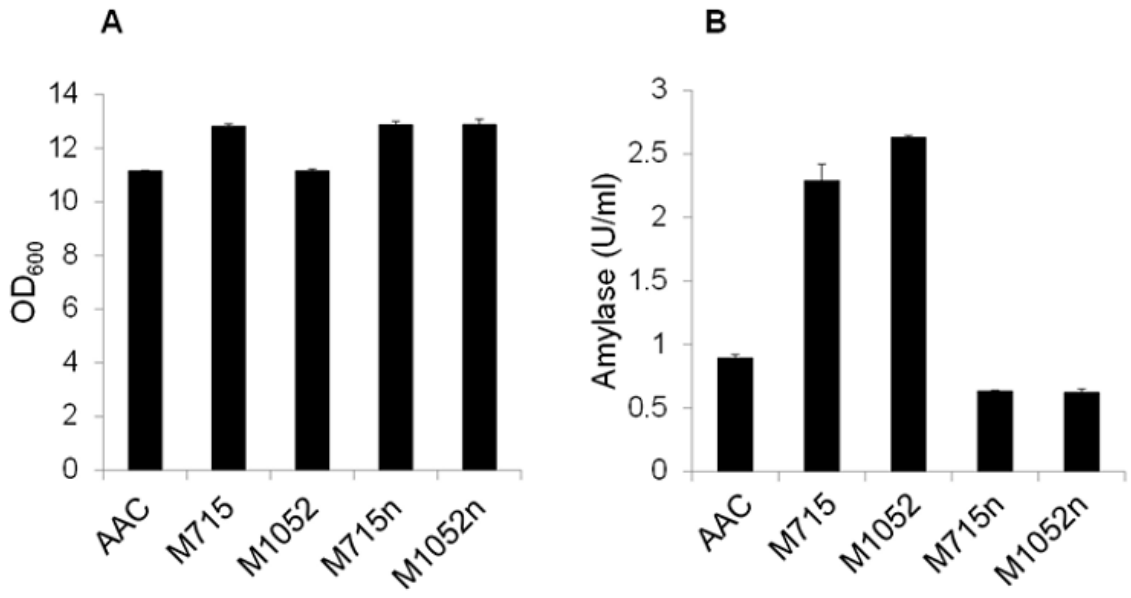

Figure S1. Effects of the plasmids extracted from the mutation strains on cell growth (A) and amylase production (B).

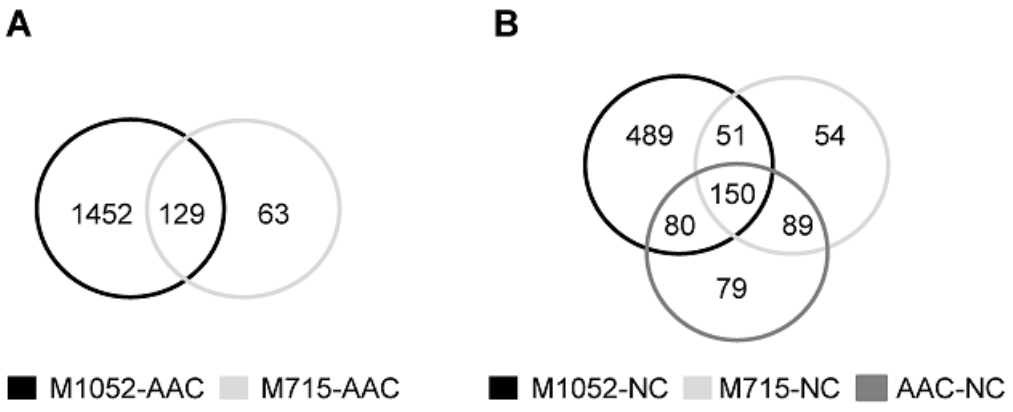

Figure S2. Venn diagrams showing significantly changed genes in the transcriptome experiment between different strains. FDR < 0.05.

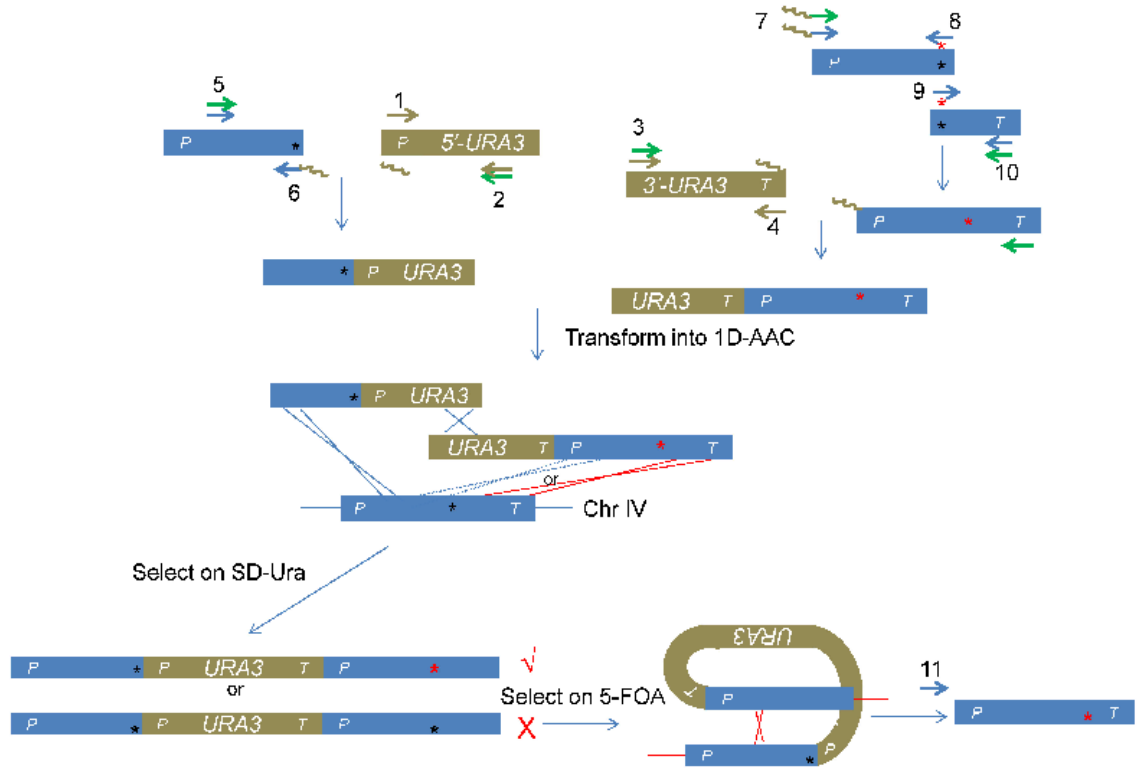

Figure S3. Procedure for Site-Directed Point Mutation. Blue bars represent fragments on the interested genes. Grey bars represent gene fragments on the *URA3* gene in *Kluyveromyces lactics*. Star marks the nucleotide to be changed (black) and will be changed to (red). Arrows represent primers used for amplification of the fragments. Primers are listed in Table S2.

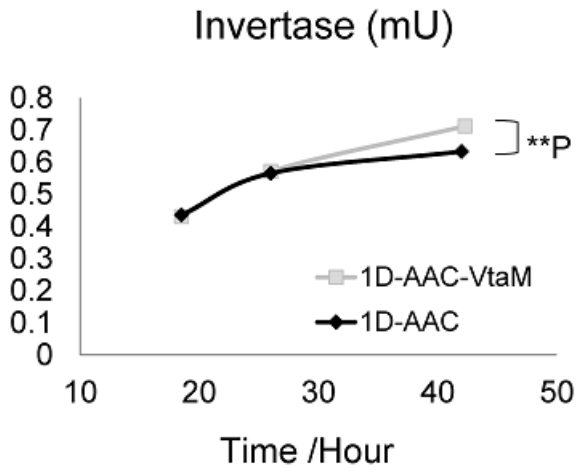

Figure S4. Effect of *Vta1S196I* mutation on periplasmic invertase production in shake flask fermentation.

Table S1. Overall statistics of the whole-genome Illumina sequencing

	M715	M1052	WT
Total reads	53846120	27382708	18413972
Coverage	443x	225x	151x
Reads mapping to genome	42388376	22603996	16067934
Mapped reads (%)	79%	83%	87%

Table S2. Primers used for amplification of fragments to apply point mutation.

No.	Primer
1	KURA_l_f: TTCGGCTTCATGGCAATTCC
2	KURA_l_r: GAGCAATGAACCCAATAACGAAATC
3	KURA_r_f: tcttgacgttcgttcgactgat
4	KURA_r_r: CGTGTCACCATGAACGACAAT
5	VTA_l_ff: gtgaaattggttccagcgac
	VTA1_l_r:
6	ACGATCCCCGGGAATTGCCATGAAGCCGAATCGATTGTCTGGTGATCAACATC
7	VTA1_m_f: tgcttaagaattgtcgttcatggtgacacgctatcggggttggtctcgtt
8	VTA1_m_r: TCGATTGTCTGGTGATCAACATC
9	VTA1_r_f: gatgttgatcaccagacaatcga
10	VTA1_r_r: CTGCCTTCAACCTCCCATGT
11	VTA_seq_2: ctattgcttagttcatattgat

Supplementary Text S1. Delft medium (defined minimal medium) for shake flask cultivation:

7.5 g/L $(NH_4)_2SO_4$; 14.4 g/L KH_2PO_4; 0.5 g/L; $MgSO_4 7H_2O$; 2 ml/L trace metal solution (per liter, pH 4.0: EDTA (sodium salt), 15.0 g; $ZnSO_4$ $7H_2O$, 0.45 g; $MnCl_2$ $2H_2O$, 1 g; $CoCl_2$ $6H_2O$, 0.3 g; $CuSO_4$ $5H_2O$, 0.3 g; Na_2MoO_4 2H2O, 0.4 g; $CaCl_2$ 2H2O, 0.45 g; $FeSO_4$ $7H_2O$, 0.3 g; H_3BO_3, 0.1 g and KI, 0.10 g). The pH of minimal medium was adjusted to 5.0 by adding 2 M NaOH and autoclaved.

Autoclaved glucose was added at a concentration of 20 g/L.

1ml/L of Vitamin solution (per liter, pH 6.5: biotin, 0.05 g; p-amino benzoic acid, 0.2 g; nicotinic acid, 1 g; Ca-pantothenate, 1 g; pyridoxine-HCl, 1 g; thiamine-HCl, 1 g and myo-inositol, 25 g) was filter sterilized and aseptically added to the medium after autoclaving.