Chromosome 7: TG572, 48cM

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PCR primer pairs were designed for RFLP probe: TG572 (Fig. 1).

Fig. 1: RFLP map of the middle of Chr. 7 (Adapted from Pan et. al., 1999).

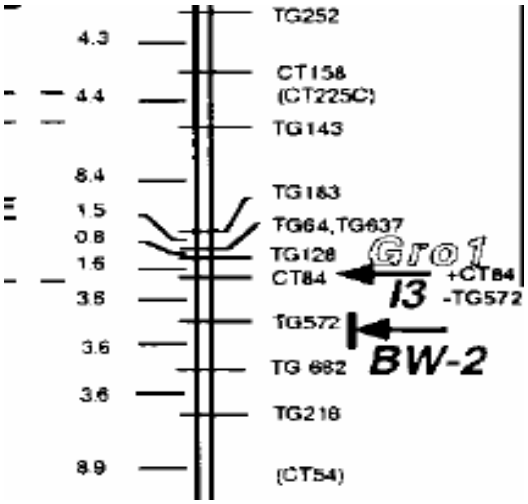

Table 1: Primers from the TG572 probe on Chr. 7

TG572	Primer Sequence (5' to 3')
P572F1	GAGACCAGCTTTGGATTTCTTTGGG
P572F2	GGAGAAGAGGATACTGCTCTAATG
P572F3	CCGGTCTGGAGTAGATTTTCTTG
P572R1	CACGGGATTCCAGAAGTAGGC
P572R2	GGAAAAGTAGAGAGAATCGCCATG

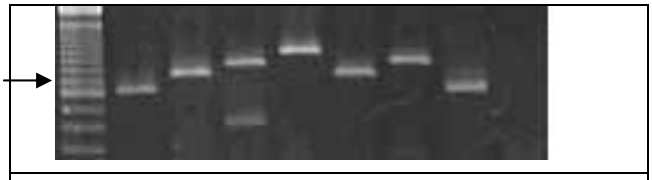

Fig. 2: Agarose gel of the PCR reactions with the TG572 primers and Heinz 1706 DNA. lane 1, 100-bp ladder; lane 2, P572F1/P572R1; lane 3, P572F1/P572R2; lane 4, P572F2/P572R1, lane 5, P572F2/P572R2; lane 6, P572F3/P572R1; lane 7, P572F3/P572R2, lane 8, positive control P301F3/P301R2; lane 9, water. The P572F1/P572R2 primer pair was chosen for sequencing reactions due to its size and the brightness of its band. Arrow marks the 700bp fragment.

TG572 RFLP Probe: Five primers were designed around the RFLP probe on chromosome 7: P572F1, P572F2, P572F3, P572R1, and P572R2 (Table 1). All possible primer combinations produced a strong band with Heinz 1706 DNA between 600 bp and 1000 bp (Fig. 2). All of the primer combinations produced single bands with the exception of P572F2/P572R1, which produced two bands one at 400 bp and one at 900 bp. The P572F1/P572R2 primer combination produced a strong single band at 800bp and was chosen for use with additional genotypes. Some primer combinations produced larger and stronger single bands, but PCR fragments larger than 900 bp are often difficult to sequence with only one primer pair and thus these primer pairs were passed over.

Upon use with the additional genotypes the P572F1/P572R2 primer pair produced a strong single band with Heinz 1706, Cerasiforme, LA1777, LA3949, LA3948, LA3950, and LA3951 at 800 bp. These PCR fragments were directly sequenced with both the forward and reverse primers. Cerasiforme (acc. no. DQ437765) produced sequence with both primer pairs. LA1777 (acc. no. DQ437772), LA3949, and LA3950 all produced good sequence with the forward primer. Heinz 1706, LA3948, and LA3951 did not produce sequence with either primer.

Upon sequence alignment the two RIL sequences matched Cerasiforme identically. These sequences differed from LA1777 by 11 SNP and one INDEL out of 600 bp of overlap (the total number of nucleotides sequenced was 700 but because only Cerasiforme gave sequence with both primers the overlap was reduced to 600 bp). Therefore, there is strong evidence that the RIL do not have an introgression from LA1777 in the region around the TG572 RFLP primer.

Table 2: Sequence differences between the RIL and LA1777 at the TG572 locus. Note that all of the RIL matched identically with Cerasiforme and are represented here by a single "RIL" label. The nt position is relative to Cerasiforme.

Line	**SNP 1**	**SNP 2**	**SNP 3**	**INDEL**	**SNP 4**	**SNP 5**	**SNP 6**	**SNP 7**	**SNP 8**	**SNP 9-10**	**SNP 11**
RIL	C	A	T	.	T	T	C	A	T	TA	T
LA1777	G	G	C	T	G	C	G	T	G	GT	A
nt Position	136 bp	141 bp	182 bp	273 bp	305 bp	506 bp	583 bp	614 bp	619 bp	636 bp	640 bp

References

Pan, Q., Liu, Y., Budai-Hadrian, O., Sela, M., Carmel-Goren, L., Zamir, D., and Fluhr, R. 1999. Comparative genetics of nucleotide binding site-leucine rich repeat resistance gene homologues in the genomes of two dicotyledons: tomato and arabidopsis. *Genetics Society of America* 88:309-322.