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APPLICATION OF NATIVE SIGNAL SEQUENCES FOR RECOMBINANT PROTEINS SECRETION IN *PICCHIA PASTORIS*

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Background

Methylotrophic yeast *Pichia pastoris* is widely used for recombinant protein production, largely due to its ability to secrete correctly folded heterologous proteins to the fermentation medium. Secretion is usually achieved by cloning the recombinant gene after a leader sequence, where alpha-mating factor (MF) prepropeptide from *Saccharomyces cerevisiae* is most commonly used. Our aim was to test whether signal peptides from *P. pastoris* native secreted proteins could be used to direct secretion of recombinant proteins.

Results

Eleven native signal peptides from *P. pastoris* were tested for their efficiency to direct secretion of reporter protein invertase SUC2 from *S. cerevisiae*. Alpha-MF prepropeptide was a reference leader sequence. All the tested *P. pastoris* signal peptides could direct secretion of invertase, two of them giving 37-44% higher activity than alpha-MF prepropeptide construct.

Additionally we developed a flexible cloning system, which consists of: a basic *Pichia* expression vector, a set of *Pichia* promoters, and a set of signal peptides. The system is based on ligation-free cloning technique Uracil-Specific Excision Reaction (USER). PCR-amplified protein coding gene is mixed with the promoter and signal peptide of choice (or a mix thereof) and the basic expression vector. The mix is treated with USER enzyme and transformed into *E. coli*. The plasmids (or plasmid mixes) are further purified, linearized and transformed into *P. pastoris*. We illustrate the commodity of the system by optimization of expression of three different proteins in *P. pastoris*.

Conclusions

Native signal peptides from *P. pastoris* can be used to direct secretion of recombinant proteins. A novel USER-based *P. pastoris* system allows easy cloning of protein-coding gene with the promoter and leader sequence of choice.