# Use of site-specific recombination to create a *Pichia pastoris* mut$^{s}$ strain

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## *Abstract*

*S.cerevisiae* site-specific recombination mechanism employs FLP recombinase and two asymmetric FLP recombination target sequences (*FRT*s) derived from *S.cerevisiae* 2 µm circle [1] [2]. Orienting two *FRT*s as direct repeats results in precise FLP protein -mediated deletion of all DNA between the *FRT*s [3]. The system has previously been reported to function in *P. pastoris* by first inserting *FRT*s with a selection marker to the genome and transforming *FLP* on a separate plasmid [4]. A simplified version of the method to introduce all parts needed to the genome in a single transformation step has been reported to function in *C. albicans* [5]. This report describes the assembly and integration of a single cassette to specifically knock out the coding sequence of *Pichia pastoris alcohol oxidase* (*AOX1*) gene from wild type *Pichia pastoris* strain without leaving any cassette components except one *FRT* (34bp) behind in the genome.

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