Recent technology developments and application for CRISPR based engineering of *Pichia pastoris*

The methylotrophic yeast *Komagataella phaffii* (syn. *Pichia pastoris*) is frequently applied as production host for industrial biocatalysts as well as biopharmaceuticals. Despite its amenability for high titer protein production, until recently genetic modifications have been substantially more difficult to achieve than in the 'classical' yeast *Saccharomyces cerevisiae*. Especially targeted replacements in the genome with newly introduced sequences (donor fragments) have been challenging.

Recently developed CRISPR-Cas systems for *P. pastoris* have greatly advanced genome editing capabilities enabling the introduction of insertions and deletions (indels) for gene knock-outs at up to 100% efficiency. Also targeted replacements were improved to up to 50% efficiency in wildtype strains by placing an autonomous replicating sequence on the donor fragment to improve availability. In *P. pastoris* strains with engineered recombination pathways even higher efficiencies have been obtained. Namely, knocking out the gene coding for the Ku70 protein results in up to 100% efficiency of specific integration of donor fragments using CRISPR-Cas, but reduces at the same time the number of transformants obtained.

With these powerful genome editing tools at hand, previously challenging metabolic engineering and synthetic biology approaches have now become substantially easier to achieve, paving the way for broad applications of *P. pastoris*.