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Establishment of paternity testing system using microsatellite markers in Chinese Holstein

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Abstract

To estimate the efficiency of microsatellite markers in paternity testing among Chinese Holstein, 30 microsatellite loci were used to differentiate 330 Chinese Holstein genotypes, according to the calculation of the allele frequency, number of alleles, effective number of alleles, genetic heterozygosity, polymorphic information content (*PIC*), and the exclusion probability in this cattle population. The results demonstrated that the exclusion probability ranged from 0.620 in locus BM1818 to 0.265 in locus INRA005 with the average of 0.472 and 11 microsatellite markers exceeding 0.5. The combined exclusion probability of nine microsatellite markers was over 0.99. The result showed that paternity testing of Chinese Holstein was basically resolved using the nine microsatellite markers selected.

Keywords: Chinese Holstein; microsatellite; paternity testing; exclusion probability

Introduction

Genetic evaluation is one of key issues in dairy breeding, as the increase of genetic progress partially depends on the exact genetic evaluation based on the entire and correct pedigree (Zhang, 1995). Because of the extensive application of artificial insemination in dairy breeding, the increase of mistakes in pedigree has been recorded. For example, the mistakes caused by repetitive insemination; the consequence of this scenario is the reduction of genetic progress. Recent studies have implicated that 3% – 4% of the decreases in genetic progresses were caused by 10% of increases in pedigree mistakes per year (Israel and Weller, 2000). However, several researchers found that the mistakes in pedigree have been elevated to 22% since the 1980s (Ron et al., 1996; Visscher et al., 2002; Geldermann et al., 2003). Therefore, to obtain the optimal genetic progress in dairy breeding, it is necessary to identify and correct the pedigree through paternity testing. So far, some developed countries, such as USA and Canada, have established the paternity testing system for their own Holstein populations.

The paternity system established in other countries might not work for Chinese Holstein cattle, because Chinese Holstein is the descent of European Holstein, North American Holstein, and local breeds. The establishment of paternity testing procedure in this dairy breed requires the selection of specific microsatellite markers. With the development of molecular technologies, paternity testing is conducted using molecular markers instead of biochemistry markers in the past decade. One type of useful molecular tool is microsatellite markers (Fan et al., 2002; Ellegren, 2004; Gong et al., 2005). Microsatellite markers are comprehensively utilized in paternity testing because of its high polymorphism, interspersing among the whole genome, and easy detection. In the present project, the polymorphic levels of 30 microsatellite markers recommended by FAO (Food and Agriculture Organization) and ISAG (International Society of Animal Genetics) were determined to establish the paternity testing system for Chinese Holstein population.

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Materials and methods

Samples

Three hundred and thirty Chinese Holstein cattle used in the experiment were from 9 dairy farms in Beijing. Blood samples were collected from jugular vein of 206 cows and 124 bulls, and semen samples were kindly provided by Beijing Breeding Bull Station.

Extraction of genomic DNA and primers

Genomic DNA was extracted from blood and semen samples using phenol-chloroform reagent. The primers of 30 microsatellite markers were designed, blasted through www.ncbi.nlm.nih.gov (Table 1), and synthesized by AOKE company in Beijing.

PCR amplification

The PCR reaction system was totally 20 μL, including 1.0 μL of 50 ng/μL DNA sample, 2.0 μL of 10 × buffer, 0.4 μL of 10 mmol/L dNTPs, 0.5–1.0 μL of 10 μmol/μL primer dilution, 0.5 μL of 2 U/μL *Taq* DNA polymerase, and ddH_2O. The amplification process was performed at 94°C for 10 min, 94 °C for 30 s, 55°C–65°C for 30 s, 72 °C for 30 s with 35 cycles, and after that extension at 72 °C for 5–10 min. The PCR products were detected by 2% agarose gel electrophoresis.

Table 1
Information of microsatellite markers used in this study

No.	Marker	Chr.	Primer sequence (5′→3′)	Detected size range (bp)
1	ETH225	9	GATCACCTTGCCACTATTTCCT ACATGACAGCCAGCTGCTACT	131–155
2	ETH152	5	TACTCGTAGGGCAGGCTGCCTG GAGACCTCAGGGTTGGTGATCAG	181–211
3	HEL1	15	CAACAGCTATTTAACAAGGA AGGCTACAGTCCATGGGATT	103–117
4	ILSTS005	10	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	184–196
5	HEL5	21	GCAGGATCACTTGTTAGGGA AGACGTTAGTGTACATTAAC	147–171
6	INRA005	12	CAATCTGCATGAAGTATAAATAT CTTCAGGCATACCCTACACC	139–147
7	INRA035	16	ATCCTTTGCAGCCTCCACATTG TTGTGCTTTATGACACTATCCG	102–114
8	INRA063	18	ATTTGCACAAGCTAAATCTAACC AAACCACAGAAATGCTTGGAAG	171–187
9	MM1220	9	CAAGACAGGTGTTTCAATCT ATCGACTCTGGGGATGATGT	105–135
10	HEL9	8	CCCATTCAGTCTTCAGAGGT CACATCCATGTTCTCACCAC	169–143
11	CSRM60	10	AAGATGTGATCCAAGAGAGAGGCA AGGACCAGATCGTGAAAGGCATAG	96–116
12	CSSM66	14	ACACAAATCCTTTCTGCCAGCTGA AATTTAATGCACTGAGGAGCTTGG	177–209
13	ETH185	17	TGCATGGACAGAGCAGCCTGGC GCACCCCAACGAAAGCTCCCAG	221–245
14	HAUT24	22	CTCTCTGCCTTTGTCCCTGT AATACACTTTAGGAGAAAAATA	109–129
15	HAUT27	26	TTTTATGTTCATTTTTTGACTGG AACTGCTGAAATCTCCATCTTA	127–155
16	ETH3	19	GAACCTGCCTCTCCTGCATTGG ACTCTGCCTGTGGCCAAGTAGG	109–133
17	ETH10	5	GTTCAGGACTGGCCCTGCTAACA CCTCCAGCCCACTTTCTCTTCTC	207–225
18	INRA032	11	AAACTGTATTCTCTAATAGCAC GCAAGACATATCTCCATTCCTTT	166–190
19	INRA023	3	GAGTAGAGCTACAAGATAAACTTC TAACTACAGGGTGTTAGATGAACTCA	197–221
20	BM2113	2	GCTGCCTTCTACCAAATACCC CTTAGACAACAGGGGTTTGG	125–143
21	BM1818	23	AGCTGGGAATATAACCAAAGG AGTGCTTTCAAGGTCCATGC	258–272
22	BM1824	1	GAGCAAGGTGTTTTTCCAATC CATTCTCCAACTGCTTCCTTG	178–192
23	HEL13	11	TAAGGACTTGAGATAAGGAG CCATCTACCTCCATCTTAAC	177–197
24	ILSTS006	7	TGTCTGTATTTCTGCTGTGG ACACGGAAGCGATCTAAACG	281–299
25	INRA037	10	GATCCTGCTTATATTTAACCAC AAAATTCCATGGAGAGAGAAAC	112–148
26	SPS115	15	AAAGTGACACAACAGCTTCTCCAG AACGCGTGTCCTAGTTTGGCTGTG	185–205
27	TGLA227	18	CGAATTCCAAATCTGTTAATTTGCT ACAGACAGAAACTCAATGAAAGCA	76–104
28	TGLA126	20	CTAATTTAGAATGAGAGAGGCTTCT TTGGTCTCTATTCTCTGAATATTCC	116–122
29	TGLA53	16	GCTTTCAGAAATAGTTTGCATTCA ATCTTCACATGATATTACAGCAGA	152–186
30	TGLA122	21	CCCTCCTCCAGGTAAATCAGC AATCACATGGCAAATAAGTACATAC	137–181