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# PCR-Based EST Mapping in Wheat (*Triticum aestivum* L.)

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## ABSTRACT

Mapping expressed sequence tags (ESTs) to hexaploid wheat is aimed to reveal the structure and function of the hexaploid wheat genome. Sixty eight ESTs representing 26 genes were mapped into all seven homologous chromosome groups of wheat (*Triticum aestivum* L) using a polymerase chain reaction technique. The majority of the ESTs were mapped to homologous chromosome group 2, and the least were mapped to homologous chromosome group 6. Comparative analysis between the EST map from this study and the EST map based on RFLPs showed 14 genes that have been mapped by both approaches were mapped to the same arm of the same homologous chromosome, which indicated that using PCR-based ESTs was a reliable approach in mapping ESTs in hexaploid wheat.



**Key words:** wheat, EST, PCR, homologous.

## INTRODUCTION

Expressed sequence tag (EST), a single-pass sequence of cDNA, can be used to study structural and functional genomics. Expressed sequence tag databases are now becoming the basis for genomic approaches to drug discovery, plant and animal genetic improvement and the study of human genetic diseases (Messing and Llaca, 1998; Saier, 1998). Expressed sequence tag databases are also valuable sources for the construction of synthetic genome linkage maps of expressed genes among related species (Cato et al., 2001).

Earlier methods for mapping ESTs involved RFLPs as hybridization probes. This approach has been augmented by more efficient PCR-based approaches where EST-specific primers are used to amplify coding sequences. A PCR-based approach is considered faster, cheaper, safer (no need radioisotopes), and need only a small amount of DNA as compared to RFLPs (Cato et al., 2001). The critical step in PCR-based EST mapping is the construction of specific primer combinations that produce a unique and polymorphic fragment among genotypes used in a study. The polymorphic band can be generated using restriction enzymes or single-stranded conformational polymorphisms (SSCPs) (Fischer and Lerman, 1983; Harry et al., 1998; Orita et al., 1989).

The more difficult situation is when primers are designed from wheat (*Triticum aestivum* L.) ESTs in projects aimed to map hexaploid wheat. Most of the genes in hexaploid wheat are present on at least two of these homologous chromosomes. To locate ESTs to a specific chromosome, the primers should amplify a specific gene, produce a single band (if possible), and only amplify from one genome. The primers should also accommodate the possibility to locate the same gene to homologous chromosomes from other genomes.

Today there are over 499,000 wheat ESTs that have been developed from different tissues from many different libraries, and available in wheat EST databases. Among those sequences, there are some EST sequences that are duplications and are derived from the same gene from the same chromosome. There are some EST sequences that show single nucleotide polymorphisms (SNPs) when they are aligned to each other, suggesting that those ESTs were derived from the same gene family from different homologous chromosomes or a different chromosome group. Sets of primer combinations could be generated to facilitate PCR-based EST mapping in the wheat genome by searching and employing the SNPs located to select ESTs.

Hexaploid wheat is comprised of the three closely related genomes that facilitate a massive amount of gene buffering. This buffering allows hexaploid wheat to tolerate deletion of gene, gene complexes, chromosome arm segments, a chromosome arm, or the entire single chromosome for particular genome.

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Sears (1954) developed a series of ditelocentric lines, which are a series of wheat lines in which each line has a chromosome arm deletion for a particular chromosome. He also developed a series of nullisomic-tetrasomic lines, which is each line missing one entire chromosome for a particular genome, and has two extra chromosomes of one of its homologous chromosomes. These aneuploid stocks could be used to locate genes to a particular chromosome and chromosome arm. Physical mapping on wheat chromosome arm can be refined into a specific chromosome arm segment by using a series of wheat deletion stocks. Endo (1988) developed a series of wheat deletion stocks by introducing *Aegilops* chromosomes into a wheat background which caused deletions to be randomly established in wheat chromosome arms. There are over 436 deletion lines that have been isolated and used to chromosome bin map in the wheat genome. In this study, those aneuploid wheat stocks, including nullisomic-tetrasomic, ditelosomic, and deletion lines were used to physically map ESTs to wheat genomes.

The objectives of this study were: (i) to develop primers from wheat ESTs that can be used to map ESTs using a PCR technique; (ii) to physically map wheat ESTs to the wheat genome using wheat deletion lines; (iii) to compare the PCR-based and RFLP-based mapping of ESTs to the wheat genome. This study was conducted in collaboration with Dr. D.J. Somers from Agriculture and Agri-Food Canada, Cereal Research Centre, Winnipeg, Canada.

## MATERIALS AND METHODS

*Plant materials*

Plant materials included 45 aneuploid lines involving 21 'Chinese Spring' wheat nullisomic-tetrasomic lines, and 24 Chinese Spring wheat ditelosomic lines from the USDA-Sears collection (University of Missouri-Columbia), and 101 Chinese Spring wheat deletion lines from Dr. B. Gill's collection (Kansas State University, USA).

*DNA isolation*

DNA was extracted from young freeze-dried leaf tissue using methodology from Saghai-Maroof et al. (1994).

*Primer design*

All primers were designed in Dr. Somers's laboratory. Designing the EST primers required several steps. First, EST sequences were selected from wheat EST-National Science Foundation (NSF) and the International Triticeae EST Consortium (ITEC) databases. Second, the selected EST sequences were aligned with other ESTs to locate any SNPs. Only alignments that showed above 95% identity over 400 bases were used. Third, the primers were designed using the 20 nucleotides before a polymorphic nucleotide, and the last base on the 3' end of each primer was the polymorphic nucleotide. Each primer pair was given code number based on the order of the time made, called a blinded name. The last step involved checking the primers by using them to amplify Chinese Spring wheat genomic DNA to see whether the primers worked or not. The working primers were mapped using the Chinese Spring nullisomic stocks to assign the chromosome location, and then bin mapped using the Chinese Spring ditelosomic and deletion lines. The blinded names, primer names, sequences of the EST primer combinations, and the size of PCR product are listed in Table 1.

*Amplification of genomic DNA using EST primers*

Polymerase chain reaction amplifications were carried out in a volume of 20 uL (non-radioactive) in a Tetrad Thermocycler. The reaction mixture contained 24 ng of template DNA, 10 pmol of each primer, 0.8 nM of dNTPs, 1 U Taq DNA polymerase in 1x reaction of PCR buffer (10 mM Tris HCl, 50 mM KCl, 1.5 mM $MgCl_2$, pH 8.3). The PCR process used a touchdown PCR program where the annealing temperature is dropped $1^o$ per cycle from $64^o$ to $55^o$C for first nine cycles then remains a constant $55^o$ C for the remaining 21 cycles. Amplification products were mixed with 1:5 volume of loading buffer (50% glycerol, 0.5% bromophenol blue, 1 mM EDTA), and then the PCR products were resolved in a 1% agarose gel in 1xTAE buffer for 1 hour.

*Chromosome bin mapping of ESTs*

Figure 1 shows how EST 152 was mapped to a specific deletion bin on wheat chromosome 2D. Nullisomic-tetrasomic lines were used to locate which chromosome had the EST. For example, N2DT2A means this line has two sets of chromosome 2A instead of one set of chromosome 2A and one set of chromosome 2D. No PCR product resulted from the N2DT2A line, indicating EST 152 resided on chromosome 2D. The ditelosomic lines were used to locate on which arm the EST was located. We used ditelo (Dt) long arm (DL) and Dt short arm (DS). For example, Dt2DS means the line has only the short arm of chromosome 2D. The absence of PCR product in a Dt2DS line indicated EST 152 was located on the long arm of chromosome 2D. The deletion lines (DL) were used to find the exact bin location of the EST. Figure 1 shows that the EST 152 band was absent on deletion 2DL3 lines, but present on 2DS1, 2DS3, 2DS5, and 2DL9. This indicated that the exact location of EST 152 is in Bin 2DL3-0.49-0.76.

*Comparative map between PCR- and RFLP-based EST mapping*

The ESTs mapped using PCR-based technology were compared with the ESTs mapped using RFLP-based technology to establish the reliability of PCR-based technology for mapping ESTs on hexaploid genome. The wheat EST-NSF database (http://wheat.pw.usda.gov/cgi-bin/westsql) was used to search for the colinearity between two EST maps.

**Table 1.** The EST accession numbers, blinded names, primer names, primer sequences, and the size of PCR-products.

| EST accession | Blinded name | Primer name | Primer sequence | Product size (bp) |
|---|---|---|---|---|
| BE500218 | 11F | F063 | CTAGCAGCTTAAGGAACTTC | 275 |
| | 11R | R271 | GCAGGATCAGCACCGTGCCC | |
| BF203011 | 21F | F174 | GCACCGTCAGCACACGCAAA | 200 |
| | 21R | R338 | TCAGGAACACCACGCGCTTC | |
| BF202619 | 22F | F073 | TCGAAGAGGCGCTCGGCATA | 325 |
| | 22R | R261 | CACCGTGCCCGGTGTGATGA | |
| BF202379 | 31F | F102 | TCGACTCTGGCAAGTCGACA | 250 |
| | 31R | R326 | TCGACTCTGGCAAGTCGACA | |
| BF483165 | 32F | F048 | TCGACTCTGGCAAGTCGACC | 300 |
| | 32R | R379 | CAATGATGAGCACAGCACAA | |
| BE500396 | 42F | F103 | CGGCGAGGAAGTCGACCGGA | 300 |
| | 42R | R334 | CGGCCTCCTGCAGCGCGGAC | |
| BE500501 | 51F | F064 | CCTTCTTCAGCGAGACTGGG | 175 |
| | 51R | R391 | CACGGGCGAAGTTGTTGGCT | |
| BF483613 | 52F | F076 | AGACTGGGGCTGGGAAGCAC | 300 |
| | 52R | R448 | TCCTGATACGATCAAGGCAG | |
| BE500375 | 53F | F278 | ATGTCCCCCGTGCTGTCTTT | 380 |
| | 53R | R546 | GGCGCTCCAGGAGAAGCGAG | |
| BF482753 | 54F | F087 | GGAAGCATGTCCCCCGCGCG | 325 |
| | 54R | R268 | AGACCAGTGCAGTTGTCTGC | |
| BF482680 | 55F | F247 | AGATGCCCGGTGACAAGACT | 425 |
| | 55R | R541 | CAGTTCCACCTCCAACAGCA | |
| BF482845 | 56F | F228 | GATGGACAGATGCCCGGTGG | 425 |
| | 56R | R520 | TGAAGACGAGGAATCCCTGA | |
| BE637755 | 61F | F071 | AATCTTATGGATCAAGGTGT | 550 |
| | 61R | R422 | TGTGGATCTGGGTGGTGTCC | |
| BF482523 | 62F | F093 | GCCGCCCCAACCCCATGACC | 400 |
| | 62R | R279 | CAGCCTCATCGATTTGGATA | |
| BF483171 | 81F | F061 | AGTACCAGGTGGTGGGTCGT | 1000 |
| | 81R | R322 | TCTGCTCCACGGCACCATTT | |
| BE499999 | 82F | F124 | TGAAGCTCTGGGCCACCAAT | 1000 |
| | 82R | R287 | TCTGCTCCACGGCACCATTC | |
| BF484421 | 83F | F091 | CGCCCGGCGATGAGCAGCCG | 1000 |
| | 83R | R287 | TCTGCTCCACGGCACCATTC | |
| BF484421 | 84F | F106 | TCTGGGCGACCAACGAGGTG | 1000 |
| | 84R | R242 | ACATGTTGTGGTAGCCGGTT | |
| BE498885 | 91F | F187 | AATCGGAAAAGGCCCCCTAT | 300 |
| | 91R | R243 | TCATCGCTGCCCTCATCGTC | |
| BM140580 | 101F | F034 | TGGTCCTTATCCTTGCCATT | 800 |
| | 101R | R526 | TGTCCCAGGCCCTTCCACTA | |
| BM140371 | 102F | F058 | TGGTCCTTATCATTGCCATC | 800 |
| | 102R | R550 | TGTCCCAGGCCCTTCCACTC | |
| BF484255 | 103F | F105 | TCCCTTCATGGTCCTTATCA | 800 |
| | 103R | R494 | TTCCCTTGATCCTTGCGAAG | |
| BM137809 | 131F | F142 | CGTATGTGCTATCTGAGTCC | 250 |
| | 131R | R295 | CGCCAGACTTCAGGTGGCCG | |
| BF202081 | 132F | F100 | CTGAGGCATCAATCTTTGCT | 375 |
| | 132R | R347 | CACCAGACTTCAGGTGGCCA | |
| BF202081 | 133F | F245 | CCAACAAGGACTTCGACTCC | 200 |
| | 133R | R211 | GGAAGATGAACATCCCACGA | |
| BM134418 | 141F | F059 | TCGACAGGAGGCCAAAGGCG | 500 |
| | 141R | R342 | AGAGGTTGGTGTCCTCGAAG | |
| BM134418 | 142F | F160 | CGAGGAAGTCTGCGCCGACG | 250 |
| | 142R | R367 | TGTCCTCGAACAGCCCCACC | |
| BE499017 | 143F | F174 | AGTCGGCGCCGACCACCGGG | 200 |
| | 143R | R318 | CGTGGCTCTGGAAGCGGAGA | |
| BE500314 | 151F | F023 | GTGGCAATGCCTATGTGATT | 250 |
| | 151R | R367 | AGGGCTCGAAGTCGAAGTCG | |
| BM138609 | 152F | F051 | CTGTCTTCTTCAAGACTCAA | 400 |
| | 152R | R384 | TGCAGTCGTATGCCTCAGCT | |
| BM138609 | 153F | F201 | CTTCAAGACTCAAGCTGATA | 250 |
| | 153R | R401 | CGCCGTAGGCCAGGGCGGAG | |
| BE499318 | 161F | F212 | AGGTTTCTACCTCGAAGACA | 400 |
| | 161R | R425 | TGAGCTGGGTCAGGATAGCA | |
| BE423540 | 172F | F097 | AAGCTCGTTCTGTTCAGGAC | 300 |
| | 172R | R296 | AGGACCTTGCACTCCTTGTC | |
| BQ240701 | 181F | F129 | GCGTGAGGGAGCGCATCGGT | 750 |
| | 181R | R394 | CCCTCACATACTCCCAGACG | |
| BQ240701 | 182F | F129 | GCGTGAGGGAGCGCATCGGT | 800 |
| | 182R | R411 | GCAAATACTCAGCGACGCTC | |
| BF202965 | 201F | F151 | CCGCCGCCTCGCCGGAGCGG | 600 |
| | 201R | R342 | AGAGGCCCTTGACGATCCCT | |
| BE499290 | 211F | F125 | AGACCGGCTGCCAGTCTCGT | 400 |
| | 211R | R512 | GGTAGTCAAACTTGCACTCA | |
| BQ620117 | 221F | F039 | GGATGTTCATCTTCCCCGGT | 400 |
| | 221R | R406 | GTCACATGGATGGTGGAGAA | |
| BF202081 | 222F | F099 | ATGTCCTGAACCGCTACTTT | 300 |
| | 222R | R599 | AGGGCTCGAAGTCGAAGTCG | |
| BF202198 | 223F | F056 | GGATGTTCATCTTCCCCGGC | 300 |
| | 223R | R341 | CCATCTCGGGAATGATGTCG | |
| BF482340 | 224F | F056 | GGATGTTCATCTTCCCCGGC | 325 |
| | 224R | R346 | AGACCTCCATCTCAGGGATA | |
| BQ619858 | 231F | F180 | ACCGCAGTTGCCTTGATATA | 300 |
| | 231R | R416 | GCTTCGACCCACTGGGCTTG | |
| BG907231 | 251F | F088 | GGTACATACGCTGGAGAAAT | 200 |
| | 251R | R291 | TCTTCTACTTCGAGGCCGGA | |
| BG904361 | 253F | F132 | ACACACCACCCCAAAGAAGA | 350 |
| | 253R | R462 | ACTTCGCCAACTTCACCGGT | |
| BG909863 | 261F | F074 | CGCCGACGCAGCGAGCTCTT | 400 |
| | 261R | R346 | CGACCTTCTCGGCGTAAAGT | |
| BF202040 | 271F | F083 | GCGCGGCCATCTCCATCTCA | 550 |
| | 271R | R372 | CCTTAGCAGCAAGGCTGGTT | |
| BF482437 | 272F | F062 | CCCCTCTATCCGACTTCGGG | 600 |
| | 272R | R362 | CCTTAGCAGCAAGGCTGGTC | |
| BQ620354 | 273F | F141 | TGAAGAGCAGCCTCTTCCTA | 700 |
| | 273R | R462 | TAGCAGAAAGGCTGGTCTTG | |
| BG906628 | 281F | F086 | CCTTGGAGACATGCCTCAGG | 200 |
| | 281R | R215 | GAGACGGCCGCCGTGCCGCA | |
| BM140514 | 283F | F067 | TTCTCTTTGTTTCTCCCTCT | 200 |
| | 283R | R226 | CCGTGCCGCCACCAGAGCGT | |
| BE422799 | 292F | F264 | GGTGTCGCTGAAGTTCCTCT | 800 |
| | 292R | R166 | TCAGGTCGTTCGGTATCAGG | |
| BE499039 | 301F | F027 | GCTAGGTTTTGGTCCTTCGT | 600 |
| | 301R | R225 | CGTCGGAGGCCTTGGTGCGT | |
| BM138684 | 302F | F096 | GGTTCAACGTCACCAAGGGT | 1150 |
| | 302R | R229 | CGTCGGAGGCCTTGGTGCGA | |
| BG904954 | 311F | F187 | CAATGTAATCATCGAAGAGG | 300 |
| | 311R | R491 | TGTACCACTCAAGACAAGGT | |
| BG906277 | 321F | F274 | AGGGCTAGGAAATTATCAAC | 200 |
| | 321R | R419 | TTCTAACCATAATGGCACGA | |
| BE424825 | 322F | F234 | AACCTCAAAGGAAGCAAGGA | 250 |
| | 322R | R026 | ATTAAGGATGCAGAGCAACT | |
| BG907633 | 331F | F205 | ACCTTCAAGTGTGGTGTCGT | 250 |
| | 331R | R431 | AGAGCGTGCTCCCTGAGGCC | |
| BG910130 | 333F | F552 | AGACCTTCAAGTGTGGTGTT | 250 |
| | 333R | R328 | AGAGCGTGCTCCCTGAGGCT | |
| BG906045 | 361F | F338 | CCTTGATCTTATCAGCATGA | 300 |
| | 361R | R560 | TCCGCCCCTTCTCTTATGGG | |
| BE424633 | 401F | F044 | GAACAAGTGTAGATGAACAT | 350 |
| | 401R | R274 | AGGGCAACGTGACCATCGCT | |
| BG904428 | 402F | F014 | AGTTGAGATGAACAGCACAG | 500 |
| | 402R | R337 | CGTTTGGCTCTGGAGTCGTG | |
| BG904212 | 403F | F170 | GCGGCGCTCAAAGATCCTCC | 600 |
| | 403R | R472 | CAGCCAGAGTGAAGCCATCA | |
| BG905429 | 411F | F139 | GCTTAACTTCTTCCACAGCA | 450 |
| | 411R | R463 | ACTAGCTGCACAGGCCACTC | |
| BG905429 | 412F | F135 | GCTTAACTTCTTCCACAGCG | 400 |
| | 412R | R459 | ACTAGCTGCACAGGCCACAA | |
| BE425039 | 421F | F209 | GAAGACTGTAAACATCTTAG | 200 |
| | 421R | R296 | ACAAGAAGAAGAGGATCGCC | |
| BQ238323 | 422F | F275 | TCTTGGCCATGCTCTCGCTG | 300 |
| | 422R | R539 | TGAGGGGACCCAAGAGGGCC | |
| BQ620599 | 431F | F080 | TACTCTCAAACTTTTCCCGG | 500 |
| | 431R | R500 | ACAGCATCCCCATCAACCTC | |
| BQ237808 | 432F | F477 | CGGCACATTCGTACTCTCAT | 1000 |
| | 432R | R407 | TTGATGACAAGCTCCACCCA | |

## RESULTS AND DISCUSSION

*Results*

**Primer design and evaluation**

Designing primers is an important and critical step with any PCR technique. In this study the primers were developed based on an SNP occurring in the EST alignment. The selected EST was aligned against other wheat ESTs pulled out from either wheat EST-NSF or ITEC databases to locate any SNPs. Twenty nucleotides were chosen upstream from the polymorphic nucleotide, including the polymorphic nucleotide, to construct forward primers. Reverse primers were constructed by translating 20 nucleotides downstream of the different polymorphic nucleotide from the same sequence.

Before mapping the EST into wheat deletion lines, the primers were evaluated to amplify Chinese Spring wheat genomic DNA. Only the primers that produced a single band were used for further analysis. The primers were then used to determine the chromosomal location of ESTs using a series of Chinese Spring wheat nullisomic stocks (Sears, 1954). In general, each evaluated primer produced single band in all nullisomic-tetrasomic lines except in the one line where the EST was physically located.

If the primers developed from the same EST family also produced a single PCR band with a different size, their chromosomal locations using nullisomic lines showed that those ESTs could be assigned to different chromosomes from the same homologous group. For example, primers EST 51, 52, and 53 were developed from the ESTs originating from the same contig (DSW03C5_contig5798.3), and produced one band but were located on different chromosomes (1A, 1B, and 1D) (Table 2).

**Table 2.** Blinded names, primer names, EST names, contig origin, location, and size of PCR products.

| Blinded names | Primer names | EST names | Contig origin | Location | Size (bp) |
|---|---|---|---|---|---|
| 51F/R | F064<br>R391 | BM140481<br>BM137664<br>BM135238<br>BE500501<br>BE500167<br>BE498208<br>BF484824<br>BF484612 | DSW03C5_5798.3 | 1AL | 175 |
| 52F/R | F076<br>R448 | BE202634<br>BF483613<br>BE498846<br>BE500808<br>BF482964 | DSW03C5_5798.3 | 1BL | 300 |
| 53F/R | F278<br>R546 | BE500375<br>BE499726<br>BE499492<br>BF484674<br>BM137850<br>BE498130 | DSW03C5_5798.3 | 1DL | 380 |

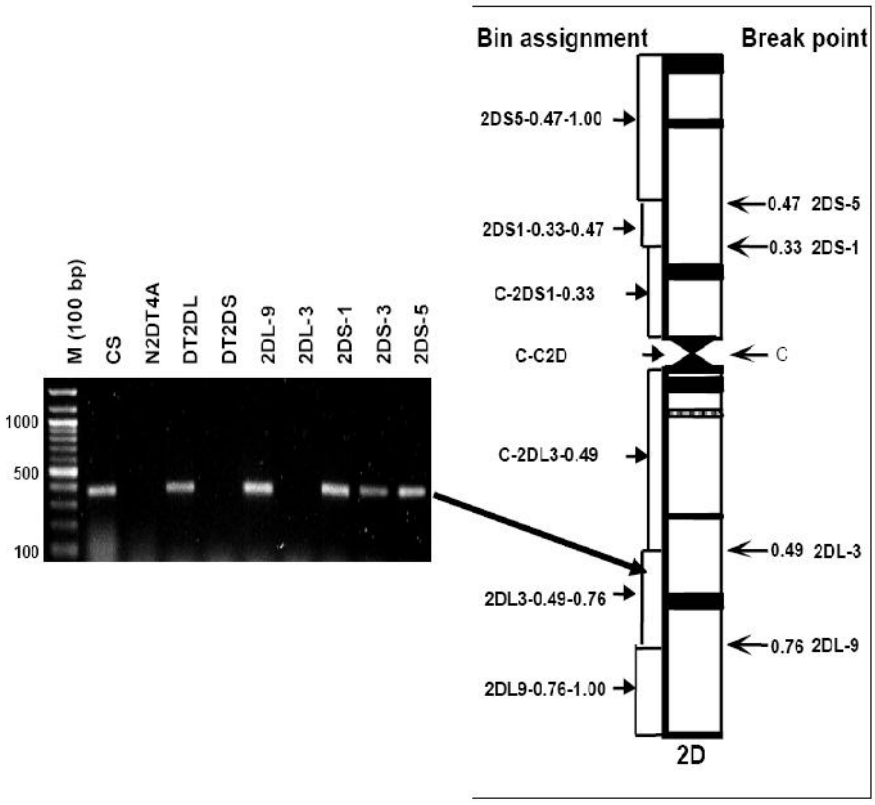


**Figure 1.** An example of mapping EST 152 to chromosome 2D using PCR technique.

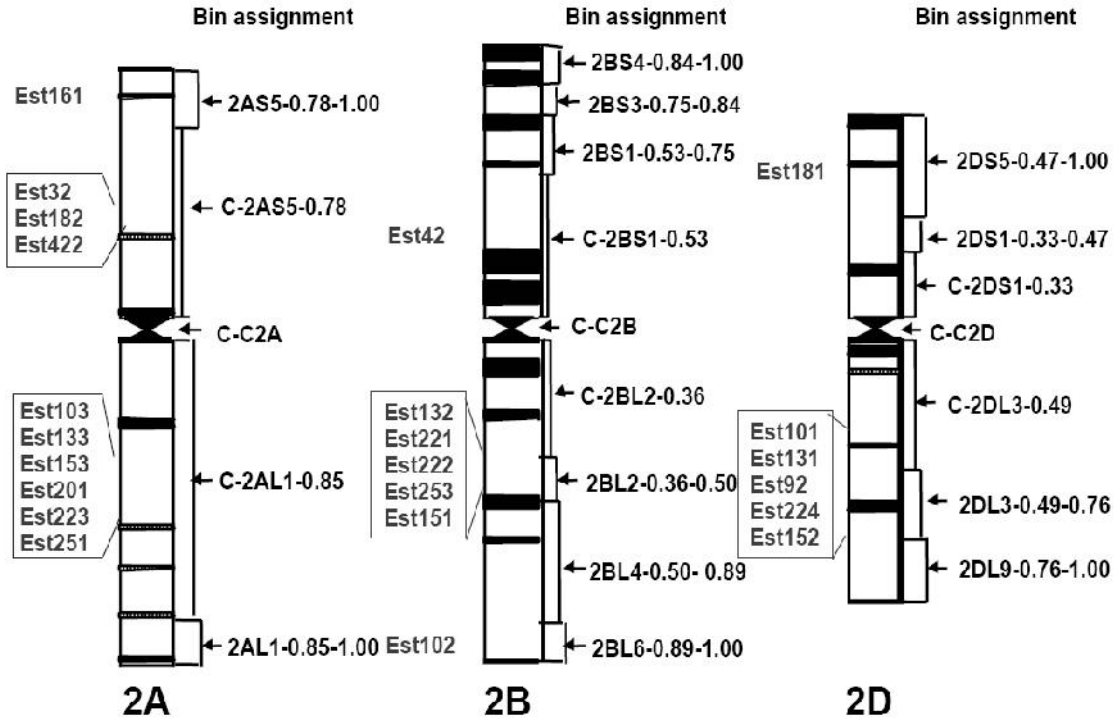


**Figure 2.** An example of EST mapping on homoeologous chromosome group 2.

**Physical mapping of ESTs**

The EST assignment to a deletion (Figure 1) was based on the presence or absence of the band among the series of deletion lines. A band that is missing in particular deletion line means the band is located to the chromosome region that has been deleted from that particular deletion line and has been assigned to a bin fraction length where the chromosome part is missing. We mapped 68 ESTs into assigned bins of the deletion lines. The blinded names of the primers, EST accessions, and assigned bins for 68 mapped ESTs are presented on Table 3. The majority of the ESTs were mapped to homologous chromosome group 2 (Figure 2), and the fewest were mapped to homologous chromosome group 6. All the long arms of the seven homologous chromosome groups were assigned by EST (50 ESTs), whereas not all of the short arms of chromosomes were assigned. There were 29, 23, and 16 ESTs that mapped into the three genomes A, B, and D, respectively (Table 4).

**Gene prediction of mapped ESTs**

We performed BLASTX (Basic Local Alignment Search Technique) analysis (Altschul et al., 1997) to establish what gene function that EST represented, and to compare the six-frame conceptual translation products of a nucleotide query sequence (both strands) against a protein sequence database. The matching gene with the highest score, lowest E-value, and the same as or a higher than 90% identity was selected as the gene that represented the EST being analyzed. All the ESTs were assigned as a known gene from the database. Some ESTs with different accession numbers that were mapped either to the same homologous chromosome group or different chromosome represented the same gene.

Many reports (Khlestkina et al., 2002; Clarke et al., 2003; Danyluk et al., 2003; Watanabe and Koval, 2003) have shown that most genes in hexaploid wheat can be found in triplicate with one copy in each genome. Some genes can also have additional copies in other chromosomes from the same or different homologous groups. Our results showed an agreement with the previous studies that 12 genes (46%) were mapped on at least two homologous chromosomes, while seven of them were specific to chromosome groups 2, 3, and 5 (Table 5). Among 26 mapped genes, 10 genes (38.5%) were mapped only to one chromosome, four genes (15.4%) were mapped to two homologous chromosomes, three genes (11.5%) were mapped to three homologous chromosomes, five genes (19.2%) were mapped to two or three homologous and other chromosomes, and the rest of the genes (15.4%) were randomly mapped, which means those genes were mapped into more than one different chromosome from a different homologous group (Figure 3).

**Table 3.** Chromosome location, bin name, blinded name of primers, and EST accession numbers.

| Chromosome | Bin name | Blinded name | EST accession |
|---|---|---|---|
| 1 AL | 1AL1-0.17-0.61 | 51 | BE500501 |
| | | 231 | BQ619858 |
| | | 302 | BM138684 |
| 1 BL | 1BL2-0.69-0.83 | 52 | BF483613 |
| | | 141 | BM134418 |
| 1 DL | 1DL2-0.41-1.00 | 53 | BE500375 |
| | | 301 | BE499039 |
| 2 AL | C-2AL1-0.85 | 103 | BF484255 |
| | | 133 | BF202081 |
| | | 153 | BM138609 |
| | | 201 | BF202965 |
| | | 223 | BF202198 |
| | | 251 | BG907231 |
| | | 311 | BG904954 |
| 2 BL | 2BL6-0.89-1.00 | 102 | BM140371 |
| | 2BL2-0.36-0.50 | 132 | BF202081 |
| | | 151 | BE500314 |
| | | 221 | BQ620117 |
| | | 222 | BE500314 |
| | | 253 | BG904361 |
| 2 DL | 2DL3-0.49-0.76 | 101 | BM140580 |
| | | 131 | BM137809 |
| | | 152 | BM138609 |
| | | 224 | BF482340 |
| 2 AS | C-2AS5-0.78 | 422 | BQ238323 |
| | | 32 | BF483165 |
| | | 182 | BQ240701 |
| | 2AS5-0.47-1.00 | 161 | BE499318 |
| 2 BS | C-2BS1-0.53 | 42 | BE500396 |
| 2 DS | 2DS5-0.47-1.00 | 181 | BQ240701 |
| 3 AL | 3AL3-0.42-0.78 | 81 | BF483171 |
| | C-3AL3-0.42 | 421 | BE425039 |
| 3 BL | C-3BL2-0.22 | 82 | BE499999 |
| | | 172 | BE423540 |
| 3 DL | 3DL2-0.27-0.81 | 83 | BF484421 |
| 3 AS | C-3AS2-0.23 | 261 | BG909863 |
| 3 BS | 3BS9-0.57-0.78 | 412 | BG905429 |
| 3 DS | 3DS6-0.55-1.00 | 411 | BG905429 |
| 4 AL | C-4AL12-0.43 | 54 | BF482753 |
| | 4AL4-0.80-1.00 | 431 | BQ620599 |
| | | 142 | BM134418 |
| | 4AL5-0.66-0.80 | 331 | BG907633 |
| 4 BL | 4BL1-0.81-1.00 | 84 | BF484421 |
| 4 BS | 4BS1-0.81-1.00 | 55 | BF482680 |
| | | 56 | BF482845 |
| | | 432 | BQ237808 |
| 5 AL | 5AL10-0.57-0.78 | 322 | BE424825 |
| | | 401 | BE424633 |
| 5 BL | 5BL14-0.75-0.76 | 402 | BG904428 |
| 5 DL | C-C5DL1-0.6 | 11 | BE500218 |
| | | 273 | BQ620354 |
| | | 283 | BM140514 |
| | 5DL-0.76-1.00 | 31 | BF202379 |
| | | 321 | BG906277 |
| | 5DL1-0.6-0.74 | 403 | BG904212 |
| 5 AS | 5AS3-0.75-0.98 | 61 | BE637755 |
| | | 271 | BF202040 |
| 5 BS | 5BS6-0.81-1.00 | 62 | BF482523 |
| | C-5BS4-0.43 | 281 | BG906628 |
| | 5BS5-0.71-0.81 | 272 | BF482437 |
| 6 AL | C-6AL4-0.55 | 21 | BF201679 |
| 6 BL | C-6BL3-0.36 | 22 | BF202619 |
| 7 AL | 7AL21-0.74-0.86 | 301 | BE499039 |
| | 7AL18-0.90-1.00 | 91 | BE498885 |
| | 7AL1-0.39-0.71 | 211 | BE499290 |
| 7 AS | 7AS1-0.89-1.00 | 333 | BG910130 |
| 7 BL | C-7BL2-0.33 | 292 | BE422799 |
| 7 BS | 7BS1-0.27-1.00 | 143 | BE499017 |
| | C-7BS1-0.27 | 361 | BG906045 |

**Table 4.** Distribution of the ESTs in each wheat chromosome group.

| Genome | Chromosome groups | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | 7 | |
| | L* | S* | L | S | L | S | L | S | L | S | L | S | L | S |
| A | 3 | 0 | 7 | 3 | 2 | 1 | 4 | 0 | 2 | 2 | 1 | 0 | 3 | 1 |
| B | 2 | 0 | 6 | 1 | 2 | 1 | 1 | 3 | 1 | 2 | 1 | 0 | 1 | 2 |
| D | 2 | 0 | 5 | 1 | 1 | 1 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 |
| Total | 7 | 0 | 18 | 5 | 5 | 3 | 5 | 3 | 9 | 4 | 2 | 0 | 4 | 3 |

Note: * L= long arm. S= short arm.

**Table 5.** Distribution of mapped genes in wheat genome.

| Genes | Chromosome location |
|---|---|
| Alpha-tubulin | 1AL, 1BL, 1DL, 4AL, 4BS |
| Chlorophyll a/b binding protein | 1AL, 2AL, 2BL, 4AL, 4BS |
| Cold shock protein-1 | 1AL, 1DL, 7AL |
| Histone H3 | 1BL, 2BS, 4BL, 6AL, 7BS |
| Plasma membrane P-type proton pump ATPase | 2AL, 2BL, 2DL |
| S-adenosylmethionine decarboxylase | 2AL, 2BL, 2DL |
| Malate dehydrogenase (MDH) | 2AL |
| DNA Binding Protein S1FA | 2AL |
| Ribosomal protein s6 RPS6-2 | 2AS, 3AL |
| Elongation Factor-1 alpha | 2AS, 5DL |
| Sucrose synthase type 2 | 2AS, 2DS |
| Translation initiation factor 5A | 2AS |
| Putative ribosomal protein L18a, cytosolic | 3AL, 3BL, 3DL, 4BL |
| NADP-specific isocitrate dehydrogenase | 3BL, 7BS |
| Putative 40S ribosomal protein S3 | 3AS |
| NADP-dependent malic enzyme | 3BS, 3DS |
| Lhcb6 protein (Lhcb6) | 4AL |
| Ribosomal protein S29-like protein | 5AL, 5DL |
| UDP-glucose pyrophosphorylase | 5AL, 5BL, 5DL |
| Lipid transfer protein 7a2b | 5DL |
| Thioredoxin M | 5DL, 5BS, 7BS |
| Methionine synthase protein | 5AS, 5BS |
| Putative 60S ribosomal protein L6 | 6 BL |
| High mobility group protein | 7AL |
| Expressed protein | 7AL |
| RL5 ribosomal protein | 7BL |

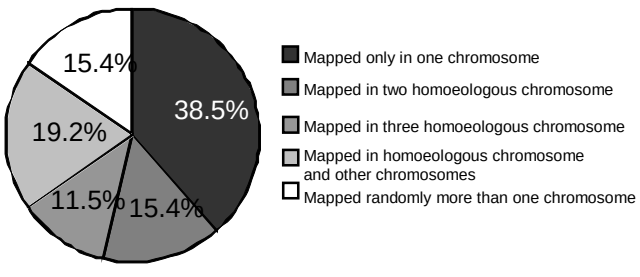


**Figure 3.** Genes distribution on wheat genome.

**Comparisons between PCR and RFLP-based EST mapping**

The reliability of PCR-based EST mapping was evaluated by comparing EST maps generated from PCR techniques to RFLP techniques. The RFLP-based mapping was performed by hybridization of cDNA clones that were used to generate ESTs. Using the PCR-based approach, before hybridization, the ESTs were screened to select singletons, which are ESTs that only represent single gene. The selected singleton was then hybridized with the *Eco*RI-digested genomic DNA from all wheat nullisomic, ditelosomic, and deletion lines. Bin assignment procedure was the same as in the PCR-based approach. The EST mapping data are publicly available through the wheat EST-NSF database (http://wheat.pw.usda.gov/westsql). We performed an EST search based on the gene names listed in the Table 5 in order to compare both maps. Any resulting ESTs, including EST accession number and bin assignment, were compared to the corresponding EST from the PCR-based EST map and mRNA from the gene to evaluate the homology and the bin location for the two ESTs.

We identified three groups of genes. First, eleven genes were mapped in the same bin. Although the EST accession numbers were different, sequence comparison between each pair of ESTs showed high identity. In the case where no homology was observed, both ESTs showed high homology to the same gene at different places indicating both ESTs originated from the same gene. Second, three genes were mapped to different bins, but on the same chromosome. Experimental errors probably can explain this situation, such as incorrect deletion lines used, poor hybridization, or gene duplications and translocations. Poor hybridization could lead to the misinterpretation when assigning the EST to a particular bin. The last group was a group of 12 genes that could not be found in the RFLP-based EST map. This is probably because those genes have not been mapped with RFLPs.

*Discussion*

Mapping ESTs to hexaploid wheat is aimed mainly at revealing the structure and function of the hexaploid wheat genome; however, it is a laborious and expensive task. Fortunately, wheat has a set of aneuploid stocks including nullisomic-tetrasomic, ditelosomic, and deletion lines (Sears, 1954; Endo and Gill, 1996) that have been widely used in physically mapping and genetic analysis of wheat. Conventional mapping approaches using RFLPs sometimes cannot be avoided, even though it is expensive, laborious, time consuming, and includes risks associated with radioisotopes. Expressed sequence tag mapping using PCR is a good alternative approach because it offers a cheap, fast, safe, and reliable approach. The successful key to any PCR technique is in designing primers that can amplify genomic DNA consistently, resulting in the expected PCR product size and polymorphic bands. In this research, PCR primers were developed from wheat ESTs, and mapped to the hexaploid wheat genome using a series of deletion lines.

Bin mapping of ESTs to hexaploid wheat using PCR requires a specific approach in constructing the primers. The primers should amplify a gene from only one chromosome. Therefore, to map a gene in hexaploid wheat with one copy from each homologous chromosome requires three different primers combinations derived from the same gene. With, an assumption that each homologous chromosome is derived from a different parental genome, the same gene, derived from each homologous group, will have at least one SNP in its coding region, which can be recognized through the alignment of EST sequences of those genes. To ascertain the amplification will occur only in one homologous chromosome, three strategies were employed when primers were designed. First, the primers were designed from the EST that has been aligned with at least three wheat ESTs showing at least one SNP in every two ESTs. Second, the primers were designed from the polymorphic nucleotide site where the polymorphic nucleotide was in the 3' end of primers to ensure that one primer combination will amplify one EST, but not the other ESTs. Finally, at least three combinations of the primers were designed from different places in the coding region specifically to produce different amplification sizes. This strategy was used to avoid one PCR product from one chromosome being confounded by another PCR product from the other homologous chromosome.

We mapped 68 ESTs that represented 26 genes into all seven homologous chromosome of wheat. On averages, more than two ESTs or primer combinations were required to map a gene in hexaploid wheat. Comparative analysis between the EST map from this study and the EST map based on RFLPs revealed that about 14 genes that have been mapped using both approaches were mapped on the same arm of the same homologous chromosome. This result indicated that the PCR-based EST mapping, including the method for designing the primers, was a reliable approach to map ESTs in hexaploid wheat.

We also observed that five genes had a different copy number between the two maps. Since the PCR-based EST mapping technique was aimed to map single band on every homologous chromosome, it was possible that with a particular gene, we could develop one pair of primers that only mapped to one chromosome. On the other hand, the RFLP-based mapping approach used one probe for a particular gene, and could produce more than one hybridization band, which was designated as locus, that could be mapped into more than one chromosome.

Beside the advantages of this PCR technique for mapping ESTs in hexaploid wheat, there was constraint that needs to be overcome in order to improve this technique. To map a gene to hexaploid wheat requires at least three properly working primer combinations, which is not easy to obtain and is time consuming.

## CONCLUSION

Sixty eight ESTs were successfully mapped into assigned bins of wheat deletion lines. The majority of the ESTs were mapped to homologous chromosome group 2, and the fewest were mapped to homologous chromosome group 6. There were 29, 23, and 16 ESTs mapped into the three genomes A, B, and D, respectively. The 68 mapped- ESTs represented 26 genes, and 14 of them were mapped to the same homologous chromosomes on the EST map, based on RFLPs. Twelve genes (46%) were mapped on at least two homologous chromosomes, while seven of them were specific to chromosome groups 2, 3, and 5. This study demonstrated that the PCR technique, including the development of primers, was reliable for mapping ESTs in hexaploid wheat.

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