

Supporting Information

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Table S1. Analysis of Multiple Sequence Alignments of ketol-acid reductoisomerases (KARIs)

Domain and loop length	Conserved residues responsible for cofactor contacts				Compared sequences, n	KARI sequences, %
	N-terminal	C-terminal antepenultimate	Penultimate	Ultimate		
Bacteria						
6	R(100%)	—	K(65%), R(20%), S(6%)	S(100%)	83	13
7	R(61%), Y(16%), K(12%)	S(78%), G(22%)	—	S(76%), T(18%)	388	61
12	R(93%)	R(87%)	—	S(99%)	117	18
Archaea						
6 + 7	R(64%)	G(77%), S(17%)	—	S(89%)	47	7
Eukaryote						
7	R(100%)	S(57%), G(43%)	—	S(100%)	7	1

Conservational degree at key residues for specificity identified in β 2 α B loop: KARI sequences were obtained from the UniProt database using SwissProt manually reviewed sequence data. To date, 643 reviewed KARI sequences are available: 588 from bacterial, 47 from archaeal, and seven from eukaryotic origin. Archaeal KARIs seem to have loop lengths of either six or seven residues, eukaryotic KARIs a loop length of seven, and bacterial ones have six-, seven-, or 12-residue loops.

Table S3. Sequence Identities (%) of KARIs Used in this Study

	Ec_IlvC	Sh_sp_KARI	Se_KARI	Ma_KARI	LI_KARI	Aa_KARI
Ec_IlvC	100	72	20	20	21	22
Sh_sp_KARI		100	19	22	19	21
Se_KARI			100	48	53	50
Ma_KARI				100	52	56
LI_KARI					100	57
Aa_KARI						100

Table S4. Crystallographic Parameters of Wild-Type Se_KARI and Variant Se_KARI^{DDV}

	Se_KARI	Se_KARI ^{DDV}
Protein Data Bank code	4KQW	4KQX
Space group	P12 ₁ 1	P2 ₁ 2 ₁ 2 ₁
Monomers per asymmetric unit	2	2
Water molecules per monomer, n	443	140
Cocrystallized cofactor	NADPH	NADH
Cocrystallized compounds	L-(+)-tartrate	N-hydroxy-N-isopropylloxamate
Unit-cell parameters (Å)	a = 52.205 b = 118.934 c = 62.158 α = 90.00 β = 101.17 γ = 90.00	a = 49.985 b = 105.346 c = 122.357 α = 90.00 β = 90.00 γ = 90.00
Resolution range (Å)	38.807–1.39	79.833–1.8
Number of unique reflections	130,511	78,635
R factor	0.1595	0.2014
R _{free}	0.1866	0.2520
Refinement parameters		
RMSD from ideal		
Bond (Å)	0.0293	0.0210
Angle (°)	2.7444	2.1084
Chirality	0.1858	0.1499
Ramachandran parameters		
Favored	95.85%	94.33%
Allowed	4.15%	5.51%
Outliers	0.00%	0.15%

Crystallographic parameters of solved structures of wild-type Se_KARI and variant Se_KARI^{DDV}.

Table S5. List of Strains and Plasmids

Strain/plasmid	Genotype	Reference
<i>E. coli</i> BL21(DE3)	<i>F ompT gal dcm lon hsdS_B(r_B⁻ m_B⁻) λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7nin5])</i>	Lucigen
pET-22b(+)	<i>PT7, bla, ori pBR322, lacI, C-term 6xHis</i>	Novagen
pETEclivC	<i>PT7::Ec_ilvC, bla, oripBR322, lacI, C-term 6xHis</i>	(1)
pETEclivC ^{6E6}	<i>PT7::Ec_ilvC_coEc^{6E6}, bla, oripBR322, lacI, C-term 6xHis</i>	(1)
pETEclivC ^{P2D1-A1}	<i>PT7::Ec_ilvC_coEc^{P2D1-A1}, bla, oripBR322, lacI, C-term 6xHis</i>	This study
pETSeKARI	<i>PT7::Se_kari_coEc, bla, oripBR322, lacI, C-term 6xHis</i>	This study
pETSeKARI ^{DD}	<i>PT7::Se_kari_coEc^{DD}, bla, oripBR322, lacI, C-term 6xHis</i>	This study
pETSeKARI ^{DDV}	<i>PT7::Se_kari_coEc^{DDV}, bla, oripBR322, lacI, C-term 6xHis</i>	This study
pETLIKARI	<i>PT7::Ll_kari_coEc, bla, oripBR322, lacI, C-term 6xHis</i>	This study
pETLIKARI ^{LPLD}	<i>PT7::Ll_kari_coEc^{LPLD}, bla, oripBR322, lacI, C-term 6xHis</i>	This study
pETLIKARI ^{LRLD}	<i>PT7::Ll_kari_coEc^{LRLD}, bla, oripBR322, lacI, C-term 6xHis</i>	This study
pETLIKARI ^{VPLD}	<i>PT7::Ll_kari_coEc^{VPLD}, bla, oripBR322, lacI, C-term 6xHis</i>	This study
pETLIKARI ^{2G6}	<i>PT7::Ll_kari_coEc^{2G6}, bla, oripBR322, lacI, C-term 6xHis</i>	This study
pETMaKARI	<i>PT7::Ma_kari_coEc, bla, oripBR322, lacI, C-term 6xHis</i>	This study
pETMaKARI ^{DD}	<i>PT7::Ma_kari_coEc^{DD}, bla, oripBR322, lacI, C-term 6xHis</i>	This study
pETAaKARI	<i>PT7::Aa_kari_coEc, bla, oripBR322, lacI, C-term 6xHis</i>	This study
pETAaKARI ^{PLD}	<i>PT7::Aa_kari_coEc^{PLD}, bla, oripBR322, lacI, C-term 6xHis</i>	This study
pETAaKARI ^{PLDA}	<i>PT7::Aa_kari_coEc^{PLDA}, bla, oripBR322, lacI, C-term 6xHis</i>	This study
pETSh_spKARI	<i>PT7::Sh_sp_kari_coEc, bla, oripBR322, lacI, C-term 6xHis</i>	This study
pETSh_spKARI ^{DD}	<i>PT7::Sh_sp_kari_coEc^{DD}, bla, oripBR322, lacI, C-term 6xHis</i>	This study
pETSh_spKARI ^{6E6}	<i>PT7::Sh_sp_kari_coEc^{6E6}, bla, oripBR322, lacI, C-term 6xHis</i>	This study

1. Bastian S, et al. (2011) Engineered ketol-acid reductoisomerase and alcohol dehydrogenase enable anaerobic 2-methylpropan-1-ol production at theoretical yield in *Escherichia coli*. *Metab Eng* 13(3):345–352.

Table S6. All Oligonucleotides Are Listed from 5' to 3' End

Oligonucleotide	Sequence
T7 promoter primer	TAATACGACTCACTATAGG
T7 terminator primer	GCTAGTTATTGCTCAGCGG
Se_DD_for	GGCTTAAGAGAAGGCGACTCTGACTGGAAAACGGCTGAG
Se_DD_rev	CTCAGCCGTTTTCCAGTCAGAGTCGCTTCTCTTAAGCC
Ll_V48NNK_for	CAA TGT TAT CAT TGG TNN KAG GCA CGG AAA ATC TTT TGA T
Ll_V48NNK_rev	ATC AAA AGA TTT TCC GTG CCT MNN ACC AAT GAT AAC ATT G
Ll_R49NNK_for	GTTATCATTGGTGTANNKACGCGAAAATCTTTTG
Ll_R49NNK_rev	CAAAAGATTTTTCCGTGMNNTACACCAATGATAAC
Ll_H50NNK_for	GTTATCATTGGTGTAAAGNNKGGAAAATCTTTTG
Ll_H50NNK_rev	CAAAAGATTTTTCCMNNCCTTACACCAATGATAAC
Ll_G51NNK_for	ATT GGT GTA AGG CAC NNK AAA TCT TTT GAT AAA GCT AAG
Ll_G51NNK_rev	CTT AGC TTT ATC AAA AGA TTT MNN GTG CCT TAC ACC AAT
Ll_K52NNK_for	GGT GTA AGG CAC GGA NNK TCT TTT GAT AAA GCT AAG GAA
Ll_K52NNK_rev	TTC CTT AGC TTT ATC AAA AGA MNN TCC GTG CCT TAC ACC
Ll_S53NNK_for	GTGTAAGGCACGCGAAAANNKTTTGATAAAGCTAAGGA
Ll_S53NNK_rev	TCCTTAGCTTTATCAAAMNNNTTTCCGTGCCTTACAC
Ll_K52NNK_S53NNK_for	GGTCTACCACACGGANNKNNKTTTGATAAAGCTAAG
Ll_K52NNK_S53NNK_rev	CTTAGCTTTATCAAAMNNMNNNTCCGTGTGGTAGACC'
Ll_L48V_for	CACAATGTTATCATTGGTGTACCGCACGGA
Ll_L48V_rev	TCCGTGCGGTACACCAATGATAACATTGTG
Ll_P49R_for	CACAATGTTATCATTGGTCTAAGGCACGGA
Ll_P49R_rev	TCCGTGCCTTAGACCAATGATAACATTGTG
Ma_DD_for	TTACGTCCGAACGATGCTGACTGGGAAAAAGCG
Ma_DD_rev	CGCTTTTCCAGTCAGCATCGTTCGGACGTAA
Aa_test_drive_for	GTGATCGGTCTGCCTCCAGGTNNKATTGGGCTAAAGCAGAA
Aa_test_drive_rev	TTCTGCTTTAGCCCAATCMNNACTGGAGGCAGACCGATCAC
Aa_R84NNK_for	GATTCTGCTGCCAGACGAANNKACGACCGCTGTTTATG
Aa_R84NNK_rev	CATAAACAGCTGGCTGMNNTTCGTCTGGCAGCAGAATC
Sh_sp_DD_for	GCACAAAAGGATGCCGATTGGCAAAAAGCGAC
Sh_sp_DD_rev	GTCGCTTTTGGCCAATCGGCATCCTTTTGTGC

T7 forward and reverse primers for DNA sequencing were provided by Laragen.