

Table S1. Oligonucleotide primers for PCR

<i>Aegilops</i> marker	Chro moso me arm	Wheat gene orthologue	Primer Forward 5'-3'	Primer Reverse 5'-3'	Amplicon size (bp)	Anealing temp. (°C)	Extension time (s)	DMSO
TC37B	4AL	TRIAE_CS42_4AL_T GACv1_289950_AA0 979330	AACTACAGATTCAT GACAGG	CTACTGCTACCTCC TCTCTT	85	60	30s	No
	4BS	TRIAE_CS42_4BS_T GACv1_328956_AA1 096100	-	-	-	-	-	-
	4DS	TRIAE_CS42_4DS_T GACv1_361737_AA1 172310	ACGGATTAGTCACA ACAAGC	GTTGGGAAGACAG ATAATGC	80	60	30s	No
KT71	4AL	TRIAE_CS42_4AL_T GACv1_288750_AA0 957220	GGARTTTGATTTCC CGCCAT	[FAM]GCAGCCCAG AAGAAAATGACA	279	59		No
	4AL	TRIAE_CS42_4AL_T GACv1_290111_AA0 981820			301			
	4BS	TRIAE_CS42_4BS_T GACv1_328355_AA1 086780			298			
	4DS	TRIAE_CS42_4DS_T GACv1_361106_AA1 161080			310			
TC30B	4AL	TRIAE_CS42_4AL_T GACv1_290077_AA0 981360	ATCTGGTACCTGAT TTCATAGTGA	CCACAACGTACCA TTATCTACTGC	265	60	30s	Yes
	4BS	TRIAE_CS42_4BS_T GACv1_328641_AA1	CAAGGACGCAATCT CACCA	GCAACGAGGAGAT GAGCC	506	60	30s	Yes

		091570							
	4DS	TRIAE_CS42_4DS_T GACv1_361093_AA1	CAAGTTCTCTACGG TTTGGAGT	TTGATCAAGAGAAT GGGGAT	311	60	30s	Yes	
		160690							
TC34	4AL	TRIAE_CS42_4AL_T GACv1_289509_AA0	ATGACACCTTTATT TCAGCCAG	AGCTCGGTCTGCAT TTGA	639	62	30s	No	
		972250							
	4BS	TRIAE_CS42_4BS_T GACv1_328517_AA1	CGCTCACCATCACC CAAG	GAGCTGAAGCGAA CGAAC	283	62	30s	No	
		089200							
	4DS	TRIAE_CS42_4DS_T GACv1_362688_AA1	TGCCATCATCGGTA GTCATT	TGGTGTGCTGTTGA TCCTT	333	62	30s	No	
		181550							
4G	4AL	TRIAE_CS42_4AL_T GACv1_289219_AA0	GATGAGCCGCCTCC CCAT	CCTTTGCTGATGCA GTTCG	304	60	30s	No	
		967530							
	4BS	TRIAE_CS42_4BS_T GACv1_330068_AA1	-	-	-	-	-	-	
		105770							
	4DS	TRIAE_CS42_4DS_T GACv1_362384_AA1	TGGAACACTGCCAT CGTG	GGTGGAGCGAGAT ATGAGATC	311	60	30s	No	
		179360							
4N5.5	4DS	TRIAE_CS42_4DS_T GACv1_363571_AA1	GGCTTTGATACTGG AACGAAT	CAGTGTAAGGCTCT GTTGCG	-	-	-	-	
		183970							

Primers were designed to wheat TGACv1 cv. Chinese Spring gene models (Clavijo et al., 2017). Marker KT71 was designed to amplify all homoeologues whilst the other markers were designed to be homoeologue-specific. For 4N5.5, only a D-genome-specific marker was designed. FastStart Taq DNA Polymerase and buffer (Roche supplied by Sigma-Aldrich) were used in PCR reactions according to the manufacturer's instructions. Primer sequences and PCR conditions are shown above. [FAM] = 5' FAM (6-fluorescein amidite) labelling of primer.

1 **Table S2. Physical map positions of markers used for genotyping deletions**

	Chromosome arm	Wheat gene orthologue/deletion	Position on 4AL (bp)		Position on 4DS (bp)	
			start	end	start	end
Deletion (exome capture data)						
	4AL		576,167,498	601,039,825		
	4DS				0	21,516,171
<i>Aegilops</i> markers						
TC37B	4AL	TRIAE_CS42_4AL_TGACv1_289950_AA0979330	591,845,338	591,852,359		
	4DS	TRIAE_CS42_4DS_TGACv1_361737_AA1172310			10,966,857	10,975,050
KT71	4AL	TRIAE_CS42_4AL_TGACv1_288750_AA0957220	590,187,733	590,179,759		
	4AL	TRIAE_CS42_4AL_TGACv1_290111_AA0981820	590,119,776	590,111,029		
	4DS	TRIAE_CS42_4DS_TGACv1_361106_AA1161080			12,457,943	12,465,627
TC30B	4AL	TRIAE_CS42_4AL_TGACv1_290077_AA0981360	589,607,862	589,602,306		
	4DS	TRIAE_CS42_4DS_TGACv1_361093_AA1160690			12,704,776	12,699,536
TC34	4AL	TRIAE_CS42_4AL_TGACv1_289509_AA0972250	589,010,812	589,016,280		
	4DS	TRIAE_CS42_4DS_TGACv1_362688_AA1181550			13,248,110	13,242,447
4G	4AL	TRIAE_CS42_4AL_TGACv1_289219_AA0967530	584,700,513	584,693,518		
	4DS	TRIAE_CS42_4DS_TGACv1_362384_AA1179360			15,877,787	15,870,454
4N5.5	4DS	TRIAE_CS42_4DS_TGACv1_363571_AA1183970			16,611,579	16,608,549

2

3 The positions of the deletions in cv. Paragon deletion lines A1 and D4, as determined by exome capture and of the genes used as PCR markers

4 (IWGSC RefSeq v1.0, cv. Chinese spring) are shown.

