*Animal Genetics,*
1997, **28,** 418–426

# AFLP™ markers for DNA fingerprinting in cattle

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## Summary

This work reports on use of the recently described amplified fragment length polymorphism (AFLP) technology for DNA fingerprinting in cattle. The AFLP technology produces molecular markers through the high-stringency polymerase chain reaction (PCR)-amplification of restriction fragments that are ligated to synthetic adapters and amplified using primers, complementary to the adapters, which carry selective nucleotides at their 3' ends. While, for plants, the double digestion of genomic DNA with *Eco*RI and *Mse*I is suggested, in mammals the enzyme combination *Eco*RI/*Taq*I produces clearer and more polymorphic AFLP patterns. In a sample of 47 Italian Holstein genotypes, 16 *Eco*RI/*Taq*I primer combinations identified 248 polymorphic bands in a species known for its low level of restriction polymorphism. In spite of the low information content carried by each AFLP polymorphism (average polymorphism information content = 0·31), the number of fragments revealed by each primer combination increased significantly the level of genetic information gained in each experiment. AFLP patterns are reproducible in independent experiments and polymorphic fragments segregate in cattle families according to Mendelian rules.

*Keywords*: PCR, restriction polymorphism, selective amplification

## Introduction

In farm animals, molecular markers are utilized for several important applications. Among these applications are the preparation of medium density linkage maps, useful for localizing genetic factors controlling economically important simple and complex traits, and the fingerprinting of the genome, necessary for paternity testing and for the investigation of animal biodiversity and evolution.

RFLPs (restriction fragment length polymorphisms), the first markers introduced, are being currently replaced by other ones based on polymerase chain reaction (PCR) amplification, like microsatellites (Tautz 1989) and RAPDs (randomly amplified polymorphic DNA; Williams *et al.* 1990). Molecular markers produced by PCR have several advantages over RFLPs: the laboratory protocols to produce them are simpler; a smaller amount of DNA is required; no extensive purification of DNA is necessary; no probe isolation and maintenance is needed; and the exchange of markers between laboratories is only based on the exchange of DNA sequences. Microsatellites, in addition, are very informative, being capable of following the segregation at multiallelic genetic loci in different crosses. However, the development of microsatellite markers is a costly process, based on sequencing and designing of locus-specific primers. Furthermore, RAPDs are very sensitive even to slight changes in the amplification conditions and are occasionally difficult to reproduce (Valentini *et al.* 1996). In addition, RAPD markers are dominant and therefore not very informative for some applications.

A new PCR technique for the production of molecular markers in a multiplex reaction has been recently described (Zabeau & Vos 1993; Vos *et al.* 1995). Amplified fragment length polymorphism (AFLP™) markers can be produced with a generic set of primers and do not depend on sequence information or probe availability. They are generated from restriction fragments ligated to double-strand adapters and are selectively amplified with primers, complementary to the adapters, which carry additional random nucleotides at their 3' end that probe the internal sequence of restriction fragments. AFLP simultaneously screens high numbers of loci for polymorphism and detects a greater number of polymorphic DNA markers than any other PCR-based detection system (Vos *et al.* 1995).

In crop plants AFLP technology has already been very successfully utilized (Becker *et al.* 1995; Maugham *et al.* 1996). For most plant species, the enzyme combination *Eco*RI/*Mse*I is recommended. The use of *Eco*RI/*Mse*I in animal DNA results in an excessive number of fragments in the restriction fragment 'library'. To reduce the number of fragments efficiently, primer extensions of three nucleotides will often not be sufficient. Therefore, in mammals the use of *Taq*I is suggested (Vos & Kuiper 1996).

In this work we used *Eco*RI/*Taq*I AFLP for fingerprinting a sample of Italian Holstein dairy

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*Accepted 26 August 1997*

cows and bulls. The results we present below demonstrate the reproducibility and suitability of AFLP analysis for the study of the cattle genome. Our results also show the AFLP efficiency in assaying, with minimal experimental effort, thousands of restriction fragments within a single cattle breed.

## Materials and methods

### *Animals*

Eleven cows, housed in the stable of the University of Piacenza, and 36 Italian Holstein bulls, randomly chosen from those tested in the national progeny testing scheme by ANAFI (National Breeders Association of Italian Holstein), were analysed.

The ND8 full-sib family (International Bovine Reference Family Panel; IBRP) was utilized for assessing AFLP Mendelian segregation. This family derives from an interspecific cross between a *Bos taurus* N'Dama sire and a *Bos indicus* Boran dam.

### *DNA extraction*

Genomic DNA from Holstein cows was extracted from whole blood and purified according to the protocol described by Sambrook *et al.* (1989). Following resuspension in 10 mM Tris, 1 mM EDTA, pH 8·0, DNA quality and concentration were tested on a 1% agarose gel in comparison with known concentrations of phage λ DNA. Genomic DNA from Holstein bulls was a gift of S. Sangalli from the Laboratorio Gruppi Sanguigni (LGS), Cremona, Italy. Genomic DNA from the IBRP was kindly provided by H. Leveziel from Laboratoire de Génétique biochimique et de Citogénétique, INRA, Jouy-en-Josas, France. We had no evidence of partial DNA digestion using *Eco*RI and *Taq*I on the three DNA sources used for AFLP analysis.

**Table 1.** Adapters and primers used in AFLP analysis

| | Name | Sequence |
|---|---|---|
| Adapters *Eco*RI | *Eco* top strand | 5'-CTCGTAGACTGCGTACC |
| | *Eco* bottom strand | 5'-AATTGGTACGCAGTCTAC |
| Adapters *Taq*I | *Taq* top strand | 5'-GACGATGAGTCCTGAC |
| | *Taq* bottom strand | 5'-CGGTCAGGACTCAT |
| Primers *Eco*RI | E01 | 5'-GAC TGC GTA CCA ATT CA |
| | E32 | 5'-GAC TGC GTA CCA ATT CAA C |
| | E33 | 5'-GAC TGC GTA CCA ATT CAA G |
| | E35 | 5'-GAC TGC GTA CCA ATT CAC A |
| | E38 | 5'-GAC TGC GTA CCA ATT CAC T |
| | E39 | 5'-GAC TGC GTA CCA ATT CAG A |
| | E42 | 5'-GAC TGC GTA CCA ATT CAG T |
| | E44 | 5'-GAC TGC GTA CCA ATT CAT C |
| | E45 | 5'-GAC TGC GTA CCA ATT CAT G |
| Primers *Taq*I | T01 | 5'-GAT GAG TCC TGA CCG AA |
| | T02 | 5'-GAT GAG TCC TGA CCG AC |
| | T32 | 5'-GAT GAG TCC TGA CCG AAA C |
| | T33 | 5'-GAT GAG TCC TGA CCG AAA G |
| | T35 | 5'-GAT GAG TCC TGA CCG AAC A |
| | T38 | 5'-GAT GAG TCC TGA CCG AAC T |
| | T48 | 5'-GAT GAG TCC TGA CCG ACA C |
| | T49 | 5'-GAT GAG TCC TGA CCG ACA G |
| | T50 | 5'-GAT GAG TCC TGA CCG ACA T |
| | T51 | 5'-GAT GAG TCC TGA CCG ACC A |

### *AFLP analysis*

The AFLP technology has been previously described in detail (Vos *et al.* 1995; Vos & Kuiper 1996): a genomic DNA digest is obtained by the use of both a rare cutter and a frequent cutter restriction endonuclease; synthetic adapters are ligated to the restriction fragments to serve as primer-binding sites; and the AFLP primers carry selective nucleotides at their 3' end, allowing the selective amplification of specific subsets of restriction fragments.

The sequence of *Eco*RI and *Taq*I adapters and primers used in the experiment described here is shown in Table 1. To produce *Eco*RI/*Taq*I AFLP markers, 400 ng of DNA was incubated for 1 h at 65°C with 5 units *Taq*I (Pharmacia Biotech Inc, Milwaukee, WI) in a volume of 25 µl containing: 10 mM Tris-HAc pH 7·5, 10 mM MgAc, 50 mM KAc, 5 mM dithiothreitol (DTT) and 50 ng/µl bovine serum albumin (BSA). In the next step, 15 µl of a solution having the same DTT, BSA and ion composition and containing 5 units of *Eco*RI (Pharmacia, Biotech Inc, Milwaukee, WI) was added and the resulting 40 µl was incubated at 37°C for 1 h. To ligate adapters, 10 µl of a solution containing 5 pmol *Eco*RI adapters, 50 pmol *Taq*I adapters, 1 unit DNA ligase (Pharmacia) and 1 mM ATP, in the same salt, DTT and BSA concentration, was added and the final 50 µl incubated at 37°C for 3 h. Template DNA was diluted 1:10 with 10 mM Tris-HCl, 0·1 mM EDTA (pH 8·0) before further use.

Five microlitres of diluted template DNA was then added to a PCR reaction mix containing: 10 mM Tris-HCl (pH 8·3), 1·5 mM $MgCl_2$, 50 mM KCl, 0·2 mM each of the four dNTPs, 1 unit Ampli*Taq* polymerase (Perkin-Elmer Cetus, Norwalk, CT) and 75 ng each of *Eco*RI and *Taq*I primers carrying one selective nucleotide (A for *Eco*RI and A or C for *Taq*I, see Table 1), in a total volume of 50 µl. Samples were subjected to 30 cycles of (pre)amplification with the following

profile: 30 s at 94°C, 1 min at 56° and 1 min at 72°C, followed by 10 min at 72°C for the completion of partial amplifications. The preamplified template was diluted 20-fold with 10 mM Tris-HCl, 0·1 mM EDTA (pH 8·0) and processed further.

Five microlitres of diluted preamplified template was added to a PCR reaction mix to a final concentration of 10 mM Tris-HCl (pH 8·3), 1·5 mM $MgCl_2$, 50 mM KCl, 0·2 mM each of the four dNTPs, 0·4 units Ampli*Taq* polymerase (Perkin-Elmer), 5 ng $^{33}P$ terminally labelled *Eco*RI primer and 30 ng unlabelled *Taq*I primer carrying arbitrarily chosen three selective nucleotides each, in a total volume of 20 µl. Amplification was performed for 36 cycles with the following profile: 30 s at 94°C for DNA denaturation, 30 s at 65°C as first annealing step and 1 min at 72°C for primer extension. In each following cycle, the annealing temperature was reduced by 0·7°C down to 56°C and thereafter kept constant until the completion of PCR run. The touchdown PCR promotes high-stringency amplification.

### *Detection and scoring of AFLP markers*

Labelled amplification products were mixed with an equal amount of formamide loading buffer, denatured and separated on a 40 cm 4·5% polyacrylamide sequencing gel in TBE buffer (100 mM Tris-HCl, 100 mM Boric acid, 2 mM EDTA). Gels were run at constant wattage (120 W). Following the run, the gel was fixed for 20 min in 10% acetic acid, rinsed with deionized water, dried and exposed to Fujix Phosphorimage screens (Raytest, Isotopenmessgeräte, Straubenhardt, Germany) for 16 h (or autoradiographed on Amersham Hyperfilm MP for 48 h (Amersham International, Little Chalfont, UK)). AFLP markers were scanned and visualized using a Fujix BAS-2000 Phosphorimage analysis system or read visually from autoradiographs.

We used an image analysis software package developed at Keygene NV for the specific analysis of AFLP pixel images. A full description of the software will be presented elsewhere (M. Kuiper *et al.* in preparation). The software allowed the identification and measurement of specific AFLP bands in a pixel image as produced by the Fujix BAS-2000. As a result, the presence/absence of a band could be scored. With refined quantification procedures, heterozygosity (corresponding to band intensity 50% of homozygous bands) could also be determined. The strict correlation between AFLP band intensity and zygosity has been thoroughly investigated in plants, where in several segregating populations (mainly F2s and backcrosses) heterozygotes and homozygotes for band intensity behaved as expected (M. Kuiper *et al.* unpublished). Therefore, AFLP markers can either be considered dominant ('0' = band absence, '1' = band presence) or codominant ('A' = homozygous band presence, 'H' = heterozygous band presence, 'B' = homozygous band absence) markers. Occasional faint bands were considered as missing data in dominant scores and classified with the ambiguity code 'C' (either 'H' or 'B') in codominant scores. Bands having intensity between heterozygous and homozygous were scored as 'D' (either 'A' or 'H') in codominant and as '1' (band presence) in dominant scoring. The computer software was designed to exclude markers having unexpected ranges of band intensities that did not fit dominant or codominant models.

### *Marker reliability*

The reproducibility of AFLP was tested across independent DNA extractions and template preparations. Profiles obtained on a set of six genotypes with six primer combinations were compared in at least three independent experiments carried out in Italy (one) and in The Netherlands (two).

### *Marker informativeness*

AFLP markers are essentially dominant, but codominant scoring allows the determination of the presence of one or two alleles at a locus. Marker informativeness was analysed after scoring the bands as codominant.

From molecular data, allelic frequencies (p and q) and observed heterozygosity ($Het_{obs}$) per marker were calculated as:

$$p_{(l)} = (nA_{(l)} + 1/2nH_{(l)})/(nA_{(l)} + nB_{(l)} + nH_{(l)});$$

$$q_{(l)} = 1-p_{(l)};$$

$$Het_{obs(l)} = nH_{(l)}/(nA_{(l)} + nB_{(l)} + nH_{(l)});$$

where $p_{(l)}$ is the frequency of the band presence allele of marker l and $q_{(l)}$ is the frequency of band absence allele of the same marker. $nA_{(l)}$, $nB_{(l)}$ and $nH_{(l)}$ refer, respectively, to the number of individuals homozygous for band presence, homozygous for band absence and heterozygous at marker l.

Expected heterozygosity ($Het_{exp}$) per marker was calculated as:

$$Het_{exp(l)} = 1-(p_{(l)}^{\ 2} + q_{(l)}^{\ 2});$$

Polymorphism information content (PIC) per marker, was calculated according to the equation

described by Botstein *et al.* (1980). All values were averaged over the codominant markers to obtain means and standard deviations. The effective number of alleles per locus (ne), an index that combines the number of alleles observed at a locus with their expected heterozygosity, was calculated according to Morgante *et al.* (1994). The total number of effective alleles surveyed by all AFLP analyses (Na) was also calculated. An assay efficiency index (Ai) of the information carried by AFLP markers per assay (corresponding to a primer combination), was calculated as:

$$Ai = Na/P$$

where Na is the total number of effective alleles surveyed and P the total number of primer combinations used.

*Mendelian inheritance*

Marker segregation was assessed in the ILRAD ND8 family belonging to IBRP. Sire, dam and 34 sons and daughters were typed with E35/T32 primer combination carrying ACA/AAC as selective nucleotides (see Table 1). Markers were tested for significant deviation from the expected Mendelian segregation using $\chi^2$-analysis.

## Results

*Marker reliability*

Independent DNA extractions and template preparations gave rise to identical AFLP profiles. Within and across laboratories, AFLP profiles proved to be highly reproducible with bands consistently identifiable even when using systems of detection of different sensitivity (autoradiography and scanner; Fig. 1).

Sixteen primer combinations generated 1098 bands with 304 of them being polymorphic. AFLP analysis software allowed 56 markers with an unexpected range of band intensity, therefore not fitting with the codominant or dominant models (see Materials and methods), to be discarded. The non-consideration of a marker band was most often the result of a migration too close to other polymorphic or monomorphic band(s) and, occasionally, because of co-migration of amplification products derived from independent loci. After the rejection of abnormal bands, 248 polymorphic bands were further considered (average of 15·5 per primer combination, range between 6 and 27; see Table 2). A high proportion of markers (160 out of 248 = approximately 65%) could be scored codominantly, while 88 markers could be scored only dominantly.

*AFLP informativeness*

Estimates of marker information content relied on data obtained from 160 codominant AFLP markers. The observed heterozygosity ranged between 0·02 and 0·78 and averaged 0·38, while the mean expected heterozygosity value was 0·40 (range 0·09–0·50). As expected, the PIC values were lower than expected heterozygosity values and averaged 0·31 (range 0·08–0·38).

A total of 272 effective alleles were uncovered by the 160 codominant markers, with a quite low mean ne per marker (ne = 1·70). However, owing to the multiplex nature of AFLP markers, the assay efficiency index was quite high (Ai = 17·0) meaning that, on average, 17·0 effective alleles were detected per primer combination. This is to be considered an underestimate because all dominant markers were excluded from calculation.

*Mendelian inheritance*

Using the E35/T32 primer combination, a total of 19 segregating markers were identified in the ND8 family (Fig. 2). Among them, eight were heterozygous in one parent and absent in the other (expected segregation ratio: 1:1 for presence of the band (heterozygous) *vs* absence), nine were heterozygous in one parent and homozygous in the other (expected segregation ratio: 1:1 for presence of the band (heterozygous) *vs* presence of a stronger band (homozygous) and two were heterozygous in both parents (expected segregation ratio: 1:2:1 for presence of the stronger band (homozygous) *vs* band presence (heterozygous) *vs* band absence). Five additional markers were found to be polymorphic between parents. However, because one parent was homozygous for the presence of the band and the second parent had no band, these markers were monomorphic in the heterozygous progeny. No marker deviated significantly from the expected Mendelian segregation ratios (Table 3).

## Discussion

The AFLP procedure amplifies a high number of restriction fragments (60–150 bands in a single gel lane) and produces markers that are scored as presence or absence of a band. As the labelled primer used in the PCR reaction is rate limiting, and it is completely consumed during the reaction (Vos *et al.* 1995), target sequences are subjected to a quasi-quantitative amplification. As a result, individuals heterozygous at a locus have a band intensity that is approximately 50% of

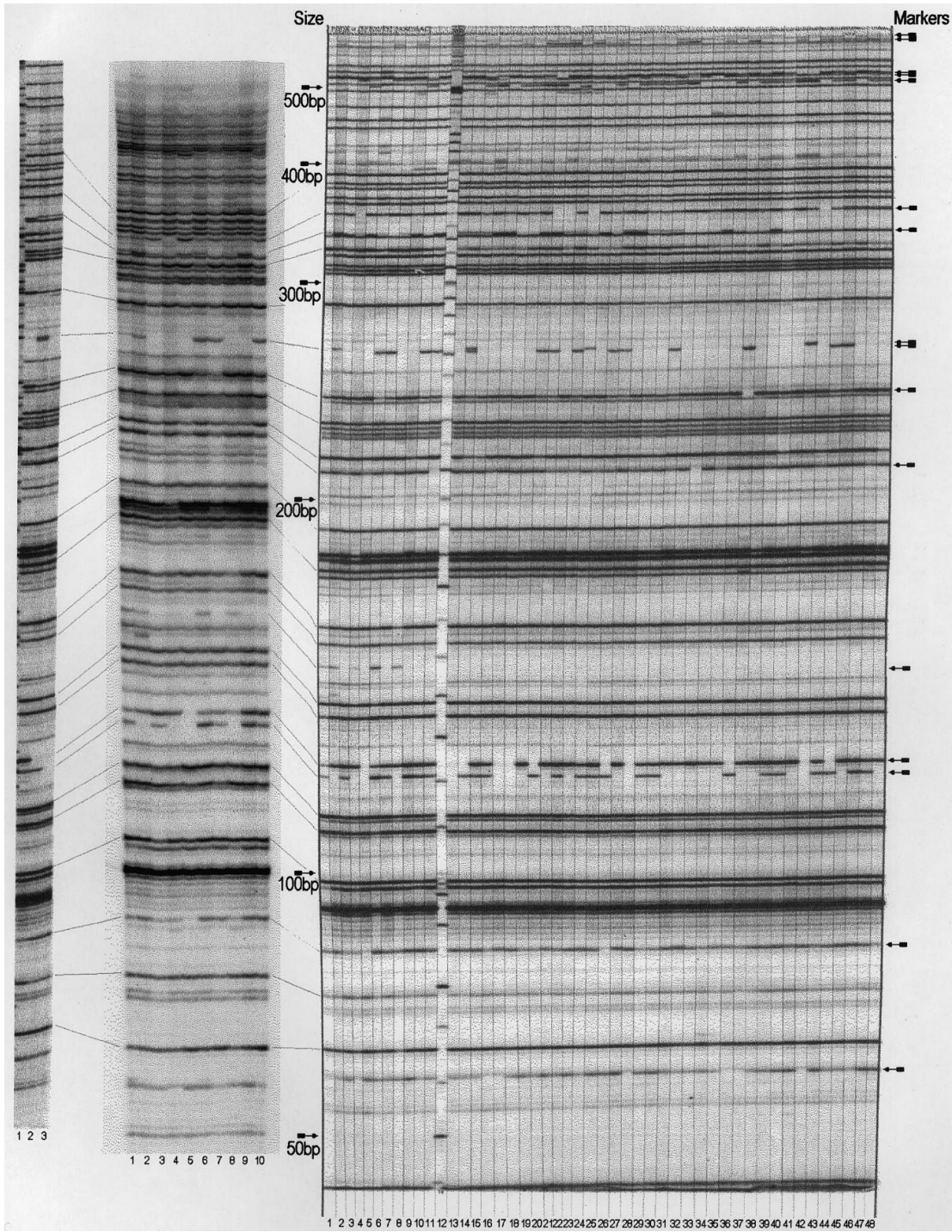


**Fig. 1.** Reproducibility of AFLP markers. Independent experiments were carried out at Keygene laboratories (left and right) and at the University of Piacenza (centre) using the primer combination E32/T32. Same genotypes are on lane 1 of the left gel, lane 3 of the centre gel and lane 3 of the right gel; lane 2 of the left gel and lane 14 of the right gel; lane 3 of the left gel and lane 13 of the right gel; lanes 1–10 of the centre gel and lanes 1–10 of the right gel. Dashed lines join corresponding bands. Arrows on the left of the right gel point to reference molecular weight marker bands (10 bp ladder, Research Genetics, run in lane 12). Arrows on the right of the right gel point to polymorphic markers.

the band intensity of homozygous genotypes. With the use of an appropriate software for densitometric analysis, the presence of one or two copies of the band presence allele can be measured and genotypes can be codominantly scored. To score a marker in a codominant way, at least one heterozygous and one homozygous individual should be assayed. The probability of observing both genotypic classes is a function of the allelic frequency of band presence and of the number of individuals analysed. Accordingly, the majority (145 out of 176 = 82%) of markers showing the presence of an AFLP band in more than 10 genotypes were scored codominantly, while only a few (15 out of 72 = 21%) markers showing the presence of an AFLP band in 10 or fewer individuals could be scored codominantly. It was presumed that at least some of the 88 markers that scored only dominantly – particularly those showing a low frequency of band presence – lacked one of the genotypic classes necessary for codominant scoring. Assuming the population in Hardy–Weinberg equilibrium, this is probably the class homozygous for the AFLP band presence. From our data, we could estimate the proportion of AFLP markers scorable in a codominant way. It ranged between a minimum of 53% (160/304), considering all polymorphisms identified, to a maximum of 82%, calculated in the subsample of markers showing the presence of the band in more then 10 genotypes. Considering the high throughput of this technology, the fact that not all AFLP markers could be codominantly scored was only a minor problem.

**Table 2.** Number of polymorphisms and number of polymorphisms that could be scored codominantly, found per primer combination, in a sample of 47 Italian Holstein genotypes

| Primer combination | No. of polymorphisms | No. of codominant polymorphisms |
|---|---|---|
| E32/T32 | 16 | 10 |
| E32/T33 | 17 | 11 |
| E32/T35 | 12 | 12 |
| E32/T38 | 15 | 8 |
| E32/T49 | 12 | 9 |
| E33/T49 | 14 | 9 |
| E35/T32 | 27 | 15 |
| E35/T48 | 16 | 10 |
| E39/T33 | 26 | 15 |
| E39/T48 | 13 | 9 |
| E42/T48 | 17 | 13 |
| E42/T49 | 9 | 8 |
| E42/T51 | 6 | 6 |
| E44/T48 | 12 | 7 |
| E45/T32 | 26 | 16 |
| E45/T48 | 10 | 2 |
| Total | 248 | 160 |

### *Reliability*

AFLP are based on a high-stringency PCR reaction that ensures the preferential amplification of template regions between annealing sites which have perfect complementarity to the primers used. Under these conditions, the technique is highly reproducible. In cattle, we observed a high consistency of AFLP profiles across independent DNA extractions and template preparations, within and across laboratories. This characteristic distinguishes AFLP from RAPD markers, which sometimes are not reproducible. RAPD markers rely, in fact, on low-stringency PCR reactions and are therefore strongly dependent on salt concentration, PCR temperature profiles, brand of thermostable DNA polymerase utilized and strategy used for band separation and detection (Valentini *et al.* 1996).

AFLPs behave as Mendelian markers in several plant species such as barley (Becker *et al.* 1995), potato (Meksem *et al.* 1995; van Eck *et al.* 1995), maize (P. Ajmone-Marsan *et al.* in preparation) and chicory (De-Simone *et al.* 1997). In cattle, evidence of the Mendelian behaviour of AFLPs was gained from a full-sib family where 19 segregating AFLPs all behaved as simple Mendelian traits.

### *Informativeness*

The AFLP technology was shown to be able to efficiently identify polymorphic markers in a sample of élite Italian Holstein genotypes. In this respect, they were more efficient than RAPDs, which detect only low-level or no polymorphism in cattle (Kantanen *et al.* 1995). Sixteen AFLP *Eco*RI/*Taq*I primer combinations were sufficient to generate almost 1100 amplified bands and to identify 248 polymorphisms. Compared with *Mse*I, commonly used to produce AFLP in plants, and experimented in rats (Otsen *et al.* 1996), the enzyme *Taq*I provides a superior level of polymorphism, presumably through the targeting of CpG dinucleotides. CpG dinucleotides are more prone to mutations through the transition of methylated cytosine to thymine. This results in a two- to fourfold increase in the detection of polymorphisms in AFLP fingerprints (M. Kuiper *et al.* unpublished).

AFLP polymorphisms originate from the same type of mutations as those detected by RFLPs

(base substitution, insertion-deletion and other rearrangements). AFLP markers are expected to be slightly more polymorphic than RFLPs, owing to the higher number of nucleotides assayed (AFLP – 16: six by the rare cutter, four by the frequent cutter and three + three by the selective nucleotides at the 3' end of each primer; RFLP – 12: six + six when a six-cutter endonuclease is used in Southern blotting).

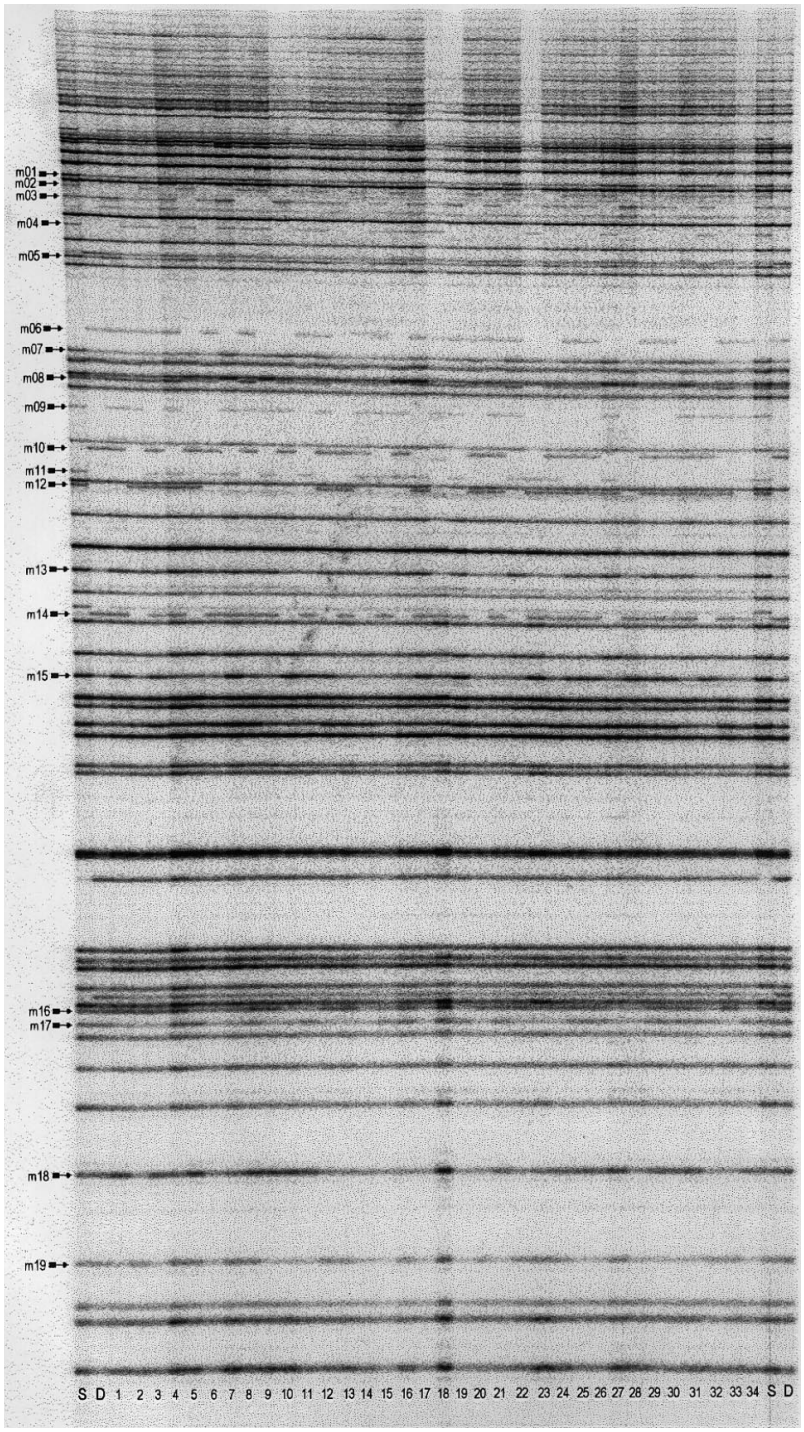


**Fig. 2.** Segregation of 19 AFLP markers (m01–m19) in 34 N'Dama × Boran full sib progenies. First and last two lanes are parental genotypes (S = sire, D = dam).

Consistently, the proportion of polymorphic AFLP markers we identified (248/1096 = 22%) was slightly higher than the one estimated from available RFLP data (69/350 = 20%; Fries *et al.* 1993). It was confirmed, moreover, that in cattle (compared with other organisms) a low level of restriction polymorphism existed. The detection of a high number of markers was therefore a result of the high number of restriction fragments assayed, rather than to the capacity of targeting highly polymorphic sequences. Compared to RFLP, however, the AFLP technique is expected to identify, very precisely, fragments of similar length, owing to a much higher resolution of sequencing compared with agarose gels. AFLP is therefore expected to permit a more accurate estimate of the genetic similarity between individuals or populations. This holds true within species and also across highly related species (J.A. Lenstra, personal communication).

In our sample, average heterozygosities, PIC values and ne per locus of individual markers were quite low (mean values 0·38, 0·31 and 1·70, respectively), in agreement with the data obtained by using RFLPs in sheep (Parsons *et al.* 1996). However, AFLP Ai was quite high (17·0). It is interesting to compare these values with those found in other genetic systems where AFLPs have been contrasted with other molecular marker classes. In maize and soybean, available data are consistent with our results: AFLP carried, per single marker, little information, less than any other system, but had quite high Ai values. In maize, an allogamous species having high genetic polymorphism, the AFLP Ai value was at least ninefold higher than Ai values observed for RFLP, RAPD and microsatellites (P. Ajmone-Marsan *et al.* unpublished), and more than double the Ai value that we observed in cattle (Ai = 45·7). Accordingly, in soybean (Powell *et al.* 1996), an autogamous species with low molecular polymorphism, the AFLP effective multiplex ratio (an index comparable to Ai, that counts the number of polymorphisms in the germplasm set of interest), was approximately 10-fold higher than that calculated on the same sample from RFLP, RAPD and microsatellite data. Its value was 19·2, a value in agreement with our results from cattle.

The application of AFLPs in farm animal studies depends on the species considered and on the goals of the research project. For fingerprinting, investigation of biodiversity and evolutionary studies, AFLP markers will probably find a wide diffusion. AFLP markers could also be adopted for map construction in species where microsatellites have not yet

**Table 3.** Number of individuals segregating 1:1 for presence *vs* absence or 1:2:1 for strong presence (homozygous = A), *vs* presence (heterozygous = H), *vs* absence (B) of AFLP bands in the N'Dama × Boran family

| | No. of individuals | | | | | |
|---|---|---|---|---|---|---|
| Marker | A | H | B | $\chi^2$1:1* | $\chi^2$1:2:1* | *P** |
| m01 | 13 | 21 | 0 | 1·176 | | 0·3 |
| m02 | 0 | 21 | 13 | 0·706 | | 0·4 |
| m03 | 5 | 18 | 11 | | 2·235 | 0·1 |
| m04 | 0 | 16 | 18 | 0·118 | | 0·7 |
| m05 | 14 | 20 | 0 | 0·706 | | 0·4 |
| m06 | 0 | 21 | 13 | 0·706 | | 0·4 |
| m07 | 13 | 21 | 0 | 1·176 | | 0·3 |
| m08 | 10 | 16 | 8 | | 0·235 | 0·6 |
| m09 | 0 | 19 | 15 | 0·118 | | 0·7 |
| m10 | 0 | 19 | 15 | 0·118 | | 0·7 |
| m11 | 0 | 16 | 18 | 0·118 | | 0·7 |
| m12 | 0 | 19 | 15 | 0·118 | | 0·7 |
| m13 | 23 | 11 | 0 | 1·765 | | 0·2 |
| m14 | 0 | 24 | 10 | 2·471 | | 0·1 |
| m15 | 16 | 18 | 0 | 0·118 | | 0·7 |
| m16 | 21 | 13 | 0 | 0·706 | | 0·4 |
| m17 | 16 | 18 | 0 | 0·118 | | 0·7 |
| m18 | 17 | 17 | 0 | 0·000 | | 1·0 |
| m19 | 17 | 17 | 0 | 0·000 | | 1·0 |

*$\chi^2$-values and probability (*P*) of the segregation to fit Mendelian ratios.

been developed, such as domesticated species of minor economical value and wild species. In addition, in main farm animal species, AFLP technology may provide a valuable tool for the saturation of existing microsatellite maps in specific genomic regions and for the quick search of markers linked to specific loci. In the same species, the potential of AFLP technology for mapping complex traits has still to be thoroughly investigated. In populations available for quantitative trait loci (QTL) studies, diallelic markers have less power of QTL detection than multiallelic markers (Soller 1990; Weller *et al.* 1990). Further research is needed to evaluate whether the combined use of AFLPs and microsatellites could enhance the efficiency of QTL analysis, and whether alternative strategies to follow the inheritance of complex traits can be designed, to better exploit a technology able to assay thousands of loci in any genome.

## Acknowledgements

Authors are grateful to F. Salamini for the revision of the manuscript. The corresponding author would like to thank Miranda Hornes for the excellent training in AFLP technology.

AFLP is a trademark filed by Keygene NV.

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