Transformasi dan ekspresi gen anti-TfR- scFv pada pichia pastoris = Transformation and expression of anti TfR-scFv gene in pichia pastoris

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Abstrak

Penelitian bertujuan untuk memperoleh Pichia pastoris transforman yang mengandung gen anti-TfR-scFv dan mengekspresikan protein rekombinan anti-TfR-scFv. Protein tersebut merupakan fragmen antibodi untai tunggal (singlechain-variable-domain, scFv) yang mengenali protein reseptor transferin yang dijumpai pada permukaan sel manusia. Gen anti-TfR-scFv telah diklon ke dalam vektor ekspresi pPICZ di bawah kontrol promoter terinduksi PAOX1 dan di fusi dengan sinyal sekresi MF- (mating factor-) dari Saccharomyces cerevisiae. Gen anti-TfR-scFv juga di fusi dengan gen EGFP (enhanced green fluorescent protein) pada ujung-C. Vektor rekombinan pPICZ_TfR_EGFP ditransformasi ke dalam P. pastoris SMD1168H menggunakan teknik elektroporasi.
Hasil penelitian menunjukkan bahwa vektor rekombinan berhasil ditransformasikan ke dalam genom P. pastoris. Sebanyak 22 koloni transforman berhasil diperoleh dengan tingkat efisiensi transformasi sebesar 0,062x103 cfu/g DNA. Proses seleksi transforman dilakukan pada medium seleksi YPD agar yang mengandung zeocin. Uji fenotipe Mut (methanol utilization) terhadap tujuh klona transforman memperlihatkan dua klona termasuk Mut+, empat klona MutS dan satu klona Mut-. Protein rekombinan telah berhasil diekspresikan secara ekstraselular. Visualisasi menggunakan mikroskop flouresen menunjukkan adanya pendaran fluoresen dari protein EGFP pada P. pastoris transforman yang mengindikasikan bahwa protein rekombinan telah terekspresi dengan benar. Analisis SDS-PAGE dan Western blotting menunjukkan protein rekombinan (±50kDa) berhasil dideteksi.
.....This research aimed to obtain Pichia pastoris transformant containing anti-TfRscFv gene which express anti-TfR-scFv recombinant protein. This recombinant protein consist of a single-chain-variable-domain (scFv) recognizing the extracellular domain of human_transferrin_receptor found on the surface of human cell. The anti-TfR-scFv gene was cloned into pPICZ expression vector under the control of inducible promoter PAOX1 and fused with MF- (mating factor-) secretion signal from Saccharomyces cerevisiae. The gene was also fused at the C-terminal with EGFP (enhanced-green-fluorescent-protein) reporter gene. The recombinant vector pPICZ_TfR_EGFP has been transformed into P. pastoris SMD1168H using electroporation technique.
The results showed that the recombinant vector has been successfully transformed into P. pastoris genome. A number of 22 transformant colonies has been obtained with a transformation efficiency number of 0,062x103 cfu/g DNA. Screening process of transformants was carried out on YPD agar medium containing zeocin. Assay on the Mut (methanol utilization) phenotype of seven transformant clones showed that two of them are Mut+, four are MutS and one is Mut-. The recombinant protein was successfully expressed and secreted from the cell. Visualization using fluorescence microscopy showed fluorescent light coming out from the transformant cells, proving that the recombinant protein has been correctly expressed. The SDS-PAGE and Western blotting analyses showed that the recombinant protein (±50kDa) has been detected.