

**Supplementary Table 1** Primers used in this study

Primer name	Sequence (5'-3')
D-ldh-FU	ATGCGT <u>CGAC</u> GCCCCGCGATCGTCTCCTTCGG
D-ldh-FL	GGAATGCTTGAAGCGTTCCAGCCAATGAGGACAATCTTGTT
D-ldh-BU	AACAAGATTGTCCTCATTGGCTGGAACGCTTCAAGCATTCC
D-ldh-BL	CCGGA <u>AAGCT</u> TGTCGTGTGCACAGTTGATGC
D-pta-ack-FU	AGGCT <u>CTAGA</u> ACTCACGCAACTCCTCAACA
D-pta-ack-FL	GGAGGCATCGGTGGAAATCACCTAGGGATATCCTCGAGGAATCCATCGAAGCT GCGGT
D-pta-ack-BU	ACCGCAGCTTCGATGGATT <u>CCTCGAGGATATCCCTAGGTGATT</u> TCCACCGATGC CTCC
D-pta-ack-BL	ATGCGT <u>CGAC</u> GACTCACCGAAATCCCCACA
D-nagD-FU	GGAGT <u>CTAGAT</u> CGATAAGCACGCTTTTAATATTT
D-nagD-FL	TGCCATGACCTACAGAATAAACACCATTGTCCCTGTTTTGGGGCGAAAT
D-nagD-BU	CCAAAACAGGGACAATGGTGTTTATTCTGTAGGTCATGGCATTTCAGACAT
D-nagD-BL	CCGGA <u>AAGCT</u> TAGTGCGTGTGTTTGTTGATGAACTCTTGG
D-butA-FU	TACTTGGATCCGTCATACGTGGTACTTCC
D-butA-BL	AGACAA <u>AAGCT</u> TACCATCCGAGAAGACTTCAGG
O-Ptuf-U	ATAT <u>CCTAGGT</u> GGCCGTTACCCTGCGAATGTC
O-Ptuf-L	TGTCATGGTTGTCCTCCTTTTTGTATGTCCTCCTGGACTTC
O-alsSD-U	GAAGTCCAGGAGGACATACAAAAGGAGGACAACCATGACA
O-alsSD-L	CGCGT <u>CTCGAGT</u> TATTCAGGGCTTCCTTCAGTTGTTTCGATATCTTT
ppc-F	CCCA <u>AAGCT</u> TGAACAGGCTCTCGATGCAGGC
ppc-L	ACGCGT <u>CGACT</u> TAGACCTTGAACCACACGCCCTC
pyc-F	CCCA <u>AAGCT</u> TCGAAGAAGGCTGGTCTGCCAG
pyc-L	ACGCGT <u>CGACT</u> TACACCAGCATGGAGTCAAAGTG
pEC-lacIq-F	GCG <u>ACTAGT</u> GCGCAACGCAATTAATGTGAGTTAG
pEC-lacIq-L	GCG <u>ACTAGT</u> ATTCACCACCCTGAATTGACTC

**Supplementary Table2** Composition of the media for optimization

Medium
CGXII ((NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> (5 g/L), Urea (5 g/L), KH <sub>2</sub> PO <sub>4</sub> (1 g/L), K <sub>2</sub> HPO <sub>4</sub> (1 g/L), MgSO <sub>4</sub> ·7 H <sub>2</sub> O (0.25 g/L), CaCl <sub>2</sub> (10 mg/L), biotin (0.4 mg/L))
CGXIIL CGXII+0.5% YE
CGXIIL +0.25%Urea
CGXIIL +1%NaAC
CGXII+1%YP
CGXII+1% CSL
CGXII+0.5% YP+0.5% CSL
CGIII (10 g/L Tryptone, 10 g/L YE, 2.5 g/L NaCl)
LBY (YE 15 g/L, Tryptone 7.5 g/L, NaCl 5 g/L)
LBRC (10 g/L YP, 50 g/L CSL, Urea 1 g/L, MgSO <sub>4</sub> 0.5 g/L, K <sub>2</sub> HPO <sub>4</sub> 0.5 g/L, NaAc 2 g/L)

YE: yeast extract, YP: yeast powder, CSL: corn steep liquor

**Supplementary Table 3** D-(-)-acetoin production from glucose using CGR7 in fed-batch fermentations.(exact data of Figure 8)

Time	OD600	Glucose (g/L)	succinate (g/L)	a-ketoglutarate (g/L)	acetate (g/L)	glycerin (g/L)	acetoin (g/L)
0.0	4.5	55.0	0.02	0.00	0.00	0.00	0.18
13.0	71.3	10.0	0.41	0.25	0.12	0.43	16.51
13.1	71.1	37.0	0.41	0.25	0.12	0.43	16.42
21.0	75.4	7.5	1.17	0.42	0.86	0.46	29.31
21.1	75.2	52.0	1.17	0.42	0.86	0.46	29.23
33.5	85.2	6.0	0.20	1.30	1.01	0.76	45.33
33.6	85.0	61.0	0.20	1.30	1.01	0.76	45.04
45.0	94.6	7.0	0.37	1.62	1.11	0.98	64.58
45.1	94.0	53.0	0.37	1.62	1.11	0.98	63.86
57.0	108.2	7.6	0.57	1.33	0.74	1.11	77.43
57.1	107.1	47.5	0.57	1.33	0.74	1.11	76.39
65.5	106.4	21.0	0.67	0.97	0.58	1.29	86.98
69.0	105.4	12.7	0.74	0.78	0.41	1.31	91.98
74.0	107.8	0.0	0.73	1.03	0.27	1.27	96.20