# ENLARGING THE SYNTHETIC BIOLOGY TOOLBOX FOR *PICHIA PASTORIS*: GOLDEN GATE CLONING AND CRISPR/CAS9

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State-of-the-art strain engineering techniques for the protein producing yeast host *Pichia pastoris* include overexpression of homologous and heterologous genes, and deletion of host genes. For this purpose overexpression vectors and gene deletion methods such as the split marker technique have been established. For metabolic and cell engineering purposes, the simultaneous overexpression of more than one gene is often needed. Previous approaches employing subsequent steps of overexpression and marker recycling were time- and labor-consuming. Therefore, efficient systems allowing multiple gene overexpression are required, that can be stably integrated into the *P. pastoris* genome. To this end, we developed a synthetic biology toolbox based on Golden Gate cloning to enable efficient construction of complex and versatile over-expression vectors. Up to five different expression cassettes, employing a library of promoters and terminators can be combined into one vector, and successfully integrated into the genomic DNA of *P. pastoris* at targeted loci in one step. Recent trends in synthetic biology, however, go into the direction of building up large and complex reaction networks. To allow for clean and unscarred genetic engineering, a CRISPR/Cas9 based method for gene insertions, deletions and replacements was developed, which paves the way for precise genomic rearrangements in *P. pastoris*. By using this technique precise genomic integrations were performed efficiently without integrative selection markers. The repertoire of genetic techniques developed so far, will provide a wide variety of possibilities to engineer *P. pastoris*. Applications for these synthetic biology tools in cell engineering of recombinant *P. pastoris* will be presented.