Validasi hasil kloning, ekspresi dan purifikasi protein ns2b-ns3 denv serotipe 3, isolat Jakarta = Validation of clone product expression and purification of ns2b-ns3 protein denv serotype 3, isolate Jakarta / Isma Nur Azzizah

Isma Nur Azzizah, author

Abstrak

ABSTRAK
Subunit protein NS2B-NS3 merupakan protein nonstruktural penyusun virus dengue. Kedua protein tersebut berperan dalam proses replikasi, memodulasi patogenesis, serta respons terhadap sel inang. Penelitian bertujuan untuk memvalidasi hasil kloning yang telah dilakukan oleh peneliti BPPT, mengekspresi dan mempurifikasi protein rekombinan NS2B-NS3 DENV serotipe 3. Vektor yang digunakan untuk kloning dan ekspresi ialah vektor plasmid pYES2/CT. Proses kloning yang telah dilakukan belum tervalidasi sehingga perlu divalidasi dengan metode digesti menggunakan enzim restriksi serta diamplifikasi menggunakan metode PCR. Plasmid yang telah tervalidasi ditransformasi menggunakan metode heat shock ke Saccharomyces cerevisiae sehingga memudahkan proses ekspresi. Hasil ekspresi kemudian divisualisasi menggunakan SDS-PAGE dan western blot. Hasil purifikasi kemudian divisualisasi menggunakan SDS-PAGE saja. Plasmid rekombinan pYES2/CT(NS2B-NS3) yang telah tervalidasi, terekspresi dan terpurifikasi kemudian dikirim untuk proses sekuensing. Hasil visualisasi ekspresi menggunakan metode SDS-PAGE dan western blot ialah terlihat pita spesifik pada ukuran 83 kDa. Hasil visualisasi purifikasi menggunakan SDS-PAGE terlihat muncul pita spesifik pada ukuran 83 kDa pada bagian flow-through dan resin. Hasil sekuensing menunjukan nilai kemiripan 96--99% antara plasmid rekombinan NS2B-NS3 dengan DENV serotipe 3 (DENV-3). Analisis homologi hasil sekuensing dengan isolat nomor 141 asal Jakarta menunjukan nilai 91--94% dan 97% untuk asam amino penyusun DENV.
<hr>
ABSTRACT
NS2B-NS3 protein subunit are nonstructural protein which construct dengue virus. Both of these proteins take a role in the replication process, modulating pathogenesis, and responding to the host cell. This research aimed to validate the cloning product that had been conducted by BPPT researchers, express and purify recombinant proteins NS2B-NS3 DENV serotypes 3. The vector used for cloning and expression was a plasmid pYES2/CT vector. Furthermore, the cloning product was validated using restriction enzyme digest and amplified using PCR method. Then the plasmid vectors that had been validated were transformed using a heat shock method into Saccharomyces cerevisiae to facilitate the expression process. The results of expression were visualized using SDS-PAGE and western blot. Whereas, the results of purification were visualized using SDS-PAGE only. Recombinant plasmid pYES2/CT (NS2B-NS3) that had been validated, expressed, and purified were proceed to the sequencing process. The visualized expression showed a band of 83 kDa. The visualized purification showed a band of 83 kDa in the flow-through and resin part. The sequencing results showed 96--99% sequence similarity between NS2B-NS3 recombinant plasmid and DENV serotypes 3 (DENV-3). The homology analysis of the sequencing results using isolates number 141 from Jakarta showed 91--94% value and 97% for the amino acid which construct DENV.