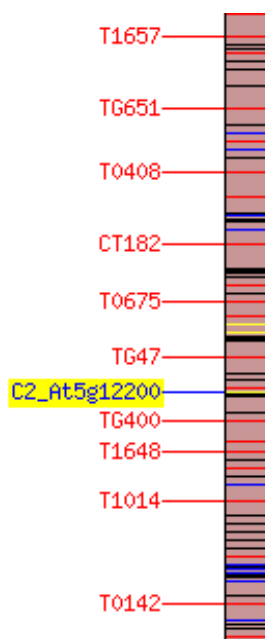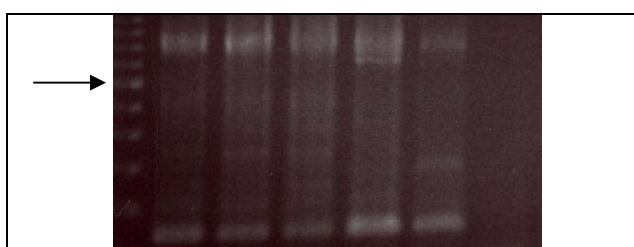# COSII Marker C2_At5g12200, 53.50cM

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

Table 1: Primers from C2_At5g12200 on Chr. 11

Primer Name	Sequence (5' to 3')
DM11 - F7	TCCTGACTTCCATTTTGCAGCAAG
DM11 - R7	TGCTCTGATTGGTGGGCTCATGAC

Fig. 2: Agarose gel of the PCR reactions with the DM11F7/DM11R7 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; 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$_2$0. 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.

<u>DM11F7-R7 Results</u>: The DM11F7/DM11R7 primer pair was chosen from the list of COSII primers on the SGN website (Solanaceae Genomics Network, 2006). The primer combination produced two distinct banding patterns (Fig. 2). Heinz 1706, Gh13, Gc9 and LA177 each produced two weak bands with one at roughly 800bp and the other at roughly 100bp. LA2779 produced three weak bands at 800bp, 200bp, and 100bp respectively. Because this primer pair produced multiple bands, the PCR product was not sequenced.

Thus, the parent plant LA2779 produced a significantly different banding pattern than the breeding lines. This indicates that none of the breeding lines have an introgression from LA2779 in this region. It is possible that the breeding lines have an introgression from LA1777 in this region. However, because the susceptible plant Heinz 1706 also shares the LA1777 banding pattern it is not likely that this area is the source of geminivirus resistance in tomato.

These primers may be useful in tracking introgressions from LA2779 in this region. The different banding patterns would allow for a quick and easy analysis of the introgression through PCR.

Table 2: Results of the PCR with the DM11F7/DM11R7 primer pair.

Plant Line	Result
Heinz, Gh13, LA1777 and Gc9	Two weak bands (800bp and 100bp)
LA2779	Three weak bands (800bp, 200bp, and 100bp)

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