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ORIGINAL PAPER

Localization of anchor loci representing five hundred annotated rice genes to wheat chromosomes using PLUG markers

Goro Ishikawa · Toshiki Nakamura · Taizo Ashida · Mika Saito · Shuhei Nasuda · Takashi R. Endo · Jianzhong Wu · Takashi Matsumoto

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Abstract PCR-based Landmark Unique Gene (PLUG) markers are EST-PCR markers developed based on the orthologous gene conservation between rice and wheat, and on the intron polymorphisms among the three orthologous genes derived from the A, B and D genomes of wheat. We designed a total of 960 primer sets from wheat ESTs that showed high similarity with 951 single-copy rice genes. When genomic DNA of Chinese Spring wheat was used as a template, 872 primer sets amplified one to five distinct products. Out of these 872 PLUG markers, 531 were assigned to one or more chromosomes by nullisomic-tetrasomic analysis. For each wheat chromosome, the number of loci detected ranged from 32 for chromosome 6A to 73 for chromosome 7D, with an average of 48 loci per chromosome. Several novel synteny perturbations were identified using deletion bin-mapping of markers. Furthermore, we demonstrated that PLUG markers can be used as probes to simultaneously identify BAC clones that contain homoeologous regions from all three genomes.

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G. Ishikawa · T. Nakamura (✉) · M. Saito
Tohoku National Agriculture Research Center,
Morioka, Iwate 020-0198, Japan
e-mail: tnaka@affrc.go.jp

T. Ashida · S. Nasuda · T. R. Endo
Graduate School of Agriculture,
Kyoto University, Kyoto 606-8502, Japan

J. Wu · T. Matsumoto
National Institute of Agrobiological Sciences,
Tsukuba, Ibaraki 305-8602, Japan

Introduction

Common wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD) is one of the most important cereal crops in the world, and breeders are constantly attempting to improve the yield, quality characteristics, stress tolerance, and disease and insect resistance of this crop. Extensive investigations of the molecular genetic basis of these traits have been undertaken in efforts to improve breeding efficiency (Marshall et al. 2001). Wheat is also of interest from an evolutionary standpoint, and the elucidation of the complex origins of this crop, which evolved by polyploidization around 10,000 years ago (Feldman and Levy 2005), is ongoing. Recent work in this area has focused on determining the degree of differentiation among the three wheat genomes (Chantret et al. 2008; Gao et al. 2007).

Over the last two decades, molecular markers have come to be regarded as useful tools for conducting genetic and genomic studies in wheat. The development of molecular markers has been aided by the large-scale chromosome mapping project led by the International Triticeae Mapping Initiative, with data organized in the form of the GrainGenes public database (Carollo et al. 2005). In common wheat, approximately 2,000 RFLP and 1,000 SSR markers have been mapped on linkage maps, and 6,426 wheat expressed sequence tags (EST) sequences have been located on physical maps of wheat using Southern hybridization analysis. However, given the large genome size of wheat (16 Gbp), the number of molecular markers available is still relatively small. The development of additional

markers would enhance the ease of identifying useful genes and would be useful in clarifying the genomic organization of wheat.

To date, more than 1,000,000 ESTs of wheat have been registered in public databases (http://www.ncbi.nlm.nih.gov/dbEST/) (Wheeler et al. 2006). Since genomic sequencing of wheat is not at an advanced stage, these abundant EST sequences represent the best templates for designing primers for PCR-based gene-specific markers. Additionally, the amount of sequence data from related species is continually increasing, and sophisticated in silico methods for comparing sequences have become available (Florea et al. 1998; Haas et al. 2003; Notredame et al. 2000; Thompson et al. 1994). Bioinformatic procedures that can be used to simplify the design of primers from conserved sequences among related species have also recently been established (Feltus et al. 2006; Fredslund et al. 2006; Wei et al. 2005; Wu et al. 2006). Through the use of these techniques, the transferability of EST-PCR markers among species has improved and marker success rates have increased.

Due to the allohexaploid nature of wheat, however, PCR products derived from orthologous genes are often amplified simultaneously, which hinders the mapping of EST-PCR markers on wheat chromosomes. To resolve this problem, we developed a system called the PCR-based Landmark Unique Gene (PLUG) marker system (Ishikawa et al. 2007). In the construction of PLUG markers, the map-based genomic sequence of rice (International Rice Genome Sequencing Project 2005; Itoh et al. 2007; Yuan et al. 2005) is used to identify single-copy rice genes, which are used as template loci for selecting conserved sequences and predicting exon–exon junctions within wheat ESTs. PLUG primer sets designed based on these ESTs usually amplify only two or three intron-containing products from genomic DNA of wheat. Products from an individual primer set share high sequence similarity, indicating they are derived from the wheat orthologues of the rice template genes. PLUG markers are useful for mapping these orthologous loci to wheat chromosomes and to deletion bins.

Here, we demonstrate the utility of the PLUG system on a large scale by designing one thousand PLUG primer sets from genes distributed across the wheat genome, thereby producing markers which were assigned to all 21 chromosomes and 215 deletion bins. We also show that PLUG markers can be used to specifically identify BAC clones containing homoeologous regions from the A, B or D genomes. This data was used to demonstrate the potential uses of PLUG markers in mapping, genome research, and comparative studies not only between wheat and other grasses, but also among the three wheat genomes.

Materials and methods

Designing PLUG primers

The PLUG system, described by Ishikawa et al. (2007), was used to design wheat primer sets. Rice pseudomolecules version 4 of TIGR and wheat UniGene build 46 from NCBI were used as sequence resources for designing primer sets. The objective was to obtain primers which would amplify products of approximately 1 kb including one or more intron(s), based on the rice genome DNA sequence. A total of 951 single-copy rice genes that showed high similarity to wheat ESTs (TaEST-LUGs) were selected as template loci for primer design. The number of TaEST-LUGs selected from each rice chromosome reflected the ratios of TaEST-LUG numbers among the 12 rice chromosomes (Fig. 1). A total of 960 primer sets were designed based on the corresponding wheat ESTs (Supplemental material 1). Although a single primer set was generally used for each locus, two primer sets were designed for seven loci and three sets for one locus. The following settings were used for primer design: Melting temperature 55–65°C (optimum: 60°C), primer length 18–25 bases (optimum: 21), and estimated size of amplified fragments based on the rice genome 200–4,000 bp (optimum: 1,000 bp).

Plant materials and DNA extraction

The common wheat cultivar Chinese Spring (CS) and its aneuploid lines and deletion stocks were used. In this study, 21 nullisomic-tetrasomic, 36 ditelosomic and 174 deletion lines were used to assign markers to chromosomes and to determine the location of markers within chromosomes (Supplemental material 2). These plant materials were from stocks that have been maintained by the National BioResources Project, Japan (NBRP-wheat). Genomic DNA was extracted from 100 mg of young leaf tissue using the automated DNA isolation system PI-50α (Kurabo Industries Ltd., Osaka, Japan) according to the manufacturer's instructions.

Molecular analysis

PCR amplification of genomic DNA was carried out using the 960 primer sets listed in Supplemental material 1. Each 25-μL PCR mixture included either 50–100 ng of DNA, 0.2 pmol of each primer, 1.5 mM $MgCl_2$, 0.2 mM dNTP (each), 1× *Ex Taq* buffer, and 0.5 U of *TaKaRa Ex Taq*™ (Takara, Osaka, Japan) or 50–100 ng of DNA, 0.2 pmol of each primer, and 1× GoTaq® Green Master Mix (Promega, WI, USA). The PCR cycle consisted of an initial 5 min denaturation at 95°C, followed by 32 cycles of 95°C for 30 s, 53–63°C (optimum 58°C) for 30 s, and 72°C for

2 min, followed by a final extension at 72°C for 7 min. DNA amplification was performed using a GeneAmp PCR system 9700 (Applied Biosystems, Foster city, CA, USA). An 8-μL aliquot of the amplification product was analyzed by electrophoresis on a 1% agarose gel in 40 mM Tris-acetate, 1 mM EDTA (TAE) buffer. For PCR–RFLP analysis, an 8-μL aliquot of the product was digested overnight with 1.0 U of *Hae*III or *Taq*I in incubators set at 37 or 65°C, respectively. Digested fragments were fractionated by electrophoresis on a 4% agarose gel in TAE buffer. Band sizes were estimated against a '1 kb plus DNA Ladder' (Invitrogen, Carlsbad, CA, USA) or a '2-Log DNA Ladder' (New England BioLabs Inc., Ipswich, MA, USA).

Chromosome assignment and deletion-bin mapping

PLUG markers were assigned to wheat chromosomes by the presence or absence of products in nullisomic-tetrasomic lines. Three to six lines were selected for this analysis based on the previously reported synteny data for rice and wheat (Fig. 1). For example, primer sets based on genes from rice chromosome 1 were subjected to analysis using the three nullisomic-tetrasomic lines of group 3 chromosomes.

The strategy used for deletion-bin mapping has been described in the NSF project homepage (http://wheat.pw.usda.gov/NSF/). Mapping was performed using 154 primer sets that produced fragments assigned to the A, B and D genomes of wheat. Markers were located to chromosomal regions by the presence or absence of the products in sequential deletion lines of the chromosome. Bin assignment was performed as described in Qi et al. (2003). The physical location of each deletion interval on a chromosome was determined by the fraction length (FL) values of the deletion lines. For example, bin 3BL2-0.22-0.50 is located in the interval between the deletion in line 3BL-2 (which has a FL value of 0.22) and that in 3BL-10 (which has a FL value of 0.50).

Wheat BAC library screening

A Chinese Spring BAC library (WCS) purchased from the John Innes Centre Genome Laboratory was used for screening experiments. The library consisted of 700,416 clones maintained on 384-well plates. For PCR screening, a DNA pool of each plate was prepared using a R.E.A.L. Prep 96 plasmid kit (QIAGEN, Hilden, Germany). DNA superpools were produced by combining 16 DNA pools, and a total of 114 superpools were used for the initial PCR. In the second PCR, each DNA pool of the positive superpool was amplified separately to determine which plate included the target clone(s). Screening was performed at the Society for Techno-innovation of Agriculture, Forestry and Fisheries (STAFF) Institute using the method described by Wu et al. (2002). Three markers, TNAC (Tohoku National Agriculture Research Center) 1248, TNAC1252 and TNAC1263, which showed distinct sizes in each wheat genome, were used in this experiment. Sequencing of products was used to confirm that they originated from the target genes. Products were purified using a SigmaSpin™ Post Reaction Purification column (Sigma-Aldrich co., St. Louis, MO, USA), and purified products were directly sequenced using an ABI3130 Genetic Analyzer (Applied Biosystems).

Results

Template rice loci for PLUG primers

The template rice loci for designing PLUG primers were distributed across the 12 rice chromosomes (Fig. 1). The

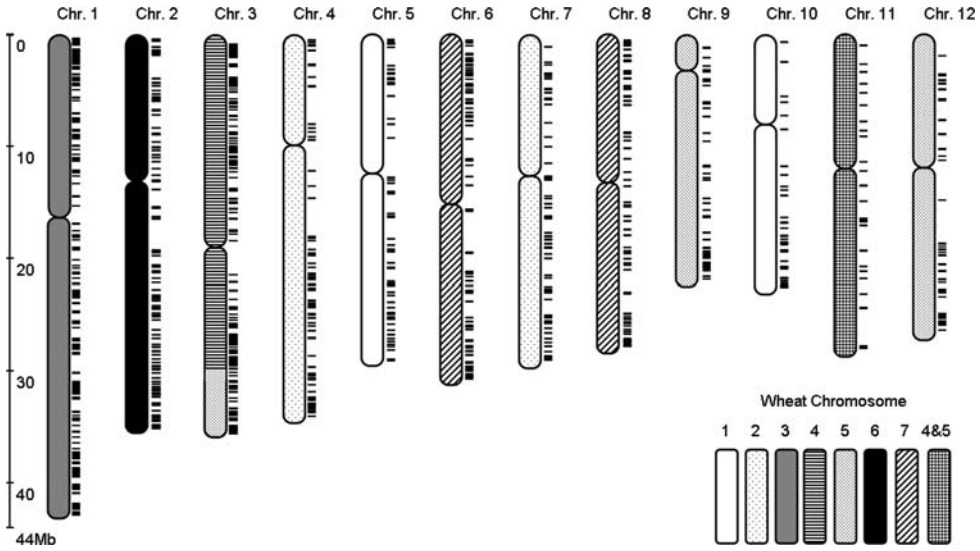

Fig. 1 Distribution of the template rice loci for designing PLUG primer sets. Template loci are indicated by *black bands* on the 12 rice chromosomes. Rice and wheat synteny, as reported by Gale and Devos (1998), La Rota and Sorrells (2004) and Sorrells et al. (2003), is indicated by different patterns for each wheat chromosome group

number of loci for each chromosome ranged from 173 for rice chromosome 1 (R1) to 29 for R11. The average interval between loci ranged from 241 kb for R3 to 980 kb for R11, with an overall average of 474 kb. The 960 primer sets extracted by the PLUG system had the following characteristics: melting temperature was 54.7–66.7°C (average: 60.8°C), primer length was 18–25 bases (average: 20.8), and the size of amplified fragments estimated from the rice genome was 273–3,197 bp (average: 951 bp).

PCR amplification with PLUG primers

Using genomic DNA from CS as a template, 872 (90.8%) out of 960 primer sets successfully amplified one to five products that were separable on a 1% agarose gel. A single band was observed with 493 primer sets (51.4% of the total), while two or three products with different sizes were obtained with 294 (30.6%) and 76 (7.9%) primer sets, respectively. Nine primer sets (0.9%) showed four or five clear bands. Unclear patterns consisting of multiple bands were obtained from 29 (3.0%) primer sets, while no detectable product was observed with 59 primer sets (6.2%), and results with these primers did not improve upon changing the annealing temperature of the reaction.

For eight randomly selected loci, we designed several primer sets to test the effect of varying the primer position. No discrepancies were observed for six loci, but two loci showed different results among the primer sets in terms of the presence or absence of amplified products. For LOC_Os06g38320 (RAP2_ID: Os06g0581300), no product was detectable using the primers associated with TNAC1816, while two clear products were amplified by the TNAC1817 primers. For LOC_Os06g39344 (Os06g0594100), TNAC1819 primers amplified multiple non-specific products, while TNAC1820 primers produced a single clear product (Supplemental material 1).

Size distribution of PCR products

The estimated size of the 1,348 products representing the 872 PLUG markers ranged from 200 to 6,000 bp, with an average size of 1,180 bp. As expected, almost all amplification products were larger than the sizes calculated from the wheat ESTs sequences (Fig. 2a), implying that the products contained introns. Only three products had a similar size to the corresponding ESTs. We found a significant correlation ($r = 0.295$, $P < 0.001$) between observed product sizes and those calculated based on the corresponding rice genomic sequence (Fig. 2b). Template loci were generally highly conserved between wheat and rice in terms of copy number and gene structure, but some exceptions were observed. Although a maximum of three bands per primer set would be expected, four or five distinct products were amplified from wheat with nine primer sets (0.9% of total primer sets). Products that were considerably larger (>3 kb) than the size calculated from the rice genomic sequence were obtained with 25 primer sets (2.6% of total). Sequence analysis of a 4 kb product associated with marker TNAC1582 indicated the presence of retroelement-like sequences (data not shown).

Assignment of PLUG markers to wheat chromosomes

The presence or absence of products in nullisomic-tetrasomic lines allowed 142 of the 872 PLUG markers to be assigned to one chromosome, whereas 32 and 24 markers were assigned to two and three chromosomes, respectively. With PCR–RFLP analysis using either *Hae*III or *Taq*I restriction enzyme, 531 markers (60.9% of the total 872 markers) were assigned to one or more chromosome(s) (Table 1). The percentage of PLUG markers assigned was high for primers designed from loci on R6 and R3-2, and low for the primers from R7 and R9 (Table 1).

A total of 341 PLUG markers could not be assigned to chromosomes using the nullisomic-tetrasomic lines selected according to the previously reported synteny

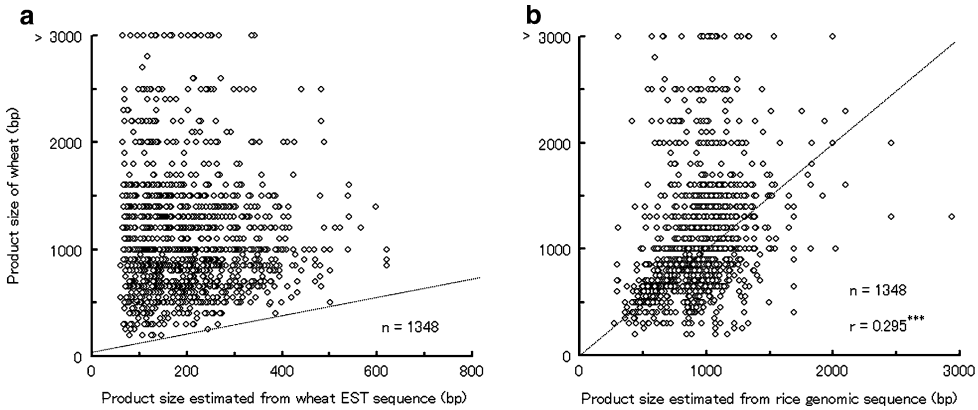

Fig. 2 Scatter diagrams of the size of PCR products amplified from wheat versus the size estimated from wheat ESTs (**a**), and versus the size estimated from rice genomic sequences (**b**). *** indicates significance at $P = 0.001$ level

Table 1 Results of genomic PCR and nullisomic-tetrasomic analyses using the PLUG primer sets

Wheat group	Syntenic rice chr.	Total primers	No. of primers producing fragments	Not assigned	Assigned	Assigned genome[d]						
						A	B	D	BD	AD	AB	ABD
1	5	57	51 (89.5)[b]	15 (29.4)[c]	36 (70.6)[c]	4	3	1	6	4	3	15
	10	39	33 (84.6)	12 (36.4)	21 (63.6)	3	2	2	5	2	0	7
2	4	80	75 (93.8)	32 (42.7)	43 (57.3)	8	2	4	5	6	5	13
	7	66	51 (77.3)	26 (51.0)	25 (49.0)	1	7	5	2	2	3	5
3	1	173	153 (88.4)	64 (41.8)	89 (58.2)	13	21	5	7	5	10	28
4	3-1[a]	121	112 (92.6)	47 (42.0)	65 (58.0)	20 (7, 5A)	12	4	5	9 (3, 5A)	2	13 (1, 5A)
	11	29	28 (96.6)	12 (42.9)	16 (57.1)	1	7 (3, 5B)	1	1	2	3	1
5	3-2[a]	31	29 (93.5)	6 (20.7)	23 (79.3)	1	3	3	2	1 (1, 4A)	2 (1, 4A)	11 (4, 4A)
	9	49	47 (95.9)	23 (48.9)	24 (51.1)	1	5	0	4	6	0	8
	12	39	38 (97.4)	12 (31.6)	26 (68.4)	3 (1, 4A)	5	0	4	2	6	6
6	2	100	93 (93.0)	39 (41.9)	54 (58.1)	5	8	6	8	7	2	18
7	6	106	99 (93.4)	25 (25.3)	74 (74.7)	10	4 (1, 4A)	7	14	10	7 (1, 4A)	22 (1, 4A)
	8	70	63 (90.0)	28 (44.4)	35 (55.6)	3	6	5	2	6	6	7
Total		960	872 (90.8)	341 (39.1)	531 (60.9)	73	85	43	65	62	49	154

[a] *3-1* and *3-2* are parts of rice chromosome 3, *3-1* from short arm terminus to 30.3 Mb, *3-2* from 30.3 Mb to long arm terminus

[b] Percentage of the total primer sets

[c] Percentage of the 872 primer sets

[d] Number of marker sets for which PCR products were assigned to translocated chromosomes is presented in parentheses together with the assigned chromosome

between rice and wheat. A complete nullisomic-tetrasomic analysis using all 21 lines lacking each pair of homologous chromosomes was conducted for six of these markers, which allowed us to assign them to one or more chromosomes (data not shown). This indicated that the chromosomal location of the genes associated with these PLUG markers did not concur with previously reported synteny relationships between rice and wheat (Gale and Devos 1998; La Rota and Sorrells 2004; Sorrells et al. 2003). Likely the same is true for a large percentage of the remaining markers which could not be assigned to chromosomes.

Among the 21 chromosomes, the number of loci detected by the PLUG markers ranged from 32 for chromosome 6A to 73 for chromosome 7D, with an average of 48 loci per chromosome (Table 2). Out of the 531 markers, 395 were developed using EST sequences that did not showed any similarity with sequences in the bin-mapped EST databases of GrainGenes 2.0 (Supplemental material 1). There were no significant differences ($P = 0.51$) among the number of loci detected from the A, B or D genomes.

Table 2 Number of orthologous loci detected by the 531 PLUG markers

Genome	Group							Total
	1	2	3	4	5	6	7	
A	38	43	56	50	51	32	71	341
B	41	42	66	41	59	36	65	350
D	42	42	45	36	47	39	73	324
Total	121	127	167	127	157	107	209	1,015

Deletion bin mapping of PLUG markers

Deletion bin mapping was performed using 154 markers that were each assigned to three homoeologous chromosomes (Table 3). Gel documentation of the nullisomic-tetrasomic analyses for these markers is provided in Supplemental material 3. Out of 215 deletion bins, we mapped one or more marker(s) into 159 bins (74.0%). For wheat group 1 chromosomes, the comparative map for rice and wheat chromosomes is shown in Fig. 3. Maps for all seven chromosome groups are provided in Supplemental material 4.

Using these markers, we found five cases where contradictions occurred between the order of the putative breakpoints and that of FL values in the deletion stocks (Endo and Gill 1996). From centromere to telomere, the orders of the breakpoints were 1AL-3 (FL = 0.61), 1AL-4 (0.47) and 1AL-6 (0.56); 1AS-1 (0.47) and 1AS-2 (0.45); 2BL-7 (0.58), 2BL-5 (0.65) and 2BL-4 (0.50); 2DL-3 (0.49) and 2DL-10 (0.47); 7DS-2 (0.73) and 7DS-4 (0.61). The FL values of these lines were therefore tentatively changed to match with the breakpoint orders indicated by the markers (Supplemental material 2).

Table 3 Results of deletion-bin mapping using 154 PLUG markers and template rice gene annotations

Marker name	Location A	Location B	Location D	RAP2 ID	RAP2 description
TNAC1001	1AS3-0.86-1.00	1BS.sat18-0.50-1.00	1DS5-0.70-1.00	Os05g0102800	Similar to AML1
TNAC1009	1AS2-0.47-0.86	1BS10-0.50-0.84	C-1DS3-0.48	Os05g0154700	Similar to Kinesin heavy chain (Fragment)
TNAC1010	1AS2-0.47-0.86	1BS10-0.50-0.84	C-1DS3-0.48	Os05g0156500	Similar to Apobec-1 binding protein 2
TNAC1019	1AS1-0.45-0.47	C-1BL6-0.32	C-1DL4-0.18	Os05g0292200	Similar to Transcription factor IIA large subunit (TFIIA-L1)
TNAC1021	C-1AL1-0.17	C-1BL6-0.32	1DL4-0.18-0.29	Os05g0299300	WD40-like domain containing protein
TNAC1026	1AL1-0.17-0.47	1BL1-0.47-0.61	1DL2-0.41-0.64	Os05g0349700	Similar to G4 protein (Chlorophyll synthetase)
TNAC1035	1AL6-0.61-1.00	1BL1-0.47-0.61	1DL9-0.64-1.00	Os05g0459400	Kinesin, motor region domain containing protein
TNAC1037	1AL6-0.61-1.00	1BL1-0.47-0.61	1DL9-0.64-1.00	Os05g0463900	HECT domain containing protein
TNAC1038	1AL6-0.61-1.00	1BL14-0.61-0.69	1DL9-0.64-1.00	Os05g0475400	Similar to Alanine:glyoxylate aminotransferase-like protein (fragment)
TNAC1041	1AL4-0.56-0.61	1BL2-0.69-0.85	1DL9-0.64-1.00	Os05g0503300	Similar to Sulfite reductase (fragment)
TNAC1043	1AL4-0.56-0.61	1BL2-0.69-0.85	1DL9-0.64-1.00	Os05g0511300	N2227-like domain containing protein
TNAC1044	1AL4-0.56-0.61	1BL2-0.69-0.85	1DL9-0.64-1.00	Os05g0512200	Conserved hypothetical protein
TNAC1048	1AL4-0.56-0.61	1BL2-0.69-0.85	1DL9-0.64-1.00	Os05g0529300	Similar to ER lumen protein retaining receptor (HDEL receptor)
TNAC1052	1AL4-0.56-0.61	1BL3-0.85-1.00	1DL9-0.64-1.00	Os05g0559900	tRNA-binding arm domain containing protein
TNAC1057	1AL3-0.47-0.56	1BL1-0.47-0.61	1DL9-0.64-1.00	Os05g0594800	Adhesion regulating molecule family protein
TNAC1063	C-1AL1-0.17	C-1BS10-0.50	C-1DS3-0.48	Os10g0320400	Similar to ATP synthase gamma chain, mitochondrial precursor (EC 3.6.3.14)
TNAC1071	1AL1-0.17-0.47	C-1BL6-0.32	1DL4-0.18-0.29	Os10g0467600	Cap-binding protein p28 [*Oryza sativa* (japonica cultivar-group)]
TNAC1076	1AL1-0.17-0.47	C-1BL6-0.32	C-1DL4-0.18	Os10g0494000	Protein of unknown function DUF789 family protein
TNAC1085	1AL1-0.17-0.47	1BL6-0.32-0.47	1DL2-0.41-0.64	Os10g0545200	Similar to 4,4-dimethyl-sterol C4-methyl-oxidase (fragment)
TNAC1086	1AL1-0.17-0.47	1BL6-0.32-0.47	1DL2-0.41-0.64	Os10g0546300	Conserved hypothetical protein
TNAC1088	1AL1-0.17-0.47	1BL6-0.32-0.47	1DL2-0.41-0.64	Os10g0564600	Similar to Calcineurin B (fragment)
TNAC1091	1AL1-0.17-0.47	1BL6-0.32-0.47	1DL2-0.41-0.64	Os10g0569000	Conserved hypothetical protein
TNAC1102	2AS5-0.78-1.00	2BS3-0.84-1.00	2DS5-0.47-1.00	Os04g0119400	Similar to Pyruvate dehydrogenase E1 component, alpha subunit
TNAC1118	2AL4-0.27-0.77	2BL2-0.36-0.50	2DL4-0.26-0.47	Os04g0376500	Similar to Eukaryotic translation initiation factor 3 subunit 3 (eIF-3 gamma) (eIF3 p38 subunit) (eIF3 h)
TNAC1195	2AL4-0.27-0.77	2BL2-0.36-0.50	2DL3-0.47-0.49	Os04g0405800	Similar to Uridylate kinase (EC 2.7.4.-) (UK) (Uridine monophosphate kinase) (UMP kinase)
TNAC1125	2AL4-0.27-0.77	2BL2-0.36-0.50	2DL3-0.47-0.49	Os04g0445400	Conserved hypothetical protein
TNAC1199	2AL4-0.27-0.77	2BL2-0.36-0.50	2DL3-0.47-0.49	Os04g0448600	ChaC-like protein family protein
TNAC1200	2AL4-0.27-0.77	2BL7-0.50-0.58	2DL3-0.47-0.49	Os04g0457200	Short-chain dehydrogenase/reductase SDR family protein
TNAC1204	2AL4-0.27-0.77	2BL7-0.50-0.58	2DL10-0.49-0.58	Os04g0496400	RNA-binding region RNP-1 (RNA recognition motif) domain containing protein
TNAC1132	2AL4-0.27-0.77	2BL5-0.58-0.65	2DL11-0.66-0.76	Os04g0517300	Protein of unknown function DUF647 family protein
TNAC1210	2AL4-0.27-0.77	2BL4-0.65-0.89	2DL11-0.66-0.76	Os04g0581000	Similar to Flavanone 3-hydroxylase-like protein
TNAC1137	2AL4-0.27-0.77	2BL4-0.65-0.89	2DL9-0.76-0.94	Os04g0612600	Similar to Coatomer-like protein, epsilon subunit

Table 3 continued

Marker name	Location A	Location B	Location D	RAP2 ID	RAP2 description
TNAC1139	2AL3-0.77-1.00	2BL6-0.89-1.00	2DL9-0.76-0.94	Os04g0628600	Conserved hypothetical protein
TNAC1140	2AL3-0.77-1.00	2BL6-0.89-1.00	2DL6-0.94-1.00	Os04g0641400	RNA-binding region RNP-1 (RNA recognition motif) domain containing protein
TNAC1142	2AL3-0.77-1.00	2BL6-0.89-1.00	2DL6-0.94-1.00	Os04g0654700	Similar to COP9 signalosome complex subunit 5b (EC 3.4.-.-) (Signalosome subunit 5b) (Jun activation domain-binding homolog 1)
TNAC1233	C-2AS5-0.78	C-2BS11-0.27	C-2DS1-0.33	Os07g0490400	Peptidylprolyl isomerase, FKBP-type domain containing protein
TNAC1176	C-2AS5-0.78	2BS11-0.27-0.53	2DS1-0.33-0.41	Os07g0568400	TB2/DP1 and HVA22 related protein family protein
TNAC1178	C-2AS5-0.78	2BS11-0.27-0.53	2DS1-0.33-0.41	Os07g0573800	Pyridoxamine 5′-phosphate oxidase-related, FMN-binding domain containing protein
TNAC1182	C-2AS5-0.78	2BS11-0.27-0.53	2DS5-0.47-1.00	Os07g0620300	Clathrin adaptor complex, medium chain family protein
TNAC1183	2AS5-0.78-1.00	2BS1-0.53-0.56	2DS5-0.47-1.00	Os07g0628500	Basic helix-loop-helix dimerisation region bHLH domain containing protein
TNAC1627	3AS4-0.45-1.00	3BS9-0.57-0.78	3DS4-0.59-1.00	LOC_Os01g01160[a]	Chaperone protein dnaJ 20, chloroplast precursor, putative, expressed[a]
TNAC1291	3AS4-0.45-1.00	3BS8-0.78-1.00	3DS4-0.59-1.00	Os01g0117900	Similar to Nodulin-like protein 5NG4
TNAC1644	3AS4-0.45-1.00	3BS8-0.78-1.00	3DS4-0.59-1.00	Os01g0132800	Peptidyl-tRNA hydrolase family protein
TNAC1648	3AS4-0.45-1.00	3BS8-0.78-1.00	3DS4-0.59-1.00	Os01g0139000	Reticulon family protein
TNAC1294	3AS4-0.45-1.00	3BS9-0.57-0.78	3DS4-0.59-1.00	Os01g0144000	Conserved hypothetical protein
TNAC1296	3AS4-0.45-1.00	3BS9-0.57-0.78	3DS4-0.59-1.00	Os01g0151700	Similar to Short-chain dehydrogenase Tic32
TNAC1300	3AS4-0.45-1.00	3BS1-0.33-0.57	3DS6-0.55-0.59	Os01g0175000	Phospholipase/Carboxylesterase family protein
TNAC1301	3AS4-0.45-1.00	3BS9-0.57-0.78	3DS4-0.59-1.00	Os01g0182600	GIGANTEA protein
TNAC1248	3AS4-0.45-1.00	C-3BS1-0.33	3DS3-0.24-0.31	Os01g0266000	Lupus La protein family protein
TNAC1314	3AS4-0.45-1.00	3BS1-0.33-0.57	3DS10-0.31-0.44	Os01g0284700	Peptidyl-prolyl *cis-trans* isomerase, cyclophilin type domain containing protein
TNAC1326	3AS4-0.45-1.00	3BS1-0.33-0.57	3DS10-0.31-0.44	Os01g0513700	Sybindin-like protein family protein
TNAC1252	C-3AL2-0.21	C-3BL2-0.22	C-3DL1-0.23	Os01g0560000	Similar to Auxin amidohydrolase
TNAC1335	C-3AL2-0.21	C-3BL2-0.22	C-3DL1-0.23	Os01g0575500	Similar to Stomatal cytokinesis defective
TNAC1341	3AL1-0.26-0.42	C-3BL2-0.22	C-3DL1-0.23	Os01g0620100	WD40-like domain containing protein
TNAC1263	3AL3-0.42-0.61	3BL2-0.22-0.41	3DL1-0.23-0.81	Os01g0681600	Similar to Splicing factor 3A subunit 3 (Spliceosome associated protein 61) (SAP 61) (SF3a60)
TNAC1267	3AL3-0.42-0.61	3BL2-0.22-0.41	3DL1-0.23-0.81	Os01g0753100	Alcohol dehydrogenase superfamily, zinc-containing protein
TNAC1356	3AL3-0.42-0.61	3BL10-0.50-0.63	3DL1-0.23-0.81	Os01g0760600	Aspartate aminotransferase, cytoplasmic (EC 2.6.1.1) (Transaminase A)
TNAC1359	3AL3-0.42-0.61	3BL3-0.41-0.50	3DL1-0.23-0.81	Os01g0770500	Similar to ABC transporter ATP-binding protein
TNAC1364	3AL4-0.61-0.78	3BL10-0.50-0.63	3DL1-0.23-0.81	Os01g0831200	Ubiquitin domain containing protein
TNAC1367	3AL5-0.78-0.85	3BL7-0.63-0.81	3DL1-0.23-0.81	Os01g0866700	Similar to Sm-like protein
TNAC1273	3AL5-0.78-0.85	3BL7-0.63-0.81	3DL1-0.23-0.81	Os01g0868300	Similar to DNA polymerase alpha catalytic subunit (EC 2.7.7.7)
TNAC1277	3AL4-0.61-0.78	3BL7-0.63-0.81	3DL1-0.23-0.81	Os01g0886600	Similar to CLP protease regulatory subunit CLPX precursor
TNAC1278	3AL5-0.78-0.85	3BL7-0.63-0.81	3DL1-0.23-0.81	Os01g0899500	Conserved hypothetical protein

Table 3 continued

Marker name	Location A	Location B	Location D	RAP2 ID	RAP2 description
TNAC1373	3AL5-0.78-0.85	3BL7-0.63-0.81	3DL1-0.23-0.81	Os01g0900900	Ovarian tumor, otubain domain containing protein
TNAC1280	3AL5-0.78-0.85	3BL7-0.63-0.81	3DL1-0.23-0.81	Os01g0911700	Similar to Regulatory protein viviparous-1
TNAC1283	3AL8-0.85-1.00	3BL11-0.81-1.00	3DL3-0.81-1.00	Os01g0928300	Prefoldin domain containing protein
TNAC1286	3AL8-0.85-1.00	3BL11-0.81-1.00	3DL3-0.81-1.00	Os01g0963300	Similar to Syntaxin 61 (AtSYP61) (Osmotic stess-sensitive mutant 1)
TNAC1383	3AL8-0.85-1.00	3BL11-0.81-1.00	3DL3-0.81-1.00	Os01g0976700	Similar to Protein phosphatase 2C-like protein
TNAC1391	5AL23-0.87-1.00	4BL10-0.95-1.00	4DL14-0.86-1.00	Os03g0123200	RNA-binding region RNP-1 (RNA recognition motif) domain containing protein
TNAC1398	4AS3-0.76-1.00	4BL5-0.86-0.95	4DL12-0.71-0.86	Os03g0169100	Ribulose-phosphate 3-epimerase, chloroplast precursor (EC 5.1.3.1) (Pentose-5-phosphate 3-epimerase) (PPE) (RPE) (R5P3E)
TNAC1403	4AS3-0.76-1.00	4BL5-0.86-0.95	4DL12-0.71-0.86	Os03g0182400	Similar to SAC domain protein 1 (FIG4-like protein AtFIG4)
TNAC1408	4AS3-0.76-1.00	4BL5-0.86-0.95	4DL11-0.61-0.71	Os03g0212200	Leucine-rich repeat, typical subtype containing protein
TNAC1412	4AS3-0.76-1.00	4BL1-0.71-0.86	4DL11-0.61-0.71	Os03g0222800	HSP40/DnaJ peptide-binding domain containing protein
TNAC1421	4AS4-0.63-0.76	C-4BL14-0.18	4DL9-0.31-0.56	Os03g0278000	UDP-glucuronic acid decarboxylase
TNAC1428	4AS1-0.20-0.63	C-4BL14-0.18	4DL9-0.31-0.56	Os03g0316900	Similar to NBD-like protein
TNAC1457	4AL12-0.43-0.66	4BS1-0.84-1.00	4DS2-0.82-1.00	Os03g0655300	HSP20-like chaperone domain containing protein
TNAC1656	4AL12-0.43-0.66	4BS1-0.84-1.00	4DS2-0.82-1.00	Os03g0667100	BTB domain containing protein
TNAC1663	4AL12-0.43-0.66	4BS1-0.84-1.00	4DS2-0.82-1.00	Os03g0683800	Similar to Proline-rich protein APG-like
TNAC1463	4AL12-0.43-0.66	4BS1-0.84-1.00	4DS2-0.82-1.00	Os03g0695600	Proteasome subunit beta type 2 (EC 3.4.25.1) (20S proteasome alpha subunit D) (20S proteasome subunit beta-4)
TNAC1464	4AL12-0.43-0.66	4BS1-0.84-1.00	4DS2-0.82-1.00	Os03g0699400	Silencing group B protein
TNAC1468	C-4AL12-0.43	4BS1-0.84-1.00	4DS2-0.82-1.00	Os03g0709000	Membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) family protein
TNAC1510	4AL12-0.43-0.66	4BS8-0.57-0.84	4DS3-0.67-0.82	Os11g0256200	Protein of unknown function DUF842, eukaryotic family protein
TNAC1559	5AL10-0.57-0.78	5BL9-0.76-0.79	5DL5-0.76-1.00	Os03g0749800	Similar to Tousled-like kinase (fragment)
TNAC1613	5AL10-0.57-0.78	5BL9-0.76-0.79	5DL5-0.76-1.00	Os03g0760600	TIP41-like protein family protein
TNAC1614	5AL10-0.57-0.78	5BL9-0.76-0.79	5DL5-0.76-1.00	Os03g0761000	SWIB/MDM2 domain containing protein
TNAC1562	5AL10-0.57-0.78	5BL9-0.76-0.79	5DL5-0.76-1.00	Os03g0772800	Cytochrome c oxidase, subunit VIa family protein
TNAC1616	5AL10-0.57-0.78	5BL9-0.76-0.79	5DL5-0.76-1.00	Os03g0774300	HAD superfamily (subfamily IG) hydrolase, 5′-Nucleotidase protein
TNAC1618	5AL17-0.78-0.87	5BL16-0.79-1.00	5DL5-0.76-1.00	Os03g0815200	Similar to Methylenetetrahydrofolate reductase (EC 1.5.1.20)
TNAC1567	5AL17-0.78-0.87	5BL16-0.79-1.00	5DL5-0.76-1.00	Os03g0818100	Histidine acid phosphatase family protein
TNAC1623	4AL12-0.43-0.66	5BL16-0.79-1.00	5DL5-0.76-1.00	Os03g0847600	Similar to GAMYB-binding protein
TNAC1624	4AL12-0.43-0.66	5BL16-0.79-1.00	5DL5-0.76-1.00	Os03g0852200	Der1-like domain containing protein
TNAC1574	4AL12-0.43-0.66	5BL16-0.79-1.00	5DL5-0.76-1.00	Os03g0854200	Exoribonuclease domain containing protein
TNAC1575	4AL12-0.43-0.66	5BL16-0.79-1.00	5DL5-0.76-1.00	Os03g0856400	Similar to Protein kinase AKINbetagamma-2
TNAC1528	C-5AL12-0.35	C-5BL8-0.52	5DL3-0.39-0.60	Os09g0237600	Protein kinase domain containing protein
TNAC1535	C-5AL12-0.35	C-5BL8-0.52	5DL3-0.39-0.60	Os09g0297000	Similar to Ferrochelatase II, chloroplast precursor (EC 4.99.1.1) (Protoheme ferro-lyase) (Heme synthetase)

Table 3 continued

Marker name	Location A	Location B	Location D	RAP2 ID	RAP2 description
TNAC1540	C-5AL12-0.35	C-5BL8-0.52	5DL3-0.39-0.60	Os09g0380000	Similar to Acetyl-CoA synthetase-like protein
TNAC1541	C-5AL12-0.35	C-5BL8-0.52	5DL3-0.39-0.60	Os09g0383400	DEAD/DEAH box helicase, N-terminal domain containing protein
TNAC1545	5AL12-0.35-0.57	C-5BL8-0.52	5DL3-0.39-0.60	Os09g0440300	Similar to Aldehyde dehydrogenase family 7 member A1 (EC 1.2.1.3) (Antiquitin 1) (Matured fruit 60 kDa protein) (MF-60)
TNAC1605	5AL12-0.35-0.57	5BL8-0.52-0.75	5DL1-0.60-0.69	Os09g0510200	Protein of unknown function DUF667 family protein
TNAC1610	5AL10-0.57-0.78	5BL8-0.52-0.75	5DL2-0.69-0.74	Os09g0533400	Peptidase S16, ATP-dependent protease La family protein
TNAC1554	5AL10-0.57-0.78	5BL8-0.52-0.75	5DL2-0.69-0.74	Os09g0535000	Similar to Triosephosphate isomerase, chloroplast precursor (EC 5.3.1.1) (TIM) (Triose-phosphate isomerase)
TNAC1864	C-5AL12-0.35	C-5BL8-0.52	5DL3-0.39-0.60	Os12g0165000	WD40-like domain containing protein
TNAC1577	5AL10-0.57-0.78	5BL8-0.52-0.75	5DL2-0.69-0.74	Os12g0182800	Conserved hypothetical protein
TNAC1485	C-5AS1-0.40	C-5BS4-0.43	C-5DS4-0.22	Os12g0207300	Similar to Clathrin coat assembly protein AP17 (Clathrin coat associated protein AP17) (Plasma membrane adaptor AP-2 17 kDa protein) (HA2 17 kDa subunit) (Clathrin assembly protein 2 small chain)
TNAC1497	5AS1-0.40-0.75	5BS4-0.43-0.56	5DS4-0.22-0.63	Os12g0548300	Similar to Nucleoside diphosphate kinase II, chloroplast precursor (EC 2.7.4.6) (NDK II) (NDP kinase II) (NDPK II)
TNAC1588	5AS3-0.75-0.98	5BS5-0.71-0.81	5DS1-0.63-0.67	Os12g0613300	Similar to Single myb histone 6
TNAC1503	5AS3-0.75-0.98	5BS5-0.71-0.81	5DS5-0.67-0.78	Os12g0616200	HUELLENLOS-like protein
TNAC1674	6AS5-0.65-1.00	6BS-Sat	6DS6-0.99-1.00	Os02g0104700	Similar to Hydroxyproline-rich glycoprotein DZ-HRGP precursor
TNAC1676	6AS5-0.65-1.00	6BS-Sat	6DS4-0.79-0.99	Os02g0121000	Similar to Glutamyl-tRNA synthetase (EC 6.1.1.17) (Glutamate–tRNA ligase) (GluRS)
TNAC1677	6AS5-0.65-1.00	6BS-Sat	6DS4-0.79-0.99	Os02g0122000	Similar to P18
TNAC1678	6AS5-0.65-1.00	6BS-Sat	6DS4-0.79-0.99	Os02g0124800	Hypothetical protein
TNAC1679	6AS5-0.65-1.00	6BS-Sat	6DS4-0.79-0.99	Os02g0127000	Protein prenyltransferase domain containing protein
TNAC1683	6AS1-0.35-0.65	6BS4-0.46-0.76	6DS1-0.54-0.79	Os02g0175900	Conserved hypothetical protein
TNAC1685	C-6AS1-0.35	6BS1-0.25-0.46	6DS1-0.54-0.79	Os02g0186700	Conserved hypothetical protein
TNAC1702	C-6AL4-0.55	C-6BL3-0.36	6DL6-0.29-0.47	Os02g0290400	Suppressor Mra1 family protein
TNAC1711	C-6AL4-0.55	C-6BL3-0.36	6DL6-0.29-0.47	Os02g0533000	Conserved hypothetical protein
TNAC1726	C-6AL4-0.55	6BL3-0.36-0.40	6DL6-0.29-0.47	Os02g0621700	Similar to Succinyl-CoA ligase [GDP-forming] beta-chain, mitochondrial precursor (EC 6.2.1.4) (Succinyl-CoA synthetase, beta chain) (SCS-beta)
TNAC1740	6AL7-0.88-0.90	6BL5-0.40-0.66	6DL6-0.29-0.47	Os02g0710900	Heat shock protein Hsp70 family protein
TNAC1741	6AL7-0.88-0.90	6BL8-0.66-0.70	6DL1-0.47-0.68	Os02g0717500	Conserved hypothetical protein
TNAC1743	6AL7-0.88-0.90	6BL8-0.66-0.70	6DL1-0.47-0.68	Os02g0725600	Protein of unknown function DUF425 family protein
TNAC1748	6AL7-0.88-0.90	6BL8-0.66-0.70	6DL1-0.47-0.68	Os02g0750500	SAM (and some other nucleotide) binding motif domain containing protein
TNAC1751	6AL8-0.90-1.00	6BL1-0.70-1.00	6DL12-0.68-0.74	Os02g0764500	Similar to Lhca5 protein
TNAC1752	6AL8-0.90-1.00	6BL1-0.70-1.00	6DL11-0.74-0.80	Os02g0768600	Similar to Chloroplast inorganic pyrophosphatase (EC 3.6.1.1)

Table 3 continued

Marker name	Location A	Location B	Location D	RAP2 ID	RAP2 description
TNAC1763	6AL8-0.90-1.00	6BL1-0.70-1.00	6DL10-0.80-1.00	Os02g0817200	WD-40 repeat containing protein
TNAC1768	6AL8-0.90-1.00	6BL1-0.70-1.00	6DL10-0.80-1.00	Os02g0833400	Conserved hypothetical protein
TNAC1776	7AS2-0.73-0.83	4AL2-0.75-0.80	7DS4-0.73-1.00	Os06g0137300	YEATS family protein
TNAC1781	7AS2-0.73-0.83	7BS2-0.27-1.00	7DS4-0.73-1.00	Os06g0153800	Beta 5 subunit of 20S proteasome
TNAC1782	7AS2-0.73-0.83	7BS2-0.27-1.00	7DS4-0.73-1.00	Os06g0158200	Conserved hypothetical protein
TNAC1941	7AS2-0.73-0.83	7BS2-0.27-1.00	7DS4-0.73-1.00	LOC_Os06g07710[a]	Sulfiredoxin-1, putative, expressed[a]
TNAC1787	7AS5-0.59-0.73	7BS2-0.27-1.00	7DS4-0.73-1.00	Os06g0185900	Similar to Phospholipid hydroperoxide glutathione peroxidase, chloroplast precursor (EC 1.11.1.12) (PHGPx)
TNAC1943	7AS5-0.59-0.73	7BS2-0.27-1.00	7DS4-0.73-1.00	Os06g0187000	Similar to Origin recognition complex 1
TNAC1948	7AS8-0.45-0.59	7BS1-0.27-0.27	7DS2-0.61-0.73	Os06g0254700	Caleosin related family protein
TNAC1803	C-7AL1-0.39	7BL2-0.38-0.63	7DL5-0.30-0.61	Os06g0338900	Nucleotidyl transferase domain containing protein
TNAC1805	7AS8-0.45-0.59	7BS2-0.27-1.00	7DS2-0.61-0.73	Os06g0472200	Similar to Isoflavone reductase homolog Bet v 6.0101 (fragment)
TNAC1806	7AS8-0.45-0.59	7BS2-0.27-1.00	7DS2-0.61-0.73	Os06g0474200	Conserved hypothetical protein
TNAC1811	7AL1-0.39-0.63	7BL2-0.38-0.63	7DL5-0.30-0.61	Os06g0561000	Similar to Myo-inositol oxygenase
TNAC1812	7AL5-0.63-0.71	7BL7-0.63-0.78	7DL5-0.30-0.61	Os06g0562600	Similar to T-complex protein 1, epsilon subunit (TCP-1-epsilon) (CCT-epsilon) (TCP-K36)
TNAC1815	7AL1-0.39-0.63	7BL2-0.38-0.63	7DL5-0.30-0.61	Os06g0574100	Similar to Coclaurine N-methyltransferase (EC 2.1.1.115)
TNAC1821	7AL1-0.39-0.63	7BL2-0.38-0.63	7DL5-0.30-0.61	Os06g0607000	Similar to Beta-1,3-glucanase-like protein
TNAC1822	7AL5-0.63-0.71	7BL2-0.38-0.63	7DL2-0.61-0.82	Os06g0632200	Conserved hypothetical protein
TNAC1825	7AL16-0.86-0.90	7BL3-0.86-1.00	7DL3-0.82-1.00	Os06g0647100	Similar to 50S ribosomal protein L35, chloroplast precursor (CL35)
TNAC1826	7AL16-0.86-0.90	7BL6-0.84-0.86	7DL3-0.82-1.00	Os06g0652000	Similar to T3/T7-like RNA polymerase (fragment)
TNAC1829	7AL16-0.86-0.90	7BL10-0.78-0.84	7DL2-0.61-0.82	Os06g0667500	Similar to 14 kDa zinc-binding protein (Protein kinase C inhibitor) (PKCI)
TNAC1956	7AL16-0.86-0.90	7BL10-0.78-0.84	7DL2-0.61-0.82	Os06g0670000	Conserved hypothetical protein
TNAC1957	7AL21-0.74-0.86	7BL10-0.78-0.84	7DL2-0.61-0.82	Os06g0678200	Similar to Geranyl diphosphate synthase
TNAC1834	7AL5-0.63-0.71	7BL10-0.78-0.84	7DL2-0.61-0.82	Os06g0702700	AIG2-like family protein
TNAC1845	7AL18-0.90-1.00	7BL3-0.86-1.00	7DL3-0.82-1.00	Os06g0727200	Catalase isozyme B (EC 1.11.1.6) (CAT-B)
TNAC1867	C-7AL1-0.39	7BL2-0.38-0.63	7DL1-0.14-0.30	Os08g0109900	Tudor domain containing protein
TNAC1868	C-7AL1-0.39	C-7BL2-0.38	7DL1-0.14-0.30	Os08g0113100	Similar to Fructokinase (fragment)
TNAC1888	C-7AL1-0.39	7BL2-0.38-0.63	7DL5-0.30-0.61	Os08g0205400	Heavy metal transport/detoxification protein domain containing protein
TNAC1903	C-7AL1-0.39	C-7BL2-0.38	7DL1-0.14-0.30	Os08g0377400	Exonuclease domain containing protein
TNAC1917	C-7AS8-0.45	7BS2-0.27-1.00	7DS3-0.15-0.36	Os08g0520900	Similar to Isoamylase (fragment)
TNAC1926	C-7AS8-0.45	7BS2-0.27-1.00	7DS3-0.15-0.36	Os08g0556200	Similar to Dihydroneopterin aldolase
TNAC1929	C-7AS8-0.45	C-7BS3-0.16	7DS3-0.15-0.36	Os08g0559600	Similar to Dihydroxy-acid dehydratase (EC 4.2.1.9) (DAD)

[a] Locus ID and annotation of the TIGR

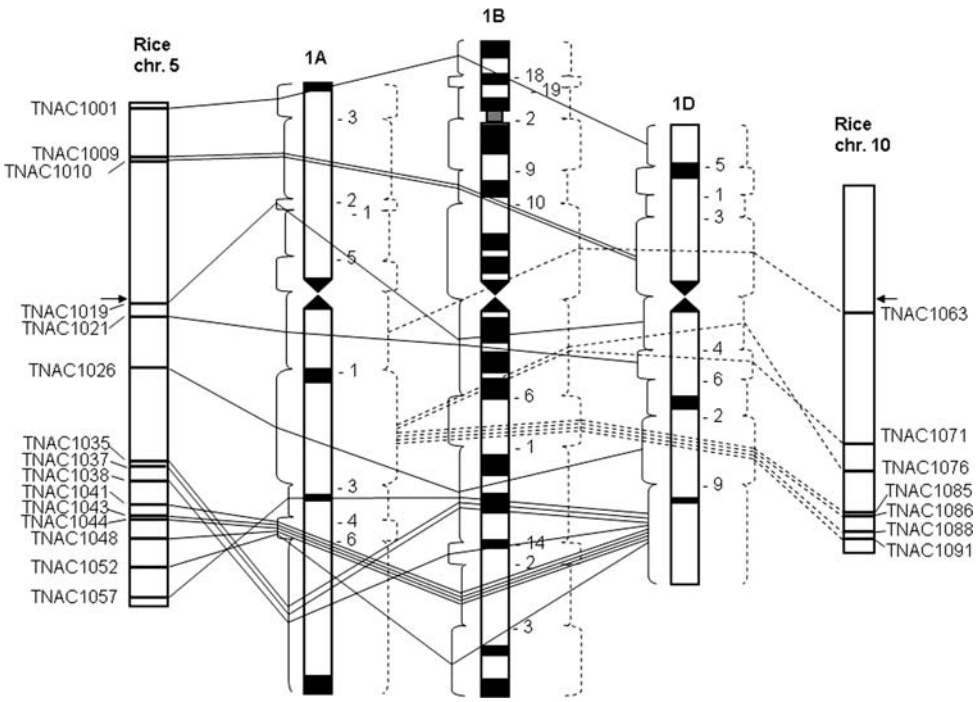

Fig. 3 Comparative map of the wheat group 1 chromosomes and rice chromosomes 5 and 10. *Arrows* beside the rice chromosomes indicate the positions of centromeres. The C banding pattern and the deletion breakpoints of Chinese Spring wheat chromosomes are based on Endo and Gill (1996)

Synteny relationships among wheat and rice chromosomes

Nullisomic-tetrasomic analysis using the three to six lines that were selected based on the synteny between rice and wheat allowed products from more than 60% of the PLUG markers to be assigned to wheat chromosomes (Table 1, Supplemental material 1). The mapping data from these markers generally supported previously reported synteny data among wheat and rice chromosomes (Gale and Devos 1998; La Rota and Sorrells 2004; Sorrells et al. 2003). However, 21 PLUG markers based on rice genes located in one region of R2 (7–20 Mb) rarely mapped to the syntenic wheat group 6 chromosomes. Similar results were observed for markers based on regions of R3 (13–26 Mb) and R7 (0–17 Mb).

There are three well known translocation regions in wheat; from 4A to 5AL (4A/5A), from 5A to 4AL (5A/4A), and from 7B to 4AL (7B/4A) (Nelson et al. 1995). A PLUG marker representing orthologous loci mapping to bins 4BL10-0.95-1.00 and 4DL14-0.86-1.00 (TNAC1391) was also found in bin 5AL23-0.87-1.00, where the 4A/5A translocation has previously been detected (Table 3, Supplemental material 4). Similarly, markers representing orthologous loci in bins 5BL16-0.79-1.00 and 5DL5-0.76-1.00 (TNAC1574, 1575, 1623 and 1624) were mapped to bin 4AL12-0.43-0.66, and a marker mapping to bin 7AS2-0.73-0.83 and bin 7DS4-0.73-1.00 (TNAC1776) was also found on the long arm of 4A (bin 4AL2-0.75-0.80).

Breakpoints of wheat translocations could be precisely identified on the corresponding regions of orthologous rice chromosomes. The breakpoint of the 4A/5A translocation was located between LOC_Os03g03510 (Os03g0126800) and LOC_Os03g07300 (Os03g0169100), that of 5A/4A was between LOC_Os03g61019 (Os03g0825400) and LOC_Os03g61220 (Os03g0827700), and that of 7B/4A was between LOC_Os06g04580 (Os06g0137300) and LOC_Os06g06030 (Os06g0153800) (Supplemental material 1).

BAC clone screening with PLUG markers

Using the genomic DNA of CS as template, primers for the TNAC1248 marker amplified 1.3, 1.1 and 0.9 kb products, and nullisomic-tetrasomic analysis indicated these fragments originated from chromosomes 3A, 3B and 3D, respectively (Fig. 4a). Using DNA pools of BAC clones as templates, three products with the same sizes as those from the genomic PCR were obtained. Therefore, the 1.3, 1.1 and 0.9 kb products from the BAC DNA superpool were likely derived from clones originating from chromosomes 3A, 3B and 3D, respectively. Subsequent screening of pools corresponding to the positive superpools allowed us to obtain individual clones bearing the 1.3 kb product (one clone), the 1.1 kb product (one clone) and the 0.9 kb product (two clones).

Primers corresponding to marker TNAC1252 produced 1 and 1.5 kb products from genomic DNA. The 1.5 kb product was directly localized to chromosome 3B. Digestion with *Hae*III indicated that the 1 kb band included two amplification products, which were assigned to chromosomes 3A and 3D (Fig. 4b). Similarly, 1.5 and 1 kb products were identified in DNA pools of BAC clones. Separate

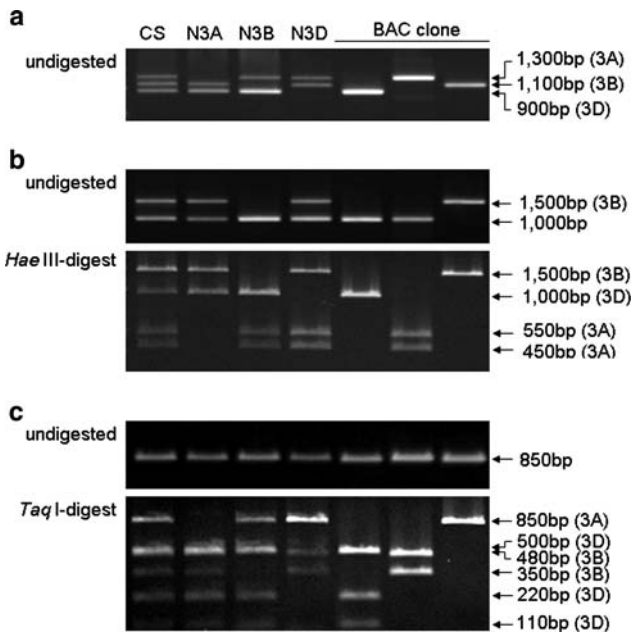

Fig. 4 PCR screening of a Chinese Spring BAC library using the markers TNAC1248 (**a**), TNAC1252 (**b**) and TNAC1263 (**c**). *CS* Chinese Spring, *N3A* nullisomic-3A tetrasomic-3D, *N3B* nullisomic-3B tetrasomic-3D, *N3D* nullisomic-3D tetrasomic-3A, *BAC clones* pattern obtained from three DNA pools extracted from a 384-well plate of BAC clones

BAC DNA pools contained 1 kb products with *Hae*III restriction-digest patterns matching those of products from chromosomes 3A and 3D, while the 1.5 kb product appeared to be derived from a clone originating from chromosome 3B (Fig. 4b).

For marker TNAC1263, the genomes from which products originated could be identified by polymorphisms in the *Taq*I-digested fragments (Fig. 4c). The restriction fragment patterns of the products amplified from BAC pools were completely consistent with those from genomic DNA of CS.

For all markers, sequencing of amplified products from BAC pools or clones confirmed that the products were derived from the target genes (data not shown).

Discussion

A TaEST-LUG is a gene which is present as a single copy in the rice genome and shows high sequence similarity with one or more wheat EST sequences. Based on previously reported genome-wide synteny data between rice and wheat (Gale and Devos 1998; La Rota and Sorrells 2004; Sorrells et al. 2003), each TaEST-LUG should correspond to a single, highly conserved gene in each of the A, B and D genomes of wheat. Such single copy genes will be useful as landmarks or anchors for navigating the huge genome of a polyploidy crop such as wheat. In our previous work, we developed the PLUG system for producing markers to detect such landmark genes on wheat chromosomes, and tested the system using 24 TaEST-LUGs. Here, we developed 872 PLUG markers from 951 TaEST-LUGs and succeeded in assigning 531 markers to one or more chromosomes (Tables 1, 2).

Previous work indicated that it should be possible to assign 70% or more markers developed by the PLUG system to at least one chromosome (Ishikawa et al. 2007). In this study, 61% of the PLUG markers were assigned to one or more chromosomes (Table 1), which was fairly close to the expected level. However, several factors were identified which might contribute to the discrepancy between predicted and observed values; the most important of these appears to be synteny perturbations between wheat and rice.

Based on data from the NSF deletion bin-mapping project, approximately 35% of wheat ESTs showing high similarity to rice genes map to non-syntenic wheat chromosomes (La Rota and Sorrells 2004). In barley, 763 of 1,032 mapped ESTs showed high similarity with rice sequences, and 288 of these (37.7%) were assigned to linkage groups that were non-syntenic to the linkage groups of rice (Stein et al. 2007). Here, approximately 40% of markers could not be assigned to chromosomes (Table 1) using an analysis based on previously reported synteny data for wheat and rice (Gale and Devos 1998; La Rota and Sorrells 2004; Sorrells et al. 2003). The percentage of markers which could not be assigned to wheat chromosomes ranged from 20.7 to 51.0% for each rice chromosome (Table 1). Since a more detailed analysis using all 21 nullisomic-tetrasomic lines with some of these markers allowed us to assign them to one or more non-syntenic chromosome(s), the unequal distribution of unassigned markers may reflect regions with different levels of conservation between wheat and rice. Recent work by Salse et al. (2008) should also be considered in future attempts to assign PLUG markers to wheat chromosomes. These authors performed an in silico analysis using annotated rice genes and wheat ESTs to identify duplications in the rice and wheat genomes. Based on 638 putatively duplicated loci that had been assigned to deletion bins, ten duplicated blocks were identified on seven chromosomes of wheat.

Although conservation of synteny among homoeologous chromosomes of wheat was generally high, there were some differences in the order of orthologous genes. In one region of 1AL, we found that eight markers mapped to bins in inverted positions compared to their locations on chromosome 1BL and the syntenic rice chromosome (Fig. 3). While is not yet known whether this chromosomal rearrangement originated in the diploid ancestor of the A genome or occurred after polyploidization, the identifica-

tion of synteny perturbations between homoeologous chromosomes can provide valuable information regarding the evolutional history of the three genomes of wheat (Akhunov et al. 2003; See et al. 2006). The development of new PLUG markers will help to clearly identify the structural divergences among wheat homoeologous chromosomes.

We detected five contradictions in the order between putative breakpoints and the FL values of the CS deletion stocks. A number of the deletion stocks used here have secondary and tertiary deletions including internal deletions (Qi et al. 2003), and it is possible that they contain as yet unreported deletions, which could lead to problems with ordering by cytological methods. However, changes to FL values based on DNA markers should be confirmed by several markers. The contradictions in order between 1AL-3 and 1AL-4, between 1AS-1 and 1AS-2, and between 2BL-5 and 2BL-4 were each only detected by single markers (Figs. 1, 2 in Supplemental material 4), and further analysis is necessary to validate the correct order of the deletion breakpoints. Since FL values are measured indirectly, they do not necessarily indicate the precise breakpoints of deletions (Endo and Gill 1996). DNA markers appear to be superior to FL values for ordering deletion stocks, and with increasing numbers of bin-mapped DNA markers, errors in FL values will continue to be detected. Furthermore, fingerprinting of deletion stocks with PLUG markers should allow simpler and more accurate validation of individual deletion lines during line multiplication and maintenance compared to the cytological methods traditionally used.

From the results described here (Fig. 2a, b), the positions of exon–intron junctions and intron sizes in wheat and rice appear to be similar. Wei et al. (2005) reported that the intron sizes of *Rhododendron* were on average 3.8 times larger than those of *Arabidopsis*, and they suggested that this difference was related to the difference in the genome sizes of these species. However, despite the four-fold difference in the genome sizes of *Brassica oleracea* and *Arabidopsis thaliana*, a sequence-based analysis indicated that the average intron lengths of the two plants were comparable (Town et al. 2006). Similarly, in this study, intron length did not increase proportionally to genome size (Fig. 2b). Nevertheless, we did observe several products that were considerably larger than expected (>3 kb). These products may represent loci where transposable elements have been inserted into genic regions. Since large insertions can result in a loss of gene function or changes in expression levels (Fu et al. 2005), further investigation of these loci may prove interesting.

PLUG markers are based on orthologous gene conservation, therefore their transferability to other Triticeae species should be high. Indeed, approximately 80% of the 960 primer sets used in this study resulted in successful product amplification when genomic DNA from rye and/or barley was used as a template (Tsuchida et al. 2008; our unpublished data), indicating that these primer sets can be widely used to directly produce anchor markers for comparing Triticeae genomes. Similarly, EST data from related species may be useful in developing additional wheat markers. Recently, more than 1,000 ESTs were mapped in three doubled-haploid lines of barley (Stein et al. 2007). This transcript map provided new anchor points for comparing grasses, and 475 markers showed a syntenic organization with the known colinearity linkage groups of rice. Some of TaEST-LUGs used in this study were orthologous to these 475 barley markers (our unpublished data). Using such common markers, additional genes located between two wheat PLUG markers can be predicted from the mapping information of the barley ESTs, which will accelerate further anchor marker production in wheat.

In a hybridization-based bin-mapping study in wheat, Hossain et al. (2004) identified 119 probes that produced only three fragments and mapped to homoeologous group 7 chromosomes. These were designated as landmark probes, with particular utility in the allocation of orthologous loci across Triticeae genomes and in the genetic mapping of orthologous genes. Here, most of the 154 bin-mapped PLUG markers amplified only three products that were assigned to homoeologous chromosomes (Supplemental material 3) and can therefore be considered landmark probes. These markers will be efficient and easy to use for identifying orthologous loci not only across Triticeae genomes but also in Poaceae genomes.

Assignment of markers to one or more wheat chromosome(s) simply based on intron size polymorphisms among orthologous genes represents a cost-effective and labor-saving means of chromosome assignment, since sequencing or RFLP analysis is not required. A total of 22.7% of PLUG markers could be assigned based on PCR product size polymorphisms alone, and increasing the percentage of markers that fall into this category would be advantageous. Previously we reported that intron regions among orthologous genes of wheat had insertion/deletion polymorphisms every 100 bp on average (Ishikawa et al. 2007). Therefore, the development of a method that can detect slight size differences, or the use of high resolution equipment such as capillary gel electrophoresis, should greatly increase the efficiency of marker development. We also noted that although most PLUG primer sets amplified specific products, a small number amplified non-specific products or did not amplify detectable products. This problem was resolved for some loci by using a second primer set (Supplemental material 1). Since PCR failure appears to be attributable to primer design, redesigning primers for problematic template loci should increase the PCR success rate.

Genes controlling important traits of wheat such as disease resistance and vernalization requirement have been

isolated by positional cloning using BAC clones (Faris et al. 2003; Feuillet et al. 2003; Yahiaoui et al. 2004; Yan et al. 2003). PCR-based BAC screening methods are easier to use than hybridization-based methods, and PCR-based contiguous probes are easier to design than hybridization-based probes. While primer sets designed from EST sequences often amplify ambiguous products from BAC DNA pools of wheat, PLUG markers were able to clearly identify pools containing all three orthologues. The ease in identifying clones containing homoeologous regions from the A, B and D genomes will aid in sequence comparisons of homoeologous regions. Such comparisons not only provide information on sequence evolution among genomes (Gu et al. 2006; Wicker et al. 2007) but also often reveal structural and functional differences among orthologous genes (Beales et al. 2007). In this study, the three BAC clones isolated with TNAC1252 (Table 3; Supplemental material 1) carried homoeologous regions of group 3 chromosomes that included the wheat orthologues of a rice auxin amidohydrolase gene. Campanella et al. (2004) previously isolated a cDNA for this gene (*TaIAR3*) from wheat based on sequence similarities with an *Arabidopsis* gene (*IAR3*), and demonstrated that the wheat gene has evolved in a functionally diverse manner compared to the corresponding *Arabidopsis* gene. The BAC clones identified here would be useful in furthering this work by simplifying the isolation of genes from the A, B and D genomes and their surrounding regulatory sequences. Similarly, rice annotation information can be used to identify genes that are potentially of interest either due to their functional annotation or their syntenic location in relationship to wheat quantitative trait loci (QTLs), and PLUG markers can be used to identify BAC clones carrying the wheat orthologues of these genes.

QTLs are generally associated with a specific genome, making the ability of PLUG markers to distinguish BAC clones originating from A, B or D genomes particularly useful. BAC sequences flanking a target gene can be used to develop new, closely linked markers for surveying polymorphisms among wheat cultivars and for identifying contiguous BAC clones. By selecting BAC clones using PLUG markers mapping close to QTLs, and then using markers designed from these BACs to survey mapping populations, we have succeeded in producing new markers linked to several QTLs (our unpublished data). To assist in the design of PLUG markers for regions targeted in specific research programs, sequences for PLUG primers and bin-mapping data are now available at the web site http://plug.dna.affrc.go.jp/ or through the RAP-DB web site http://rapdb.dna.affrc.go.jp/.

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