Konstruksi Plasmid Rekombinan untuk Pengekspresian Epitop PD1 EP2 dalam Sistem Ekspresi E. coli = Construction of Recombinant Plasmid for Expression of PD1 EP2 Epitope in E. coli Expression System

Pauline Phoebe Halim, author

Abstrak

Imunoterapi merupakan metode terapi kanker yang sedang berkembang namun belum tersedia bebas di Indonesia karena biayanya yang mahal. Salah satu cara kerjanya adalah dengan menargetkan bagian spesifik dari sistem imun untuk membentuk respon terhadap sel ganas dan meningkatkan apoptosis sel menggunakan antibodi monoklonal. Contohnya reseptor PD1 yang berfungsi untuk menghalangi ligan PD1 yang banyak terdapat pada permukaan sel kanker. Riset ini bertujuan untuk mendapatkan plasmid rekombinan epitop PD1 EP2 yang akan dipergunakan dalam riset payung tim PRVKP. Rekombinasi DNA dilakukan dengan memasukan fragmen DNA PD1 EP2 yang telah diperbanyak dan dipotong ke dalam plasmid pQE-80L. Hasil ligasi kemudian ditransformasi dalam bakteri E.coli TOP10 dan dianalisa dengan PCR koloni. Produk PCR terbukti mengandung plasmid rekombinan PQE EP2. Konfirmasi selanjutnya dengan analisa sekuens DNA memastikan kandungan basa pada fragment insert telah terekspresi dalam plasmid. Sekuensing koloni ke 12 menunjukan bahwa insert telah terekspresi tanpa mutasi. Beberapa protokol ditemukan berpotensi mengurangi kemungkinan keberhasilan studi ini namun penelitian lebih lanjut. Dikarenakan hasil analisa ulang situs restriksi HindIII pada sekuens DNA tidak dapat memberi kepastian, sekuensing ulang disarankan untuk dilakukan. Protokol PRVKP disarankan untuk disempurnakan kembali. <hr /> Immunotherapy is a flourishing method for cancer treatment that is not available in Indonesia due to its expensive cost. One of its working method is by targeting a specific part of immune system to induce response against cancer cell and increase cell apoptosis with monoclonal antibodies. As example, PD1 receptor which function is to inhibit PD1 ligands abundantly found at the surface of cancer cell. This research focused on obtaining PD1 EP2 epitope recombinant plasmid to create the PD1 monoclonal antibody that is being develop by PRVKP team. DNA recombination was performed by inserting PD1 EP2 epitope's fragment into pQE-80L plasmid. Ligation product was then transformed into E.coli TOP10 bacteria and analyzed with colony PCR. PCR product had been proven to yield several colonies which contain PQE EP2 recombinant plasmid. Next, sequence analysis was conducted to confirm correct insert fragment was successfully expressed in plasmid. The 12th colony sequence was confirmed to contain non-mutated bases. Several protocol were found to potentially decrease the chance of success, yet further examination was not executed as it was not the focus of the research. As reanalysis of HindIII restriction site in recombinant sequence remained inconclusive, resequencing was suggested. PRVKP protocols adjustment was advised.