Transformasi dan ekspresi gen metCSF3syn menggunakan vektor rekombinan pGAPZ? dengan promotor konstitutif PGAP pada Picha pastoris SMD1168H = Transformation and expression of metCSF3syn gene using pGAPZÎ± recombinant vector with PGAP constitutive promoter on Pichia pastoris SMD1168H

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Abstrak

Gen CSF3syn adalah gen sintetis yang dibangun secara in vitro menggunakan teknik PCR, yang mengkode Human Granulocyte Colony-Stimulating Factor (hG-CSF) yang termasuk dalam hematopoiesis sitokin. Protein hG-CSF berperan dalam merangsang proliferasi, diferensiasi, dan pematangan sel progenitor granulosit. Penelitian sebelumnya telah berhasil memasukkan kodon metionin ke ujung 5 'dari gen CSF3syn, menghasilkan gen metCSF3syn. Penelitian ini bertujuan untuk mengubah vektor rekombinan pGAPZ-metCSF3syn menjadi strain Pichia pastoris SMD1168H, untuk mengekspresikan protein met-hG-CSF sebagai biosimilar dari filgastrim. Vektor rekombinan pGAPZ-metCSF3syn diisolasi dari Escherichia coli DH5, kemudian dilinierisasi menggunakan enzim restriksi BamHI. Vektor rekombinan diubah menjadi P. pastoris menggunakan teknik elektroporasi. Hasil penelitian menunjukkan bahwa koloni P. pastoris transforman tumbuh pada media YPD yang mengandung 100 g / mL zeosin. Koloni transforman kemudian diseleksi pada medium yang sama dengan konsentrasi zeosin 500 g / mL. Semua koloni yang diuji tumbuh dengan baik pada media seleksi, menunjukkan bahwa sel transforman secara genetik stabil dan resisten terhadap zeosin. Verifikasi gen metCSF3syn dilakukan dengan teknik koloni PCR, diperoleh 7 dari 8 klon positif yang menunjukkan pita gen metCSF3syn sebesar 532 bp yang menunjukkan klon tersebut mengandung gen target dalam genomnya. Analisis SDS-PAGE menunjukkan klon yang membawa gen metCSF3syn berhasil mengekspresikan protein met-hG-CSF dengan berat molekul 18,8 kDa, sesuai dengan bobot molekul teoritisnya. Hasil kuantifikasi protein pada analisis Western blot menunjukkan bahwa klon nomor 1 memiliki tingkat ekspresi tertinggi. Peningkatan ekspresi protein met-hG-CSF dan jumlah sel P. pastoris transforman berbanding lurus dengan waktu kultur menggunakan metode kultur sistem fed-batch. .....CSF3syn gene is a synthetic gene constructed in vitro using PCR technique, which encodes Human Granulocyte Colony-Stimulating Factor (hG-CSF) which is included in cytokine hematopoiesis. The hG-CSF protein plays a role in stimulating the proliferation, differentiation, and maturation of granulocyte progenitor cells. Previous research has succeeded in inserting a methionine codon into the 5 'end of the CSF3syn gene, resulting in the metCSF3syn gene. This study aims to convert the recombinant vector pGAPZ-metCSF3syn into a strain of Pichia pastoris SMD1168H, to express the met-hG-CSF protein as a biosimilar from filgastrim. The recombinant vector pGAPZ-metCSF3syn was isolated from Escherichia coli DH5, then linearized using BamHI restriction enzyme. The recombinant vector was converted into P. pastoris using electroporation techniques. The results showed that P. pastoris transforman colonies grew on YPD media containing 100 g / mL zeosin. Transformant colonies were then selected on the same medium with a zeosin concentration of 500 g / mL. All the colonies tested grew well on the selection media, indicating that the transformant cells were genetically stable and resistant to zeosin. Verification of the metCSF3syn gene was carried out using PCR colony technique, obtained 7 out of 8 positive clones which

showed the metCSF3syn gene band of 532 bp, indicating that the clone contained the target gene in its genome. SDS-PAGE analysis showed a clone carrying the metCSF3syn gene was successful in expressing the met-hG-CSF protein with a molecular weight of 18.8 kDa, according to its theoretical molecular weight. The results of protein quantification in Western blot analysis showed that clone number 1 had the highest expression level. The increase of met-hG-CSF protein expression and the number of transforman P. pastoris cells were directly proportional to the culture time using the fed-batch system culture method.