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APPLICATION OF NATIVE SIGNAL SEQUENCES FOR RECOMBINANT PROTEINS SECRETION IN PICHIA PASTORIS

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Background
Methylo trophic 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.