

1 **1: Amplification of full length LcMYB2**

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3 **A: 5'-RACE for *LcMYB2***

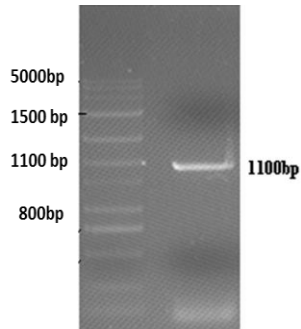
4 The first-strand cDNA was synthesized with SMARTer™ RACE cDNA Amplification Kit (Clontech). A
5 gene specific primer and a universal primer was used for 5'-end amplification.

6 Primers:

7 5'GSP: 5'- GACAGGCATATAGGGTATTTTC -3'

8 UPM: 5'- CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT -3'

9 5'- CTAATACGACTCACTATAGGGC -3'

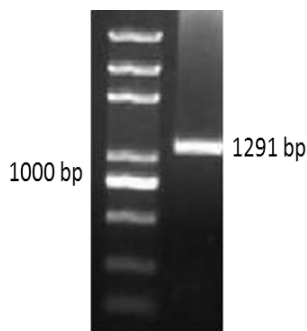


12 **B: Full length amplification of *LcMYB2***

13 A putative full-length sequence of *LcMYB2* was amplified using the specific pair of primers:

14 LcMYB2-F: 5'-ATGACGAGGCGGTGCTCGCAC-3'

15 LcMYB2-R: 5'-GACAGGCATATAGGGTATTTTC-3'

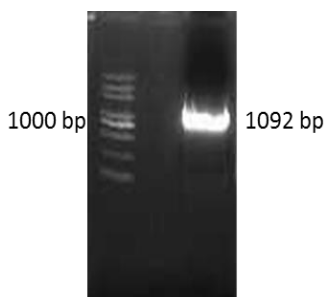


19 **C: OFR region amplification**

20 Primers:

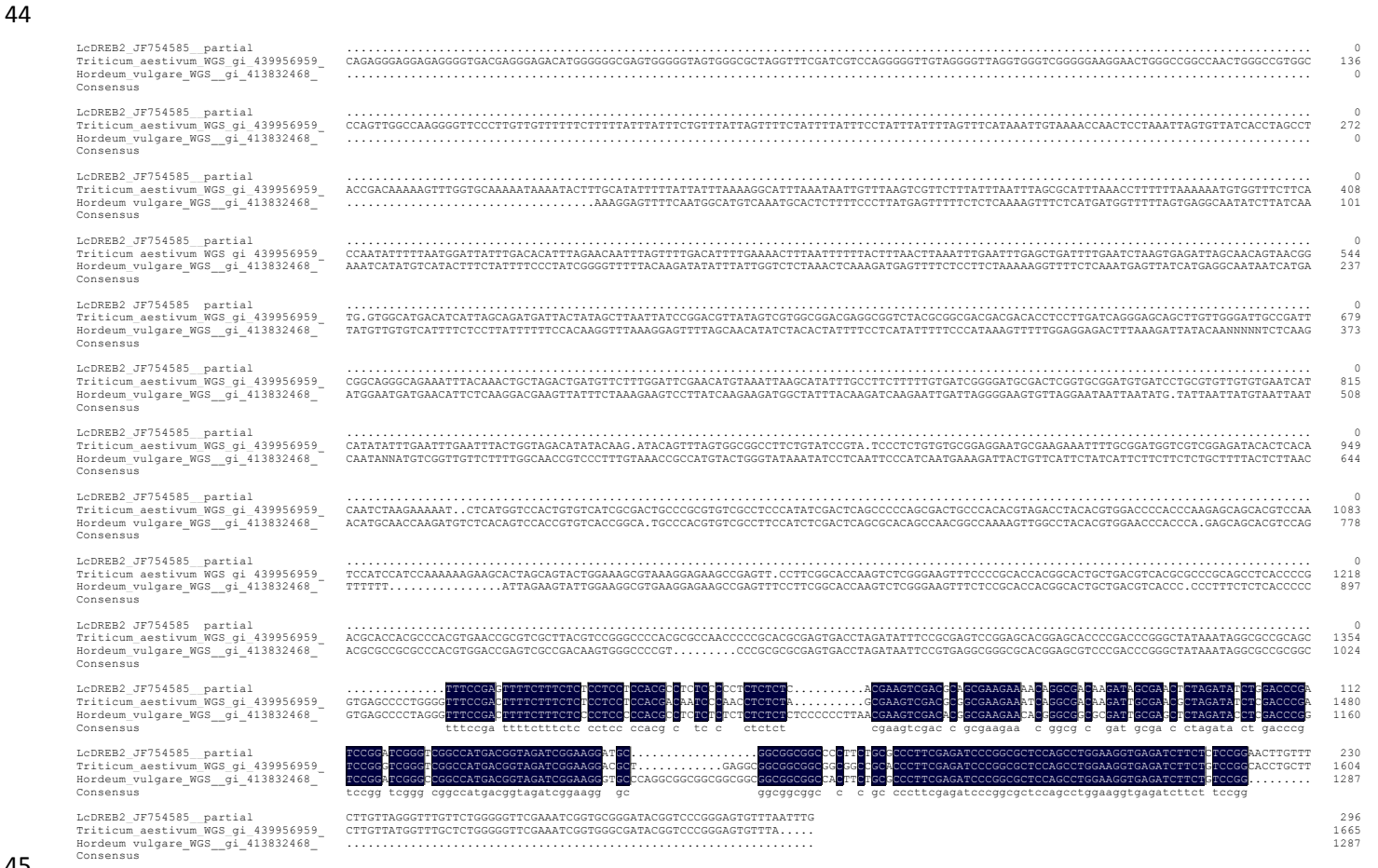
21 LcMYB2-ORF-F: 5'-ATGACGAGGCGGTGCTCGCAC-3'

22 LcMYB2-ORF-R: 5'-TCAAACGTCATGGATTGGGCTG-3'

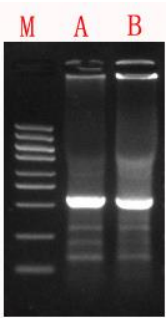


26 **2: Amplification of LcDREB2 promoter sequence**

27 Previous studies revealed that *Leymus chinensis* has closer relationship to *Hordeum vulgare* and *Triticum*
 28 *aestivum* [1], therefore, we search the whole-genome shotgun contigs (WGS) of *Hordeum vulgare* and
 29 *Triticum aestivum* with *LcDREB2* (JF754585) and find that partial sequence locating at 5'-end of *LcDREB2*
 30 is aligned to gi_413832468 (*Hordeum vulgare*) and gi_439956959 (*Triticum aestivum*) (Fig. 1). Two primers
 31 (P1, P4) were designed according to the conserved sequence of *Hordeum vulgare* and *Triticum aestivum* and
 32 were used to amplify the putative promoter of *LcDREB2* using total genomic DNA of sheepgrass as template.
 33 A fragment about 500bp was amplified and cloned into pMD18-T vector for sequencing (Fig. 2). Three
 34 independent clones were sequenced and used to assemble with *LcDREB2* (Fig. 5). According to the
 35 assembling sequence (AS1), two primers (P190, P268) were designed to amplify the upstream unknown
 36 sequence based the Self-Formed Adaptor PCR [2]. A fragment of about 1000bp was obtained (Fig. 3),
 37 ligated into pMD18-T vector, sequenced and assembled with AS1 to get the new sequence (AS2) (Fig. 5).
 38 Based on the same method, we acquired another fragment using primers P561 and P784 (Fig. 4). At last, all
 39 the sequence were assembled together to form a putative complete *LcDREB2* sequence (Fig. 5). At this time,
 40 a primer (F7) picked out in the putative promoter region and another primer (R1821) picked from the coding
 41 sequence of *LcDREB2* were used to confirm our previous assembly result. We successfully amplified a
 42 1500bp fragment and sequence analysis revealed that about 200bp of this fragment is the same as the 5'-end
 43 of *LcDREB2* (JF754585) (Fig. 6, 7).

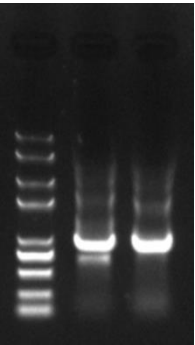


46 Fig. 1 Sequence alignment of *Leymus chinensis* (*LcDREB2*), *Hordeum vulgare* (gi_413832468) and *Triticum*
 47 *aestivum* (gi_439956959)



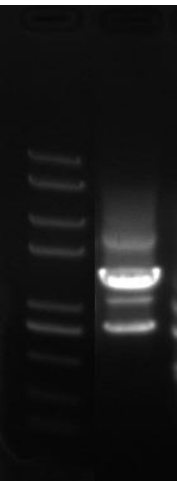
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Fig. 2 The single main band on 1% agarose gel showing the products amplified with P1 and P4
M: BMD5000+1.5k ladder, the bands indicate 5, 3, 2, 1.5, 1, 0.75, 0.5, 0.25, 0.1 (kbp) from up to down



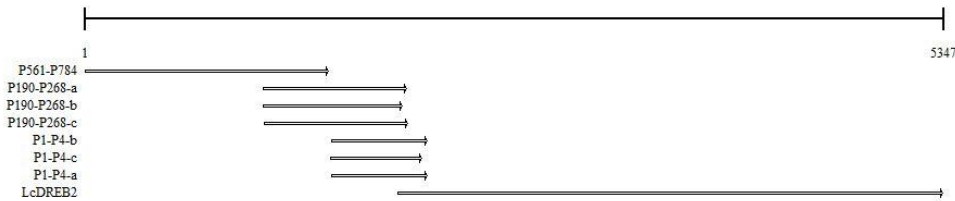
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Fig. 3 The single main band on 1% agarose gel showing the products amplified with P190 and P268



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Fig. 4 The single main band on 1% agarose gel showing the products amplified with P561 and P784



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Fig. 5 The assembly result of putative promoter sequence and transcriptional region of *LcDREB2*

90 GAGCGTCTGCTCTGCGCTGGAGCATCAGGGGCTTGGGGCACGCTGCTGGGGTGGAAACCCGGA
91 GGAGAGGAGGCGGCGGGGCTAGTTGCGGTGGCGCCGACATCGCAGCTACGTGCTAACGTGGGG
92 TGGCAGTGGCGGCGTAACCCTAGCGCTCGGGCTGGAGAAAGAACGAGAGGAAGAAGTCATGC
93 GTGACCAGAGATGGCAGCCCCTTGTTCCTCGGCGGTGGCATGCTTTGAAATTTTAGCGAAGTAC
94 CAAACCGTGCATTTTTTCACTTGGCGGTGGTAGGGGGTGTAAATTTGTGTGGACTCCATGAAAATG
95 GAGAATTCGTGGCGCTGGGAATACCCAGCTTCTCCAACCTCCGCCAAAAAATGCACTGAGGCCCA
96 ATACAGCTTCTCGAATTTAGCTTCGCAGAGCTCAGGCGTTCGGCCCCAGCTTCACACGCGGAGT
97 TCGCGAAGTGCAGAGCTGGAGAAGGTTTCAGAACAAGACCCTTGGGGGACGGATTTGTCACGAA
98 TGTAGATGCTCTAAGGAAAAGGAAAAGGTGTTGGAGTTGCTCTCGGGACCGGAGAGTGGACTC
99 CACGCTTGCCACCACTGATCCATGCAACAAAGATGTCTCACAATCCATCTTGTCATCCGACTGCA
100 CACGTGTCGCCTCCCATATCGACCCAGCCCCAGCAATTGGCCATACGTGGGCCTACACGTGGA
101 ACCCACCCAGAGGAGCACGTCCAATCATACAAAAAAAGGAAGTATACAAAATACTGGAAAGC
102 GTAAAGGAGAGGCCGAGTTTCTTCGGCACCAAGTCTCCGGAAGTTTCCCCGCACCACGGCACT
103 ACTTACGTCACGCGCCCGCAGTCTCACCTCCCACGCACCGCGCCACGTGGACCGTGTCACTTA
104 CGACTGGGCCCCACGCGCCAACGTCCGGACGCGAGTGACCTAGATAATTCCGCGGGGCAGGAG
105 CACGAAGTATCCCGACCCGGGCTATAAATAGGCGACGCGCCGCGTGCCCCCTGGGGTTTCCGAGT
106 TTTCTTTCTCTCCTCCTCCACGCCTCTCCCCCTCTCTCTCACGAAGTCGACGCAGCGAAGAAAAC
107 AGGCGACAAGATAGCGAACTCTAGATATCTGGACCCGATCCGGATCGGGTCGGCCATGACGGTA
108 GATCGGAAGGATGCGGCGGCGGCCCTTCTGCGCCCTTCGAGATCCCGGCGCTC

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