Cloning of dengue virus type 3 (Indonesian strain D3-1703) non structural-1 gene into pYES2/CT vector

Vanny Narita, author

Abstrak

ABSTRACT
Dengue is an infectious disease caused by dengue virus. Dengue endemic region includes America, Western Pacific,
Africa, East Mediterranian, and South East Asia including Indonesia. An early diagnostic system specific for Indonesia
is needed to control dengue in Indonesia. In this research, cloning of Non Structural 1 (NS1) gene from dengue virus
type 3 (Indonesian strain D3-1703) into pYES2/CT vector was performed. In the long run, NS1 recombinant protein
will be expressed in
Saccharomyces cerevisiae
for diagnostic materials. Polymerase Chain Reaction (PCR)
amplification of NS1 gene fragments were done with optimal annealing temperature at 55 ºC. NS1 gene fragment and
pYES2/CT were cut by Bam H I and Not I enzymes. The digested pYES2/CT was dephosphosrylated using Calf Intestine Alkaline Phospatase enzyme. Ligation with the vector:insert
ratio of 1:12 and 1:20 resulted in 6 and 5 recombinant colony candidates respectively. Restriction enzyme and PCR verifications showed that 5 recombinant plasmids contained NS1 gene. Sequencing of the first 600 bp of one recombinant plasmid was performed. The blastn
analysis showed that it had a 99% identity with dengue virus type 3 strain FW06. Finally, it was shown that NS1 clone
within pYES2/CT was in the correct Open Reading Frame and ready to be expressed in S. cerevisiae.