Co-dominant SCAR Marker, P6-25, for Detection of *Ty-3*, *Ty-3a*, and *Ty3b* introgressions from three *Solanum chilense* accessions at 25 cM of Chromosome 6 of Begomovirus-Resistant Tomatoes

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Breeding for resistance to begomoviruses in tomato can be greatly aided by the availability of PCR-based markers for the various resistance loci. Four begomovirus-resistance loci or regions have been mapped to chromosome 6 (Agrama and Scott, 2006; Chagué et al., 1997; Ji and Scott, 2006b; Ji et al., 2007; Zamir et al., 1994). The *Ty-1* locus, which is part of the introgression derived from *Solanum chilense* LA1969, is located between markers TG297 (4 cM) and TG97 (8.6 cM) (Zamir et al., 1994). Agrama and Scott (2006) reported three regions that contributed to resistance in breeding lines with introgressions from *S. chilense* LA2779 or LA1932. One region corresponded to the region having the *Ty-1* locus. Another region was the *Ty-3* locus, which was mapped to a region between cLEG-31-P16 (20 cM) and T1079 (27 cM) (Ji and Scott, 2006b; Ji et al., 2007). The third region was near the self-pruning (*sp*) and potato leaf (*c*) loci. Another begomovirus-resistance QTL, derived from an introgression from *Solanum pimpinellifolium*, was mapped near the marker TG153 (33 cM; Chagué et al., 1997).

Previously, Ji and Scott (2006a, 2006b; Ji et al., 2007) reported the development of SCAR and CAPS markers linked to begomovirus resistance genes derived from *S. chilense* on chromosome 6, and they determined that the *Ty-3* locus mapped to a region that included the *FER* locus (25 cM, BAC clone 56B23, AY678298). Jensen and Maxwell (Maxwell et al., 2007) found that the sequences for the G8 gene of the BAC clone 56B23 are different for lines derived from *S. chilense* LA2779 and LA1932. To differentiate the two introgressions, the one from LA2779 is designated *Ty-3* and the one from LA1932, *Ty-3a*. A co-dominant SCAR marker, FLUW25, was reported by Ji et. (2007), which would detect ty3 (*S. lycopersicum*) and *Ty3* loci. This report describes a set of PCR primers, P6-25F2/P6-25R5, that provide co-dominant SCAR markers for detection of the *Ty-3* and *Ty-3a* introgressions and a newly discovered introgression from *S. chilense* LA1969.

Primer Design:

Initially, PCR primers were designed to amplify sequences near the 5' end of the BAC clone 56B23. These primers were used to amplify PCR fragments from the begomovirus-susceptible heritage tomato, *S. lycopersicum* 'Purple Russian', and a begomovirus-resistant breeding line, Gc43-3, from the tomato breeding program at San Carlos University, Guatemala with an introgression in this region from *S. chilense* LA2779 (Mejía et al., 2005). These sequences were aligned, and forward and reverse primers designed from conserved regions: forward primer FLUW25F (5' CAAGTGTGCATATACTTCATA(T/G)TCACC) and reverse primer, FLUW25R (5' CCATATATAACCTCTGTTTCTATTTCGAC). As expected, these primers gave PCR fragments for *S. lycopersicum* and the *S. chilense* introgression of 475 and 641 bp, respectively. A third primer

pair was designed to give smaller PCR fragments from the aligned sequences of the FLUW25 fragment for the forward primer and sequences 3' of the FLUW25R primer for the reverse primer: forward primer, P6-25-F2, 5' GGT AGT GGA AAT GAT GCT GCT C, and reverse primer, P6-25-R5, 5' GCT CTG CCT ATT GTC CCA TAT ATA ACC. The P6-25-F2/P6-25-R5 primers were expected to give fragment sizes for *S. lycopersicum* and the *S. chilense* LA2779 introgression of 320 bp and 453 bp, respectively.

DNA Extraction and PCR Methods: DNA was extracted from fresh leaves of plants with PUREGENE® DNA Purification Kit (Gentra Systems, Inc., Minneapolis, MN) and DNA adjusted to approximately 10 ng/µl. PCR parameters were for 25-µl reactions containing 2.5 µl 2.5 mM dNTPs, 5 µl 5x buffer, 2.5 µl 2.5 mM $MgCl_2$, 0.1 µl (0.5 units) Go*Taq* DNA polymerase (Promega Corp., Madison, WI), 2.5 µl each forward and reverse primer at 10 µM, 2-5 µl of DNA extract, and water. PCR cycles were 94 C for 4 min, 35 cycles of 94 C for 30 sec, 53 C for 1 min, and 72 C for 1 min. These cycles were followed by 72 C for 10 min, and then the reaction was held at 4 C. PCR reactions were performed in the MJ DNA Engine PT200 Thermocycler™ (MJ Research Inc., Waltham, MA). PCR-amplified fragments were separated by gel electrophoresis with 1.5% agarose in 0.5 X TBE buffer, stained with ethidium bromide, and visualized with UV light. ssDNA was digested in PCR reactions with shrimp alkaline phosphatase (Promega Corp.) and exonuclease I (Epicentre, Madison, WI) and the PCR-fragments were directly sequenced with Big Dye Sequencing Kit™ and analyzed by the Biotechnology Center, University of Wisconsin-Madison.

Results and Discussion:

The FLUW25 primer pair amplified fragments of 480 bp and 640 bp from the susceptible line *S. lycopersicum* Heinz 1706 and from the begomovirus-resistant breeding line, Gc43-3, with an introgression from *S. chilense* LA2779, respectively. Surprisingly, the Gh25-3 breeding line, which was derived from the begomovirus-resistant line Ih902 (see line 902 in Vidavsky and Czosnek, 1998) with resistance reported to be from an introgression from *Solanum habrochaites*, also gave a 640-bp fragment. The heterozygous plant, Gh228-1, with resistance from Ih902, gave the two sizes, 480 and 640 bp, for the *Ty3/ty3* genotype (Fig. 1).

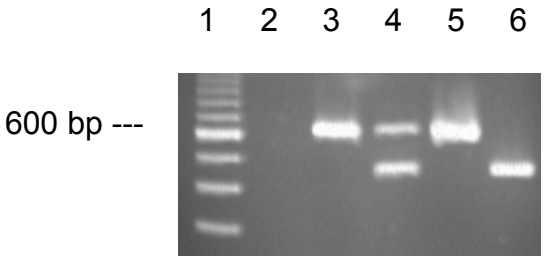

Fig. 1. PCR fragments with primers FLUW25F/FLUW25R; Lane 1, 100-bp marker, Invitrogen, bright band, 600 bp; Lane 2, water control; Lane 3, Gc43-3 (resistant); Lane 4, Gh228-1 (resistant, heterozygous); Lane 5, Gh25-3 (resistant); Lane 6, Heinz 1706 (susceptible).

The sequences for the FLUW25-PCR fragments from Heinz (475 bp) and Gc43-3 (641 bp) were aligned; and there were 18 SNPs and 5 indel differences (Maxwell et al., 2007). Single indels of 3, 6, 42, and 143 nt and two indels of 5 nt were present. The sequence for the fragment from Heinz had 100% nt identity with the sequence of the comparable region of the BAC clone 56B23. In another susceptible line, M82-1-8, the sequence for the 475-bp fragment was identical to that of Heinz. Surprisingly, the sequence for the 640-bp fragment from Gh25-3 with resistance from Ih902 had 100% nt identity with the fragment from Gc43-3. The two fragments from Gh228-1 had 100% nt identity with the 475- and 641-bp fragments from Heinz and Gc43-3, respectively. Additionally, the sequence of the 640-bp fragments from 11 other begomovirus-resistant breeding lines with

resistance from either *S. chilense* LA2779 or Ih902 had 99-100% nt identity with Gc43-3. These FLUW25 primers were used to evaluate the presence of the *Ty-3* locus in 102 breeding lines from the tomato breeding program at San Carlos University, Guatemala (Mejía et al., 2005), and the results were as expected except for the Gc171 line. This line was known to have an introgression, *Ty-3a*, in this region derived from *S. chilense* LA1932, but no PCR fragment was amplified by these primers. van Betteray and Smeets (unpublished) determined that the FLUW25R primer did not anneal to *S. chilense* LA1932 sequences. Thus, several more reverse primers were designed from the same area of the BAC clone 56B23 sequence. These were evaluated with Gc171 or lines selected from Gc171 crosses. One primer, P6-25-R5, gave fragments with FLUW25F for *S. chilense* LA1932 derived lines.

An additional primer set, P6-25-F2 and P6-25-R5, was designed to include the 143-nt *ty-3/Ty-3* indel and to give smaller fragments than the FLUW25 primer set (Fig. 2). With begomovirus-resistant breeding lines derived from either the *S. chilense* LA2779 source, Gc9, or the Ih902 line (Vidavsky and Czosnek, 1998), the expected 450-bp *Ty-3* fragment was obtained. A 320-bp *ty-3* fragment was amplified from breeding lines lacking the introgression from either of these two begomovirus-resistance sources. A 630-bp *Ty-3a* fragment was obtained from lines derived from *S. chilense* LA1932, such as Gc171. Heterozygous hybrids were easily detected with these primers which amplified two fragments corresponding to the *S. lycopersicum ty-3* fragment (320 bp) and either the *Ty-3* (450 bp) or the *Ty-3a* (630 bp) fragment (Fig. 2). No F1 hybrids were available to test for fragments with the *Ty3/Ty3a* genotype, but it is expected that this primer pair would also amplify two fragments (450 and 630 bp) with this genotype.

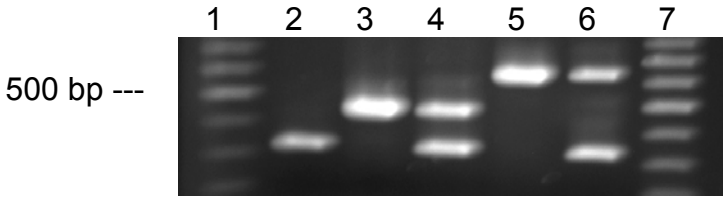

Fig. 2. PCR fragments with primers P6-25-F2/P6-25-R5. Lane 1, 100-bp Brenchtop DNA ladder, Promega; Lane 2, M82-1-8 (ty-3/ty-3); Lane 3, Gc9 (Ty-3/Ty-3); Lane 4, Romelia, F1 hybrid, (Ty-3/ty-3); Lane 5, Gc171 (Ty-3a/Ty-3a); Lane 6, GTc191-3, F1 hybrid, (Ty-3a/ty-3); Lane 7, 100-bp marker.

The three sizes of the P6-25-F2/P6-25-R5 fragments were sequenced (Maxwell et al., 2007). The 320-bp and the 450-bp fragments corresponded to the sequences of *S. lycopersicum* and of the *Ty-3* locus associated with lines derived from *S. chilense* LA2779, respectively. The 650-bp fragment from Gc171 had two large inserts, when compared with the *S. lycopersicum* sequence.

The P6-25F2/P6-25R5 primer pair was used to screen several begomovirus-resistant hybrids from different commercial seed companies. Yet, another size PCR fragment of 660 bp was obtained with three commercial hybrids (Fig. 3). This fragment was sequenced and had 100% nt identity with the fragment from *S. chilense* LA1969.

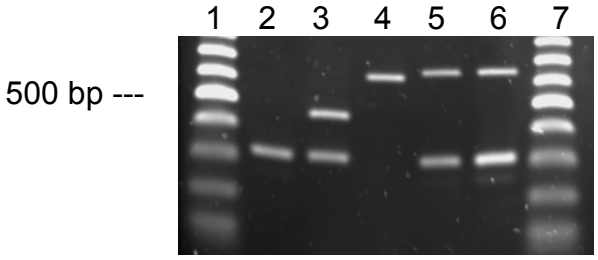

Fig. 3. 1.0% agarose gel for the PCR products with primers, P6-25F2/P6-25R5. Lane 1, Promega, 100-bp ladder, Lane 2, susceptible (ty-3/ty-3), Lane 2, resistant hybrid (ty-3/Ty-3), Lane 4, resistant breeding line (Ty-3a/Ty-3a), Lane 5 and 6, two commercial hybrids (ty-3/Ty-3b). All bands were extracted from the gel and sequenced. Note sizes: ty-3 = 320 bp, Ty-3 = 450 bp, Ty-3a = 630 bp, and Ty-3b = 660 bp.

Conclusions: These two sets of primers detect co-dominant SCAR markers, FLUW25 and P6-25, for the *ty-3*, *Ty-3*,*Ty-3a* and *Ty-3b* loci. It is not known how closely these markers are to the functional *Ty-3* gene (Ji et al., 2007), so it is possible that some breeding lines would give false negative or false positive results. It is expected that these markers will be evaluated in various tomato breeding programs.

It is of interest that the introgressions from three different *S. chilense* accessions have different size introgressions. Only the *Ty-3b* introgression has 100% nt identity with one of these accessions, LA1969, and these accession has been used as a source of the *Ty-1* gene in several different laboratories.

Acknowledgements: This project was funded in part by USAID-CDR (TA-MOU-05-C25-037) and USAID-MERC (GEG—G-00-02-00003-00) grants to D. P. Maxwell, by the College of Agricultural and Life Sciences at University of Wisconsin-Madison, and by grants from Unilever Bestfoods Ltd. and the Florida Tomato Committee to J. W. Scott.

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HUJ-VF: 320 bp;ty3/ty3, source Hebrew University of Jerusalem, susceptible to begomoviruses.

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ORIGIN
1      GGTAGTGGAA ATGATGCTGC TCAAATTATT GTGTGAACAT ATTATGAGAG GTAGGATTAA
61     GAATGAAGTT ATATAAGATA AAGTGGAAGT TACTTTTCGA AAAAAAAAGA AAGACGAAAA
121    AAATGAGATT GAAATGGATT GAATACGTGA AGAAGAGATG CATGGGTTCA CCAATAAAAA
181    GGTTTGAGAG TTTGACTTAA GAAGAGGTAG AAGTAGGTTG AAAAACAACT AGGTAAAGTT
241    TTACTTTTAG TTTTGTTTTG ATTGCACATT TTTTTAGTCG AAATAGAAAC AGAGGTTATA
301    TATGGGACAA TAGGCAGAGC
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Gc143-2: 453 bp; Ty3/Ty3, introgression from *S. chilense* LA2779, resistant to begomoviruses.

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ORIGIN
1      GGTAGTGGAA ATGATGCTGC TCAAATTAAT GTGTGAACAT GAGAGGTAGG ATTAGAAATG
61     AAGTTATATA AGATAAAGTG GAAGTAACTT CCAATAAAAA AAGACGAAAA AAATGAGATT
121    GAAATGGGTT GAATACGTGA AGAAGAGATG CATGGATTCA CCAATAAAAA GGTATGAGAG
181    TTTGACTTAA GAAGATGTAG AAGTAGGTTG AAAAAAAACT ACGTAAAGAT GATTAGATAA
241    GATATATCAC GAGGACACGA CTATAGCAAG ATATGGCAGC AGAGTTTTGT CGTATTGTTA
301    CATGGAAGAG GTAAGGGACT TGTCTCTGCT TTTCATGCAC ATTGCTTCAA TTTACTTTGT
361    TAGACTTGTT ATTTTACTTT TAGTTCTGTT TTGATTGCAC ATTTTTTTAG TCGAAATAGA
421    AACAGAGGTT ATATATGGGA CAATAGGCAG AGC
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Gc171: 623 bp;Ty3a/Ty3a, introgression from S. chilense LA1932, resistant to begomoviruses.

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ORIGIN
1      GGTAGTGGAA ATGATGCTGC TCAAATTAAT GTGTGAACAT ATTATGAGAG GTAGGATTAG
61     AAATGAAGAT ATGTAAGATA AAGTGGGTGA CTTTCAAAAA AAAAAAAGAC GAAAAAAATG
121    AGATTGAAAT GGATTGAATA CGTGAAGAAG AGATGCATGG ATTCATCAAT AAAGAGGTGT
181    GAGAGTTTCA CTTAAGAAGA AATAGAAGTA GGTTGAAGAA CAACTGAAGA TGATTAGATA
241    AGATATATCA CAATTTCAAT AAATGAGGAC ACGACTATAG CAAGATATGG TAGCAGAGTT
301    TTATCGTATT GTTACGTGGA AGAGGTAAGG TACTTGTCTC TACTTTTCAT GCACATTGCT
361    TCAGTTTACT TTGTTAGACT TGTTATTTTA ATGAGATTCG AACCTTGGTA CAACAATATT
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421 AAAAGAGTTT TACCCATCTT AGAATCATTG GGTCAACTAT ATGATTTATT CATTAGCTGC
481 TTTATGTTAA TTTTATACAA ATATCTATCG ATTTCTACAT AGATATATAT ATTTCGTAAC
541 AAAGTTAATG AGTGCTCGAG CACCCAGCGG ACAACACGTG GGTCCGCCCT GACAGAGGTT
601 ATATATGGGA CAATAGGCAG AGC

Commercial Hybrid AH: 660 bp; Ty3b, resistance from *S. chilense* LA1969, slightly resistant to begomoviruses.

ORIGIN
1 GGTAGTGGAA ATGATGCTGC TCAAATTAAT GTGTGATATG AGAGGTAGGA TTAGAAATAA
61 AGTTATATAA GAAAGTGGGT GACTTTCAAA AAAGAAAAAA AAGGAAGACT AAAAAATGAG
121 ATTGAAATAA GTTGAATACG TTAAGAAGAG ATGCATGGAT TCACCAATAA AGAGGTGTGA
181 GAGTTTGGCT TAAGAAGAGG TAGAAGTAGA TTGAAGAACA ACTAGGTGAA AGATGATTAG
241 ATAAGATATA TCACAATTTC AATAAATGAG GACGCGACTA TAGCAAGATA TGAAAGCAGA
301 GTTTTGTCGT ATTGTTACGT GGAAGAGGTA AGGGACTTGT CTCTACTTTT CATGCACATT
361 GCTTCAATTT ACTTTGTTAG ACTTGTTATT TTACTTTTAG TTTTGATTGC ATTATGTGTT
421 AACAATCAGA TTCGAATTTT GCTACAATAA CATTAAAAGA GTTTTACCCA TTCTAGAACT
481 ATTGAGTCAA CTATATGATT TATTCATTGA GTGCTTTATG TTAATTTTAT ACAAATACCT
541 ATCGATTTCT ATATAGATAT ATATATATTT CGTAACGGAG TTAATGGGTG TTCGAGCACC
601 CAGCGGACAC CACGTGGGTC CACCCCTGAC AGAGGTTATA TATGGGACAA TAGGCAGAGC

Sequence alignment of the four alleles at P6-25 marker—Gc171 (Ty3a), com. AH (Ty3b), Gc143-2 (Ty3), HUJ-VF (ty3).

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ORIGIN
Gc171      GGTAGTGGAAATGATGCTGCTCAAATTAATGTGTGAACATATTATGAGAGGTAGGATTAG     60
Com. AH    GGTAGTGGAAATGATGCTGCTCAAATTAATGTGTGA......TATGAGAGGTAGGATTAG     54
Gc143-2    GGTAGTGGAAATGATGCTGCTCAAATTAATGTGTGAACAT.....GAGAGGTAGGATTAG     55
HUJ-VF     GGTAGTGGAAATGATGCTGCTCAAATTAtTGTGTGAACATATTATGAGAGGTAGGATTAa     60
Consensus  ggtagtggaaatgatgctgctcaaatta tgtgtga         gagaggtaggatta

Gc171      AAATGAAGATATGTAAGATAAAGTGG..GTGACTTTC...AAAAAAAAAAAAGACGAAA.    114
Com. AH    AAATaAAGtTATaTAAGA..AAGTGG..GTGACTTTC...AAAAAAgAAAAAaAaGgAAg    107
Gc143-2    AAATGAAGtTATaTAAGATAAAGTGGaaGTaACTTcC.....AAtAAAAAAAGACGAAA.    109
HUJ-VF     gAATGAAGtTATaTAAGATAAAGTGGaaGTtACTTTtcgaAAAAAAAAgAAAGACGAAA.    119
Consensus   aat aag tat taaga  aagtgg  gt actt       aa a a aaa a g aa

Gc171      .....AAAATGAGATTGAAATGGATTGAATACGTGAAGAAGAGATGCATGGATTCATCAA    169
Com. AH    actaaAAAATGAGATTGAAATaagTTGAATACGTtAAGAAGAGATGCATGGATTCAcCAA    167
Gc143-2    .....AAAATGAGATTGAAATGGgTTGAATACGTGAAGAAGAGATGCATGGATTCAcCAA    164
HUJ-VF     .....AAAATGAGATTGAAATGGATTGAATACGTGAAGAAGAGATGCATGGgTTCAcCAA    174
Consensus       aaaatgagattgaaat   ttgaatacgt aagaagagatgcatgg ttca caa

Gc171      TAAAGAGGTGTGAGAGTTTCACTTAAGAAGAAATAGAAGTAGGTTGAAGAACAACTG...    226
Com. AH    TAAAGAGGTGTGAGAGTTTggCTTAAGAAGAggTAGAAGTAGaTTGAAGAACAACTaggt    227
Gc143-2    TAAAaAGGTaTGAGAGTTTgACTTAAGAAGAtgTAGAAGTAGGTTGAAaAAaAACTacgt    224
HUJ-VF     TAAAaAGGTtTGAGAGTTTgACTTAAGAAGAggTAGAAGTAGGTTGAAaAACAACTaggt    234
Consensus  taaa aggt tgagagttt  cttaagaaga  tagaagtag ttgaa aa aact

Gc171      ..AAGATGATTAGATAAGATATATCACAATTTCAATAAATGAGGACACGACTATAGCAA.    283
Com. AH    gaAAGATGATTAGATAAGATATATCACAATTTCAATAAATGAGGACgCGACTATAGCAA.    286
Gc143-2    .aAAGATGATTAGATAAGATATATCA.............cGAGGACACGACTATAGCAA.    269
HUJ-VF     .aAAG.TttTacttTtAGtTtTgTtt.............TGAttgCACattTtTttagtc    279
Consensus    aag t  t    t ag t t t               ga   c c   t t

Gc171      GATATGGTAGCAGAGTTTTATCGTATTGTTACGTGGAAGAGGTAAGGTACTTGTCTCTAC    343
Com. AH    GATATGaaAGCAGAGTTTTgTCGTATTGTTACGTGGAAGAGGTAAGGgACTTGTCTCTAC    346
Gc143-2    GATATGGcAGCAGAGTTTTgTCGTATTGTTACaTGGAAGAGGTAAGGgACTTGTCTCTgC    329
HUJ-VF     GAaATaGaAaCAGAGgTT.....................................        297
Consensus  ga at   a cagag tt

Gc171      TTTTCATGCACATTGCTTCAGTTTACTTTGTTAGACTTGTTATTTTA.............    390
Com. AH    TTTTCATGCACATTGCTTCAaTTTACTTTGTTAGACTTGTTATTTTActttttagttttga    406
Gc143-2    TTTTCATGCACATTGCTTCAaTTTACTTTGTTAGACTTGTTATTTTActttttagttctgt    389
HUJ-VF     ..........................................................      297
Consensus

Gc171      .................ATGAGATTCGAACCTTGGTACAACAATATTAAAAGAGTTTTA    432
Com. AH    ttgcattatgtgttaacaATcAGATTCGAAttTTGcTACAAtAAcATTAAAAGAGTTTTA    466
Gc143-2    tttgatt.....................................................    396
HUJ-VF     ..........................................................      297
Consensus

Gc171      CCCATCTTAGAATCATTGGGTCAACTATATGATTTATTCATTAGCTGCTTTATGTTAATT    492
Com. AH    CCCATtcTAGAActATTGaGTCAACTATATGATTTATTCATTgagTGCTTTATGTTAATT    526
Gc143-2    ..........................................................      396
HUJ-VF     ..........................................................      297
Consensus
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Gc171      TTATACAAATATCTATCGATTTCTACATAGATATATATAT..TTCGTAACAAAGTTAATG    550
Com. AH    TTATACAAATAcCTATCGATTTCTAtATAGATATATATATATatTTCGTAACggAGTTAATG    586
Gc143-2    ..........................................................      396
HUJ-VF     ..........................................................      297
Consensus

Gc171      AGTGCTCGAGCACCCAGCGGACAACACGTGGGTCCGCCC.TGACAGAGGTTATATATGGG    609
Com. AH    gGTGtTCGAGCACCCAGCGGACAcCACGTGGGTCCaCCCcTGACAGAGGTTATATATGGG    646
Gc143-2    ................gcacatttttTaGtcgaaatagaaACAGAGGTTATATATGGG    439
HUJ-VF     ..................................................ATATATGGG    306
Consensus                                                     atatatggg

Gc171      ACAATAGGCAGAGC                                                 623
Com. AH    ACAATAGGCAGAGC                                                 660
Gc143-2    ACAATAGGCAGAGC                                                 453
HUJ-VF     ACAATAGGCAGAGC                                                 320
Consensus  acaataggcagagc
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