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## Selection systems for a-type and $\alpha$ -type yeasts using cell-type-specific transcriptional regulation machinery

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Sake yeasts belong to the budding yeast species *Saccharomyces cerevisiae* and have high fermentation activity and ethanol production. Although the traditional crossbreeding of sake yeasts is a time-consuming and inefficient process due to the low sporulation rates and spore viability of these strains, considerable effort has been devoted to the development of hybrid strains with superior brewing characteristics. In the current study, we constructed growth selection systems for 'a' and  $\alpha$ -type cells derived from parental  $MATa/\alpha$  yeasts and confirmed that the generated cells possess suitable mating abilities for the production of hybrid yeasts. To achieve it, we designed suitable genetic circuits for expression of the *kanMX4* selection marker gene to permit isolation of 'a' and  $\alpha$ -type cells, respectively, on solid medium. And also, we prevented autopolyploidization of yeast cells derived from the same parent via forced expression of the a1 or  $\alpha$ 2 gene to increase hybridization efficiency in crossbreeding. Industrially-used strains Kyokai No. 6 and No. 7 were selected as parental sake yeasts and we generated a hybrid strain, designated K76, using the constructed selection systems, which inherited the genetic characteristics of both parents. Notably, because all of the genetic modifications of the yeast cells were introduced using plasmids, these traits can be easily removed. The approach described here has the potential to markedly accelerate the crossbreeding of industrial yeast strains with desirable properties.

## Biography

Nobuo Fukuda has completed his PhD from Kobe University. He is a Senior Research Scientist of Biomedical Research Institute, AIST. He has published more than 15 papers in reputed journals.

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