

Application of Next Generation Sequencing of a Begomovirus-Resistant Inbred to Design a KASPar® Assay for SNP Detection of the Ty1-Ty3 Region

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Begomoviruses are a major threat to tomato production in many areas of the subtropics and tropics. In the past decade great advances have been made in breeding tomato hybrids with resistance to begomoviruses. Sources of resistance have been primarily from *Solanum chilense*, *S. peruvianum* and *S. habrochaites*. Several resistance loci have been mapped to chromosomes 3 (Ty4), 4 (ty5), 6 (Ty1, Ty3, Ty3a, Ty3b), and 11 (Ty2). Verlaan *et al.* (2011. *Plant J.* 68:1093) provided data that Ty1 and Ty3 are located in an overlapping region of chr. 6 between 30.6 – 30.9 Mbp. Next-generation Illumina whole genome sequencing (WGS) of Gh13, a begomovirus-resistant inbred derived from the hybrid FAVI 9 (Vidavsky and Czosnek. 1998. *Phytopathology* 88:910), was performed. A comparison of SNP density (10 kbp bin) plots of Gh13 and Heinz 1706 showed that Gh13 has an introgression from a wild species from 30.6 – 34.2 Mbp on chr. 6. Since the overlap region for Ty1-Ty3 is within this region, putative genes within this region were selected as targets for development of an AS-PCR protocol. These gene sequences from Heinz 1706 were compared using BLAST with the WGS of Gh13 to find suitable regions for PCR primer design. PCR fragments were sequenced from a representative group of inbred lines known to have Ty1, Ty3, Ty3a or Ty3b genes, as well as from lines lacking introgressions in this region. Sequences were aligned and several sets of primers designed for detecting SNPs with a KASPar® genotyping assay (www.lgccgenomics.com/genotyping), and the SNP assay was performed at Ag Biotech Inc. (San Juan Bautista, California). Three sets of primers (two SNP-discriminating primers and one common primer per set) were tested on 27 lines or hybrids, which had been previously characterized with standard markers,

and the SNP assay for primer set ST-Ty1-3 gave the expected calls for each line or hybrid. There were no false positives for germplasm having the Mi1.2 gene for root-knot nematode resistance. Primer set ST-Ty1-3 detected the introgressions for Ty1, Ty3, Ty3a and Ty3b and gave no false positives or false negatives. The ST-Ty1-3 primer set should therefore be useful for tracking this begomovirus resistance locus in breeding populations.

