# Chromosome 4: TG208, 68.00cM

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PCR primer pairs were designed for RFLP probe: TG208 (Fig. 1).

**Fig. 1**: RFLP map of the middle of Chr. 4 (Adapted from Pan et. al., 1999).

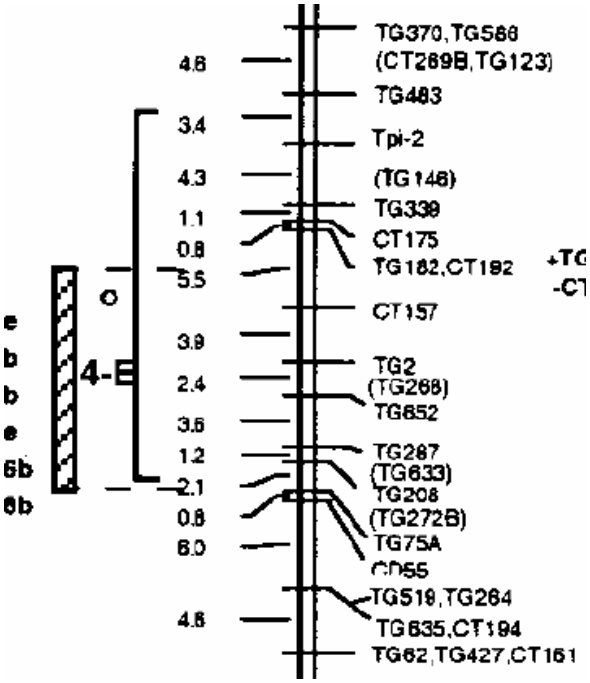


**Table 1**: Primers from the TG208 probe on Chr.4.

| TG208 | Primer Sequence (5' to 3') |
|---|---|
| PTG208F1 | CCCTCTCCAGATCTACTTTTATTGGGTACG |
| PTG208F2 | GCATTCACAAAGTACGGACAAGGTTAGG |
| PTG208R1 | CATATCCAACAGCATGGCTATCAG |
| PTG208R2 | GAAGCAGAGAATAACCGGTGAAGACTC |

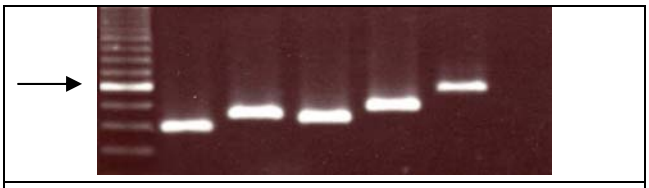

**Fig. 2**: Agarose gel of the PCR reactions with the four TG208 primers and Heinz 1706 DNA. lane 1, 100-bp ladder; lane 2, PTG208F1/PTG208R1; lane 3, PTG208F1/PTG208R2; lane 4, PTG208F2/PTG208R1; lane 5, PTG208F2/PTG208R2; lane 6, control, primer pair PTG301F3/PTG301R2 with Heinz 1706 DNA; lane 7, water. The PTG208F2/PTG208R2 gave the largest band and was chosen for use with additional genotypes. Arrow marks the 600-bp fragment.

TG208 RFLP Probe: Four primers were designed from the TG208 RFLP probe: PTG208F1, PTG208F2, PTG208R1, and PTG208R2 (Table 1). All four primer combinations gave intense single bands with Heinz 1706 DNA that were from 400 bp to 500 bp (Fig. 2). The PTG208F2/PTG208R2 gave the largest band and was chosen for use with additional genotypes. Heinz1706, Gc9, Gh13, and Sheriff each gave intense single bands at 500 bp. Gc173 gave a weak band at 500 bp. These PCR fragments were sequenced with both the forward and reverse primers. When these sequences were aligned three distinct patterns were found. The sequences were different due to SNPs in three different places. Gc9 (acc. no. DQ120939), Gh13, and Gc173 each had one pattern while Heinz 1706 (acc. no. DQ120940) had a distinctly different pattern (3 SNPs). Sheriff (acc. no. DQ223930) was heterozygous for both patterns (Table 2). In order to determine the significance of this pattern, sequence was obtained for 20 additional plant lines with the PTG208F2/PTG208R2 primer pair (Dominique, Gpim10, Marina, Celebrity, Gc16, Gh902a, H7996, Moneymaker, Naenemor, Silverado, LA1968a, LA0386, Gp11, Ih902a, LA1777, Hc7880, Motelle, LA0462b, LA3900, and Toro). Gpim10, Dominique, and Marina each had the same pattern as Gc9, Gh13, and Gc173. Celebrity, Gc16, Gh902a, H7996, Moneymaker, Naenemor, Silverado, and LA 1968a each had the same pattern as Heinz 1706. LA0386, Gp11, Ih902a, and LA1777 each had the same sequence as Sheriff. Hc7880, Motelle, LA0462b, LA3900, and Toro failed to sequence, despite being run in the same reaction as all of the other DNAs (Table 2). The groups of plant lines do not correlate with any known morphological difference or any known resistance gene, geminivirus related or otherwise. Thus, there is no evidence of a molecular marker for the begomovirus resistance gene at the TG208

locus. It is possible that the groups of patterns that we found correlate with an as yet to be found morphological difference or resistance gene, and these correlations may warrant additional research.

**Table 2:** Sequence patterns associated with the TG208 RFLP probe. The first SNP is at 37 bp, the second is at 230 bp, and the third is at 290 bp in reference to Heinz 1706.

| **Pattern (TGT)** | **Pattern (CAC)** | **Pattern (Heterozygous)** | **Failed to Sequence** |
|---|---|---|---|
| Gc9 | Heinz | Sheriff | Hc7880 |
| Gh13 | Celebrity | LA0386 | Motelle |
| Gc173 | Gc16 | Gp11 | LA0462b |
| Gpim10 | Gh902a | Ih902a | LA3900 |
| Dominique | H7996 | LA1777 | Toro |
| Marina | Moneymaker | | |
| | Naenemor | | |
| | Silverado | | |
| | LA1968a | | |

# References

Pan, Q., Liu, Y., Budai-Hadrian, O., Sela, M., Carmel-Goren, L., Zamir, D., and Fluhr, R. 1999. Comparative genetics of nucleotide binding site-leucine rich repeat resistance gene homologues in the genomes of two dicotyledons: tomato and arabidopsis. *Genetics Society of America* 88:309-322.