

Analysis of Vaginal Swabs for Paternity Testing and Marker-Assisted Selection in Cattle

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ABSTRACT

Blood is the standard source for DNA analysis, but requires venipuncture of cows by veterinarian and tedious and costly DNA extraction. A procedure was developed for sampling of vaginal cells from cows, establishing a cell lysis protocol using robotics, and applying fluorescent analysis of genetic markers. Two insemination technicians collected vaginal cells from 254 elite Israeli Holstein cows located in 152 herds using commercial Catch-All sample collection brushes. Cells were lysed in a 400- μ l solution, and 5 μ l was used as template for polymerase chain reaction (PCR). Sensitivity of the PCR was enhanced using only 1 μ l of lysed cells. Eight markers of the International Society of Animal Genetics paternity panel were amplified in four separate PCR. ILSTS039, a marker for a quantitative trait loci on BTA14, was amplified in a separate reaction. Genotypes from one to nine genetic markers were obtained for 253 out of 254 samples, and 244 cows had genotypes for at least three markers (96%). Cows that did not inherit either paternal allele for at least two loci were considered not to be daughters of the sire listed. Fifteen cows met this criterion, for a paternity misidentification rate of 6.25%. The frequency of allele 225 of ILSTS039, which was associated with increased milk fat content, was 11.1% in the bull-dam population, similar to the 12% found in the cow population in Israel. The use of vaginal cells for genetic analysis is accurate, as demonstrated by replicated analysis and by comparison to individual and population analysis based on DNA derived from blood.

(Key words: genetic marker, DNA microsatellite, parentage exclusion, marker-assisted selection)

INTRODUCTION

A variety of bovine specimens have been used successfully for PCR including cell biopsy, hair, blood, milk, and semen (Ron et al., 1995). Common applications of

DNA analysis in cattle are paternity and forensic testing, veterinary diagnosis, and gene mapping and screening. Genome scans for QTL in cattle based on the daughter design (Weller et al., 1990) and implementation of marker-assisted selection are dependent on simple and robust collection of cells from specific cows dispersed in large number of herds. In large genome-scan projects thousands of cows were sampled by blood (Ron et al., 2001) and milk (Mosig et al., 2001).

Blood is the standard source for DNA analysis, but requires puncture of cows by veterinarian and tedious and costly DNA extraction. Milk can only be used as template for PCR with radioactive labeling, due to the low fluorescent signal (Mosig et al., 2000; Ron, unpublished data). We therefore sought an alternative sampling method of biological material from cows that would be amenable for PCR, robotics, and fluorescent detection, without the need for prior extraction of DNA.

Human female genital specimens have traditionally been used for PCR-based detection of infectious diseases, population genetics, and forensic applications (Weisenfeld et al., 1996; Brennan et al., 2001). The objectives of this study were to develop a procedure for sampling of vaginal cells from cows, establish a cell lysis protocol using robotics, and apply fluorescent detection of genetic markers for paternity testing and marker-assisted selection.

Two hundred seventy-five Israeli Holstein cows with the highest genetic evaluations for the Israeli breeding index were selected for sampling of vaginal cells. The cows were born between 1992 and 1998. Two inseminators collected vaginal cells from 254 of these cows located in 152 herds using the following procedure. The cows in the herd were chest locked against the feeding lot, the tail of the selected cow was raised to allow cleaning of the skin surrounding the vagina by a paper towel, and the commercial Catch-All sample collection brush (Epicentre Technologies, Wisconsin, USA) was placed inside the cow's vagina and twirled three times. The handle of the brush was cleaned before it was returned to its pre-labeled container with the cow's number. The swabs were kept in their containers at ambient temperature until arrival at the laboratory, and at 4°C thereafter.

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Swabs were organized in batches of 91 in racks of 12 × 8 positions, allowing two empty positions and three positions with DNA of a known sire. These samples served as negative and positive controls, and also for verification of plate orientation. The swabs were removed from their packages and placed in the respective positions in a deep 1-ml 96-plate (#267001, Beckman) with a 400- μ l lysis buffer in each pore. The lysis buffer included the following ingredients at final concentration: Tween, 0.0025% (Sigma-Aldrich, St. Louis, MO); NP40, 0.0036% (Sigma-Aldrich); 1× PCR buffer with 1.5 mM MgCl₂; and Proteinase K (Roche, Mannheim, Germany) at 120 μ g/ml. After 5 min, each swab was squeezed gently against the walls of the pore and discarded. The plate was vortexed for 15s, and centrifuged at 4000 rpm at room temperature (Eppendorf, Germany) for 1 min (vortex/spin thereafter), incubated in water bath at 60°C for 30 min, vortex/spin, incubated at 95°C water bath for 8 min, vortex/spin, incubated at 95°C water bath for 8 min, put on ice, and vortex/spin for 15 min at 4°C. Using a Hydra dispenser robot (Robbins Scientific, www.robosc.com) 5 μ l was transferred to standard 96-well replica plates. The plates were dried overnight in laminar hood and kept dry in room temperature until subsequent PCR reactions following Ron et al. (2001). We used the same protocol to compare the lysis buffer prepared in our laboratory to a commercial lysis solution designed for buccal swabs (Epicentre Technologies, Madison, WI). Both solutions yielded similar results (data not shown), thus only our own laboratory's lysis buffer was used in this study.

Eight markers of the International Society of Animal Genetics paternity panel were amplified in four separate PCR: ETH10 and ETH225, BM2113 and BM1824, TGLA126 and TGLA227, and SPS115 and TGLA122. An additional locus, ILSTS039, which is a marker for a QTL on BTA14, was amplified in a separate reaction (Weller et al., 2003). All nine microsatellites are located on different chromosomes. Annealing temperatures of PCR ranged from 55 to 64°C. Information on all the markers, except for ILSTS039, is at: <http://sol.marc.usda.gov/>.

Two microliters from each PCR were pooled and loaded on ABI 377 DNA sequencer gel, as described by Ron et al. (2001).

The number of valid genotypes per cow is given in Table 1. Genotypes from one to nine genetic markers were obtained for 253 out of 254 vaginal swabs. Thus, virtually all swabs contained cells that were amenable for lysis, and subsequent PCR of single or duplex marker systems. Cows (n = 244) had genotypes for at least three markers (96%). The lysed cells of the remaining nine cows were analyzed in triplicates with 25 and 1 μ l of lysate, separately, as opposed to the 5 μ l in the original reaction. Successful amplification for nearly all the markers was obtained with the lower volume of 1 μ l of lysate. Apparently excess of DNA or substances in the lysate were inhibiting PCR, and a five-fold dilution of the lysate restored PCR efficiency.

The number of alleles per marker varied from seven (BM1824, BM2113, ETH10, TGLA126) to 19 (TGLA122), with an average of 10 alleles per marker. The frequency of allele 225 of ILSTS039, which was associated with increased milk fat content, was 11.1% in the bull-dam population, similar to the 12% found in the cow population in Israel (Weller et al., 2003). This exemplifies the potential use of vaginal cells for genetic analysis and marker-assisted selection. Identical results were obtained for four cows with both swabs and blood, and for nine cows with triplicate analysis of individual swabs.

The 253 cows with at least a single genotype were listed as daughters of 52 sires. Semen samples for DNA analysis were collected for 48 of the listed sires. Each of the remaining four bulls was the listed sire for only a single cow in the sample. Thus, paternity could be verified for 249 cows. DNA was extracted from the semen samples, and the 48 bulls were each genotyped for all nine microsatellites as described (Ron et al., 1995). Paternity misidentification was deduced for the 244 cows with valid genotypes for at least three markers. Cows that did not inherit either paternal allele for at least two loci were considered not to be daughters of

Table 1. Number of markers with valid genotypes per cow.

Number of markers	Number of cows	Percent	Cumulative number of cows	Cumulative number of genotypes
1	5	2.0	5	5
2	4	1.6	9	13
3	6	2.4	15	31
4	9	3.6	24	67
5	11	4.4	35	122
6	16	6.3	51	218
7	47	18.6	98	547
8	70	27.7	168	1107
9	85	33.6	253	1872

Table 2. The number of cows with single discrepancies, by number of valid genotypes per cow.

Number of valid genotypes	Number of cows	Cumulative number of cows
1	2	2
2	1	3
3	0	3
4	1	4
5	4	8
6	3	11
7	9	20
8	10	30
9	26	56

the sire listed. Fifteen cows met this criterion, for a paternity misidentification rate of 6.25%.

The rate of 6.25% is somewhat lower than the misidentification rate of 11.6% for cows in the general Israeli population born between 1990 and 1997 that were genotyped based on DNA extracted from blood (Ron et al., 1996; Ron, unpublished data). The true misidentification rate in the current sample is probably slightly higher, because exclusion probabilities (Jamieson and Taylor, 1997) were low for the cows with only a few valid genotypes, and some of the cows with single discrepancies were also probably not daughters of the listed sire. Furthermore, the mean birth year of the current elite cows' sample is about 2.5 yr younger than the previous sample.

There were 56 cows with discrepancies for a single marker. The number of cows with single discrepancies, by number of valid genotypes per cow is given in Table 2. Forty-five of these cows had valid genotypes for at least seven markers. With this number of valid genotypes it is expected that in most cases the number of markers without correspondence between the cow and the putative sire would be greater than one, if paternity is in fact incorrect. Thus most of the single discrepancies are probably due to genotyping mistakes. There were a total of 1654 valid genotypes for cows with genotypes for at least seven markers (Table 1). If all of these single discrepancies are due to mistakes, this would amount to 2.7% of the total genotypes for these cows, which is similar to a previous estimate based on analysis of DNA derived from blood (Ron et al., 1996, 2001).

The use of vaginal cells for genetic analysis is accurate, as demonstrated by replicated analysis, and by

comparison to individual and population analysis based on DNA derived from blood. The data presented in this study indicate that the amount of lysed cells in vaginal swabs is sufficient for 80 to 400 PCR reactions, using 5 to 1 μ l of lysate for PCR, respectively. Sensitivity of the PCR was enhanced using only 1 μ l lysed cells, as opposed to 5 μ l. Thus, vaginal cells provide a robust template for PCR that may be applied to a variety of PCR-based assays in cows. The potential for molecular analysis based on vaginal swabs of other animals warrant further study.

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