

Precise CRISPR-Cas9 based genome editing in methylotrophic yeast

Ogataea polymorpha

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Abstract: Methanol expands the biorefinery substrate spectrum by using methylotrophic microbes. *Ogataea (Hansenula) polymorpha*, as a representative methylotrophic yeasts, increasingly attracts numerous attentions due to its thermotolerance, but low homologous recombination (HR) efficiency may block genetic manipulation in metabolic engineering. Here, we provided a workable CRISPR/Cas9 system with an enhanced HR activity, and first achieved a reasonable regulation of HR activity and NHEJ strength in *O. polymorpha*. Both the overexpression of HR-related proteins and down-regulated NHEJ increased HR rates from 20~30% to 60~70%, which could be attributed to the competitive relationship between HR and NHEJ. Our novel CRISPR/Cas9 system had been applied in homologous integration of large fragments and pathway assembly, and even in-vivo self-assembly of 20 kb plasmid, to achieve the first production of fatty acid-derived chemicals in *H. polymorpha*. These findings will simplify constructions of genetically engineered strains, and facilitate the adoption of *H. polymorpha* as an attractive cell factory for industrial-scale production.

Key words: CRISPR/Cas9; *Hansenula polymorpha*; Homologous recombination; Non-homology end joining; Cell factory

Brief Biography

Jiaoqi Gao, **Ph.D.**, assistant professor. He received his bachelor's degree in Biological Engineering at Dalian University of Technology in 2012, and a doctor's degree in Biochemical Engineering at Dalian University of Technology in 2017, during which he was engaged in ethanol fermentation from Jerusalem artichoke and regulatory mechanisms of inulinase. In 2015, he went to School of Medicine of University of Pittsburgh as a joint student, conducting research on yeast signal pathways. He participated in Dr Zhou's lab of Dalian Institute of Chemical Physics, Chinese Academy of Sciences in September, 2017, and his research interest is related to construction of yeast cell factories and methanol biotransformation. Until now, he has already published over 10 articles in *Biotechnology for Biofuels*, *Biomass & Bioenergy*, *Microbial Cell Factories*, *FEMS Yeast Research*, and so on.

Brief CV

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Education and Professional Career:

2008-2012	Dalian University of Technology	Bachelor degree
2012-2017	Dalian University of Technology	Doctor degree
2015-2016	University of Pittsburgh	Joint PhD student
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Research Interests:

1. Genetic tools construction
2. Yeast cell factory construction
3. Methanol biotransformation

Selected publications

1. Gao J.Q., et al. ***Biotechnol. Biofuels***, 2017, 10:79.
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