DNA profiling in sheep

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ABSTRACT

DNA profiles, or fingerprints as they are often called, provide the basis for a powerful parentage test that has a number of applications for the animal breeding industry. Two examples of its use are presented here. During a breeding program where single sires were being mated to groups of ewes, two groups became mixed, compromising the experiment. Using DNA profiling results, each of the progeny could be assigned to one of the two rams. The second example involves the auditing of pedigrees in which the Booroola gene is segregating. In searching for a DNA marker that segregates with the Booroola gene it is essential that flock records are accurate. In one pedigree a number of individuals that were wrongly assigned as daughters of a heterozygous Booroola sire were identified. In another pedigree all daughters were correctly assigned.

Keywords DNA profiling, progeny test, minisatellite, sheep pedigree analysis.

INTRODUCTION

DNA profiling is a technique used to analyse DNA from an individual by producing a series of bands not unlike a supermarket 'bar code'. These banding patterns or profiles, first demonstrated by Jeffreys *et al.*, (1985a), are unique to each individual (except identical twins). Repetitive DNA probes hybridise to dispersed autosomal hypervariable minisatellites in restriction digests of genomic DNA. Minisatellites or VNTRs (variable number tandem repeats) are found dispersed through out the genome of all animals studied so far. They are usually GC rich, short 9-64 bp sequences that are repeated in tandem. The number of repeats in tandem shows allelic variation thought to be brought about initially by mis-alignment and cross over at meiosis. The frequency of this event has been estimated at approximately 10^{-4} per kilobase of DNA per generation (Jarman and Wells, 1989) which is low enough to follow the inheritance of bands from either parent.

The applications of DNA profiling are far reaching and well documented in forensics (Gill *et al.*, 1985), immigration law (Jeffreys *et al.*, 1985b), paternity testing, transplant screening, ecological genetics (Kuhnlein *et al.*, 1989), segregation analysis with disease (Jeffreys *et al.*, 1986) and gene mapping (Wells *et al.*, 1989). In this paper we demonstrate the application of DNA profiling to the sheep breeding industry.

MATERIALS AND METHODS

Pedigrees and Probes.

The first pedigrees analysed were part of a breeding program for leaness in sheep. Two single sire mating groups became mixed such that either ram could have been the sire of the progeny. A sample of blood was taken from each ram and all progeny. The dams were not available at the time samples were taken for the experiment. The other two pedigrees were derived from progeny tests of Booroola rams heterozygous for the F gene. These pedigrees are being used to find genetic linkage between a DNA or blood protein marker and the Booroola gene. In these pedigrees only the sires and the female progeny were bled for DNA purification and analysis.

A total of five probes were used with these particular pedigrees.

1) M13 (Varssart *et al.*,1987).

2) Orf, which is a repeated DNA sequence from the parapoxvirus Orf (Fraser *et al.*, (1990), Crawford *et al.*, (in press)).

3) pUCJ, (Georges *et al.*, 1988).

4) pV4 and

5) pV20 which are probes derived from a lambda ZAP library of sheep genomic DNA prepared in this laboratory.

Labelling of Probes.

The M13 probe was labelled by primer extension as described by Jeffreys *et al.* (1985a). The other minisatellite probes, Orf, pUCJ, pV4 and pV20 were contained in recombinant plasmids, pVU34, pUCJ, pV4, and pV20 respectively. Digestion of pVU34 with the restriction enzymes Bst EII and Bam HI yielded a 523bp fragment. Digestion of pUCJ with Hind III and Eco R1 yielded a 250bp fragment. Digestion of pV4 with Eco R1 and Bam HI yielded a 3.8kbp fragment and digestion of pV20 with Eco R1 yielded a 6kbp fragment. These fragments were purified from preparative agarose gels using "Geneclean" according to the manufacturers instructions (Bio 101, La Jolla, CA). Between 20 and 50ng of these fragments were labelled with $\alpha[^{32}P]$dCTP using a random priming kit (Amersham, U.K.) according to the manufacturers instructions.

DNA Profiling

Sheep DNA was purified from white blood cells (Montgomery and Sise, 1990) and digested with either Alu I or a combination of Alu I and Hae III. The digested DNA was loaded on a 25 x 20 cm 0.7% agarose gel in TBE(Tris 0.089 pH 8.0, 0.089 Boric acid, 5mM EDTA) and electrophoresed at 55V for 40 hours. The DNA from the gel was transferred to Hybond N+ membrane by capillary action using the manufacturers instructions (Amersham, U.K.). The DNA was fixed to the membrane by treatment with 0.4M NaOH for 10 minutes. The membrane was then briefly washed in 2X SSC prior to placing in prehybridisation buffer (7% SDS, 1% BSA, 1% dextran sulphate, 1mM EDTA, 0.263M phosphate buffer pH 7.2) at 62°C. Hybridisation was performed at 62°C overnight in the same prehybridisation buffer containing labelled heat denatured probe. Membranes were washed consecutively in 2X SSC + 0.1% SDS at 62°C for 5, 15, and 30 minutes before being blotted dry, wrapped in "Gladwrap" and autoradiographed with intensifying screens at -80°C.

RESULTS

The DNA profiles in Figure 1a are from two rams and their progeny using the Orf probe. Bands were identified in each sire that were not present in the profile of the other. In ram 120, 6 such bands were identified (small arrows) whereas ram 342 had 7 bands (large arrowheads). The presence or absence of each of these bands was then scored in the progeny lambs (Table 1).

TABLE 1 Assignment of sires using band sharing from DNA profiles probed with Orf.

Progeny	Ram 120 bands	Ram 342 bands	Sire assignment
4	4/6	0/7	120
54	4/6	1/7	120
27	4/6	1/7	120
26	4/6	1/7	120
233	1/6	5/7	342
232	1/6	4/7	342
272	0/6	5/7	342
271	2/6	7/7	342
227	1/6	6/7	342
228	0/6	5/7	342
281	0/6	3/7	342
13*	2/6(3/3)	0/7(1/3)	120
80	0/6	4/7	342

* daughter that was probed twice, () = bands shared with sire when the DNA profiles was probed with M13.

For all but one of the progeny there is a clear majority of bands inherited from the one sire. Some of the progeny however appear to have inherited one or two bands from the alternate sire, but it is likely that these bands will have been inherited from the dams, which were not available for analysis.

In one case, lamb 13 showed only 2 bands were inherited from sire120 and none from sire 342. The inheritance of only two bands was not considered sufficient evidence of parentage. We therefore used a second probe, M13, which confirmed lamb 13 was the progeny of sire 120 as 3 further bands were inherited using this probe (Figure 1b).

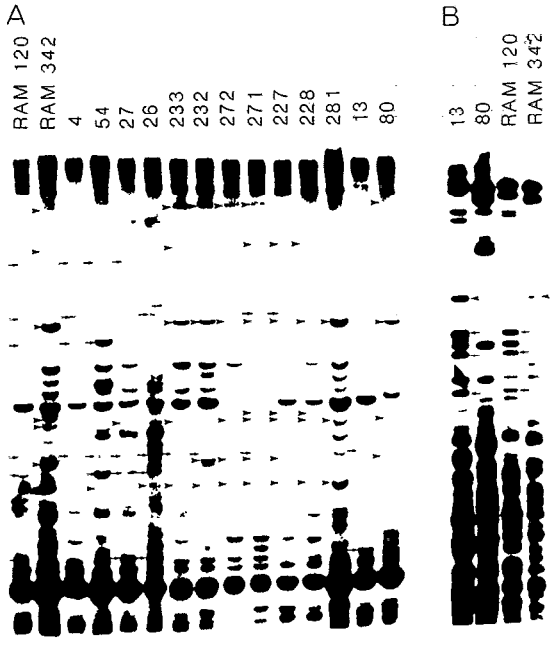

FIG 1 a: DNA profile of the two rams 120, 342 and the 13 progeny probed with Orf. Bands scored from Ram 102 are represented by a arrow and bands scored from Ram 342 are represented by a filled in arrow head. **b:** DNA profiles of the two Rams, 102 and 342, with progeny 13 and 80, probed with M13.

TABLE 2 Band sharing in DNA profiles from Booroola pedigree 153

Daughter	Proportion of sires bands found					
	M13	pV20	pV4	Orf	Total	
1	16/23	6/10	2/4	2/3	26/40	65%
2	10/23	2/10	0/4	1/3	13/40	33%
3	5/23	2/10	1/4	1/3	9/40	23%
4	14/23	8/10	1/4	1/3	24/40	60%
5	14/23	7/10	4/4	3/3	28/40	70%
6	15/23	7/10	0/4	3/3	25/40	63%
7	17/23	9/10	2/4	3/3	31/40	78%
8	18/23	6/10	1/4	3/3	28/40	70%
9	5/23	7/10	3/4	0/3	15/40	38%
10	13/23	7/10	1/4	1/3	22/40	55%
11	2/23	4/10	3/4	2/3	11/40	28%

Table 2 summaries the results from screening a pedigree containing a ram and eleven putative daughters. Once again the dams were not available for testing. The DNA profiles from each individual were probed with M13, pV20, pV4 and Orf. The numbers of scoreable bands from the sire in each instance were 23, 10, 4, and 3 respectively. The progeny were scored for these bands. One would expect all daughters of the ram to inherit approximately 50% of the bands from their sire. Some bands are however in common to all individuals so one would expect all daughters to have more than 50% of bands in common with their sire. Four of the daughters had less than 50% of the paternal bands indicating an incorrect assignment as daughters of this ram.

In another larger pedigree (Table 3) all daughters contained greater than 50% of their putative sire's bands and their pedigree assignment could be confirmed.

TABLE 3 Band sharing in DNA profiles from Booroola pedigree 109

Daughter	Proportion of sires bands found				
	M13	pV20	pUCJ	Total	
1	7/11	14/18	2/4	23/33	70%
2	6/11	11/18	3/4	20/33	61%
3	6/11	10/18	2/4	18/33	55%
4	7/11	13/18	2/4	22/33	67%
5	6/11	9/18	3/4	18/33	55%
6	7/11	9/18	3/4	19/33	58%
7	7/11	11/18	3/4	21/33	64%
8	8/11	12/18	4/4	24/33	73%
9	7/11	13/18	3/4	23/33	70%
10	7/11	11/18	3/4	21/33	64%
11	7/11	8/18	3/4	18/33	55%
12	6/11	9/18	4/4	19/33	58%
13	7/11	11/18	3/4	21/33	64%
14	8/11	11/18	4/4	23/33	70%
15	6/11	12/18	3/4	20/33	61%
16	8/11	12/18	2/4	22/33	67%
17	5/11	13/18	3/4	21/33	64%
18	7/11	11/18	2/4	20/33	61%
19	8/11	13/18	1/4	22/33	67%
20	7/11	13/18	3/4	23/33	70%
21	9/11	10/18	2/4	21/33	64%
22	7/11	11/18	2/4	20/33	61%
23	7/11	11/18	3/4	21/33	64%
24	7/11	16/18	2/4	25/33	76%

DISCUSSION

Results from these experiments demonstrate that repetitive DNA probes identify multiple bands in sheep DNA and the collective use of these probes provides a powerful parentage test that can solve practical problems that occur in sheep breeding. While all breeders and animal scientists strive to avoid the mixing of animals our two examples show that despite the best intentions accidents can happen. Mismothering during spring storms will always be a problem. DNA profiling provides a solution for these problems as it can be used retrospectively to determine genetic relationships.

At present we have no idea how accurate flock records are in the New Zealand sheep breeding industry. It is possible that most records are accurate and do not retard the genetic improvement of breeders' flocks, but the reverse may also be true. Clearly it is important, now that we have the technology, that this question be addressed.

Until the advent of DNA profiling, blood typing was the only method available for checking the parentage of offspring. Blood typing has the advantage of being a relatively simple and cheap test but it cannot always give an unequivocal answer. DNA profiling is essentially unlimited in the number of genetic differences it can detect. Not only do the DNA probes detect more loci, the loci they detect often have multiple alleles. Each of these highly variable bands can be regarded as an inherited trait of each individual so results from a number of probes can be combined to give sufficient numbers of observations to assess inheritance. New probes are continually being isolated, and in fact three of the five probes used in this study have been isolated in our own laboratory.

In all the pedigrees used in this study the dams were unavailable for analysis. Because we could examine large numbers of loci this was not an impediment whereas the small number of loci available for blood typing analysis limited its use. For example, in the first test (Figure1, Table 1) only one of the progeny could be assigned using blood typing (M. L. Tate, personal communication). The ram in the Booroola pedigree (Table 2) was heterozygous at most of the blood typing loci and as a result, three of the four daughters excluded by their DNA profiles could also be excluded by their blood typing results (M. L. Tate, personal communication).

The adoption of this test by the animal breeding industry will to a large extent be dependent on its cost. At present the cost of the test is very high. We estimate that the cost of labour and materials is approximately $35 per individual tested. This will limit its present uses to situations where parentage information is at a premium. The pedigrees in this paper are examples of this. The leaness pedigrees were part of long term breeding program and correct parentage information is absolutely essential for the genetic linkage studies being undertaken to find the Booroola gene It is also possible that owners of particularly valuable breeding animals may wish to authenticate their pedigree records with this test but our challenge must be to reduce the cost so DNA profiling will be available to all breeders.

One strategy which we intend using immediately is to combine blood typing with DNA profiling. Blood typing will resolve many parentage assignments cheaply. The remainder will still require a DNA profile but the overall cost is reduced. Our ultimate goal however is to simplify the DNA profiling itself so we can offer a cheap and reliable test of parentage.

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