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UTILIZING SINGLE NUCLEOTIDE POLYMORPHISM ANALYSIS IN DETERMINING PARENTAGE OF CATTLE

by

Nicole M. Elbert

Thesis submitted in partial fulfillment of the requirements for the degree

of

DEPARTMENTAL HONORS

in

Animal, Dairy, and Veterinary Science in the Department of Animal, Dairy, and Veterinary Science

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ABSTRACT

Utilizing Single Nucleotide Polymorphism Analysis

In Determining Parentage of Cattle

by

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Utah State University, 2013

Departmental Honors Advisor: Dr. Lee F. Rickords
Department: Animal, Dairy and Veterinary Sciences

Parentage identification within cattle herds is an important aspect of record keeping. It is essential for accurate registration within a purebred association and decision making for production purposes, such as replacement heifer and sire selection. Methods used to identify parentage have evolved from utilizing blood protein antigens, restriction fragment length polymorphism (RFLP) and microsatellites to the current technology of analyzing DNA profiles for differing single nucleotide polymorphisms (SNPs). In this preliminary study we analyzed genotype data from 1,066 samples obtained from various cattle breeds. These breeds included Holstein, Angus, Hereford, Irish Black, Jersey, Red Angus, Limousine, Charolais, Brown Swiss and Maine-Anjou. Samples were analyzed using parentage calling software (Cervus 3.0 and SireMatch 2.0) utilizing the 88 SNP parentage-testing panel from Fluidigm®. Of the 1,066 offspring samples submitted, 789 were verified by DNA identification to match herd records submitted by producers, indicating that 277 herd records were incorrect. Beyond parentage identification, future efforts employing SNP technology will include creating

reduced SNP panels to determine the heritability of qualitative traits desirable to the beef cattle industry, such as marbling and tenderness.

For Jesse C. Busch & Grandma Elbert

ACKNOWLEDGMENTS

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CHAPTER I

LITERATURE REVIEW

1.1 Blood Antigens & Early Paternity Determination

In the early 1900s Karl Landsteiner discovered that different blood groups exist within the human species[4]. Landsteiner utilized serological methods to determine that proteins in different individuals varied but were specific for each species[4]. In later years, Todd and White conducted studies in the area of haemolytics. Utilizing oxen they concluded that cells within an individual are unique[5]. They also revealed that proteins (red blood cells) of closely related oxen exhibited similarities which suggested they were somehow linked genetically[5].

Drawing on these early discoveries, erythrocyte antigens were identified in humans and animals[2]. These antigens were used to test individuals serologically, which created blood type profiles, which were compared to profiles of potential parents.

Parentage determination methods have significantly advanced since the early days of blood antigen testing. The discovery of DNA and the role it plays in genetics assisted in significantly advancing methods used, by streamlining the processes and making them more cost effective. This discovery also allowed for a wide variety of samples to be collected, not just blood[7].

1.2 Restriction Fragment Length Polymorphisms (RFLPs)

Use of RFLP analysis in conjunction with Southern blotting was the next advancement in parentage testing[3]. A RFLP occurs when a restriction site is eliminated or created, which is due to a base-pair change.

This technique is applied to parentage determination by using restriction enzymes to digest DNA, which is run through electrophoresis on an agarose gel. From here the Southern blot method is employed to probe DNA fragments with homologous oligonucleotides[20]. After this is complete, radioactive bands of offspring and potential parents are compared[20].

Although this technique is more accurate than blood typing, it is difficult to definitively trace where the alleles came from; also restriction enzymes are not completely without fault[21]. To overcome the shortcomings of RFLP, microsatellites or single tandem repeats were developed.

1.3 Microsatellites/Short Tandem Repeats

Microsatellites, also known as short tandem repeat polymorphism (STR), are an ideal tool in genetic mapping analyses due to the versitility[9] and high frequency of occurrence in the genome, occurring about every 300 to 500 kb[22]. In addition, STR is easy to use, low in cost and exhibits high specificity, making it the choice method of parentage testing[7] for the past few decades. The technique of microsatellites involves amplifying STR regions followed by electrophoresis of amplified regions utilizing fluorescence. The amount of DNA required for STR analysis is minimal and can be of lesser quality, adding to the other advantages of using STR[8].

Advancements created by STR lead to development of a new technology called single nucleotide polymorphism (SNP) which is becoming more widely used in determining parentage.

1.4 Single Nucleotide Polymorphism

A SNP is a single base change at a single, specific nucleotide in a DNA sequence, which is different between members of a species or paired chromosomes within individuals. Since SNPs are located at a specific nucleotide they can be sequenced and used to determine parentage. Thus far millions of SNPs have been identified in livestock species[7], appearing in the genome about every 100 to 1000 bp, making them very abundant[1].

The advantages of using SNPs are abundant. SNPs have proven to have low genotyping errors[6] and require only a small amount of DNA, which makes it easier to degrade samples[7]. Furthermore, a SNP profile can be developed in a shorter amount of time than STR profiles and is easier to interpret[1]. Finally, utilizing SNP analysis is more cost effective and allows for testing of non-coding and coding regions of DNA[7].

1.5 Objectives

The objective of this study was two-fold; first, to learn techniques associated with genetic testing, such as DNA extraction and single nucleotide polymorphism analysis, and second, to determine parentage of cattle by utilizing single nucleotide polymorphism analysis.

CHAPTER II

METHODOLOGY

2.1 Sample Collection

A total of 1,066 samples were collected from beef cattle ranches and dairies in Northern Utah, Southern Idaho and Southwestern Wyoming. Samples represented various cattle breeds, including Holstein, Angus, Hereford, Irish Black, Jersey, Red Angus, Limousine, Charolais, Brown Swiss and Maine-Anjou. Samples were collected from known sire/dam/offspring trios and sire/offspring or dam/offspring duo groups. Hair was collected from the switch (tail) tip by pulling against the grain of the tail hair. This ensured the DNA containing bulb at the base of the hair remained in tack allowing for the next step, DNA extraction.

2.2 DNA Extraction

DNA was extracted from at least 10 hair follicles, cut to size and placed in microcentrifuge tubes (follicle end down), by following the Agencourt® DNAdvance™ Genomic DNA Isolation Kit[14] (Agencourt® Bioscience Corp., Beckman Coulter, Beverly, MA, USA). Manufacture's protocol was followed, with the exception of reducing reagent volumes by half. Dithiothreitol (DTT) was omitted from Lysis Buffer, as it is too destructive for hair follicles (the kit is designed for DNA extraction from rodent tail tips). 98 µl Lysis Buffer and Proteinase K was added to each tube containing hair. Lysis Buffer 'bursts' open cells making DNA available while Proteinase K inactivates nucleases, which degrade DNA during purification and aids in the degradation of cell

membrane proteins. Tubes were placed in a shaking incubator for 15 minutes at 55ºC. Supernatant was aspirated and transferred to a 96 well plate.

Bind1 Buffer, Bind2 Buffer and 70% Ethanol were used during binding and washing steps. 50 µl Bind1 Buffer and 85 µl Bind2 Buffer were added to the wells and mixed by pipetting up and down. Bind2 Buffer contains magnetic particles that bind to DNA. During the mixing process air bubble introduction was kept at a minimum by mixing slowly and carefully. Air bubbles trap magnetic beads and keep them from falling to the bottom of the well, which decreases DNA yields. The 96 well tray was incubated at room temperature for one minute then placed on an Agencourt SPRIPlate® 96 Ring Super Magnet.

The 96 well tray was placed on the Agencourt SPRIPlate® 96 Ring Super Magnet for four minutes. DNA bound to the magnetic beads was pulled to the bottom of the well. The supernatant was aspirated from each well and discarded. The 96 well tray was removed from the magnet and 170 µL of Ethanol added to each well and mixed thoroughly by pipetting up and down. The 96 well tray was again placed on the magnetic plate and the supernatant aspirated and discarded from well. The Ethanol wash step was repeated twice for a total of three washes.

30 µl of Elution Buffer was added and mixed by pipetting up and down until magnetic beads were re-suspended. Elution Buffer releases DNA from the magnetic beads so the final volume pipetted from the wells only contains DNA and Elution Buffer. The 96 well tray was covered and placed in a shaking incubator for 15 minutes at 55º C. After the incubation period, the 96 well tray was placed back on the magnetic plate and

25 µl of eluent was transferred into a clean 96 well plate. Samples were stored at -20 °C for future use and evaluation.

Samples were evaluated with a spectrophotometer (NanoDrop® 2000, Thermo Scientific, Wilmington, DE, USA), which measures the concentration and purity of DNA samples. A minimum of 10 ng/µl, with a 260/280 purity score of 1.7-2.1 was required for use in Specific Target Amplification (STA).

2.3 Specific Target Amplification

STA was performed by following Fluidigm® SNPtype™ Assays for SNP Genotyping on the Dynamic Array™ IFCs protocol[13].

10X SNPtype™ STA Primer Pool was created by mixing 100 µM SNPtype™ Assay STA Primer and 100 µM SNPtype™ Assay Locus Specific Primer (for each assay in the 88 SNP panel) with DNA Suspension Buffer (10 mM Tris (pH 8), 0.1 mM EDTA) for a final primer concentration of 500 nM.

STA Pre-Mix aliquots were prepared by combining Qiagen® 2X Multiplex PCR Master Mix (PN 206143, Qiagen® Inc., Valencia, CA, USA) with the STA Primer Pool and PCR-certified water for a final concentration of 1X each for the Qiagen® Master Mix and SNPtype™ Primer Pool.

STA Pre-Mix was added to each genomic DNA sample (1.25 ul) in a 96-well Polymerase Chain Reaction (PCR) plate to make a final volume of 5 µl. Thermal cycling proceeded with a 95°C hold for 15 minutes followed by 14 cycles of 95°C denaturation for 15 seconds and 60°C annealing/extension for 4 minutes. bSTA

products were diluted 1:100 in DNA Suspension Buffer and stored at -20°C until genotyping.

2.4 Genotyping Reaction & Calls

SNPtype™ Assay Mixes were prepared by mixing 100 µM each of SNPtype™ Assay Allele Specific Primers (ASP1/ASP2), 100 µM SNPtype™ Assay Locus Specific Primer and DNA Suspension Buffer.

10X Assays were created for each of the 88 SNPs (total 5 µl) by combining SNPtype™ Assay Mix, 2X Assay Loading Reagent (final concentration of 1X) and PCR-certified water. For the eight unused assay wells on the chip, 2X Assay Loading Reagent was mixed with water for a final concentration of 1X in 5 µl.

Sample Mix was prepared by preparing a Sample Pre-Mix with final reagent concentrations of 1 X. Reagents consisted of Biotium 2X Fast Probe Master Mix (PN 31005, Biotium Inc., Hayward, CA, USA), SNPtype™ 20X Sample Loading Reagent, SNPtype™ 60X Reagent, ROX (PN 12223-012, Invitrogen™, Life Technologies, Grand Island, NY, USA) and water. 3.5 µl Sample Pre-Mix was added to 2.5 µl 1:100 diluted STA products. The Dynamic Array™ IFC chip was primed by injecting control line fluid into accumulators on the IFC chip, removing protective blue film and running the Prime (138x) script on the IFC Controller HX. After priming chip, 4 µl 10X Assay Mix was pipetted into each assay inlet and 5 µl of Sample Mix was pipetted into each sample inlet on IFC chip. Load Mix (138x) script on IFC Controller HX was run and chip was transferred to the Biomark™ HD for thermal cycling. SNPtype™ 96x96 v1 was run

according to protocol, which included four cycles of touchdown PCR, followed by thirty-four additional PCR cycles and was finally completed by a cooling cycle.

After thermal cycling was complete, information on the chip (IFC barcode and type) was loaded into the chip run file and saved.

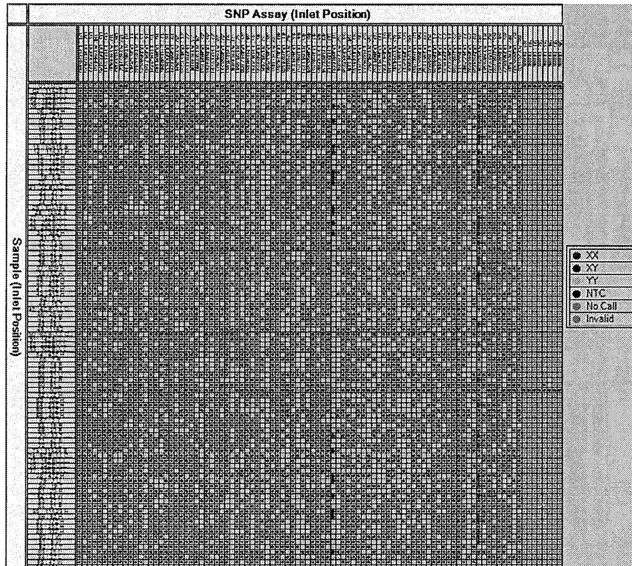

Figure 1. Call Map View. This represents the chip after it has been processed. Assay IDs are located across the top of the chip and sample IDs are located down the left side of the chip. Each colored square represents a genotype call for one sample of DNA against one assay.

Each Dynamic Array™ IFC Chip was processed individually using Fluidigm's® Genotyping Analysis Software. Fluorescence data from each chip was analyzed and grouped into color-coded scatter plots using the Auto-Call Analysis feature.

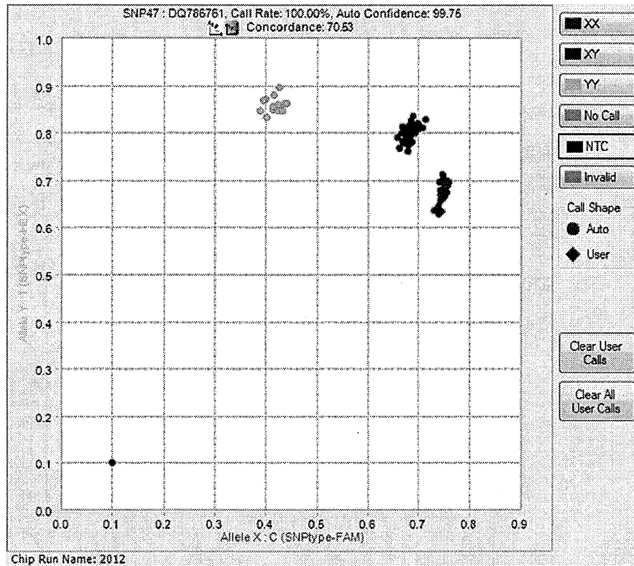

Figure 2. Scatter Plot. Each dot on the scatter plot represents one genotype call taken from the chip. Green and red represent homozygous calls and blue represents heterozygous calls.

2.5 Parentage Determination

CERVUS 3.0 and SireMatch 2.0 softwares were used to compare records collected from cattle producers to genotype results for each sample group to determine parentage of each offspring.

Calf	# Bulls w 0 Excl	Most Likely	# Excl	Prob	Compared Loci	Excl List	# Bulls w <= 2 Excl	Most Likely	# Excl	Prob	Compared Loci	Excl List	# Loci Genotyped
10Z BULL CALF	1	11 S	0	100	87		1	11 S	0	100	87		87 / 88
11Z H	1	2 N	0	100	88		1	2 N	0	100	88		88 / 88
12Z BULL CALF	1	27 U	0	100	88		1	27 U	0	100	88		88 / 88
13Z H	1	59 L	0	100	87		1	59 L	0	100	87		88 / 88
14Z BULL CALF	1	19 R	0	100	88		1	19 R	0	100	88		88 / 88
15Z H	1	15 T	0	100	88		1	15 T	0	100	88		88 / 88
16Z BULL CALF	1	81 P	0	100	88		1	81 P	0	100	88		88 / 88
17Z H	1	5 T	0	100	88		1	5 T	0	100	88		88 / 88
18Z BULL CALF	1	53 J	0	100	87		1	53 J	0	100	87		87 / 88
19Z H	1	55 W	0	100	88		1	55 W	0	100	88		88 / 88
1Z H	1	61 L	0	100	88		1	61 L	0	100	88		88 / 88
20Z BULL CALF	1	5 S	0	100	88		1	5 S	0	100	88		88 / 88
21Z H	2	9 W	0	100	88		2	9 W	0	100	88		88 / 88
22Z BULL CALF	1	25 U	0	100	88		1	25 U	0	100	88		88 / 88
23Z H	1	37 S	0	100	88		1	37 S	0	100	88		88 / 88

Table 1. Data snapshot from SireMatch 2.0

CHAPTER III

RESULTS

Of the 1,066 offspring samples submitted, 789 were verified by DNA identification to match herd records submitted by producers, indicating that 277 herd records were incorrect.

Herd records may be incorrect for a number of reasons. Simple errors in record keeping are the main errors seen on dairies due to the high percentage of cows artificially inseminated. For example semen or cow may have been recorded incorrectly or not at all. Also, the wrong semen may have been deposited in the wrong cow. The commercial beef industry generally turns out multiple bulls per pasture of cows. This makes it impossible for beef ranchers to know which bull bred which cow. On other purebred or smaller operations cows and bulls mixing and the rancher unaware is a reasonable possibility.

CHAPTER IV

DISCUSSION

4.1 Benefits & Future Use in the Industry

Parentage identification within cattle herds is an important aspect of record keeping. By utilizing SNP technology to determine parentage producers are able to keep more accurate records. It also allows for ranchers to accurately determine the sire, therefore providing a means to select specific replacement heifers based on the genetics and phenotype of specific bulls. In typical range production practices with multiple breeding sires, it is difficult to impossible to know who the sire is for each offspring. Use of SNP technology will allow producers to obtain a more rapid rate of genetic improvement.

Genetic Testing is a technology that has recently been applied in the livestock industry to determine parentage of offspring as well as determine the presence of markers that produce phenotypes of interest, such as marbling. In the future we would like to use Genetic Testing to determine if favorable genes have been passed on to offspring and more importantly if the sire we are interested in using carries those favorable genes and the likelihood he would pass those on to his offspring.

CHAPTER V

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