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Design and construction of a vector for recombinant protein expression in the yeast *Hansenula polymorpha* and expression of granulocyte colony stimulating factor (GCSF) as a candidate protein

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Objectives

Production of recombinant proteins has a market of billion dollars. Production of a new product starts with the selection of a suitable host. Among different expression systems, yeast cells serve as single cell factories with simple genetic manipulations and specific secretion pathways which result in correct protein production and post translational modifications. *Saccharomyces cerevisiae, Pichia pastoris* and *Hansenula polymorpha* are the most studied yeast host cells which the two latters have a common pathway in methanol metabolization. Technology of *Hansenula* attracted global attention due to its high expression level of approved pharmaceutics including Hepatitis B vaccine, insulin, interferon alpha 2a and 2b. The aim of this study was to design an expression platform of *Hansenula* polymorpha and evaluation of GSCF heterologous expression in this system as a candidate protein.

Methods

Required gene cassette consists of an inducible promoter (pFMD), pre-pro-sequence of *Saccharomyces cerevisiae* mating alpha factor (as a secretion signal sequence), transcription termination sequence and an auxotrophic selection marker were designed and the synthetic fragment (approximately 3500bp) was cloned into an *E. coli* cloning vector. Coding sequence of *granulocyte stimulating growth factor* gene (*gcsf*) (the cDNA; approximately 600bp) was also used as a synthetic fragment and cloned into another *E. coli* cloning vector after codon optimization for the yeasts. Digested *gcsf* gene fragment was ligated with digested *Hansenula* vector and ligation reactions were transformed into *E. coli* TOP10F'.

Results

The identity of synthetic vectors was confirmed through restriction digestions with designed restriction enzymes and observation of expected fragments on agarose gel. *gcsf* cloning procedure was also confirmed on the grown colonies through restriction digestion analysis and sequencing.

Conclusions

The next step of the study is transformation of the recombinant vector into *Hansenula polymorpha* as the host by electroporation and induction of protein expression by methanol.