

AABB 2005

Poster Presentation

CLONING OF WEST NILE VIRUS INTERNAL CONTROL AND NUCLEOTIDE FRAGMENTS SPANNING THE FULL-LENGTH VIRAL GENOME FOR PRODUCTION OF STABLE RNA STANDARDS (SP423)

S Nguyen (steve_nguyen@chiron.com), V Shyamala, H Huang, Chiron Corporation, Emeryville, CA; M Badgett, C WalkerPeach, Ambion Diagnostics, Austin, TX; D Chien, B H Phelps, S Pichuantes, Chiron Corporation, Emeryville, CA

Background:

West Nile Virus (WNV) is a linear, positive, single stranded, RNA virus of the Flaviviridae family commonly found in Africa, Eastern Europe, West Asia and the Middle East. West Nile virus was first found in the United States in 1999 in the New York metropolitan area and has continuously expanded across the continent. Because of the possibility of recurrent WNV epidemics in the US, blood collections agencies have implemented WNV nucleic acid testing to screen all donations and quarantine and retrieve potentially infectious blood components.

Objective:

Synthesize and clone WNV fragments of a New York isolate for further cloning and preparation of Armored RNA[®] Quant[™], a ribonuclease resistant RNA that could be used as an external or internal positive control in nucleic acid testing. Armored RNA Quant is a complex of MS2 bacteriophage coat protein and *in vitro* transcribed RNA quantitated by NIST-traceable phosphate determination and confirmed by HPLC analysis of nucleoside composition.

Methods:

A recombinant plasmid containing the full-length WNV genome of strain NY385-99 was constructed and used to synthesize an internal control with altered probe-binding sequences and a set of five DNA fragments spanning the full-length WNV genome. The fragments were introduced into bacterial cloning vectors and their nucleotide sequences were confirmed by DNA sequencing.

Results:

A WNV internal control fragment of 972 bp containing a 22-nucleotide capsid region substituted for a target binding sequence and five WNV fragments ranging from 2.3 kb to 2.7 kb spanning the entire WNV genome were synthesized and cloned into pGEM-4z or pCR2.1. Production of an armored RNA Quant was completed for the internal control and its evaluation was performed using an in-house WNV TaqMan assay. Results showed that the internal control was properly captured, detected and quantitated by the in-house WNV TaqMan assay.

Conclusion:

The availability of well-characterized, quantitative, non-infectious, RNase resistant WNV internal control and genomic fragments will assist in the improvement and performance evaluation of WNV nucleic acid tests.