey (n=4). Holstein call rates averaged lower, with 57% (Table 4), than the Jerseys that averaged 63% (Table 5). But with the 260/280 ratios, the Holsteins averaged higher with 1.67 (Table 4) and the Jerseys averaged 1.49 (Table 5). In regards to the ng/ul, Holsteins had much higher amounts of DNA averaging 438.34ng/ul (Table 4) compared to the Jerseys that averaged 138.76ng/ul (Table 5). This high number may be skewed in the Holsteins from contamination upon initial collection.

Table 4. Analyses of DNA that was not able to be tested by Holstein (n=4)

	ng/ul	A260	A280	260/280	Call Rate
avg	56.62	1.13	0.67	1.71	0.98
median	57.03	1.14	0.65	1.70	0.99
stdev	41.56	0.83	0.49	0.08	0.02
min	10.68	0.21	0.12	1.62	0.95
max	101.75	2.04	1.24	1.80	1.00

Table 5. Analyses of DNA that was unable to be tested by Jersey (n=4)

	ng/ul	A260	A280	260/280	Call Rate
avg	111.98	2.24	1.30	1.57	0.88
median	53.53	1.07	0.70	1.58	0.93
stdev	150.98	3.02	1.65	0.20	0.15
min	9.08	0.18	0.12	1.33	0.68
max	331.78	6.64	3.68	1.81	1.00

Interpretation

These results show some valuable information in regards to effectiveness of the use of Performagen™LIVESTOCK. This new simple way of collecting DNA needs to be able to obtain at least the same amount of DNA as traditional blood or hair sampling. From my results, of the call rates from the 56K chip on 102 samples, only 8 of 102 were rejected because less than 80% of the SNPs were recognized. This means that 7.8% of the samples didn't pass quality, but 92.2% did meet quality standards.

Critical Analysis

There were numerous different ways DNA could have been collected however nasal swabbing is the newest method for extraction that was still being tested for the reliability of quality and quantity DNA. Other tissues targeted for DNA extraction were the blood, milk, and semen. Blood sampling is an invasive way to collect quality DNA, while milk, semen, and nasal swabbing are non invasive. Blood collection has been a major method used because of the quality and quantity of DNA collected. Also to be considered when collecting was the physiology of the animal. Semen DNA can only be collected from bulls, and milk DNA can only be collected from lactating cows. These limit the animals that were available to test. The perk of the nasal swab is that there is no limitation on the animals that can be tested. In an experiment performed by Foley he compared ng/ul of DNA collected and the 260/280 ratios from blood, semen, milk, and the nasal passage. Results showed that nasal swabs collected just as much or more in terms of DNA quantity, and quality is then based on the 260/280 ratios. The nasal swab ratios compared directly to that of blood with a ratio of 1.8 for the swab, and 1.9, 1.7, and 1.6 for the different tests used on the blood to extract DNA. Based on the results of this

test and my test nasal swabs through DNA Genotek were the most simple collection method which was noninvasive and also yields quality and quantity of DNA that was genotyped.

Alternate Circumstances

This experiment was performed on very cooperative animals. In order to get an actual representation on the effectiveness of the nasal swabs, this experiment would benefit being run on a number of other herds. Cal Poly's herds of Holsteins and Jerseys were very tame and comfortable around people. The animals were not startled when they were handled around the neck and nasal passage. This indicated a need to sample normal herds so actual ease of collection could be determined.

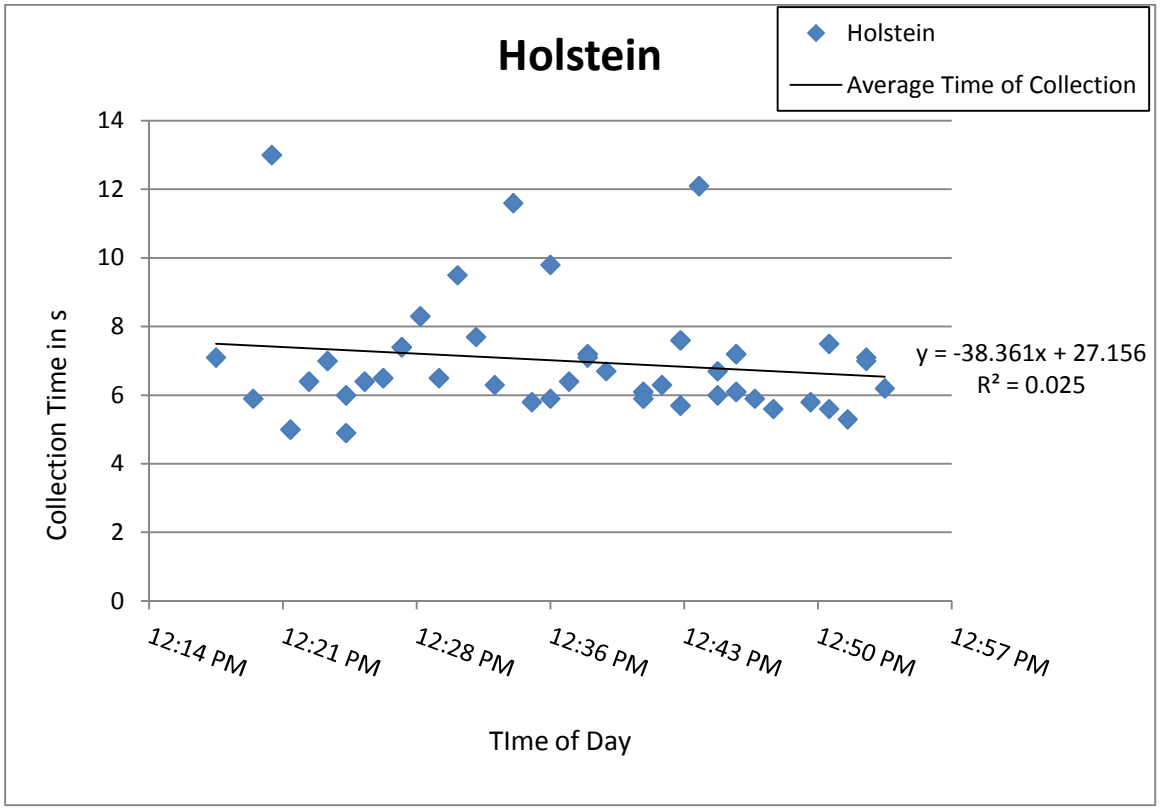

Figure 3. DNA collection time per Holstein in s on May 4, 2011 from 12:18pm-12:55pm (n=44).

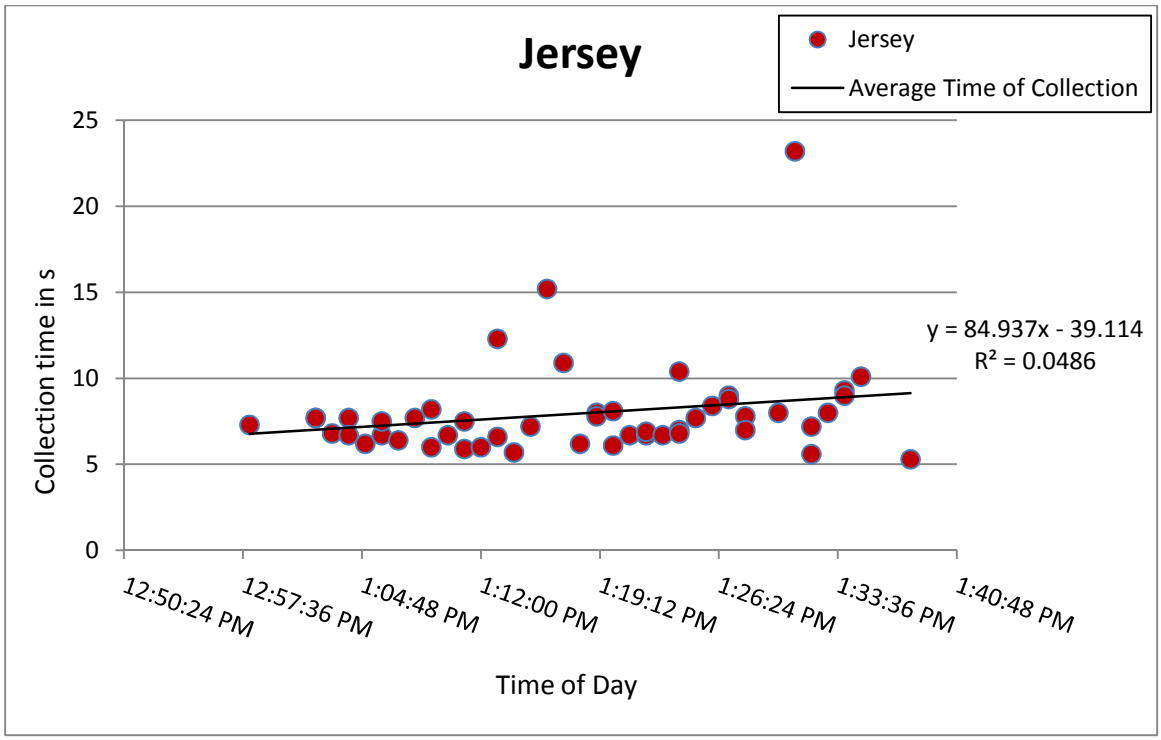

Figure 4. DNA collection time per Jersey in s on May 4, 2011 from 12:58pm-1:38pm (n=50).

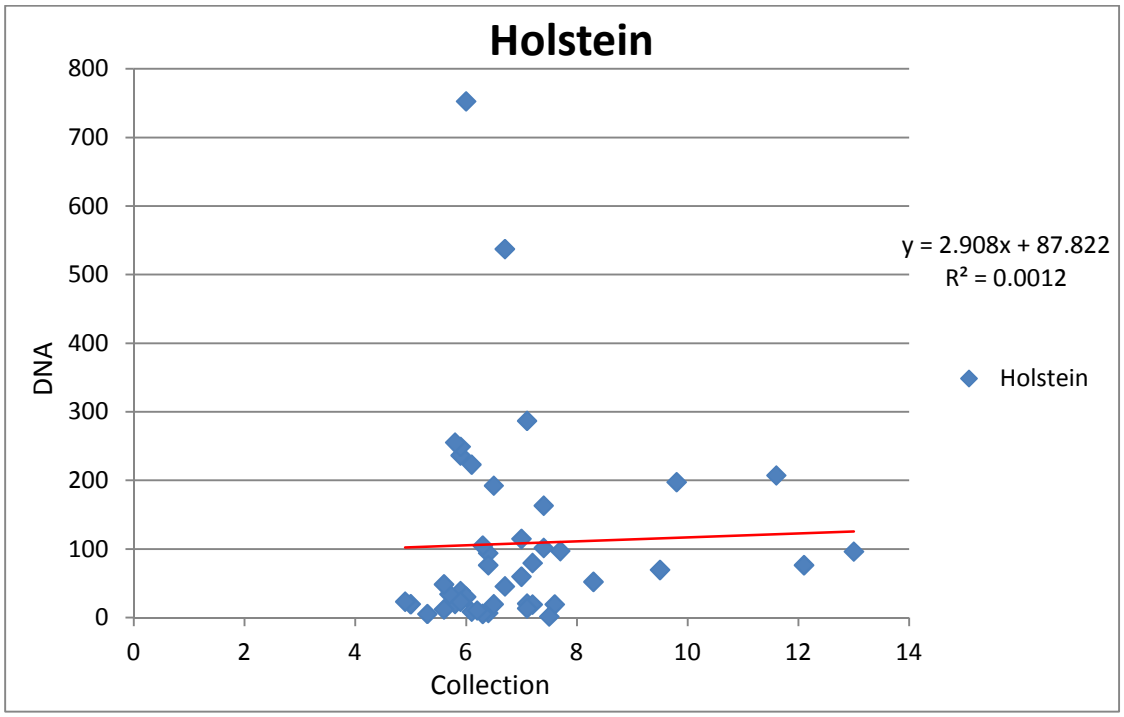

Figure 5. Quantity of Holstein DNA comapred to collection time (n=47).

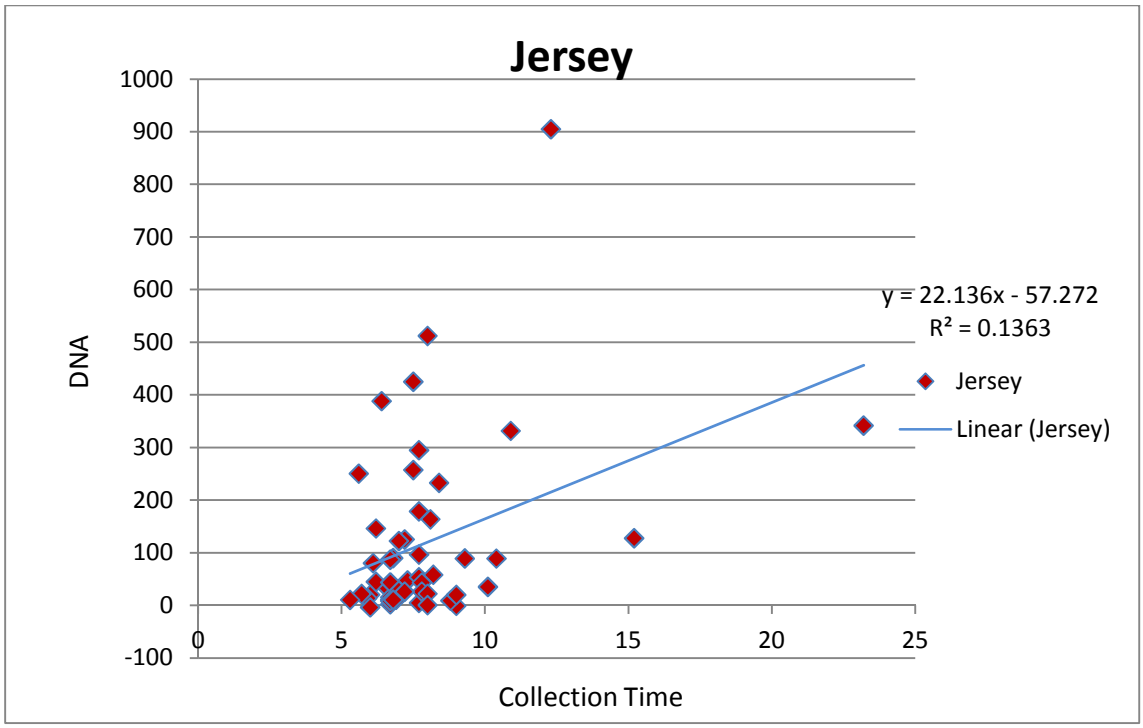

Figure 6. Quantity of Jersey DNA compared to collection time (n=53).

CONCLUSION

In conclusion results indicate that DNA sampling with the use of nasal swabs yields both quantity and quality DNA. Samples varied by breed but also yielded samples which did not have a call rate greater than 80%. This experiment proved the Performagene™ LIVESTOCK product requires little training and experience. An individual with basic animal handling skills can collect quality DNA from cattle. In addition, the use of the nasal swab resulted in call rates similar to that of blood and hair sampling while also being non-invasive to the animal. However, a hair card SNP chip run can be re-ran from a punch of new hair from the hair card because hair samples last indefinitely.

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