Advancements in *Pichia pastoris* expression platform: improving targeting efficiency for the introduction of new auxotrophies

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Basic expression systems for recombinant protein production in *Pichia pastoris* have been commercially available in the recent past. We have developed a new independent and well characterized expression platform with improved vectors and production strains. The versatility of this expression system enables the production of a wide variety of proteins with different requirements concerning promoter efficiency, expression cassette location, choice of markers or co-expression of chaperones.

To illustrate the potential of the improved platform in protein and strain engineering, we have successfully utilized the high targeting efficiency of a *P. pastoris* Δ*Ku70* strain to screen essential metabolic routes in search for new auxotrophies to be used as selection markers free of antibiotics. For the biopharmaceutical environment avoiding the use of antibiotic resistant strains, introduction and combination of new auxotrophies enables safe co-expression of multiple proteins.