Real-Time PASA-based Genotyping Method for Detection of Pyrethroid Resistance in *Anopheles sinensis* Wiedemann (Diptera: Culicidae)

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We have reported that the *kdr*-like L1014F mutation in the sodium channel gene is mainly responsible for pyrethroid resistance in *Anopheles sinensis*, the most important malaria vector in Korea. To investigate the status of *kdr* allele frequency in field populations, we have developed a PASA (PCR amplification of specific alleles) genotyping protocol. The performance of the genotyping technique was evaluated by using a field-collected population from Ansan, a typical urban residential area. The Ansan population was composed of 28% resistant homozygotes, 31% susceptible homozygotes and 41% heterozygotes in terms of the L1014F allele genotype, indicating substantial level of *kdr*-like resistance (53.5% L1014F allele in total). To facilitate a population-based genotyping, we have developed a real-time PASA protocol. For the prediction of allele frequency, a plot of allele frequency versus cycle threshold value (Ct-value) was generated using standard DNA mixtures of susceptible (without L1014F mutation) and resistant (with L1014F mutation) alleles in various ratios. The semi log plot was linear within the 10-80% range of resistance allele frequencies with a high correlation coefficient ($r2$ = 0.943). The *kdr* allele frequency of the Ansan population predicted from the regression line agreed well with that estimated from the individual PASA using 100 larvae, demonstrating its reliability and accuracy. By employing this real-time PASA protocol, we are currently analyzing the *kdr* allele frequencies of several field populations from Paju, Junju, Gurye, Gwangju, and Ulsan.