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RAPID COMMUNICATION

Development of novel SNP system for individual and pedigree control in a Japanese Black cattle population using whole-genome genotyping assay

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ABSTRACT

Individual identification and parentage analysis using DNA markers are essential for assuring food identity and managing livestock population. The objective of this study was to develop a single nucleotide polymorphism (SNP) panel system for individual effective identification and parentage testing in a Japanese Black cattle population using BovineSNP50 BeadChip. On the basis of SNP frequencies, 60 unlinked informative SNPs were finally selected as candidate markers. The allelic frequencies for each SNP were estimated using additional individuals by PCR-RFLP (restriction fragment length polymorphism). A total of 87 SNP markers added in conjunction with previously developed 27 SNPs were evaluated to assess the utility of the test. The estimated identity power was 2.01×10^{-34}. Parentage exclusion probabilities, when both suspected parents' genotypes were known and when only one suspected parent was genotyped, were estimated as 0.99999997 and 0.99998010, respectively. This developed SNP panel was quite powerful and could successfully exclude false sires with a probability of >0.9999 even if the dam's genotype information was not obtained. The SNP system would contribute to management of the beef industry in Japan.

***Key words**: individual identification, Japanese Black cattle, parentage test, single nucleotide polymorphism.*

INTRODUCTION

DNA-based identification and parentage tests are essential for genetic identity and pedigree of animal populations. In recent years, single nucleotide polymorphism (SNP) has attracted attention as a new marker to replace microsatellite markers for those tests. The SNP is abundant in genomes, genetically stable and amenable to high-throughput automated analysis (Heaton *et al*. 2002; Baruch & Weller 2008). Therefore, SNP markers are considered to be more efficient anda future tool for genomic analyses. Development of SNP systems for individual identification and pedigree tests have been reported in US and European cattle (Heaton *et al*. 2002; Werner *et al*. 2004). However, additional development of SNP panels for each breed would be necessary since the efficiency of the tests depends on allelic frequencies of the SNPs in each breed, especially for high inbreeding populations such as Japanese Black cattle.

In our previous study, 29 SNP markers for Japanese Black cattle were developed by amplified fragment length polymorphisms (AFLP) method. The SNP panel possessed sufficient probability (2.73×10^{-12}) for individual identification in Japanese Black populations (Hara *et al*. in press). However, the parentage exclusion probabilities were 0.96929 (when both suspected parents' genotypes were known) and 0.99693 (when only one suspected parent was genotyped), suggesting that additional SNPs were required to investigate more accurate parentage relationships. The problems of the AFLP method in SNP development are that it was difficult to obtain the chromosomal location of the SNPs and it is not able to select the unlinked SNP markers.

Recently, different genome-wide, high-throughput SNP genotyping technologies such as Affymetrix GeneChip arrays and Illumina BeadChip, have been available after discovery of numerous SNP through-genome sequencings. These were widely applied as efficient tools for linkage disequilibrium analysis

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(Khatkar *et al*. 2008), association studies (Nijman *et al*. 2008), assessment of population structure (Mckay *et al*. 2008) and genetic evaluations (Wiggans *et al*. 2009). The Illumina BeadChips with Infinium whole-genome genotyping assay was used as a major array platform (Staaf *et al*. 2008) and BovineSNP50 BeadChip powered by Infinium II assay containing 54 001 SNPs is also available. This assay enables us to expect allele frequency of the SNPs and effectively select candidate SNPs in each cattle breed.

The objective of this study was to select additional SNP markers and to develop an SNP panel system with sufficient parentage exclusion probabilities for Japanese Black cattle populations. We employed BovineSNP50 BeadChip to select candidate SNPs for the population in this study.

MATERIALS AND METHODS

Samples

Japanese Black cattle ($n = 161$) were collected from diverse areas in Japan. They were selected based on pedigree information and geographic criteria to avoid having close relationships. Genomic DNA was extracted from tissue and blood samples according to standard phenol and chloroform methods (Sambrook & Russell 2001).

Detection of candidate SNP

The 54 001 SNPs genotyped with the Illumina BovineSNP50 BeadChip (Illumina Inc., San Diego, CA, USA) were investigated. The 54 001 SNPs were distributed in an average SNP spacing of 51.5 Kb across whole genomes, and their chromosome positions were known for approximately 97% SNPs. In this analysis, 50 Japanese Black samples were used. Candidate SNPs were selected on the basis of genotype data and their allele frequencies. In order to obtain flanking sequences of the SNPs, homology searches were performed using the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/) running the BLAST programs (Altschul *et al*. 1990). Based on the flanking sequences, primers were designed to perform PCR-RFLP (restriction fragment length polymorphism) (Table 1).

PCR-RFLP condition

PCR reactions were performed in a volume of 20 μL, containing 20 ng genomic DNA as a template, 2.0 μL reaction buffer (100 mmol/L Tris-HCl, 15 mmol/L $MgCl_2$, 500 mmol/L KCl), 1.6 uL dNTP Mix (2.5 mmol/L), 0.5 μL of each primer (10 nmol/mL) and 1.0 U of EX Taq polymerase (Takara Shuzo Co., Tokyo, Japan). PCR reactions were carried out using a standard PCR program with 5 min denaturation at 94°C, 30 cycles for 1 min at 94°C, 30 s annealing at temperatures listed in Table 1, 30 s extension at 72°C, and final extension for 7 min at 72°C. PCR-RFLP method was performed in a volume of 15 μL, containing 5 μL PCR products, 1.5 μL 10 × reaction buffer and 1.0 U of adequate restriction endonuclease (Table 1).

Statistical analyses

P_I, the index of power in individual identification, was defined as the estimated probability that two unrelated individuals selected at random would possess identical multilocus genotypes. This probability for a marker is equal to summation of the square of each genotypic frequency (Holt *et al*. 2000; Heaton *et al*. 2002). To calculate P_I, the multiple product of each individual marker probability was computed. Parentage exclusion probabilities when both suspected parents' genotypes were known (P_{E2}) and parentage exclusion probabilities when only one suspected parent was genotyped (P_{E1}) were computed as described (Jamieson & Taylor 1997). Detail s of formulas of P_I, P_{E2} and P_{E1} were described in our previous study (Hara *et al*. in press).

Analyses of Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) tests were calculated utilizing ARLEQUIN (Excoffier *et al*. 2005).

RESULT AND DISCUSSION

In order to obtain candidate SNPs, Illumina BovineSNP50 BeadChip was applied for 50 Japanese Black cattle in this study. Out of 54 001 SNPs, 43 122 SNPs were polymorphic and their allele frequencies were calculated. The 15 692 SNPs revealed a minor allele frequency (MAF) of more than 0.3 and were considered as primary candidate markers.

An assumption for statistical analyses of individual identification and parentage test was that all markers were independent. Khatkar *et al*. (2008) described that significant LD in cattle was estimated as 8.2 Mb using *D′* measure. Therefore, we applied an unlinked assumption of 20 Mb physical distances in this study and this was considered to have little effect on the power of the tests. On the basis of the unlinked assumption and MAFs, 60 SNPs out of 15 692 were finally selected as final candidate markers (TSB0101-TSB2901, Traceability SNP marker from BeadChip).

PCR-RFLP using an additional 111 Japanese Black individuals was conducted to investigate genotype and allele frequencies of the candidate SNPs. Table 2 shows chromosomal locations, observed MAFs and expected heterozygosities of 60 SNPs. The MAFs ranged from 0.297 (TSB1103) to 0.500 (TSB1802), as expected from BeadChip SNP genotype data. An average MAF and expected heterozygosity was 0.433 and 0.486, respectively. The results suggested that developed SNP markers in the current study were quite informative and that employment of BovineSNP50 BeadChip was a useful tool to select efficient SNPs for individual identification and parentage tests.

We also added informative 27 SNPs derived from previously developed 29 SNPs into the current SNP panel. The chromosomal locations were determined in a previous study (Hara *et al*. 2010), but we re-analyzed the locations using the latest version of the NCBI database (Table 2). According to the location, novel unlinked SNP markers were selected in the present study. Association of LD was estimated between all pairs of SNPs on the same chromosome. The LD tests demonstrated no significant deviation from expectations ($P < 0.05$) for all pairs of SNPs. The HWE test

Table 1 Marker information for PCR-RFLP

Marker	Forward primer 5′-3′ Reverse primer 5′-3′	PCR annealing temperature	Variation	RE†
TSB0101	AGG CAA ACT AAC ACA TCT TCC C GCT ATA TCA CCA TCT CCC ACC TT	62	C/T	*Msp* I
TSB0102	TAG ATA CAG GGC ACA TAG CTC AA CTT GTC CTA ATC CTT CCT AAA CC	62	G/T	*Mse* I
TSB0103	TGG GAG CAC AAG ACG GCA CAA GAT TCC CCC CAA CAG ATA GCC	62	C/T	*Alu* I
TSB0104	TTT GGG GAC ATT TCA GAC AGT TG CTA ATC CTA CTA AAG CAA CTG GG	62	G/T	*Mse* I
TSB0105	TGC CCA GCC CAA AGT AGA GTA GA TAC TCT CCA TCT CCC AGG TTC CA	62	G/T	*Msp* I
TSB0106	GGG TTC TTT TGT CTG GTC TTG CT TCA GAG GAC GAA GAG GAA GCT AT	62	A/G	*Msp* I
TSB0201	GCA ACC CTC CAG TAA TCC CAC TGC CTT CCT CCC ACA TGA TCG	62	C/T	*Alu* I
TSB0202	AAC AGC ATA CAA TAC AGC CCA GT GAA CTG TAA ATG GAA TCC CCT TG	62	C/T	*Msp* I
TSB0203	CGG CTC CTC CCA AAT AAA GAC TT CAG TGC AGG TTT CTC TCT CTT GT	62	A/G	*Taq* I
TSB0301	GTC CAT TGT TTT TTT CAC CCC AG TCA GTT CCT AGA GGT TGC TTA TG	62	C/T	*Taq* I
TSB0302	ATC CTA GAG TCT TCC TGT CCA CA CGG ATA CGA TTG AGG GAC TAA GT	62	A/C	*Msp* I
TSB0401	CTT CTT GCT GTG GTT AAT TTG CC GAT TGA ACC TGG GTC TCC TGT AT	62	A/G	*Hpy*CH4IV
TSB0402	TCC AGG ATG CTT CAG TCA CGT CT AGC CTC GTG GAT GTG TTG TTG AC	62	C/T	*Mse* I
TSB0403	CCA CAG AAA AAA ACT AGA GTC GC GAT TCC TCT TAC GTG CCT CAT TT	62	C/T	*Alu* I
TSB0501	TTA GCA GCA GCA GGA GCA AGA TGT GCT ACC TGT GAC TAT CCC	62	A/G	*Alu* I
TSB0502	AAC CAA GGG GAA CTG TAG GAT AA GAT GAG AAC TAT TAC CAG ACA CG	62	A/G	*Msp* I
TSB0503	ATT CCT TTC TCA ACA GCA GCT CA TTG GTA GTA AAG CAG GGA AGT TG	62	A/G	*Taq* I
TSB0601	GAG GTC AGA AAA AGA GAG AGA TC AAT TGG AGT GCG TAA GCC TTT GT	62	A/G	*Mse* I
TSB0602	ATT CTC CCT ATT GCA CCC TGT CT TAG TGG TAA AGA ATC TGC CTG CC	62	C/T	*Mse* I
TSB0603	CAA TGA AAA GTC CAG GCA CCA CA GAC TCT CCC TCT TGG TAT CTT TC	62	A/G	*Hpy*CH4IV
TSB0604	GGA CAC TAA GCC TGA GGA TTT CT TGA GGA GGA GAC CGT TAG AAG TT	62	A/G	*Hpy*CH4IV
TSB0701	CTA CCA TTT GAC TCA GAC ACA GC GGA GAG CCA CTG TGA AAG AAA GA	62	A/G	*Taq* I
TSB0702	TTG GTT CAG TTC AGT TCA GTT GC CTT CCA TCA TTC ACT CCT TAC CT	62	C/T	*Msp* I
TSB0703	GTA ACC ACA CAT CTA GCT GCT GA GAT GGC ACT TCT TTT TAC CTG GC	62	A/G	*Msp* I
TSB0801	TGT TGT CTT ATA GGT GGG TAG GC GAC TAA TGT GTA ATG CGG GCT TC	62	A/G	*Mse* I
TSB0802	CAG AGT GAA GCC AGA TGG ACA TT GTC TAC ACT GCC ATC TTC TCC TT	62	C/T	*Taq* I
TSB1001	TCT GTC TTT CCT CTG TGA TAT GC CAC AAC AGG TAT GAA GGC AGA TT	62	C/T	*Msp* I
TSB1002	AGG AGG GCA GGT GTG ATT ATG AG CTC CAC TGA CCA GGG TTA GAC TT	62	C/T	*Taq* I
TSB1101	CTT TGT TTA CTG GAC TTT GCC TG ACA AAA TTA GCC AGG GAG AAC AG	62	C/T	*Msp* I
TSB1102	TGT GGG AGT CCG ATG AAA TGA AA AAA GAA GCA AAG AGA GTG TGT CC	62	C/T	*Msp* I

Table 1 *Continued*

Marker	Forward primer 5′-3′ Reverse primer 5′-3′	PCR annealing temperature	Variation	RE†
TSB1103	CGC ACC CAG GTT GTT TAA GCA GA TGG CCT GTA ATG GAT GGG TGA GA	62	A/G	*Mse* I
TSB1201	AAC TCA CTT GCA GCA CGA TTC CT ACC AAC ACA AAG CTA CCT CCT GA	62	C/T	*Msp* I
TSB1202	TGT GAG TGA GTT CCC AGG CAA AG AGG TGG TGC TAG TGG TGA ACA AC	62	C/T	*Mse* I
TSB1301	AGG ACC AGA GGA GGC AAA ACT TT AAA AGT TCC CCA GAG GTC ACC AT	62	A/G	*Msp* I
TSB1302	GAC CTA AAG CTG TGC CTG GAA GA ACA ATC ATG CCA CCT CTC CGA CA	62	A/G	*Msp* I
TSB1303	TCC AGA ACC CAA AAT GCC ACA G AGC ATC AGC ATC AGT CCT TCC A	62	C/T	*Msp* I
TSB1401	TGC TGC AAG TCG TAA AGA AAG GA CGC ATC CAG TGT CTT CTT ATC AA	62	C/T	*Taq* I
TSB1402	GGC AGA GGA AGA ATT TGA GTA AG GCA AAC AAA CTA CCC TTC TTC CT	62	G/T	*Mse* I
TSB1403	TGA GCC TTA GAT TTC CGC ATT TC GGA AAG ATT GAA GGT GAG AGG AA	62	C/T	*Taq* I
TSB1501	GAA GGT CAA GTC TCC ACA AGC TA CCA GCA CCT TCA ACA TTT CCT AC	62	A/C	*Taq* I
TSB1502	CAG AAA ACA GAG AGA GAA CAA GC TAC TCA GAC ACG GGC ATT TCA AT	62	C/T	*Msp* I
TSB1601	CAG ATA ACA GTC CAT GAG ATT GC CAG CCA CTC CAG TTA ATC TTT CA	62	C/T	*Taq* I
TSB1602	ACT TCC CTA TTG CTT ATG CCT TC GCG ACT AAC ACT TTC CTC TTT TG	62	A/T	*Taq* I
TSB1701	CCC TAG AAC AGA CCT TTT TAT GC AGC AGT ATG TGG GAT CTT AGT TC	62	A/G	*Taq* I
TSB1801	TTC CAT CTT GTT GCC AGT CTA CT CAG GAA CAG GTA TCA AAA GCT CT	62	A/G	*Hpy*CH4IV
TSB1802	TAG CCG AGA AAG CGA TCA GCA GA CAT TCA GAG TCC TTA CCC ACA GC	62	A/G	*Taq* I
TSB1901	GGG AAA AAG AGA ACA GGT GCC A TTG GGT CAT GGA TTC CTC TAG G	62	C/T	*Alu* I
TSB2001	GTC TCT TGG GTG AAT TTG TTA GC TTT CTC TGG TGA TAT CGC TAA GC	62	C/T	*Msp* I
TSB2002	TTC ATT TGC TAT CTG CCC ACC TC AAG CAC AGA TGG ACA AAT GAC CG	62	C/T	*Msp* I
TSB2201	CTG ATA ATG CCC TGG TAC TTC GG CAG AGG ATG AGA TGG TTG GAT GG	62	C/T	*Msp* I
TSB2202	TGA AAT GAC CAC CCT GTA GGA AG TTA CCC CAC GCC TAG CTT TTA CA	62	A/G	*Msp* I
TSB2301	GGG TTG ACC TAC AGA GAT GAC AT AGG CTG CTC AAG AAA GTT AGG AC	62	G/T	*Mse* I
TSB2401	CTT CCA TCC CAA TCA TCT GTT CT CAT TCA CAA ACA CAC ATC TCC AG	62	A/G	*Taq* I
TSB2402	AGG ACA CAC ACC AAC GAT ACC AT GAA GAC TGC CTC CAA ATA CCA CT	62	A/G	*Msp* I
TSB2501	AGG AGA GAG GGC AGA CAA AAG AA TGA ACT TGA CCT GGA TGT GAT GG	62	C/T	*Msp* I
TSB2601	GGT GTC AGG GTT ACT TCA TCA AA TTG CTG CCT GTG TTA ATT TGG GA	62	A/G	*Msp* I
TSB2602	GGT CAG CAG GCA CAT ATT CCA TT CTC CTG TGT AGT GGT GAT TAA GC	62	A/G	*Msp* I
TSB2701	GTT GAA TAC TCC AGC ACT GAT AC CAC CTA ATT TTC ATC CCT GCT AC	62	C/T	*Taq* I
TSB2801	GAG CCC CTT ACA TTC ACT TCA CA AGA AAG GAT CAA GCA GCA GGG AA	62	C/T	*Msp* I
TSB2901	GGG TAT TTT TGT TAG GTC TGT GG AAG GCA TCA GGA AAA GGG AGA GA	62	A/T	*Taq* I

†Restriction enzyme.

Table 2 Chromosomal location and genetic indices for 87 single nucleotide polymorphism markers

Marker	BTA	Position (Mb)	Observed allele frequency	Ho†	He‡
TSB0101	1	1	0.387	0.468	0.475
TSB0102	1	41	0.350	0.477	0.455
TSB0103	1	61	0.421	0.598	0.487*
TSB0104	1	100	0.356	0.546	0.459
TSB0105	1	132	0.444	0.551	0.494
TSB0106	1	160	0.466	0.433	0.498
TSB0201	2	1	0.414	0.234	0.485*
TSA1	2	51	0.477	0.514	0.499
TSA2	2	83	0.417	0.431	0.486
TSB0202	2	113	0.450	0.569	0.495
TSB0203	2	140	0.420	0.651	0.487*
TSA4	3	37	0.424	0.619	0.488*
TSB0301	3	66	0.360	0.523	0.461
TSA5	3	91	0.365	0.532	0.463
TSB0302	3	119	0.482	0.651	0.499*
TSB0401	4	25	0.459	0.739	0.497*
TSB0402	4	47	0.455	0.545	0.496
TSA6	4	75	0.358	0.459	0.460
TSB0403	4	123	0.361	0.556	0.461
TSB0501	5	5	0.391	0.436	0.476
TSA7	5	46	0.324	0.360	0.438
TSB0502	5	102	0.385	0.385	0.474
TSB0503	5	124	0.401	0.462	0.480
TSB0601	6	4	0.491	0.691	0.500*
TSB0602	6	36	0.423	0.595	0.488
TSB0603	6	70	0.486	0.713	0.500*
TSB0604	6	122	0.458	0.613	0.496*
TSB0701	7	31	0.436	0.600	0.492*
TSB0702	7	52	0.481	0.623	0.499*
TSB0703	7	106	0.455	0.550	0.496
TSB0801	8	25	0.495	0.559	0.500
TSB0802	8	88	0.433	0.562	0.491
TSA10	9	20	0.438	0.476	0.492
TSA11	9	51	0.367	0.440	0.465
TSA12	9	87	0.354	0.406	0.457
TSA13	10	3	0.450	0.486	0.495
TSB1001	10	53	0.495	0.573	0.500
TSB1002	10	75	0.477	0.482	0.499
TSB1101	11	19	0.459	0.555	0.497
TSB1102	11	68	0.468	0.445	0.498
TSB1103	11	110	0.297	0.462	0.418
TSB1201	12	32	0.394	0.459	0.478
TSB1202	12	83	0.351	0.468	0.456
TSB1301	13	6	0.405	0.523	0.482
TSB1302	13	61	0.364	0.564	0.463*
TSB1303	13	84	0.472	0.505	0.498
TSB1401	14	1	0.467	0.523	0.498
TSB1402	14	51	0.459	0.505	0.497
TSB1403	14	80	0.421	0.729	0.487*
TSA15	15	28	0.414	0.541	0.485
TSB1501	15	50	0.452	0.481	0.495
TSB1502	15	84	0.454	0.541	0.496
TSB1601	16	8	0.392	0.405	0.477
TSA17	16	32	0.486	0.541	0.500
TSB1602	16	54	0.347	0.565	0.453*
TSA20	16	76	0.400	0.520	0.480
TSA21	17	8	0.281	0.402	0.404
TSB1701	17	30	0.495	0.639	0.500*
TSA22	17	55	0.395	0.486	0.478
TSB1801	18	2	0.434	0.453	0.491
TSA23	18	27	0.396	0.505	0.479
TSB1802	18	53	0.500	0.578	0.500

Table 2 *Continued*

Marker	BTA	Position (Mb)	Observed allele frequency	Ho†	He‡
TSA24	19	6	0.486	0.495	0.500
TSB1901	19	37	0.468	0.541	0.498
TSB2001	20	21	0.396	0.604	0.478*
TSB2002	20	74	0.486	0.523	0.500
TSA25	21	17	0.382	0.582	0.472*
TSA27	21	64	0.422	0.437	0.488
TSA28	22	2	0.438	0.468	0.491
TSB2201	22	23	0.477	0.536	0.499
TSB2202	22	58	0.467	0.514	0.498
TSB2301	23	24	0.423	0.450	0.488
TSA29	23	46	0.458	0.509	0.497
TSB2401	24	3	0.327	0.473	0.440
TSA31	24	43	0.495	0.520	0.500
TSB2402	24	63	0.482	0.618	0.499*
TSB2501	25	3	0.396	0.486	0.479
TSA32	25	40	0.315	0.370	0.431
TSB2601	26	9	0.427	0.491	0.489
TSB2602	26	45	0.491	0.509	0.500
TSB2701	27	21	0.430	0.505	0.490
TSA33	27	45	0.500	0.570	0.500
TSA35	28	7	0.323	0.409	0.437
TSB2801	28	41	0.491	0.560	0.500
TSB2901	29	19	0.450	0.445	0.495
TSA37	29	42	0.405	0.486	0.482
TSA39	X	99	0.441	0.569	0.493

*Hardy-Weinberg equilibrium deviation at $P < 0.05$ level. †observed heterozygosity. ‡expected heterozygosity. BTA: *Bos taurus* chromosome number.

revealed that 20.7% (18/87) of the SNP loci showed significant departure from HWE at $P < 0.05$ (Table 2). Most of the SNPs (17/18) showed heterozygote excess, while heterozygote deficiency was observed in only TSB0201. Such minor HWE deviations would not have any crucial effect on the degree of accuracy for calculated exclusion probabilities (Schnabel *et al*. 2000). However, we should appraise the trend of the allele frequency thoughtfully in future generations and in each cattle population.

A total of 87 SNPs were evaluated regarding their utility for individual identification and parentage testing. Based on the allele and genotype frequencies of each SNP, combined P_I was calculated as 2.01×10^{-34}. The SNP panel could be an extremely powerful system for individual identification. Out of all SNPs, 15 most informative SNPs yielded sufficient probability (2.58×10^{-7}) to identify all Japanese Black cattle with approximately 1.6 million populations in Japan. In general, exclusion probability of >0.9999 is required for parentage testing. In the current study, P_{E1} and P_{E2} were estimated as 0.99998010 and 0.99999997, respectively. This developed SNP panel is quite a powerful tool and could successfully exclude false sires with a probability of >0.9999 even if the dam's genotype information was not obtained.

Recently, high-throughput genotyping methods for a modest number of SNPs have been developed, such as TaqMan assay (Holland *et al*. 1991), matrix-assisted laser desorption ionization – time-of-flight (MALDI-TOF) mass spectrometry (Ross *et al*. 1998), flow cytometric analysis performed on a Luminex 100 flow cytometer (Taylor *et al*. 2001), SNPlex assay (Tobler *et al*. 2005) and the DigiTag2 assay (Nishida *et al*. 2007). Rapid, cost-effective, highly reliable and high-throughput genotyping methods are essential for practical individual identification and parentage testing. The application of the techniques would be useful in practical inspection.

In conclusion, we developed a novel SNP system consisting of 87 SNP markers for Japanese Black cattle. This system could be powerful for individual identification and parentage testing, and may contribute to assurance of food identity and management of Japanese Black populations.

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