

**Analysis of sequences from the TG105 marker of
Chromosome 11 as a tag for Ty-2 locus from *S.*
*habrochaites***

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Recently, Drs. E. Graham and P. Hanson reported (2007) that the Ty-2 locus, which was derived from *S. habrochaites*, was located between RFLP markers TG36 (84.0 cM) and TG26 (92.0 cM) on chromosome 11 and that the TG105A marker is closely associated with the introgression and could be used to follow the introgression in breeding populations. The PCR primers for TG105A (90 cM) (TG105AF, 5' CTTGAGAATTCCTGTTTTAGTCAGTTGAACC 3', and TG105AR, 5' ATGTCACATTTGTTGCTTGGACCATCC 3', at an annealing temperature of 55°C) were shown by L. Mejia and D. P. Maxwell (2002) to give different sequences for H24 (Ty-2 gene) and a susceptible line, M82. Also, the introgression associated with H24 was nearly identical to the sequence from *S. habrochaites* LA1223 (DNA from D. Spooner, UW-Madison). Graham and Hanson showed that the restriction enzyme digestion with *TaqI* of the PCR products generate polymorphic bands for H24 (introgression from *S. habrochaites*) and *S. lycopersicum* (susceptible).

As part of a study to detect the Ty-2 gene in different begomovirus-resistant breeding lines being developed in Guatemala, the TG105AF/TG105AR primers were used to amplify fragments from the following germplasm: H24 (Ty-2 locus, derived from an unknown *S. habrochaites*), Gc143-2 [resistant in Guatemala to begomoviruses, derived from a cross of Gc9 (introgression for resistance from *S. chilense* LA2779) x Marina], Glh902b (resistant in Guatemala, introgression from *S. habrochaites* LA1777 and/or LA0386), and M82 (susceptible in Guatemala).

Alignment of these sequences showed that H24 had a unique introgression that was nearly identical to the sequence from *S. habrochaites* LA1223 (see below). Gc143-2 and Glh902b had **identical sequences** and they also had an introgression that was different than the introgression associated with H24.

The next step was to analyze these sequences for their restriction patterns with *TaqI*. There were four *TaqI* restriction sites associated with M82 and the approximate fragment sizes are 15, 30, 90, and 200 bp. Two restriction sites are in the H24 sequence giving fragment sizes of approximately 40, 100, and 315 bp. Two restriction sites are also associated with the introgression present in Gc143-2 and Glh902b, which give fragment sizes of approximately 40, 90 and 310 bp. Thus, it would be **difficult** to distinguish between the introgression associated with H24, which has the Ty-2 locus, and the

introgression in Gc143-2 and Glh902b, which does *NOT* have the Ty-2 locus using normal agarose gel electrophoresis.

The M82 sequence was identical to the sequence from TY50 (*S. lycopersicum* sequence) and from *S. lycopersicum* var. *cerasiforme*. These data provide additional evidence that M82 sequence is that of *S. lycopersicum*.

Sequence alignment:

M82_105F-R	CTTCAGAATTCCTGTTTTAGTCAGTTGAACCCCTCACTTGTTCCTTTTTGAAACCTGCAGA	60
Gc143-2-105FR	CTTCAGAATTCCTGTTTTAGTCAGTTGAACCCCTCACTTGTTCCTTTTTGAAACCTGCAGA	60
902b-105FR	CTTCAGAATTCCTGTTTTAGTCAGTTGAACCCCTCACTTGTTCCTTTTTGAAACCTGCAGA	60
H24-105FR	CTTCAGAATTCCTGTTTTAGTCAGTTGAACCCCTCACTTGTTCCTTTTTGAAACCTGCAGA	60
Consensus	cttcagaatTCCTGTTTTAGTCAGTTGAACCCCTCACTTGTTCCTTTTTGAAACCTGCAGA	
M82_105F-R	ATTTCAACATGACAATAAATGTGAAAAAGACCATAATATAAGCTACATCGAT...ATG	116
Gc143-2-105FR	ATTTCAAtATGACAATAAATGTGAAAAAGACCATAATATAAGCTACAcCGAT...ATG	116
902b-105FR	ATTTCAAtATGACAATAAATGTGAAAAAGACCATAATATAAGCTACAcCGAT...ATG	116
H24-105FR	ATTTCAACATGACAATAAATGTGAAAAAGACCATAATATAAGCTACATCaATcaatATG	120
Consensus	atTTCAA atgacaataaAtGTGAAAAAGaccataatataagctaca c at atg	
M82_105F-R	CACTGCGGACGCAGGATTTTCATTAAGGAGTACAGTAAAAAAATAGGCACACACGACC	176
Gc143-2-105FR	CACTaGCGGACGCAGGATTTTCATTAAGGAGTACAGTAAAAAAATAGGCACACACGACC	176
902b-105FR	CACTaGCGGACGCAGGATTTTCATTAAGGAGTACAGTAAAAAAATAGGCACACACGACC	176
H24-105FR	CAgTGGCGGACGCAGaATTTTCATTAAGGAGTACAGTAAAAAAATAGGCACACACcACC	179
Consensus	ca t gcggacgcag atTTTCATTAaggagTAcagTAAAAAA t ggcacacac acc	
M82_105F-R	TCATATAGTGTTTGCAAACCCCTAACAAATAGACTAAGCCCTTTAACTTGTTTTAAGAGA	236
Gc143-2-105FR	TCATATAGTGTTTGCAAACCCCTAACAAATAGACTAAGCCCTTTAACTTGTTTT.AAGAGA	235
902b-105FR	TCATATAGTGTTTGCAAACCCCTAACAAATAGACTAAGCCCTTTAACTTGTTTT.AAGAGA	235
H24-105FR	TCATAcAgTGTTTGtAAACCCCTAACAAATAGAcAAaCCCTTcAACTTGTTTTAAGAcA	239
Consensus	tcata agTgTttg aaacccctaacaAatagac aa ccctt aactTgTtt aaga a	
M82_105F-R	TGTCaAATTTATGTATTTATATTTATAAACTAAAAATTTAATCACTATATACAACGTAATT	296
Gc143-2-105FR	TGTCaAATTTATGTATTTATATTTAcAAAATTTAATCACTATATACAACGTAATT	295
902b-105FR	TGTCaAATTTATGTATTTATATTTAcAAAATTTAATCACTATATACAACGTAATT	295
H24-105FR	TGTaAATTTATGTATTTATATTTATAAACTAAAAATTTAAcCACTATATACAACGTAATT	299
Consensus	tgt aatTTatgtatTTtatTTta aaactaaatTTta cactatatacaacgtaatt	
M82_105F-R	TTCCGACAAAGGAGTGTGCG.....AGCCAAGGTAGGTCCTGCCTGCTGCGAT	342
Gc143-2-105FR	TTCCGACAAAGGAGTGTGCG.....AGCCAAGGTAGGTCCTGCCTGCTGCGAT	341
902b-105FR	TTCCGACAAAGGAGTGTGCG.....AGCCAAGGTAGGTCCTGCCTGCTGCGAT	341
H24-105FR	TTCCGACgAAGGAGTGTGCGctcgacaccccttggacCAAGGTAGGTCCTcCACTGCTGCGAT	359
Consensus	ttccgac aaggagTgtcg ccaaggtaggtcct cactgTcgat	
M82_105F-R	ATGCTATGTTGCTCGAACTCTTCAAAAATCTGTCCGTTTTTAGAGAATCTAACACAGATA	402
Gc143-2-105FR	ATGCTATGTTGCTtGgACTCTTCAAAAATCTGTCCGTTTTTAGAGAATCTAACACAGATA	401
902b-105FR	ATGCTATGTTGCTtGgACTCTTCAAAAATCTGTCCGTTTTTAGAGAATCTAACACAGATA	401
H24-105FR	ATGCTATGTTGCTCGgACTCTTCAAAAATCTGTCCGTTTTTAGAGAATCTAACACAGATt	419
Consensus	atgctatgTtGct g actcttcaaaaatctgtccgTTTTtagagaatctaacacagat	
M82_105F-R	CAGCAAAACTTTTGGATGGTCCAAGCAACAAATGTGACAT	442
Gc143-2-105FR	CAGCAAAACTTTTGGATGGTCCAAGCAACAAATGTGACAT	441
902b-105FR	CAGCAAAACTTTTGGATGGTCCAAGCAACAAATGTGACAT	441
H24-105FR	CAGCAAAACTTTTGGATGGTCCAAGCAACAAATGTGACAT	459
Consensus	cagcaaaactTTTggatGGTccaagcaacaAatgtgacat	

Computer generated restriction analysis for *TaqI* (TCGA):

Restriction sites on H24-105FR

1 CTTCAGAATTCCTGTTTTAGTCAGTTGAACCCTCACTTGTTTCCTTTTTGAAACCTGCAGA
GAAGTCTTAAGGACAAAATCAGTCAACTTGGGAGTGAACAAGGAAAAACTTTGGACGTCT

61 ATTTCAACATGACAATAAATGTGAAAAAAGACCATAATATAAGCTACATCAATCAATATG
TAAAGTTGACTGTTATTTTACACTTTTTCTGGTATTATATTTCGATGTAGTTAGTTATAC

121 CAGTGGCGGACGCAGAATTTTCATTAAGGAGTACAGTAAAAAATCGGCACACACCACCT
GTCACCGCCTGCGTCTTAAAAGTAATTCCTCATGTCAATTTTTTAGCCGTGTGTGGTGGA

181 CATAAGTGTGTTGTAACCCCTAACAAATAGACAAAACCTTCAACTTGTTTTAAGACAT
GTATGTCACAAACATTTGGGGATTGTTTATCTGTTTTGGGAAGTTGAACAAAATTCTGTA

241 GTAAATTTATGTATTTATATTTATAAAACTAAAATTTAACCCTATATACAACGTAATTT
CATTTAAATACATAAATATAAATATTTTGATTTTAAATTGGTGATATATGTTGCATTA

301 TCCGACGAAGGAGTGTGCGT**TCGA**CACCCCTTGGACCAAGGTAGGTCCTCCACTG**TCGATA**
AGGCTGCTTCCTCACAGCGAGCTGTGGGGAACCTGGTTCATCCAGGAGGTGACAGCTAT

361 TGCTATGTTGCTCGGACTCTTCAAAAATCTGTCCGTTTTTAGAGAATCTAACACAGATTC
ACGATACAACGAGCCTGAGAAGTTTTTAGACAGGCAAAAATCTCTTAGATTGTGTCTAAG

421 AGCAAACTTTTGGATGGTCCAAGCAACAAATGTGACAT
TCGTTTTGAAACCTACCAGTTCGTTGTTTACACTGTA

Restriction sites on 902b-105FR

1 CTTCAGAATTCCTGTTTTAGTCAGTTGAACCCTCACTTGTTTCCTTTTTGAAACCTGCAGA
GAAGTCTTAAGGACAAAATCAGTCAACTTGGGAGTGAACAAGGAAAAACTTTGGACGTCT

61 ATTTCAATATGACAATAAATGTGAAAAAAGACCATAATATAAGCTACACCGATATGCACT
TAAAGTTATACTGTTATTTTACACTTTTTCTGGTATTATATTTCGATGTGGCTATACGTGA

121 AGCGGACGCAGGATTTTCATTAAGGAGTACAGTAAAAAATAGGCACACACGACCTCAT
TCGCCTGCGTCTTAAAAGTAATTCCTCATGTCAATTTTTTTATCCGTGTGTGCTGGAGTA

181 ATAGTGTGTTGCAAACCCCTAACAAATAGACTAAGCCCTTTAACTTGTTTAAAGAGATGTCA
TATCACAACGTTTGGGGATTGTTTATCTGATTCCGGAAATTGAACAAATTCTCTACAGT

241 ATTTATGTATTTATATTTACAAAATTTAATCACTATATACAACGTAATTTTCCG
TAAATACATAAATATAAATGTTTTGATTTTAAATTAGTGATATATGTTGCATTAAGGC

301 ACAAAGGAGTGT**TCGAG**CCAAGGTAGGTCCTGCACTG**TCGAT**ATGCTATGTTGCTTGGACT
TGTTTCCTCACAGCTCGGTTCCATCCAGGACGTGACAGCTATACGATACAACGAACCTGA

361 CTTCAAAAATCTGTCCGTTTTTAGAGAATCTAACACAGATACAGCAAACTTTTGGATGG
GAAGTTTTTAGACAGGCAAAAATCTCTTAGATTGTGTCTATGTCGTTTTGAAACCTACC

421 TCCAAGCAACAAATGTGACAT
AGGTTTCGTTGTTTACACTGTA

M82_105F-R	TATGCTATGTTGCTCGAACTCTTCAAAAATCTGTCCGTTTTTAGAGAATCTAACACAGAT	401
H24-105FR	TATGCTATGTTGCTCGgACTCTTCAAAAATCTGTCCGTTTTTAGAGAATCTAACACAGAT	418
1223-105s	TATGCTATGTTGCTCGgACTCTTCAAAAATCTGTCCGTTTTTAGAGAATCTAACACAGAT	348
Consensus	tatgctatgttgctcg actcttcaaaaatctgtccgTTTTtagagaatctaacacagat	
M82_105F-R	ACAGCAAAACTTTTGGATGGTCCAAGCAACAAATGTGACAT	442
H24-105FR	tCAGCAAAACTTTTGGATGGTCCAAGCAACAAATGTGACAT	459
1223-105s	tCAGCAAAACTTTTGGATGGTCCAAGCAACAA	380
Consensus	cagcaaaactTTTggatggTccaagcaacaa	

References:

TG105A PCR-based protocol will occur in the following chapter of the book entitled:

Tomato yellow leaf curl virus disease

Management, molecular biology, breeding for resistance

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Sources of resistance, inheritance, and location of genetic loci conferring resistance to members of the tomato-infecting begomoviruses

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Hanson, P., Green, S.K., and Kuo, G. 2006. Ty-2, a gene on chromosome 11 conditioning geminivirus resistance in tomato. TGC Report 56:17-18.