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QTL analysis of black rot resistance in cabbage using newly developed EST-SNP markers

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Abstract

One hundred sixty-one EST-SNP markers were newly developed for analysis of QTLs for resistance to black rot caused by *Xanthomonas campestris* pv. *campestris* by determining EST sequences of a resistant line obtained from cabbage 'Early Fuji' and a susceptible broccoli line. A linkage map consisting of nine linkage groups was constructed with a total of 209 markers, including these new SNP markers and previously reported DNA markers. F_2 plants grown in a field for one month were inoculated by spraying bacteria of race 1, and disease severity of each plant was recorded. Three QTLs, i.e., QTL-1, QTL-2, and QTL-3, were detected on linkage group C2, C4 and C5, respectively. QTL-1, which showed the highest LOD score and additive effect, was again detected in another F_2 population used the next year, suggesting QTL-1 to be a major QTL. QTL-2 and QTL-3 could be minor QTLs influenced by environmental factors. The genomic region harboring QTL-1 showed synteny with a region from 5.3 Mb to 7.4 Mb from the short arm end of chromosome 5 of *Arabidopsis thaliana*, which is rich in TIR-NBS-LRR family genes. The identified SNP markers in QTL-1 are considered to be useful in marker-assisted selection for black rot resistance in *B. oleracea* lines.

Keywords: *Brassica oleracea*; dot-blot-SNP markers; marker-assisted selection; synteny; *Xanthomonas campestris* pv. *campestris*

Introduction

Black rot of cabbage, *Brassica oleracea* L. *capitata* group, caused by *Xanthomonas campestris* pv. *campestris* (Xcc) is a serious disease epidemic in the world. It is transmitted by contact, water splash, and also through seeds. Xcc infects through hydathodes, wounds, and rarely stomata, and is spread by rainfall. Xcc infection causes V-shaped yellowing along veins, followed by browning and black rot (Alvarez 2000). Infected cabbages lose market value and symptom development brings about complete loss of production. Therefore, control of black rot disease is important for cabbage production. No cabbage cultivar not infected by Xcc has been reported, but the level of disease resistance is different among cabbage cultivars (Williams et al. 1972).

Based on virulence in Wirosa F_1 (*B. oleracea*), Just Right Hybrid Turnip (*Brassica rapa*), Seven Top Turnip (*B. rapa*), PI 199947 (*Brassica carinata*), Florida Broad Leaf Mustard (*Brassica juncea*), and Miracle F_1 (*B. oleracea*), Xcc is classified into different races (Vicente et al. 2001), nine races having been reported (Fargier et al. 2007). Most Xcc races causing black rot in *B. oleracea* have been identified as race 1 and race 4 (Vicente et al. 2001). These races have also been isolated from cabbage plants infected by black rot in Japan (Ignatov et al. 1998).

Several studies for identification of black rot resistance genes in *B. oleracea* have been performed. Dickson and Hunter (1987) have reported that one recessive gene and two modifying genes control black rot resistance in PI 436606, a cabbage line from China. Quantitative trait locus (QTL) analysis of black rot resistance in 'Badger Inbred-16' using RFLP markers has revealed four QTLs on three linkage groups (Camargo et al. 1995). Analysis of resistance in 'Reiho' using sequence-related amplified polymorphism (SRAP) and cleaved amplified polymorphic sequence (CAPS) markers has detected two QTLs having major effects and one QTL having a minor effect (Doullah et al. 2011). Although resistance of cauliflower line SN455 from India has been reported to be determined by a recessive allele of a single gene (Jamwal and Sharma 1986), black rot resistance of most *B. oleracea* lines is considered to be controlled by at least three genes (Williams et al. 1972, Camargo et al. 1995). However, resistance genes have not been identified, probably because the number of mapped DNA markers has not been sufficient.

Recently, many DNA markers, especially single nucleotide polymorphism (SNP) markers, which are the most frequent DNA polymorphism in the genomes of living organisms, have become usable. Various techniques for detecting SNPs have been developed, but rapid, efficient techniques generally require special equipment or high running costs. Among them, the dot-blot-SNP technique developed by Shiokai et al. (2010) enables efficient analysis of SNPs at low cost and without high-priced equipment. In the present study, we constructed a linkage map of 161 new SNP markers in cabbage using this technique and analyzed QTL for black rot resistance.

Materials and Methods

Plant material and source of pathogen

A cabbage inbred line CY resistant to black rot, developed from black rot resistant cultivar 'Early Fuji' (Kaneko Seeds Co. Ltd), was crossed with a broccoli inbred line BB susceptible to black rot, derived from 'Green Dome 115' (Kaneko Seeds Co. Ltd). One F_1 plant was self-pollinated, and obtained F_2 plants were used for inoculation tests and QTL analysis.

Xanthomonas campestris pv. *campestris* (Xcc) used for inoculation tests was isolated from a black rot infected cabbage in Isesaki, Gunma in 2008. Isolated Xcc was identified to be race 1 by the method of Vicente et al. (2001) (data not shown).

Inoculation test

Inoculation tests using 140 and 142 F_2 plants were performed in October 2009 (09Au) and October 2010 (10Au), respectively. F_2 plants were grown on a 128-cell tray for one month and transplanted to an isolated field. The tests were performed in Isesaki, Gunma, Japan. The average temperature and total precipitation in a period from inoculation to recording were 17.7°C and 110 mm in 09Au test, and 17.0°C and 176 mm in 10Au test. Xcc was grown on potato sucrose agar medium for 48 h at 28°C. Xcc culture from the surface of the medium was suspended in distilled water with 0.03% spreader (Mix Power, Syngenta Japan) and the concentration was adjusted to about 10^7 cfu/ml by serial dilution method. Xcc was inoculated into plants about one month after transplanting using an engine power sprayer. The severity of the black rot symptoms was recorded by visual scale taking in account the entire plant about one month after inoculation. Disease indices were as follows: 1, less than 25% of the leaf showing black rot symptoms; 2, 25 to 49% of leaf edge having black rot symptoms; 3, 50 to 74%; 4, more than 76% of leaf edge having the black rot symptom. In 09Au test, two susceptible checks ('Wirosa F_1' and 'Miracle F_1') (Vicente *et al.* 2001) were also inoculated and both had black rot symptoms. In 09Au and 10Au tests, there was no plant without black rot symptoms.

DNA polymorphism analysis

DNA was extracted from leaves using the CTAB method (Murray and Thompson, 1980). Primers were designed from expressed sequence tag (EST) sequences of radish, which belongs to the same family as cabbage and broccoli. Polymerase chain reaction (PCR) was performed in a 20 µl reaction mixture containing about 10 ng of DNA, 0.5 mM of forward and reverse primers, 1 x *Ex Taq* buffer, 4 nmol of dNTP, and 1 unit of *Ex Taq* DNA polymerase (TAKARA BIO INC., Japan). The PCR conditions were initial denaturation at 94°C for 30 sec followed by 45 cycles of 94°C for 30 sec, 58°C for 30 sec, and 72°C for 1 min, and final extension at 72°C for 3 minutes. PCR products amplified as a single fragment were sequenced by the Sanger method and sequences were analyzed to find SNPs using SEQUENCHER software (Gene Codes Cooperation, MI, USA). The sequences having SNPs between CY and BB were used for producing probes for dot-blot-SNP analysis according to Shiokai et al. (2010). In case of SNP at a recognition site of a restriction enzyme, primer pairs were used as CAPS markers. If the sizes of PCR products were clearly different between CY and BB, primer pairs were used as sequence characterized amplified region (SCAR) markers.

Simple sequence repeat (SSR) markers (Brassica info (http://www.brassica.info/); Piquemal et al. 2005; Okazaki et al. 2007; Iniguez-Luy et al. 2009; Nagaoka et al. 2010) and CAPS markers (Okazaki et al. 2007; Nagaoka et al. 2010) were used to assign a linkage group according to the internationally agreed nomenclature of the *B. oleracea* reference linkage group.

F_2 genotyping, linkage map construction, and QTL analysis

For F_2 genotyping, PCR was performed in a 10 µl reaction mixture containing about 5 ng of DNA, 0.5 µM of forward and reverse primers, 1 x reaction buffer, 2 nmol of dNTP, and 0.5 units of KAPA Taq Extra (Nippon Genetics Co. Ltd., Japan) or HybriPol (BIOLINE, UK). Dot-blot-SNP analysis was carried out according to Shiokai et al. (2010). SCAR, CAPS, and SSR markers were electrophoresed using agarose gel or polyacrylamide gel and visualized by ethidium bromide staining. From F_2 genotyping data, a linkage map was constructed using the JoinMap 4.0 software (van Ooijen, 2006). The marker order was determined by a regression mapping algorithm and eight linkage groups were made on the basis of a minimum LOD score of 2.5. Kosambi mapping function was used to convert recombination values to genetic distances. QTL analysis was performed using QTL Cartographer ver. 2.5 by composite interval mapping (Wang et al. 2007). The 1,000 times permutation tests at 5% significant level were performed to determine LOD thresholds. LOD threshold values for 09Au and 10Au tests were 3.9 and 3.5, respectively.

Results

Inoculation test

In 09Au and 10Au, 142 and 140 F_2 plants, respectively, were inoculated with Xcc. In each test, five plants of each CY, BB and F_1 were tested simultaneously. Results of inoculation tests are shown in Fig. 1. Disease severities of F_2 plants were distributed continuously.

Linkage map construction

Out of 1,907 primer pairs designed from radish EST sequences, 690 primer pairs amplified single DNA fragments from both CY and BB, and the amplified fragments were sequenced. In 537,024 sequenced bases, SNP sites between CY and BB were 606 (1/886 bases) containing 762 SNP bases (1/704 bases) and Indel sites were 69 (1/7783 bases) containing 409 Indel bases (1/1013 bases). Polymorphic DNA fragments were 245 (35.5%). To construct a linkage map, new markers of 161 SNPs, 7 CAPS, and 2 SCAR were developed in this study (Supplementary Table 1). Nine SNP markers (Ashutosh et al. 2012), 24 SSR markers (Brassica info; Piquemal et al. 2005; Iniguez-Luy et al. 2009), and six CAPS

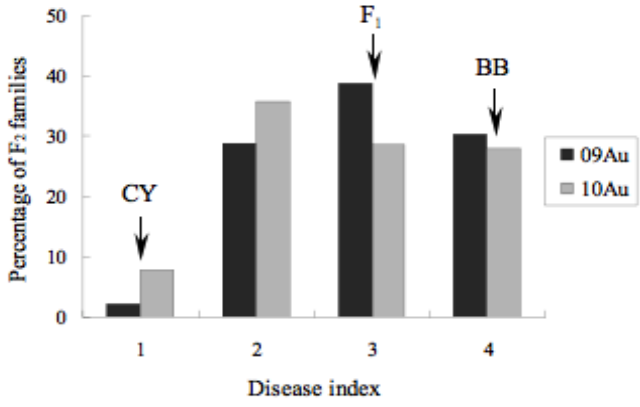

Fig. 1. Disease index distribution of F_2 families.
Black and gray bars indicate 09Au and 10Au population, respectively.

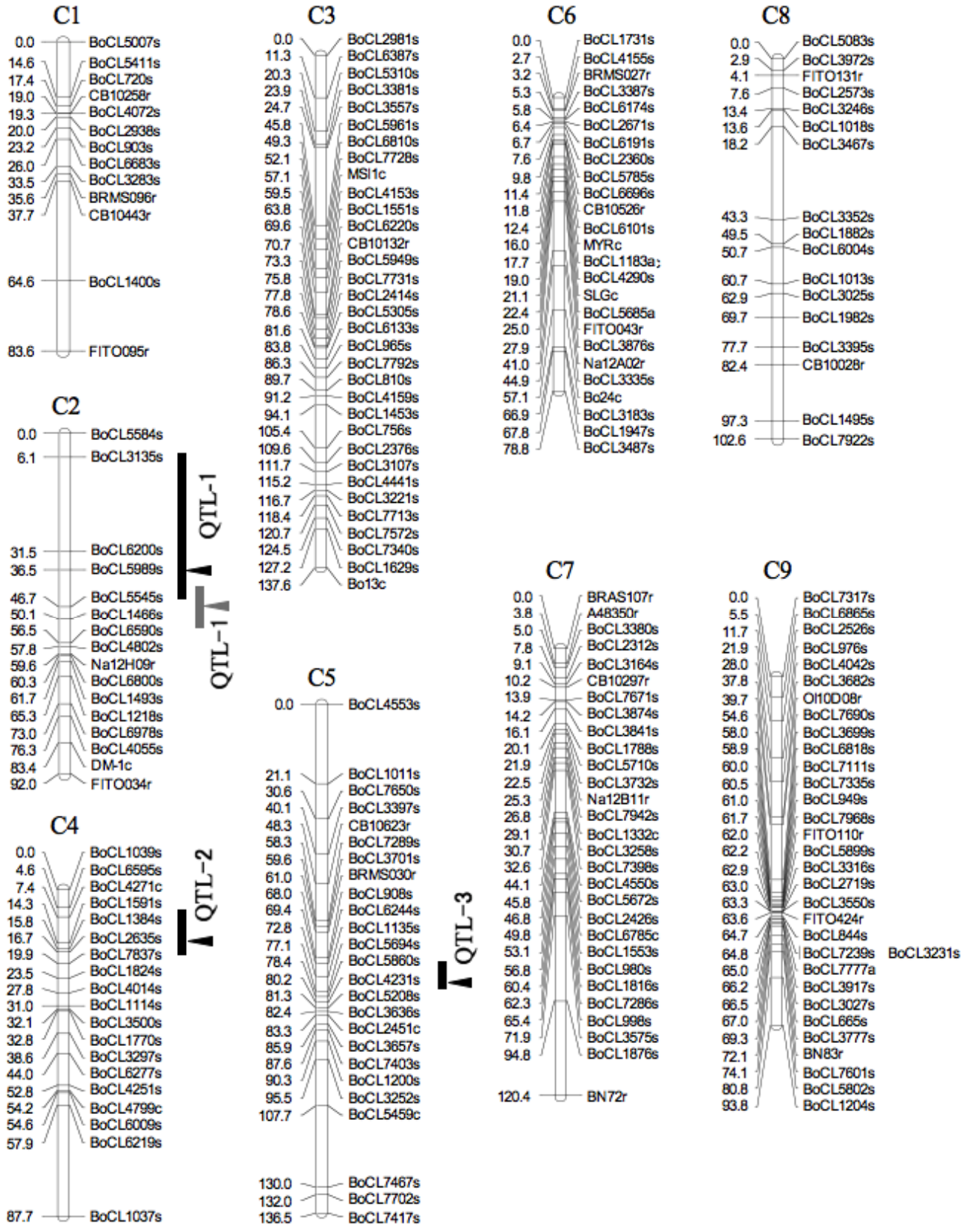

Fig. 2. Linkage map and detected QTLs for a *Brassica oleracea* F_2 population derived from a cross between CY and BB lines. Detected QTLs in 09Au and 10Au are shown by black bars and gray bar, respectively. The arrow heads indicate the peak of LOD score in the QTLs. The last letters of s, c, a, and r represent dot-blot-SNP markers, CAPS markers, SCAR markers, and SSR markers, respectively.

Table 1. Cabbage QTLs for traits related to resistance to race 1 of *Xanthomonas campestris* pv. *campestris*, position of the QTL on the map, LOD scores, additive and dominant effects, and percentage of variance explained

Inoculation group	QTL name	Linkage group	Marker interval	Nearest maker of peak LOD score	LOD[a]	Additive effect[b]	Dominance effect[b]	Variance explained (%)[c]
09Au	QTL-1	C2	BoCL3135s-BoCL5545s	BoCL5989s	6.04	-0.46	0.003	15.05
09Au	QTL-2	C4	BoCL4271s-BoCL2635s	BoCL1591s	4.86	-0.19	-0.55	12.21
09Au	QTL-3	C5	BoCL908s-BoCL5694s	BoCL1135s	5.13	-0.39	-0.26	10.77
10Au	QTL-1	C2	BoCL5989s-BoCL4802s	BoCL5545s	3.68	-0.38	0.054	9.88

[a] Peak LOD score of the QTL.

[b] Additive or dominant effect of CY allele.

[c] Percentage of variance explained at the peak of QTL.

markers (Okazaki et al. 2007; Nagaoka et al. 2010) were also used for construction of a linkage map (Fig. 2). The linkage map had nine linkage groups with a total of 209 markers. The total length was 928.7 cM and the average marker interval was 4.4 cM. The chromosome of each linkage group was determined according to Brassica info or Piquemal et al. (2005). All the linkage groups except for one were assigned to the *B. oleracea* reference linkage groups. The remaining one linkage group did not have markers corresponding to ones in the reference linkage groups. Assembling the present map with our previously reported map of *B. oleracea* (Ashutosh et al. 2012) using JoinMap 4.0 software revealed this linkage group to be C4 (Fig. 2).

QTL analysis

QTL analyses were performed using the data of disease indices of 09Au and 10Au and the genotyping data of F_2 plants. In 09Au analysis, a major QTL was detected on C2 and named QTL-1. QTL-1 had 6.04 of the maximum LOD (logarithm of the odd) score, -0.46 of the additive effect by CY, and 15.05% of variance explained (Table 1). QTL-2 and QTL-3 were detected on C4 and C5, respectively, but with smaller LOD scores, additive effects, and variances explained than those of QTL-1. In 10Au, one QTL was detected near the QTL-1 of 09Au. The regions of QTL-2 and QTL-3 showed 2.67 and 1.06 of the maximum LOD score, -0.25 and -0.31 of the additive effect, -0.26 and -0.04 of the dominance effect by CY, and 7.0% and 2.7% variance explained, respectively, in 10Au. Although these regions had higher LOD scores than other regions, they did not reach a threshold value.

Discussion

Several studies on genetics of black rot resistance in cabbage have been reported, and multiple genes have been considered to be responsible for the resistance (Camargo et al. 1995; Doullah et al. 2011). In the present study, disease severities of F_2 plants showed a continuous distribution, indicating participation of multiple genes in disease resistance, and various QTLs were detected. QTL-1 on C2 was detected in both 09Au and 10Au populations, and is considered to be a major QTL. On the other hand, QTL-2 and QTL-3 on C4 and C5, respectively, were detected in 09Au, but the LOD scores were lower than the threshold value in 10Au, suggesting that QTL-2 and QTL-3 were largely influenced by environmental factors.

QTLs for black rot resistance have been detected on LG2 and LG9 of *B. oleracea* by Doullah et al. (2011). Since Bo13 marker (=BOHM13) on LG9 of Doullah et al. (2011) was mapped on C3 in the present study, their LG9 is considered to correspond to C3. *CAM1, CO, DGAT1, GSA*, and *GA1* on LG2 of Doullah *et al.* (2011) have been mapped on O9 (=C9) (Okazaki et al. 2007). LG1 of Camargo et al. (1995), which contains QTL for black rot resistance, was regarded as LG9 by Doullah et al. (2011). QTLs on C3 and C9 were not detected in the present study, and other QTLs were found. The difference of these results is probably due to the difference of disease resistant lines used in these studies. In the present study, race 1 was used, while a used race was not described by Camargo et al. (1995) and Doullah et al. (2011). The difference of detected QTLs might be also due to difference of used races.

BoCL5989 and BoCL5545 near QTL-1 on C2 had high homology with At5g16360 and At5g22400 of *Arabidopsis thaliana* L., respectively. These sequences are on 5.3 Mb and 7.4 Mb, respectively, from the end of the short arm of chromosome 5 of *A. thaliana*. Synteny of a long region between C2 and *A. thaliana* chromosome 5 has been reported (Ashutosh et al. 2012). The region between At5g16360 and At5g22400 is a region rich in TIR-NBS-LRR family genes (Mayers et al. 2003). Analysis of TAIR (The Arabidopsis Information Resource, http://www.arabidopsis.org/index.jsp) revealed the presence of nine TIR-NBS-LRR family genes and other disease resistance-related genes in this region. *RPS4* (Gassmann et al. 1999) and *RRS1-R* (Deslandes et al. 2003), which are genes conferring resistance to bacteria of *Pseudomonas syringae* and *Ralstonia solanacearum*, respectively, belong to the TIR-NBS-LRR family. It has been reported that resistance of *A. thaliana* to Xcc is controlled by *RXC1* (*RXC4*), *RXC2*, and *RXC3* (Buell and Somerville, 1997), the latter two having been reported to be mapped on chromosome 5. *RXC2* has been located near the markers, mi138 and mi90, which are located at 7.6 Mb and 7.9 Mb, respectively, from the top of the short arm of chromosome 5 according to PHYSICAL_KAZUSA map (TAIR; mi138, http://www.arabidopsis.org/servlets/TairObject?accession=Clone:14886; mi90, http://www.arabidopsis.org/servlets/TairObject?accession=Clone:1

4986). Since BoCL5545s has homology to At5g22400 near mi138, an ortholog of *A. thaliana RXC2*, which has not been identified at the molecular level, might be contained in the QTL-1 region. BLAST search for *B. rapa* genes indicated the highest homology of BoCL5989s with KBrB068I03 on A10 and also homologies with KBrB027O09 on A7 and KBrB018H04 on A3. On the other hand, BoCL5545s had the highest homology to KBrH065B20 on A2. Genome rearrangement might have occurred in this region after divergence of *B. oleracea* and *B. rapa*. It is considered to be difficult to use the *B. rapa* genome information for identification of a candidate gene for Xcc resistance in *B. oleracea*.

B. oleracea lines resistant to black rot disease have been selected in the field infected by Xcc or by inoculation tests. However, disease severity depends on environmental factors and plant conditions. Use of DNA markers enables reliable selection of resistant plants even at the seedling stage. DNA marker-assisted selection became popular in breeding of crops, in which marker information is rich. In tomato breeding, DNA markers for disease resistance determined by a single gene are commonly used (Barone and Frusciante 2007). Since the selection for disease resistance controlled by multiple genes requires a larger field, longer time, and higher breeding cost than selection for that controlled by a single gene, development of DNA markers is especially important. Recently, techniques for genotyping of DNA markers have rapidly advanced. For example, SNP genotyping, which had been costly or laborious, can be performed rapidly without high cost. Further analysis of the three QTLs identified in the present study will enable development of SNP markers useful in *B. oleracea* breeding for black rot resistance.

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Supplementary Data

Supplementary Table 1. Dot-blot-SNP, CAPS, and SCAR markers newly developed in this study

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Supplementary Table 1. Dot-blot-SNP, CAPS, and SCAR markers newly developed in this study

SNPs markers	Primer sequence (5'-3')[a]	Genotype	Probe sequence[b]	Hybridization and washing condition	
				Temperature (°C)	SSC[c]
BoCL665s	TTGAAGAACCAGGAGTTGAAGG	BB	GAAGAAGAGAACCAAAA	40	0.1
	GTCTTGCTCTCCCTTTCCCATT	CY	GAAGAAGATAACCAAAA	40	0.1
BoCL720s	CAAAAAGGAAGATCTGGTGCAG	BB	CTGAAGCATTTTTGTTA	40	0.2
	GGAACATGCCCATTATCAGACA	CY	CTGAAGCACTTTTGTTA	40	0.1
BoCL756s	CAACCAGAAGGATGAAATCACG	BB	GCGGATCCAAACGCAAT	40	0.1
	CAAGAGCCTGAGCAAGAAAACA	CY	GCGGATCCGAACGCAAT	40	0.1
BoCL810s	CAAGCACACAAGAACAGACCAA	BB	AGCAGAAGCACTTGGTC	40	0.2
	ACGACCACGGTCACTGAGAATA	CY	AGCAGAAGAACTTGGTC	40	0.2
BoCL844s	CTCTGCAAGTAATCGTGCATCC	BB	ACACTTCCTTACACAAG	40	0.5
	TCGAGCTCACTATCGATCAAGC	CY	ACACTTCCATACACAAG	40	0.5
BoCL903s	ACGGCTCTTCGGGAACATATAC	BB	CTCGCATGCAACGGTTT	40	0.2
	CTCTCTCTCACTGTCGGCAAAA	CY	CTCGCATGTAACGGTTT	40	0.2
BoCL908s	CGTTTTAACTGTTCAGGCGACA	BB	CTTCACCAGAAGCACGA	50	0.1
	GGATCGGTGAAGCTTTTGGA	CY	CTTCACCATAAGCACGA	50	0.1
BoCL949s	GCTCTGTACATGGAGCAACTGA	BB	CTCAAAGATGTGATGAA	40	0.2
	ATGACAATGGCACCAAAGCA	CY	CTCAAAGACGTGATGAA	40	0.2
BoCL965s	TGGAGAGACAGCAAGAAACCAA	BB	GCATTGTCTGGTGAAGA	40	0.1
	AGCCACATGAAATGCTTAGCTG	CY	GCATTGTCGGGTGAAGA	40	0.1
BoCL976s	CTTTAGCCAGCTTCCCATTTTC	BB	AGCTTCTAACAGAACTA	40	0.5

	CCTTCACGCTCCTCATTCTCTT	CY	AGCTTCTACCAGAACTA	40	0.5
BoCL980s	TGGAACTCCACGTACAAGATCA	BB	GCAACTATTTTCAGTCT	40	0.1
	AATTGAAGGTCACGTGATGGAG	CY	GCAACTATCTTCAGTCT	40	0.1
BoCL998s	TGGCCACAGTGTTGGTTCTATT	BB	AAAGACCAAGAGGAATC	40	0.2
	TACCGGAGAAAGCACACTTCTG	CY	AAAGACCATGAGGAATC	40	0.2
BoCL1011s	GAAGCCCTAAAAGCCGATCTCT	BB	GAGTACACATGTGGTCA	40	0.1
	CTTGAGAACAACCGCAAATACG	CY	GAGTACACGTGTGGTCA	40	0.1
BoCL1013s	AAAGAGAACGGAGACGAGGTTG	BB	TGATAGTGGTGATGATG	40	0.1
	GAAAGCCAAAGAAGCTGGTGAT	CY	TGATAGTGGGGATGATG	50	0.1
BoCL1018s	CGTCCACTGACTTTGACGATGT	BB	AATCTACACCTGAAACC	40	0.5
	ATATAACACGGGCCTCATTGCT	CY	AATCTACAGCTGAAACC	40	0.5
BoCL1037s	CAGCATGGAAAATATGGGGAAC	BB	AGAGAGAGTGTGGTTAA	40	0.5
	AAAAGCATCTACTCGGCTCCA	CY	AGAGAGAGCGTGGTTAA	40	0.5
BoCL1039s	TCTCCGCTGGGTTATAGGGTTA	BB	CTCCTTCTTCTTCTTCT	40	0.5
	CATCGGGTTCCAGAGATTCTTC	CY	CTCCTTCTCCTTCTTCT	40	0.2
BoCL1114s	AGCCGTCATGGGTTTTCTACAG	BB	ACTATAAAAGGAATGTA	40	1
	TGGGACACTGAAACGAAGAAGA	CY	ACTATAAAGGGAATGTA	40	1
BoCL1135s	TACAAGTACCGGCCATAGGTGA	BB	TTCATATTTGAACGGCT	40	0.1
	GCATGCTGAAAGATTCTCTGTG	CY	TTCATATTGGAACGGCT	40	0.5
BoCL1200s	CCCTTCCTCAGAGTTGGTTTTG	BB	GTTTTCTTACCAGAAAC	40	0.1
	GATGATGTCTTCGCCGATGTTA	CY	GTTTTCTTGCCAGAAAC	40	0.1
BoCL1204s	TCCCAAATCTCCTTACGAGTGG	BB	CCTTCTGTCGATTTCAG	40	0.1
	GCAAAGCACACAACAGAGGAAC	CY	CCTTCTGTGGATTTCAG	40	0.1

BoCL1218s	CGGTCATCAATACGCTCATCAT	BB	CTCACAGAAGTGACTGT	40	0.2
	ATGTGCTCGTTGACGATTCAGT	CY	CTCACAGAGGTGACTGT	40	0.2
BoCL1384s	GAAGAACAAAGTGGCGGCTATT	BB	CAGCGTCGGATTGTTAG	50	0.1
	CATGGTTGATGGCTTCATACG	CY	CAGCGTCGTATTGTTAG	40	0.5
BoCL1400s	CTAGAACGGCTGGCTGATGATA	BB	CAATGGTTACTATGGTA	40	0.1
	ACCATGAAAGGGTTCGAGTGTT	CY	CAATGGTTGCTATGGTA	40	0.1
BoCL1453s	GAATTGCAGCCGTCAGATAACA	BB	GAAGCTATGGACGAGAT	40	0.2
	GGGACCAATGGCGATAAGTAGT	CY	GAAGCTATTGACGAGAT	40	0.2
BoCL1466s	AGGTCGGTTTCTGAGGAAGATG	BB	CGATTAACGTTGAGGAT	40	1
	ACCCATCAGAGATTGCAAGACA	CY	CGATTAACATTGAGGAT	40	1
BoCL1493s	CGTGTTCATGTGTCTTGCCATA	BB	GAAAAAAAAATCAAGAA	40	0.5
	AAGACGGAGAGTGGGTTAACGA	CY	GAAAACAAAATTAAGAA	40	0.5
BoCL1495s	CATGGACGATCCATACTCATCA	BB	CAGGGACTATTGTCATC	40	0.5
	TTGCCATTACAGGCTTCACATC	CY	CAGGGACTGTTGTCATC	40	0.5
BoCL1551s	GGAGGAAGACGTATTGGTTTCG	BB	GAGGATAGTATGGCGGA	50	0.1
	TTATTTCAAGCAACGGGGAGAG	CY	GAGGATAGCATGGCGGA	50	0.1
BoCL1553s	AACCCTTTGGTGTTATGCATCC	BB	CTTACCGTCGCTGTTCA	55	0.1
	TGGCAACTCCCCAAGATAAACT	CY	CTTACCGTTGCTGTTCA	55	0.1
BoCL1591s	TTCCTTCACCCCTCCACAAT	BB	TATGTCACCGTTTTGAC	40	0.5
	CGGTGCAGTAGACAAGGATGAA	CY	TATGTCACTGTTTTGAC	40	0.5
BoCL1629s	CCTGCTTTTTCTCCTCACTGGT	BB	TCTGGTACAGTTTCGGT	40	0.5
	CATTCAAACTCCGTGGTTCAAG	CY	TCTGGTACCGTTTCGGT	40	0.2
BoCL1731s	AAAGGAGGAGATGGACTGGTGA	BB	AAATCTCTCAAGAATTG	40	0.5

	GATTACACCGCCAATGAAACG	CY	AAATCTCTTAAGAATTG	40	0.5
BoCL1770s	GCTTCCTTTCACATGCTCCTCT	BB	ATGACGATCTGCATGAT	55	0.2
	CCTGGAATCGTGCTTGATGTT	CY	ATGACGATATGCATGAT	45	0.2
BoCL1788s	GCTGCTGATCCAAAGAAAGGTT	BB	CAAGGTCGTTCGGACCA	50	0.1
	GGACATCAAACATACCCAAGCA	CY	CAAGGTCGCTCGGACCA	50	0.1
BoCL1816s	TGCTCGAGCTGCTACTATTGCT	BB	GCAAGTGGGTTGAACAC	50	0.1
	CAAGGGCCTATATTCGAGGATG	CY	GCAAGTGGATTGAACAC	50	0.1
BoCL1824s	GGAACTTCCCTCGAGAGTCAAA	BB	AAGATTGTGAAGCTCGA	40	0.1
	AAACTTCAGTTCAGGGCATGG	CY	AAGATTGTTAAGCTCGA	40	0.1
BoCL1876s	AAGCTCTTTGTCGGGATGATTC	BB	GTTTTGTTCTATCGTTC	40	0.1
	AAAATCCCTACATCGGAGAGCA	CY	GTTTTGTTATATCGTTC	40	0.1
BoCL1882s	AAGCGGTGAAGATTGGTATCGT	BB	GGACGGCCATGGAAACC	40	0.1
	TCCCAAAATGCCTAGAACCCTA	CY	GGACGGCCGTGGAAACC	40	0.1
BoCL1947s	GATTGACGAGAACCGTACTGGA	BB	AGATTCTCAGGTACTCA	40	0.1
	CTCGATCGGATGGTACAAACAA	CY	AGATTCTCCGGTACTCA	40	0.1
BoCL1982s	CTTTTTCCCAGTGAAAGCTTGG	BB	AGAGCTACCACTTGCTA	55	0.1
	AAGTTGTGCCTGAACCTGAACC	CY	AGAGCTACTACTTGCTA	50	0.1
BoCL2312s	GCTGGGGTAGGATCATCAAGAA	BB	GCATATGCAGTGCAGAA	50	0.1
	GGTAGATCCCAACTCCGTTTTG	CY	GCATATGCGGTGCAGAA	50	0.1
BoCL2360s	CATCAGCAGCTTGATTCTCCAG	BB	GAAGTTGAGTATCAACT	40	0.5
	CAATGGAAGTGGAAGGGAGAGT	CY	GAAGTTGATTATCAACT	40	0.5
BoCL2376s	GATACCTTGCCTTCCTTGGAGA	BB	TTCCACGGCTTCTTGAT	50	0.2
	CTACTCGTTTCTTTCGCAATGG	CY	TTCCACGGTTTCTTGAT	40	0.2

BoCL2414s	TGCTTCAGGGAGATGCTTGATA	BB	TCGAACCCATCTCATGG	40	0.1
	CATCCATAGCGGATCAACGA	CY	TCGAACCCGTCTCATGG	50	0.2
BoCL2426s	TTGTCCAGAGCATCTTTTGCAG	BB	ACTATGTCTGCTAGAGA	40	0.2
	TATCCATTACATTCGCGTGGTC	CY	ACTATGTCAGCTAGAGA	40	0.5
BoCL2526s	CGACACCATTTGCAGATAAAGC	BB	TTGGGATGTAAGCAAGC	40	0.1
	CAAACAACCAGAGAGCGAGAGA	CY	TTGGGATGCGAGCAAGC	40	0.1
BoCL2573s	CCAGAGAGCATCGCTAAATCCT	BB	TCGCTTCCATCGCCGCG	50	0.1
	AGTTTAACGGACGAGCGAGAAG	CY	TCGCTTCCGTCGCCGCG	50	0.1
BoCL2635s	AAAGGATGAGGACCATGCAACT	BB	GTGGTTCTACCAATGGA	40	0.1
	CTTTACCCACACGTGCATCATT	CY	GTGGTTCTGCCAATGGA	40	0.1
BoCL2671s	AATGCAAACACTCTGCGTCATC	BB	TCCATCACTCCACACAA	50	0.2
	TTGTCCTGAAACACGTCGAACT	CY	TCCATCACACCACACAA	50	0.2
BoCL2719s	GACATTGTTGGGAGACGACTTG	BB	CCATCCCCTTAACCTCT	40	0.1
	TACAACATTGCACCCAACTGC	CY	CCATCCCCGTAACCTCT	40	0.1
BoCL2938s	TACGTTCCCATGATGAACCAAC	BB	CCGTTTTGATAACCCAA	40	0.1
	CTGCAGAGAAGACGGTGTCATT	CY	CCGTTTTGCTAACCCAA	40	0.1
BoCL2981s	CCGAAGCTCAAAAAGCTTCATC	BB	ACCGGAACGGTGGCTCA	40	0.1
	CGTTGTGCGTTAGGAGAAGAGA	CY	ACCGGAACAGTGGCTCA	40	0.1
BoCL3025s	CCCAATTGCATCGTGAAGAAG	BB	TCCAGACACGTACTTAT	40	0.5
	CCATCACACCACCCCAATTA	CY	TCCAGACACATACTTAT	40	0.2
BoCL3027s	AGAGGAAGTGGATCCAAACGAG	BB	AAGAAAGATGAGGATGT	40	0.5
	TTTTCCTCAGGCTCATCCTTCT	CY	AAGAAAGAAGAGGATGT	40	0.5
BoCL3107s	TGGACGGATTGACTATGGAGAA	BB	TACATATCTGTCTTTGG	40	0.5

	AAACCCAAAAGAGGGTCAAAGC	CY	TACATATCAGTCTTTGG	40	0.5
BoCL3135s	GTGTTCTCCGTATTGCCACATT	BB	TTGTTTGAATAACCAAT	40	1
	CAGCTTGTCTCTCTTCCGTTTC	CY	TTGTTTGAGTAACCAAT	40	1
BoCL3164s	AATGAGGCGAAGAGAGCAAGAC	BB	GTCCATAATCTTTCGTT	40	0.2
	TTGCTGTGCACATACACAAACC	CY	GTCCATAACCTTTCGTT	40	0.2
BoCL3183s	TCCTGAACGTCCAGAACAAGAA	BB	GATGAAGAGGAAGAAGT	40	0.1
	GACAAGGCATTGTGAAGGAAAG	CY	GATGAAGAAGAAGAAGT	40	0.1
BoCL3221s	AGATGGCAAGTCTCCTTCCAAA	BB	AAATTCAAAAAGTTTAG	40	1
	GTGAACGTCAAGGAAGTTGTGG	CY	AAATTCAAGAAGTTTAG	40	1
BoCL3231s	GAAGAAGAAAGGACCCATCGTG	BB	TCACCGTTCGATCTCAT	40	0.1
	TCCATTGATCGTAGTCCACTCA	CY	TCACCGTTAGATCTCAT	40	0.1
BoCL3246s	TGAAGCAATATAGACCGGTTCG	BB	CTGGCCCACCTTCAAAG	50	0.1
	AGTCAGAAGGGACTTTGCCATC	CY	CTGGCCCATCTTCAAAG	50	0.1
BoCL3252s	ATAAACCCTAAATCCGGGAGGA	BB	TGCGTTGGGGTCTTAGA	50	0.1
	CTTCCATGATCCCTGGAAAGAC	CY	TGCGTTGGAGTCTTAGA	50	0.1
BoCL3258s	CTCTGGTCTCGGATTTGGTTTC	BB	GCGATCTTAGTTAGTCA	55	0.1
	TCGAGATGTATTCCGATCGTGT	CY	GCGATCTTGGTTAGTCA	50	0.1
BoCL3283s	TGTATCGGTTGAGTTGGGTAGG	BB	TTGGTAGCTATCCCTTT	40	0.2
	TTCTGGACACTCACTCCAGGTT	CY	TTGGTAGCCATCCCTTT	40	0.2
BoCL3297s	ATAGCGAGAGCGCAAGAGAGAT	BB	GTTCCTGTCTCTCTGTT	45	0.5
	ATCAGCTGCATTTCTGCAAGAC	CY	GTTCCTGTATCTCTGTT	45	1
BoCL3316s	AGAAGGTGAGGAACTGCAGAAA	BB	CTCCAGTTAGCATATAG	40	0.1
	TGTTTGCCAGCAACAAAAGC	CY	CTCCAGTTCGCATATAG	40	0.1

BoCL3335s	ACACAGACAAAGCAAAGGCAAG	BB	ACCAGACCGAAGAGATA	40	0.2
	CATTAGAGGCAACGGGAAGAAC	CY	ACCAGACCAAAGAGATA	40	0.2
BoCL3352s	AAAACCGGGATTGAGTTGACAG	BB	GAAGTAGATGAGGCCGG	50	0.1
	TTCCTCGTCGATACAAGTTTGC	CY	GAAGTAGACGAGGCCGG	55	0.1
BoCL3380s	GAATGGGTGTAACATCCGTGTG	BB	AAGAAAAACGCAGCTCT	40	0.2
	TGACTTCGGAGGCTGATACAAA	CY	AAGAAAAATGCAGCTCT	40	0.2
BoCL3381s	ACATATGGCACAGATCGACAGG	BB	GATTATGATTTACCTT	40	0.2
	TTTGCCTTCCACTTATGGGTTC	CY	GATTATGATTTTACCTT	40	0.2
BoCL3387s	GGTAAGAAGGCGACAGCTTTTC	BB	CTATCGATGCGTGGACC	50	0.1
	GCTGGAGTGACAACTGACTGAA	CY	CTATCGATCCGTGGACC	50	0.1
BoCL3395s	GGTTTCAGTTCCAAGGCAATTC	BB	GTGGCCTTTGTGTTGTT	55	0.1
	GTAACATAAAGCCGGGCCATAA	CY	GTGGCCTTGTGTTGTT	50	0.1
BoCL3397s	GACTGTGAAAGCGCAGATATGG	BB	TGAGAATGTAAGCAGGT	40	0.2
	TGATAAGCCCCTTTCAGGAAGA	CY	TGACAATGTTAGCCGGT	40	0.1
BoCL3467s	CATGATCCTCACTATCGCTGCT	BB	AGCTCTGGAGCTGGGAT	55	0.1
	ACAGCCGATATCAAAGCCGTAT	CY	AGCTCTGGTGCTGGGAT	55	0.1
BoCL3487s	AACCGGTAGCGAAGTTCATCAT	BB	TGGAACCATCATGTGGG	55	0.1
	ACTGATGAGAAGCCGAGTCAGA	CY	TGGAACCACCATGTGGG	50	0.1
BoCL3500s	GCAACATGATTCGTGGTTTAGC	BB	TGTCGCACTTGTTTAAA	40	0.1
	GAAGAGAGTCAACACGCGAGAA	CY	TGTCGCACGTGTTTAAA	40	0.1
BoCL3550s	TCTCATCGCTCCACTCTCTCAT	BB	ACATGTTCTGTTGCTAC	50	0.2
	CTTCATGACGTCTCGGTAGCAA	CY	ACATGTTCCGTTGCTAC	50	0.2
BoCL3557s	GAAGGCGAGAAGGGAAGCTTAT	BB	TTCGATGTGTATATCCA	40	0.5

	AACCTCCAGGGATGATAGCAAG	CY	TTCGATGTTTATATCCA	40	0.2
BoCL3575s	AATCAGCGACCAGAGATCATCA	BB	TGATCCTGCCACTCTAC	50	0.2
	TCACTAGCTTGCCTGAAAGTGG	CY	TGATCCTGTCACTCTAC	40	0.2
BoCL3636s	CCATCAGCGAGATAAGCTCCAT	BB	TAGGACCCATTAATTAC	40	0.5
	TCATCTTCGTTGATGACGGAGT	CY	TAGGACCCGTTAATTAC	40	0.2
BoCL3657s	TATGGAGTTTCAACGGATGCAC	BB	AGCTACTTGAGCATCTG	40	0.2
	GTGGGTAACATTCACGTGCTTT	CY	AGCTACTTAAGCATCTG	40	0.2
BoCL3682s	ATCCCCTTCCTTCATCTGAGTG	BB	GGTCTTCTTTCCATAGG	40	0.1
	CACCAGGTACACGTCATCATCA	CY	GGTCTTCTCTCCATAGG	40	0.1
BoCL3699s	ATAAAGCTGACCAGATGGGAGA	BB	CGAGATCCGTTGTTGTT	40	0.5
	GTACATGGAAAGCATGCAACAG	CY	CGAGATCCATTGTTGTT	40	0.2
BoCL3701s	AGAATGCCTAGGGTCAGATTCG	BB	GGTCCGTATACTCACCA	40	0.1
	GTTGGAAGGCAACAAAATGG	CY	GGTCCGTAAACTCACCA	40	0.1
BoCL3732s	CAATGGAGCTGTTGCTGATTCT	BB	CTCATGGATGTCTTGTT	40	0.5
	TAGTGACAGCAAGTGCAGCAGA	CY	CTCATGGACGTCTTGTT	40	0.5
BoCL3777s	TAGGACTTCGTGCTGCAGATTC	BB	TGATTACAATAGAAGGA	40	0.5
	ATGGTGAGTGCACCACTCTGAT	CY	TGATTACAGTAGAAGGA	40	0.5
BoCL3841s	CGGTTGGTTATGTCGCATGTAT	BB	TCACTTCACAAGGATAG	40	0.2
	TGTGGTCGTGGTGAGATCTTTT	CY	TCACTTCAAAAGGATAG	40	0.2
BoCL3874s	ACGGGAAGCCAGTTTCAAGA	BB	AGAAAAAAAATTGTTCT	40	1
	TAACGAAAACCAGAGGATCAGC	CY	AGAAAAAAGATTGTTCT	40	1
BoCL3876s	CGCACAAGGAGGGAGATACTTT	BB	TGAAGCCGGCATCACTT	55	0.5
	CGGCTTTCCAATGTAACCTCTT	CY	TGAAGCCGCCATCACTT	45	0.5

BoCL3917s	GGGCCTAACGTTCAGTGGAATA	BB	GAATGGAGTTATCATGG	40	0.5
	AAGCCACCAACACATGTACGTT	CY	GAATGGAGGTATCATGG	40	0.2
BoCL3972s	CGCTATAGCTTGCGGTTACACA	BB	CTCTTCTCGCTGCAATT	40	0.1
	TTTACACAACACGGCAAGAAGC	CY	CTCTTCTCACTGCAATT	40	0.1
BoCL4014s	GCTCGTGAGTTGCTGAAACTTG	BB	AAAGATGTAGCGACCAG	40	0.5
	TGGTAGAACCACCAACAAGGAA	CY	AAAGATGTCGCGACCAG	40	0.5
BoCL4042s	AGGAGGAAGAAGCCAAGACTGA	BB	AAGAAGAATCCGAAATG	40	0.2
	GATGCAAGTTTCTGGGGAAAAC	CY	AAGAAGAAACCGAAATG	40	1
BoCL4055s	GATTAACATGGCGGCTTGTCTT	BB	GGAGGCGGGTTCGCCAA	50	0.2
	CAAAGCCGAGATCAGTGAGAAG	CY	GGAGGCGGATTCGCCAA	50	0.2
BoCL4072s	TCTTTGACGCCTCAGTGATTTG	BB	ATCTCCAAAATTCATAT	40	0.5
	AGCTAGAAGACGGGACAACCTT	CY	ATCTCCAAGATTCATAT	40	0.1
BoCL4153s	TAAACGGAGCGTCACGAGACTA	BB	TGGAACTACAATTACGG	40	0.2
	TTGCAACCTTACATGTGTGTGC	CY	TGGAACTATAATTACGG	40	0.2
BoCL4155s	GCTAAATCGAGCAAAGCTGGTT	BB	AGGAAGACGTAAAGGAC	40	0.2
	GCATTTCTTCCCAGTTTCTTGG	CY	AGGAAGACATAAAGGAC	40	0.2
BoCL4159s	TGGTGTTGGAAGTGTTCTTTGC	BB	TTTGTATTGTCGTTGAT	40	0.1
	ATGTTGTCTCCAGTTCGACCAA	CY	TTTGTATTTTCGTTGAT	40	0.2
BoCL4231s	GGCTCGTGATCAACAGTCATCT	BB	CTCACCATCGCTGTTTT	40	0.1
	GAGCTTCTGTTGCTTCGGTTCT	CY	CTCACCATTGCTGTTTT	40	0.1
BoCL4251s	TGCGTAAAGCAGGATACAATGG	BB	TTGCACCATCAAACTCC	40	0.2
	GTTGCGTTTTCAGAGAATGGTG	CY	TTGCACCACCAAACTCC	40	0.2
BoCL4290s	CAGAGTCGCTAACCCTTTGACA	BB	ATCGAAGGTTTTGATTG	40	0.5

	ACCCGAGAAAACTGCGTACTTC	CY	ATCGAAGGGTTTGATTG	40	0.5
BoCL4441s	GGAAAGGACACGACTTTGAGGT	BB	AGGTGAAGTAATGGAGA	45	0.2
	AGACTCCGCTTCTCATCTTTCC	CY	AGGTGAAGCGATGGAGA	45	0.5
BoCL4550s	CACATCCATAGCTCTCGAAGGA	BB	CAAATCAGCGAGACAGA	40	0.5
	TGTTCTCCACCGTCTACCTTTG	CY	CAAATCAGTGAGACAGA	40	0.1
BoCL4553s	TAGGGATGACTATGACCGAGCA	BB	TGGTCAATGATGCAGCA	40	0.5
	CTTTTCCTGGAGGGATGACAAC	CY	TGGTCAATAATGCAGCA	40	0.5
BoCL4802s	AAAGAAGGGCTGCAAGAAGATG	BB	AGAGTAAGTGACTGTAA	40	0.2
	GCTTGAGCAGCAATCAAATCAG	CY	AGAGTAAGAGACTGTAA	40	0.2
BoCL5007s	GTGTGTCGGCTGTGGAAATAAA	BB	AAGGTAGCCTGTGCGGA	40	0.1
	ATCCTGCAATTAGGTTCGTGGT	CY	AAGGTAGCATGTGCGGA	40	0.1
BoCL5083s	ACGGAGTTTGAGGAACAGAAGG	BB	GATGATTATAGATCCAT	40	0.5
	TCCTTCCGAGAATGCCTAACTC	CY	GATGATTACAGATCCAT	40	0.5
BoCL5208s	GACGCAAATGTAAGACGGGTTT	BB	ACCACAAACGGAGTCAC	40	0.2
	TACTGCTATCAAACACCGTTGG	CY	ACCACAAATGGAGTCAC	40	0.1
BoCL5305s	GAAGAGGATGAGGCTTTTTGGA	BB	GATCACTTCTTTAAGAA	40	0.5
	TCAGGAACCCTTGACAAAAGAC	CY	GATCACTTATTTAAGAA	40	0.5
BoCL5310s	CAACGAGAATCCAGATGCTGAG	BB	GTCGCTGACGCTCTCTT	50	0.5
	TTCAAGACCAGTCCCATAAGCA	CY	GTCGCTGATGCTCTCTT	50	0.5
BoCL5411s	GGGCAGAACTGGTGTTCTGTAA	BB	TTATTCTCCGAGTTTTG	40	0.5
	CAACAAACACAAGGTTGGAAGC	CY	TTATTCTCTGAGTTTTG	40	0.5
BoCL5545s	TACGCGGTTCAAGTGATGAACT	BB	GTCCAAGCAGCAGTGAG	50	0.1
	TGCTCTGCTCCTTTGTCTTCAC	CY	GTCCAAGCGGCAGTGAG	40	0.1

BoCL5584s	CAAGAGCACAATCTCGGTCCTA	BB	GGTACCACACAGGAGAA	40	0.1
	ATGACACGCGTTTACACTCTGC	CY	GGTACCACTCAGGAGAA	40	0.1
BoCL5672s	AGATGGATATGGGGATCAATCG	BB	TTTGGTTCTTGTTACCT	40	1
	CCCCAAACATAATAAGCCAAGC	CY	TTTGGTTCGTGTTACCT	40	1
BoCL5694s	GCCGGCAAGTAAGAGATCAAAG	BB	TGGGAAAAGAATAGCTT	40	0.5
	GCAAAGGCTATAAGCCAGCAGA	CY	TGGGAAGAGGATAGCTT	40	0.2
BoCL5710s	CAAGGCATGTCCGTAACGTAAG	BB	TTAGTTGAGTTTAACGT	40	0.2
	GGGTCTCGCATTTACATACACG	CY	TTAGTTGAATTTAACGT	40	0.5
BoCL5785s	AGATTGTGATGTGGGCTGAGAA	BB	GATGTTAAGGGCTCTGG	40	1
	TCTCGTTTAGCAACTCCACTGC	CY	GATGTTAAAGGCTCTGG	40	1
BoCL5802s	AAGAGCAAGACTCACCAAGACG	BB	AGGACATGAACTTATCC	40	0.2
	GCTTTCACCAACATTGTTCACG	CY	AGGACATCAGCTTATCC	40	1
BoCL5860s	GCGTGTGGTGCATCAAGATACT	BB	GAGGAACCGCGGTAACG	40	0.1
	TGTCTCCACAAAGCTCCCTTTT	CY	GAGGAACCTCGGTAACG	40	0.1
BoCL5899s	TCTACGACATTGGACCTCAGGA	BB	GCTTTGTTCCCAGAGAA	40	0.1
	TACAGAGGAGGGAACCATGTGA	CY	GCTTTGTTTCCAGAGAA	40	0.1
BoCL5949s	TGGAGAAACCGAAGAAGAGGAC	BB	GTGTGTGTGTGTTTATT	40	0.1
	AGGTGAAATGCGAAGGTGAATC	CY	GTGTGTGTATGTTTATT	40	0.2
BoCL5961s	ACAGCTACGGCTACCATGATGA	BB	GGTTCTGGCTCTAGTTC	50	0.1
	TGGAAGTGGGTGGTAGCTTTTT	CY	GGTTCTGGTTCTAGTTC	50	0.1
BoCL5989s	TCGGTGAGTACCATCTCTTGGA	BB	TGCTTCAAAGAGTGCTC	40	0.2
	TCGACGTCTGATTTCCCTTGTA	CY	TGCTTCAACGAGTGCTC	40	0.2
BoCL6004s	AAGAGACAAGCCCACGAATCAT	BB	AAGTAAGGAAAGAGGAG	40	0.5

	GTCCTAAAGACCCATCGCAATC	CY	AAGTAAGGGAAGAGGAG	40	0.5
BoCL6009s	TGTGAGCAAGGTTACCGTCTTG	BB	ACCTGGTTGCTAGATAA	40	0.5
	TTACCATGGCTTCCTCATCTTG	CY	AACTGGTTACTAGATAA	50	0.5
BoCL6101s	CACTTCAAGAATCCAGCCAAGA	BB	TTTAATTATTCGTTCTA	40	1
	GAGCAACGCAAAAGTCAATCAC	CY	TTTAATTAGTCGTTCTA	40	1
BoCL6191s	TAGGATTGGCTGGTCAACAAGA	BB	CTGGAAGCTTTTGAAGT	40	0.1
	ACCATGGTTGGTTTGTCAACTG	CY	CTGGAAGCGTTTGAAGT	40	0.1
BoCL6200s	GGTTGGAAAGCAATTGGTGAAC	BB	AGAAGGAATGAGAAGTC	45	0.5
	GGTTCGACACACAAAGAAACCA	CY	AGAAGGAACGAGAAGTC	55	0.5
BoCL6133s	CGAACTGAATCAGCATCAAAGG	BB	TCTCAGCTCTGTTCACG	50	0.1
	GGAGCCCTTTTACCTCATCAAA	CY	TCTCAGCTATGTTCACG	55	0.1
BoCL6174s	ACAAGGGCTTTCTAATGGCTGA	BB	GGATGCTTAGACAACGG	40	0.2
	AGTGCTTCAACTTGCTCAGGTG	CY	GGATGCTTGGACAACGG	40	0.2
BoCL6219s	GAGAAACAAGGCATGTCACCAG	BB	GTGATGTTTGGCGAGAT	40	0.1
	AATGGGCCAGCAACAATAACTC	CY	GTGATGTTAGGCGAGAT	40	0.1
BoCL6220s	GCAAGGGGATAGCAAAGAGACT	BB	CTTCAAGTCCAAGGCAA	40	0.5
	TTTAAGACCACAAGCGCCACTA	CY	CTTCAAGTTCAAGGCAA	40	0.5
BoCL6277s	CCGATATGGTGGAGATGGTACT	BB	ATACTGCTCTTTGTCTT	45	0.5
	CAACGTCCAAAACACACTATGC	CY	ATACTGCTGTTTGTCTT	45	1
BoCL6244s	CTATGTCGATAATGCCGGTGAA	BB	GGTAACCCGCTCACCTG	40	0.1
	TGTGATCTTAACGGCGATGGT	CY	GGTAACCCACTCACCTG	40	0.1
BoCL6387s	TTGATGCGCTTAAAGGTGGTC	BB	TGGCAGGCGGCTACAAG	55	0.5
	CCCTGATCTCTTCTGTTGCTTC	CY	TGGCAGGCAGCTACAAG	55	0.1

BoCL6590s	GTCTTCATTGGAGCCTCTGGAT	BB	AGTAAAGCCTACATTTT	45	1
	ACCGAGGCTCTTTCTTCTATCG	CY	AGTAAAGCATACATTTT	45	0.5
BoCL6595s	ATGCTCACCAAAGGAGACATCA	BB	GTGTTAGTTGTTGGTTA	40	0.1
	CGGGAGATTCACAATGGAAAG	CY	GTGTTAGTGGTTGGTTA	40	0.1
BoCL6683s	GAAGAAAGTCGAAATGCGTGTG	BB	TTGCCAAACCTAAACAG	40	1
	GATTCCACGCAAACTCTCAATG	CY	TTGCCAAAGCTAAACAG	40	1
BoCL6696s	TTGCGGGTCTTCTTGAAGGTAT	BB	GCTTGAGTGTGAAGAAA	40	0.2
	CTGTGTTCCTCACTGCACACAA	CY	GCTTGAGTATGAAGAAA	40	0.5
BoCL6800s	GGAGAATCCCATTCCATCAAGA	BB	ACGTTAACGAATCATCG	40	0.1
	CCATTGAGCTTGGCGTATACAA	CY	ACGTTAAC-AATCATCG	40	0.1
BoCL6810s	GCTTCAGGAATCCATACGATCA	BB	AAATGGCAATGGCCATG	40	0.1
	GAACATTTGGCACACGACCATA	CY	AAATGGCACTGGCCATG	40	0.1
BoCL6818s	GAGGTTGCGGTACTCTGCATAA	BB	TTTGGATTTGTTTGTTT	40	0.5
	GGCCAACCCTTGTGTAATCATA	CY	TTTGGATATTTTTGTTT	40	0.5
BoCL6865s	ACTCCATCGTTAAACCCCCAAT	BB	TCTCACCCGATGGATCC	40	0.1
	CGTTGTCGAATGTGAGCTCTTT	CY	TCTCACCCAATGGATCC	40	0.1
BoCL6978s	CTCTCTCTCAGAATGGCTGCAA	BB	CCTTCTCACAAGCTCAA	40	0.1
	TTGATCCTAGCAGCCTCAATCA	CY	CCTTCTCAGAAGCTCAA	40	0.1
BoCL7111s	GATGTGTATGGGTTTGGTGTGG	BB	GAACATAACGCAGCTCG	50	0.1
	CACCACATTCACAAGGCATTTC	CY	GAACATAATGCAGCTCG	50	0.1
BoCL7239s	AACATGGGAGCATTCAGCTACA	BB	TACACAACATTGACAGA	40	0.5
	TATAAACCTGCAGCACAAGACG	CY	TACACAACACTGACAGA	40	0.2
BoCL7286s	ATGGTTTGATCCGCTCAAGG	BB	AAAGGCATTTAGAAAAA	40	1

	GAAGCTGAAGCTAAAACGCATC	CY	AAAGGCATCTAGAAAGA	40	0.1
BoCL7289s	CGTGTATGAGAAGGGGAGGAAT	BB	AACTGGCAGAGCAACTC	40	0.2
	ATCAAGGCCTTCTGCAAAACC	CY	AACTGGCAAAGCAACTC	40	0.2
BoCL7317s	CGGGTTGATGTGGTATGACACT	BB	TTTCGATATACTTTGTT	40	0.5
	TGCTACGCAGAAGGTAGCATGT	CY	TTTCGATACTTTGTTCT	40	0.2
BoCL7335s	ACAGGAACCTCATCCTCCAAAC	BB	ATCCCGATGATCTTCCT	50	0.2
	ATCTTAGCTAACGCGACGAGGA	CY	ATCCCGATCATCTTCCT	50	0.2
BoCL7340s	CGAAAAAGTCTGAACGGTGATG	BB	GCATGTATTCAAAGCGT	40	0.1
	GTAAGGGCCGACTTTGTTTGAG	CY	GCATGTATGCAAAGCGT	40	0.1
BoCL7398s	AAAGCAGAGGCCTACCATGTTT	BB	GAGCTCATAGTTCGCTG	40	0.5
	ACCAAACAAGTGGCTGTTCTGA	CY	GAGCTCATTGTTCGTTG	40	0.5
BoCL7403s	ACTCTGTGGACCAGGTGAAACA	BB	TTCAATGGCTATACTCA	40	1
	TTAACAACCGTGACCACGAAAC	CY	TTCAATGGATATACTCA	40	1
BoCL7417s	TATAGTTCCCAGCTGCCACAAA	BB	ATGTCATCACTTCAAAA	40	0.5
	CTCACCGCGAATATGACGATAA	CY	ATGTCATCGCTTCAAAA	40	0.2
BoCL7467s	GAGTCCTCTTCACGCTTTTTGG	BB	TAATAACATCGAGAGAG	40	1
	TGTCCGGTCAGCTTTTTAACCT	CY	TAATAACAACGAGAGAG	40	1
BoCL7572s	AATGGAGAACTCGCCCAGATAC	BB	AAGCCGATACCACTTCC	50	0.1
	AATCGAGGATGCTTGGAGAGAG	CY	AAGCCGATGCCACTTCC	50	0.1
BoCL7601s	ATAGATCATGCCTGTGGAGCAA	BB	ATCAGGTAGATGATGCG	40	0.2
	ACCATAACGATCCCACGAGTCT	CY	ATCAGGTACATGATGCG	40	0.2
BoCL7650s	AAGTTCCTGGCTGCAGCTCTAT	BB	AAGAAGAATGGAAAGAA	40	0.2
	AATGGTGGAACCGAGTTCTGTC	CY	AAGAAGAACGGAAAGAA	40	0.2

BoCL7671s	CGTTTAAAGCAAGCCACCTCTT	BB	GATTATGAATTGACGGG	40	0.2
	CGACTGCCTGAAAATCAATCTG	CY	GATTATGAGTTGACGGG	40	0.2
BoCL7690s	AATCTCTGCAACAGCACGGTTA	BB	GCGGTTGCAGGTGGGGA	40	0.1
	CCACTCTCTCTCAACTGCCTTT	CY	GCGGTTGCGGGTGGGGA	50	0.1
BoCL7702s	GGAGCCCAGAAAAACCCTAAAA	BB	AAGCTTGAGACACAAAG	40	0.5
	GCGTGGTACATTTTCCTCAAGA	CY	AAGCTTGAAACACAAAG	40	0.5
BoCL7713s	AGGCTTGACGACCCGTCTATAA	BB	GTAAGATGCTGTGGTTC	40	0.5
	ACCCGACATTAAAACCAGAACC	CY	GTAAGATGTTGTGGTTC	40	0.5
BoCL7728s	CGCGGAGATGAAACCGTTAT	BB	CTTGCCATCAGGTTCAG	40	0.5
	CTCTCAGATTTGCGGAAAAAGC	CY	CTTGCCATGAGGTTCAG	40	0.5
BoCL7731s	AGTACGATGTTCACGTGGGATG	BB	GAGTCGTTGAGGAATGC	40	0.2
	TCTAGGTTCATCCCCAAAATGG	CY	GTCGTTAAGGAATGCTC	40	0.5
BoCL7792s	GCTAAAGAAGGACCGAGATCCA	BB	TTTGAACATTATCTACA	40	1
	CGAAGTTGACGTTTGTACACGA	CY	TTTGAACACTATCTACA	40	1
BoCL7837s	AAGATGCGGATTATGCAGTGG	BB	ACCCCAAATGTGAATAC	40	0.5
	AACATCGTCGTTGCGTATTCAC	CY	ACCCCAAAAGTGAATAC	40	0.5
BoCL7922s	ACATGGACGATCCATACACACC	BB	TAAAAGACGGGGCATCT	40	0.2
	ACATGCCTTTGCCATTACAGG	CY	TAAAAGACAGGGCATCT	40	0.2
BoCL7942s	GTTAGCTTCCCATTTCGCTTTC	BB	GAATCTGTATCATGAGA	40	0.5
	TGGATAGGATCAGGTCCATTTG	CY	GAATCTGTTTCATGAGA	50	0.5
BoCL7968s	ACAAGACGCATCAATGTCACCT	BB	GAGCTATCGTGGAGGTG	50	0.1
	GAAACCCCCTTAGCCTCTTTTG	CY	GAGCTATCATGGAGGTG	50	0.5

CAPS markers	Primer sequence (5'-3')[a]	Restriction enzyme
BoCL1183c	TAAAGGTGTGATCCCAATGCAC	Mbo I
	AAACGGTATGACCAACTCAGGA	
BoCL1332c	TTGGATGGCGTCAAATATGG	Hae III
	AATCGGATGCTCAGCTTCTACG	
BoCL2451c	CAGCTGTTGGAACCATCAAGAC	Hae III
	CAAAGGGTTCGTCACAAGAGTG	
BoCL4271c	ACGGGCTTAAACGTTGTTGACT	Mbo I
	CGAAAAAGCAGAGCAGGAGATT	
BoCL4799c	AACACAGGACTCTTCGGGACAT	Afa I
	GCGTGGGAAAGACAGTGTAAAG	
BoCL5459c	AGGACTACATCAAGAGGCAGCA	Hae III
	CGTCTTGGTGCTTTGTGCTT	
BoCL6785c	GAGGATAAAATTGCGGAGCTGT	Hae III
	GTATTTCTTGTCGCGCGATGTA	

SCAR markers	Primer sequence (5'-3')[a]
BoCL7777a	GGGAAGAAAAGTGAGGAGACGA
	ATCCCGATGGACTTGCTATCAC
BoCL5685a	GAGACGTGTTGGTTGCTATTGG
	CTCGATACACACTCGCCATCTT

[a] Upper lalyer is forward primer, lower layer is reverse primer sequence.

[b] The bridge probe was constructed according to Shiokai *et al*. (2010). SCR-52 sequence was added to CY probe sequence, and SCR-27 sequence with was added to BB probe sequence.
SCR sequence opposite to other probe was added toBoCL6009s, BoCL6200s and BoCL6590s.
Each probe have spacer seqence between probe and SCR sequence.

[c] Washing SSC concentration with 0.1% SDS. 1 x SSC contains 0.15 M sodium chloride and 15 mM sodium citrate.