Identification of Single Nucleotide Polymorphism Markers in the Laccase Gene of Shiitake Mushrooms (Lentinula edodes)

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Abstract  We identified single nucleotide polymorphism (SNP) markers in the laccase gene to establish a line-diagnostic system for shiitake mushrooms. A total of 89 fungal isolates representing four lines, including Korean registered, Korean wild type, Chinese, and Japanese lines, were analyzed. The results suggest that SNP markers in the laccase gene can be useful for line typing in shiitake mushrooms.

Keywords  Laccase gene, Lentinula edodes, Mushroom, Shiitake, SNP
Table 1. PCR primer pairs and sequencing primer pairs for the *Letinula edodes* laccase gene

| Purpose          | Name            | Sequences            | mer | Ta (°C) |
|------------------|-----------------|----------------------|-----|---------|
| PCR, sequencing  | Lac-forward     | 5'-ATG CTT CCC TTC GTT TCT TCT-3' | 21  | 52      |
| PCR, sequencing  | Lac-reverse     | 5'-TCA AGG TAA TTG AGC AGG GGT-3' | 21  | 52      |
| Sequencing       | Lac-internal 1  | 5'-ATC CCG AGC GAC CTG AAT-3' | 18  | 50      |
| Sequencing       | Lac-internal 2  | 5'-AAG GGT GCA GCA TCG ATT-3' | 18  | 50      |
| Sequencing       | Lac-internal 3  | 5'-TTT CTT TGA CCC TAC TGC-3' | 18  | 50      |

Ta is annealing temperature.

*The primer pairs designed to amplify the LELCC gene were based on its open reading frame.*

Table 2. TaqMan probes for SNP genotyping of the *Letinula edodes* laccase gene

| Probe name | Primer and TaqMan probe sequences |
|------------|-----------------------------------|
| SNP1       | VIC→ATC ATC ATA TCT GTAT CAT TT (5→3) |
| SNP2       | FAM→TCC ATG TTC TCT CAT ATG T |
| SNP3       | VIC→CTG AGC CCC TGT TTG A |
| SNP4       | FAM→CTG ATC CAC GGT CAG CAT A |
| SNP5       | VIC→AAA AGT TGT GAT TGT CAT C (3→5) |
| SNP6       | FAM→AAG TGT TGA TAG GCA TCC |
| SNP7       | VIC→CTG GCG TGG TGT TGA |
| SNP8       | FAM→CTG GCG TGG TGT TGA |
| SNP9       | VIC→ACT GCT GTG GGA CA |

Each allele specific TaqMan probe is labeled with a reporter dye at the 5’ end.

*VIC dye is linked to the 5’ end of the allele 1 probe.*

*FAM dye is linked to the 5’ end of the allele 2 probe.*
genotyped in an ABI StepOnePlus Real-Time PCR System (Applied Biosystems) using the following conditions: an initial denaturation of 10 min at 95°C, and 40 cycles of 92°C for 15 sec and 60°C for 1 min. StepOnePlus software ver. 2.0 (Applied Biosystems) was used to detect the fluorescence generated from each sample and to perform an automatic or a manual determination of SNP type for each assay.

In order to quantify SNP variation within four shiitake lines, we calculated genetic parameters such as expected heterozygosity ($H_e$), observed heterozygosity ($H_o$), polymorphism information content (PIC) [17], and Hardy-Weinberg equilibrium (HWE) $p$-values using PowerMarker ver. 3.25 [18]. The $p$-values for HWE were adjusted using the sequential Bonferroni correction [19]. Allelic and genotypic frequencies were calculated for the samples analyzed. The genetic variability of the sample as a whole was estimated as described for each of the four lines.

To examine if the observed SNPs represent synonymous (silent) or non-synonymous (replacement) substitutions, the full-length sequence of the LELCC gene for each shiitake line was prepared in a single contig created according to the international unit base (IUB) code by multiple sequence alignment using Clustal W. We also translated the nucleotide sequences of the LELCC gene contigs into amino acid

![Fig. 1. Allelic discrimination plot using allele specific probes for the *Letinula edodes* laccase gene. Red color indicates a GG homozygote that was labeled with fluorescent VIC, blue color indicates a CC homozygote that was labeled with fluorescent FAM, and green color indicates a GC heterozygote that was labeled with both fluorescent VIC and FAM. ■, negative control; ×, undetermined.](image)

### Table 3. Genotype frequencies at eight SNP markers in four different lines of shiitake (*Letinula edodes*)

| Probe locus | Korea registered 20 lines | Korea wild type 29 line | Japan 20 lines | China 20 lines |
|-------------|--------------------------|-------------------------|----------------|----------------|
| SNP1        | H_e  0.79 0.36 | H_e  0.24 0.27 | H_e  0.71 0.35 | H_e  0.76 0.38 |
| Probe name  | SNP type       | H_o  0.07          | H_k  0.42      | H_k  0.12      |
| SNP2        | G 0.21         | G 0.69             | G 0.29        | G 0.12        |
| SNP3        | G 0.68 0.38    | G 0.18             | G 0.59 0.37   | G 0.11 0.37   |
| SNP4        | G 0.16         | G 0.14             | G 0.12        | G 0.12        |
| SNP5        | G 0.58 0.38    | G 0.30             | G 0.61 0.36   | G 0.08        |
| SNP6        | G 0.42         | G 0.28             | G 0.32        | G 0.11        |
| SNP7        | G 0.53         | G 0.62             | T 0.58        | T 0.33        |
| SNP8        | G 0.05         | G 0.10             | A 0.42 0.28   | A 0.17        |
| SNP9        | G 0.29 0.32    | A 0.20             | A 0.76 0.36   | A 0.81 0.37   |
| SNP10       | A 0.16         | A 0.51             | A 0.24        | A 0.13        |
| SNP11       | G 0.34 0.30    | G 0.76             | G 0.24        | G 0.06        |
| SNP12       | A 0.16         | A 0.56             | A 0.34 0.36   | A 0.05        |
| SNP13       | GA 0.45 0.28   | AA 0.55             | GA 0.69 0.35  | GA 0.80 0.38  |
| SNP14       | GA 0.79 0.37   | AA 0.31             | GA 0.31        | GA 0.10        |
| SNP15       | GA 0.16         | AA 0.51             | GA 0.05        | AA 0.10        |

$H_o$, homozygote frequency; $H_e$, heterozygote frequency; PIC, polymorphism information content: number of polymorphic loci/total number of loci analyzed; SNP, single nucleotide polymorphism; -, value zero.
Table 4. Summary statistic of SNP markers from pooled lines of shiitake

| SNP locus | $H_e$ | $H_s$ | HWE $\chi^2$ | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ |
|-----------|-------|-------|--------------|----------|----------|----------|
| SNP 1     | 0.5733| 0.4488| 0.0084      | −0.33    | −0.33    | 0.060    |
| SNP 2     | 0.5652| 0.4962| 0.3338      | −0.13    | −0.13    | 0.001    |
| SNP 3     | 0.5797| 0.4873| 0.1500      | −0.21    | −0.22    | 0.034    |
| SNP 4     | 0.5970| 0.4972| 0.0782      | −0.19    | −0.19    | −0.003   |
| SNP 5     | 0.4444| 0.4132| 0.7706      | −0.07    | −0.07    | 0.0003   |
| SNP 6     | 0.6579| 0.4830| 0.0020      | −0.38    | −0.38    | 0.0174   |
| SNP 7     | 0.6506| 0.4738| 0.0011      | −0.42    | −0.42    | 0.0442   |
| SNP 9     | 0.6567| 0.4598| 0.0005      | −0.48    | −0.48    | 0.0484   |
| Mean      | 0.5906| 0.4699| −0.28       | −0.25    | −0.25    | 0.0256   |

SNP, single nucleotide polymorphism.

$H_e$: observed heterozygosity is the proportion of heterozygous individuals in the population.

$H_s$: expected heterozygosity is defined as the probability that two randomly chosen alleles from the population are different.

HWE: probability estimated from likelihood ratio (G2) tests for Hardy-Weinberg equilibrium at each locus. Significance levels at $\alpha = 0.05$, 0.01, and 0.001 are indicated by ** and ***, respectively. ns, non-significance.

$F$-statistics describe the amount of inbreeding-like effects within subpopulation ($F_{IS}$), among subpopulations ($F_{IT}$), and within the entire population ($F_{ST}$).

sequences in accordance with open reading frame identification.

We successfully recovered a total of 2,249 bp sequences of the partial LELCC gene from all 89 samples of the four shiitake lines (GenBank accession No. HQ662226–HQ662271). From our alignment of the sequences from all shiitake lines, we identified the nucleotide positions of SNPs (Supplementary Fig. 1). Although our analyses yielded a total of 48 SNPs, we restricted to only 10 SNP markers (SNP 1 to SNP 10) based on the accuracy, amount of within-line polymorphism (i.e., frequency), and designed TaqMan probes.

Allelic discrimination was accomplished using real-time PCR. The probes for SNP 8 and SNP 10 were excluded from this analysis owing to technical issues (e.g., low reproducibility of PCR). An example of SNP genotyping using the SNP 2 probe is shown in Fig. 1. The red color indicates the GG homozygote, which was labeled by fluorescent VIC, the blue color denotes the CC homozygote, labeled by FAM, and the green color indicates the GC heterozygote, fluorescently labeled by both VIC and FAM. All eighty-nine shiitake lines were genotyped with the eight SNP markers (Supplementary Table 1). The SNP markers we developed allowed the discrimination of 81 out of 89 lines, with only eight lines that could not be distinguished (KFRI 401 = FMRI137, China 482 = Japan 754, China 488 = China 494, China 490 = Japan 812) (see Supplementary Table 1).

We performed statistical analyses on each of the four lines separately (Korean registered lines, Korean wild type lines, Japanese lines, and Chinese lines). The analysis classified genotypes of the LELCC gene by SNP markers and calculated the frequency of each genotype and the PIC values (Table 3). According to this analysis, the mean frequency of heterozygote individuals was 0.76 (Chinese) > 0.68 (Korean registered) > 0.55 (Japanese) > 0.29 (Korean wild type). However, the mean PIC values, which indicate the number of polymorphic loci/total number of loci analyzed, were similar between lines, ranging from 0.32 to 0.37.

To assess genetic diversity across the shiitake lines, we estimated observed heterozygosity ($H_e$), expected heterozygosity ($H_s$), HWE $p$-values, and the amount of inbreeding-like effects within subpopulation ($F_{IS}$), among subpopulations ($F_{IT}$), and within the entire population ($F_{ST}$). The $H_e$ and $H_s$ of all samples pooled were 0.5906 and 0.4699, respectively. The genotypes at four out of eight SNP markers (SNPs 1, 6, 7, 9) were significantly different from Hardy-Weinberg expectations (Table 4), which suggests non-random mating at these markers.

We determined the structure of the LELCC gene by identifying 11 exons and 10 introns, based on the splicing sites (AG/GT) in the sequences (Fig. 2). Among the observed eight SNP markers, SNPs 1, 3, and 9 were located within introns and the other five within exons (Fig. 2). We suggest that SNP 2 located in exon 3, SNP 4 in exon 4, and SNPs 5, 6, and 7 in exon 9 can be good probe candidates for the identification of specimens in the four shiitake lines. GGG (glycine: nonpolar) was converted to GCC (alanine: nonpolar) in SNP 2, and AAT (asparagine: neutral) was converted to TAT (tyrosine: aromatic) in SNP 5. In both cases a purine was substituted by a pyrimidine (G or A to C or T) and the corresponding amino acid was altered, i.e., replacement (nonsynonymous) polymorphisms. In contrast, the remaining polymorphisms were synonymous: GAC (aspartate: negative) converted to GAT (aspartate) in SNP 4, GTG (valine: nonpolar) converted to GTA (valine) in SNP 6, and TTG (leucine: nonpolar) converted to CTG (leucine) in SNP 7.

Until recently, line typing and breeding of shiitake (Lentinula edodes) cultivars have been depended on traditional DNA markers like restriction fragment length polymorphism [20], randomly amplified polymorphism DNA [21], amplified
fragment length polymorphism (AFLP) [4], and microsatellites [22]. However, these markers have limitations in that they often show low levels of reproducibility and usually lack specific genetic information (e.g., AFLP; genetic loci showing DNA polymorphisms are usually unknown). Kim et al. [22] used co-dominant microsatellite DNA markers (GenBank accession No. DQ231475–DQ231479) to discriminate 89 lines of shiitake from East Asia, including Korea, China, and Japan, but they found that precise cultivar typing was difficult because of insufficient genetic information. The SNP markers that we developed here from the laccase gene, a gene known to be functionally important in basidiomycetes, can complement these limitations and allow for more reliable typing of shiitake mushrooms and possibly for cultivar development through marker association selection. It is worth noting that a combined analysis of SNP markers (that were developed for this study) and microsatellites (that were developed in our previous study [22]) allows all 89 shiitake lines to be distinguished.

Moreover, it will be interesting to examine the possible relationships between SNP markers and phenotypic traits, particularly shiitake pigmentation. We found two motifs using MotifFinder software (http://wwwgenome.jp/tools/motif). First, the position of serpin was located between 120 bp and 130 bp at nucleotide sequences and was registered as PS00284 in PROSITE. Second, the position of multicopper oxidase 1 was between 137 bp and 157 bp and was registered as PS00079 in PROSITE. Serpins are a group of proteins with similar structures and were first identified as a set of protease inhibitors. The acronym “serpin” was coined because many serpins inhibit chymotrypsin-like serine proteases (serine protease inhibitors) [23]. A single fungal serpin has been characterized to date: celpin from *Piromyces* spp. line E2. *Piromyces* is an anaerobic fungus found in ruminant guts and is important for digesting plant material. Celpin is predicted to be an inhibitory molecule and contains two N-terminal dockerin domains in addition to the serpin domain. Dockerins are commonly found in proteins that localize to the fungal cellulosome, a large extracellular multiprotein complex that breaks down cellulose [23]. It is therefore suggested that celpin protects the cellulosome against plant proteases. Interestingly, certain bacterial serpins also localize to the cellulosome. Laccases are copper-containing oxidases that are found in many plants, fungi, and microorganisms. Our analysis of SNPs in shiitake laccases is expected to be useful in future genotyping or protein function studies.

**ELECTRONIC SUPPLEMENTARY MATERIAL**

Supplementary data including one table and one figure can be found with this article online at http://www.mycobiology.or.kr/src/sm/mb-43-75-s001.pdf.

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Supplementary Fig. 1. Multiple sequence alignment of the LELCC (*Letinula edodes* laccase) gene sequences (2,249 bp) of 89 shiitake lines, illustrating locations of the single nucleotide polymorphism polymorphisms identified. R = A or G, Y = C or T, K = G or T, M = A or C, S = G or C, and W = A or T.
## Supplementary Table 1. Genotyping at eight SNPs in the LELCC (*Letinula edodes* laccase) gene from each shiitake line

| KFRI No. | Origin | SNP1 | SNP2 | SNP3 | SNP4 | SNP5 | SNP6 | SNP7 | SNP9 |
|----------|--------|------|------|------|------|------|------|------|------|
| 401      | KFRI   | A/G  | G/C  | G/A  | G/A  | T/A  | A/G  | G/A  | G/A  |
| 402      | KFRI   | ??   | G/C  | G/A  | T/T  | G/A  | G/A  | G/A  |
| 403      | KFRI   | G/G  | C/C  | A/A  | G/G  | T/T  | G/G  | A/A  | G/G  |
| 404      | KFRI   | A/G  | G/G  | G/G  | A/A  | T/T  | A/G  | G/A  | G/A  |
| 405      | KFRI   | A/G  | A/G  | T/T  | G/G  | A/A  | A/A  | G/G  | A/A  |
| 406      | KFRI   | ?/?  | G/C  | G/A  | G/A  | T/T  | A/G  | G/A  | G/A  |
| 407      | KFRI   | G/G  | C/C  | A/A  | G/G  | T/T  | G/G  | A/A  | G/G  |
| 408      | KFRI   | G/G  | C/C  | A/A  | G/G  | T/T  | G/G  | A/A  | G/A  |
| 299      | KFRI   | ??   | G/C  | G/A  | A/G  | T/A  | A/G  | G/A  | G/A  |
| 169      | KFRI   | A/G  | G/G  | G/G  | A/A  | T/T  | A/G  | G/A  | G/A  |
| 1       | FMRI   | ??   | G/C  | G/A  | T/A  | A/G  | G/A  | G/A  | G/A  |
| 2       | FMRI   | ??   | G/C  | G/A  | T/A  | A/G  | G/A  | G/A  | G/A  |
| 3       | FMRI   | A/G  | G/G  | A/A  | T/A  | A/G  | G/A  | G/A  | G/A  |
| 5       | FMRI   | A/G  | G/G  | A/A  | T/T  | A/G  | G/A  | G/A  | G/A  |
| 137      | FMRI   | A/G  | G/C  | G/A  | T/A  | A/G  | G/A  | G/A  | G/A  |
| 180      | FMRI   | A/G  | G/C  | G/A  | A/G  | T/T  | A/G  | G/A  | G/A  |
| 504      | FMRI   | A/G  | G/C  | A/A  | T/A  | A/G  | G/A  | G/A  | G/A  |
| 689      | FMRI   | ??   | G/C  | G/A  | A/G  | T/A  | A/G  | G/A  | G/A  |
| 192      | RDA    | A/G  | G/C  | G/A  | G/A  | T/T  | A/G  | G/A  | G/A  |
| 36       | Wild   | G/G  | C/C  | A/A  | G/G  | T/T  | G/G  | A/G  | G/G  |
| 37       | Wild   | G/G  | ?/?  | ?/?  | ?/?  | ?/?  | G/G  | A/A  | ?/?  |
| 38       | Wild   | G/G  | C/C  | A/A  | G/G  | ?/?  | G/G  | A/A  | G/G  |
| 42       | Wild   | G/G  | ?/?  | ?/?  | ?/?  | T/T  | G/G  | A/A  | ?/?  |
| 51       | Wild   | G/G  | ?/?  | ?/?  | ?/?  | ?/?  | G/G  | A/A  | ?/?  |
| 53       | Wild   | G/G  | ?/?  | ?/?  | ?/?  | T/T  | ?/?  | A/A  | G/G  |
| 55       | Wild   | A/G  | G/G  | G/A  | T/A  | A/G  | G/A  | G/A  | G/A  |
| 57       | Wild   | A/G  | G/C  | G/A  | T/A  | A/G  | G/A  | G/A  | G/A  |
| 60       | Wild   | A/G  | ?/?  | ?/?  | ?/?  | G/G  | ?/?  | G/A  | G/A  |
| 62       | Wild   | A/G  | G/C  | G/A  | T/A  | A/G  | G/A  | G/A  | G/A  |
| 63       | Wild   | A/G  | G/G  | A/A  | T/A  | A/A  | G/A  | G/A  | G/A  |
| 64       | Wild   | G/G  | C/C  | A/A  | G/G  | T/T  | G/G  | A/A  | G/G  |
| 128      | Wild   | G/G  | ?/?  | ?/?  | ?/?  | ?/?  | G/G  | A/A  | ?/?  |
| 129      | Wild   | A/A  | ?/?  | ?/?  | ?/?  | T/T  | ?/?  | ?/?  | G/G  |
| 135      | Wild   | G/G  | ?/?  | ?/?  | ?/?  | T/T  | ?/?  | A/A  | G/G  |
| 136      | Wild   | G/G  | ?/?  | ?/?  | T/T  | ?/?  | ?/?  | A/A  | G/G  |
| 176      | Wild   | G/G  | ?/?  | A/A  | ?/?  | T/T  | G/G  | A/A  | G/G  |
| 177      | Wild   | ?/?  | G/C  | G/A  | G/A  | T/A  | A/G  | G/A  | G/A  |
| 188      | Wild   | G/G  | C/C  | A/A  | G/G  | T/T  | G/G  | A/A  | G/A  |
| 369      | Wild   | G/G  | C/C  | A/A  | G/A  | T/A  | A/G  | G/A  | G/A  |
| 370      | Wild   | G/G  | C/C  | A/A  | G/A  | T/A  | A/G  | G/A  | G/A  |
| 411      | Wild   | A/G  | G/G  | ?/?  | A/A  | ?/?  | A/A  | G/A  | G/A  |
| 665      | Wild   | A/A  | ?/?  | ?/?  | T/T  | ?/?  | A/A  | G/A  | G/A  |
| 666      | Wild   | G/G  | C/C  | A/A  | A/A  | A/A  | A/A  | G/G  | G/A  |
| 669      | Wild   | ?/?  | G/G  | A/A  | T/T  | A/A  | G/A  | ?/?  | G/A  |
| 672      | Wild   | G/G  | ?/?  | ?/?  | T/T  | G/G  | A/A  | G/G  |
| 674      | Wild   | A/G  | G/G  | G/A  | A/A  | T/A  | A/A  | G/A  | G/A  |
| 675      | Wild   | G/G  | C/C  | A/A  | G/G  | T/T  | G/G  | A/A  | G/G  |
| 731      | Wild   | G/G  | C/C  | A/A  | G/G  | T/T  | G/G  | A/A  | G/A  |
| 478      | China  | A/G  | G/C  | G/A  | T/A  | A/G  | G/A  | G/A  | G/A  |
| 480      | China  | A/G  | G/C  | G/A  | T/A  | A/G  | G/A  | G/A  | G/A  |
| 481      | China  | G/G  | ?/?  | A/A  | ?/?  | T/T  | G/G  | A/A  | G/G  |
| 482      | China  | A/G  | G/C  | G/A  | T/A  | A/G  | G/A  | G/A  | G/A  |
| 483      | China  | A/G  | G/C  | G/A  | T/T  | A/G  | G/A  | G/A  | ?/?  |
| 484      | China  | A/G  | G/C  | G/A  | T/A  | A/G  | G/A  | G/A  | ?/?  |
| 485      | China  | A/A  | G/G  | G/A  | A/A  | A/A  | A/A  | G/G  | G/G  |
| 486      | China  | A/G  | G/C  | G/A  | T/T  | A/G  | G/A  | G/A  | ?/?  |
| 487      | China  | ?/?  | ?/?  | ?/?  | ?/?  | ?/?  | ?/?  | ?/?  | ?/?  |
| 488      | China  | A/G  | G/C  | G/A  | T/A  | A/G  | G/A  | G/A  | ?/?  |
| KFRI No. | Origin | SNP1 | SNP2 | SNP3 | SNP4 | SNP5 | SNP6 | SNP7 | SNP9 |
|----------|--------|------|------|------|------|------|------|------|------|
| 489      | China  | A/G  | G/C  | G/A  | G/A  | A/A  | A/G  | G/A  | G/A  |
| 490      | China  | A/G  | G/C  | G/A  | G/A  | T/T  | A/G  | G/A  | G/A  |
| 491      | China  | A/G  | G/C  | G/A  | G/A  | T/A  | A/G  | G/A  | G/A  |
| 494      | China  | A/G  | G/C  | G/A  | G/A  | T/A  | A/G  | G/A  | ?/?  |
| 495      | China  | A/G  | G/C  | G/A  | G/A  | T/A  | A/G  | G/A  | ?/?  |
| 497      | China  | A/G  | G/C  | G/A  | G/A  | T/A  | A/G  | G/A  | G/A  |
| 816      | China  | A/A  | G/G  | G/G  | A/A  | A/A  | G/G  | A/A  |
| 817      | China  | G/G  | C/C  | A/A  | G/G  | T/T  | G/G  | A/A  | G/G  |
| 754      | Japan  | A/G  | G/C  | G/A  | T/A  | A/G  | G/A  | G/A  |
| 755      | Japan  | A/G  | G/C  | G/A  | G/A  | T/A  | A/G  | G/A  | G/A  |
| 760      | Japan  | A/G  | G/C  | G/A  | G/A  | T/A  | A/G  | G/A  | ?/?  |
| 761      | Japan  | A/G  | G/G  | G/G  | T/T  | G/G  | A/A  | G/G  |
| 751      | Japan  | A/G  | G/C  | G/A  | G/A  | T/A  | A/G  | G/A  | ?/?  |
| 756      | Japan  | G/G  | C/C  | A/A  | G/G  | T/T  | G/G  | A/A  | G/G  |
| 803      | Japan  | A/G  | G/C  | G/A  | T/A  | A/G  | G/A  | G/A  |
| 804      | Japan  | G/G  | C/C  | A/A  | G/G  | T/T  | G/G  | A/A  | G/G  |
| 805      | Japan  | G/G  | C/C  | A/A  | G/G  | T/A  | A/G  | G/A  | G/A  |
| 806      | Japan  | A/G  | G/C  | G/A  | G/A  | T/T  | A/G  | G/A  | G/A  |
| 807      | Japan  | A/G  | G/C  | G/A  | G/A  | T/T  | A/G  | G/A  | G/A  |
| 808      | Japan  | G/G  | C/C  | A/A  | G/G  | T/T  | G/G  | A/A  | G/G  |
| 809      | Japan  | A/G  | G/G  | G/G  | A/A  | T/A  | A/G  | G/A  | G/A  |
| 810      | Japan  | A/G  | G/C  | G/A  | T/A  | A/G  | G/A  | G/A  |
| 812      | Japan  | A/G  | G/C  | G/A  | G/A  | T/T  | A/G  | G/A  | G/A  |

Shiitake lines highlighted in bold could not be distinguished by the SNP markers developed.

SNP, single nucleotide polymorphism; KFRI, Korea Forest Research Institute; FMRI, Forest Mushroom Research Institute, Korea; RDA, Rural Development Administration, Korea; Wild: Korea wild type; ?/?, amplification failure.