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UNFOLDED PROTEIN RESPONSE BIOSENSORS FOR RECOMBINANT PROTEIN EXPRESSION

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The yeast *Pichia pastoris* is regarded as an attractive workhorse for the synthesis of valuable products. One of its key features as an industrial protein production host, is its ability to secrete high amounts of recombinant proteins. However, the secretion efficiency also depends on the protein target. Therefore, strain engineering and improvement is paramount to generating high producing, industrially applicable strains. The unfolded protein response (UPR) significantly impacts the recombinant protein production and secretion in eukaryotic cell factories, including *P. pastoris*. This cellular stress response is triggered when unfolded proteins accumulate inside the endoplasmic reticulum (ER) lumen, exceeding the protein folding capacity of the ER. The UPR modulates the transcription of UPR-responsive genes to help restore the ER homeostasis. The transcription of one of these UPR-responsive gene, the KAR2 gene has been used as an indicator to evaluate the manifestation and strength of the UPR in yeast cells. Here, the putative promoter sequence of KAR2 is coupled with a fluorescent marker gene (eGFP) to generate a UPR biosensor strain that can be used as chassis for recombinant protein expression. The UPR biosensor provides a signal for folding stress and thus for target protein secretion, which can be used for clone selection during screening, scaling up to bioreactor and during fermentation development by better understanding the cell folding stress during recombinant protein production. The UPR biosensor will give elements to guide rational engineering strategies towards optimal heterologous protein production.