

## PCR protocol for the co-dominant SCAR marker, FLUW-25, for detection of the introgression at 25 cM (*Ty-3* locus) of chromosome 6

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### Design of FLUW-25 primers:

Ji and Scott (2006a, 2006b; Ji et al., 2007) mapped the *Ty-3* gene from LA2779 (*S. chilense*) for resistance to *Tomato yellow leaf curl virus* to an introgression near the *FER* locus (25 cM) on chromosome 6. The BAC clone 56B23 (AY678298) contains the *FER* gene. PCR primers were designed by Ji and Scott (Lend, Ji et al. 2007. Mol. Breeding, in press) to amplify sequences near the 5' end of the BAC clone. These primers were used to amplify PCR fragments from the begomovirus-susceptible heritage tomato, *S. lycopersicum* 'Purple Russian', and a begomovirus-resistant breeding line, Gc43, from a tomato breeding program in Guatemala with an introgression in this region (Mejía et al., 2005). These sequences were aligned, and forward and reverse primers designed: FLUW-25F (5' CAAGTGTGCATATACTTCATA(T/G)TCACC) and a reverse primer, FLUW-25R (5' CCATATATAACCTCTGTTTCTATTTTCGAC). It was expected that these primers would give PCR fragments for *S. lycopersicum* and the LA2779 introgression of 475 and 641 bp, respectively (M. S. Salus, C. T. Martin, and D. P. Maxwell, personal communication).

### **FLUW-25F (DM6-F30)**

5' CAAGTGTGCATATACTTCATA(T/G)TCACC

### **FLUW-25R (DM6-R30)**

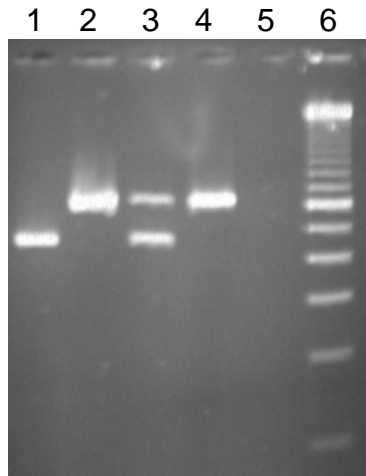
5' CCA TAT ATA ACC TCT GTT TCT ATT TCG AC 3'

PCR conditions: 25 µl reaction: 2.5 µl 2.5 mM dNTPs, 2.5 µl 10X buffer, 2.5 µl 25 mM MgCl<sub>2</sub>, 0.1 µl *Taq* polymerase (Promega Corp., Madison, WI), 2.5 ul each forward and reverse sense primer at 10 µM, 5 µl of 15 ng/µl DNA extract and H<sub>2</sub>O. PCR cycler (MJ DNA Engine PT200 Thermocycler™, MJ Research Inc., Waltham, MA) parameter: denaturation at 94 C for 3 min, then 35 cycles at 94 C for 30 sec, annealing at 53 C for 1 min, and extension at 72 C for 1 min, followed by 72 C for 10 min, then the reaction is held at 4 C.

### Results:

These FLUW-25 primers amplify fragments of 480 bp for *S. lycopersicum* and about 640 bp for the introgression in lines from HUJ (Ih902-derived lines from *S. habrochaites* LA1777 and LA0386, Vidavsky and Czosnek, 1998) and U of FL (derived from LA2779). The two fragment sizes were sequenced from a putative heterozygous plant (228-1), and the sequences were as expected for each fragment size. For an F2 population of

Gh13 (resistant) x M82 (susceptible), the ratio of introgression-homozygous: heterozygous: homozygous *S. lycopersicum* was 18:29:17, respectively.



Lane 1, Heinz (susceptible)  
Lane 2, Gh25  
Lane 3, 228-1(lh902 x Daniella), selected in Morocco  
Lane 4, Gc43 (Gc9 x Marina)  
Lane 5, water control  
Lane 6, 100-bp Invitrogen marker, bright band=600 bp

Note: Gh25, 228-1, and Gc43 are resistant to begomoviruses in Guatemala.

When these primers were used with begomovirus-resistant lines derived from LA1932 *S. chilense*, no fragment was produced. It was known that these lines had an introgression at FER locus from sequence of the G8 gene of the BAC clone. A new set of primers was designed (P6-25 locus), which amplified difference size fragments from *S. lycopersicum*, LA2779-derived lines, and LA1932-derived lines (P6-25 co-dominant SCAR marker, this web site).

Surprisingly, the sequence of the 600-bp fragment from lines derived from the resistance sources (902, *S. habrochaites*) from Hebrew University of Jerusalem, e.g., Gh25, was identical to the 600-bp fragment from LA2779-derived lines. It is unexpected that the sequences from *S. habrochaites* and *S. chilense* would be identical, as these species fall into distinct phylogenetic clades (Salus and Maxwell, unpublished)

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- Ji, Y. and Scott, J.W. 2006b. *Ty-3*, a begomovirus resistance locus linked to *Ty-1* on chromosome 6. Rept. Tomato Genetics Coop. 56: (in press).
- Ji, Y., Schuster, D.J., and Scott, J.W. 2007. *Ty-3*, a begomovirus resistance locus near the Tomato yellow leaf curl virus resistance locus *Ty-1* on chromosome 6 of tomato. Mol. Breeding (in press)
- Mejía, L., Teni, R.E., Vidavski, F., Czosnek, H., Lapidot, M., Nakhla, M.K., and Maxwell, D.P. 2005. Evaluation of tomato germplasm and selection of breeding lines for resistance to begomoviruses in Guatemala. Acta Hort. 695:251-255.

Vidavsky, F., and Czosnek, H. 1998. Tomato breeding lines immune and tolerant to tomato yellow leaf curl virus (TYLCV) issued from *Lycopersicon hirsutum*. *Phytopathology* 88:910-914.

Sequences:

Heinz 1706 (*S. lycopersicum*)

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1      CAAGTGTGCA TATACTTCAT AGTCACCCAA CCACTATTTTTC TTCCAAACCT TCAACCTTAC
61     CATCGTATTA GCATAGGGTG AGTGAAATGT AGGATTATAC ATGGGGTATT CAGTCGTAAG
121    AACGTGTTAT AAAGGCTAAA AGGGAAGTTC TACTTCTTGT AAAATATAAA GGTAGTGGAA
181    ATGATGCTGC TCAAATTATT GTGTGAACAT ATTATGAGAG GTAGGATTAA GAATGAAGTT
241    ATATAAGATA AAGTGGAAGT TACTTTTTCGA AAAAAAAGA AAGACGAAAA AAATGAGATT
301    GAAATGGATT GAATACGTGA AGAAGAGATG CATGGGTTC AATAAAAAA GGTTTGAGAG
361    TTTGACTTAA GAAGAGGTAG AAGTAGGTTG AAAAACAACT AGGTAAAGTT TTACTTTTGT
421    TTTTGTTTTG ATTGCACATT TTTTGTAGTCG AAATAGAAAC AGAGGTTATA TATGG

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Gc43 (35a) (resistance from LA2779)

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1      CAAGTGTGCA TATACTTCAT AGTCACCCCA CCACTATTTTTC TTCCAAACCT TAAACCTTAC
61     CCTTAAGTTT AAAGTGACAT GGAGATTGAT GATGATCTTG TACATTGTAT TAGCATAGGG
121    TGAGTGAAAT GTATACATGG GGTATTCAGT CGTAAGAACG TGTTATAAAG GCTTAAAGGG
181    AAGTTCTACT TGTAATAATAT AAAGGTAGTG GAAATGATGC TGCTCAAATT AATGTGTGAA
241    CATGAGAGGT AGGATTAGAA ATGAAGTTAT ATAAGATAAA GTGGAAGTAA CTCCAATAA
301    AAAAAGACGA AAAAAATGAG ATTGAAATGG GTTGAATACG TGAAGAAGAG ATGCATGGAT
361    TCACCAATAA AAAGGTATGA GAGTTTGACT TAAGAAGATG TAGAAGTAGG TTGAAAAAAA
421    ACTACGTAAA GATGATTAGA TAAGATATAT CACGAGGACA CGACTATAGC AAGATATGGC
481    AGCAGAGTTT TGTCGTATTG TTACATGGAA GAGGTAAGGG ACTTGTCTCT GCTTTTCATG
541    CACATTGCTT CAATTTACTT TGTTAGACTT GTTATTTTAC TTTTGTCTCT GTTTTGATTG
601    CACATTTTTT TAGTCGAAAT AGAAACAGAG GTTATATATG G

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# Alignment of Heinz (susceptible) by Gc43 (LA2779-derived resistance to begomoviruses in Guatemala) for the co-dominant SCAR marker, FLUW-25

FLUW-25 (25 cM)Chromosome 6: Sept. 14, 2006 - Heinz = Heinz 1706 and 35a = Gc43 (introgression from *S. chilense* LA2779)

35a_F30-R30	CAAGTGTGCATATACTTCATAGTCACCCCACCCACTATTTCTTCCAAACCTTAAACCTTAC	60
HeinzF30-R30	CAAGTGTGCATATACTTCATAGTCACCC <b>a</b> ACCCTATTTCTTCCAAACCTT <b>c</b> AACCTTAC	60
Consensus	caagtgtgcataatacttcatagtcaccc accactatttcttccaaacctt aaccttac	
35a_F30-R30	<b>CCTTAAGTTTAAAGTGACATGGAGATTGATGATGATCCTTGTAC</b> ATTGTATTAGCATAGGG	120
HeinzF30-R30	C.....AT <b>c</b> GTATTAGCATAGGG	78
Consensus	c at gtattagcataggg	
35a_F30-R30	TGAGTGAAATGTA.....TACATGGGGTATTTCAGTCGTAAGAACGTGTTATAAAGGCTT	174
HeinzF30-R30	TGAGTGAAATGTA <b>aggatta</b> TACATGGGGTATTTCAGTCGTAAGAACGTGTTATAAAGGCT <b>a</b>	138
Consensus	tgagtgaaatgta t acatggggatttcagtcgtaagaacgtgttataaaggct	
35a_F30-R30	AAAGGGAAGTTCTACTT...GTAATAATATAAAGGTAGTGGAATGATGCTGCTCAAATTA	231
HeinzF30-R30	AAAGGGAAGTTCTACTT <b>ctt</b> GTAATAATATAAAGGTAGTGGAATGATGCTGCTCAAATTA	198
Consensus	aaaggggaagttctactt gtaataatataaaggtagtggaatgatgctgctcaaatta	
35a_F30-R30	ATGTGTGAACAT.....GAGAGGTAGGATTAGAAATGAAGTTATATAAGATAAAGTGGAA	286
HeinzF30-R30	<b>t</b> TGTGTGAACAT <b>atttat</b> GAGAGGTAGGATTA <b>ag</b> AATGAAGTTATATAAGATAAAGTGGAA	258
Consensus	tgtgtgaacat gagaggtaggatta aatgaagttatataagataaagtgga	
35a_F30-R30	GTAACCTCC.....AATAAAAAAAGACGAAAAAATGAGATTGAAATGGGTGAATACGT	341
HeinzF30-R30	GT <b>t</b> ACTTT <b>tcgaaa</b> AAAA <b>g</b> AAAGACGAAAAAATGAGATTGAAATGG <b>a</b> TTGAATACGT	318
Consensus	gt actt aa aaa aaagacgaaaaaatgagattgaaatgg ttgaatacgt	
35a_F30-R30	GAAGAAGAGATGCATGGATTACCAATAAAAAAGGTATGAGAGTTTGACTTAAGAAGATGT	401
HeinzF30-R30	GAAGAAGAGATGCATGG <b>g</b> TTACCAATAAAAAAGGT <b>t</b> TGAGAGTTTGACTTAAGAAG <b>g</b> GT	378
Consensus	gaagaagagatgcatgg ttcaccaataaaaaggt tgagagtttgacttaagaaga gt	
35a_F30-R30	AGAAGTAGGTTGAAAAAAACTACGTAAAG <b>ATGATTAGATAAGATATATCACGAGGACAC</b>	461
HeinzF30-R30	AGAAGTAGGTTGAAAA <b>ca</b> ACT <b>ag</b> GTAAG.....	408
Consensus	agaagtaggttgaaaaa aacta gtaaag	
35a_F30-R30	<b>GACTATAGCAAGATATGGCAGCAGAGTTTTGTCTGTTTACATGGAAGAGGTAAGGGA</b>	521
HeinzF30-R30	.....	408
Consensus		
35a_F30-R30	<b>CTTGTCTCTGCTTTTCATGCACATTGCTTCAATTTACTTTGTTAGACTTGTATTTTACT</b>	581
HeinzF30-R30	.....TTTTACT	415
Consensus	ttttact	
35a_F30-R30	TTTAGTTCTGTTTTGATTGCACATTTTTTTAGTCGAAATAGAAACAGAGGTTATATATGG	641
HeinzF30-R30	TTTAGTT <b>t</b> GTTTTGATTGCACATTTTTTTAGTCGAAATAGAAACAGAGGTTATATATGG	475
Consensus	tttagtt tgttttgattgacacatTTTTTTAGTCGAAATAGAAACAGAGGTTATATATGG	