

Multiple gene disruption and marker rescue in the yeast *Kluyveromyces marxianus*

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Gene disruption is one of the most powerful techniques for the study of gene function and metabolic engineering. After the integration of the disruption cassette in the desired locus it's convenient to remove the selection marker from the organism's genome. The Cre-loxP system meets this purpose of marker rescue by expression of Cre recombinase. In the particular case of *Kluyveromyces marxianus* the availability of disruption tools is very limited or even inexistent for this biotechnological important and interesting yeast, making necessary the development of new methods. As the Cre-loxP system works in some non-conventional yeasts, namely *Kluyveromyces lactis*, we wished to know whether it also works in *K. marxianus*. In this study, we validated the Cre-loxP system in *K. marxianus* by disrupting two copies of the beta-galactosidase gene (*LAC4* gene), using a gene disruption cassette that combines the advantages of the *kanMX* gene (which confers resistance to the antibiotic geneticin, G418) with those of the Cre-loxP system. Besides testing the application of the Cre-loxP system in *K. marxianus*, one of the purposes of this work was to construct a disruption cassette that offered a high percentage of correct transformants. Even using a disruption cassette with long flanking homologous sequences (723 bp upstream and 712 bp downstream), the gene targeting efficiency in *LAC4* loci was very low, 34% and 15% for the first and second round of transformation, respectively. The low gene targeting efficiency presented by this yeast indicates that it is necessary to construct gene disruption cassettes with long-flanking homology regions, when *K. marxianus* is the organism under investigation.