Pengklonaan gen pengekspresi protein retinoblastoma (RB1) manusia ke dalam vektor plasmid pQE-80L = Cloning of human retinoblastoma gene (RB1) into pQE-80L vector

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Abstrak

Peran protein retinoblastoma (pRb) dalam pencegahan pembentukan tumor diinhibisi oleh interaksinya dengan protein E7 HPV pada kanker serviks. Oleh sebab itu, perlu dilakukan strategi pengembangan uji in vitro untuk analisis interaksi pRb dan E7, terutama dalam pengembangan vaksin HPV berbasis antigen E7. Protein pRb dapat diperoleh dalam bentuk protein rekombinan yang diproduksi pada bakteri Escherichia coli. Penelitian bertujuan untuk memperoleh klona gen RB1 dalam vektor pQE_80L. Sintesis gen RB1 (2787 pb) dilakukan dengan metode PCR overlap extension. Fragmen gen RB1 dan vektor didigesti dengan enzim restriksi BamHI dan SalI kemudian diligasikan dengan enzim T4 ligase. Hasil ligasi ditransformasi ke dalam Escherichia coli TOP10 secara kejut panas. Hasil transformasi diseleksi menggunakan PCR koloni untuk mengidentifikasi keberadaan DNA sisipan. Sebanyak 1 dari 27 koloni yang diseleksi mengandung plasmid rekombinan. Plasmid rekombinan kemudian diisolasi dan diverifikasi dengan digesti dan sekuensing. Hasil analisis digesti dan sekuensing menunjukkan gen RB1 berhasil disisipkan ke vektor pQE_80L. Namun terdapat beberapa mutasi, yaitu substitusi (c.117G>A dan c.2316T>C) serta mutasi delesi (c.719_724delAAACAG).

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The role of human retinoblastoma protein (pRb) as tumor suppressor is inhibited by its interaction with HPV E7 protein in cervical cancer. Therefore, it is interesting to develop strategy for development of in vitro assay to analyze pRb and E7 interaction, especially in the development of therapeutic HPV vaccine that is based on E7 antigen. The pRb protein can be provided in the form of recombinant protein that is poduced in Escherichia coli. The study objective was to obtain RB1 gene clone in pQE_80L vector. The synthesis of RB1 gene (2787 pb) was performed by using overlap extension PCR. The RB1 gene fragment and vector was digested by BamHI and SalI restriction enzyme then ligated by T4 ligase enzyme. The ligation product was transformed into Escherichia coli TOP10 with heat shock. The transformation result was screened using colony PCR to identify the presence of insert DNA. There was 1 out of 27 selected colonies that carried the recombinant plasmid. The recombinant plasmid then isolated and verified with digestion and sequencing. The results of digestion and sequencing analysis showed that RB1 gene was successfully inserted into pQE_80L vector. However, there were mutations which were substitution (c.117G>A and c.2316T>C) and deletion (c.719_724delAAACAG).