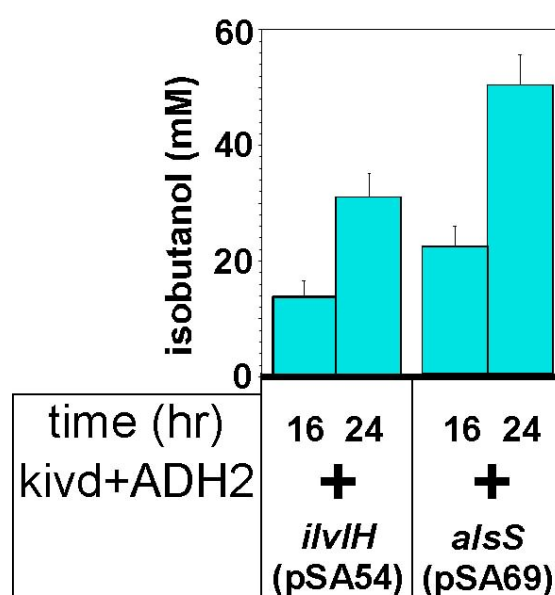


Non-Fermentative Pathways for Synthesis of Branched-Chain Higher Alcohols as Biofuels

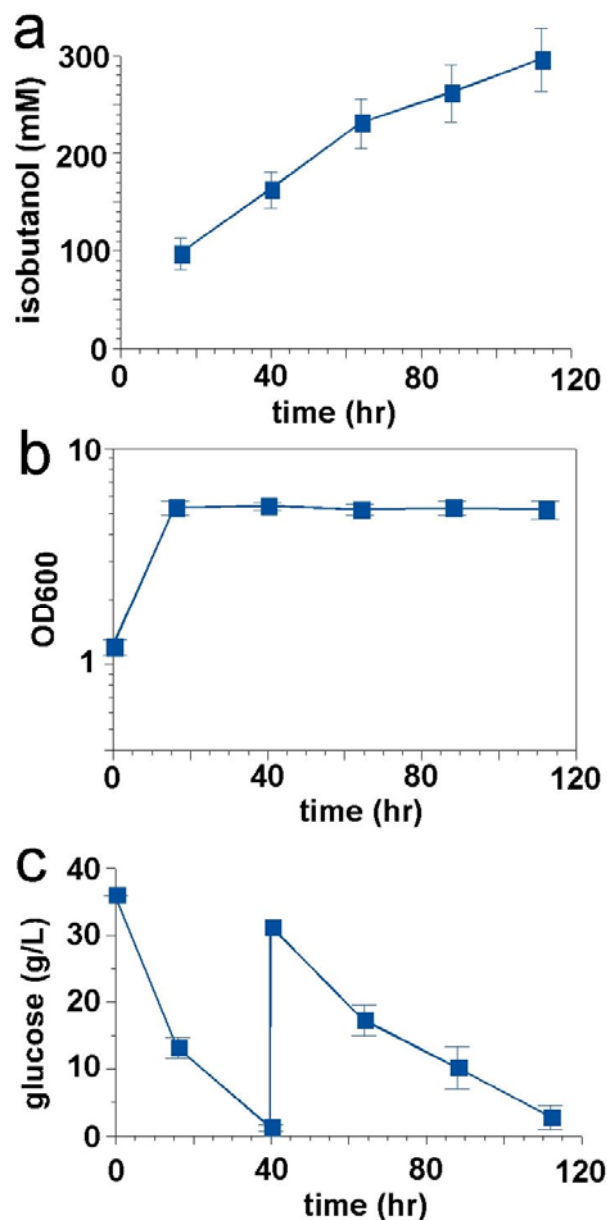
S.Atsumi, T. Hanai and J.C. Liao

Supplementary Fig. 1



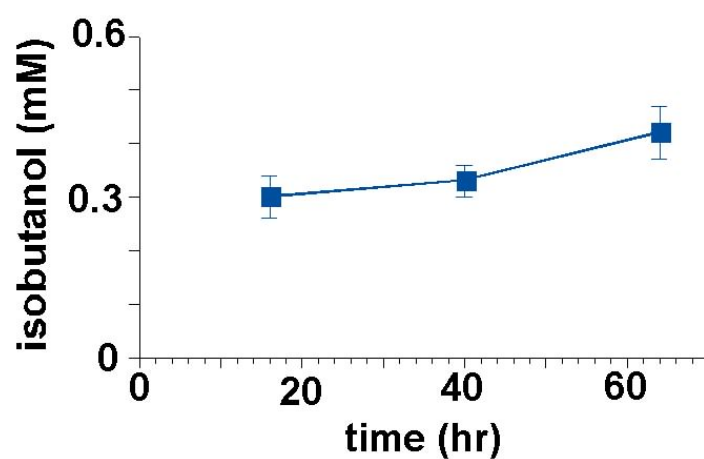
Supplementary Fig. 1 Comparison of isobutanol production with the engineered *ilvIHCD* (strain JCL88/pSA55/pSA54) or *alsS-ilvCD* (strain JCL88/pSA55/pSA69) pathway in M9 medium with 3.6% glucose. Error bars: standard deviations from three independent experiments.

Supplementary Fig. 2



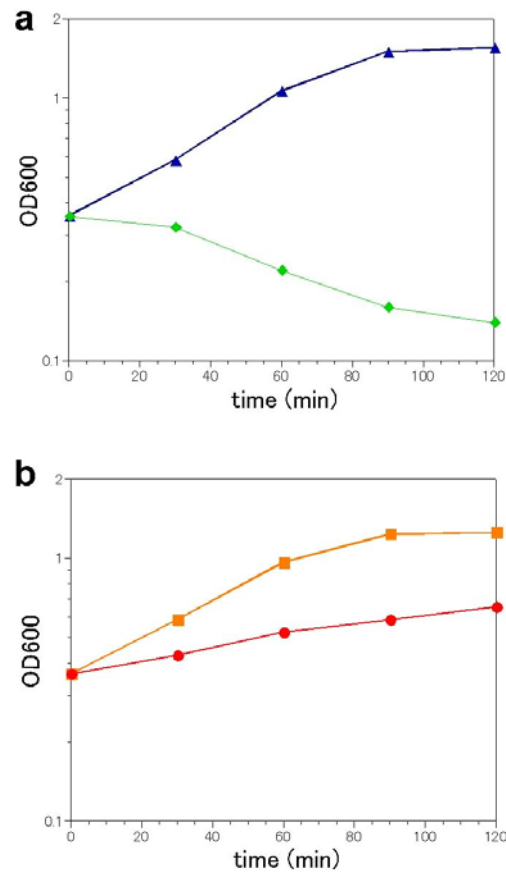
Supplementary Fig. 2. Isobutanol production with the engineered *alsS-ilvCD* pathway in M9 medium with 3.6% glucose and 0.5 % yeast extract. The strain is JCL260/pSA55/pSA69. At 40 hr, 3.0% glucose was added to the culture. Error bars: standard deviations from three independent experiments. **a:** Time profiles of isobutanol production; **b:** Time profiles of cell growth. **c:** Time profiles of glucose concentration in medium.

Supplementary Fig. 3



Supplementary Fig. 3. Isobutanol production in M9 medium with 0.5 % yeast extract only, without glucose. The strain was JCL260/pSA55/pSA69. Error bars: standard deviations from three independent experiments.

Supplementary Fig. 4

**Supplementary Fig. 4. Effect of isobutanol on growth.**

Time course for cell growth of the wild type (JCL16) and the high tolerant mutant, with or without 2 % isobutanol. Both strains were grown in LB to exponential phase. At OD600 about 0.35, 2% isobutanol was added to the medium. **a**, Wild type strain, Blue triangle: without isobutanol; green diamond: with isobutanol; **b**, High tolerant mutant, Orange square: without isobutanol; Red circle: with isobutanol.

Supplementary Table 1. Strains used in this study

Strain	Relevant genotype
BW25113	<i>rrnB</i> _{T14} Δ <i>lacZ</i> WJ16 <i>hsdR</i> 514 Δ <i>araBAD</i> _{AH33} Δ <i>rhaBAD</i> _{LD78}
JCL16	BW25113/F' [traD36, proAB ⁺ , lacI ^q Z Δ M15]
JCL88	Same as JCL16 but with Δ <i>adhE</i> , Δ <i>ldhA</i> , Δ <i>frdBC</i> , Δ <i>fnr</i> , Δ <i>pta</i>
JCL260	Same as JCL88 but with Δ <i>pflB</i>
SA203	Same as JCL16 but with Δ <i>ilvD</i>

Supplementary Table 2. Synthetic oligonucleotides for the plasmids

name	sequence
A65	CGAGCGGTACCATGTCTGAAATTACTCTTGAAAAAT
A66	GCCTGCGCATGCTTATTGTTTGGCATTGTAGCGGCA
A67	GCCTGCGCATGCAGGAGATATACCATGTCTATTCCAGAAACTCAAAAAG
A68	GCTCTAGATTATTTAGAAGTGTCAACAACGTAT
A70	ACGCAGTCGACTCAACGCATTATTTTATCGCCGCGC
A71	ACGCAGTCGACGAGGAATCACCATGGCTAACTACTT
A72	AATAACCCGGGTAAACCCGCAACAGCAATACGTTTC
A74	CGAGCACGCGTTTAAACCCCCAGTTTCGATTTATCG
A83	GCCACCGGTCTCCGTACCATGGAGATGTTGTCTGGAGCCGAGA
A84	GGCTCCGGAAGGAGATATACCATGCCTAAGTACCGTTCCGCCACCA
A96	CGAGCGGTACCATGTATACAGTAGGAGATTACCTAT
A97	GCCTGCGCATGCTTATGATTTATTTTGTTCAGCAAAT
A98	CGAGCGGTACCATGGCACCTGTTACAATTGAAAAGT
A99	GCCTGCGCATGCCTATTTTTTATTTCTTTTAAGTGCCGC
A100	CGAGCGGTACCATGAATTCTAGCTATACACAGAGAT
A101	GGTCAGTATCCAACCTGATTTTTTTTTTAGAAG
A102	CGAGCGGTACCATGAAGAGTGAATACACAATTGGAAG
A103	GCCTGCGCATGCCTAATTATTTTGATTTGCAAAACGT
A104	CGAGCGGTACCATGGCTGACTCGCAACCCCTGTCCG
A105	CCGCTCGAGCTAACCCGCCAAAAAGAACCTGAAC
A106	ACGCAGTCGACAAGAGACAAGGACCCAAACCATGAGCCAG
A109	GGAAGATCTTTAATTCATAAACGCAGGTTGTTTTGC
A123	GCCACCCGTCTCCGTACCATGTTGACAAAAGCAACAAAAGAAC
A124	ACGCAGTCGACCTAGAGAGCTTTCGTTTTTCATGAGT
A125	CGAGCTGTACAATGTTGACAAAAGCAACAAAAGAAC
A126	TCTCTAGAAAGGTACCGGCAGCTTG

Supplementary Table 3. Alcohol productions with the ilvIHCD pathway overexpression

KDC	kivd	kivd	kivd	kivd	kivd	kivd
Strain	JCL16	JCL16	JCL16	JCL16	JCL88	JCL88
plasmid	pSA55	pSA55	pSA54 pSA55	pSA54 pSA55	pSA54 pSA55	pSA54 pSA55
Time (hr)	16	24	16	24	16	24
Product (μ M)						
ethanol	1277	1744	2047	2228	850	1430
1-propanol	321	420	612	808	210	493
isobutanol	2069	2172	12979	20813	13794	30910
1-butanol	67	112	146	198	89	127
2-methyl-1-butanol	223	629	262	345	195	239
3-methyl-1-butanol	1096	1696	1103	1577	727	1333
2-phenylethanol	545	1220	282	425	454	820

Culture was performed in M9 medium with 0.2 M glucose + 0.1 mM IPTG at 30°C.

Supplementary Table 4. Alcohol productions with the threonine pathway overexpression

KDC	kivd	kivd	kivd	kivd
plasmid	pSA55	pSA55 pSA62	pSA55 pSA62	pSA54 pSA62
strain	JCL16	JCL16	JCL16	SA203
0.8% L-threonine	-	-	+	+
Product (μM)				
ethanol	2450	2343	3243	1493
1-propanol	520	1356	7592	9849
isobutanol	2242	4322	1061	ND
1-butanol	220	583	3157	9232
2-methyl-1-butanol	766	1444	2002	ND
3-methyl-1-butanol	1495	4074	1349	ND
2-phenylethanol	324	358	269	524

Culture was performed in M9 medium with 0.2 M glucose + 0.1 mM IPTG at 30°C for 40 hr. “ND” indicates “not detectable”.