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Delimitation of a QTL region controlling cold tolerance at booting stage of a cultivar, 'Lijiangxintuanheigu', in rice, *Oryza sativa* L.

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Abstract

Low temperature at the booting stage of rice causes male sterility resulting in severe yield loss. Cold tolerance has long been an important objective in rice breeding. We identified a QTL for cold tolerance on the long arm of chromosome 3 from the cold-tolerant breeding line 'Ukei 840' by using F_2 and BC_1F_2 populations from crosses between 'Ukei 840' and 'Hitomebore'. The cold tolerance of 'Ukei 840' is derived from the Chinese cultivar 'Lijiangxintuanheigu'. The effect of this QTL on cold tolerance was confirmed by developing 'Hitomebore' chromosome segment substitution lines having 'Lijiangxintuanheigu' alleles on chromosome 3. By producing recombinants in chromosome 3, the QTL region for cold tolerance was delimited to the region of about 1.2-Mb region between RM3719 and RM7000. All lines heterozygous for the QTL showed seed fertilities as low as that of 'Hitomebore', suggesting that the 'Lijiangxintuanheigu' allele for cold tolerance in the QTL region is recessive. Determination of a 1.2-Mb nucleotide sequence of 'Ukei 840' and comparison with the published genomic sequence of 'Nipponbare' showed 254 SNPs, of which 11 were in coding regions of genes, seven in five genes being non-synonymous. SNPs were detected in the 5-kb upstream regions of 89 genes, but no differences of gene expression levels were detected between alleles of these genes. Although further delimitation is required to identify the gene responsible for cold tolerance of 'Lijiangxintuanheigu', SNP markers developed here will be useful for marker-assisted selection in a breeding program using 'Lijiangxintuanheigu' as a donor of cold tolerance.

Keywords: SNP markers, marker assisted selection, seed fertility, rice breeding

Introduction

High temperature is required for normal development of pollen grains at the booting stage in rice. Low temperature, lower than 19°C, during the period of microspore development causes male sterility, resulting in severe loss of yield (Satake and Hayase 1970). Such damage to gamete development by low temperature is observed only in male organs, while development of female organs is normal (Hayase et al. 1969). In the northern region of Japan and some countries in the temperate zone where the temperature is not consistently high enough for rice production, rice yield is reduced dramatically once every several years. Thus, rice cultivars with high tolerance to low-temperature stress at the booting stage are required for stable rice production in such regions.

In the development of pollen grains, the callose wall, which covers haploid cells of a tetrad, is digested by callase secreted by the tapetum. The haploid cells are released as microspores, and mitotic divisions occur twice in the microspores during development to mature pollen grains. Simultaneously, tapetal cells are degraded. Under low temperature, the tapetal cells expand abnormally, and anther locules are invaded (Nishiyama 1970). A decrease of inorganic phosphate, an increase of nonreducing sugar (Ito 1978), and a decrease of acid phosphatase activity (Nishiyama 1978) have been observed in anthers of rice under low temperature, suggesting that abnormal sugar metabolism causes expansion of the tapetal cells due to a change of osmotic pressure and results in abnormality of pollen development.

Rice breeding for cold tolerance has long been performed in Japan. A deep-water irrigation system using cold water with controlled temperature has been developed as a reliable screening method for cold tolerance and is widely used for development of cold-tolerant cultivars (Matsunaga 2005). 'Koshihikari' has been identified as a highly cold-tolerant cultivar, but its flowering time is too late for cultivation in the northern region of Japan. Therefore, 'Hitomebore', which has high tolerance and an early flowering trait, has been developed by crossing 'Koshihikari' with 'Hatsuboshi' (Sasaki 2005). 'Hitomebore' shows high seed fertility, more than 70%, after cultivation in the deep-water irrigation system with cold water controlled at 19°C. However, even these cold-tolerant cultivars exhibit low seed fertilities, less than 50%, under cold-deep-water irrigation of 18.5°C. Thus, further improvement of cold tolerance is required.

Cold tolerance of rice at the booting stage is a quantitative trait controlled by multiple genes. Since it is difficult to combine many genes responsible for cold tolerance by investigating plant phenotypes, identifying each gene, and combining the genes by marker-assisted selection are considered to be effective means of developing cold-tolerant cultivars. Since there is a large variation of cold tolerance in rice cultivars (Toriyama and Futsuhara 1960), quantitative trait locus (QTL) analyses using various cultivars have been carried out. 'Silewah' and 'Padi Labou Alumbis', which have been identified as highly cold-tolerant cultivars by a Japan-China cooperative project for rice genetic resources in Yunnan Province of China (International Rice Research Institute 1977), have been used as parents for developing 'Norin PL8' and 'Norin PL11', respectively. QTLs for cold tolerance of 'Norin PL8' have been

on chromosome 3 and 4 (Saito et al. 1995, 2001, 2004), and that of 'Norin PL11' has been mapped on chromosome 8 (Kuroki et al. 2007). The QTL on chromosome 4 derived from 'Silewah' has been delimited to a 56-kb region, and a gene encoding F-box protein among seven genes in this region has been suggested to be the gene responsible for cold tolerance (Saito et al. 2004; 2010). QTLs for cold tolerance of the Japanese leading cultivar 'Koshihikari' have been found on chromosomes 1, 7, and 11 (Takeuchi et al. 2001), and those of 'Kunmingxiaobaigu' have been detected on chromosomes 3, 6, and 7 (Dai et al. 2004). The presence of many different QTLs in different rice cultivars suggests a complicated mechanism of cold tolerance controlled by many genes.

'Lijiangxintuanheigu' ('LTH' hereafter), a local variety of Yunnan province in China, has been reported to be one of the most cold-tolerant cultivars among 148 cultivars (Horisue et al. 1988). Recently, Ye et al. (2010) have detected one QTL for cold-tolerance at the booting stage of 'Lijiangheigu', which has a name similar to 'LTH', on the short arm of chromosome 10. Investigating the effects of low temperature at the booting stage on pollen development in a leading cultivar in Australia, 'Doongara', and the highly cold-tolerant cultivar 'R31', Oliver et al. (2005) have revealed that sucrose content increases in the anthers and that starch does not accumulate in the pollen grains of 'Doongara', while sucrose does not accumulate in anthers having fertile pollen grains in 'R31' and that expression of a gene encoding cell-wall-bound acid invertase, which has an important role in transport of sucrose to sink tissues, is suppressed in 'Doongara' but is high in 'R31'. An increase in the level of abscisic acid (ABA) has been observed in 'Doongara' treated by low temperature, while not in similarly treated 'R31' (Oliver et al. 2007). Treatment of 'Doongara' with ABA has been found to result in a change of expression of an invertase gene and in male-sterility similar to that caused by low temperature. ABA has been suggested to function as a signaling molecule participating in male-sterility due to low-temperature stress by controlling the transport pathway of sugar (Oliver et al. 2007).

'LTH' is a useful breeding material for further improvement of cold tolerance in the present cold-tolerant cultivars such as 'Hitomebore'. Analysis using progeny plants from a cross between 'LTH' and 'Hitomebore' may enable identification of genes for cold-tolerance. However, 'LTH' is tall, which makes it difficult to treat plants with cold water by the deep-water irrigation system. The height of 'Ukei 840', which is a cold-tolerant line selected from BC_1F_6 progeny plants between 'LTH' as a donor parent and 'Hitomebore' as a recurrent parent (Fig. 1), is as great as that of 'Hitomebore'. In the present study, we analyzed QTLs for cold-tolerance using progeny from a cross between 'Ukei 840' and 'Hitomebore', and delimited the QTL region for identification of a candidate gene responsible for cold tolerance

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Materials and methods

Plant materials and isolation of DNA

'Lijiangxintuanheigu' ('LTH'), 'Hitomebore', and 'Ukei 840',

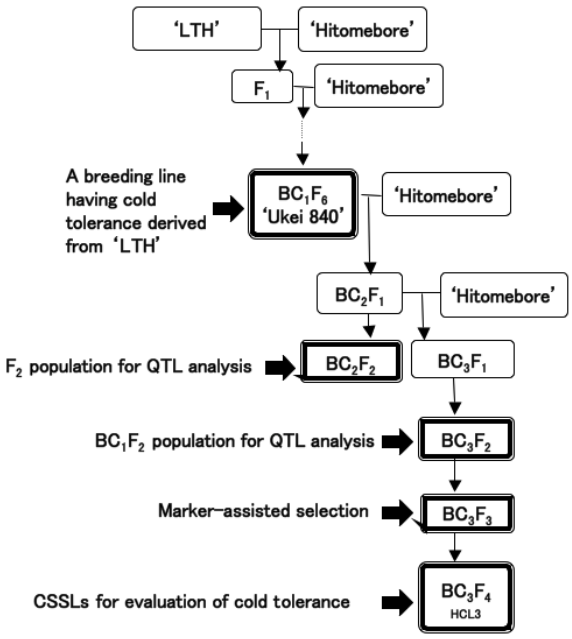

Fig.1 Lineage of 'Ukei 840' and progeny population used for analysis of a QTL for cold tolerance

which was later renamed 'Ouu PL4', were used for developing DNA markers and evaluation of seed fertility after low temperature treatment. The F_2 population of 192 individuals between 'Ukei 840' and 'Hitomebore', which are BC_2F_2 plants from a cross between 'LTH' as a donor parent and 'Hitomebore' as a recurrent parent (Fig. 1), were used for analysis of QTL for cold tolerance at the booting stage in 2005. For reevaluation of QTL for cold tolerance, 192 BC_1F_2 (BC_3F_2 between 'LTH' and 'Hitomebore') individuals were used in 2006. Genomic DNAs were extracted from leaves of 'LTH', 'Ukei 840' and 'Hitomebore' by the CTAB method (Murray and Thompson 1980) and leaves of populations for QTL analyses by the method of Edwards et al. (1991).

Examination of cold tolerance of plants

Populations for QTL analysis and parental lines were grown in a paddy field (Daisen Research Station, National Agricultural Research Center for Tohoku Region, Akita, Japan) and treated by the cold-deep-water irrigation method (Matsunaga 2005) for about two months from the panicle initiation stage to full heading. The cold-deep-water irrigation method was developed as a method for examining cold tolerance of rice about 30 years ago, and is widely used for selecting cold tolerant lines because of its high reliability (Matsunaga 2005, Suh et al. 2010). In the cold-deep-water irrigation method for low temperature treatment at the booting stage, the water temperature was controlled at 18.4 to 18.5°C and its depth was 20 to 25 cm. Seed fertility, which is the percentage of the number of fertile seeds in the number of florets, of plants grown under low-temperature stress was used as an index of cold tolerance.

Graphical genotyping of 'Ukei 840'

We used 684 DNA markers including 251 SSR (simple sequence repeat) (McCouch et al. 2002), 12 SCAR (sequence-characterized amplified region), 19 CAPS (cleaved amplified polymorphic region) (Shirasawa et al. 2004a), 306 PCR-RF-SSCP (PCR-restriction fragment-single strand conformation polymorphism) (Shirasawa et al. 2004b), and 96 dot-blot-SNP (single nucleotide polymorphism) markers (Shirasawa et al. 2006) for polymorphism analysis between 'LTH' and 'Hitomebore'. The dot-blot-SNP markers were primer pairs for specific amplification of a single DNA fragment and allele-specific oligonucleotide probes, which are hybridized to dot-blotted PCR products on nylon membrane together with competitive oligonucleotides having sequences of the other alleles. Eighty SNP markers were also developed by genome-wide sequencing of 'LTH' using a 454 sequencer (Genome Sequencer FLX System, Roche, USA) and by comparison of sequence data with the published genome sequence of 'Nippponbare' (International Rice Genome Sequencing Project 2005). These 80 SNP markers were dot-blot-SNP markers using the bridge hybridization method (Shiokai et al. 2010). A total of 275 markers (Supplementary Table 1), which can detect polymorphisms between 'LTH' and 'Hitomebore', were used for graphical genotyping of 'Ukei 840'.

QTL analysis

Forty-eight markers, which were mapped on chromosome segments derived from 'LTH' in 'Ukei 840', were used for genotyping of the 192 F_2 (BC_2F_2 between 'LTH' and 'Hitomebore') plants (Fig. 1). A linkage map was constructed using Mapmaker 3.0 (http://www.broad.mit.edu/ftp/distribution/software/mapmaker3/). Analysis of QTL for cold tolerance was performed by composite interval mapping (CIM) (Jansen and Stam 1994; Zeng 1994) using Windows QTL cartographer 2.5 (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm). For determining threshold value, a permutation test was carried out 1,000 times.

Delimitation of a region containing a QTL for cold tolerance

Genotypes of 1,504 BC_1F_3 individuals (BC_3F_3 between 'LTH' and 'Hitomebore') were determined using seven makers within a QTL region (Fig. 1). For preparation of PCR templates for a large number of plants, the leaf-punch method (Shiokai et al. 2009) was used. Ten individuals having recombination in the QTL region were selected. Six types of recombinant lines were selfed, and genotypes of DNA markers in the QTL region of 64 BC_1F_4 progenies (BC_3F_4 for 'LTH') of each recombinant line were determined. Eighteen individuals homozygous for the QTL region in each recombinant line were selected and seed fertility was evaluated for delimiting the QTL region.

Sequencing of the QTL region for cold tolerance

The QTL region for cold tolerance of 'Ukei 840' was amplified by PCR using 152 pairs of primers, which were designed for amplifying each 5 kb of the candidate region using published sequence data of 'Nipponbare'. A 20 µl PCR mixture consisting of 20 ng template genomic DNA, 20 pmol primers, 1 × PCR buffer, 0.4 mM dNTPs, and 0.4 units DNA polymerase (KOD-FX: TOYOBO, Japan) was used. PCR was carried out as follows: 2 min denaturation at 94˚C, 40 cycles of 10 sec denaturation at 98˚C and 6 min extension at 68˚C. After isolation by agarose gel electrophoresis, PCR products were purified using ULTRACLEAN 15 DNA PURIFICATION KIT (MO BIO, USA). All purified products were mixed and concentrated by ethanol precipitation. Nucleotide sequences of the PCR products were determined by Genome Analyzer IIx (Illumina, USA). Sequence data of 'Ukei 840' were compared with the published nucleotide sequence of 'Nipponbare' in the corresponding region. SNPs between 'Ukei 840' and 'Nipponbare' were detected.

Gene expression analysis

Total RNAs were extracted using the SV Total RNA Isolation System (Promega, USA) from 0.03 g spikelets of young panicles at the booting stage of 'Hitomebore' and 'HCL3-homo' grown in the cold-deep-water paddy field under normal growing conditions. First-strand cDNA was synthesized using First-Strand cDNA Synthesis Kit (GE Healthcare, USA). Genes having SNPs between 'Ukei 840' and 'Nipponbare' within 5 kb upstream regions of translation initiation sites were analyzed by RT-PCR using the first-strand cDNA as a template. Sequences of primer pairs are shown in Supplementary Table 2. The rice actin gene was used as a control. PCR was performed under the following conditions: 1 min denaturation at 94˚C, 30 cycles of 30-sec denaturation at 94˚C, 30-sec annealing at 58˚C, and 30-sec extension at 72˚C, and 1-min extension at 72˚C.

Results

Graphical genotyping of 'Ukei 840'

DNA polymorphism between 'LTH' and 'Hitomebore' was detected by analysis using 297 DNA markers including 144 SSR, 8 SCAR, 4 CAPS, 100 PCR-RF-SSCP, and 41 dot-blot-SNP markers from among 251 SSR, 12 SCAR, 19 CAPS, 306 PCR-RF-SSCP, and 96 dot-blot-SNP markers tested. To develop SNP markers for the regions in which the distances between markers exceed 3 Mb, nucleotide sequences of genomic DNA of 'LTH' were determined using a 454 nucleotide sequencer, and 80 SNPs between 'LTH' and 'Nipponbare' were selected. Removing closely linked DNA markers, 275 markers were used for genotyping of 'Ukei 840'. 'Ukei 840' was homozygous for 'LTH' alleles in 42 markers (ca. 20%), homozygous for 'Hitomebore' alleles in 225 markers (ca. 75%), and heterozygous in 8 markers (ca. 5%) (Fig. 2). Regions homozygous for 'LTH' alleles were 12 regions in short and long arms of chromosomes 3, 5, and 11 and long arms of chromosomes 4, 8, 9, and 12. Heterozygous regions were in the long arms of chromosomes

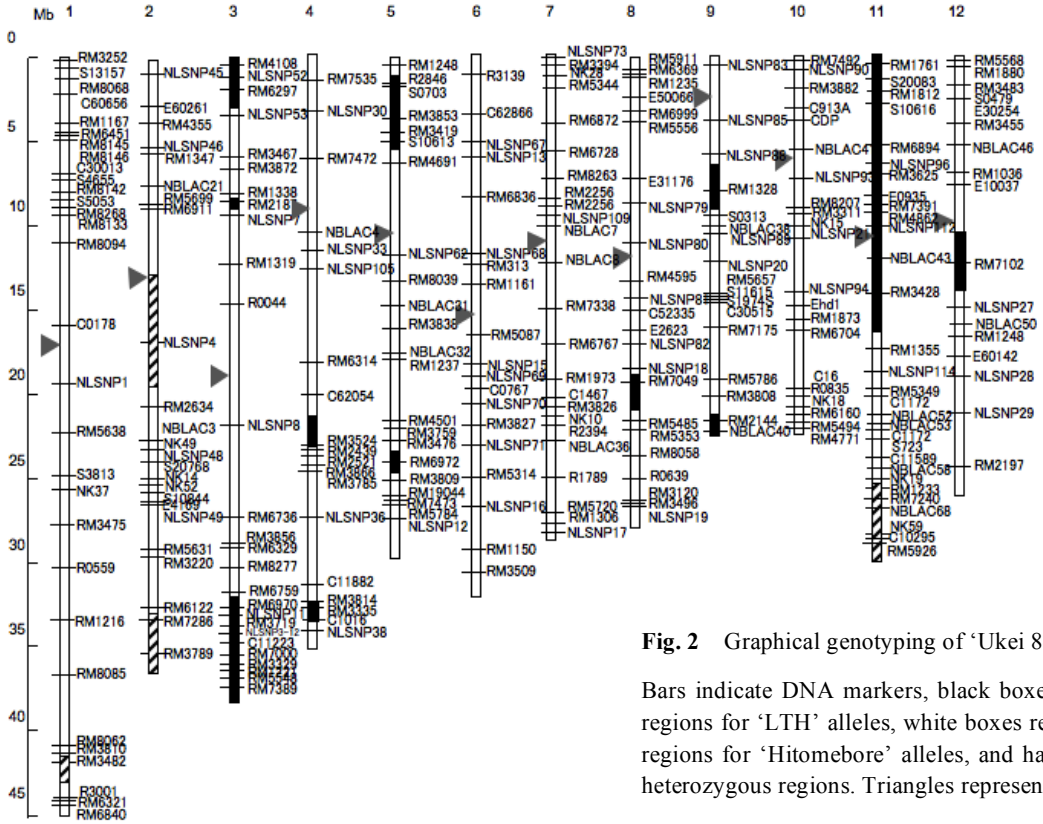

Fig. 2 Graphical genotyping of 'Ukei 840'.

Bars indicate DNA markers, black boxes show homozygous regions for 'LTH' alleles, white boxes represent homozygous regions for 'Hitomebore' alleles, and hatched boxes indicate heterozygous regions. Triangles represent centromeres.

1, 2, and 11. Genotypes of all the tested DNA markers on the short arm of chromosome 10 of 'Ukei 840', on which a QTL for cold-tolerance of 'LTH' has been mapped by Ye et al. (2010), were homozygous for 'Hitomebore' alleles.

QTL analysis of cold tolerance

Seed fertilities of 192 F_2 plants derived from a cross between 'Ukei 840' and 'Hitomebore' grown in a deep-water paddy field with water controlled at 18.5˚C showed continuous distribution from 17.4% to 84.2% (Fig. 3). Genotypes of the 192 F_2 plants were analyzed using the 48 DNA markers in the regions homozygous for 'LTH' alleles and the heterozygous region, and QTLs for the seed fertilities were investigated. One QTL with an LOD score of 7.1, higher than the threshold value of 5% significance represented as LOD score 2.5, was detected at marker C11223 on the long arm of chromosome 3. The phenotypic variance explained by this QTL was 24.4%. The additive effect was 7, indicating that substitution of this region from homozygous 'Hitomebore' alleles to homozygous 'LTH' alleles increases seed fertility by 14% (Table 1). This QTL was named qLTB3 (QTL for Low-temperature Tolerance at Booting stage on chromosome 3).

An F_1 plant obtained from a cross between 'LTH' and 'Hitomebore' was backcrossed with 'Hitomebore' to obtain 192 BC_1F_2 plants, which are BC_3F_2 plants for 'LTH' and 'Hitomebore' (Fig. 1). These BC_1F_2 plants had 'LTH' alleles of the DNA markers on the long arms of chromosomes 3 and 8 (Supplementary Fig. 1). Seed fertilities of these BC_1F_2 plants grown in the cold-deep-water paddy field were distributed continuously from 0% to 60.3% (Supplementary Fig. 1). Analyzing genotypes of these plants using the DNA markers in these regions, QTLs were again investigated. A significant QTL was detected at marker RM7000 in qLTB 3. The LOD score of this QTL was 6.9, and the explained phenotypic variance was 18.1% (Table 1).

Development of chromosome segment substitution lines (CSSLs) and delimitation of the QTL region for cold tolerance

A BC_1F_2 plant, which is heterozygous for DNA markers in qLTB3 and homozygous for 'Hitomebore' alleles in the other genomic regions, named 'HCL3-hetero' ('Hitomebore' Chromosome segment substitution line with qLTB3-hetero), was selected, and 'HCL3-homo' was obtained from its selfed progeny. Six recombinants between RM6970 and RM7389 in qLTB3 were selected from 1,504 selfed progeny of 'HCL3-hetero'. Determining genotypes of selfed progeny obtained from the six recombinant lines using seven DNA markers in qLTB3, we selected plants homozygous for 'LTH' alleles (Fig. 4). These lines, 18 plants for each line, were grown in the cold-deep-water paddy field together with 'HCL3-homo' and 'Hitomebore', and seed fertility of each plant was investigated in 2008. 'HCL3-homo' and 'Hitomebore' showed seed fertilities of 31% and 21%, respectively. The difference of seed fertility between the tested lines was significant at the 1% level by ANOVA. Lines with significantly higher seed fertility than that of 'Hitomebore' at the 1% level by the Dunnett test were 'HCL3-3', 'HCL3-6', 'HCL3-7', and 'HCL3-homo', while seed fertilities of 'HCL3-5', 'HCL3-8', and 'HCL3-10' were not significantly different from that of 'Hitomebore'. Cold

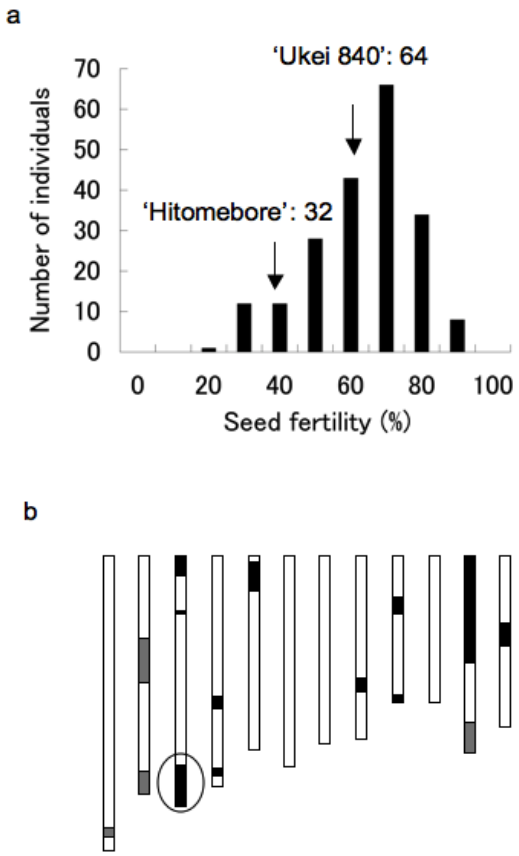

Fig. 3 QTL analysis using an F_2 population.

(a) Distribution of seed fertilities of F_2 plants between 'Ukei 840' and 'Hitomebore' cultivated in the cold-deep-water irrigation field at the booting stage.

(b) Position of a QTL detected by analysis using an F_2 population. Black boxes represent homozygous regions for 'LTH' alleles, white boxes indicate homozygous regions for 'Hitomebore' alleles and gray boxes show heterozygous regions. A circle indicates a QTL region.

tolerances of these lines were again examined using selfed progeny of these lines in 2009. 'Hitomebore' showed 18% seed fertility, while 'HCL3-homo' exhibited 32% seed fertility. Seed fertilities of 'HCL3-3', 'HCL3-6', 'HCL3-7', and 'HCL3' were significantly higher than that of 'Hitomebore' at the 1% level, while those of the other lines were comparable to that of 'Hiromebore' (Fig. 4). These results suggest that the QTL for cold tolerance can be delimited to the region from RM3719 to RM7000.

The dominance effect of the QTL was investigated using heterozygous plants of 'HCL3-3', 'HCL3-6', and 'HCL3-hetero'. All these heterozygotes showed seed fertilities as low as that of 'Hitomebore', suggesting that the 'LTH' allele for cold tolerance in qLTB3 is recessive (Fig. 5).

Identification of SNPs in qLTB3

The 'Nipponbare' genome sequence of the region between RM3719 and RM7000 is 1,161,293 bp. Genomic DNA of 'Ukei 840' in this region was amplified by long PCR for ca. 5 kb using 216 primer pairs. For amplification of the regions which could not be amplified by these primer pairs, 12 other primer pairs were designed. Genomic DNA fragments of 'Ukei 840' covering the whole region between RM3719 and RM7000 were obtained. Nucleotide sequences of these PCR products were determined using an Illumina genome analyzer, and 172 times coverage on average was obtained. The obtained 1,161,293-bp sequence of 'Ukei 840' was compared with the 'Nipponbare' genome sequence. In this nucleotide sequence analysis, many short sequences of ca. 30 nt were aligned with the published 'Nipponbare' genome sequence, and therefore insertions in 'LTH' genome could not be detected. However, all the DNA fragments amplified from 'Ukei 840' had the same sizes as those from 'Nipponbare', indicating that there is no large insertion in this genomic region of 'Ukei 840'. Deletions of nucleotides in 'Ukei 840' were not detected. Detected SNPs were 254, of which 223 were present outside of the assigned gene regions. Among the 31 SNPs identified in the gene regions, seven SNPs detected in five genes were variations causing amino acid changes in encoded proteins. In Os03g0790700, Ile at 728 from the first Met, which is a conserved amino acid residue among genes for aldehyde oxidase-2, was replaced by Asn in 'Ukei 840'. Leu at 59 and Glu at 439 in Os03g0793700 were replaced by Phe and Gly, respectively (Table 2).

SNPs were detected in the 5-kb upstream regions of 89 genes. In spikelets of young panicles at the booting stage, gene expression of 57 genes was detected, but no differences of gene expression levels were detected in these genes between 'HCL3-homo' and 'Hitomebore' nor between plants grown in the cold-water paddy field and those under the normal growing conditions.

Seven dot-blot-SNP markers, i.e., qLTB3-1 to -7, were developed with SNPs identified in Os03g0789800, Os03g0790700, Os03g0793700, Os03g0800500, and Os03g0806700 (Table 3). All the markers showed clear dot-blot signals for each allele. In all the markers, 'Ukei 840' showed genotypes of 'LTH' type, and 'Hitomebore' did genotypes of 'Nipponbare' type (Fig. 6).

Table 1 Analysis of QTLs using F_2 and BC_1F_2 populations

Population	The nearest marker	Chr.	LOD of peak	Additive effect	Variance explained (%)
F_2 (2005)	C11223	3	7.1	7	24.4
BC_1F_2 (2006)	RM7000	3	6.9	6.5	18.1

* Additive effect of the 'LTH' allele. The value is in percentage.

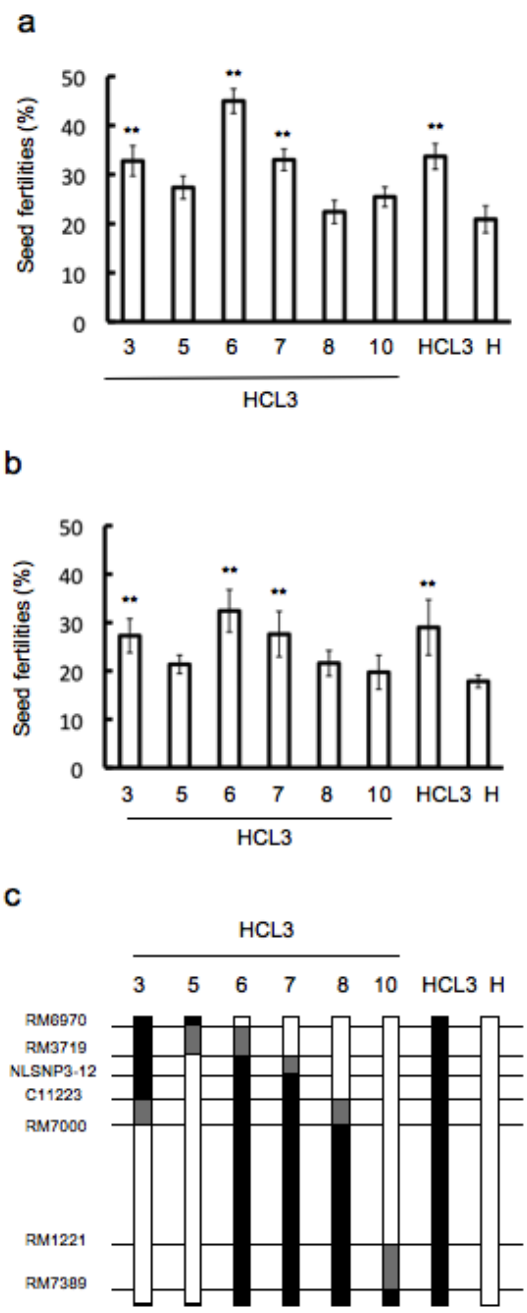

Fig. 4 Seed fertilities investigated in 2008 (a), in 2009 (b) and graphical genotypes of six CSSLs (HCL3-3, 5, 6, 7, 8 and 10), HCL3-homo (HCL3) and 'Hitomebore' in the QTL region (c).
Two asterisks indicate that lines showed significant differences in seed fertilities at 1% level against 'Hitomebore'. Black boxes show homozygous regions for 'LTH' alleles and white boxes represent homozygous regions for 'Hitomebore' alleles. Gray boxes show regions containing recombination break points.

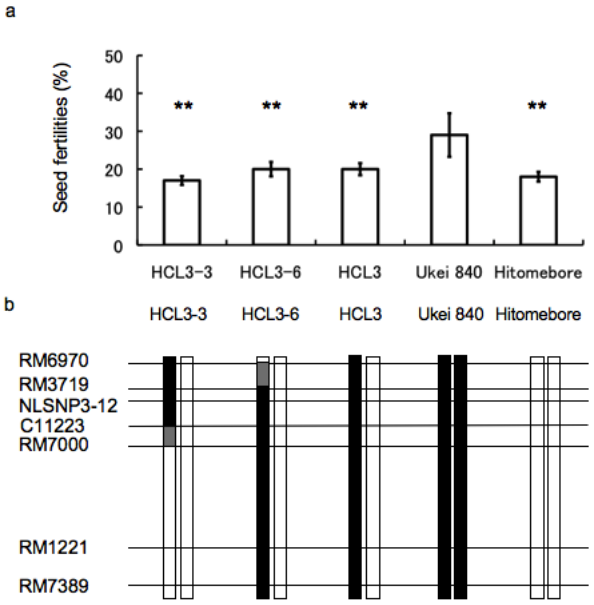

Fig. 5 Seed fertilities (a) and graphical genotypes of heterozygous CSSLs of HCL3-3, HCL3-6 and HCL3, 'Ukei 840', and 'Hitomebore' in the QTL region (b).
Two asterisks indicate that lines showed significant differences in seed fertilities at 1% level against 'Ukei 840' and one asterisk indicates no significant difference at 5% level against 'Hitomebore'. Black boxes show regions of 'LTH' alleles and white boxes represent regions of 'Hitomebore' alleles.

Table 2. Missense variations caused by SNPs between 'Ukei 840' and 'Nipponbare'

Gene name	Amino acid		Annotation by RAP-DB
	Nipponbare	Ukei 840	
Os03g0789800	Ser	Asn	Hypothetical protein
Os03g0790700	Arg Ile	Lys Asn	Similar to Aldehyde oxidase-2
Os03g0793700	Leu Glu	Phe Gly	Cupin 1 domain containing protein
Os03g0800500	Ile	Val	Putative small multi-drug export family protein
Os03g0806700	Leu	Ser	Protein of unkn own function DUF868, plant f amily protein

Discussion

Repeated QTL analyses using 'Hitomebore' and 'Ukei 840', which is a cold-tolerant breeding line derived from a crossing of 'LTH' with 'Hitomebore', revealed the presence of a QTL for cold tolerance at the booting stage on the long arm of chromosome 3. Although only one QTL with a significant effect was detected, phenotypic variance explained by this QTL was only 24.4%. A CSSL having a region of chromosome 3 derived from 'LTH' with a genetic background of 'Hitomebore' developed by backcrossing showed significantly higher cold tolerance than 'Hitomebore', but QTL analysis using a backcrossed population again showed this region to have a small explained phenotypic variance, 18.1%. The presence of many other QTLs with minor effects might be one of the reasons for this low explained phenotypic variance, but backcrossing to remove allelic variations at other QTLs did not increase the phenotypic variance explained by the QTL in chromosome 3, suggesting that most of the remaining phenotypic variance, ca. 80%, is due to environmental factors. Although further improvement

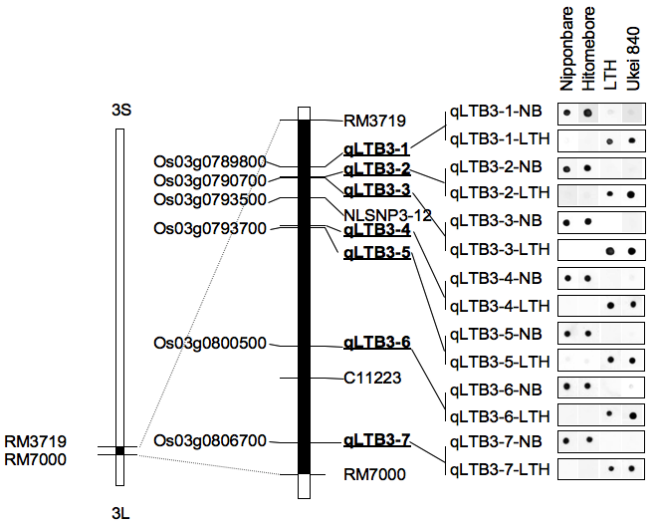

Fig. 6 A map of SNPs used for developing dot-blot-SNP markers.
Dot-blot signals detected by the SNP markers are shown to the right. qLTB3-n-NP and qLTB3-n-LTH are probes for 'Nipponbare' alleles and 'LTH' alleles, respectively.

is required to develop a more reliable method for testing genetic effects on the cold tolerance of breeding lines in rice, detection of significant QTLs at the same region, i.e., qLTB3, by repeated analysis and significant differences of seed fertilities between the chromosome segment substitution lines having qLTB3, i.e., 'HCL3' lines, and 'Hitomebore' suggests the reliability of the effect of qLTB3.

Three, four, three, and one QTLs for cold tolerance at the booting stage have been observed by Takeuchi et al. (2001), Dai et al. (2004), Suh et al. (2010), and Ye et al. (2010), respectively, explained phenotypic variance of each QTL being less than 22% with summed explained phenotypic variances of 32%, 45%, 27%, and 20.5%, respectively. Since reevaluations of QTLs using chromosome segment substitution lines or progeny populations were not carried out in these studies, it cannot be speculated whether these low values of summed explained phenotypic variances are due to large environmental effects. Only one QTL for cold tolerance at the booting stage of 'Hokkai-PL9' detected on chromosome 8 has explained the higher phenotypic variance, i.e., 26.6% (Kuroki et al., 2007), than the QTL in the present study. In the previous QTL studies, cold-tolerance QTLs have been detected on every chromosome from chromosome 1 to 12, and a few QTLs shared by different cold-tolerant cultivars have been found (Andaya and Mackill, 2003; Dai et al. 2004; Takeuchi et al. 2001, Ye et al., 2010). It can be inferred that many genes participate in cold tolerance at the booting stage of rice.

In the chromosomal region of about 1.2 Mb containing a gene for cold tolerance delimited by analysis using CSSLs, 143 genes have been annotated in the 'Nipponbare' genome (International Rice Genome Sequencing Project 2005) and full-length cDNA clones of them have been isolated. In the present study, SNPs were detected in 5-kb upstream regions of 89 genes. No difference of gene expression of these genes was detected between the CSSL having qLTB3 and 'Hitomebore' nor between plants grown in the cold-water paddy field and those under normal growing condition.

Determining the nucleotide sequence in this region of 'Ukei 840', we revealed seven SNPs in five genes causing amino acid substitutions. Among them, one SNP in Os03g0790700 was found to be responsible for alteration of a conserved amino acid residue. Os03g0790700 is a possible candidate of the cold-tolerance gene of 'LTH'. Os03g0790700 is similar to AAO2 in *A. thaliana*, which is considered to be a member of aldehyde oxydase functioning in ABA biosynthesis (Koiwai et al. 2004; Seo et al. 2004). Os03g0806700, which has been reported to be expressed in the anther (RiceXPro: Sato et al. 2011), encodes DUF family protein, the function of which is unknown. Proteins having an amino acid sequence similarity to Os03g0806700, e.g., SORBI_01g004400 of *Sorghum bicolor*, 100381620 of *Zea mays*, and RCOM_0557310 of *Ricinus communis*, have serine at 244 aa similar to a protein encoded by the 'LTH' allele, not leucine encoded by the 'Nipponbare' allele.

Table 3 Sequences of primers and probes of dot-blot-SNP markers for marker-assisted selection of cold tolerance

Gene name	Maker name	Chr.	SNP position	Primer sequence (5'–3')	Probe sequence (5'–3')
Os03g0789800	qLTB3-1	3	33730737	Forward: CTACACCAGGACCCACTCATGC	CAAGGGCGCTGCTGCCG[a]
				Reverse: GGCTTCTTTCGTCGCCTTTCTT	CAAGGGCGTTGCTGCCG[b]
Os03g0790700	qLTB3-2	3	33779883	Forward: CGAAGATGGTGACGAGGCTGTA	GGTTCTTCCTCCTGATG[a]
				Reverse: CCTTCGACATCAAGCTCTGCAA	GGTTCTTCTTCCTGATG[b]
	qLTB3-3	3	33781220	Forward: GCTCCTTTCATTCTTAGCACCTG	CATCTATGATCTTGTGA[a]
				Reverse: CCCCCATTTTTAGCTCCTACGC	CATCTATGTTCTTGTGA[b]
Os03g0793700	qLTB3-4 3	3	3918756	Forward: GCTCATCTCCCTGTGCTTCCTC	TACCACTTAGGGGAGGA[a]
				Reverse: AACCTCTCCAGCACGCTGAACC	TACCACTTCGGGGAGGA[b]
	qLTB3-5	3	33920833	Forward: AGGAGGAACAGGGAGAGGAGGA	TCGGGAGGAGTCGGTGA[a]
				Reverse: CGAGGAACACCTTCTCGTCGT	TCGGGAGGGGTCGGTGA[b]
Os03g0800500	qLTB3-6	3	34289263	Forward: GAAGCCGGACGCCTTGATCG	CCCCGCGATGGCGCGAG[a]
				Reverse: ACAAATCCCAGGCTCGCTCTC	CCCCGCGACGGCGCGAG[b]
Os03g0806700	qLTB3-7	3	34611594	Forward: GTGAAGCGTCTGGCTTGGAAAT	GTTTGGATTGACGACAA[a]
				Reverse: CGAGAAACCAAGACCCTGCAAC	GTTTGGATCGACGACAA[b]

a 'Nipponbare' type

b Variant type

Although there is a possibility that the SNPs identified in Os03g0790700 and Os03g0806700 are variations responsible for cold tolerance in 'Ukei 840', further analysis is required to demonstrate participation of these SNPs in cold tolerance. Since *Tos17* insertion lines of Os03g0790700 and Os03g0806700 are available (http://tos.nias.affrc.go.jp/), we are transforming these *Tos17* insertion lines with 'LTH' alleles and 'Hitomebore' alleles of Os03g0790700 and Os03g0806700 to produce transgenic plants for testing their cold tolerance. However, such transgenic plants should be cultivated in an isolated greenhouse in line with requirements of the Biodiversity Convention. Although many plants per line should be examined for cold tolerance to minimize errors, it is not easy to test many transgenic plants in such an isolated greenhouse. Production of recombinants only in upstream and downstream intergenic regions by meiotic recombination may enable a large-scale evaluation of cold tolerance using a deep-water field with controlled water temperature for demonstrating the function of a candidate gene.

The only gene so far identified as a gene for cold tolerance at the booting stage in rice is the F-box protein gene from 'Silewah' (Saito et al. 2010). Although a 30% increase of seed fertility under low temperature has been shown in transgenic plants having the F-box protein gene from 'Silewah', there is neither a nucleotide sequence variation nor a difference of gene expression levels between 'Silewah' and a cold-sensitive cultivar. Since 687 genes have been reported as F-box protein genes in rice (Jain et al. 2007), it is not easy to elucidate the 'LTH' is required for elucidation of the cold-tolerance gene. However, the SNP markers developed in the present study would be useful for marker assisted selection of cold tolerant lines in rice breeding programs using 'LTH' as a gene source of cold tolerance.

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Supplementary Data
Supplementary Table 1. Sequences of primers and probes used for QTL analysis
Supplementary Table 2. Sequences of primers used for RT-PCR analysis
Supplementary Fig. 1. QTL analysis using a backcrossed population. (a) Seed fertilities of backcross population after cultivation under low temperature conditions at the booting stage. (b) Position of a QTL detected using a backcrossed population. Black boxes indicate regions having segregated genotypes and white boxes show regions having a fixed genotype of homozygous 'Hitomebore' alleles. A circle represents a QTL region. A gray bar indicates candidate region of the QTL and a triangle shows the peak of the LOD score.

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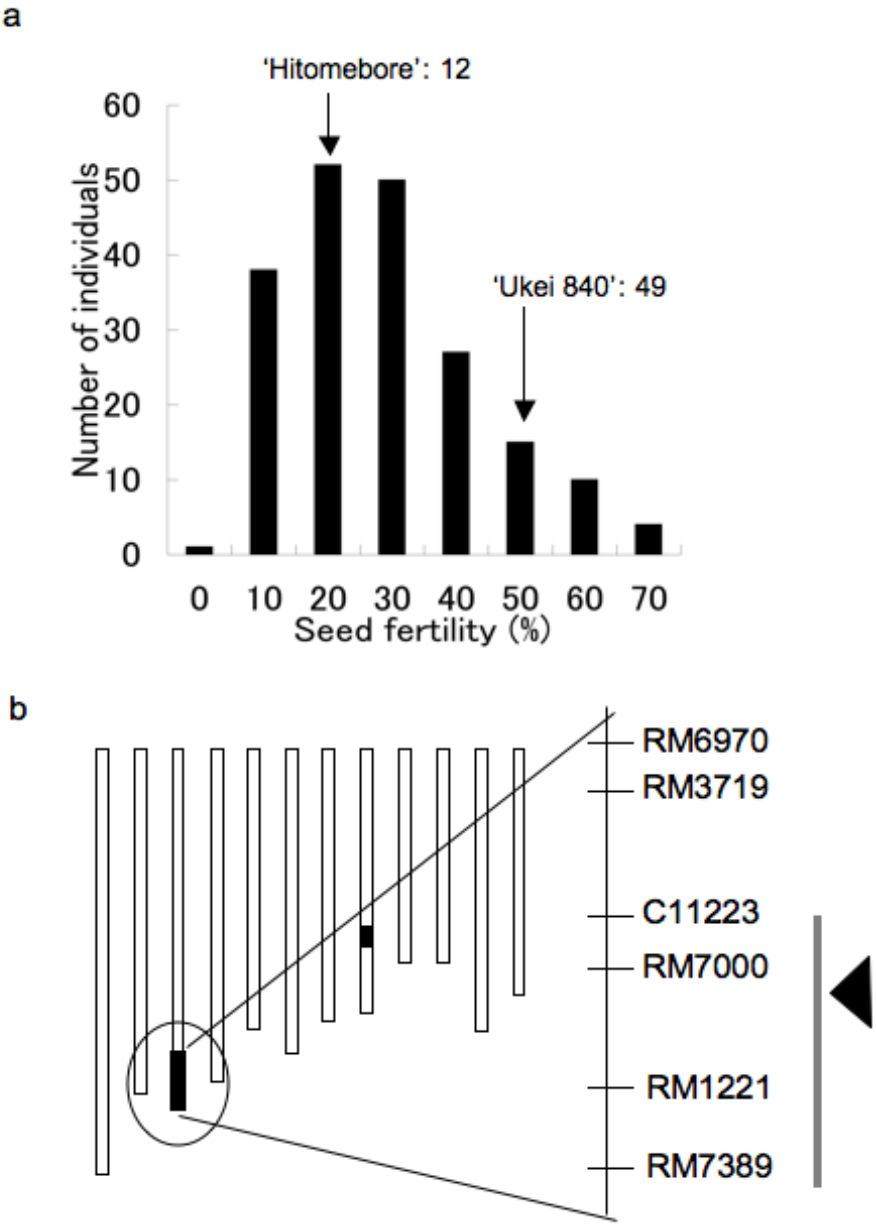

Supplementary Fig. 1

Supplymentary table 1 Sequences of primers and probes used for QTL analysis

Marker name	Chr.	SNP position	Marker type	Reference	Forward primer sequence (5'–3')	Reverse primer sequence (5'–3')	Probe sequence of 'Nipponbare' type (5'–3')	Probe sequence of variant type (5'–3')
RM3252	1	302050	SSR	1	GGTAACTTTGTTCCCATGCC	GGTCAATCATGCATGCAAGC		
S13157	1	920089	dot	–	GCATGCATTCATACAGATGAAG	CGAATGCGGAGAATTCAAGC	GCATACtaTCCATCAC	GCATACaTGACAAGAA
RM8068	1	1659209	SSR	1	AAACCTCTCGCTGTAATTAG	TGAACATTTATTGATATGGTA		
C60656	1	2215405	dot	5	GTTCGACGAGCCAAGTTCTC	GCTGCTACAAACACGCCATA	TCGGGTAGtGGAATGTA	TCGGGTAGcGGAATGTA
RM1167	1	4239164	SSR	1	GAACATAAACCATGCGGGAG	AGCTAGTGGCAAAAGTGTGC		
RM6451	1	4796393	SSR	1	TATGACATTGACCGTGGGC	TCTCCCCATGTTTGATCCTC		
RM8145	1	4885915	SSR	1	CGGCATGAGAGTCTGTGATG	AAGCAAGCCCTGGGATTC		
RM8146	1	4908081	SSR	1	GACTCCTCCAAGTGCAACG	GTAGCTTCCCCACAATGTCA		
C30013	1	4912281	dot	5	ACGAACCCTAGCCACATGAC	CTCCCCTCTCTCTCTTGTTCC	CCATCCAAcACGCCAAA	CCATCCAAtACGCCAAA
S4655	1	7268206	dot	5	TATCCGGCACTTGTTTGTCAC	GCCTCATAAAGCCCAAGATG	GAGACATCaTGCAACTA	GAGACATCgTGCAACTA
RM8142	1	7457477	SSR	1	ATTCATATATATTGGCTAGAGTGGCTGT	AAGCCAATATATGTAGAGATGAATGGAA		
S5053	1	7852785	PRS	4	CATTTATGGGCATGCAACAG	TGTGCGTGATGTGTTAGCAA		
RM8268	1	9190446	SSR	1	AAATCGACATTCTCTGTTGC	ATGGCTTACCTGCTGTCTC		
RM8133	1	9390236	SSR	1	AAAACTGACTGTTTGTTTAAATGAAAT	GTTACTGCTGTAATGTGAATTGCT		
RM8094	1	11286841	SSR	1	AAGTTTGTACACATCGTATACA	CGCGACCAGTACTACTACTA		
C0178	1	15849958	PRS	4	CACGCGTCATACAAAGCTAAGTC	CCAGTTAACCTAGTGCAGGTTTG		
TUSNP1	1	19714968	dot	2	GTACGTCATCCATAGTCCGATTC	AAAAATGCGTGGACGTTAGC	CGTGCATGtGTGAGGCG	CGTGCATGcGTGAGGCG
RM5638	1	22592885	SSR	1	GGCTTCCTCATCGCCATC	CTGAGCAGCATTCCAGTCTG		
S3813	1	24980344	dot	5	CCTCTTTAGTACCACCTCGACAG	GCAGCAGCCTGTTATCCTTC	CTCGCCTAcCCTGCCTC	CTCGCCTAtCCTGCCTC
NK37	1	25536345	CAPS	3	AATTTGTTGCATGTGGGTGTAG	CACGCTCTTCGTCTTTATCACTT		
RM3475	1	27797947	SSR	1	GTCGGTTTGCCTAGTTGAGC	TTCCTCGGTGTATGGGTCTC		
R0559	1	30312642	PRS	4	AGTCTGGAGGTCTTCACCATTCT	GTGCCAGACCTACTAATTGTTGC		
RM1216	1	33864286	SSR	1	TTCCCCAATGGAACAGTGAC	AGGGTCTACCACCCGATCTC		
RM8085	1	36618931	SSR	1	TGCGTTTCGATTTCTTTTTA	GGAAAGTTGTGTTCTTTGGC		
RM8062	1	40928887	SSR	1	CAAATTACTCCATCCATTATT	GAGGAGATGCTAGTTAATTAC		
RM3810	1	41248012	SSR	1	ACGAAGGAACTACCCGTGTG	CGCACATGTTACTCTAGCGG		
RM3482	1	41475886	SSR	1	TTGTTGTCAAGCTACGGTGG	CTGCTTCGTGATGTTGTTGG		
R3001	1	44648503	PRS	4	ACGCAACTCTAGTATCAGGTGGA	TGGGCGCTTACTCTTGTAGTAGT		
RM6321	1	44719165	SSR	1	GGCTCTACCTCGCTGTTGTC	ACGAATATAACCTGCGGCAG		
RM6840	1	44967764	SSR	1	TACCAAGACTCCGCTATGGC	GAAGAAGGGATCATGGATCG		
TUSNP45	2	977553	dot	2	CTGGGACTGGATGAGACGTT	CGGAGCTGGATCAGACCTAC	TGCTGCCGgCGAACCGC	TGCTGCCGaCGAACCGC
E60261	2	2997852	PRS	4	CTTCTGAGTTGAGCTGCTTTCA	GCACACTTTGTTTTGTGTGG		
RM4355	2	4264267	SSR	1	GGGATGAGAGTAGAAGGCA	TATATGGCAAGCCTAGCG		
SCSNP46	2	5253801	SSCP	–	GGGCTGATGCTGAATTTTCT	CATCGAGGTACGAGGTAGCAG		
RM1347	2	5314517	SSR	1	AACAAATTAAACTGCCAAG	GTCTTATCATCAGAACTGGA		
NBLAC21	2	7645937	dot	–	ATGGCGTGAGTAGCGACTTC	CCACTGAAATGTCCCAGTTT	AGTTGAGTcGAAAGATC	AGTTGAGTtGAAAGATC
RM5699	2	9010409	SSR	1	ATCGTTTCGCATATGTTT	ATCGGTAAAAGATGAGCC		
RM6911	2	9038132	SSR	1	GGTGATTGCTATTTAACTTC	ACTTTTTCCAAATTATGCT		
TUSNP4	2	17056398	dot	2	GTTACCGTGGGTACCAGCACTAT	GGCTTGCAGAAGAGTGAAGAAT	TCTTTGAAtGCTTGATT	TCTTTGAAgGCTTGATT
RM2634	2	21359015	SSR	1	GATTGAAAATTAGAGTTTGCA	TGCCGAGATTTAGTCAACTA		
P0812F	2	23257255	dot	–	TGGCAAGCCGAAGATCTCTC	CTACCCTAATGCAAGGAAAA	TGGGTGACcCATTTGCA	TGGGTGACgCATTTGCA
NK49	2	23935700	CAPS	3	TATGGTCACGCCTATCATACACA	GGCTCAATACCTTTTTGCATCTA		
SCSNP48	2	24279890	SSCP	–	CCCCAAGTATTGATGCCAAC	CTCCAAAGGAAAGCCACAAC		
S20768	2	25025944	dot	5	CCTGTGTACCTCGAAGAGTCAA	TAGCTGATGTGCCACGTGAA	TCTGTACCaTGGTAAAT	TCTGTACCtTGGTAAAT
NK14	2	25153369	SCAR	3	CGGTTTAGCACGATCTATCAAG	TAGAGAGGAAGATTTCAAGTGG		
NK52	2	25321909	SCAR	3	ATTTCATTCTCCCATTTCTCCTC	CCCCTGTAGAGTGGCAAATAATA		
R2559	2	25827732	dot	5	GCAATTGTGCCTTATACCGAG	GAGCTTCTGTCTTTGGTAACACG	ATATGTGGtTGGGTTAG	ATATGTGGcTGGGTTGG
E3295	2	25940259	dot	5	ACGTACGTCACACAACATCTGTC	GCTGCAGTTCGTGATATGGA	TGCAAGCGcCGTCGTGA	TGCAAGCGaCGTCGTGA
SCSNP49	2	26630912	SSCP	–	ATAAGCGCAATGGGCTAGAG	GTGCATGGGAGCACATTACA		
RM5631	2	29160338	SSR	1	CGTCCAAGAAATATTGCAGT	GTGAGACAGAATCCTTACGC		
RM3220	2	29350959	SSR	1	TTGAGTTTTCCTGGCCAGTC	CTCGCTTTACAGGCCAGAAC		
RM6122	2	33081236	SSR	1	CCGCCCTCTTCTTCTTCTTC	TAGACACCAACAATGGCGTC		
RM7286	2	33887388	SSR	1	CAGAACAATTCGACCGCTTC	GGCTTGAGAGCGTTTGTAGG		
RM3789	2	35577595	SSR	1	ATTAAGGGCAGGGGCATATC	CATTGACTGGTGTGGTCAGG		
RM4108	3	515005	SSR	1	GTCCCTCGCTTTATATCTAG	CAACTCTGCTAAACGAATTA		
SCSNP52	3	1008965	SSCP	–	CCAGAAGCCAAATAAGTGGTG	ACGATGTCTACGACGCGATA		
RM6297	3	1744274	SSR	1	TTCTTCTCTCCTCTCGCTCG	CCAAAGCAACCCATCTCAAC		
SCSNP53	3	3577015	SSCP	–	GCCTGTCCCTCGTGAACTTA	GTTCATCCCCGATAGCTCAA		
RM3467	3	6031935	SSR	1	ATAATGGCAGGGTTGTCTCG	CTCGGTGAGCCTCCTACAAC		
RM3872	3	6902498	SSR	1	GGAAGAAAGGATCTATATCA	TACGATTTGTTTAAGTTCAA		
RM1338	3	8444141	SSR	1	AGAGGGAATTAGATTGGATT	GGTCCACTTCTTCCTTCTAT		
RM2187	3	8876841	SSR	1	GTCATTTGAAGTAAATCCGT	GGTCTACTTGCGAAATAAGT		
TUSNP7	3	9640009	dot	2	GCTGGGTGATAGAGCTACCTTC	CTTGGCACACAGAACAAAGA	ACTTGCTCcATCTATTT	ACTTGCTCtATCTATTT
RM1319	3	13078979	SSR	1	GTGCTAAGCTTCTTCTGTGC	GCCAGTTAGCCCTTAAATC		
R0044	3	14984339	PRS	4	GCCATCGTAGACAAAGAGTATGG	ACCACAAGAACAGCTCTAACCAG		
TUSNP8	3	21926296	dot	2	gaacggaggaagtaCATGAGAAAC	AGGATGCATGAGGGGATATCTA	TAGAGTCAcGTAGAGAT	TAGAGTCAtGTAGAGAT
RM6736	3	28084003	SSR	1	TGGAGGATGAAGATTAAGTA	ATTTCGTGAAAAAAATTCTA		
RM3856	3	29542776	SSR	1	TTGCATAACAAGATGAACAA	TTAATTGCGGATTTTTTATT		
RM6329	3	29572249	SSR	1	CCCTGGATGAAAAGCACAAG	GAAGTTGTAGATGCCCCATC		
RM8277	3	29572261	SSR	1	AGCACAAGTAGGTGCATTTC	ATTTGCCTGTGATGTAATAGC		
RM6759	3	33081236	SSR	1	TGGAAAATTTGATGACATAA	TATGGGGTATCCATAATCTC		
RM6970	3	33342605	SSR	1	TCGCTTGTGTTTTCTGGGTC	TGGAGAATTTGGAGGCTGC		
TUSNP11	3	33369280	dot	2	CCAGATTTTGTCTTGGCCCTAA	GGTGGATGCAAATAGGTACAAC	ATCAGTACaTGCTAAGT	ATCAGTACgTGCTAAGT
RM3719	3	33540013	SSR	1	GTAGGTCAACTTACACGCAGTG	GTACATACGACCAACGCTGC		
NLSNP3-13	3	33900516	dot	–	CCACTATAAGGGTCGTTTGGGAGA	GCAAGCAACCATATGGGCAGA	AATAAATTATTTTATTC	AATAAATTCTTTTATTC
C11223	3	34347216	dot	5	ATTGTACCCTGACGATCGAA	CTGTCTTTCCAGACAGAAGAACC	CGCTTGACaGTGAATGG	CGCTTGACgGTGAATGG
RM1373	3	34567215	SSR	1	AGTTGGATATATAATGCAGG	CATGCTATGTTTTCATGATA		
RM7000	3	34653353	SSR	1	CCCTTCTTTTCAACTGAATA	TTGTAACAATGAACTCGTTC		
RM3329	3	36446341	SSR	1	GCACATACAGAAATGGTGAA	GGCAAGGGACATGTAGTAAC		
RM1221	3	36527459	SSR	1	GAGTAGAGAGAGATGGCGGC	AGGATTAGCAGCGTTAAGCG		
RM5548	3	36693701	SSR	1	GGTGCAGAGTGATGCAATTC	AACATTAGGGATGAGGCTGG		
RM7389	3	37013036	SSR	1	AGCGACGGATGCATGATC	TTGAGCCGGAGGTAGTCTTG		
RM7535	4	1159365	SSR	1	GACGAAAACCGGTCGAATTC	TCCAACAAGAGTGAGCATGC		
SCSNP30	4	3413358	SSCP	–	CCTTGATCCGGTGTCAGAAC	CTCACACCCTCTCGATCTCTGT		
RM7472	4	7087972	SSR	1	GCCACGTGACGGTTTAAGAG	CAAGTGGCACGTATGAGTCC		

P1255F	4	11851141	dot	−	TACATCACATCATAAAGCTA	TGGGTTGCACTTACATTTAA	ACAATAGGtTGTTTTCA	ACAATAGGcTGTTTTCA
SCSNP33	4	12292847	SSCP	−	CAGTTACTCAGTGGAGCTTGTGA	TGAATACAGGTACCACCAAGAGG		
NLSNP105	4	13477923	dot	−	AGCCTTGGCACCCCCGGCTG	GCCATCACCAACCAATGGGC	TCCTTGCAcGTGGCAAC	TCCTTGCAgGTGGCAAC
RM6314	4	18638026	SSR	1	GATTCGTGTCGGTTGTCAAG	GGTTCAGGGACGAATTTCAG		
C62054	4	20456093	dot	5	TGATGCACCGTCCTCAATTA	TGTTGGAAGGGAACATGTCA	AAACTTGTgACCAATGA	AAACTTGTcACCAATGA
RM3524	4	23292432	SSR	1	CGGAGCTGGTCTAGCCATC	GTCTCCGTCTTCCTCACTCG		
RM2439	4	23496189	SSR	1	ATGTTTAGATTCTTAGCACT	GCTCATATCCATATAAATGT		
RM2521	4	23616994	SSR	1	TACGACTGCCTACATGATAT	GTTGCCAGTTTTTTTATGTC		
RM3866	4	23757049	SSR	1	AGTTGGTCATCTACCAGAGC	GATCTTCTTGCCTCAGAAAG		
RM3785	4	24646204	SSR	1	ACCTTTTCTTGGCTTGAGGG	GCTTTTGCTACTTTTGGGGG		
TUSNP36	4	28171432	dot	2	GGTACCTTGGTTACTTGGGCTA	CTCCTCCTCTCTCTTGGGGTAT	CTGGTacACTGGGCTAG	CTtGGTtACTGGGCTAG
C11882	4	32381472	dot	5	ATTGTGATGTCCCAGGAAGC	CACAGTGCTACATGTGACATTCC	TGACAAAAtTGAGAACT	TGACAAAAcTGAGAACT
RM3814	4	33083061	SSR	1	GCGTCTTTCGTCTCTGTGTG	GTCCATCATCCCTATGGTCG		
RM3335	4	33425009	SSR	1	TAATCCACTGTGTCATTTAA	ACCATCATCTTGTACCTAGT		
C1016	4	33713844	PRS	4	TTCCTGTGGTGGTGGTTATAGAG	GGGTAGGTTCAGTGAATCAGATG		
SCSNP38	4	34432656	SSCP	−	CTGATGATGAAGATGACGAGGA	CGTCACAATAACGAAGATCGAG		
RM1248	5	72127	SSR	1	ACAAGCAGCTAATGGTTGGG	GTGATTTTGGCTCAGGTCAG		
R2846	5	1991425	dot	5	CGTCAAACTTTTCACGGAACTAC	GTAGGGAAACGAATTGAATCCTG	ATCACAAAaCTACAGAT	ATCACAAAgCTACAGAT
S0703	5	2027570	dot	5	TGGATCATTCCTGCTTATCC	CACCACCCATTCCAATTCA	CTCAATTGgTTGCTGAT	CTCAATTGtTTGCTGAT
R2846	5	2027592	dot	5	CAGATTTAATGCACCATATCAC	GTAGGGAAACGAATTGAATCCTG	ATCACAAAaCTACAGAT	ATCACAAAgCTACAGAT
RM3853	5	4082433	SSR	1	AACATATGCTATGTGCCCTT	GGAGTTATCAGCAAATGCTC		
RM3419	5	5267135	SSR	1	ATCTTGGTGAAACAGTGCTC	CTGCTGCTATTCCTCAAGAC		
S10613	5	5454769	PRS	4	CATATTGGCCCATCAGTTCC	AACATCATCGGGTGGAAGAC		
RM4691	5	7008395	SSR	1	GGTTTCCGTTATTTTTATCG	CATCAAGAGATAGTGCTCCA		
SCSNP62	5	11952692	SSCP	−	CGAGAGTTCCAAGATGAGGAA	AGGATCCAGTCAGCTGAGGA		
RM8039	5	13406414	SSR	1	CGTACGTACTTATATCTCAT	AAATCTAATGTATCTGAGGT		
NBLAC31	5	14208505	dot	−	TTAGCCAGCGTATGTGTT	CGGTACTTAATTACCTTGT	GAtCTTATGAGCCTaAC	GAgCTTATGAGCCTcAC
RM3838	5	16531553	SSR	1	AGATGTTGCCAGTTTGCCTG	TAGTGTCTTTGTGCAAGCCG		
NBLAC32	5	17833379	dot	−	CAATTCATTAAGGACTCCCAG	ATAACCCTGAAATGCTCAAA	ACAAAATGcCATTTTAA	ACAAAATGtCATTTTAA
RM1237	5	18023520	SSR	1	CTCCGCGAGCTTTAGAAGAG	CACATACTCTGGCTCTCCCG		
RM4501	5	22159668	SSR	1	GCACAAATGTCTCTGTCTAA	AGAGTACGAAACGGTACAAG		
RM3759	5	22437811	SSR	1	CGTGCACAAACGAATTGACAAGG	CAGATGCTGGTGCGGATCTGG		
RM3476	5	23953842	SSR	1	GATTCTCGTCGTAATCAAGA	ATCCACGGTTAAGATAAATG		
RM6972	5	25469993	SSR	1	GCTCTCCTGTGGGTTTTCAG	CATGGTGCTCCTACTGGTTG		
RM3809	5	26712245	SSR	1	AAATATCTATCGGCCTCTCCAAGC	GGAGGAATCGAACCAGAGAAGC		
RM19044	5	27038393	SSR	1	GGAACATCTATCCCTGTCCATGC	CCATGGAAGATGAAACTGCAACC		
RM7473	5	27069221	SSR	1	CCGAGAATATCAAATCCCTACC	TAGATAGACAGACGAACCGATGG		
RM5784	5	27939360	SSR	1	GAACGCACAAACGTCCATTC	TTCACTCCAGTTCCTCCACC		
SCSNP12	5	28161272	SSCP	−	CTGCCTAAACTCCACTTCACATC	CTGAAGCTAGAGCTCAACCAAAC		
R3139	6	1371037	PRS	4	ATGGAGGTCTGGGTAACACTGTA	CAACAGCTTGGTTCAGGTCTC		
C62866	6	3358870	PRS	4	CTCGTCATGTCTAACTTCCCTTTC	ACTTGTCCCCAATAGACACTTCC		
TUSNP67	6	5476999	dot	2	GGCGGTAATAGTGGGACTGA	CCGGTATGATGGATGGTTTC	ATGCAAGGcCAACCTCT	ATGCAAGGtCAACCTCT
TUSNP13	6	6458563	dot	2	ACTCCACGAGGTAGTCCTCGAC	GGCCCATCTACAGTATACACACC	TTCGCTTGcTTCCAGAC	TTCGCTTGtTTCCAGAC
RM6836	6	9309089	SSR	1	TTGTTGTATACCTCATCGAC	AGGGTAAGACGTTTAACTTG		
TUSNP68	6	12252705	dot	2	GGCGTAGATGTGGGTAGTGG	GATCATGTCCGCGTATGTTG	AACACACAcacacttgg	AACACACAggcttggac
RM3183	6	12516179	SSR	1	GCTCCACAGAAAAGCAAAGC	TGCAACAGTAGCTGTAGCCG		
RM1161	6	13821128	SSR	1	AAACTGTTTTACCCCTGGCC	ATCCCCTTCTGCGGTAAAAC		
RM5087	6	17554814	SSR	1	AAGGAGTTAGTGGGGGATAA	GAGATGAGATCCGAACCTCT		
TUSNP15	6	18992003	dot	2	GAATGCTTACCGAGATTCCATC	GACTACATGAGGTCCAGCTATGC	CATCGGTAgAGCTAGAC	CATCGGTAcAGCTAGAC
TUSNP69	6	19354468	dot	2	CAAAAATTCTCCGGACCACA	CCATCTTTGCAGGTTCATTG	CTACCTCTgACAAATCC	CTACCTCTaACAAATCC
C0767	6	20214495	PRS	4	CAGAAATTTGGCCACTGTTACC	GTGTGTGTGGTTTTCTGCAACT		
TUSNP70	6	21979144	dot	2	ACAAGCCTCTGGTGCTTTTT	TGCAGGCCACTGACTTAACA	GTTGAATTcAATTTTTA	GTTGAATTtAATTTTTA
RM3827	6	23175146	SSR	1	TAGTCCTCGAGGACGGATTG	CTGGCCTTTCTTCAATCTGC		
SCSNP71	6	24708544	SSCP	−	GCTAAGTGTGGGGGCTAAGTC	TGCGACCACTTAACAAACG		
RM5314	6	25720796	SSR	1	ATCCCACCAATAACCCTTGC	TGGTTGAGAGGTTTGGATGG		
SCSNP16	6	27542447	SSCP	−	AGGCTGGTATGTACACCGAAAT	GGAGACACACATTGGTCTGAGAT		
RM1150	6	31258391	SSR	1	ACAGTGGCCACAGTGTGTTG	GGATTCGGGAGGTTGACG		
RM3509	6	31848997	SSR	1	GTGGTACATCCTCAAGGATCG	GTTGAGGAAGGGGGCTAGAG		
SCSNP73	7	150574	SSCP	−	AGACCGGATCACCACATAGC	GTTGCTTTTACAAGGGAGATGG		
RM3394	7	653262	SSR	1	CCCTTACGTGCAGTACATTG	ATGCAGGCTACTTACTAGCG		
NK23	7	1531355	SCAR	3	AAACAACAGTGCCACCATGTAG	CAAGTCGGTCATGTATCAAACAG		
NK28	7	1591816	SCAR	3	CCTGACAGCTTGTTTTCTTTGTC	GGAGGCGTAATAGATTGACTTGA		
RM5344	7	1937749	SSR	1	ACGAACGGGAGCAAGGTC	CTCTCAACCAAGACGCCTTC		
RM6872	7	4692642	SSR	1	GGATGAACACTGATGATGGC	ACCTCCACCACGATATCCAC		
RM6728	7	5762308	SSR	1	GGGTATGTGTCGCTATTTTA	GAAATCTGGAATTTTCCCTA		
RM8263	7	7718435	SSR	1	TTTGCTGTCCCTTTGTTC	TGCAATTCAAAGTCTTAGGG		
RM2256	7	9184403	SSR	1	GTGCTTGCATATAACCTATA	AGATCAACCTTCTTATTCAG		
RM8006	7	9290613	SSR	1	TGCCGGTTCTTAATTTTATC	AATGGTCCACATTACTCCAC		
NLSNP109	7	10777360	dot	−	ACACTAACCAATACCATTGC	TACTTGTCTCGAAATAATTA	TCTCCAGTtTGCTAGCT	TCTCCAGTcTGCTAGCT
NBLAC7	7	11072974	dot	−	TTCTTTTTTATCCCTTGTTC	ACTATTGTTACGTTAGTACT	GCTCTCACtAACAAGAA	GCTCTCACaAACAAGAA
NBLAC8	7	13205067	dot	−	ATACATCATGTGCCACGGTG	CGAGCCTTAGCACGCTTCTG	TGATTGAAaACAGAACT	TGATTGAAgACAGAACT
RM7338	7	16049090	SSR	1	CTTATCTCTCGGCAAGCAGC	CTCACACGCATGGATCAATC		
RM6767	7	18131297	SSR	1	ACATTCTTGATCTACGTGGC	AATTATGGTTGCTAGGTTGG		
RM1973	7	20825003	SSR	1	GAGTTGCAAGGATATTTTAA	TGGAGCCTAGAGAATACATA		
C1467	7	21395095	dot	5	CAATCGTCTGGAGAGCTCTATG	AAGCAAATCGGAAGAACAGG	AATTCATGaAAACAGTT	AATTCATGgAAACAGTT
RM3826	7	21468510	SSR	1	TTAGCTTTCCTCCAGTCTCC	ACGGGTATCTGAAACACAAC		
NK10	7	23503072	SCAR	3	CGAGGTTCCCTAATGACCAA	CTTGTACTTCGCCCCTCTTG		
R2394	7	23520106	dot	5	CTACTATTGAAAAGCCATAGTTAGG	GCTTGAAAATGTGGTGGTTC		
NBLAC36	7	24963340	dot	−	GCTGGCCTAATAGTCTGCAAT	ACGGCTCATCACATGCGCACA	AAGAATTCgGTAGGACG	AAGAATTCaGTAGGACG
R1789	7	27190213	PRS	4	GGCAATACAAAGAGCACATAG	GCACAAATCATAAATACACTGG		
RM5720	7	29330369	SSR	1	CCTGATAAATTGACAGTTAC	GAGAGTAGGAGTTGATAACA		
RM1306	7	29608200	SSR	1	TGCCAATTACCTTCCCGTAC	TGCTCCGTATTGCTGCTATG		
SCSNP17	7	30269280	SSCP	−	GGAGATGACTCTCGATGTATTGC	CACCTCCTGTCCTTTGCTATG		
RM5911	8	74440	SSR	1	CCCTCTTTTTAAGTCTGGGG	GGTGCCTCCTTTCAAAGTTG		
RM6369	8	124747	SSR	1	CAAGCTAGGGCTGCATAAGC	GCTTCACCTACCTACCTCACC		
RM1235	8	1208745	SSR	1	GAAAACTAAAAAGCAGAGGA	AAGCTATCCATTTTGGATTA		
E50066	8	3147604	dot	5	ATGCAGCAGTTTAGGCATGA	CTGAAGACCAGGGGATAGGG	AAGTTAGCgCTAAGCCT	AAGTTAGCcCTAAGCCT
RM6999	8	3984397	SSR	1	TTATCTGGGATCCATCGAGC	GTGAATTTCCTTGGAGGGAC		

RM5556	8	4588509	SSR	1	ATCTCCCTCCCTCTCCTCAC	TCCACACCTTCACAGTTGAC		
E31176	8	8343288	PRS	4	CGCGAGCAATCAAATCGAAATC	TCACATGACAATCTCAGGTTTC		
SCSNP79	8	9714494	SSCP	–	AATGACGAGCGTACGAGGAG	GCAATAGCCCAATGGGTAGA		
TUSNP80	8	12194374	dot	2	GCGATTTCGAAGGCAAGATA	AACAACCATAGCCGAAGTGG	TGTTACATaTTTTGTTC	TGTTACATgTTTTGTTC
RM4595	8	14424588	SSR	1	AATAGTTGTTGTTTTGGACA	AAATTTAAGTGATTTTGTGC		
TUSNP81	8	15829286	dot	5	CCCCACTGAGACCAAAAAGA	ATCGTCGGTCGATCTGTGTT		
C52335	8	16627858	dot	5	CCCATCATATCACCCATTCC	AAGCCCTTGTTCCTTATCCAAC	AAACAATAtATGCATGA	AAACAATAgATGCATGA
E2623	8	17608326	PRS	4	GTGGTCAAACCAACTCACCATAC	GTGCCACTAACTGCTGAAGCTAT		
SCSNP82	8	18365995	SSCP	–	AATGCAGGATTACCGACGAT	GCTCGAGAGTACAGCCAAGG		
TUSNP18	8	20864104	dot	2	CAGTTAACCACTTCGACAGAAGG	GGGAGGGATTTGCAGATTAAC	AATAGATAtCCACAAGC	AATAGATAcCCACAAGC
RM7049	8	20903095	SSR	1	AACCTAGATCTAATCCGTGG	CATCTCTGAGTTGAGCAAAC		
RM5485	8	24160909	SSR	1	CTTCCACAAGCTTGGCTAGG	AATGCCATCCCCTACTCATG		
RM5353	8	24209761	SSR	1	ACCCTCGATCTCCTAGGCTG	TCTACTCCAAACCCATTGCC		
RM8058	8	24658119	SSR	1	ATATGATTTTCTCAAACAAC	CCAAACTACTAAACAGTACA		
R0639	8	26999621	PRS	4	GAGCTAACCTCGCCTCTAAGTTC	GACGTGGTCAGGAGGTACAAC		
RM3120	8	27908245	SSR	1	ATCGATGGAAGCTCTTTGCC	GGATGTACAAGAGCTTAGGAG		
RM3496	8	27930203	SSR	1	CGCTGAAAATACTGAATTGA	AGATGCATTTATTCCGAAAG		
SCSNP19	8	28049807	SSCP	–	CTAACGGGCTGCACTCATTATT	TGCTAAGGTTCATCCTCATGCT		
SCSNP83	9	586242	SSCP	–	CTGGTCCCACCTGACATACA	TGGGTTTCCGTGTCCAATTT		
SCSNP85	9	4331290	SSCP	–	GTTCAACAGCCCAAACCCTA	GATCCCGCTTCAATCCCTCT		
TUSNP88	9	8418691	dot	2	CAGGCTTCCTGTTCTTCGTC	TTCGCTACTGGGTCCACTC	CAACTACAaCAACTTGG	CAACTACAgCAACTTGG
RM1328	9	9772096	SSR	1	GAATGGGATTAGACGATTTG	CCATGAGTGACATCAAAAGG		
S0313	9	10196552	dot	–	GGTGAATTCATGGCACTGGT	GGAACGATGTGACGGAAAAG	AATTCTACaTGTATGCT	AATTCTACgTGTATGCT
NBLAC38	9	11062947	dot	–	TCAACCAACACGGTGATCAA	AGGACTAAATCCAAAAAGGA	TCTGTCATcGTTTATTT	TCTGTCATtGTTTATTT
TUSNP89	9	11464861	dot	2	CTCGCCATGTTCAAAGAAGC	CTGGGTGTGAGAGACGAACA	AAGAAGCTgAGCGACGC	AAGAAGCTaAGCGACGC
TUSNP20	9	13754905	dot	2	GATAAAGCAGAGAGGGGAAGATG	cccTCATTTGCACATTCAGA	TGGCACTCcGAGGCTCC	TGGCACTCtGAGGCTCC
RM5657	9	15021542	SSR	1	TATGTGCATTTGTAAGGTGA	GCTTTAGATTATTGAGCGAG		
S11615	9	15789179	dot	5	CTGCAGCAAACTCACTCACTCTA	ACAACTACTGCTGGGGCTTC	AGCACGGTgGTGGCTCT	AGCACGGTaGTGGCTCT
S1974S	9	15814650	dot	5	TTGTTCGCCTCCGCTTCTTC	AAAACCACTTCCCCCAATTC	TTTGATTCcTCCAAGCT	TTTGATTCaTCCAAGCT
C30515	9	15818384	dot	5	CCTGCAAGAACAACCTGATG	ACTATTACCTTGGTCCGAAGAGC	TTGACAGCaACCATCAC	TTGACAGCtACCATCAC
RM7175	9	17526525	SSR	1	ACAGTAAACGTGGTGCCTCC	AGAAGTAGCCTCGAGGACCC		
RM5786	9	20442348	SSR	1	AAATCAGGAAAGTTTCTCAGC	AGAGACACAGGCAAGTCATC		
RM3808	9	21379507	SSR	1	CGTTAGCGAAACGAACAGTG	CAGTGGCTCGGTAATCGC		
RM2144	9	23041614	SSR	1	ACATTATGAAACGGAGGAAG	GAAATGATGCATCAGCATTA		
NBLAC40	9	23765863	dot	–	CGCCGGGAGCCCGATGAATA	GCGCCACAGCTGGGTGTAGA	CGGCAAGGtCTTCCGCA	CGGCAAGGcCTTCCGCA
RM7492	10	34023	SSR	1	AGATGGTTGCCAAGAGCATG	GTCACGTGGCGATTTAGGAG		
SCSNP90	10	543488	SSCP	–	TAGGGCCGATGTGGATAAAG	ACTCCGAGAAGTCGAAGACG		
RM3882	10	2717552	SSR	1	GGTGCCCAATTTAGCAGAAC	CGGTGGGTTCCGAAATTTC		
RM7217	10	4308039	SSR	1	TTTGTAGGATGACACGTGGC	CGGGATTTCAGTACCTCACG		
C913A	10	4320668	dot	–	GCTTCTTAGGATTGAGGAGGAG	AGAACACGCAAGCTCAGAAC	ATGCTGTcTTCACCAT	GCATGATaTTCACCAT
CDP	10	4347142	dot	5	GTCTTGCGAAATGTGACGTG	CCTGCATTCGAAGAATTCCA	GCTGGTGCgGCGCCTTG	GCTGGTGCaGCGCCTTG
NBLAC41	10	7942955	dot	–	AGAGCCGTTCAAAGCCACAG	ATCACCCAAATGGTCAACCG	GTTCCGTTtGCCTATTT	GTTCCGTTcGCCTATTT
TUSNP93	10	9665031	dot	2	CGTGTGGGAGGGAAAAATAA	AGGACTTCCTCCGTCTCAAA	CGACAAAAcCCTATAAT	CGACAAAAgCCTATAAT
RM8207	10	10139191	SSR	1	TTCATCGACATCATCAACTG	CAGTTTGGGATGAAGTGTTC		
RM3311	10	10950850	SSR	1	AAGTATCCCTGTCTCACGCC	AGGTAGAGGGAGGGAGGGAG		
NK15	10	11738209	CAPS	3	TGGTGGAGACAAAAGGTTCGAG	TGGTGGGTGTGTTGATGATGG		
TUSNP21	10	12100820	dot	2	ATATTTCAGCAGTGGGTTGTGG	CGACTCTTTCTTCTCTCCATCTTC	GGTGTCGAtGCTGTGCT	GGTGTCGAgGCTGTGCT
TUSNP94	10	16148652	dot	2	AAATTGGCAGCAGTGTCCTT	TTGGACAGAGCCAACCTACA	ACCAAGCAtGCATCTGC	ACCAAGCAaGCATCTGC
Ehd1	10	17535095	dot	–	GAGGATCGAAGAGCTGAGCA	CTTCTTTGGAACTGCCTCTGC	ACCACCTCgGAGAAGAC	ACCACCTCaGAGAAGAC
RM1873	10	18352141	SSR	1	CTGACAGGACATTAAAAAAC	CCTCATCCTTAATCTCTTTA		
RM6704	10	18462922	SSR	1	CACACATTGCATTACGAGGG	CAGGGGCAGCTTGAATACTG		
C16	10	21624964	dot	5	ACATCTTGAGGATTGGGAGC	GAGCGTCTTCTTGGCAATCT	TGTATGGAaAAGTTAAT	TGTATGGAgAAGTTAAT
R0835	10	22116792	dot	5	GTCCGTCCGGTATCAAGAGT	ACCGGATAAAATCAGCCACA	TAAACCTAcTATTAAAT	TAAACCTAaTATTAAAT
NK18	10	22845285	CAPS	3	GATGATAGACACAGCTTGACG	AGCTGGTGGGGATTTAAACGG		
RM6160	10	23023039	SSR	1	AATAGAATCCCGAACCGTCC	CGCGAGAAAGACAGCGAC		
RM5494	10	23055210	SSR	1	CCAACAACATGCCACTTCTG	TTGCTCCTGATTCTCGTGTG		
RM4771	10	23540814	SSR	1	ACGTTGATTTCATTCAGGTC	ACGCTAACTGAGAAACATGG		
RM1761	11	305110	SSR	1	ACGCTTAAAGAACATTTGAT	GCGATTAACTTTTAACCATT		
S20083	11	2107262	PRS	4	GCTGAGAACGACATGTGGAG	GCAGCTTTCACCGACTTGAT		
RM1812	11	2391251	SSR	1	CAGCTAGTGAGCTCCTAGTG	GCTAACCCACCAACTTATTC		
S10616	11	3978112	dot	5	CACCCTTGTGGTCACACTTG	CGGTGAACACCAGGTTCATT	GATCCGCTgACGGAGGG	GATCCGCTaACGGAGGG
RM6894	11	5901535	SSR	1	AATCTCCACTGCAGCGATTC	CGAATGGTCAAACGTAGGTG		
TUSNP96	11	6180907	dot	2	CAGCGCGTGGTAACTATGAC	ATCTCATCCCCTCCTTACCC	CCAGTCCAtATCTGGAT	CCAGTCCAgATCTGGAT
RM3625	11	6652024	SSR	1	CTTGCAATTCAATTGCTTAC	GGTGGCCTAGTGAAACTAAA		
E0935	11	9119856	PRS	4	TTGCAAGAACTTCCCCTGAT	GGTTCGTTTTCACTCCGTTTTC		
RM7391	11	9985225	SSR	1	GATGCCACATAGCGACTTAG	GTCAATGAGTTCTTCAATTCC		
RM4862	11	10030624	SSR	1	CAACTTTCTGGCATAAACTA	TGGTGAAAGATATTTCAGAC		
NLSNP112	11	11446647	dot	–	CTATTGGCTGTGCAATACTG	AAAGATCACAACATTTAGTA	GTCGATTTcGACACATA	GTCGATTTtGACACATA
NBLAC43	11	14507234	dot	–	CGATATTGAGCGAAAGCCCT	GTCCATTGATCGAGATCAGC	TCGATCcCGTcCTGAAC	TCGATCtCGTtCTGAAC
RM3428	11	15769270	SSR	1	ATTCATGCTTCCTTTCAGTG	GATTACTGGTTTGCCATTTG		
RM1355	11	19497166	SSR	1	GTTGACGCCCAGAAGAGGTATCC	TTTCCTCCACATAAGCGAAGAGC		
NLSNP114	11	20111696	dot	–	CACAATAGTATTTGGTGTAT	AGTATAGGATAACCTTTTTTAA	ACTTCCCTaTCATCATT	ACTTCCCTtTCATCATT
RM5349	11	21478113	SSR	1	AGGGCATGCTTACATCCAAC	CATTTGCTTCTATGCCCCAG		
C1172	11	21900333	dot	–	CAGGAAGCTCTGGCATTGAG	GTACAAGAAACCCGGCTGTG	TATCTATAgTCAGCAAG	TATCTATAaTCAGCAAG
NBLAC52	11	23078628	dot	–	GGGCGATGAGGAGAGAGACAGA	GCACAGCTCTGGCTCGAAGTAA	TGAGCCATaGTTAGGGC	TGAGCCATcGTTAGGGC
NBLAC53	11	23740362	dot	–	TTTGGATGGTGTGTGGGTGTAGA	GATATTCGGTGCGCAACTCTGA	GTTCATGAtGATGTCTT	GTTCATGAcGATGTCTT
S723(Pb1)	11	24061028	dot	5	GTGGGATTTAGGAGGACAAG	AGGCCTGTATGTGCCAATCG	GTGTTCGCaAACAAGCA	GTGTTCGCgAACAAGCA
C11589	11	25737984	dot	5	TCTTGCAGGTCACTACAAGCAT	GGTCAGCTATATGCATCAGGGTA	GAATTCAAaCAACAGGC	GAATTCAAgCAACAGGC
NK19	11	28063367	SCAR	3	CAAGAAACATAGGCGAGGTCG	GTCGGTTTGATGAAAGATTCG		
RM1233	11	28848386	SSR	1	GTGTAAATCATGGGCACGTG	AGATTGGCTCCTGAAGAAGG		
NBLAC68	11	29270751	dot	–	TGGGTGGAGGAGGTGAGGAT	ACCAGTAGCCGCACCGACAT	TTGTGGATaATTACTTG	TTGTGGATgATTACTTG
RM7240	11	28872420	SSR	1	GCGACGACGAAGCTACCTAC	ACGTTTCGGGTTCATGCC		
NK59	11	30213450	CAPS	3	TCCTTGTTCTAGAGCACAATCAAT	GGAGGTAGGAAAAGGACCAGTTA		
C10295	11	30561744	dot	5	GGGTCAACCAGATCTTACGGTAG	GGATTGTGAAATGCCTGACC	CATGTATTgAAATCAGT	CATGTATTcAAATCAGT
RM5926	11	30766118	SSR	1	ATATACTGTAGGTCCATCCA	AGATAGTATAGCGTAGCAGC		
RM5568	12	711976	SSR	1	ATTATTGCTTGCGCCTTTAG	AACGGAACAGATCCAAATG		
RM1880	12	747083	SSR	1	ACCACTAAATAAGCACATAC	GGCATCATACATTAAAATAC		
RM3483	12	1611954	SSR	1	CCTAGCTTTCAGGAGCAAG	CCCACAATGAGAAACAGTTG		

S0479	12	3576638	dot	–	CGAGCAATTTGCCTCATTTC	CGTTACATGGCTACAAGGTCA	TGAGACCTgTAAGGCTG	TGAGACCTaTAAGGCTG
E30254	12	3983133	dot	5	TTCGTCTCGATGTCGATCTG	CGATGGCCAGGTATCTACTCTC	ACTTAAAAaGCTTAAGC	ACTTAAAAgGCTTAAGC
RM3455	12	4919688	SSR	1	TGAATCCACACTCGCAGATC	GCCAGTCCACGATTGGTC		
NBLAC46	12	6863682	dot	–	ATTTACCACTACTTTGAG	TTTGCAATTTTCTAGTGG	TCATTATTaCAAGACTG	TCATTATTgCAAGACTG
RM1036	12	8796300	SSR	1	CTCATTTGTCGATTGCCGTC	ATGGGAGGAGTGATCAAACG		
E10037	12	9396624	dot	5	GCATCCTATATCCGGGTTTT	GTCAACAAACAAGAGGGGATGT	GGGTTTTTtCGATACCA	GGGTTTTTgCGATACCA
RM7102	12	14868524	SSR	1	CGGCTTGAGAGCGTTTTTAG	TACTTGGTTACTCGGGTCGG		
SCSNP27	12	16153136	SSCP	–	ATGCATGCTGGATTTACCTG	GGAGGCCCTCGTGTACTACT		
NBLAC50	12	17443881	dot	–	CCGTTTAAGATGACTTCGT	ATGCCCCTCACATGCCAG	TGTGACGGcCCACAAAA	TGTGACGGtCCACAAAA
RM1246	12	19260113	SSR	1	AGCTCGATCCCCTAGCTCTC	TTGGAGAAGGTCACCTGCC		
E60142	12	19306962	dot	5	CGTATATCCTGCTCGGGTTC	CTTATCATTCAGGTGAACTCC	AAAGACCCaTTTGCAGA	AAAGACCCgTTTGCAGA
SC28	12	21022066	SSCP	–	TGGCAGGGATTTTAGAGAAG	CTGGTCCAAGAAATTCTCCACT		
TUSNP29	12	24186101	dot	2	ACTGATGACAGGTGAGACCAAGT	TACGTGCTCCGTACGTATGCTAT	GGTTCACTaTACTATTA	GGTTCACTgTACTATTA
RM2197	12	27610890	SSR	1	ACTGAGAACTTTAATCATCG	GAACAACTTTGAAGAGAAAC		

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Supplymentary table 2 Sequences of primers used for RT-PCR analysis

Marker name	Chr.	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
Os03g0793500	3	GACTGCAGCAACTCCTGACCAA	AGATTTGATCGGTTGGCAGCTC
Os03g0793700	3	CTTCTCCAACAACCACGGCAAG	GTGGGCACGCCATCTCAAAGTA
Os03g0794700	3	GGGAGGTTCTCCCAGGTTGTCT	GCTTCATCAAGTCTCGGGTGGT
Os03g0794800	3	AATAGTCGGCGTCAGCCAAGAG	CACCCCTGTATGGCATGTCAAG
Os03g0794900	3	TGAGCTGAATCGCTGATGGTG	ACGAGGGTCCAGGGACAACA
Os03g0795100	3	GGCTCCGCTGAAGAGTAACCTG	ATTGGATAGCGCAAACGCATTC
Os03g0795200	3	TGGGTGAGGAGGAGAAAGCAAC	CCACAAGAGCAGACCCCATCTT
Os03g0796900	3	CTCGCTGTTAAGGCGTCTTGCT	GTTGCTTCAGATGGGGAGCTGT
Os03g0797100	3	CGCACTCGACCAAGGGACTT	TGGCACAGTTGCTGGGAGAA
Os03g0797300	3	GGGTCCCCGTCCAAAAGTAAAC	TAGCTGCCTCACCATTGCACTC
Os03g0797400	3	GAGGCATGCTCTTTCAGGAGTGA	CACGTCTTGGCGAGATCCTGTA
Os03g0797500	3	TGCATTCCCTAAGGTGGCTACA	CCAGGCACCAGCTGCTAAAAA
Os03g0797800	3	ACTTCGCCGTCGCCTATGAG	TTCTGTGAGATGTCTACTCCATGC
Os03g0798200	3	CCAACTTTACCACAACTACCAGCA	CGGCTCTTTGGTCCTTTCTCAA
Os03g0799100	3	TCCTTAGTCATCAGGGCAAAGC	GTTCGCAATGCTGTGACGATGT
Os03g0799600	3	GCTGTATTGCTGAAAATGGAAAGG	AAGCAGCCTTGCACAGGAAGAG
Os03g0799700	3	AAACGGAAATACAGGCGAACAT	GCCTCTGGTATGCTGGCTGACT
Os03g0799900	3	GGCAATGCTGCAGATTTTTGAA	TTCCAAATGGACCACGTGTTTG
Os03g0800000	3	AAGGGGGACATCTACGCCAAGT	TAGCAGCAAGCCTACCATTTTT
Os03g0801700	3	CCTCTTGGGCTCCATACTGTAAGA	TGTGTCCATGACTGACCGTACAA
Os03g0801900	3	AGAGACGGGCCTCCATGACATA	TCATTCCACCCAATTCGCTACC
Os03g0802600	3	TCCTACCGCCTCGTCTTCCA	CCCTCTTCTTCTCCGGATCCAT
Os03g0802700	3	TCTTGGCATACGACTCACACTCC	GTAGGGATTTGCAGCGCTGATG
Os03g0803500	3	CGAGTCGCCACTGTGCTTATGT	GAAGACTGGCTGGATCCGTTGT
Os03g0803600	3	TATGCTAATGGGCCAGGCTACG	TAGCCATCAAAGCAACCGGACT
Os03g0805100	3	GTGGCTATTGTTTTGGGCATGA	GGTTTAGGGTGAACAGATAGGTGA
Os03g0805200	3	TCTGGCCAGTAATGCCTCGTC	ATCAGCCTGGTTGGCATGGT
Os03g0805300	3	GCTATCGCTGTGGGAACATGG	TATCTGCAACCAGGGAACTTCA
Os03g0805500	3	AGCTGGCTATCTTGGCTGTGCT	CATAGCTTGCTGCGATTCGAGA
Os03g0806700	3	GCAATTGCCTCCGTTTTAGTGC	TTTCTTGGAGCATTGCCACAG
Os03g0807200	3	CCGAAGAAGAGGCCAAAGTTGA	GCCGCTCAGATCTGTCTAGTTAATG
Os03g0807800	3	CGGCATTGAGGATGTCTTCACC	GGTCGGTGTACTCCTGGAATGG
Os03g0808300	3	AAGCAGCAGAGGGCAACAGC	CAGCCAGCCTCTGCAAACCT
Rice Actin 1	3	GGACCCAAGAATGCTAAGCCAAG	GGCCGGTTGAAAACTTTGTCC