

GENETIC POLYMORPHISM OF SOME MICROSATELLITES ON CHROMOSOME SEVEN IN THE EGYPTIAN BUFFALO

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SUMMARY

Five bovine microsatellites were tested in the Egyptian buffalo genome to determine the amplification as well as the number of alleles if amplification was successful. These microsatellites, BM143, TGLA37, FBN13, ILSTS035 and ILSTS093 were tested in 70 lactating Egyptian buffaloes. All microsatellites tested amplified the buffalo DNA and produced a PCR product. Characterization of the PCR products on polyacrylamide gels revealed that three of the microsatellites tested were monomorphic (TGLA37, FBN13, ILSTS035), while the other two were polymorphic (BM143, ILSTS093). BM143 microsatellite had nine alleles with size ranging from 99 and 135 bp. Allele frequency ranged from 0.015 to 0.431. ILSTS093 showed eight alleles ranging in size from 193 to 217 bp with allele frequency ranging between 0.009 and 0.293. The results obtained indicated the successful genotyping of bovine microsatellites in the buffalo genome and suggested that some cattle microsatellites can be a valuable source for genome analysis in the Egyptian buffalo.

Keywords: Egyptian buffalo, chromosome 7, microsatellites, polymorphism

INTRODUCTION

The Egyptian buffalo has been an integral part of livestock agriculture in Egypt for over 1200 years (Bhat, 1992) producing milk, meat, leather, manure, and draft power for agricultural operations. Today, about four million buffaloes are raised in Egypt, providing 2.3 million tons of milk and 270,000 tons of meat annually (FAOSTAT, 2005). Moreover, the buffalo plays a vital role in socioeconomic life of farmers in Egypt.

Microsatellite markers are powerful research tools but their development is labor intensive and costly. Consequently, researchers have tried to use microsatellite markers developed from one species in another (Moore *et al.*, 1991). Microsatellite markers developed for *Bos taurus* have been successfully used in closely related species (buffalo: *Bubalus bubalis*) (Navani *et al.*, 2002 and Soysal *et al.*, 2005)

Despite the great advances in genomic technology observed in several animal species, the availability of molecular tools such as microsatellite markers typing or characterization is limited in the Egyptian buffalo where few studies were performed.

The aim of the present study was to investigate the polymorphism, the number and sizes of the obtained alleles for some bovine microsatellites in the buffalo

chromosome 7 equivalent to chromosome 6 in cattle, namely, BM143, TGLA37, FBN13, ILSTS093 and ILSTS035.

MATERIALS AND METHODS

Blood sample collection and DNA extraction

Blood samples were collected from 70 lactating unrelated buffaloes from the herd maintained at the Agriculture Experimental Station, Faculty of Agriculture, Cairo University. Blood was collected in 10 ml tubes containing EDTA as an anticoagulant matter. The samples were kept at 4°C and processed for DNA extraction in a period not exceeding 3 days from its arrival to the laboratory. DNA was extracted and purified using the standard Phenol–Chloroform technique described by Sambrook *et al.* (1989). DNA concentration was measured on U.V. spectrophotometer at a wave length of 260 nm.

PCR run with specific microsatellite primers:

All microsatellites chosen were located in cattle chromosome 6 which are correlated with milk yield and constituents in cattle (Georges *et al.*, 1995; Spelman *et al.*, 1996; Freyer *et al.*, 2002; Olsen *et al.*, 2002; Freyer *et al.*, 2003; Freyer *et al.*, 2004; Peter *et al.*, 2005; Schnabel *et al.*, 2005 and Chen *et al.*, 2006). Details of these microsatellites are presented in Table (1).

Table 1. Details of microsatellites chosen in cattle chromosome 6

Microsatellite	Primer sequence	Reference
BM143	ACCTGGGAAGCCTCCATATC CTGCAGGCAGATTCTTTATCG	Bishop <i>et al.</i> , 1994
TGLA37	CATTCCAATCCCCTATCCTGAG TGAATGATTCTATGAAGACCTGTA	Yoneda <i>et al.</i> , 1999
FBN13	ACTTTCATTAGATTGCTGCAAATAG AAATATGGAAACGACCTGTGG	Weikard <i>et al.</i> , 1995
ILSTS093	TGAAATATACCTGAGTAGCAGC TTGTTTTAACTCCCCACCCC	Kemp <i>et al.</i> , 1995
ILSTS035	TTGACCATAACAGCTACTCC TAGGTCCATGAATCACAGGG	Kemp <i>et al.</i> , 1995

Polymerase chain reaction (PCR) was carried out on 50 ng of genomic DNA in a 25 µl reaction of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 200 µM dNTP, 1.5 mM MgCl₂, 1 mM tetra-ammonium-chloride, 0.1% Triton X-100, 0.01% gelatin, 4.5 pmol of each primer and 0.25 U *Taq* DNA polymerase. The standard PCR run cycle was usually as: primary denaturation: 95 °C for 3 minute then 35 cycles as: 95 °C for 15 sec.; 55-60 °C for 30-60 sec.; 72°C for 30 sec, final extension: 72 °C for 5 minute and storage: 15 °C forever. The success of the PCR was detected on 2% agarose after running in a horizontal electrophoresis set and staining with ethidium bromide. For

optimization the PCR, the temperature and time of annealing temperature were changed.

The products of the successful PCR were characterized under denaturing conditions on 12 % polyacrylamide vertical electrophoresis (Sambrook *et al.*, 1989). After the end of the run, the gel was stained in an ethidium bromide solution (0.5 µg/ml TBE buffer). The gel image was captured electronically using Biometra gel documentation system. The allele sizes were determined using free software named Lab. image V2.7. It is dispersed free from Proland company (Germany), from the internet through the web page: <http://www.labimaging.com/servlet/engine/home/start.html>.

RESULTS AND DISCUSSION

The results of the prescreening by running the PCR products on 2% agarose gel electrophoresis were used to test the success of amplification. All microsatellites tested amplified the buffalo DNA and produced a PCR product. The PCR products were subjected to characterization on vertical polyacrylamide gel for identification of the polymorphism as well as the number and sizes of alleles if polymorphism is present.

The results showed that only two microsatellites tested were polymorphic (BM143; ILSTS093) meaning that they could be used further in parentage and QTL mapping studies. While the other three were monomorphic (TGLA37; FBN13; ILSTS035).

Polymorphic microsatellites:

BM143:

Figure (1) shows the characterization of microsatellite BM143 products on polyacrylamide gel.

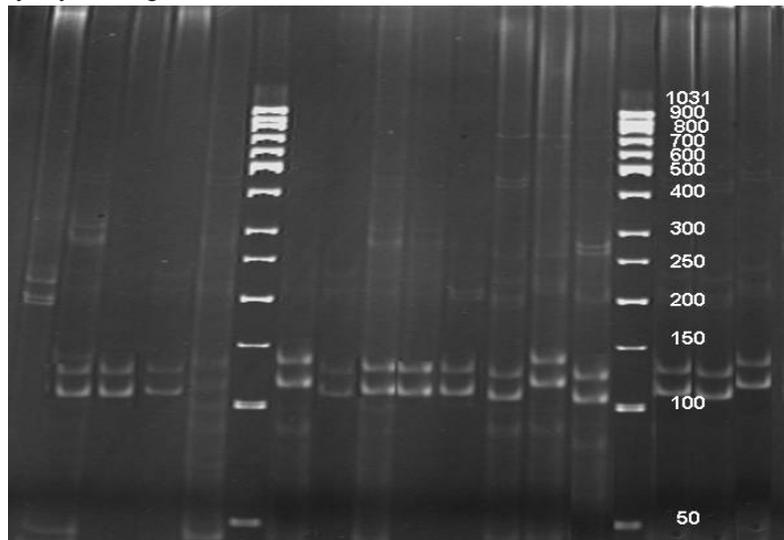


Fig. 1 Ethidium bromide stained polyacrylamide gel for the two separated alleles of microsatellite BM143 after running vertically

In general, this microsatellite showed totally nine alleles. The minimum allele size was 99 bp while the maximum was 135 bp. The frequencies of these alleles ranged from 0.015 to 0.431. The observed allele sizes of the BM143 microsatellite marker and their frequencies in the population under study are presented in Table (2).

Table 2. The observed allele size of BM143 microsatellite marker and their frequencies in the population under study

Allele number	Allele size (bp)	Allele frequency
1	99	0.031
2	105	0.030
3	109	0.431
4	115	0.023
5	119	0.015
6	121	0.023
7	125	0.408
8	131	0.023
9	135	0.015

The highest allele frequency was observed for the allele no 3 (109 bp) with a frequency of 0.431. While alleles no 5, 9 (119, 135 bp) were the lowest allele frequencies observed (0.015).

The observed heterozygosity was 1.00 and the expected heterozygosity was 0.644. Accordingly, the value of the expected heterozygosity was very high and the value of the observed heterozygosity was higher than the expected heterozygosity indicating much of genetic variability between individuals. Unfortunately, no previous studies for this marker were conducted on the buffalo genome. On the other hand, this microsatellite was reported to be polymorphic in cattle and produced many alleles (Bishop *et al.*, 1994; Kappes *et al.*, 1997; Ron *et al.*, 2001; Olsen *et al.*, 2004; Baruch *et al.*, 2006 and D'Angelo *et al.*, 2006).

Previous analysis of BM143 marker in cattle (*Bos taurus*) agreed with our results. Bishop *et al.* (1994) tested this marker in four breed crosses consisting of Gelbvieh, Simmental, Nellore, Hereford, Angus and Brahman. Crosses were designed to maximize heterozygosity of one or both parents and obtain samples of diverse gene pools. Also, Olsen *et al.* (2004) studied this marker in Norwegian dairy cattle breeds. They found that there were 13 alleles ranging in size between 90 and 122 bp. BM143 marker was studied likewise by Baruch *et al.* (2006) in the Israeli Holstein cattle where 13 alleles were found ranging in fragment length from 90 to 118 bp. Moreover, Bishop *et al.* (1994); Kappes *et al.* (1997); Ron *et al.* (2001) and D'Angelo *et al.* (2006) reported that this microsatellite was polymorphic in cattle.

BM143 microsatellite marker was not only studied in bovidae species but also in ovine and caprine species such as sheep and goats (De Gortary *et al.*, 1997 and Maudet *et al.*, 2004). The number of observed alleles ranged from 3 to 4 and allele size ranged from 102 to 128 bp in crosses came from three sheep breeds (Romanov, Rambouillet and Suffolk). Also, BM143 marker was analyzed in six indigenous Spanish sheep breeds Churra, Latxa, Manchega, Rasa-Aragonesa, Castellana and Merino by Arranz *et al.* (2001). The authors found that there were 11 alleles in

Churra and Latxa breeds, 10 alleles in Manchega and Merino breeds, 9 alleles in Rasa-Aragonesa breed and 6 alleles in Castellana breed. In goats, there were 9 alleles, and allele size ranged between 93 and 121 bp (Maudet *et al.*, 2004).

ILSTS093:

Microsatellite ILSTS093 was shown to be, also, a polymorphic. Figure (2) shows the characterization of its products on polyacrylamide gel.

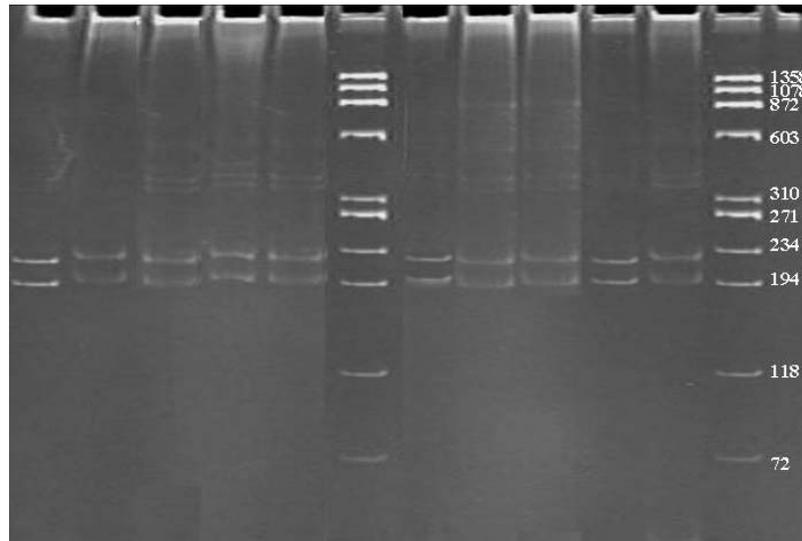


Fig. 2 Ethidium bromide stain for the two separated alleles of ILSTS093 marker, run vertically on polyacrylamide gel electrophoresis.

In general, this microsatellite showed a total of eight alleles. The minimum allele size was 193 bp while the maximum allele size was 217 bp. The frequencies of these alleles ranged from 0.009 to 0.293. The observed allele size of the ILSTS093 microsatellite marker and their frequencies in the population under study are presented in Table (3).

All animals under study were heterozygotes, accordingly, the observed heterozygosity was 1.00 and the expected heterozygosity was 0.789. Alleles no 1 and 5 (193, 207 bp) were the least frequent (0.009). While allele no 7 (213 bp) was the most frequent allele (0.293).

No previous studies for this allele were conducted on the river buffalo. ILSTS093 microsatellite was monomorphic in the African buffalo (*Syncerus caffer*) (Van Hooft *et al.*, 1999) and gave only one allele with the size of 166 bp. Navani *et al.* (2002) tested this marker in Indian buffalo (Murrah, Nili-Ravi and Mehsana breeds) but no amplification happened.

Previous analysis of ILSTS093 marker in cattle (*Bos taurus*) agreed with our results. Kemp *et al.* (1995) reported 8 alleles in cattle with a size range from 193 to 207 bp. Ma *et al.* (1996) tested this marker in four beef breeds of cattle (Angus,

Devon, Gelbvieh, Simmental) and found that, there were 11 alleles ranging in size from 186 to 212 bp. Vallejo *et al.* (2003) reported seven alleles in Holstein cattle. These differences in number of alleles are due to the type of cattle breed studied and the genetic polymorphism within the breed itself.

Table 3. The observed allele sizes of ILSTS093 microsatellite marker and their frequencies in the population under study

Allele number	Allele size (bp)	Allele frequency
1	193	0.009
2	195	0.094
3	199	0.292
4	203	0.094
5	207	0.009
6	209	0.104
7	213	0.293
8	217	0.104

Monomorphic Markers

ILSTS035:

Figure (3) shows the result of the amplification after running the product on 2% agarose and staining with ethidium bromide.

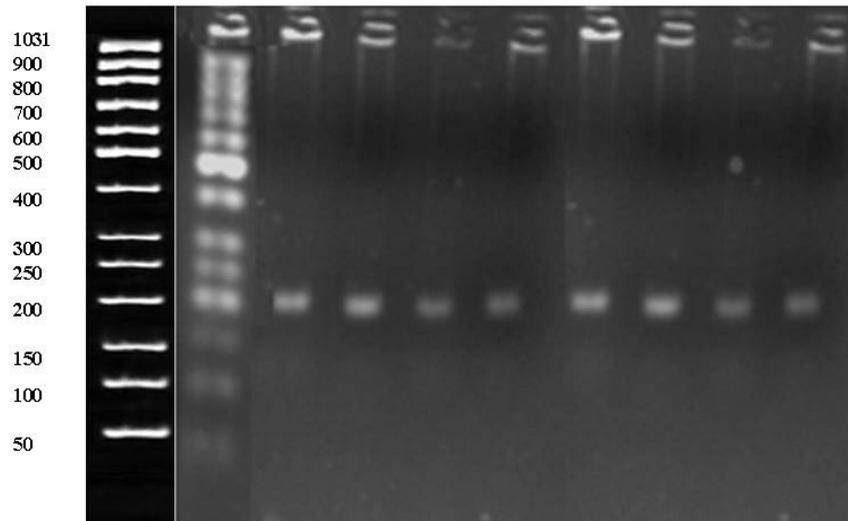


Fig. 3 Ethidium bromide stained agarose gel for the PCR product of microsatellite ILSTS035 after running horizontally

Characterization of the PCR products on polyacrylamide gel revealed the presence of only one allele with 189 bp size length. Figure (4) shows the ethidium bromide stained for the polyacrylamide gel. Monomorphic markers probably reflect the absence of allelic variation rather than primer slippage (Hayden and Sharp, 2001).

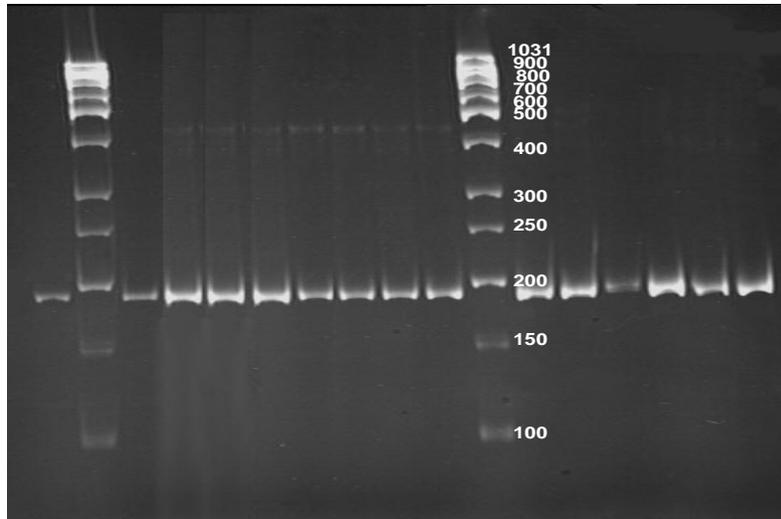


Fig. 4 Ethidium bromide stained polyacrylamide gel for the ILSTS035 microsatellite after running vertically

Our results for this marker agree with those reported by Navani *et al.* (2002) after testing it on Murrah, Nili-Ravi and Mehsana Indian river buffalo breeds, the authors found it monomorphic microsatellite with allele size of 145 bp. Moreover, Van Hooft *et al.* (1999) found that this microsatellite was also monomorphic when typed in African buffalo (*Syncerus caffer*), with allele size 186 bp. This microsatellite was shown to be polymorphic in cattle. Ma *et al.* (1996) after typing it in four beef breeds of cattle (Angus, Devon, Gelbvieh and Simmental), found that it produced 9 alleles with a size range: 236-266 bp. Vallejo *et al.* (2003) reported 5 alleles for it in the North American Holstein cattle. Yeo *et al.* (2004) observed nine alleles for it with a size range from 210 to 266 bp in Hanwoo Korean cattle. The authors used this microsatellite to detect and locate quantitative trait loci (QTL) for traits in Hanwoo cattle. Likewise, nine alleles were recorded for this marker in Chinese Holstein cattle in a study of quantitative trait loci affecting milk production traits on cattle chromosome 6 by the daughter design (Chen *et al.*, 2006).

This microsatellite cross hybridized with sheep genome and it was found to be also monomorphic giving one allele with a size 191 bp. (De Gortary *et al.*, 1997).

Results of the amplification of the bovine microsatellite in buffalo and sheep genomes may be referring to the sharing of a common ancestry for cattle, buffalo and sheep after the divergence of subfamily Bovinae (*Bos taurus*) from the family Bovidae (Mattapallil and Ali, 1999).

TGLA37:

Figure (5) shows the results of the amplification after running the product on 2% agarose and staining with ethidium bromide.

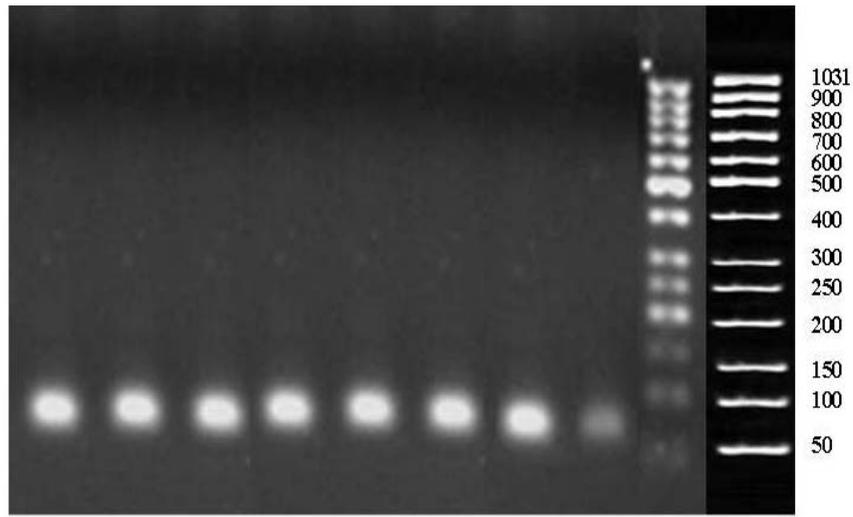


Fig. 5 Ethidium bromide stained agarose gel for the PCR product of microsatellite TGLA37 after running horizontally

Characterization of the PCR products on polyacrylamide gel revealed the presence of only one allele with 86 bp size length. Figure (6) shows the ethidium bromide stained for the polyacrylamide gel.

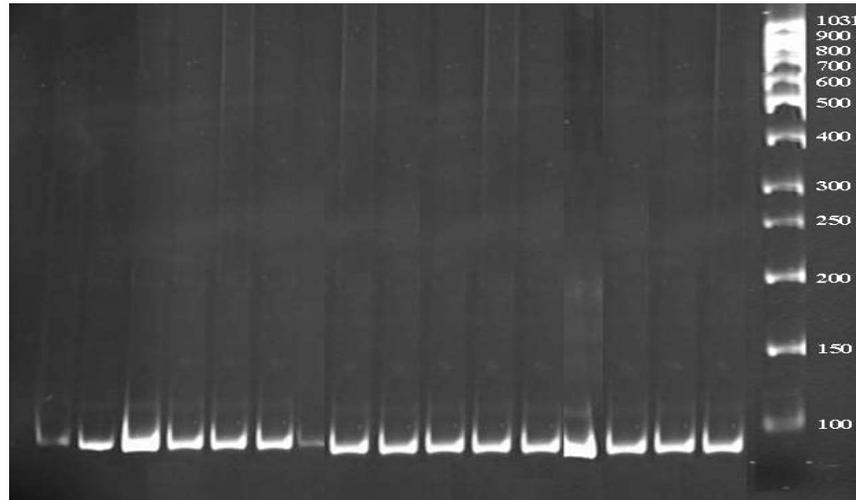


Fig. 6 Ethidium bromide stained polyacrylamide gel for the TGLA 37 microsatellite after running vertically

No previous studies were conducted with this microsatellite on buffalo to compare with our results. In contrast, Georges and Massey (1992); Georges *et al.* (1995) and Ihara *et al.* (2004) found that, there were 5 observed alleles, their size ranging between 108 to 126 bp in American Holstein cattle.

Several of the previous studies have indicated the presence of a QTL variously affecting milk yield and milk traits on cattle chromosome 6 located near marker TGLA37. QTL were found between markers BM1329 and TGLA37, as well as in the interval between TGLA37 and FBN13 in American Holsteins (Georges *et al.*, 1995), Dutch Holsteins (Spelman *et al.*, 1996), German Holsteins (Freyer *et al.*, 2002; and Freyer *et al.*, 2004). In addition, Freyer *et al.* (2003) found that there was highly significant impact of TGLA37 marker on fat yield of milk in German Holstein Friesian.

FBN13:

Figure (7) shows the results of the amplification after running the product on 2% agarose and staining with ethidium bromide.

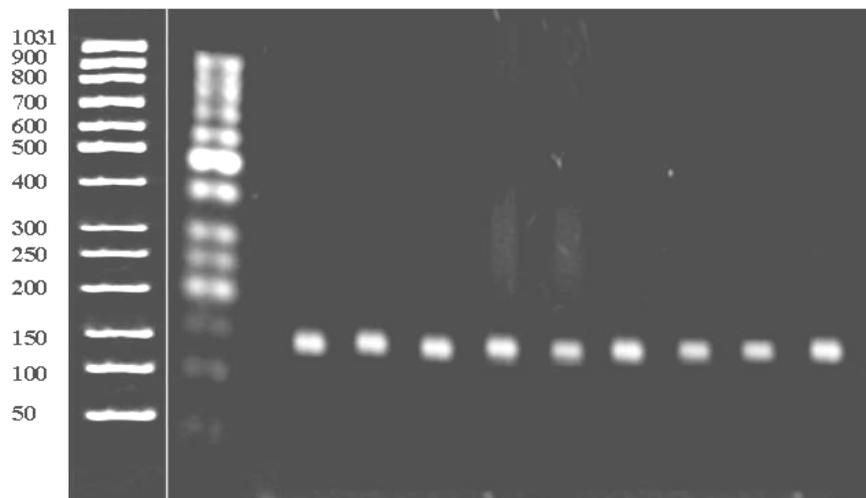


Fig. 7 Ethidium bromide stained agarose gel for the PCR product of microsatellite FBN13 after running horizontally

The characterization of the PCR products on polyacrylamide gel revealed the presence of only one allele with 140 bp size length. Figure (8) shows the ethidium bromide stained for the polyacrylamide gel.

Limited studies regarding typing this microsatellite were conducted in cattle. Olsen *et al.* (2004) reported that, FBN13 was a polymorphic marker and gave nine alleles in American Holstein Cattle. Studies on this marker indicated the presence of a QTL affecting milk fat and protein percentage in the interval between FBN9 and FBN13 in Norwegian dairy cattle (Olsen *et al.*, 2002). In addition, Freyer *et al.* (2004) obtained a significant QTL between TGLA37 and FBN13 for protein yield of

milk in German Holstein–Friesian. Likewise, FBN13 marker had highly significant effect on milk yield traits (Peter *et al.*, 2005) in German Holstein–Friesian.

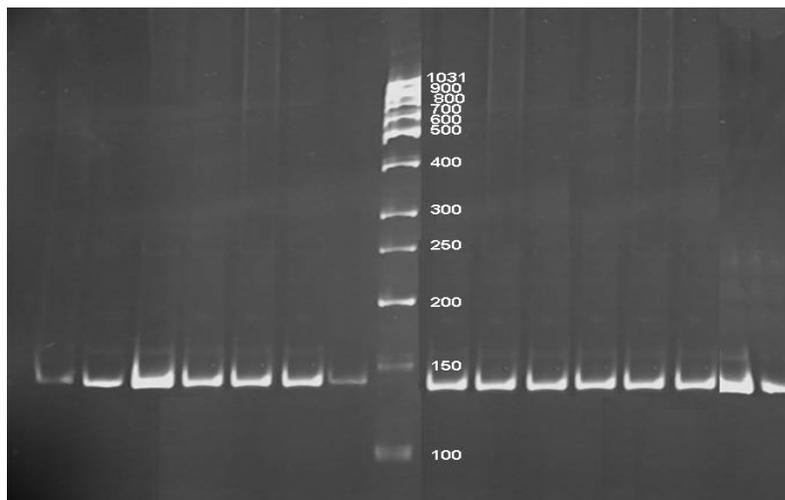


Fig. 8 Ethidium bromide stained polyacrylamide gel for the FBN13 microsatellite after running vertically

he similarities in chromosomal banding patterns between cattle and buffalo were shown in the genetic content either with type I or type II markers. Concerning type I markers which is a coding sequence mapped by *in situ* hybridization or somatic cell hybridization, great similarities were found in the chromosomal localization (for review see Iannuzzi, 1998). This similarity of the genetic content extended also to the type II markers such as microsatellites. Many reports indicate that the microsatellites are conserved between cattle and buffalo, and all the microsatellite PCR primers are working in buffalo (Moore *et al.*, 1991; Pepin *et al.*, 1995; Hassanane *et al.*, 2000 and Moioli *et al.*, 2001). It is well established that chromosome 6 in cattle carries many markers correlated with milk yield and milk constituents. Chromosome 7 in the buffalo is equivalent to chromosome 6 in cattle, so studying the genetic content for this chromosome will be helpful in understanding genes controlling milk and their contents in buffalos.

CONCLUSIONS

The results of the current study demonstrate that some cattle specific primers can be a valuable source for genome analysis in *Bubaline* species. Microsatellites BM143 and ILSTS093 are polymorphic in the Egyptian buffalo, so they are suggested for further productive genomic studies, such as gene mapping, marker-assisted selection, and genetic diversity in the Egyptian buffalo. Although ILSTS035, FBN13 and TGLA37 microsatellites are polymorphic markers in cattle, they did not show polymorphism in the present sample of Egyptian buffalo.

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تعدد المظاهر الوراثية لبعض التوابع الوراثية الدقيقة على الكروموسوم السابع في الجاموس المصري

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أجريت هذه الدراسة على 70 من إناث الجاموس المصري في مزرعة الجاموس التابعة لمحطة التجارب الزراعية بكلية الزراعة، جامعة القاهرة بهدف التعرف على إمكانية تكبير خمسة من التوابع الوراثية الدقيقة من أصل بقرى (ILSTS093, ILSTS035 ، FBN13، TGLA37، BM143) لجينوم الجاموس، وكذلك الكشف عن تعدد المظاهر الوراثية لهذه التوابع و عدد الاليليات المشاهدة لكل تابع منها. وقد أوضحت الدراسة أن جميع هذه التوابع الوراثية الدقيقة قامت بتكبير جينوم الجاموس المصري المستخدم في الدراسة، وبالتفريد الكهربائي الرأسي على مادة البولي أكريلاميد لم تبد ثلاثة منها (ILSTS035 ، FBN13 ، TGLA37) أي تعدد في المظاهر الوراثية أي نتج عنها أليل واحد فقط (homozygous) ، بينما أبدى التابع الوراثي الدقيق (BM143) تعدداً واضحاً للمظاهر الوراثية و كان عدد الاليليات المشاهدة له هو تسعة أليلات تراوح الوزن الجزيئي بين 99 و135 زوج من القواعد النيتروجينية، و تراوحت تكرارات الاليليات بين 0.015 و 0.431 . كما أبدى أيضا التابع الوراثي الدقيق (ILSTS093) تعددا للمظاهر الوراثية و كان عدد الاليليات المشاهدة له ثمانية اليلات تراوح الوزن الجزيئي لها بين 193 و 217 زوج من القواعد النيتروجينية و تراوحت تكراراتها بين 0.009 و 0.293 . أقترحت الدراسة إمكان استخدام بعض التوابع الوراثية الدقيقة من أصل بقرى في دراسة الجينوم الجاموسى وعلى ذلك يمكن استخدام هذين التابعين اللذين أبديا تعددا للمظاهر الوراثية في الدراسات التي تجرى بهدف تحديد منشأ السلالة، إيجاد العلاقة بين السلالات و بعضها (التباين الوراثي) ، كذلك في الدراسات التي تجرى بهدف رسم الخرائط الوراثية للمواقع المسؤولة عن الصفات الكمية على الجينوم (QTLs) في الجاموس المصري.