PCR-based Landmark Unique Gene (PLUG) markers are a useful tool for comparative genomic analysis and BAC clone screening in wheat

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ABSTRACT

Using a proprietary program, a total of 960 primer sets were designed based on 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 of these primer sets which amplified one to five distinct products were defined as PLUG (PCR-based Landmark Unique Gene) markers. Nullisomic-tetrasomic analyses were used to determine the location of the products on wheat chromosomes. In total, 1,016 products from 533 primer sets were assigned to chromosomes. The number of loci detected for each chromosome ranged from 32 for chromosome 6A to 74 for chromosome 7D, with an average of 48 loci per chromosome. Deletion-bin mapping was performed with 154 primer sets that had been successfully assigned to the A, B and D genomes. The mapping data from these markers clearly supported previously reported synteny data between wheat and rice. By using these markers as anchors, we found some large differences in sizes among the corresponding parts of homoeologous chromosomes and identified several novel synteny perturbations. Furthermore, we report that BAC clones containing homoeologous regions from the A, B or D genomes can be simultaneously picked up from a library using a PLUG marker as a probe.

INTRODUCTION

PCR-based Landmark Unique Gene (PLUG) markers are EST-PCR markers developed based on the orthologous gene conservation between rice and wheat, and the intron polymorphisms among the three orthologous genes of wheat. We have shown the potential of PLUG markers for distinguishing the three wheat orthologs of a template rice gene (Ishikawa et al, 2007). Here, we describe the development of one thousand PLUG markers distributed among the three genomes of wheat. These markers will be useful in genetic and genomic analyses of wheat.

MATERIALS AND METHODS

Primer design: The PCR-based Landmark Unique Genes (PLUG) system (Ishikawa et al, 2007) was used to design wheat primers. Rice pseudomolecules version 4 of the TIGR and wheat UniGene build 46 from NCBI were used as sequence resources for designing primer sets. In designing primers, the Tm value was set at 55 to 65°C with an optimum of 60°C. Primer length was set from 18 to 25 bp, with 21 bp set as the optimum size.

Plant materials: The wheat cultivar Chinese Spring (CS), and the nullisomic-tetrasomic, ditelosomic and deletion lines (Endo and Gill, 1996) of CS used in these experiments were obtained from National BioResources Project, Japan (NBRP-Wheat).

DNA extraction and PCR: DNA was extracted using the automatic DNA isolation system PI-50α (Kurabo Industries Ltd.). Each PCR was performed in a volume of 25 μL. An 8-μL aliquot of the PCR mixture was separated on a 1% agarose gel in TAE buffer. For PCR-RFLP analysis, an 8-μL aliquot was digested overnight with 1.0U of either *Hae*III or *Taq*I at 37 or 65°C, respectively. Digested fragments were separated on a 4% agarose gel.

BAC clone screening: Screening of BAC clones was performed at the Society for Techno-innovation of Agriculture, Forestry and Fisheries (STAFF) using the method described in Wu et al. (2002). The test was conducted using three PLUG markers, TNAC1280, 1283 and 1300 as probes.

RESULTS AND DISCUSSION

We designed a total of 960 primer sets that were based on wheat ESTs showing high similarity with single-copy rice gene loci. When genomic DNA of CS was used as a template, 872 primer sets amplified distinct products (Table 1). To determine the location of the products on wheat chromosomes, nullisomic-tetrasomic analyses were conducted based on the previously reported synteny between wheat and rice (Gale and Devos, 1998; Sorrells et al, 2003). Of the 872 primer sets tested, products from 533 (61.1%) were assigned to one or more wheat chromosome(s) (Table 1).

A total of 1,016 products from these 533 primer sets were assigned to chromosomes (Table 2). The number of loci detected for each of the 21 chromosomes ranged from 32 for chromosome 6A to 74 for chromosome 7D, with an average of 48 loci per chromosome. There were no notable differences in the number of loci detected for corresponding chromosomes from the A, B or D genomes.

Deletion bin mapping was performed using 154 primer sets that amplified distinguishable products from the A, B and D genomes. The mapping data from these markers clearly supported previously reported synteny data between wheat and rice (data not shown). However, using these markers as anchors, we detected inversion-like synteny perturbations between parts of the long arm of wheat group 7 chromosomes and rice chromosome 6, and between wheat chromosome 1A and rice chromosome 5 (data not shown).

A PCR-based nullisomic-tetrasomic analysis indicated that the primer set TNAC1280 amplified 1.3kb, 1.1kb and 0.9kb products from 3A, 3B and 3D chromosomes, respectively (Figure 1a). Products of these three sizes were also obtained when DNA pools of BAC clones were used as templates (Figure 1a). For the TNAC1283 and TNAC1300 markers, PCR-RFLP analysis of nullisomic-tetrasomic lines identified three polymorphic products which were also detected in BAC clones (Figure 1b and c). For each of these three markers, the sequences of the three orthologous products from the BAC clones showed 83-94% similarities (data not shown), indicating we could identify BAC clones containing individual orthologous loci from the A, B and D genomes. Sequence comparison among orthologous regions provides information on evolution among genomes (Gu et al, 2006; Wicker et al, 2007) and often reveals structural and functional differences among orthologous genes (Beales et al, 2007). In this study, we have succeeded in identifying BAC clones which harbour a targeted genomic region

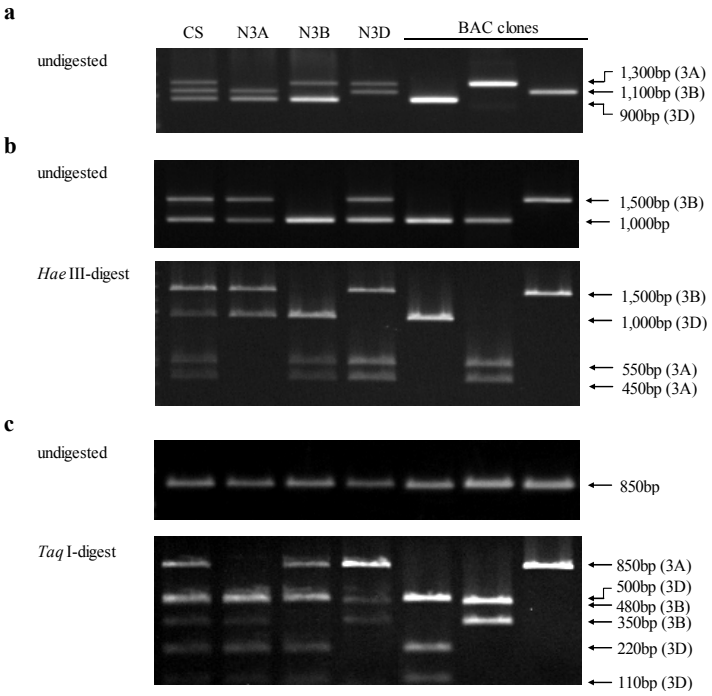

Figure 1. PCR screening of a Chinese Spring BAC library using the TNAC1280 (a), TNAC1283 (b) and TNAC1300 (c) markers. CS: Chinese Spring, N3A: Nullisomic-3A tetrasomic-3D, N3B: Nullisomic-3B tetrasomic-3D, N3D: Nullisomic-3D tetrasomic-3A, BAC clones: PCR was conducted using DNA pools extracted from each 384-well plate of BAC clones.

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

Rice chr.	Syntenic wheat chr. group[2]	Total primers	No. of primers producing fragments	Not assigned	Assigned	Assigned genome A/B/D	Assigned genome BD/AD/AB	Assigned genome ABD	Transloc. region[5]
1	3	173	153 (88.4)[3]	63 (41.2)[4]	90 (58.8)[4]	40	22	28	
2	6	100	93 (93.0)	39 (41.9)	54 (58.1)	19	17	18	
3a[1]	4	121	112 (92.6)	46 (41.1)	66 (58.9)	37	16	13	11(4A→5A)
3b[1]	5	31	29 (93.5)	6 (20.7)	23 (79.3)	7	5	11	6 (5A→4A)
4	2	80	76 (95.0)	33 (43.4)	43 (56.6)	14	16	13	
5	1	57	51 (89.5)	15 (29.4)	36 (70.6)	8	13	15	
6	7	106	99 (93.4)	24 (24.2)	75 (75.8)	22	31	22	3 (7B→4A)
7	2	66	51 (77.3)	25 (49.0)	26 (51.0)	14	7	5	
8	7	70	63 (90.0)	28 (44.4)	35 (55.6)	14	14	7	
9	5	49	46 (93.9)	23 (50.0)	23 (50.0)	6	9	8	
10	1	39	33 (84.6)	12 (36.4)	21 (63.6)	7	7	7	
11	4	29	28 (96.6)	13 (46.4)	15 (53.6)	8	6	1	
12	5	39	38 (97.4)	12 (31.6)	26 (68.4)	8	12	6	1 (5A→4A)
Total		960	872 (90.8)	339 (38.9)	533 (61.1)	204	175	154	

[1] 3a and 3b are parts of rice chromosome 3, 3a: from short arm terminal to 30.3Mb; 3b: from 30.3Mb to long arm terminal.

[2] Simplified rice and wheat synteny with reference to Gale and Devos (1998) and Sorrells et al. (2003).

[3] percentage of the total primer sets; [4] percentage of the 872 primer sets.

[5] number of primer sets that amplified products from previously reported translocation region (Nelson et al. 1995).

Table 2. Number of wheat loci detected by the PLUG markers

Genome	Group 1	2	3	4	5	6	7	Total
A	38	43	56	51	50	32	71	341
B	41	43	66	43	56	36	65	350
D	42	42	46	36	46	39	74	325
Total	121	128	168	130	152	107	210	1,016

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