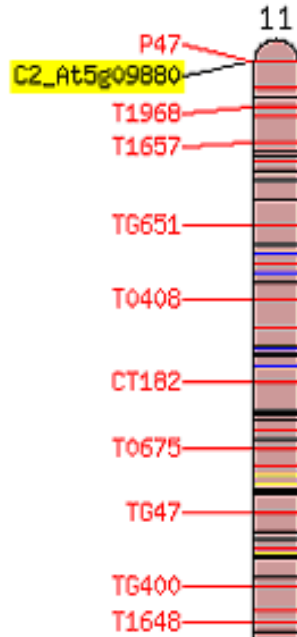


# COSII Marker C2\_At5g09880, 0.00cM

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



**Table 1:** Primers from At5g09880 on Chr. 11

Primer Name	Sequence (5'-3')
DM11 - F1	AGGGTCAAGCATATCCATGTGGAC
DM11 - R1	TCTCTTGTGCATGGCTTGTGAGC



**Fig. 2:** Agarose gel of the PCR reactions with the DM11F1/DM11R1 primers from Chr. 11. lane 1, 100-bp ladder; lane 2, Heinz 1706; lane 3, Gh13; lane 4, Gc9; lane 5, LA1777; lane 6, LA2779; lane 7, water. 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; Ozminowski, 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. Ozminowski, 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- $\mu$ l reactions containing: 5- $\mu$ l 2.5mM deoxynucleotide triphosphates (dNTPs), 5- $\mu$ l 10X buffer, 5- $\mu$ l 25 mM MgCl<sub>2</sub>, 0.2- $\mu$ l *Taq* DNA polymerase, 5- $\mu$ l each forward and reverse sense primer at 10 $\mu$ M, 5-7  $\mu$ l of DNA extract, and H<sub>2</sub>O. Some PCR reactions were run with 25- $\mu$ l reactions. When this was the case, the concentrations of all chemicals were exactly half of what appeared in the 50- $\mu$ l 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.

**DM11F1-R1 Results:** The DM11F1/DM11R1 primer pair was obtained from a search of all COS II primers on the SGN website (Solanaceae Genomics Network, 2006). The primer combination produced three distinct banding patterns (Fig. 2). Heinz 1706 and Gh13 produced a strong band at roughly 300bp and a weak band at roughly 180bp. Gc9 produced a strong band at roughly 300bp, whereas LA1777 produced a strong band at roughly 600bp. LA2779 did not produce a band with the DM11F1/DM11R1 primer pair (Table 2). Because multiple bands were obtained, these primers were not used for sequencing reactions. However, the different banding patterns may be indicative of sequence variations that correlate with important disease resistance or morphological characteristics.

**Table 2:** Results of the PCR reactions with the DM11F1/R1 primer pair.

Plant Line	Result
Heinz 1706 and Gh13	Strong band at 300bp, weak band at 180bp
Gc9	Strong band at 300bp
LA1777	Strong band at 600bp
LA2779	No Band

## References

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