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A Microsatellite Map of Wheat

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ABSTRACT

Hexaploid bread wheat (*Triticum aestivum* L. em. Thell) is one of the world's most important crop plants and displays a very low level of intraspecific polymorphism. We report the development of highly polymorphic microsatellite markers using procedures optimized for the large wheat genome. The isolation of microsatellite-containing clones from hypomethylated regions of the wheat genome increased the proportion of useful markers almost twofold. The majority (80%) of primer sets developed are genome-specific and detect only a single locus in one of the three genomes of bread wheat (A, B, or D). Only 20% of the markers detect more than one locus. A total of 279 loci amplified by 230 primer sets were placed onto a genetic framework map composed of RFLPs previously mapped in the reference population of the International Triticeae Mapping Initiative (ITMI) Opata 85 × W7984. Sixty-five microsatellites were mapped at a LOD >2.5, and 214 microsatellites were assigned to the most likely intervals. Ninety-three loci were mapped to the A genome, 115 to the B genome, and 71 to the D genome. The markers are randomly distributed along the linkage map, with clustering in several centromeric regions.

WHEAT (*Triticum aestivum* L. em. Thell.) is one of the most important food crops in the world, and understanding its genetics and genome organization using molecular markers is of great value for genetic and plant breeding purposes. It is an allohexaploid ($2n = 6x = 42$) with the three genomes A, B, and D and has an extremely large genome of 16×10^9 bp/1C (Bennett and Smith 1976) with more than 80% repetitive DNA. Detailed RFLP (restriction fragment length polymorphism) linkage maps (Chao *et al.* 1989; Devos and Gale 1993; Xie *et al.* 1993; Nelson *et al.* 1995a,b,c; Van Deynze *et al.* 1995; Marino *et al.* 1996) and physical maps (Gill *et al.* 1993; Kota *et al.* 1993; Hohmann *et al.* 1994; Ogihara *et al.* 1994; Delaney *et al.* 1995a,b; Mickelson-Young *et al.* 1995; Gill *et al.* 1996) have been published for all seven homoeologous groups.

Although the progress in building wheat genetic maps has been steady, the use of RFLP markers in gene mapping has been slow because of the very limited level of polymorphism in wheat (Chao *et al.* 1989; Kam-Morgan *et al.* 1989; Liu *et al.* 1990; Cadalen *et al.* 1997). Because of this limited polymorphism, gene and genome mapping has required the use of populations derived from wide crosses. However, mapping many agronomically important genes or QTL (quantitative trait loci), a major goal in plant breeding, requires informative markers in an intraspecific context. This is particularly true for marker-assisted selection. RFLPs detected with single-copy genomic and cDNA clones are extremely powerful for comparative mapping approaches (Ahn *et al.* 1993; Moore *et al.* 1995; Sherman *et al.* 1995; Yu *et al.* 1996). They are only of limited use for intraspecific molecular analysis of agronomic traits, however, because usually <10% of all RFLP loci are polymorphic in wheat.

The genomes of all eukaryotes contain a class of sequences, termed microsatellites (Litt and Luty 1989) or simple sequenced repeats (SSRs) (Tautz *et al.* 1986). Microsatellites with tandem repeats of a basic motif of <6 bp have emerged as an important source of ubiquitous genetic markers for many eukaryotic genomes (Wang *et al.* 1994). The analysis of microsatellites is based on the polymerase chain reaction (PCR), which is much easier to perform than RFLP analysis and is highly amenable to automation. In plants, it has been demonstrated that microsatellites are highly informative, locus-specific markers in many species (Condit and Hubbell 1991; Akkaya *et al.* 1992; Lagercrantz *et al.* 1993; Senior and Heun 1993; Wu and Tanksley 1993; Bell and Ecker 1994; Saghai-Maroof *et al.* 1994; Rongwen *et al.* 1995; Liu *et al.* 1996; Mörchen *et al.* 1996; Provan *et al.* 1996; Szewc-McFadden *et al.* 1996; Taramino and Tingey 1996; Smulders *et al.* 1997). Because they are multiallelic, microsatellites have high potential for use in evolutionary studies (Schloetterer *et al.* 1991; Buchanan *et al.* 1994) and studies regarding genetic relationships.

Microsatellites show a much higher level of polymor-

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The primer sequences described in this article are available for public research only. Requests for commercial use of the primer pairs should be directed to the corresponding author.

phism and informativeness in hexaploid bread wheat than any other marker system (Plaschke *et al.* 1995; Röder *et al.* 1995; Ma *et al.* 1996; Bryan *et al.* 1997). However, due to the large genome size, the development of microsatellite markers in wheat is extremely time-consuming and expensive. Only 30% of all primer pairs developed from microsatellite sequences are functional and suitable for genetic analysis (Röder *et al.* 1995; Bryan *et al.* 1997). The majority of such markers are inherited in a codominant manner and, in most cases, they are chromosome-specific. This is a useful feature in a hexaploid genome. In this article, we present the development of 230 polymorphic primer sets and a genetic map of the wheat genome containing 279 microsatellites covering the seven homoeologous chromosome groups.

MATERIALS AND METHODS

Plant material and DNA extraction: The variety Chinese Spring was used as the DNA source for the development of wheat microsatellites. Mapping was performed on 70 recombinant inbred (RI) lines from the International Triticeae Mapping Initiative (ITMI) population. This population was derived by single seed descent (F_8) from the cross of W-7984, an amphihexaploid wheat synthesized from *Triticum tauschii* (DD) and the *T. durum* (AABB) variety Altar 84, with the Mexican wheat variety Opata 85 from CIMMYT (Centro Internacional de Mejoramiento de Maiz y Trigo). The plant material was described in Van Deynze *et al.* (1995), and seeds were kindly provided by M. Sorrells, Cornell University. DNA was extracted from whole seeds as described in Plaschke *et al.* 1995.

Microsatellite marker development: For microsatellite isolation, various phage λ libraries were constructed by cloning Chinese Spring genomic DNA. After digestion with the restriction enzyme *Acs*I, DNA was cloned into the *Eco*RI site of the vector Lambda Zap II (Stratagene, La Jolla, CA) or, alternatively, after digestion with *Mbo*I or *Sau*3A, into the *Bam*HI site of the vector Lambda Zap express (Stratagene) according to the manufacturer's instruction. Initially, total genomic DNA was completely digested and used without size selection. Later, genomic wheat DNA (500 μg) was predigested with the methylation-sensitive restriction enzyme *Pst*I. *Pst*I-digested DNA was separated on preparative agarose gels, and the size range of 2–5 kb was excised and isolated using the GeneClean kit (Dianova). The size-selected DNA was further digested with *Mbo*I and cloned as described above. Unamplified libraries were plated and phage filters were probed with synthetic polymers of GA and GT (Pharmacia, Piscataway, NJ) and then washed to a stringency of 0.5× SSC, 0.1% sodium dodecyl sulfate (SDS) at 65° (Röder *et al.* 1995). Positive plaques were purified and converted into plasmids by *in vivo* excision. Plasmid clones were reconfirmed by colony hybridization and sequenced according to standard procedures using automated laser fluorescence (ALF) DNA sequencers (Pharmacia). Primer pairs flanking the microsatellite motifs were designed using the program Primer 0.5, which was kindly provided by E. Lander (Massachusetts Institute of Technology). The program Primer 0.5 allows checking for known repetitive sequences and exclusion of these sequences in the designated primers. For this purpose a data file was created consisting of published repetitive wheat sequences and of sequences of microsatellite markers that had resulted in a smear after PCR amplification. This data file was routinely used to check for repeated sequences when new primer pairs were developed. One primer was always labeled with fluorescein. If it was not possible to design both primers simultaneously, one fluorescein-labeled primer was designed close to the microsatellite, and further sequence information was obtained in another sequencing reaction using that primer.

A list of all primer sequences and mapped microsatellites, including the microsatellite motif, annealing temperatures (T_m), and allele sizes in the parent lines are presented in the appendix.

Polymerase chain reaction and fragment analysis: PCR reactions were performed in a volume of 25 μl in Perkin-Elmer (Norwalk, CT) thermocyclers. The reaction mixture contained 250 nm of each primer, 0.2 mm of each deoxynucleotide, 1.5 mm $MgCl_2$, 1 unit *Taq* polymerase, and 50–100 ng of template DNA. The mapping reactions were set up using a pipetting robot (Biomek 1000; Beckman, Fullerton, CA). After 3 min at 94°, 45 cycles were performed with 1 min at 94°, 1 min at either 50, 55, or 60° (depending on the individual microsatellite), 2 min at 72°, and a final extension step of 10 min at 72°.

Fragment analysis was carried out on automated laser fluorescence (ALF) sequencers (Pharmacia) using short gel cassettes. Denaturing gels (0.35 mm thick) with 6% polyacrylamide were prepared using SequaGel XR (Biozym). The gels were run in 1× TBE buffer [0.09 m Tris-borate (pH 8.3) and 2 mm EDTA] with 600 V, 50 mA, and 50 W with 2 mW laser power and a sampling interval of 0.84 sec. The gels were reused four to five times. In each lane, fragments with known sizes were included as standards. Fragment sizes were calculated using the computer program Fragment Manager Version 1.2 (Pharmacia) by comparison with the internal size standards.

Approximately 30 microsatellites were mapped using conventional sequencing gels and visualization by silver staining as described by Sourdille *et al.* (1998).

Genetic mapping: The microsatellites were integrated into a framework map composed of 302 RFLP markers. The data for the RFLP markers were kindly provided by C. Nelson and M. Sorrells (Cornell University) and are based on previously published RFLP maps (Nelson *et al.* 1995a,b,c; Van Deynze *et al.* 1995; Marino *et al.* 1996). As far as possible, the RFLP framework was constructed at a LOD of 3.0, and the microsatellite markers were assigned to chromosomes using the "PLACE" command of the computer program MAPMAKER 2.0 (Lander *et al.* 1987). Marker position within the respective chromosome was determined with the "TRY" and "RIPPLE" commands. Centimorgan units were calculated using the Kosambi mapping function (Kosambi 1944). In a few ambiguous cases, additional nulli-tetrasomic analysis of the microsatellite markers was performed as described previously (Röder *et al.* 1995). Mapped wheat microsatellite loci were designated *Xgwm* for "*G*atersleben *w*heat *m*icrosatellite."

RESULTS

Marker development: *Efficacy of microsatellite isolation:* Microsatellite-containing clones were purified from various genomic phage λ libraries containing small inserts (see materials and methods). Primer pairs could be designed for ~54% of the sequenced clones containing GA or GT microsatellites based on hybridization of the plasmid clones. It was not possible to design two primers for the other 46% because of the following reasons: First, 36% of the clones did not contain microsatellite

TABLE 1

Efficiency of different libraries

Restriction enzyme	Functional primer pairs (total tested primer pairs)	Functional primer pairs (%)
*Acs*I	19 (61)	31
*Mbo*I	10 (32)	31
*Pst*I / *Mbo*I	76 (148)	51
*Pst*I / *Acs*I	81 (120)	67
*Eco*RII / *Mbo*I	39 (117)	33

arrays in the sequenced region (usually 400–500 bp from either side). This was due to the fact that a number of clones were much larger than the sequenced region or contained multiple inserts. Second, for 4% of the microsatellites it was not possible to design both primers because the microsatellite was too close to one of the cloning sites. Finally, 6% of the clones contained repeated DNA regions close to the microsatellite site that were detected with the program Primer 0.5.

Functionality of primer pairs: As previously reported (Röder *et al.* 1995; Bryan *et al.* 1997), only ~30% of the primer pairs designed from wheat microsatellite sequences yield functional microsatellite markers. Functionality is defined as amplification of a fragment of the same size as the sequence of the respective clone. Nonfunctional primer pairs amplified either a smear (large numbers of fragments), nothing, or fragments of the wrong size. Fragments with unexpected sizes were usually monomorphic.

Effects of different libraries: The *Acs*I and *Mbo*I libraries yielded a large number of primer pairs that produced a smear after PCR amplification. We assumed that, due to the large genome size of wheat, such a smear was created from microsatellites harbored in repeated DNA.

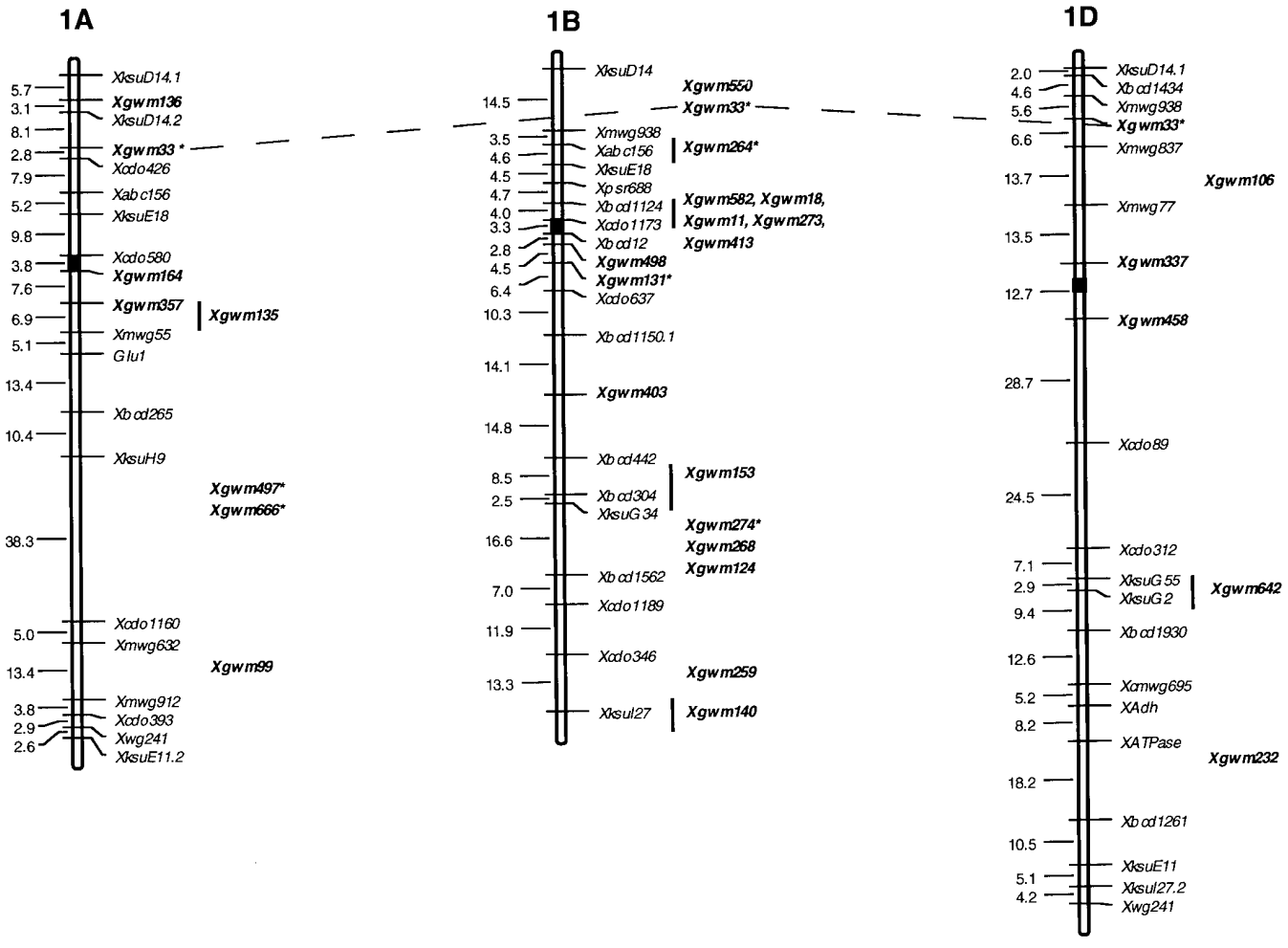


Figure 1.—Molecular linkage map of wheat. Short arms of chromosomes are at the top. The microsatellite loci are indicated in bold and carry the lab designator "*gwm*" (*G*atersleben *w*heat *m*icrosatellite). Microsatellite loci mapped with a LOD >2.5 are integrated in the framework; the other microsatellites were placed in the most probable interval. The centromeres are indicated in black. Primer sets that amplify more than one locus are marked by an asterisk. Dashed lines connect orthologous loci amplified by one microsatellite primer set.

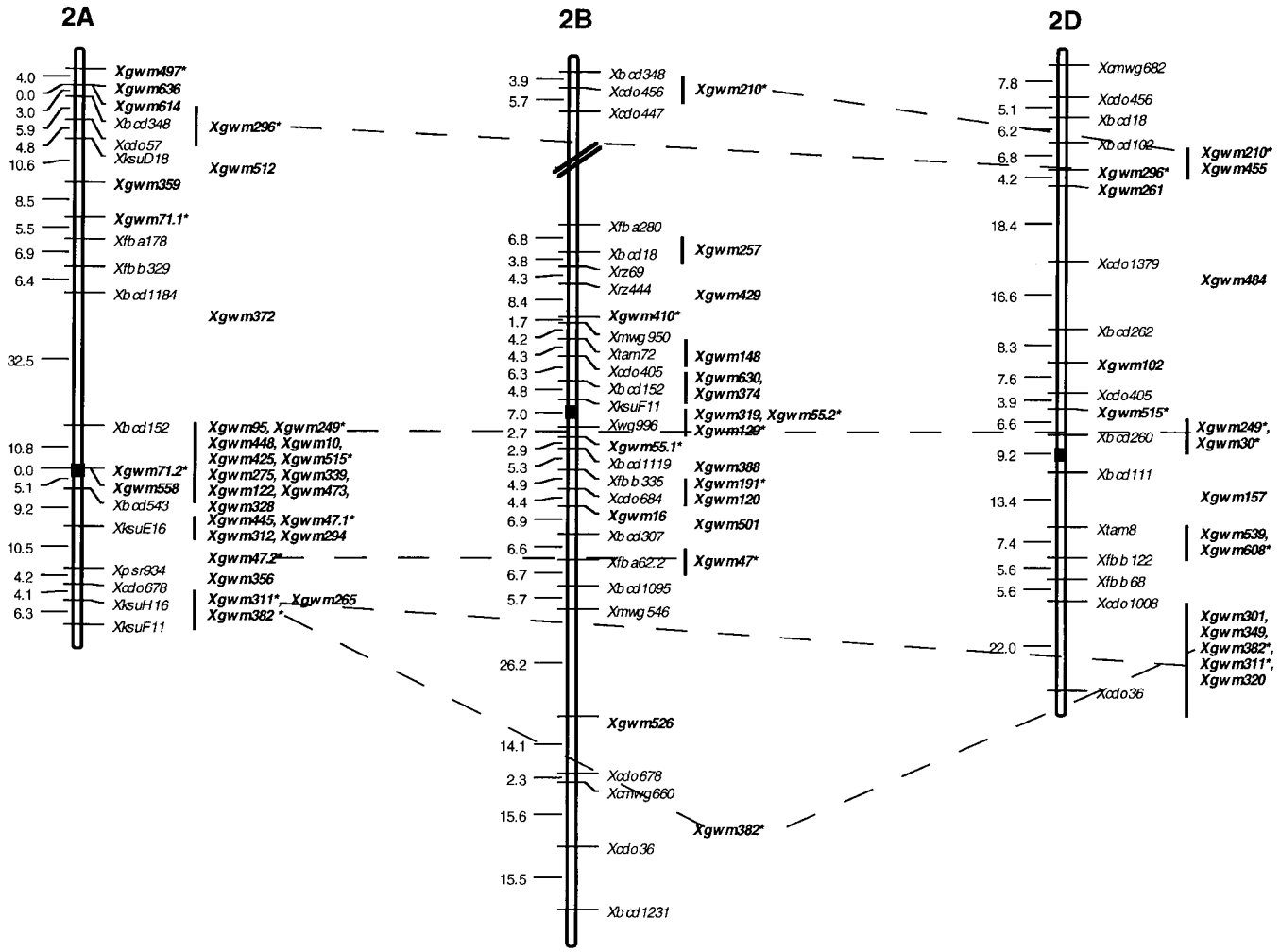


Figure 1.—*Continued.*

We have investigated this by predigesting wheat DNA with the methylation-sensitive restriction enzyme *Pst*I. This enzyme is known to cut preferentially in single-copy DNA of many plant species. By predigestion with *Pst*I and subsequent isolation of the fragments in the size range of 2–5 kb before digestion with a 4-bp restriction enzyme (*Mbo*I or *Sau*3A) and cloning, it was possible to increase the success rate of functional primers from 31 to 67% (Table 1). Using this procedure, the number of primer pairs yielding a smear was reduced significantly. Interestingly, this increase in effectiveness was only obtained by predigestion with *Pst*I. The use of *Eco*RII, another CNG methylation-sensitive restriction enzyme, did not produce this increase in effectiveness. In total, 1380 clones were sequenced, and primer pairs were designed for 720 clones. A total of 294 primer pairs (41%) yielded a discrete fragment of the expected fragment size.

Number and polymorphism of amplified PCR fragments: Eighty percent of the primer pairs amplifying a fragment of the expected size detected polymorphism between Opata 85 and the synthetic wheat W7984, the parents of the RI lines. Of these, ~40% exclusively amplified the expected fragment, 40% amplified mostly one or, in a few cases, several additional monomorphic fragments, and 20% amplified one or several additional polymorphic fragments. Therefore, only one site could be mapped for 80% of the markers, and two or more sites were mappable for 20% of the markers.

The wheat microsatellite map: *Map construction:* The polymorphic microsatellites were integrated into a framework RFLP map of all chromosomes. Only those markers that could be ordered at a LOD score of >2.5 were directly included in the RFLP framework. All other markers were assigned to the most likely interval according to Nelson *et al.* (1995a,b,c). The linkage map is shown in Figure 1. In total, 230 primer sets amplified 279 microsatellites, 65 of which were mapped at a LOD score >2.5 and 214 of which were assigned to intervals on the RFLP map.

In two cases, independently isolated microsatellites appeared to be duplicates that cosegregated and consisted of identical or almost identical sequences. This was the case for *Xgwm213* and *Xgwm335* on chromosome 5B and for *Xgwm269* and *Xgwm565* on chromosome 5D.

The centromeres were positioned according to previously published RFLP maps (Nelson *et al.* 1995a,b,c;

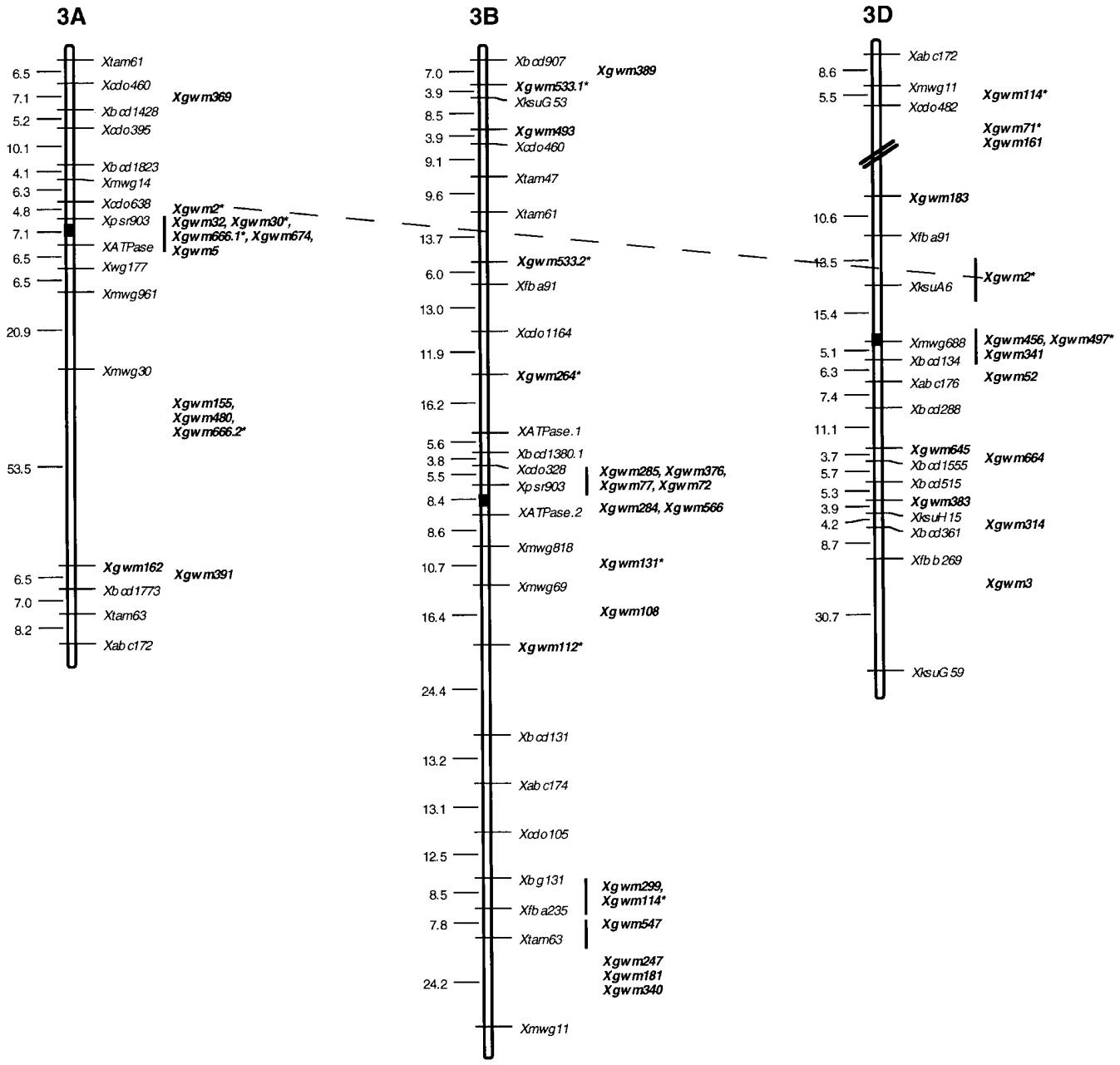


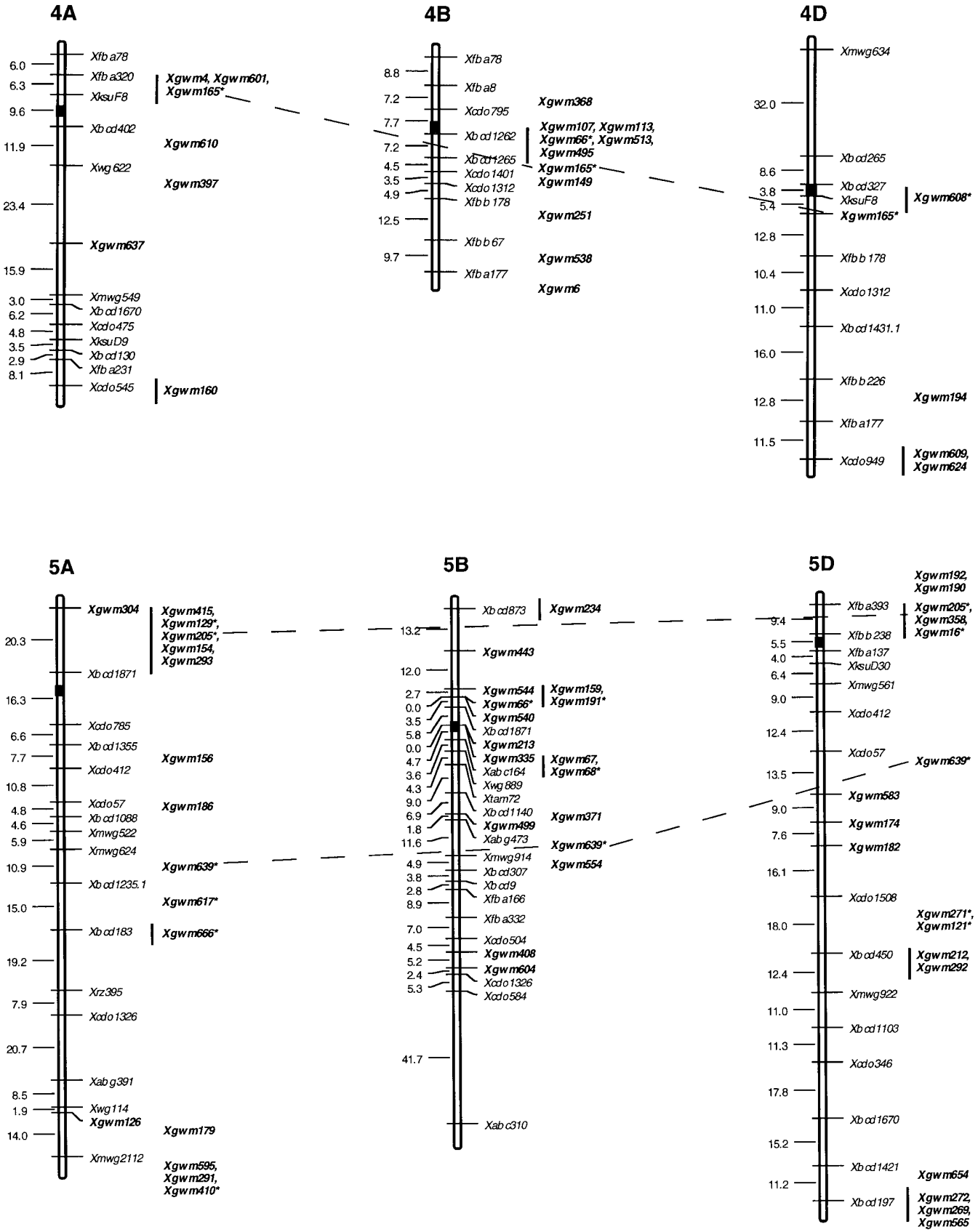
Figure 1.—*Continued.*

Van Deynze *et al.* 1995; Marino *et al.* 1996). In cases where microsatellites mapped in the centromeric region, their chromosomal arm locations were determined by analysis with the respective ditelosomic lines of Chinese Spring.

Compared to the previously published RFLP maps, three changes were made in the framework. These were suggested by new results of nulli-tetrasomic analyses of RFLP markers in the respective chromosomal regions (J. C. Nelson, personal communication). The end of the 2AS linkage group from *Xbcd348.1* to *Xcdo447* was moved to the end of the 2BS linkage group, the end of the 3AL linkage group ranging from *Xabc172.2* to *Xbcd451* was moved to the end of the 3DS linkage group, and the 4AL linkage group from *Xbcd129* to *Xbcd1975* was moved to the end of the 7DS linkage group. These changes were corroborated by nulli-tetrasomic analysis of the microsatellites mapping to the respective chromosomal regions: *Xgwm210-2B* mapped to chromosome 2B, *Xgwm114-3D* to 3D, and *Xgwm635-7D* to chromosome 7D.

The original RFLP framework map was extended by microsatellites mapping outside the outermost RFLP locus on the ends of the 2AS, 5AS, 5AL, 5DS, 6BS, 7AS, 7BS, and 7BL linkage groups.

Genome specificity of microsatellite markers: Only 37 of 230 primer sets produced more than one mappable locus. The majority of 193 microsatellite markers constitute



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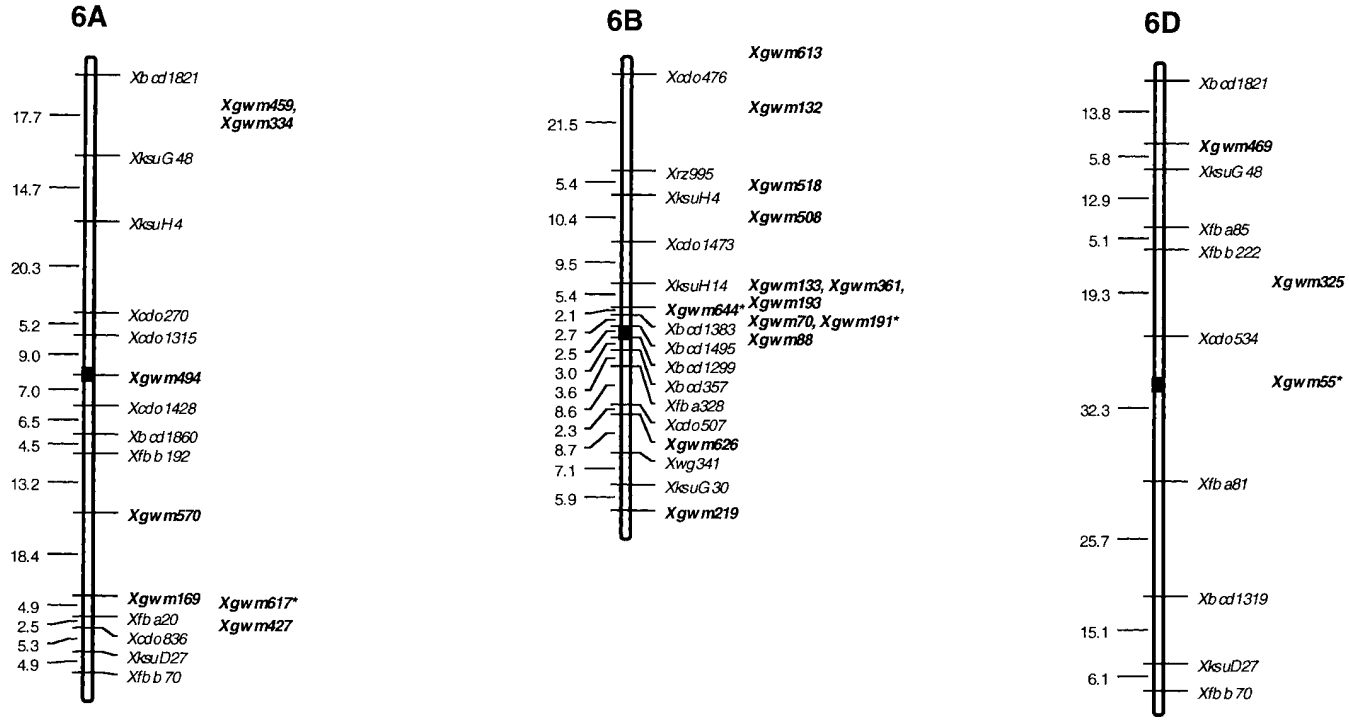

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genome-specific markers. The highest number of loci was detected by *Xgwm666* with five sites, all mapping to the A genome. The primer sets that amplified two or more loci mapped to homoeologous as well as to nonhomoeologous sites. In nine cases, microsatellites mapped to two homoeologous sites, and in four cases they mapped to three homoeologous sites (Figure 1). *Xgwm165* mapped to chromosome arms 4AS, 4BL, and 4DL, thus marking the known chromosome 4A pericentric inversion (Nelson *et al.* 1995c).

The B genome contains the highest number of microsatellites, 115, the A genome 93, and the D genome only 71. Low numbers of microsatellite markers were found in chromosomes 1A, 4A, 6A, 1D, 4D, 6D, and 7D. Along the individual linkage groups, the mapped markers were evenly distributed with no significant clustering except in the centromeric regions of some chromosomes.

DISCUSSION

We present here the first genetic map of the wheat genome based on microsatellites. The development of wheat microsatellites is a tedious task. Primer pairs can be developed for only 54% of the sequenced plasmid clones containing microsatellites. Also, using short insert libraries developed from digestion with 4-bp recognition restriction enzymes, the percentage of useful primer pairs that amplify a polymorphic fragment of the expected size is in the range of 30%. Thus, on average, one out of six purified microsatellite-containing clones yields a functional primer pair. From these data, it is obvious that the development of wheat microsatellites is a tedious process that requires optimization. One possible way to increase the rate of microsatellite-containing clones for which primer pairs can be designed might be the use of libraries that are enriched for microsatellites and/or are size-selected for clones below an insert size of 1000 bp. However, a disadvantage of such enrichment procedures, which is associated with smaller inserts, is the increased frequency of microsatellites too close to one of the cloning sites. Furthermore, enriched libraries carry a considerable risk of obtaining duplicate clones.

We found that an effective way to increase the efficiency of functional primer pairs is to use the undermethylated fraction of the wheat genome as a source for microsatellite isolation. As has been shown for the isolation of single-copy RFLP clones from plants with large genomes, predigestion with the CNG methylation-sensitive restriction enzyme *Pst*I creates a fraction that is highly enriched for low- and single-copy DNA. Using this DNA fraction as a source for microsatellite clones, it was possible to reduce the number of microsatellite clones derived from repeated DNA and thus effectively double the number of functional microsatellites isolated from the wheat genome. Interestingly, the use of the similarly CNG methylation-sensitive enzyme *Eco*RII did not yield this increase in effectiveness. At the moment, it is not clear why such differences between CNG methylation-sensitive restriction enzymes exist.

The identification and mapping of 279 microsatellites

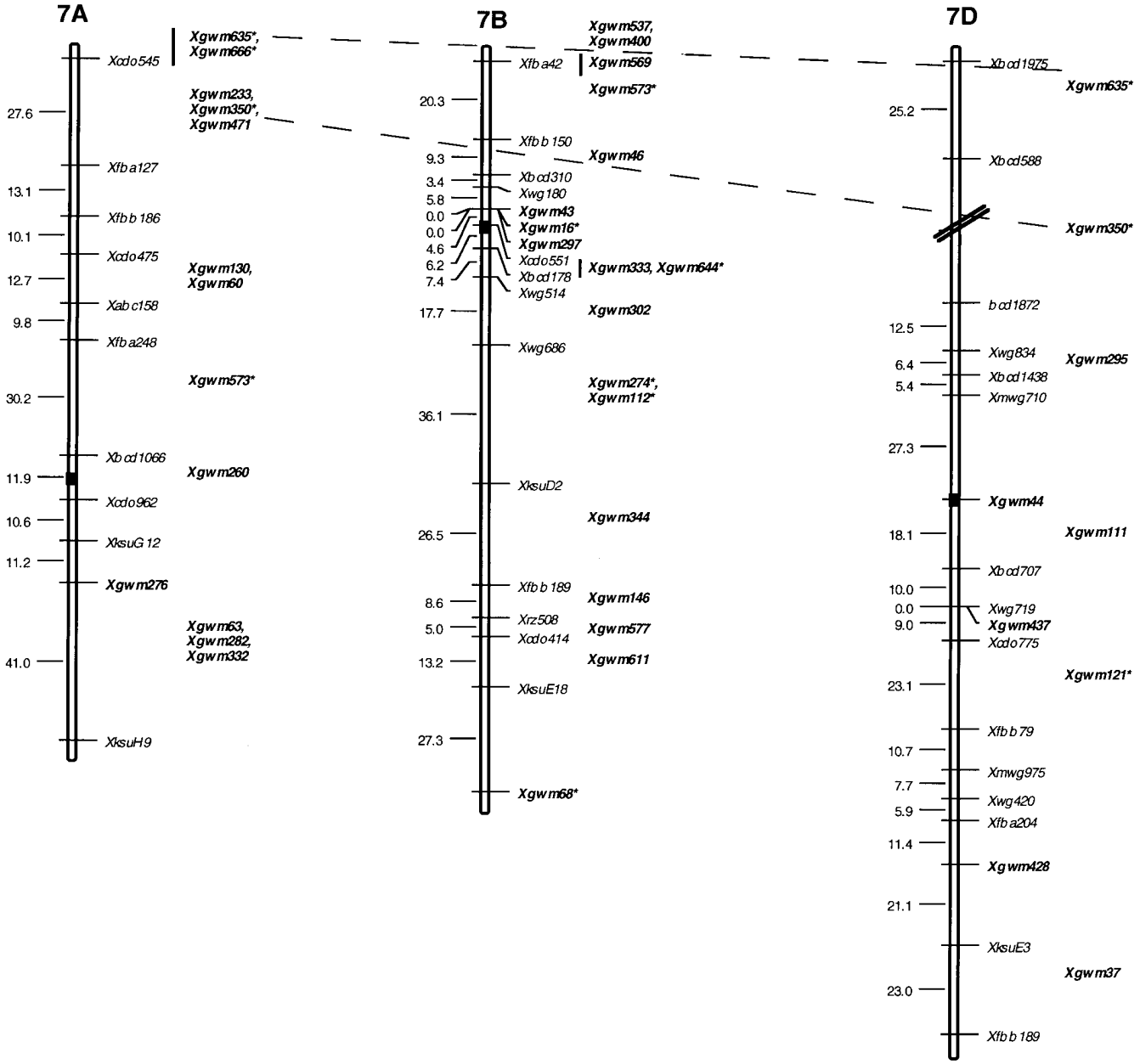

Figure 1.—*Continued.*

amplified with 230 primer sets demonstrates that wheat microsatellites are mainly genome-specific and that microsatellite primer sets usually amplify only a single locus from one of the three genomes. Wheat microsatellite primer sets were successfully used for the amplification of DNA from wild progenitors or relatives of bread wheat *T. monococcum*, *T. boeoticum*, *T. urartu* (V. Korzun and M.-H. Tixier, unpublished data), *T. dicoccoides* (Fahima *et al.* 1998), *T. durum*, and *T. aethiopicum* (Plaschke *et al.* 1995). This indicates that microsatellite sequence diversity between the genomes is much higher than between each genome and its diploid and tetraploid ancestors. Only 20% of all primer sets amplify more than a single locus. Of these, approximately one-half amplify orthologous loci. The other one-half amplify loci from nonhomoeologous regions in the wheat genome. One possible explanation for this is that microsatellite markers can be derived from moderately repeated DNA sequences, provided that their primer sequences are sufficiently specific to amplify only a single or very few loci. It is known that a large portion of the Gramineae genomes is composed of ancestral transposable elements such as inactive retrotransposons. If a microsatellite marker resides within such a moderately repetitive element, nonorthologous loci could be amplified.

Of 279 microsatellites, 65 could be integrated into the RFLP framework with a LOD >2.5, whereas 214

microsatellites were assigned to intervals. In the previously published RFLP maps of wheat also <50% of the RFLP markers were mapped with a LOD >3.0 (Nelson *et al.* 1995a,b,c; Van Deynze *et al.* 1995; Marino *et al.* 1996). One reason for the occurrence of low LOD scores in the mapping population may be, besides very close distances of the markers, a considerable amount of residual heterozygosity in the recombinant inbred (RI) lines. For mapping of the RFLPs and the microsatellites, different generations of RIs were used, which might lead to different levels of heterozygosity in the same RI lines. Furthermore, for the mapping of microsatellites, only 70 plants were used, although the RFLP framework is composed of data for 114 plants. This results in a reduced amount of mapping information for the microsatellite markers related to the RFLPs.

Microsatellites in hexaploid wheat are fairly evenly distributed along the linkage groups. We have not observed a significant clustering of such markers, with the exception of several centromeric regions on chromosomes 2A, 3A, 3B, 4B, 5B, and 6B. Thus, microsatellites are useful for complete coverage of the wheat genome in the same way as RFLP markers. Data from physical mapping of microsatellites on deletion stocks of group 2 chromosomes (Röder *et al.* 1998) confirm that microsatellites are not physically clustered in specific regions of the wheat chromosomes. This situation is similar to the results found for other Gramineae and is clearly different from their chromosomal location in sugar beet and tomato. In these two species, microsatellites are heavily clustered around the centromeres (Schmidt and Heslop-Harrison 1996; T. Areshchenkova and M. W. Ganal, unpublished results).

Of the 279 microsatellites, 93 mapped to the A genome, 115 to the B genome, and 71 to the D genome. The percentage of markers assigned to the respective genomes and chromosomes is in good agreement with the numbers obtained for RFLP markers (Marino *et al.* 1996) and thus reflects mainly the amount of polymorphism within the different genomes in the ITMI mapping population, rather than an unequal distribution of microsatellites. In order to increase the number of A or D genome microsatellites, they could be isolated from *T. monococcum* or *T. tauschii*. Preliminary data suggest that by using the diploid ancestors as a source for microsatellite isolation, it is possible to specifically enrich for microsatellites from the D genome (M. S. Röder, unpublished results).

Most of the published molecular maps of wheat include only a few mutant loci and agronomically important genes. The main reason for this is that the use of RFLPs and isozyme markers for mapping has been inefficient because of a low level of allelic variation (<10%) among cultivated varieties (Chao *et al.* 1989; Kam-Morgan *et al.* 1989). In addition, RFLP assays require large quantities of DNA and are technically demanding and laborious, and the most common detection method uses radioisotopes. In contrast, microsatellites are abundant, highly polymorphic, evenly distributed over the genome, and require only small amounts of genomic DNA for analysis. Therefore, they are highly suitable as genetic markers in wheat for mapping agronomically important genes. Furthermore, the analysis of microsatellites can easily be automated and applied to large plant numbers, as has been shown for microsatellite analysis in the human genome (Mansfield *et al.* 1994).

The map presented here provides a good starting point for the production of a saturated map of the wheat genome based on microsatellites. Microsatellites provide readily detectable markers for agronomically important genes and quantitatively inherited traits and facilitate their handling in segregating breeding populations. Examples for this are the use of microsatellites for molecular mapping of known genes of bread wheat, including the dwarfing genes *Rht8* (Korzun *et al.* 1998) and *Rht12* (Korzun *et al.* 1997) in chromosome arms 2DS and 5AL and the major vernalization genes *Vrn1*, *Vrn2*, and *Vrn3* (V. Korzun, unpublished data) in chromosome arms 5AL, 5BL, and 5DL, respectively.

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APPENDIX

Description of wheat microsatellite primer sets and loci

Locus	Left primer	Right primer	Repeat	An. temp.	Opata (bp)	Synth. (bp)
Xgwm2-3A	CTG CAA GCC TGT GAT CAA CT	CAT TCT CAA ATG ATC GAA CA	(CA)18	50°	128	130
Xgwm2-3D	CTG CAA GCC TGT GAT CAA CT	CAT TCT CAA ATG ATC GAA CA	(CA)18	50°	265	267
Xgwm3-3D	GCA GCG GCA CTG GTA CAT TT	AAT ATC GCA TCA CTA TCC CA	(CA)18	55°	84	—
Xgwm4-4A	GCT GAT GCA TAT AAT GCT GT	CAC TGT CTG TAT CAC TCT GCT	(CA)13(TA)26	55°	257	255
Xgwm5-3A	GCC AGC TAC CTC GAT ACA ACT C	AGA AAG GGC CAG GCT AGT AGT	(TC)23(T)4(GT)12(GA)10	50°	171	158
Xgwm6-4B	CGT ATC ACC TCC TAG CTA AAC TAG	AGC CTT ATC ATG ACC CTA CCT T	(GA)40	55°	207	196
Xgwm10-2A	CGC ACC ATC TGT ATC ATT CTG	TGG TCG TAC CAA AGT ATA CGG	(AT)5(GT)15	50°	138	143
Xgwm11-1B	GGA TAG TCA GAC AAT TCT TGT G	GTG AAT TGT GTC TTG TAT GCT TCC	(TA)6CATA(CA)19(TA)6	50°	202	213
Xgwm16-2B	GCT TGG ACT AGC TAG AGT ATC ATA C	CAA TCT TCA ATT CTG TCG CAC GG	(C)12ACAAA(CA)14(GA)18	50°	181	176
Xgwm16-5D	GCT TGG ACT AGC TAG AGT ATC ATA C	CAA TCT TCA ATT CTG TCG CAC GG	(C)12ACAAA(CA)14(GA)18	50°	224	225
Xgwm16-7B	GCT TGG ACT AGC TAG AGT ATC ATA C	CAA TCT TCA ATT CTG TCG CAC GG	(C)12ACAAA(CA)14(GA)18	50°	206	204
Xgwm18-1B	TGG CGC CAT GAT TGC ATT ATC TTC	GGT TGC TGA AGA ACC TTA TTT AGG	(CA)17GA(TA)4	50°	188	182
Xgwm30-2D	ATC TTA GCA TAG AAG GGA GTG GG	TTC TGC ACC CTG GGT GAT	(AT)19(GT)15	60°	—	156
Xgwm30-3A	ATC TTA GCA TAG AAG GGA GTG GG	TTC TGC ACC CTG GGT GAT	(AT)19(GT)15	60°	196	205
Xgwm32-3A	TAT GCC GAA TTT GTG GAC AA	TGC TTG GTC TTG AGC ATC AC	(GA)19	55°	169	173
Xgwm33-1A	GGA GTC ACA CTT GTT TGT GCA	CAC TGC ACA CCT AAC TAC CTG C	(GA)19	60°	116	—
Xgwm33-1B	GGA GTC ACA CTT GTT TGT GCA	CAC TGC ACA CCT AAC TAC CTG C	(GA)19	60°	—	119
Xgwm33-1D	GGA GTC ACA CTT GTT TGT GCA	CAC TGC ACA CCT AAC TAC CTG C	(GA)19	60°	—	158
Xgwm37-7D	ACT TCA TTG TTG ATC TTG CAT G	CGA CGA ATT CCC AGC TAA AC	(AG)8GG(AG)21	60°	189	—
Xgwm43-7B	CAC CGA CGG TTT CCC TAG AGT	GGT GAG TGC AAA TGT CAT GTG	(CA)22	60°	184	176
Xgwm44-7D	GTT GAG CTT TTC AGT TCG GC	ACT GGC ATC CAC TGA GCT G	(GA)28	60°	178	176
Xgwm46-7B	GCA CGT GAA TGG ATT GGA C	TGA CCC AAT AGT GGT GGT CA	(GA)2GC(GA)33	60°	186	179
Xgwm47.1-2A	TTG CTA CCA TGC ATG ACC AT	TTC ACC TCG ATT GAG GTC CT	(CT)7TT(CT)16	60°	—	170
Xgwm47.2-2A	TTG CTA CCA TGC ATG ACC AT	TTC ACC TCG ATT GAG GTC CT	(CT)7TT(CT)16	60°	150	—
Xgwm47-2B	TTG CTA CCA TGC ATG ACC AT	TTC ACC TCG ATT GAG GTC CT	(CT)7TT(CT)16	60°	—	188
Xgwm52-3D	CTA TGA GGC GGA GGT TGA AG	TGC GGT GCT CTT CCA TTT	(GT)4AT(GT)20	60°	142	128
Xgwm55.1-2B	GCA TCT GGT ACA CTA GCT GCC	TCA TGG ATG CAT CAC ATC CT 3	(TC)3(T)3(CT)17	60°	122	118
Xgwm55.2-2B	GCA TCT GGT ACA CTA GCT GCC	TCA TGG ATG CAT CAC ATC CT 3	(TC)3(T)3(CT)17	60°	161	149
Xgwm55-6D	GCA TCT GGT ACA CTA GCT GCC	TCA TGG ATG CAT CAC ATC CT 3	(TC)3(T)3(CT)17	60°	128	132
Xgwm60-7A	TGT CCT ACA CGG ACC ACG T	GCA TTG ACA GAT GCA CAC G	(CA)30	60°	190	224
Xgwm63-7A	TCG ACC TGA TCG CCC CTA	CGC CCT GGG TGA TGA ATA GT	(CA)17(TA)21	60°	269	271
Xgwm66-4B	CCA AAG ACT GCC ATC TTT CA	CAT GAC TAG CTA GGG TGT GAC A	(CA)30(TA)21	60°	—	218
Xgwm66-5B	CCA AAG ACT GCC ATC TTT CA	CAT GAC TAG CTA GGG TGT GAC A	(CA)30(TA)21	60°	158	137
Xgwm67-5B	ACC ACA CAA ACA AGG TAA GCG	CAA CCC TCT TAA TTT TGT TGG G	(CA)10	60°	94	92
Xgwm68-5B	AGG CCA GAA TCT GGG AAT G	CTC CCT AGA TGG GAG AAG GG	(GA)3(G)3(GA)25	60°	—	166
Xgwm68-7B	AGG CCA GAA TCT GGG AAT G	CTC CCT AGA TGG GAG AAG GG	(GA)3(G)3(GA)25	60°	—	180
Xgwm70-6B	AGT GGC TGG GAG AGT GTC AT	GCC CAT TAC CGA GGA CAC	(GT)7GC(GT)11	60°	197	194
Xgwm71.1-2A	GGC AGA GCA GCG AGA CTC	CAA GTG GAG CAT TAG GTA CAC G	(GT)20	60°	126	124
Xgwm71.2-2A	GGC AGA GCA GCG AGA CTC	CAA GTG GAG CAT TAG GTA CAC G	(GT)20	60°	120	118
Xgwm71-3D	GGC AGA GCA GCG AGA CTC	CAA GTG GAG CAT TAG GTA CAC G	(GT)20	60°	—	101

(*continued*)

APPENDIX

(Continued)

Locus	Left primer	Right primer	Repeat	An. temp.	Opata (bp)	Synth. (bp)
Xgwm72-3B	TGG TCC CTC TCC CTT TCT CT	ACA GAA TTG AAG ATT GTC GGT C	(CT)48imp	55°	148	136
Xgwm77-3B	ACA AAG GTA AGC AGC ACC TG	ACC CTC TTG CCC GTG TTG	(CA)10(GA)40imp	60°	—	135
Xgwm88-6B	CAC TAC AAC TAT GCG CTC GC	TCC ATT GGC TTC TCT CTC AA	(GT)18TT(GA)4	60°	162	—
Xgwm95-2A	GAT CAA ACA CAC ACC CCT CC	AAT GCA AAG TGA AAA ACC CG	(AC)16	60°	128	116
Xgwm99-1A	AAG ATG GAC GTA TGC ATC ACA	GCC ATA TTT GAT GAC GCA TA	(CA)21	60°	117	120
Xgwm102-2D	TCT CCC ATC CAA CGC CTC	5′TGT TGG TGG CTT GAC TAT TG	(CT)15	60°	153	145
Xgwm106-1D	CTG TTC TTG CGT GGC ATT AA	AAT AAG GAC ACA ATT GGG ATG G	(GA)24	60°	—	81
Xgwm107-4B	ATT AAT ACC TGA GGG AGG TGC	GGT CTC AGG AGC AAG AAC AC	(CT)21	60°	188	—
Xgwm108-3B	CGA CAA TGG GGT CTT AGC AT	TGC ACA CTT AAA TTA CAT CCG C	(GT)35imp	60°	135	137
Xgwm111-7D	TCT GTA GGC TCT CTC CGA CTG	ACC TGA TCA GAT CCC ACT CG	(CT)32(GT)17	55°	206	184
Xgwm112-3B	CTA AAC ACG ACA GCG GTG G	GAT ATG TGA GCA GCG GTC AG	(CT)8GT(CT)20	55°	83	81
Xgwm112-7B	CTA AAC ACG ACA GCG GTG G	GAT ATG TGA GCA GCG GTC AG	(CT)8GT(CT)20	55°	101	99
Xgwm113-4B	ATT CGA GGT TAG GAG GAA GAG G	GAG GGT CGG CCT ATA AGA CC	(GT)12	55°	148	156
Xgwm114-3B	ACA AAC AGA AAA TCA AAA CCC G	ATC CAT CGC CAT TGG AGT G	(GA)53	60°	168	142
Xgwm114-3D	ACA AAC AGA AAA TCA AAA CCC G	ATC CAT CGC CAT TGG AGT G	(GA)53	60°	134	181
Xgwm120-2B	GAT CCA CCT TCC TCT CTC TC	GAT TAT ACT GGT GCC GAA AC	(CT)11(CA)18	60°	162	174
Xgwm121-5D	TCC TCT ACA AAC AAA CAC AC	CTC GCA ACT AGA GGT GTA TG	(CAAA)2(CA)28	50°	107	104
Xgwm121-7D	TCC TCT ACA AAC AAA CAC AC	CTC GCA ACT AGA GGT GTA TG	(CAAA)2(CA)28	50°	141	143
Xgwm122-2A	GGG TGG GAG AAA GGA GAT G	AAA CCA TCC TCC ATC CTG G	(CT)11(CA)31	60°	147	131
Xgwm124-1B	GCC ATG GCT ATC ACC CAG	ACT GTT CGG TGC AAT TTG AG	(CT)27(GT)18imp	60°	190	197
Xgwm126-5A	CAC ACG CTC CAC CAT GAC	GTT GAG TTG ATG CGG GAG G	(CA)15	60°	196	—
Xgwm129-2B	TCA GTG GGC AAG CTA CAC AG	AAA ACT TAG TAG CCG CGT	(GT)8(N)28(GT)16	50°	—	223
Xgwm129-5A	TCA GTG GGC AAG CTA CAC AG	AAA ACT TAG TAG CCG CGT	(GT)8(N)28(GT)16	50°	217	220
Xgwm130-7A	AGC TCT GCT TCA CGA GGA AG	CTC CTC TTT ATA TCG CGT CCC	(GT)22	60°	126	121
Xgwm131-1B	AAT CCC CAC CGA TTC TTC TC	AGT TCG TGG GTC TCT GAT GG	(CT)22	60°	165	157
Xgwm131-3B	AAT CCC CAC CGA TTC TTC TC	AGT TCG TGG GTC TCT GAT GG	(CT)22	60°	—	95
Xgwm132-6B	TAC CAA ATC GAA ACA CAT CAG G	CAT ATC AAG GTC TCC TTC CCC	(GA)24(GAA)6imp	60°	118	116
Xgwm133-6B	ATC TAA ACA AGA CGG CGG TG	ATC TGT GAC AAC CGG TGA GA	(CT)39imp	60°	128	124
Xgwm135-1A	TGT CAA CAT CGT TTT GAA AAG G	ACA CTG TCA ACC TGG CAA TG	(GA)20	60°	153	176
Xgwm136-1A	GAC AGC ACC TTG CCC TTT G	CAT CGG CAA CAT GCT CAT C	(CT)58	60°	278	321
Xgwm140-1B	ATG GAG ATA TTT GGC CTA CAA C	CTT GAC TTC AAG GCG TGA CA	(CT)42	55°	223	233
Xgwm146-7B	CCA AAA AAA CTG CCT GCA TG	CTC TGG CAT TGC TCC TTG G	(GA)5GG(GA)20	60°	174	—
Xgwm148-2B	GTG AGG CAG CAA GAG AGA AA	CAA AGC TTG ACT CAG ACC AAA	(CA)22	60°	165	167
Xgwm149-4B	CAT TGT TTT CTG CCT CTA GCC	CTA GCA TCG AAC CTG AAC AAG	(GA)23imp	55°	161	152
Xgwm153-1B	GAT CTC GTC ACC CGG AAT TC	TGG TAG AGA AGG ACG GAG AG	(GA)18	60°	183	195
Xgwm154-5A	TCA CAG AGA GAG AGG GAG GG	ATG TGT ACA TGT TGC CTG CA	(GA)37imp	55°	102	120
Xgwm155-3A	CAA TCA TTT CCC CCT CCC	AAT CAT TGG AAA TCC ATA TGC C	(CT)19	60°	143	127
Xgwm156-5A	CCA ACC GTG CTA TTA GTC ATT C	CAA TGC AGG CCC TCC TAA C	(GT)14	60°	300	279
Xgwm157-2D	GTC GTC GCG GTA AGC TTG	GAG TGA ACA CAC GAG GCT TG	(CT)14	60°	106	110
Xgwm159-5B	GGG CCA ACA CTG GAA CAC	GCA GAA GCT TGT TGG TAG GC	(GT)15	60°	189	187

(*continued*)

APPENDIX

(Continued)

Locus	Left primer	Right primer	Repeat	An. temp.	Opata (bp)	Synth. (bp)
Xgwm160-4A	TTC AAT TCA GTC TTG GCT TGG	CTG CAG GAA AAA AAG TAC ACC C	(GA)21	60°	184	196
Xgwm161-3D	GAT CGA GTG ATG GCA GAT GG	TGT GAA TTA CTT GGA CGT GG	(CT)15	60°	154	145
Xgwm162-3A	AGT GGA TCG ACA AGG CTC TG	AGA AGA AGC AAA GCC TTC CC	(CA)14AA(CA)4	60°	202	208
Xgwm164-1A	ACA TTT CTC CCC CAT CGT C	TTG TAA ACA AAT CGC ATG CG	(CT)16	55°	122	128
Xgwm165-4A	TGC AGT GGT CAG ATG TTT CC	CTT TTC TTT CAG ATT GCG CC	(GA)20	60°	188	193
Xgwm165-4B	TGC AGT GGT CAG ATG TTT CC	CTT TTC TTT CAG ATT GCG CC	(GA)20	60°	257	261
Xgwm165-4D	TGC AGT GGT CAG AGT TTT CC	CTT TTC TTT CAG ATT GCG CC	(GA)20	60°	197	—
Xgwm169-6A	ACC ACT GCA GAG AAC ACA TAC G	GTG CTC TGC TCT AAG TGT GGG	(GA)23	60°	220	193
Xgwm174-5D	GGG TTC CTA TCT GGT AAA TCC C	GAC ACA CAT GTT CCT GCC AC	(CT)22	55°	233	204
Xgwm179-5A	AAG TTG AGT TGA TGC GGG AG	CCA TGA CCA GCA TCC ACT C	(GT)15	55°	181	—
Xgwm181-3B	TCA TTG GTA ATG AGG AGA GA	GAA CCA TTC ATG TGC ATG TC	(GA)28	50°	150	168
Xgwm182-5D	TGA TGT AGT GAG CCC ATA GGC	TTG CAC ACA GCC AAA TAA GG	(CT)18	60°	163	187
Xgwm183-3D	GTC TTC CCA TCT CGC AAG AG	CTC GAC TCC CAT GTG GAT G	(GA)21(N)51(C)25	55°	—	105
Xgwm186-5A	GCA GAG CCT GGT TCA AAA AG	CGC CTC TAG CGA GAG CTA TG 5′	(GA)26	60°	132	106
Xgwm190-5D	GTG CTT GCT GAG CTA TGA GTC	GTG CCA CGT GGT ACC TTT G	(CT)22	60°	201	253
Xgwm191-2B	AGA CTG TTG TTT GCG GGC	TAG CAC GAC AGT TGT ATG CAT G	(CT)19	60°	117	122
Xgwm191-5B	AGA CTG TTG TTT GCG GGC	TAG CAC GAC AGT TGT ATG CAT G	(CT)19	60°	110	107
Xgwm191-6B	AGA CTG TTG TTT GCG GGC	TAG CAC GAC AGT TGT ATG CAT G	(CT)19	60°	128	134
Xgwm192-5D	GGT TTT CTT TCA GAT TGC GC	CGT TGT CTA ATC TTG CCT TGC	(CT)46	60°	191	232
Xgwm193-6B	CTT TGT GCA CCT CTC TCT CC	AAT TGT GTT GAT GAT TTG GGG	(CT)24imp(CA)8	60°	171	182
Xgwm194-4D	GAT CTG CTC TAC TCT CCT CC	CGA CGC AGA ACT TAA ACA AG	(CT)32imp	50°	136	131
Xgwm205-5A	CGA CCC GGT TCA CTT CAG	AGT CGC CGT TGT ATA GTG CC	(CT)21	60°	158	152
Xgwm205-5D	CGA CCC GGT TCA CTT CAG	AGT CGC CGT TGT ATA GTG CC	(CT)21	60°	—	143
Xgwm210-2B	TGC ATC AAG AAT AGT GTG GAA G	TGA GAG GAA GGC TCA CAC CT	(GA)20	60°	303	—
Xgwm210-2D	TGC ATC AAG AAT AGT GTG GAA G	TGA GAG GAA GGC TCA CAC CT	(GA)20	60°	—	182
Xgwm212-5D	AAG CAA CAT TTG CTG CAA TG	TGC AGT TAA CTT GTT GAA AGG A	(CT)20	60°	102	117
Xgwm213-5B	TGC CTG GCT CGT TCT ATC TC	CTA GCT TAG CAC TGT CGC CC	(GA)35	60°	162	198
Xgwm219-6B	GAT GAG CGA CAC CTA GCC TC	GGG GTC CGA GTC CAC AAC	(GA)35imp	60°	184	153
Xgwm232-1D	ATC TCA ACG GCA AGC CG	CTG ATG CAA GCA ATC CAC C	(GA)19	55°	140	144
Xgwm233-7A	TCA AAA CAT AAA TGT TCA TTG GA	TCA ACC GTG TGT AAT TTT GTC C	(CT)24	50°	256	264
Xgwm234-5B	GAG TCC TGA TGT GAA GCT GTT G	CTC ATT GGG GTG TGT ACG TG	(CT)16(CA)20	55°	250	229
Xgwm247-3B	GCA ATC TTT TTT CTG ACC ACG	ATG TGC ATG TCG GAC GC	(GA)24	55°	187	198
Xgwm249-2A	CAA ATG GAT CGA GAA AGG GA	CTG CCA TTT TTC TGG ATC TAC C	(GA)11(GGA)8	55°	177	180
Xgwm249-2D	CAA ATG GAT CGA GAA AGG GA	CTG CCA TTT TTC TGG ATC TAC C	(GA)11(GGA)8	55°	154	150
Xgwm251-4B	CAA CTG GTT GCT ACA CAA GCA	GGG ATG TCT GTT CCA TCT TAG	(CA)28	55°	110	109
Xgwm257-2B	AGA GTG CAT GGT GGG ACG	CCA AGA CGA TGC TGA AGT CA	(GT)30	60°	190	192
Xgwm259-1B	AGG GAA AAG ACA TCT TTT TTT TC	CGA CCG ACT TCG GGT TC	(GA)17	55°	105	—
Xgwm260-7A	GCC CCC TTG CAC AA TC	CGC AGC TAC AGG AGG CC	(GA)20	55°	169	165
Xgwm261-2D	CTC CCT GTA CGC CTA AGG C	CTC GCG CTA CTA GCC ATT G	(CT)21	55°	164	194
Xgwm264-1B	GAG AAA CAT GCC GAA CAA CA	GCA TGC ATG AGA ATA GGA ACT G	(CA)9A(CA)24	60°	157	165

(*continued*)

APPENDIX

(Continued)

Locus	Left primer	Right primer	Repeat	An. temp.	Opata (bp)	Synth. (bp)
Xgwm264-3B	GAG AAA CAT GCC GAA CAA CA	GCA TGC ATG AGA ATA GGA ACT G	(CA)9A(CA)24	60°	—	226
Xgwm265-2A	TGT TGC GGA TGG TCA CTA TT	GAG TAC ACA TTT GGC CTC TGC	(GT)23	55°	179	204
Xgwm268-1B	AGG GGA TAT GTT GTC ACT CCA	TTA TGT GAT TGC GTA CGT ACC C	(GA)17TA(GA)27	55°	204	198
Xgwm269-5D	TGC ATA TAA ACA GTC ACA CAC CC	TTT GAG CTC CAA AGT GAG TTA GC	(CA)29	60°	148	126
Xgwm271-5D	CAA GAT CGT GGA GCC AGC	AGC TGC TAG CTT TTG GGA CA	(CT)4imp(GA)10	60°	—	179
Xgwm272-5D	TGC TCT TTG GCG AAT ATA TGG	GTT CAA AAC AAA TTA AAA GGC CC	(CA)17	50°	138	140
Xgwm273-1B	ATT GGA CGG ACA GAT GCT TT	AGC AGT GAG GAA GGG GAT C	(GA)18	55°	171	165
Xgwm274-1B	AAC TTG CAA AAC TGT TCT GA	TAT TTG AAG CGG TTT GAT TT	(GT)27	50°	184	177
Xgwm274-7B	AAC TTG CAA AAC TGT TCT GA	TAT TTG AAG CGG TTT GAT TT	(GT)27	50°	—	154
Xgwm275-2A	AAT TTT CTT CCT CAC TTA TTC T	AAC AAA AAA TTA GGG CC	(CT)21	50°	110	113
Xgwm276-7A	ATT TGC CTG AAG AAA ATA TT	AAT TTC ACT GCA TAC ACA AG	(CT)24	55°	109	101
Xgwm282-7A	TTG GCC GTG TAA GGC AG	TCT CAT TCA CAC ACA ACA CTA GC	(GA)38	55°	274	193
Xgwm284-3B	AAT GAA AAA ACA CTT GCG TGG	GCA CAT TTT TCA CTT TCG GG	(GA)17	60°	121	117
Xgwm285-3B	ATG ACC CTT CTG CCA AAC AC	ATC GAC CGG GAT CTA GCC	(GA)27	60°	222	227
Xgwm291-5A	CAT CCC TAC GCC ACT CTG C	AAT GGT ATC TAT TCC GAC CCG	(CA)35	60°	160	158
Xgwm292-5D	TCA CCG TGG TCA CCG AC	CCA CCG AGC CGA TAA TGT AC	(CT)38	60°	214	188
Xgwm293-5A	TAC TGG TTC ACA TTG GTG CG	TCG CCA TCA CTC GTT CAA G	(CA)24	55°	—	205
Xgwm294-2A	GGA TTG GAG TTA AGA GAG AAC CG	GCA GAG TGA TCA ATG CCA GA	(GA)9TA(GA)15	55°	96	102
Xgwm295-7D	GTG AAG CAG ACC CAC AAC AC	GAC GGC TGC GAC GTA GAG	(GA)25	60°	254	258
Xgwm296-2D	AAT TCA ACC TAC CAA TCT CTG	GCC TAA TAA ACT GAA AAC GAG	(CT)28	55°	182	—
Xgwm296-2A	AAT TCA ACC TAC CAA TCT CTG	GCC TAA TAA ACT GAA AAC GAG	(CT)28	55°	165	157
Xgwm297-7B	ATC GTC ACG TAT TTT GCA ATG	TGC GTA AGT CTA GCA TTT TCT G	(GT)12(GA)18	55°	150	168
Xgwm299-3B	ACT ACT TAG GCC TCC CGC C	TGA CCC ACT TGC AAT TCA TC	(GA)31(TAG)4	55°	206	215
Xgwm301-2D	GAG GAG TAA GAC ACA TGC CC	GTG GCT GGA GAT TCA GGT TC	(GA)31(G)12	55°	—	171
Xgwm302-7B	GCA AGA AGC AAC AGC AGT AAC	CAG ATG CTC TTC TCT GCT GG	(GA)21	60°	277	286
Xgwm304-5A	AGG AAA CAG AAA TAT CGC GG	AGG ACT GTG GGG AAT GAA TG	(CT)22	55°	202	208
Xgwm311-2A	TCA CGT GGA AGA CGC TCC	CTA CGT GCA CCA CCA TTT TG	(GA)29	60°	—	120
Xgwm311-2D	TCA CGT GGA AGA CGC TCC	CTA CGT GCA CCA CCA TTT TG	(GA)29	60°	157	143
Xgwm312-2A	ATC GCA TGA TGC ACG TAG AG	ACA TGC ATG CCT ACC TAA TGG	(GA)37	60°	216	219
Xgwm314-3D	AGG AGC TCC TCT GTG CCA C	TTC GGG ACT CTC TTC CCT G	(CT)25imp	55°	182	171
Xgwm319-2B	GGT TGC TGT ACA AGT GTT CAC G	CGG GTG CTG TGT GTA ATG AC	(CT)11(N)23(CT)6	55°	170	168
Xgwm320-2D	CGA GAT ACT ATG GAA GGT GAG G	ATC TTT GCA AGG ATT GCC C	(GT)9(GA)15	55°	—	226
Xgwm325-6D	TTT CTT CTG TCG TTC TCT TCC C	TTT TTA CGC GTC AAC GAC G	(CT)16	60°	133	138
Xgwm328-2A	GCA ATC CAC GAG AAG AGA GG	CAC AAA CTC TTG ACA TGT GCG	(GT)14	55°	191	193
Xgwm332-7A	AGC CAG CAA GTC ACC AAA AC	AGT GCT GGA AAG AGT AGT GAA GC	(GA)36	60°	290	211
Xgwm333-7B	GCC CGG TCA TGT AAA ACG	TTT CAG TTT GCG TTA AGC TTT G	(GA)19	55°	154	166
Xgwm334-6A	AAT TTC AAA AAG GAG AGA GA	AAC ATG TGT TTT TAG CTA TC	(GA)19	50°	114	110
Xgwm335-5B	CGT ACT CCA CTC CAC ACG G	CGG TCC AAG TGC TAC CTT TC	(GA)14(GCGT)3	55°	203	240
Xgwm337-1D	CCT CTT CCT CCC TCA CTT AGC	TGC TAA CTG GCC TTT GCC	(CT)5(CACT)6(CA)43	55°	191	182
Xgwm339-2A	AAT TTT CTT CCT CAC TTA TT	AAA CGA ACA ACC ACT CAA TC	(CT)22	50°	162	166

(*continued*)

APPENDIX

(Continued)

Locus	Left primer	Right primer	Repeat	An. temp.	Opata (bp)	Synth. (bp)
Xgwm340-3B	GCA ATC TTT TTT CTG ACC ACG	ACG AGG CAA GAA CAC ACA TG	(GA)26	60°	159	—
Xgwm341-3D	TTC AGT GGT AGC GGT CGA G	CCG ACA TCT CAT GGA TCC AC	(CT)26	55°	166	157
Xgwm344-7B	CAA GGA AAT AGG CGG TAA CT	ATT TGA GTC TGA AGT TTG CA	(GT)24	55°	121	—
Xgwm349-2D	GGC TTC CAG AAA ACA ACA GG	ATC GGT GCG TAC CAT CCT AC	(GA)34	55°	243	—
Xgwm350-7A	ACC TCA TCC ACA TGT TCT ACG	GCA TGG ATA GGA CGC CC	(GT)14	55°	215	209
Xgwm350-7D	ACC TCA TCC ACA TGT TCT ACG	GCA TGG ATA GGA CGC CC	(GT)14	55°	178	—
Xgwm356-2A	AGC GTT CTT GGG AAT TAG AGA	CCA ATC AGC CTG CAA CAA C	(GA)36	55°	216	—
Xgwm357-1A	TAT GGT CAA AGT TGG ACC TCG	AGG CTG CAG CTC TTC TTC AG	(GA)18	55°	123	120
Xgwm358-5D	AAA CAG CGG ATT TCA TCG AG	TCC GCT GTT GTT CTG ATC TC	(GA)18(G)2(GA)4	55°	164	162
Xgwm359-2A	CTA ATT GCA ACA GGT CAT GGG	TAC TTG TGT TCT GGG ACA ATG G	(CT)20(CTT)13imp	55°	212	—
Xgwm361-6B	GTA ACT TGT TGC CAA AGG GG	ACA AAG TGG CAA AAG GAG ACA	(GA)20imp	60°	125	123
Xgwm368-4B	CCA TTT CAC CTA ATG CCT GC	AAT AAA ACC ATG AGC TCA CTT GC	(AT)25	60°	259	271
Xgwm369-3A	CTG CAG GCC ATG ATG ATG	ACC GTG GGT GTT GTG AGC	(CT)11(T)2(CT)21	60°	184	—
Xgwm371-5B	GAC CAA GAT ATT CAA ACT GGC C	AGC TCA GCT TGC TTG GTA CC	(CA)10(GA)32	60°	191	176
Xgwm372-2A	AAT AGA GCC CTG GGA CTG GG	GAA GGA CGA CAT TCC ACC TG	(GA)>51	60°	310	309
Xgwm374-2B	ATA GTG TGT TGC ATG CTG TGT G	TCT AAT TAG CGT TGG CTG CC	(GT)17	60°	210	192
Xgwm376-3B	GGG CTA GAA AAC AGG AAG GC	TCT CCC GGA GGG TAG GAG	(CA)16(GA)22imp	60°	143	147
Xgwm382-2A	GTC AGA TAA CGC CGT CCA AT	CTA CGT GCA CCA CCA TTT TG	(GA)26	60°	—	86
Xgwm382-2B	GTC AGA TAA CGC CGT CCA AT	CTA CGT GCA CCA CCA TTT TG	(GA)26	60°	—	184
Xgwm382-2D	GTC AGA TAA CGC CGT CCA AT	CTA CGT GCA CCA CCA TTT TG	(GA)26	60°	—	108
Xgwm383-3D	ACG CCA GTT GAT CCG TAA AC	GAC ATC AAT AAC CGT GGA TGG	(GT)27	60°	188	199
Xgwm388-2B	CTA CAA TTC GAA GGA GAG GGG	CAC CGC GTC AAC TAC TTA AGC	(CT)4(CA)11(CA)12	60°	174	168
Xgwm389-3B	ATC ATG TCG ATC TCC TTG ACG	TGC CAT GCA CAT TAG CAG AT	(CT)14(GT)16	60°	117	128
Xgwm391-3A	ATA GCG AAG TCT CCC TAC TCC A	ATG TGC ATG TCG GAC GC	(CA)17(GA)9	55°	—	148
Xgwm397-4A	TGT CAT GGA TTA TTT GGT CGG	CTG CAC TCT CGG TAT ACC AGC	(CT)21	55°	175	193
Xgwm400-7B	GTG CTG CCA CCA CTT GC	TGT AGG CAC TGC TTG GGA G	(CA)21	60°	143	150
Xgwm403-1B	CGA CAT TGG CTT CGG TG	ATA AAA CAG TGC GGT CCA GG	(CA)13	55°	140	—
Xgwm408-5B	TCG ATT TAT TTG GGC CAC TG	GTA TAA TTC GTT CAC AGC ACG C	(CA)>22(TA)(CA)7(TA)9	55°	182	148
Xgwm410-2B	GCT TGA GAC CGG CAC AGT	CGA GAC CTT GAG GGT CTA GA	(CA)11(CA)10(CA)8	55°	335	367
Xgwm410-5A	GCT TGA GAC CGG CAC AGT	CGA GAC CTT GAG GGT CTA GA	(CA)11(CA)10(CA)8	55°	157	151
Xgwm413-1B	TGC TTG TCT AGA TTG CTT GGG	GAT CGT CTC GTC CTT GGC A	(GA)18	60°	91	95
Xgwm415-5A	GAT CTC CCA TGT CCG CC	CGA CAG TCG TCA CTT GCC TA	(GA)25imp	55°	133	131
Xgwm425-2A	GAG CCC ACA AGC TGG CA	TCG TTC TCC CAA GGC TTG	(CT)21	60°	141	120
Xgwm427-6A	AAA CTT AGA ACT GTA ATT TCA GA	AGT GTG TTC ATT TGA CAG TT	(CA)31(CA)22	50°	195	184
Xgwm428-7D	CGA GGC AGC GAG GAT TT	TTC TCC ACT AGC CCC GC	(GA)22	60°	137	133
Xgwm429-2B	TTG TAC ATT AAG TTC CCA TTA	TTT AAG GAC CTA CAT GAC AC	(CT)25	50°	211	209
Xgwm437-7D	GAT CAA GAC TTT TGT ATC TCT C	GAT GTC CAA CAG TTA GCT TA	(CT)24	50°	109	111
Xgwm443-5B	GGG TCT TCA TCC GGA ACT CT	CCA TGA TTT ATA AAT TCC ACC	(CA)20(GA)22	55°	209	—
Xgwm445-2A	TTT GTT GGG GGT TAG GAT TAG	CCT TAA CAC TTG CTG GTA GTG A	(CT)19	55°	188	190
Xgwm448-2A	AAA CCA TAT TGG GAG GAA AGG	CAC ATG GCA TCA CAT TTG TG	(GA)29	60°	203	243

(*continued*)

APPENDIX

(Continued)

Locus	Left primer	Right primer	Repeat	An. temp.	Opata (bp)	Synth. (bp)
Xgwm455-2D	ATT CGG TTC GCT AGC TAC CA	ACG GAG AGC AAC CTG CC	(GT)19imp	55°	147	—
Xgwm456-3D	TCT GAA CAT TAC ACA ACC CTG A	TGC TCT CTC TGA ACC TGA AGC	(GA)21	55°	138	165
Xgwm458-1D	AAT GGC AAT TGG AAG ACA TAG C	TTC GCA ATG TTG ATT TGG C	(CA)13	60°	115	119
Xgwm459-6A	ATG GAG TGG TCA CAC TTT GAA	AGC TTC TCT GAC CAA CTT CTC G	(GA)>28	55°	118	126
Xgwm469-6D	CAA CTC AGT GCT CAC ACA ACG	CGA TAA CCA CTC ATC CAC ACC	(CT)19(CA)10	60°	172	170
Xgwm471-7A	CGG CCC TAT CAT GGC TG	GCT TGC AAG TTC CAT TTT GC	(CA)34	60°	—	130
Xgwm473-2A	TCA TAC GGG TAT GGT TGG AC	CAC CCC CTT GTT GGT CAC	(GT)14(TTGG)(GT)8	55°	228	248
Xgwm480-3A	TGC TGC TAC TTG TAC AGA GGA C	CCG AAT TGT CCG CCA TAG	(CT)16(CA)13	60°	172	168
Xgwm484-2D	ACA TCG CTC TTC ACA AAC CC	AGT TCC GGT CAT GGC TAG G	(CT)29	55°	153	143
Xgwm493-3B	TTC CCA TAA CTA AAA CCG CG	GGA ACA TCA TTT CTG GAC TTT G	(CA)43imp	60°	179	171
Xgwm494-6A	ATT GAA CAG GAA GAC ATC AGG G	TTC CTG GAG CTG TCT GGC	(CA)13	60°	194	196
Xgwm495-4B	GAG AGC CTC GCG AAA TAT AGG	TGC TTC TGG TGT TCC TTC G	(GA)20	60°	160	178
Xgwm497-1A	GTA GTG AAG ACA AGG GCA TT	CCG AAA GTT GGG TGA TAT AC	(GT)29imp	55°	—	147
Xgwm497-2A	GTA GTG AAG ACA AGG GCA TT	CCG AAA GTT GGG TGA TAT AC	(GT)29imp	55°	137	—
Xgwm497-3D	GTA GTG AAG ACA AGG GCA TT	CCG AAA GTT GGG TGA TAT AC	(GT)29imp	55°	—	103
Xgwm498-1B	GGT GGT ATG GAC TAT GGA CAC T	TTT GCA TGG AGG CAC ATA CT	(CA)10(TA)4	55°	159	161
Xgwm499-5B	ACT TGT ATG CTC CAT TGA TTG G	GGG GAG TGG AAA CTG CAT AA	(GA)32	60°	131	177
Xgwm501-2B	GGC TAT CTC TGG CGC TAA AA	TCC ACA AAC AAG TAG CGC C	(CA)33	60°	176	—
Xgwm508-6B	GTT ATA GTA GCA TAT AAT GGC C	GTG CTG CCA TGA TAT TT	(GT)19imp	50°	—	170
Xgwm512-2A	AGC CAC CAT CAG CAA AAA TT	GAA CAT GAG CAG TTT GGC AC	(GT)16	60°	185	—
Xgwm513-4B	ATC CGT AGC ACC TAC TGG TCA	GGT CTG TTC ATG CCA CAT TG	(CA)12	60°	152	146
Xgwm515-2A	AAC ACA ATG GCA AAT GCA GA	CCT TCC TAG TAA GTG TGC CTC A	(GT)17(TCAT)(GT)6	60°	130	116
Xgwm515-2D	AAC ACA ATG GCA AAT GCA GA	CCT TCC TAG TAA GTG TGC CTC A	(GT)17(TCAT)(GT)6	60°	109	119
Xgwm518-6B	AAT CAC AAC AAG GCG TGA CA	CAG GGT GGT GCA TGC AT	(CA)34	55°	166	154
Xgwm526-2B	CAA TAG TTC TGT GAG AGC TGC G	CCA ACC CAA ATA CAC ATT CTC A	(CT)16	55°	148	138
Xgwm533.1-3B	AAG GCG AAT CAA ACG GAA TA	GTT GCT TTA GGG GAA AAG CC	(CT)18(CA)20	60°	—	316
Xgwm533.2-3B	AAG GCG AAT CAA ACG GAA TA	GTT GCT TTA GGG GAA AAG CC	(CT)18(CA)20	60°	120	—
Xgwm537-7B	ACA TAA TGC TTC CTG TGC ACC	GCC ACT TTT GTG TCG TTC CT	(CA)18(TA)13	60°	207	203
Xgwm538-4B	GCA TTT CGG GTG AAC CC	GTT GCA TGT ATA CGT TAA GCG G	(GT)6(T)(GT)10	60°	168	149
Xgwm539-2D	CTG CTC TAA GAT TCA TGC AAC C	GAG GCT TGT GCC CTC TGT AG	(GA)27	60°	143	157
Xgwm540-5B	TCT CGC TGT GAA ATC CTA TTT C	AGG CAT GGA TAG AGG GGC	(CT)3(CC)(CT)16	55°	133	117
Xgwm544-5B	TAG AAT TCT TTA TGG GGT CTG C	AGG ATT CCA ATC CTT CAA AAT T	(CT)12(ATCT)5(CT)16	55°	197	175
Xgwm547-3B	GTT GTC CCT ATG AGA AGG AAC G	TTC TGC TGC TGT TTT CAT TTA C	(CA)12	60°	171	—
Xgwm550-1B	CCC ACA AGA ACC TTT GAA GA	CAT TGT GTG TGC AAG GCA C	(CT)8(GT)18	55°	156	158
Xgwm554-5B	TGC CCA CAA CGG AAC TTG	GCA ACC ACC AAG CAC AAA GT	(CT)13(GT)14	60°	148	164
Xgwm558-2A	GGG ATT GCA TAT GAG ACA ACG	TGC CAT GGT TGT AGT AGC CA	(CA)15	55°	121	117
Xgwm565-5D	GCG TCA GAT ATG CCT ACC TAG G	AGT GAG TTA GCC CTG AGC CA	(CA)10	60°	142	150
Xgwm566-3B	TCT GTC TAC CCA TGG GAT TTG	CTG GCT TCG AGG TAA GCA AC	(CA)21(GA)2(TA)8	60°	131	122
Xgwm569-7B	GGA AAC TTA TTG ATT GAA AT	TCA ATT TTG ACA GAA GAA TT	(GT)36	47°	130	126
Xgwm570-6A	TCG CCT TTT ACA GTC GGC	ATG GGT AGC TGA GAG CCA AA	(CT)14(GT)18	60°	149	143

(*continued*)

APPENDIX

(Continued)

Locus	Left primer	Right primer	Repeat	An. temp.	Opata (bp)	Synth. (bp)
Xgwm573-7A	AAG AGA TAA CAT GCA AGA AA	TTC AAA TAT GTG GGA ACT AC	(CA)30	50°	178	170
Xgwm573-7B	AAG AGA TAA CAT GCA AGA AA	TTC AAA TAT GTG GGA ACT AC	(CA)30	50°	210	212
Xgwm577-7B	ATG GCA TAA TTT GGT GAA ATT G	TGT TTC AAG CCC AAC TTC TAT T	(CA)14(TA)6	55°	164	155
Xgwm582-1B	AAG CAC TAC GAA AAT ATG AC	TCT TAA GGG GTG TTA TCA TA	(CA)27imp(TA)6	50°	126	135
Xgwm583-5D	TTC ACA CCC AAC CAA TAG CA	TCT AGG CAG ACA CAT GCC TG	(CA)27	60°	165	161
Xgwm595-5A	GCA TAG CAT CGC ATA TGC AT	GCC ACG CTT GGA CAA GAT AT	(GA)39imp	60°	—	146
Xgwm601-4A	ATC GAG GAC GAC ATG AAG GT	TTA AGT TGC TGC CAA TGT TCC	(CT)17	60°	152	142
Xgwm604-5B	TAT ATA GTT CAA TAT GAC CCG	ATC TTT TGA ACC AAA TGT G	(GA)29	50°	133	127
Xgwm608-2D	ACA TTG TGT GTG CGG CC	GAT CCC TCT CCG CTA GAA GC	(GA)16	60°	166	181
Xgwm608-4D	ACA TTG TGT GTG CGG CC	GAT CCC TCT CCG CTA GAA GC	(GA)16	60°	151	144
Xgwm609-4D	GCG ACA TGA CCA TTT TGT TG	GAT ATT AAA TCT CTC TAT GTG TG	(CA)23	50°	100	—
Xgwm610-4A	CTG CCT TCT CCA TGG TTT GT	AAT GGC CAA AGG TTA TGA AGG	(GA)17imp	60°	172	162
Xgwm611-7B	CAT GGA AAC ACC TAC CGA AA	CGT GCA AAT CAT GTG GTA GG	(GA)32imp	55°	166	143
Xgwm613-6B	CCG ACC CGA CCT ACT TCT CT	TTG CCG TCG TAG ACT GG	(CT)23	60°	114	118
Xgwm614-2A	GAT CAC ATG CAT GCG TCA TG	TTT TAC CGT TCC GGC CTT	(GA)23imp	60°	126	—
Xgwm617-5A	GAT CTT GGC GCT GAG AGA GA	CTC CGA TGG ATT ACT CGC AC	(GA)43	60°	154	164
Xgwm617-6A	GAT CTT GGC GCT GAG AGA GA	CTC CGA TGG ATT ACT CGC AC	(GA)43	60°	133	—
Xgwm624-4D	TTG ATA TTA AAT CTC TCT ATG TG	AAT TTT ATT TGA GCT ATG CG	(GT)26	50°	129	—
Xgwm626-6B	GAT CTA AAA TGT TAT TTT CTC TC	TGA CTA TCA GCT AAA CGT GT	(CT)5(GT)13	50°	101	128
Xgwm630-2B	GTG CCT GTG CCA TCG TC	CGA AAG TAA CAG CGC AGT GA	(GT)16	60°	120	—
Xgwm635-7A	TTC CTC ACT GTA AGG GCG TT	CAG CCT TAG CCT TGG CG	(CA)10(GA)14	60°	109	—
Xgwm635-7D	TTC CTC ACT GTA AGG GCG TT	CAG CCT TAG CCT TGG CG	(CA)10(GA)14	60°	99	93
Xgwm636-2A	CGG TAG TTT TTA GCA AAG AG	CCT TAC AGT TCT TGG CAG AA	(GA)28imp	50°	112	84
Xgwm637-4A	AAA GAG GTC TGC CGC TAA CA	TAT ACG GTT TTG TGA GGG GG	(CA)18	60°	159	157
Xgwm639-5A	CTC TCT CCA TTC GGT TTT CC	CAT GCC CCC CTT TTC TG	(GA)19	55°	141	137
Xgwm639-5B	CTC TCT CCA TTC GGT TTT CC	CAT GCC CCC CTT TTC TG	(GA)19	55°	166	170
Xgwm639-5D	CTC TCT CCA TTC GGT TTT CC	CAT GCC CCC CTT TTC TG	(GA)19	55°	130	—
Xgwm642-1D	ACG GCG AGA AGG TGC TC	CAT GAA AGG CAA GTT CGT CA	(GT)14	60°	187	179
Xgwm644-6B	GTG GGT CAA GGC CAA GG	AGG AGT AGC GTG AGG GGC	(GA)20	60°	152	—
Xgwm644-7B	GTG GGT CAA GGC CAA GG	AGG AGT AGC GTG AGG GGC	(GA)20	60°	193	—
Xgwm645-3D	TGA CCG GAA AAG GGC AGA	GCC CCT GCA GGA GTT TAA GT	(CT)23imp	55°	161	145
Xgwm654-5D	TGC TGA TGT TGT AAG AAG GC	TGC GTC AGA TAT GCC TAC CT	(GT)28	55°	129	138
Xgwm664-3D	CAG TCA GTG CCG TTT AGC AA	AGC TTT GCT CTA TTG GCG AG	(GA)22	55°	148	146
Xgwm666-1A	GCA CCC ACA TCT TCG ACC	TGC TGC TGG TCT CTG TGC	(CA)13	60°	98	100
Xgwm666.1-3A	GCA CCC ACA TCT TCG ACC	TGC TGC TGG TCT CTG TGC	(CA)13	60°	96	92
Xgwm666.2-3A	GCA CCC ACA TCT TCG ACC	TGC TGC TGG TCT CTG TGC	(CA)13	60°	106	—
Xgwm666-5A	GCA CCC ACA TCT TCG ACC	TGC TGC TGG TCT CTG TGC	(CA)13	60°	110	114
Xgwm666-7A	GCA CCC ACA TCT TCG ACC	TGC TGC TGG TCT CTG TGC	(CA)13	60°	87	—
Xgwm674-3A	TCG AGC GAT TTT TCC TGC	TGA CCG AGT TGA CCA AAA CA	(CT)16CCC(GT)4	60°	162	172

An. temp. = Annealing temperature.
Synth. = Synthetic wheat.
imp = imperfect repeat.