Abstract

Lactic acid is a valuable platform chemical used in the food, biomedical, and bioplastics industries, particularly as a precursor for biodegradable polylactic acid. *Komagataella phaffii* is a promising industrial yeast due to its robust growth, ability to utilize methanol as a carbon source, and suitability for genetic engineering. The native *adh2* gene encodes an alcohol dehydrogenase involved in ethanol metabolism. This study aimed to establish a CRISPR/Cas9-based genome editing strategy for the integration of the *Pediococcus acidilactici lactate dehydrogenase-l* (*Paldh*) gene into the *K. phaffii adh2* locus. A donor DNA construct consisting of the *K. phaffii adh2* upstream flanking region promoter, *Paldh* coding sequence, and *K. phaffii adh2* downstream flanking region terminator was assembled using overlap extension PCR. A single-guide RNA was designed using *CHOPCHOP* tools and cloned into the BB3cK CRISPR plasmid to direct Cas9-mediated cleavage at the *adh2* locus. A successfully constructed donor DNA and sgRNA have 2972 bp and 251 bp length respectively. The recombinant BB3cK_sgRNA plasmid was validated using PCR, which showed the expected 251 bp amplicons, indicating the presence of sgRNA construct inside the BB3cK CRISPR plasmid. The donor DNA and sgRNA_BB3cK plasmid were co-transformed into *K. phaffii*. Transformants were subsequently screened by colony PCR using *Paldh*-specific primers to identify colonies carrying the integrated gene. Out of five single colonies screened, one colony showed a DNA band of a apporimately 1,027 bp DNA fragment, indicating successful integration of *Paldh* into the engineered *K. phaffii* strain.

Keywords: Metabolic engineering; CRISPR-Cas9; Komagataella phaffii; Lactate dehydrogenase; Lactic acid production