

Sequence analysis for 84 cM on Chromosome 3

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Background: Our UW-Team has been searching for introgressions associated with begomovirus-resistant tomato inbred lines for several years. Below is data for the marker designed from Atg05350 (84.5 cM, chromosome 3) and the unigene U322707. The sequence of the unigene was compared with sequences in GenBank in January 2008, and there was a match with *Vitis vinifera*, AM43538. Primers were designed which corresponded to exons.

PCR primers:

P3-84F1 5' cat tgt ctg cat gaa cag ggt ttt ctg c

P3-84R1 5' gtg ctg cat cac tac cta cta caa tgg

PCR program: annealing temperature 53 C

Partial sequence for Gc143-2 (resistance from *S. chilense* LA2779), 788 bp:

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1      AGAGGTCACC ATAAATATC AGAACTTCAA CTTTTTTAGG NAATAAAGAT GACCTCACTT
61     GTAGGACCAT GGACACTATA CCTGTCGTTT CAAACAAGCA GCATTTGAAC ATTGTGAGTT
121    TGGCTTCATC TCCATTGTTG GAAAATAATC TTTGAGAGCA TTGTACCCCT AAAACAAGCA
181    ACAAAAAAGA AACTATAAAC ATCAAAAGTG TTGAGCTACA CTTAATTTCT GTCTTCACCT
241    TGTCTCCTT TTATAGGAGG CAAGTTCACT TCTGTTCTTG ACCAGTTATC TGCTAAGAAA
301    GTCCTCCGTA CTCCAGAGGG TTGAAAGACT CAAGTCTTTG ACTAAAAAAA AGTCCAAGAA
361    AGAACTTAAA GAGTGATAAA CATTCTCTCA CATCTACAGC CTTAGTGGGG GAGAAGGGGA
421    GAGAGAGAGC AAAAAAGAGA TGGCAAGCTT TACCAGATAG CGTGACACCT GTCCGAACCT
481    CAACAAGTAC TTCAATGTGT TTTGAACTAA AAGTCCAGCA ACAACTCCCT ATCAAAGAGA
541    GTTCAAATGT TAATTTACCA AATCAAATAA TGAATTTGTA ATACAGCTTC AATCAAATCA
601    TAAACAAGAT TCTAGTATTT TTGCTAGAAG AGAAGTTCAT ATTATTCTTC AAAAGAAAGT
661    GGGAGCATAA ACATGAGAAA AGTAACCAA TTAGCAGCAA ATGCGGCTAT AAAGAGAGAG
721    AATAATTTTG AAGATGAGAA GAGCCAAACT TCAACCACAA TTTTCAGTGT TTCTTTTTAC
781    AATTACAA
```

Blast searches at SGN and GenBank:

>[SGN-E368981](#) [TUS-25-N17]

Length = 695



Score = 174 bits (88), Expect = 3e-42
Identities = 88/88 (100%), Frame = +1 / -1

```
Query: 82 ctgtcgttccaacaagcagcatttgaacattgtgagtttggcttcatctccattgttgg 141
          |||
Sbjct: 226 ctgtcgttccaacaagcagcatttgaacattgtgagtttggcttcatctccattgttgg 167
```

```
Query: 142 aaaataatctttgagagcattgtacccc 169
          |||
Sbjct: 166 aaaataatctttgagagcattgtacccc 139
```

Score = 153 bits (77), Expect = 1e-35
 Identities = 77/77 (100%), Frame = +1 / -1
 Query: 453 ccagatagcgtgacacctgtccgaacttcaacaagtacttcaatgtgttttgaactaaaa 512
 |||
 Sbjct: 140 ccagatagcgtgacacctgtccgaacttcaacaagtacttcaatgtgttttgaactaaaa 81

Query: 513 gtccagcaacaactccc 529
 |||
 Sbjct: 80 gtccagcaacaactccc 64

 [emb|AM439538.1](#)  Vitis vinifera, whole genome shotgun sequence, contig VV78X097748.17,
 clone ENTAV 115
 Length=25375

sequence by: Sort alignments for this subject

<u>position</u>	<u>E value</u>	<u>Score</u>	<u>Percent identity</u>	<u>Query start position</u>	<u>Subject start</u>
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Score = 113 bits (124), Expect = 2e-21
 Identities = 88/104 (84%), Gaps = 1/104 (0%)
 Strand=Plus/Plus

Query 79 TACCTGTCGTTCCAAACAAGCAGCATTTGAACATTGTGAGTTTGGCTTCATCTCCATTGT 138
 |||
 Sbjct 8222 TACCTGTCGTTCCAGACAGGAAGCATTTGAACATTGAGGGTTTGGCCTCATTTCCATGGT 8281

Query 139 TGGAAAATAATCTTTGAGAGCATTGTACCCT-AAAACAAGCAA 181
 |||
 Sbjct 8282 TGGAAAGTAGTCTTTAAGAGCATTATATCCCTGCAAGCAAGCAA 8325

Score = 86.0 bits (94), Expect = 2e-13
 Identities = 82/104 (78%), Gaps = 1/104 (0%)
 Strand=Plus/Plus

Query 445 AAGCTTTACCAGATAGCGTGACACCTGTCCGAACCTCAACAAGTACTTCAATGTGTTTTG 504
 |||
 Sbjct 8447 AAGGTTTACCAAGTATGGGGAGACATGTCCAAACTTTAGCAAGAATTTAAGTGTGTTTTG 8506

Query 505 AACTAAAAGTCCAGCAACAACCTCCCTATCAAAGAGAGTTCAAAT 548
 |||
 Sbjct 8507 AACTAGAAGCCCAGCAACAACCTCCCTA-CAAAGTCCATTCAAAT 8549

Conclusions: It was expected that this PCR-fragment (ca. 1,000 bp) would have two exons and this is what was found from the SGN and GenBank searches. The sequence for the Gc143-2 contains introns and no intron sequence (other genomic sequence) is currently available for other tomato inbreds. Our group will obtain sequence for Gc171 (resistant) and HUJ-VF (susceptible).