This Sheet Has Been Replaced

ABSTRACT

The design (or synthesis) of two new consensus primers (DVCU and DVCD) for the polymerase chain reaction (PCR) is based on conserved motifs within the serine protease and RNA helicase domains encoded by the NS3 genes of dengue and other flaviviruses. Target fragments of 470 base pairs are amplified on cDNA templates synthesized from RNAs of dengue types 1, 2, 3 and 4, Japanese encephalitis, Kunjin and yellow fever viruses. As few as 10^3 copies of dengue viral RNA can be detected. DVCU and DVCD primers can also serve as DNA sequencing primers. Direct DNA sequencing of PCR products of reference strains of dengue 2 (NGC), Kunjin (MRM61C) and yellow fever (17D) viruses demonstrate complete concurrence with published data. Differences at 21, 16 and 11 nucleotide positions are noted between dengue 1 Hawaii and S275/90; dengue 4 H241 and 814669; Japanese encephalitis Nakayama and JaOArS982 viral strains. Four new unique type-specific primers (DSP1, DSP2, DSP3 and DSP4) are also designed for typing (and sequencing) dengue virus types 1, 2, 3 and 4, yielding PCR products of diagnostic sizes 169, 362, 265 and 426 base pairs respectively, when used with DVCU primer. When applied in PCR and DNA sequencing techniques, these new consensus and type-specific primers represent useful tools for the molecular diagnosis and epidemiology of dengue and other flaviviral infections.