

Illumina GoldenGate SNP Marker For LMV Resistance

Description:

The *elf4e* gene sequence, with the polymorphism in DNA sequence between the wild-type *elf4e* (LMV-susceptible) and the *mo1²* allele of *elf4e* (LMV-resistant) noted (Nicaise et al., 2003), was submitted to Illumina, Inc. (San Diego, CA) for oligonucleotide and assay design for use with the Illumina Golden Gate® SNP assay (<http://www.illumina.com> ; Table 1).

AlleleA_Probe	ACTTCGTCAGTAACGGACGCCAAGCGACTTGCTTGGACTTAG [G]
AlleleB_Probe	GAGTCGAGGTCATATCGTGCCAAGCGACTTGCTTGGACTTAG [C]
IllumicodeSeq	GTTGCACCATAACATCGAAGTGAGA
TopGenomicSeq	ACGGCCATAGCTCAGCATCCGCTCGAGCATTCTTGGACTTTCTGGTTTCGATACTC CCTCT [C/G] CTAAGTCCAAGCAAGTCGCTTGGGGTAGTTCCATGCGCCCTATCT ACACTTTCTCCTCCG

Table 1. Oligonucleotides synthesized by Illumina, Inc. for the GoldenGate SNP assay of *mo1²*. AlleleA_Probe and AlleleB_Probe comprise 24 nucleotides of the wild-type *elf4e* sequence plus the G or C, respectively, that distinguishes the resistant from the susceptible allele plus, on their respective 3' ends, Illumina primers that are used to “tag” these sequences with different fluorescent labels. IllumicodeSeq is a unique sequence assigned to this particular polymorphic locus (*mo1²*) that binds to a complementary sequence attached to a bead on a “sentrix array matrix” (SAM); genotypes are “called” when the SAM (with attached fluorescent tags) is analyzed by the Illumina BeadStation or BeadXpress. TopGenomicSeq is the *elf4e* sequence containing the polymorphism that is distinguished between by using this marker assay. The resistance/susceptibility-conferring nucleotide polymorphism is indicated by brackets.

As an example, the GoldenGate assay was performed as per the manufacturer’s protocol (and as described in Fan et al., 2003 and Hyten et al., 2008) to analyze an F₂ population derived from a cross between LMV-susceptible *L. sativa* cv. La Brillante and LMV-resistant *L. sativa* cv. Salinas 88. The assay effectively labels each homozygous resistant sample with one fluorescent tag, the homozygous susceptible lines with another fluorescent tag and heterozygous lines with both tags. The variously labeled products were then hybridized by virtue of the IllumicodeSeq to a “sentrix array matrix” (SAM) and the SAM analyzed using the Illumina BeadStation or BeadXpress (Illumina, San Diego, CA) at the DNA Technologies Core Facility at the UC Davis Genome Center (http://genomecenter.ucdavis.edu/dna_technologies/). Genotypes (fluorescence channels) were called using GenCall software (Illumina, San Diego, CA) (Figure 1).

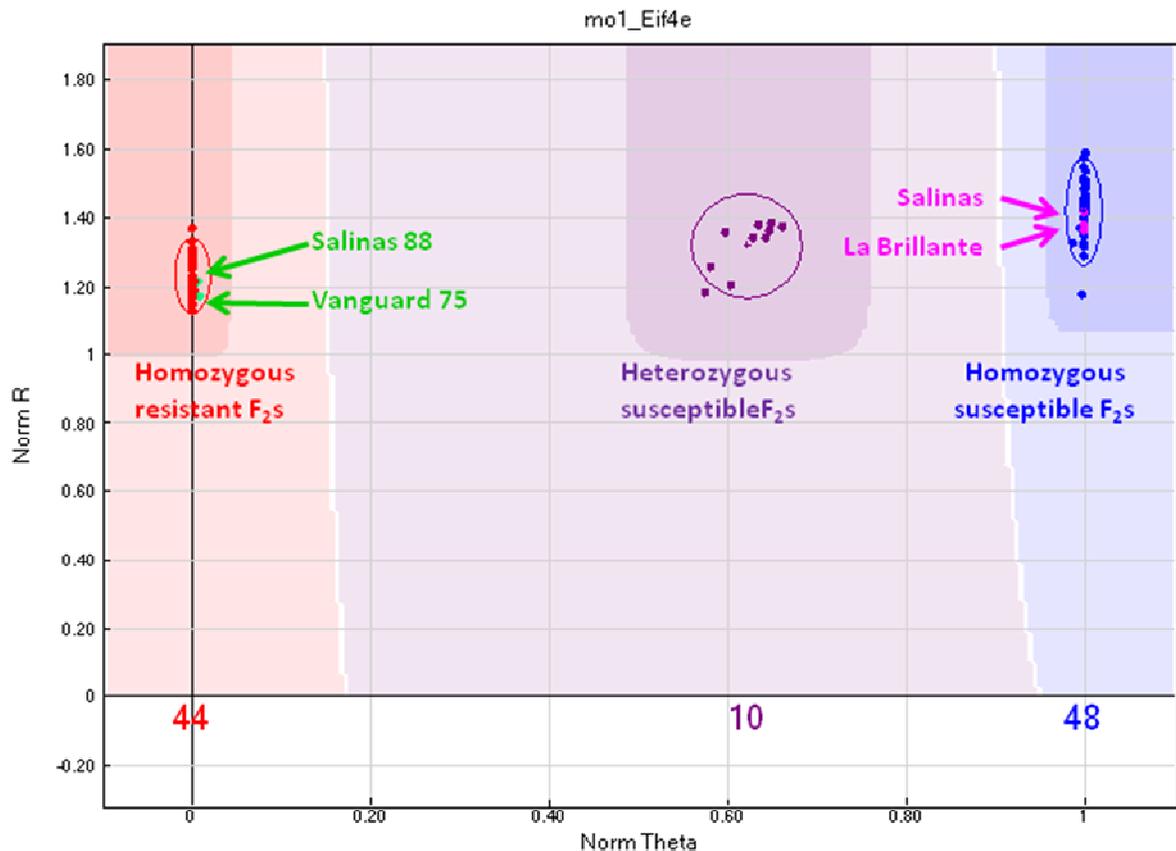


Figure 1. Normalized ‘SNP graph’ of $mo1^2$ Illumina GoldenGate SNP Assay outputted from BeadStudio 3.2 (www.illumina.com). An F_2 population derived from a cross between *L. sativa* cv. La Brillante (LMV-susceptible) and *L. sativa* cv. Salinas 88 (LMV-resistant) was genotyped using the $mo1^2$ SNP assay. Progeny were easily categorized as **homozygous resistant**, **heterozygous susceptible**, or **homozygous susceptible**. **Resistant controls** (*L. sativa* cv. Salinas 88, *L. sativa* cv. Vanguard 75) and **susceptible controls** (*L. sativa* cv. Salinas, *L. sativa* cv. La Brillante) were also assayed.

References:

- Fan JB, Oliphant A, Shen R et al. (2003) Highly parallel SNP genotyping. *Cold Spring Harb Symp Quant Biol* 68:69-78
- Hyten DL, Song Q, Shoi I-Y et al. (2008) High-throughput genotyping with the GoldenGate assay in the complex genome of soybean. *Theor Appl Genet* 116:945-952
- Nicaise V, German-Retana S, Sanjuan R, Dubrana MP, Mazier M, Maisonneuve B, Candresse T, Caranta C, LeGall O (2003) The eukaryotic translation initiation factor 4E controls lettuce susceptibility to the Potyvirus Lettuce mosaic virus. *Plant Physiol* 132:1271-1282

(Marker developed by Leah McHale)