Konstruksi plasmid pengekspresi mRNA bisistronik untuk translasi simultan protein hemagglurirzin (HA) dan ncuraminidase (NA) virus influenza A HSNI melalui sistem IRES-MLV dan IRES-HIV = Construction of bicistronic mRNA expression plasmid for simultaneous translation of H5N1 influenza a virus Hemagglutin (HA) and Neuraminidase (NA) proteins through MLV-IRES and HIV-IRES system

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Abstrak

Expression of Virus Like Particles (VLP) containing the Influenza A haemagglutinin (HA) and neuraminidase (NA) may require simultaneous expression ofthe genes that encodes the proteins in order to obtain a balanced composition of I-IA and NA molecules in the VLP for optimal induction of protective immune response due to the increase of NA molecules in the VLP vaccine preparation. Such a condition can be more easily achieved by placement of the genes in the same vector DNA. A DNA construct for simultaneous expression of H5N1 Irriluenza A HA and NA proteins was thus constructed utilizing a pre constructed vector as the initial backbone DNA, which was a pcDNA3.I/His A plasmid that contained an insertion of the H5N1 NA gene. The sequences of internal ribosomal entry site (IRES) belonging to Murine Leukemia Virus (MLV) and Human Immrmodeficiency Virus (HIV), the HA gene of H5N1 influenza A, and the bsf gene of HIV IRES were introduced into the backbone DNA, such that the resulting DNA contains the MLV IRES upstream to the NA gene and the HIV IRES upstream to the HA gene, while the bsf gene was placed in between the NA gen and the HIV IRES. The IRES sequences were introduced for simultaneous expression of the HA and NA genes during the G2/ Mitosis phase of the cell cycle, while the bsf gene was introduced to prevent reinitiation process after termination of translation. The orientation and the accuracy of the nucleotide sequences of the inserted DNA fragments were analyzed using PCR and nucleotide sequencing. A clone that bears the inserted DNA fragments in the correct orientations with nucleotide sequences that have been confirmed by nucleotide sequencing to support proper expression of the HA and NA proteins, was obtained.
<hr>Ekspresi Virus Like Particles (VLP) mengandung haemagglutinin (HA) dan neuraminidase (NA) Iniluenza A mungkin memerlukan ekspresi gen yang mengkode protein secara simultan supaya memperoleh komposisi molekul HA dan NA dalam VLP untuk induksi optimal respon imun protektif karena peningkatan molekul NA dalam penyiapan vaksin VLP. Kondisi tersebut dapat dengan mudah dicapai melalui penempatan gen dalam vektor DNA yang sama. Rancangan DNA untuk ekspresi protein HA dan NA Influenza H5Nl, kemudian dibuat menggunakan vektor pra konstruksi sebagai DNA backbone awal, yaitu plasmid pcDNA3.l/His A yang mengandung gen sisipan NA HSN1. Sekuen internal ribosomal entry site (IRES) Murine Leukemia Vims (MLV) dan Human Immimodeiicieney Virus (HIV), gen HA Influenza A HSN1, dan gen bsf-IRES HIV dimasukkan ke dalam DNA backbone, sehingga menghasilkan DNA yang mengandung IRES MLV pada hulu gen NA dan IRES HIV pada hulu gen HA, sedangkan gen bsf terletak di antara gen NA dan IRES HIV. Sekuen IRES dimasukkan untuk ekspresi gen HA dan NA secara simultan selama fase G2 / mitosis dalam siklus sel, sedangkan gen bsf dimasukkan untuk menoegah proses reinisiasi

setelah terminasi translasi. Orientasi dan akurasi sekuen nukleotida iiagmen DNA sisipan dianalisis menggunakan pobrmerase chain reaction (PCR) dan sequencing nukleotida. Klona yang membawa fragmen DNA sisipan dengan selcucn nukleotida dalam orientasi benar telah dikonfirmasi melalui sequencing nukleotida untuk mendukxmg kebeuaran ekspresi protein HA dan NA yang diperoleh.