# COSII Marker C2_At5g20890, 76.00cM

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**Fig.1**: Map of the bottom of Chr. 11. (Modified from Solanaceae Genomics Network, 2006).

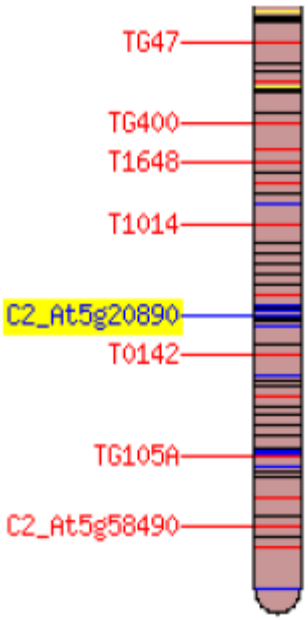


**Table 1**: Primers from C2_At5g20890 on Chr. 11

| Primer Name | Sequence (5' to 3') |
|---|---|
| DM11-F10 | AACTTATCGAGGAGATCATGATTGG |
| DM11-R10 | AGCTGAGCAATCAGCTCAGCACTGTC |

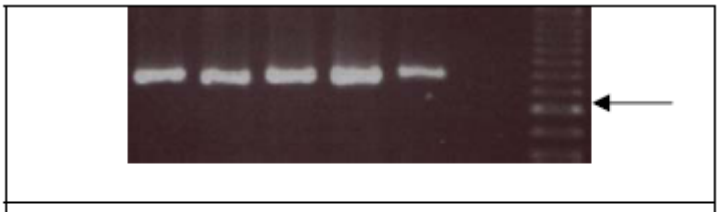

**Fig. 2**: Agarose gel of the PCR reactions with the DM11F10/DM11R10 primers from Chr. 11. lane 1, Heinz 1706; lane 2, Gh13; lane 3, Gc9; lane 4, LA1777; lane 5, LA2779; lane 6, water; lane 7, 100-bp ladder. Arrow marks the 600-bp fragment.

Background: The purpose of this project was to locate molecular markers for disease resistance in tomato. To accomplish this goal, primers were obtained from the Solanaceae Genomics Network (SGN) website (Solanaceae Genomics Network, 2006), and used with five different tomato lines.

We used the tomato breeding lines, Gh13 and Gc9 which are resistant to the bipartite begomoviruses in Guatemala (Mejía *et al*., 2004; Nakhla *et al*., 2004). Gh13 is the F7 generation and is a homogeneous breeding line with resistance derived from *L. hirsutum*. Gc9 is at least an F8 breeding line with resistance genes introgressed from *L. chilense* by J. W. Scott (Scott *et al*., 1995). LA1777 is the *L. hirsutum* parent, and is thought to be the source of the introgression in Gh13. LA2779 is the *L. chilense* parent and is thought to be the source of the introgression in Gc9 (Maxwell, D., pers. com.)

As a control, we used the breeding line Heinz 1706. Heinz 1706 is the tomato cultivar being sequenced in an international sequencing project (Budiman *et al*., 2000; Ozminkowski, 2004), and is susceptible to geminiviruses (Hapidat, M., pers. com.). The susceptibility of Heinz 1706 to geminiviruses was confirmed through testing with *Tomato Yellow Leaf Curl Virus*, which is a begomovirus (Maxwell, D., pers. com.).

The begomovirus resistant lines, Gh13 and Gc9 were supplied by Dr. L. Mejía, Universidad de San Carlos, Guatemala City. The susceptible line, Heinz 1706, was supplied by Dr. R. Ozminikowski, Heinz Seed Co., Stockton, CA.

Polymerase Chain Reaction (PCR): PCR fragments from each set of primers, for each of the five genotypes, were obtained using methods developed in the Maxwell lab (Czosnek *et al*., 2004). PCR parameters were for 50-µl reactions containing: 5-µl 2.5mM deoxynucleotide triphosphates (dNTPs), 5-µl 10X buffer, 5-µl 25 mM $MgCl_2$, 0.2-µl *Taq* DNA polymerase, 5-µl each forward and reverse sense primer at 10µM, 5-7 µl of DNA extract, and $H_20$. Some PCR reactions were run with 25-µl reactions. When this was the case, the concentrations of all chemicals were exactly half of what appeared in the 50-µl reactions. PCR cycle parameters for fragment amplification were as follows: denaturation at 94°C for 3 min, then 35 cycles at 94°C for 30 sec each, annealing at 53°C for 1 min, and extension at 72°C for 1 min. These cycles were followed by a reaction at 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). The PCR-amplified DNA was run on an electrophoresis gel of 1.5% Seakem LE™ agarose (BioWhittaker Molecular Applications Rockland, ME) in 0.5X TBE buffer, stained with ethidium bromide, and visualized with a Kodak Gel Logic 200 Imaging System.

DM11F10-R10 Results: The DM11F10/DM11R10 primer pair was chosen from the list of COSII primers on the SGN website (Solanaceae Genomics Network, 2006). The primer combination produced a strong single band at approximately 800 bp with all tested samples (Fig. 2). These PCR products were directly sequenced with both the forward and reverse primer pairs. Heinz 1706, Gc9, Gh13, and LA1777 each produced good sequence with both the forward and reverse primer pairs. LA2779 appeared to be heterozygous at this location. Upon alignment, the breeding lines matched exactly with Heinz 1706. Thus, there were no SNP or INDEL that separated the begomovirus breeding lines from the susceptible control Heinz 1706. LA1777 differed from the other lines by 18 SNP and 2 INDEL out of 777 bp. Thus, there is no indication that a molecular marker for begomovirus resistance could be found at this location.

SEQ Heinz DM11F10-R10, Genbank Accession DQ855128, 778 bp;

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ORIGIN
1      AACTTATCGA GGAGATCATG ATTGGCGAAG ATAAGCTGAT CCACTTCTCT GGGGTTGCAA
61     TGGGACAGGC ATGTACAATT GTTCTGAGAG GTGCAAGGTG AGTTGGTGTT ATGGTGCCTG
121    CCTTTTATTT CATATGTTTC ACTTGCTTGT GCAAGCTTGT TATGCATAAC TGTGATGTTC
181    CATTGTGTGA GTATTAAACT ATGACATTGT AGACGCTTCA TTTTTACCTA CCCCCTCTTA
241    AAGGTAGTTT GAACAATTAA ATTTGTTTTA GAAGTCTTGG GATAATTCTT TTTTTTGTAA
301    AAGAAAAAAA GAAGCATTGC TCGACTGTTT GTTTTACTTT TACTCGAGGA CCGGCTCGAT
361    TCCTTAGTGC TTGTGTTCTG TAATTGGACC TGATGGTAAT TGTCCCACAT TGCATTCTTC
421    TGGACTTTTT GTGTTTTCCT TACTGGGTTT TAAATATGAT GATATGTTCT ACTTGTGATG
481    TGTTTCCTTG TTAATGTCTA TTTTTGTTCT TTCTTTCAGC CCTCATGTAC TGGATGAAGC
541    TGAAAGATCT CTGCACGATG CATTGTGTGT ACTATCTCAG ACGGTAAATG ACAGCAGGGT
601    TCTACTTGGA GGTGGATGGC CCGAGATGGT GATGGCTAAG GCGGTTGATG AACTAGCTAA
661    GAAGACTCCA GGTAAAAGGT CTCATGCAAT TGAGGCTTTC ACCCGTGCAC TTCTGGCAAT
721    TCCAACCACC ATTGCCGACA ATGCTGGGTT AGACAGTGCT GAGCTGATTG GCTCAGCT
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SEQ LA1777_DM11F10-R10, Genbank Accession DQ855116, 763 bp;

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ORIGIN
1      AACTATTCGA GGAGATCATG ATTGGNGAAG ATAAGCTGAT CCACTTCTCT GGGGTTGCAA
61     TGGGACAGGC ATGTACAATT GTTCTGAGAG GTGCAAGGTG AGTTGGTGTT ACGGTGTCTG
121    CCTTTTATTT CATATGTTTC ACTTGCTTGT GCAAGCTTGT TATGCATAAC TGTGATGTTC
181    CCTTGTGTGA GTATTAAACT ATGACATTGT AGACACTTCA TTTTTACCTA CCCCTTTTAA
241    AGGTAGTTTG AACAATTAAA TTTGTTTTAG AAGTCTTGGG ATAATTTTTT TTATGTAAGA
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301    GAAAAAAAGA AGCATTGTTC GACTGTTTGT TTTACTTATA CTCGAGGACC GGCTCAATTC
361    CTTAGTGCTT GTGTTCTGAA ATTGGACCTG ATGGTAATTG TCCCACATTG CATTCTTCTG
421    GACTTTTTGG TTTTTCCTTC ATGGGTTTTA AATATGATGA TATGTTCTAC TTGTGATGTG
481    TTTCCTTGTT AACGTCTCTG TTTGTTCTTT CTTTCAGCCC TCATGTACTG GATGAAGCTG
541    AAAGATCTCT GCACGATGCA TTGTGTGTAC TATCTCAGAC GGTAAATGAC AGCAGGGTTC
601    TACTTGGAGG TGGATGGCCC GAGATGGTGA TGGCTAAGGC GGTTGATGAA CTAGCTAAGA
661    AGACTCCAGG TAAAAGGTCT CATGCAATTG AGGCTTTCAC CCGTGCACTT CTGGCAATTC
721    CAACCACCAT TGCCGACAAT GCTGGGTTAG ACAGTGCTGA GCT
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