# A Method for Discriminating a Japanese Brand of Chicken, the Hinai-jidori, Using Microsatellite Markers[1]

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**ABSTRACT** The Hinai-dori is a native breed of chicken from the Akita Prefecture in Japan. A cross between the Hinai-dori and Rhode Island Red breeds has been commercialized as the Hinai-jidori chicken, one of the most popular brands of chicken in Japan. Here, a method of discriminating between the Hinai-jidori and other chickens is described. Individuals (555) of the Hinai-dori breed were analyzed by using 37 microsatellite markers on the Z chromosome. Fourteen of the marker loci (**ABR1003**, **ADL0250**, **ABR0241**, **ABR0311**, **ABR1004**, **ABR1013**, **ABR0633**, **ABR1005**, **ABR0089**, **ABR1007**, **ABR1001**, **ABR1009**, **ABR1010**, and **ABR1011**) were fixed in the Hinai-dori breed. So, the Hinai-jidori chicken, $F_1$ of the Hinai-dori breed, must have at least one of the alleles with all fixed loci. When these alleles on 14 loci from the Hinai-dori breed were not detected in meat samples, it would be judged that the samples were not the Hinai-jidori chicken. Thus, the use of these 14 microsatellite markers provides a practical method of accurately discriminating the Hinai-jidori chicken from other chickens on the market.

**Key words**: chicken, brand discrimination, microsatellite marker, Hinai-dori breed, $F_1$ meat

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## INTRODUCTION

Food traceability is defined as the ability to follow particular foods through all stages of the food chain, from production to sale (Committee of the Guidelines for Introduction of Food Traceability Systems of Japan, 2003), and it is increasingly becoming standard across the agriculture-food industry. The DNA identification technology has been playing an important role in refining existing traceability systems because it ensures that meat products can be traced to the animal of origin. Microsatellite markers, amplified fragment length polymorphism (**AFLP**) markers, and single nucleotide polymorphism (**SNP**) markers have been used for individual identification and parentage testing in cattle (Glowatzki-Mullis et al., 1995; Usha et al., 1995; Heyen et al., 1997; Ajmone-Marsan et al., 1997; Heaton et al., 2002). It is possible to discriminate individual chickens by using these DNA markers, but individual management is not realistic in the case of commercial chicken production; breed or brand identification with conclusive proof is more desirable.

The Hinai-dori is a breed of chicken native to Akita Prefecture, in northern Honshu, Japan. The taste of Hinai-dori meat is well recognized and has been used for many years as an ingredient in the indigenous dish, Kiritanpo stew, of Akita Prefecture (Introduction to Akita Prefecture, 1997). The Hinai-dori breed decreased in numbers under the influence of exotic breeds introduced in the Meiji period (1868 to 1912) and for a time was at risk for extinction. In 1942, the Hinai-dori was designated a national treasure of Japan. For efficient conservation and effective use of the Hinai-dori breed, the Akita Prefectural Livestock Experiment Station (now the Livestock Experiment Station of the Akita Prefectural Agriculture, Forestry, and Fisheries Research Center) performed single-crossing tests with Hinai-dori male parents (Hatakeyama et al., 1978). Taste tests revealed that $F_1$ meat from a cross between the Hinai-dori and Rhode Island Red breeds was best. In addition, the $F_1$ individuals had a resemblance to the Hinai-dori breed. Therefore, the crossbred (Hinai-dori sire × Rhode Island Red dam) was commercialized as the Hinai-jidori chicken. The Hinai-jidori chicken is a popular brand in Japan, and sales continue to increase year after year. The market price of Hinai-jidori chicken meat is much higher than that of contemporary broiler meat. However, consumers cannot easily distinguish cuts of Hinai-jidori meat from those of other chickens simply by appearance. Consequently, with the continued expansion of sales, the need to check the validity of labeling of the Hinai-jidori chicken has arisen. The objective of the


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current study was to develop a method of discriminating between the Hinai-jidori chicken and all other chicken on the market by using microsatellite markers.

## MATERIALS AND METHODS

Three hundred sixty individuals (40 male and 320 female) at the Livestock Experimental Station, Akita Prefectural Agriculture, Forestry, and Fisheries Research Center, and 195 individuals (94 male and 101 female) collected from members of the Association for Preservation of Native Chickens, in Akita Prefecture, Japan, were studied. Chicken genomic DNA for PCR amplification was extracted from blood and myocardium by the conventional phenol-chloroform extraction method or by using a DNA extraction kit (Sepagene, Sanko-Junyaku, Tokyo, Japan).

The Hinai-jidori chicken is a crossbred between the male of the Hinai-Dori breed and the female of the Rhode Island Red breed. Because the taste of female meat is more suitable for the indigenous dish, Kiritanpo stew, than that of male meat, almost 100% of Hinai-jidori chickens sold commercially are females. Female individuals have 1 Z chromosome from the Hinai-dori breed and 1 W chromosome from the Rhode Island Red breed. Thus, priority was given to the microsatellite markers on chromosome Z, and 37 markers were tested. Details of each marker are summarized in Table 1.

The PCR amplifications were performed in a 6-μL reaction volume, which included 2.5 pmol of each primer, 100 μ*M* of each deoxynucleotide triphosphate, 1.2 m*M* $MgSO_4$, 0.0125 units of KOD plus DNA polymerase (KOD-201, Toyobo, Tokyo, Japan) isolated from *Thermococcus kodakaraensis*, 1 × reaction buffer provided by the supplier, and 30 ng of genomic DNA in a 384-well plate on an iCycler Thermal Cycler (BioRad Laboratories, Hercules, CA). The PCR was performed as follows: hot start 75 s at 94°C followed by 10 cycles of 15 s at 94°C, 30 s at 60°C, and 60 s at 68°C, followed by 10 cycles with the same conditions except that the annealing temperature was 55°C, and then 30 cycles with an annealing temperature of 50°C, and finally elongation for 9 min at 68°C. The PCR products were run with the internal size standard GENESCAN 400HD [ROX] Size Standard (Perkin-Elmer, Foster City, CA) on an ABI PRISM 3100 DNA Sequencer (Perkin-Elmer). The size of fragments was analyzed by using the GeneScan (Version 3.7) and GeneMapper (Version 2.0) programs (Perkin-Elmer). Alleles were designated according to PCR product size, and allele frequencies were calculated directly from observed genotypes.

In the case of female chickens, the exclusion probabilities ($\mathbf{P_E}$) of the Hinai-jidori chicken were calculated from the allele frequencies observed as

$$\mathbf{P}_{\#} = 1 - \prod_{i=1}^{n} p_i,$$

where $p_i$ = the allele frequencies of the *i*th locus, which shows 1 fixed allele in the Hinai-dori breed, and n = the number of loci that show 1 fixed allele in the Hinai-dori breed.

In the case of male chickens, the expected genotype frequencies showing the Hinai-jidori (*HJ*)-type were calculated as

$$HJ = p_i^2 + 2\,p_i(1 - p_i).$$

The $P_E$ of the Hinai-jidori chicken were calculated as

$$P_E = 1 - \prod_{i=1}^{n} HJ_i,$$

where n = the number of loci that show 1 fixed allele in the Hinai-dori breed.

## RESULTS

Of the 37 microsatellite markers examined on the Z chromosome, 14 markers (*ABR1003*, *ADL0250*, *ABR0241*, *ABR0311*, *ABR1004*, *ABR1013*, *ABR0633*, *ABR1005*, *ABR0089*, *ABR1007*, *ABR1001*, *ABR1009*, *ABR1010*, and *ABR1011*) showed 1 fixed allele each in the Hinai-dori breed (Table 2). The Hinai-jidori chicken cross between the Hinai-dori and Rhode Island Red breeds must have 1 Z chromosome from the Hinai-dori breed. Thus, these 14 markers were used to determine whether they could discriminate between the Hinai-jidori and other chickens. Samples (420) belonging to 9 breeds (11 populations): Japanese Game A (13 individuals), Japanese Game B (42), Satuma-dori (17), White Leghorn (19), Rhode Island Red (18), New Hampshire (30), White Plymouth Rock A (42), White Plymouth Rock B (20), Barred Plymouth Rock (30), White Cornish (51), Red Cornish (43), and broiler chickens (95) were analyzed by using the 14 markers. Allele frequencies for all populations by locus are shown in Table 2. Of the 14 markers, 10 markers (*ABR1003*, *ADL0250*, *ABR0241*, *ABR0311*, *ABR1004*, *ABR1013*, *ABR0633*, *ABR1005*, *ABR0089*, and *ABR1007*) were polymorphic, and 4 markers (*ABR1001*, *ABR1009*, *ABR1010*, and *ABR1011*) were monomorphic in the samples. From the data on allele frequencies of the 10 polymorphic loci in Table 2, genotype frequencies showing Hinai-jidori-types in various chicken populations and the $P_E$ of the Hinai-jidori chicken were calculated (Table 3). The expected $P_E$ in the 9 purebreds and broiler chicken was 100% because the samples were not the Hinai-jidori chickens.

## DISCUSSION

The PCR-based marker systems such as random amplified polymorphic DNA, AFLP, and microsatellites are widely used for cultivar identification in autogamous plants such as rice (Garland et al., 1999; Ohtsubo et al., 2002; Shirasawa et al., 2004). Breed identification in livestock is much more difficult than cultivar identification in autogamous plants because each individual is heterozygous and each breed has genetic diversity. In swine, Okumura et al. (2000) reported that polymorphic informa-

**Table 1.** Microsatellite markers used in the current study

| Marker | Forward primer (5′ → 3′) | Reverse primer (5′ → 3′) | References | Map position in the draft sequence (May 2006 Assembly) | | |
|---|---|---|---|---|---|---|
| ABR0080 | TTGCCCTGGGGCAGAACACG | CAACAGCTTTCGACGAGACGG | Takahashi et al. (2005) | chrZ | 20142891 | 20143063 |
| ABR0082 | TCCTGAATTTCCAAATAAGTTTTA | TAATCACAGCCCAAATCAAAG | Takahashi et al. (2005) | chrZ | 29886108 | 29886328 |
| ABR0089 | ATAATCACAGCCCAAATCAA | CCTGAATTTCCAAATAAGTTTTA | Takahashi et al. (2005) | chrZ | 29886107 | 29886327 |
| ABR0112 | TACTTTTATCCTGCTTCTCA | GCTTGTAGGGTAATCCAATG | Takahashi et al. (2006) | chrZ | 21804512 | 21804742 |
| ABR0241 | ATACACTCGGCAAGCCAGAC | CCCGGATCAGCTCATAAAGAC | Takahashi et al. (2006) | chrZ | 48390709 | 48390824 |
| ABR0254 | TTTGGTAACTGAGTAAATAGC | ACTTTGTAGGAAATGGACTT | Takahashi et al. (2005) | chrZ | 38054130 | 38054456 |
| ABR0289 | TTCTCAAACTGTTAAGGTCCAC | AACTCCCACTCCACCACAAC | Takahashi et al. (2005) | chrZ | 63376080 | 63376379 |
| ABR0311 | CCTAAAGCAGGAAGGCAGAA | TTGGAGCATTTGTGGAGAAG | Takahashi et al. (2005) | chrZ | 31395698 | 31395907 |
| ABR0376 | AGGGTATGGATGTCTTACTA | CACAAAGTTCCTGAATAATA | Takahashi et al. (2006) | chrZ | 43199764 | 43199933 |
| ABR0505 | TTATTTATGGCACTCCACTG | TATTCCTTGTTTTGCTTTGA | Takahashi et al. (2006) | chrZ | 32134968 | 32135193 |
| ABR0524[1] | TCCTACCGAAGGCAACAGAA | GGCCCACTTAGCAGATGGAGAAT | Takahashi et al. (2005) | Un_random | 6580137 | 6580414 |
| ABR0588 | ATACAATCCAGCATCTCACA | CCCATTATTCGTTATTCTTACTT | Takahashi et al. (2005) | chrZ | 17778232 | 17778361 |
| ABR0598 | CAGGTCCTTTGCTACTTACA | GTACTCCGCAGACTTTCACT | Takahashi et al. (2005) | chrZ | 34260619 | 34260921 |
| ABR0608 | GCAGGAAGGTTCACAGAAAG | TTGGCAATAGCTTCAAAACA | Takahashi et al. (2005) | chrZ | 8313971 | 8314196 |
| ABR0620 | GCCAGCTTCAGGGAACAAAA | TGAAACGCAAAATCAACGGA | Takahashi et al. (2005) | chrZ | 23271403 | 23271669 |
| ABR0621 | ACTTTCCCTCTTGCTGGACT | GTTGGCATGACTTTGTTGCT | Takahashi et al. (2005) | chrZ | 40284779 | 40285147 |
| ABR0633 | AGTATGTTATTGCCTGTGGC | TTTGGGAGAAGGAATGTTGT | Takahashi et al. (2005) | chrZ | 13031126 | 13031391 |
| ABR0651 | TGGGAAAAGTCAGTAGAACA | TGCATTATTACATCCCATCT | Takahashi et al. (2005) | chrZ | 72120493 | 72120683 |
| ABR0657 | CAGCAACAAACAAATACAAA | AGTAAGGTATCATCAGAGGG | Takahashi et al. (2005) | chrZ | 63335383 | 63335579 |
| ADL0250 | AAGCCGTACTGAGAAGCACT | CAGGCACAGTAGAAAAGAAC | Hu et al. (2001) | chrZ | 36707738 | 36707898 |
| LEI0121 | TTGACGTCCTGGATAGATTAC | ATTATCCAGAACTAACATCAAC | Crooijmans et al. (1997) | chrZ | 49858361 | 49858680 |
| ABR1001[2] | TTGAGATGTTGATGTGGAAAACG | CAAGAAGGTGAGGAGAACAAGGA | | chrZ | 12997930 | 12998258 |
| ABR1002 | TAGGAAAGATGCCCGATAAAA | CAAGGGTTGGGAATGTAGTGA | | chrZ | 13089627 | 13089974 |
| ABR1003 | AGAGGTAGGCGATGGACCAAA | ATGCACCAAGTGACCAGGGAC | | chrZ | 23015564 | 23015724 |
| ABR1004 | TGTACTCAACTAAGACGGGATT | TGTTATGTGATGTGAAACCTGA | | chrZ | 30217729 | 30217945 |
| ABR1005 | GTAACACTTCACATTCAAGAGGCAT | AACCAGCATTTCCTTCAGCAA | | chrZ | 28357290 | 28357508 |
| ABR1006 | GCATCGCATTTAGGGTAAGTAT | AGTTTCACTCACGGGAGTTTTA | | chrZ | 28617283 | 28617471 |
| ABR1007 | GTCCCTCCCCTTTGCCACAAC | TGCTGAAGACAGACTGCTGATAG | | chrZ | 28871952 | 28872182 |
| ABR1008 | GGGCTCTTTAGGACAACTCAC | ATCAATATGAAGGCAGTTACAAGA | | chrZ | 29369940 | 29370207 |
| ABR1009 | GCATTTGATTAAGTGTGCTC | CAGGTAAGTGTCATGGTTGG | | chrZ | 33592107 | 33592309 |
| ABR1010 | CTGCTTCAGCCAGTCTCAGTAT | CTTTTCACAAGTTTTCCCTTTT | | chrZ | 32746964 | 32747232 |
| ABR1011 | TATCACCTGTGACTGAGGCATT | CTTGGAGAGATTTTTTGGAAGC | | chrZ | 32449118 | 32449338 |
| ABR1012 | CACCACGATACTTTCCCTTTA | AACAATCTTTTCCCCCCATAC | | chrZ | 32135019 | 32135286 |
| ABR1013 | GAACAAGGTAGAACTCGTCGGT | TGCTCGGGGAAGTATCACAAC | | chrZ | 31442224 | 31442362 |
| ABR1014 | GGACAGCCAGTTGCTAGCCTTG | TGCCTGCTTGACCACAAACCAC | | chrZ | 31365234 | 31365373 |
| ABR1015 | CATCGCACCAACATCCACCTT | TGCATAAAGCCTGCTATGACC | | chrZ | 36624037 | 36624356 |
| ABR1016 | CCTTGAACAGAAAGCAGGTGG | TCGTGGAAAACATGAGATGGC | | chrZ | 36706061 | 36706195 |

[1]ABR0524 was mapped on the Z chromosome in the linkage map of Takahashi et al. (2005).

[2]PCR primers for ABR1001 to 1016 were newly designed from the draft sequence of the chicken genome [UCSC Chicken Genome Browser (2004) and Ensembl Chicken Genome Browser (2004)].

**Table 2.** Allele frequencies for all populations[1] by locus

| Name and size of alleles (bp) | Hinai-Dori | JG-A | JG-B | STM | WL | RIR | NH | WR-A | WR-B | BPR | WC | RC | Broiler chicken |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ABR1003 | | | | | | | | | | | | | |
| 148 | 0 | 0 | 0 | 0 | 0 | 0.368 | 0.100 | 0 | 1.000 | 0 | 0.600 | 0.234 | 0.293 |
| 153 | 0 | 0 | 0 | 0.952 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 155 | 0 | 0 | 0 | 0 | 0 | 0.474 | 0.100 | 0 | 0 | 0 | 0.033 | 0.128 | 0.012 |
| 157 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.267 | 0.404 | 0.439 |
| 159 | 0 | 0.944 | 0.250 | 0 | 1.000 | 0.105 | 0.800 | 0.409 | 0 | 0 | 0 | 0.234 | 0.165 |
| 161 | 1.000 | 0.056 | 0.750 | 0.048 | 0 | 0.053 | 0 | 0.591 | 0 | 1.000 | 0 | 0 | 0.091 |
| ADL250 | | | | | | | | | | | | | |
| 155 | 0 | 0 | 0 | 0 | 0 | 0.000 | 0 | 0 | 0.04 | 0 | 0 | 0 | 0 |
| 156 | 0 | 0 | 0.591 | 0 | 0 | 0.176 | 0 | 0.474 | 0.160 | 0 | 0.639 | 0.444 | 0.176 |
| 157 | 0 | 0.278 | 0 | 0.261 | 0 | 0.471 | 0.222 | 0.526 | 0.520 | 1.000 | 0 | 0.311 | 0.165 |
| 158 | 0 | 0 | 0 | 0.130 | 0 | 0.353 | 0.222 | 0 | 0.280 | 0 | 0 | 0 | 0.082 |
| 159 | 1.000 | 0.722 | 0.409 | 0.609 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.200 | 0 |
| 162 | 0 | 0 | 0 | 0 | 1.000 | 0 | 0.556 | 0 | 0 | 0 | 0.361 | 0.044 | 0.576 |
| ABR241 | | | | | | | | | | | | | |
| 98 | 1.000 | 0 | 0 | 0 | 0.111 | 0.059 | 0.381 | 0.982 | 0.048 | 0 | 0.403 | 0.936 | 0.060 |
| 113 | 0 | 0.778 | 0.500 | 1.000 | 0 | 0 | 0 | 0.018 | 0.952 | 0 | 0 | 0.021 | 0.179 |
| 118 | 0 | 0.222 | 0.500 | 0 | 0 | 0.941 | 0.095 | 0 | 0 | 0.235 | 0 | 0 | 0 |
| 122 | 0 | 0 | 0 | 0 | 0.889 | 0 | 0 | 0 | 0 | 0.765 | 0.125 | 0.043 | 0.143 |
| 124 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.083 |
| 126 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.472 | 0 | 0.464 |
| 128 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.071 |
| 130 | 0 | 0 | 0 | 0 | 0 | 0 | 0.524 | 0 | 0 | 0 | 0 | 0 | 0 |
| ABR311 | | | | | | | | | | | | | |
| 194 | 0 | 0 | 0.408 | 0 | 0.05 | 0.550 | 0.600 | 0 | 0.636 | 0 | 0 | 0.128 | 0 |
| 202 | 0 | 0 | 0 | 0 | 0 | 0 | 0.267 | 0.581 | 0.364 | 0.667 | 0.000 | 0.051 | 0 |
| 204 | 0 | 0.294 | 0 | 0 | 0.950 | 0 | 0 | 0.233 | 0 | 0 | 0.100 | 0.282 | 0.097 |
| 208 | 1.000 | 0.353 | 0 | 0 | 0 | 0.450 | 0.133 | 0.186 | 0 | 0.333 | 0.900 | 0.385 | 0.903 |
| 210 | 0 | 0.353 | 0.592 | 1.000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.154 | 0 |
| ABR1004 | | | | | | | | | | | | | |
| 214 | 0 | 0.647 | 0.574 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.16 | 0.006 |
| 216 | 1.000 | 0.353 | 0.426 | 0.500 | 0 | 0 | 0.200 | 1.000 | 0 | 0.759 | 0.067 | 0.340 | 0.774 |
| 217 | 0 | 0 | 0 | 0 | 1.000 | 1.000 | 0.800 | 0 | 0.389 | 0.241 | 0.933 | 0.480 | 0.220 |
| 218 | 0 | 0 | 0 | 0.500 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 220 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.611 | 0 | 0 | 0.02 | 0 |
| ABR1013 | | | | | | | | | | | | | |
| 133 | 1.000 | 0.722 | 0.250 | 0 | 0.136 | 0.375 | 0.133 | 0.086 | 1.000 | 0.536 | 0.946 | 0.532 | 0.564 |
| 134 | 0 | 0 | 0.750 | 1.000 | 0.818 | 0.333 | 0.600 | 0 | 0 | 0.464 | 0.054 | 0.064 | 0.248 |
| 135 | 0 | 0.278 | 0 | 0 | 0 | 0.292 | 0.267 | 0.914 | 0 | 0 | 0 | 0.404 | 0.180 |
| 141 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.008 |
| 143 | 0 | 0 | 0 | 0 | 0.045 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ABR633 | | | | | | | | | | | | | |
| 261 | 1.000 | 0 | 0.015 | 0 | 0 | 0.842 | 0.067 | 1.000 | 1.000 | 1.000 | 0.775 | 0.340 | 0.675 |
| 267 | 0 | 1.000 | 0.985 | 1.000 | 0 | 0 | 0 | 0 | 0 | 0 | 0.197 | 0.149 | 0.151 |
| 269 | 0 | 0 | 0 | 0 | 0.900 | 0 | 0 | 0 | 0 | 0 | 0.028 | 0.426 | 0 |
| 271 | 0 | 0 | 0 | 0 | 0.100 | 0.158 | 0.933 | 0 | 0 | 0 | 0 | 0.085 | 0.175 |
| ABR1005 | | | | | | | | | | | | | |
| 203 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.019 |
| 205 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.056 | 0 | 0 | 0.043 | 0 |
| 207 | 1.000 | 0.400 | 0.682 | 0.278 | 0 | 0.708 | 0.967 | 0.340 | 0.944 | 0.885 | 0 | 0.522 | 0.683 |
| 213 | 0 | 0 | 0 | 0.722 | 0 | 0.292 | 0.033 | 0 | 0 | 0.115 | 0 | 0.196 | 0.269 |
| 218 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.029 |
| 224 | 0 | 0.600 | 0.318 | 0 | 1.000 | 0 | 0 | 0.660 | 0 | 0 | 1.000 | 0.239 | 0 |
| ABR89 | | | | | | | | | | | | | |
| 214 | 0 | 0 | 0 | 0 | 0.048 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 215 | 0 | 0 | 0 | 0.588 | 0 | 0 | 0 | 0 | 0 | 0 | 0.771 | 0.022 | 0.051 |
| 216 | 1.000 | 0.471 | 0.424 | 0.412 | 0.952 | 0.368 | 0.862 | 1.000 | 1.000 | 1.000 | 0.171 | 0.891 | 0.808 |
| 217 | 0 | 0.529 | 0.424 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.057 | 0.022 | 0 |
| 218 | 0 | 0 | 0 | 0 | 0 | 0.632 | 0.138 | 0 | 0 | 0 | 0 | 0.087 | 0.141 |
| 220 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ABR1007 | | | | | | | | | | | | | |
| 224 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.006 |
| 228 | 1.000 | 0.600 | 0.623 | 0.786 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.980 | 0.994 |
| 239 | 0 | 0 | 0 | 0.214 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 247 | 0 | 0.400 | 0.377 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.020 | 0 |
| ABR1001 | | | | | | | | | | | | | |
| 327 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |

*Continued*

**Table 2 (Continued).** Allele frequencies for all populations[1] by locus

| Name and size of alleles (bp) | Hinai-Dori | JG-A | JG-B | STM | WL | RIR | NH | WR-A | WR-B | BPR | WC | RC | Broiler chicken |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ABR1009 | | | | | | | | | | | | | |
| 199 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| ABR1010 | | | | | | | | | | | | | |
| 265 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| ABR1011 | | | | | | | | | | | | | |
| 217 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |

[1]JG-A = Japanese Game A; JG-B = Japanese Game B; STM = Satuma-dori; WL = White Leghorn; RIR = Rhode Island Red; NH = New Hampshire; WPR-A = White Plymouth Rock A; WPR-B = White Plymouth Rock B; BPR = Barred Plymouth Rock; WC = White Cornish; RC = Red Cornish. The White Leghorn and Rhode Island Red breeds have been maintained at the Aichi-ken Agricultural Research Center. The Barred Plymouth Rock and New Hampshire breeds have been maintained at the Livestock Experiment Station, Akita Prefectural Agriculture, Forestry and Fisheries Research Center. The Japanese Game A and Satuma-Dori breeds have been maintained at the Kochi Prefectural Livestock Experiment Station. The Japanese Game B, White Plymouth Rock A and B, White Cornish, and Red Cornish breeds were introduced from Hyogo station, National Livestock Breeding Center of Japan. White Plymouth Rock A and B are meat- and layer-type, respectively. Hearts of broiler chickens were bought in the market.

tion in *MC1R* (Melanocortin Receptor 1) and *KIT* genes was useful for distinguishing pig breeds that are commercially produced in Japan. Alves et al. (2002) reported the usefulness of AFLP markers to discriminate between purebred and crossbred Iberian pigs. In cattle, markers to discriminate between Japanese Black and $F_1$ (Japanese Black × Holstein) breeds have been reported (Sasazaki et al., 2004). The AFLP and 6 SNP markers absent in Japanese Black but present in Holstein were identified. From the allelic frequencies of the 6 markers in both breeds, the combined probabilities of identifying $F_1$ and of misjudgment were estimated at 88.2 and 2%, respectively.

In commercial chickens, Nakamura et al. (2006) reported a method for discriminating a Japanese chicken breed, the Nagoya, from other chicken breeds. The Nagoya, a dual-purpose breed for eggs and meat, is a popular native chicken in Aichi Prefecture, Japan. Five microsatellite markers, each of which has a single allele in the Nagoya breed, were identified. Because commercial Nagoya chickens must have fixed alleles for the 5 markers, these markers can be used for discriminating between the Nagoya and all other chickens. The expected probability of exclusion of the Nagoya breed in purebreds and broiler chicken was almost 100%.

**Table 3.** Expected genotype frequencies showing Hinai-jidori-types and the exclusion probabilities (PE) for all populations[1] by locus

| | | Locus | | | | | | | | | | PE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | Sex | ABR1003 | ADL0250 | ABR0241 | ABR0311 | ABR1004 | ABR1013 | ABR0633 | ABR1005 | ABR0089 | ABR1007 | (%) |
| JG-A | Female | 0.056 | 0.722 | 0 | 0.353 | 0.353 | 0.722 | 0 | 0.400 | 0.471 | 0.600 | 100 |
| | Male | 0.108 | 0.923 | 0 | 0.581 | 0.581 | 0.923 | 0 | 0.640 | 0.720 | 0.840 | 100 |
| JG-B | Female | 0.750 | 0.409 | 0 | 0 | 0.426 | 0.250 | 0.015 | 0.682 | 0.424 | 0.623 | 100 |
| | Male | 0.938 | 0.651 | 0 | 0 | 0.671 | 0.438 | 0.030 | 0.899 | 0.669 | 0.858 | 100 |
| STM | Female | 0.048 | 0.609 | 0 | 0 | 0.500 | 0 | 0 | 0.278 | 0.412 | 0.786 | 100 |
| | Male | 0.093 | 0.847 | 0 | 0 | 0.750 | 0 | 0 | 0.478 | 0.654 | 0.954 | 100 |
| WL | Female | 0 | 0 | 0.111 | 0 | 0 | 0.136 | 0 | 0 | 0.952 | 1.000 | 100 |
| | Male | 0 | 0 | 0.210 | 0 | 0 | 0.254 | 0 | 0 | 0.998 | 1.000 | 100 |
| RIR | Female | 0.053 | 0 | 0.059 | 0.450 | 0 | 0.375 | 0.842 | 0.708 | 0.368 | 1.000 | 100 |
| | Male | 0.102 | 0 | 0.114 | 0.698 | 0 | 0.609 | 0.975 | 0.915 | 0.601 | 1.000 | 100 |
| NH | Female | 0 | 0 | 0.381 | 0.133 | 0.200 | 0.133 | 0.067 | 0.967 | 0.862 | 1.000 | 100 |
| | Male | 0 | 0 | 0.617 | 0.249 | 0.360 | 0.249 | 0.129 | 0.999 | 0.981 | 1.000 | 100 |
| WR-A | Female | 0.591 | 0 | 0.982 | 0.186 | 1.000 | 0.086 | 1.000 | 0.340 | 1.000 | 1.000 | 100 |
| | Male | 0.833 | 0 | 1.000 | 0.337 | 1.000 | 0.165 | 1.000 | 0.564 | 1.000 | 1.000 | 100 |
| WR-B | Female | 0 | 0 | 0.048 | 0 | 0 | 1.000 | 1.000 | 0.944 | 1.000 | 1.000 | 100 |
| | Male | 0 | 0 | 0.093 | 0 | 0 | 1.000 | 1.000 | 0.997 | 1.000 | 1.000 | 100 |
| BPR | Female | 1.000 | 0 | 0 | 0.333 | 0.759 | 0.536 | 1.000 | 0.885 | 1.000 | 1.000 | 100 |
| | Male | 1.000 | 0 | 0 | 0.556 | 0.942 | 0.784 | 1.000 | 0.987 | 1.000 | 1.000 | 100 |
| WC | Female | 0 | 0 | 0.403 | 0.900 | 0.067 | 0.946 | 0.775 | 0 | 0.171 | 1.000 | 100 |
| | Male | 0 | 0 | 0.643 | 0.990 | 0.129 | 0.997 | 0.949 | 0 | 0.313 | 1.000 | 100 |
| RC | Female | 0 | 0.200 | 0.936 | 0.385 | 0.340 | 0.532 | 0.340 | 0.522 | 0.891 | 0.980 | 100 |
| | Male | 0 | 0.360 | 0.996 | 0.621 | 0.564 | 0.781 | 0.565 | 0.771 | 0.988 | 1.000 | 100 |
| Broiler chicken | Female | 0.091 | 0 | 0.060 | 0.903 | 0.774 | 0.564 | 0.675 | 0.683 | 0.808 | 0.994 | 100 |
| | Male | 0.175 | 0 | 0.116 | 0.991 | 0.949 | 0.810 | 0.894 | 0.899 | 0.963 | 1.000 | 100 |

[1]JG-A = Japanese Game A; JG-B = Japanese Game B; STM = Satuma-dori; WL = White Leghorn; RIR = Rhode Island Red; NH = New Hampshire; WPR-A = White Plymouth Rock A; WPR-B = White Plymouth Rock B; BPR = Barred Plymouth Rock; WC = White Cornish; RC = Red Cornish.

In Japan, consumer demand for DNA identification of jidori (Japanese old-style native) chicken meat is increasing, especially since publication of the paper by Nakamura et al. (2006). The Hinai-jidori chicken is very popular in Japan, but it is a crossbred, whereas the Nagoya is a purebred. Therefore, the Hinai-jidori chicken cannot be identified by the strategy adopted for the Nagoya breed. On the basis of the fact that almost 100% of Hinai-jidori chickens sold commercially are females, the strategy described here was conceived for the utilization of microsatellite markers on the Z chromosome. In the Hinai-dori breed, 14 of the markers (*ABR1003*, *ADL0250*, *ABR0241*, *ABR0311*, *ABR1004*, *ABR1013*, *ABR0633*, *ABR1005*, *ABR0089*, *ABR1007*, *ABR1001*, *ABR1009*, *ABR1010*, and *ABR1011*) on the Z chromosome each had a single allele. Because the Hinai-jidori has a Z chromosome from the Hinai-dori male parent, the Hinai-jidori chicken must have at least 1 set of fixed alleles for the 14 markers. The expected probability of exclusion of the Hinai-jidori chicken among purebred populations or hybrid broilers in Japan was 100%. In the market, it is suspected that some hybrid chickens (i.e., broilers) are being falsely labeled as Hinai-jidori. This is why a way is needed of identifying the Hinai-jidori chicken from commercial broiler stocks. In practice, it is not necessary to examine all markers to discriminate the Hinai-jidori from other chicken breeds because sufficient exclusion probabilities can be obtained by using a combination of several markers, e.g., *ABR1003*, *ADL0250*, *ABR0241*, *ABR0311*, and *ABR1004*.

In conclusion, a method is described for discriminating the Hinai-jidori chicken from other chicken breeds by using microsatellite markers on the Z chromosome. Because this method is useful for discrimination between the Hinai-jidori and broiler chickens on the market and can help to check the validity of Hinai-jidori labeling, it is now being applied to the Japanese market.

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## REFERENCES

Ajmone-Marsan, P., A. Valentini, M. Cassandro, G. Vecchiotti-Antaldi, G. Bertoni, and M. Kuiper. 1997. AFLP markers for DNA fingerprinting in cattle. Anim. Genet. 28:418–426.

Alves, E., C. Castellanos, C. Ovilo, L. Silio, and C. Rodriguez. 2002. Differentiation of the raw material of the Iberian pig meat industry based on the use of amplified fragment length polymorphism. Meat Sci. 61:157–162.

Committee of the Guidelines for Introduction of Food Traceability Systems of Japan. 2003. Guidelines for Introduction of Food Traceability Systems (in Japanese), Tokyo, Japan.

Crooijmans, R. P. M. A., R. J. M. Dijkhof, J. J. van der Poel, and M. A. M. Groenen. 1997. New microsatellite markers in chicken optimized for automated fluorescent genotyping. Anim. Genet. 28:427–437.

Ensembl Chicken Genome Browser. 2004. European Bioinformatics Institute. Cambridge, UK. http://www.ensembl.org/Gallus_gallus/index.html Accessed Mar. 2007.

Garland, S. H., L. Lewin, M. Abedinia, R. Henry, and A. Blakeney. 1999. The use of microsatellite polymorphisms for the identification of Australian breeding lines of rice (*Oryza sativa* L.). Euphytica 108:53–63.

Glowatzki-Mullis, M. L., C. Gaillard, G. Wigger, and R. Fries. 1995. Microsatellite-based parentage control in cattle. Anim. Genet. 26:7–12.

Hatakeyama, Y., T. Katsuura, and A. Akagawa. 1978. Hybrid use of the Hinai-Dori breed—the third report (in Japanese). Res. Bull. Akita Prefectural Livest. Exp. Stn. (1977 fiscal year):81–89.

Heaton, M. P., G. P. Harhay, G. L. Bennett, R. T. Stone, W. M. Grosse, E. Casas, J. W. Keele, T. P. Smith, C. G. Chitko-McKown, and W. W. Laegreid. 2002. Selection and use of SNP markers for animal identification and paternity analysis in U.S. beef cattle. Mamm. Genome 13:272–281.

Heyen, D. W., J. E. Beever, Y. Da, R. E. Evert, C. Green, S. R. Bates, J. S. Ziegle, and H. A. Lewin. 1997. Exclusion probabilities of 22 bovine microsatellite markers in fluorescent multiplexes for semiautomated parentage testing. Anim. Genet. 28:21–27.

Hu, J., C. Mungall, A. Law, R. Papworth, J. P. Nelson, A. Brown, I. Simpson, S. Leckie, D. W. Burt, A. L. Hillyard, and A. L. Archibald. 2001. The ARKdb—genome databases for farmed and other animals. Nucleic Acids Res. 29:106–110.

Introduction to Akita Prefecture. 1997. Akita Prefectural Government, Akita, Japan. http://www.pref.akita.jp/industry-location/english/about/craft10.html Accessed Mar. 2007.

Nakamura, A., K. Kino, M. Minezawa, K. Noda, and H. Takahashi. 2006. A method for discriminating a Japanese chicken, the Nagoya breed, using microsatellite markers. Poult. Sci. 85:2124–2129.

Ohtsubo, K., S. Nakamura, and T. Imamura. 2002. Development of the primer sets for identification of a rice cultivar, Koshihikari, by PCR (in Japanese). Nippon Nogeikagaku Kaishi 76:388–397.

Okumura, N., E. Kobayashi, H. Suzuki, T. Morozumi, N. Hamashima, and T. Mitsuhashi. 2000. Breed specific mutations in melanocortin receptor 1 (MC1R) and KIT genes in pigs (in Japanese). Anim. Sci. J. 71:J222–J234.

Sasazaki, S., K. Itoh, S. Arimitsu, T. Imada, A. Takasuga, H. Nagaishi, S. Takano, H. Mannen, and S. Tsuji. 2004. Development of breed identification markers derived from AFLP in beef cattle. Meat Sci. 67:275–280.

Shirasawa, K., S. Kishitani, and T. Nishio. 2004. Conversion of AFLP markers to sequence-specific markers for closely related lines in rice by use of the rice genome sequence. Mol. Breed. 14:283–292.

Takahashi, H., A. Nakamura, and K. Rikimaru. 2006. Identification of brand chicken meat derived from Japanese native chickens (in Japanese). Pages 207–213 in Final Reports for Research Grants for Meat and Meat Products. Vol. 24. Ito Foundation, Tokyo, Japan.

Takahashi, H., M. Tsudzuki, O. Sasaki, J. Niikura, M. Inoue-Murayama, and M. Minezawa. 2005. A chicken linkage map based on microsatellite markers genotyped on a Japanese Large Game and White Leghorn cross. Anim. Genet. 36:463–467.

UCSC Chicken Genome Browser. 2004. University of California, Santa Cruz, CA. http://genome.ucsc.edu/cgi-bin/hgGateway?org=Chicken&db=galGal3 Accessed Mar. 2007.

Usha, A. P., S. P. Simpson, and J. L. Williams. 1995. Probability of random sire exclusion using microsatellite markers for parentage verification. Anim. Genet. 26:155–161.