#### **Supplementary Information**

# Versatile selective evolutionary pressure using synthetic defect in universal metabolism

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**Supplementary Figure 1. Enzymatic activity assays for CBADH and TBADH variants. a-f**, Initial reaction rates obtained for each enzyme by varying the isopropanol concentration at a fixed concentration of NAD<sup>+</sup> or NADP<sup>+</sup>. A Michaelis-Menten model was fitted in all cases. Data points represent mean values, with error bars showing standard deviation. n=3 biologically independent assays for all substrate concentrations with both enzymes. Source data are provided as a Source Data file. g, h, SDS-PAGE confirming the purity of CBADH<sub>WT</sub>, CBADH<sub>S</sub>, TBADH<sub>WT</sub>, TBADH<sub>S1</sub> and TBADH<sub>S2</sub> used for enzymatic assays. Active, pure proteins were successfully obtained with the described procedure from three independent preparations.



Supplementary Figure 2. Anaerobic cultures of AL, ALS, ALP and ALPS cells containing various NAD and NADP-dependent oxidoreductases. For each E. coli strain, anaerobic growth with (right panels) and without (left panels) acetone supplemented to the culture media was followed. **a.** AL mutant ( $\Delta adhE \Delta ldhA$ ). **b.** ALS mutant ( $\Delta adhE$ Δ*ldhA* Δ*sthA*). **c.** ALP mutant (Δ*adhE* Δ*ldhA* Δ*pntA*). **d.** ALPS mutant (Δ*adhE* Δ*ldhA* Δ*pntA* Δ*sthA*). Anaerobic growth of cells with at least one active transhydrogenase was recovered upon transformation of either an NAD or an NADPdependent oxidoreductase. However, in the case of ALPS cells, where both transhydrogenase genes were deleted, only the NAD-dependent enzyme restored anaerobic growth, indicating that metabolic complementation by NADPdependent enzymes is mediated by transhydrogenases. Data points of growth curves represent mean values, with error bars showing standard deviation; n=3 biologically independent cultures for all timepoints of growth curves. Source data are provided as a Source Data file.



Supplementary Figure 3. Overview of mutations in  $\mathsf{TBADH}_{S1}$  and  $\mathsf{TBADH}_{S2}$ . Absolute positions are shown above each sequence. Point mutations are shown in red.  $\mathsf{TBADH}_{S1}$  contains substitutions at all positions targeted for saturation mutagenesis (198, 199, 200 and 218).  $\mathsf{TBADH}_{S2}$  contains substitutions at all targeted positions and a duplication (shown in pink) of residues 191-241 inserted between positions 241 and 242 of the original sequence. Positions relative to  $\mathsf{TBADH}_{WT}$  are shown below  $\mathsf{TBADH}_{S2}$  with a prime symbol. The insertion contains a second copy of the targeted positions, two of which were also substituted. These were positions 249 and 251, corresponding to positions 198' and 200' of  $\mathsf{TBADH}_{WT}$ .

### **a.** $CBADH_s$



### **b**. TBADH<sub>S1</sub>



Supplementary Figure 4. Density for the NAD<sup>+</sup> cofactor in the crystallographic maps of CBADH<sub>s</sub> and TBADH<sub>S1</sub>. Left panels display the density for the nicotinamide part of the cofactor, while right panels show the density for the adenine part. The density for the cofactor was stronger in CBADH<sub>S</sub> (**a**) than in TBADH<sub>S1</sub> (**b**), due to partial occupancy of the cofactor in the latter.  $2F_o$ - $F_c$  maps are shown at sigma level of 1 for CBADH<sub>S</sub> and 0.8 for TBADH<sub>S1</sub>. The NAD+ cofactors were refined with an approximate occupancy of 80%.



**Supplementary Figure 5. Enzymatic activity assays for MsIRED variants. a-c**, Initial reaction rates obtained for each enzyme by varying the 2-methyl-1-pyrroline concentration at a fixed concentration of NADH or NADPH. A Michaelis-Menten model equation modified to account for substrate inhibition was fitted in all cases. Data points represent mean values, with error bars showing standard deviation. n=3 biologically independent assays for all substrate concentrations with both enzymes. Source data are provided as a Source Data file. **d**, SDS-PAGE confirming the purity of wild-type MsIRED, MsIRED<sub>C</sub> and MsIRED<sub>S</sub> used for enzymatic assays. Active, pure proteins were successfully obtained with the described procedure from three independent preparations.



**Supplementary Figure 6. Enzymatic activity assays for EntNfsB variants. a-e**, Initial reaction rates obtained for each enzyme by varying nitroaromatic substrate (4-NBA, 2-NBA or 4-NBALC) concentration at a fixed concentration of NADH. A Michaelis-Menten model was fitted in all cases. Data points represent mean values, with error bars showing standard deviation. n=3 biologically independent assays for all substrate concentrations with both enzymes. Parameters that could not be determined due to a too low activity are indicated with an asterisk (\*). Source data are provided as a Source Data file. f, SDS-PAGE confirming the purity of wild-type EntNfsB, EntNfsB<sub>S1</sub> and EntNfsB<sub>S2</sub> used for enzymatic assays. Active, pure proteins weresuccessfully obtained with the described procedure from three independent preparations.



**Supplementary Figure 7. Characteristic <sup>1</sup>H-NMR signals used to quantify each of the analyzed metabolites.** Protons contributing to the characteristic signal of each metabolite are highlighted in red.



**Supplementary Figure 8. Uncropped gels.** The shown gels correspond, from left to right, to Supplementary Fig. 1g, Supplementary Fig. 1h, Supplementary Fig. 5d and Supplementary Fig. 6f.

### Supplementary Table 1. <sup>1</sup>H-NMR analysis of fermentation broths. Mean values and standard deviations are shown for each metabolite.

Strain	Plasmid	Encoded enzyme	[Substrate] (mM)	[Product] (mM)	[Ethanol] (mM)	[Lactate] (mM)	[Succinate] (mM)	[Acetate] (mM)	[Formate] (mM)
BW25113	-	-	-	-	15.4 ± 0.5	7.1 ± 0.4	2.3 ± 0.1	14.5 ± 0.5	26.2 ± 0.8
AL	pLS1	ADHE	-	-	16.9 ± 0.4	0	2.1 ± 0.1	14.3 ± 0.3	25.6 ± 0.6
AL	pUC19	-	3.2 ± 0.02 (Acetoin)	5.9 ± 0.1 (2,3-butanediol)	0	0	1.2 ± 0.03	6.7 ± 0.1	4.0 ± 0.1
AL	pLS2	BDHA	0 (Acetoin)	8.8 ± 0.2 (2,3-butanediol)	0	0	0.6 ± 0.03	7.5 ± 0.3	4.5 ± 0.1
AL	pLS3	BUDC	0.1 ± 0.002 (Acetoin)	8.5 ± 0.1 (2,3-butanediol)	0	0	0.7 ± 0.1	7.9 ± 0.2	5.7 ± 0.2
AL	pLS12	TADH	0 (Cyclohexanone)	9.1 ± 0.2 (Cyclohexanol)	0	0	0.8 ± 0.05	9.9 ± 0.1	7.9 ± 0.2
AL	pLS12	TADH	4.3 ± 0.1 (3-methylcyclohexanone)	3.8 ± 0.1 (3-methylcyclohexanol)	0	0	0.4 ± 0.01	3.7 ± 0.1	3.1 ± 0.04
AL	pLS6	CBADH <sub>WT</sub>	0.2 ± 0.008 (Acetone)	10.8 ± 0.2 (Isopropanol)	0	0	1.2 ± 0.02	8.3 ± 0.2	6.1 ± 0.1
AL	pLS10_3	CBADH₅	0.6 ± 0.1 (Acetone)	12.5 ± 0.8 (Isopropanol)	0	0	1.3 ± 0.1	12.8 ± 0.9	9.9 ± 0.7
ALPS	pLS69	TBADH <sub>WT</sub>	8.4 ± 0.2 (Acetone)	0 (Isopropanol)	0	0	0	0	0
ALPS	pLS73_2	TBADH₅1	0.1 ± 0.01 (Acetone)	14.8 ± 0.3 (Isopropanol)	0	0	1.6 ± 0.1	15.2 ± 0.4	11.2 ± 0.7
ALPS	pLS73_1	TBADH₅2	0.1 ± 0.04 (Acetone)	15.4 ± 0.4 (Isopropanol)	0	0	1.7 ± 0.1	17.2 ± 0.5	12.5 ± 0.3
AL	pLS130	MsIRED <sub>wt</sub>	5.6 ± 0.1 (2-methyl-1-pyrroline)	6.5 ± 0.1 (2-methylpyrrolidine)	0	0	1.1 ± 0.1	4.3 ± 0.3	2.1 ± 0.1
AL	pLS131	MsIRED <sub>c</sub>	3.3 ± 0.009 (2-methyl-1-pyrroline)	9.3 ± 0.03 (2-methylpyrrolidine)	0	0	1.7 ± 0.01	10.4 ± 0.2	8.5 ± 0.1
AL	pLS133_1	MsIREDs	0.8 ± 0.1 (2-methyl-1-pyrroline)	13.3 ± 0.2 (2-methylpyrrolidine)	0	0	1.9 ± 0.1	15.6 ± 0.3	13.6 ± 0.7
AL	pLS168	EntNfsBwr	0 (4-nitrobenzoic acid)	0.4 ± 0.008 (4-aminobenzoic acid)	0	0	0.3 ± 0.02	2.9 ± 0.1	2.3 ± 0.2

AL	pLS168	EntNfsB <sub>WT</sub>	8.0 ± 0.02 (2-nitrobenzoic acid)	0 (2-aminobenzoic acid)	0	0	0	0	0
AL	pLS168	EntNfsBwr	0.2 ± 0.02 (4-nitrobenzyl alcohol)	0.5 ± 0.03 (4-aminobenzyl alcohol)	0	0	0.5 ± 0.1	6.3 ± 0.1	5.8 ± 0.1
AL	pLS169_1	EntNfsB₅1	3.2 ± 0.4 (2-nitrobenzoic acid)	3.5 ± 0.04 (2-aminobenzoic acid)	0	0	1.2 ± 0.1	12 ± 0.4	8.9 ± 0.4
AL	pLS169_3	EntNfsB <sub>s2</sub>	0 (4-nitrobenzyl alcohol)	0.8 ± 0.3 (4-aminobenzyl alcohol)	0	0	0.4 ± 0.1	6.1 ± 0.9	5.0 ± 1.2
BW25113	pStA212	-	0 (Acetone)	0 (Isopropanol)	0	0	0	0	0
BW25113	pLS60_1	AtoB/AtoA /AtoD/ ADC/CBA DHwt	12.0 ± 3.6 (Acetone)	62.3 ± 5.1 (Isopropanol)	0	0.8 ± 0.3	2.6 ± 1.6	3.5 ± 1.7	3.6 ± 2.3

Supplementary Table 2. Summary of kinetic parameters of wild-type and evolved enzymes. Parameters that could not be determined due to a too low activity are indicated with an asterisk (\*). Cases for which substrate inhibition was not observed are indicated with a hyphen (-).

Enzyme	Substrate	Cosubstrate	Km	<b>k</b> cat (min⁻¹)	k <sub>cat</sub> /K <sub>m</sub>	K <sub>i</sub> (mM)
					204.4 ± 9.1	
CBADHwt	Isopropanol	NADP⁺	5.80 ± 0.25 mM	1185.6 ± 14.0	min <sup>-1</sup> mM <sup>-1</sup>	-
					19.0 ± 2.6	
CBADHs	Isopropanol	NAD⁺	17.49 ± 2.30 mM	333.0 ± 12.8	min <sup>-1</sup> mM <sup>-1</sup>	-
					4.2 ± 0.25	
TBADH <sub>WT</sub>	Isopropanol	NADP⁺	119.4 ± 6.9 mM	498.0 ± 9.2	min <sup>-1</sup> mM <sup>-1</sup>	-
					29.8 ± 4.6	
TBADH <sub>S1</sub>	Isopropanol	NAD⁺	3.74 ± 0.54 mM	111.5 ± 5.7	min⁻¹mM⁻¹	-
					10.8 ± 1.4	
TBADH <sub>S2</sub>	Isopropanol	NAD⁺	22.07 ± 2.70 mM	238.5 ± 8.7	min <sup>-1</sup> mM <sup>-1</sup>	-
					4.2 ± 0.2	
TBADH <sub>S2</sub>	Isopropanol	NADP <sup>+</sup>	55.15 ± 2.57 mM	231.4 ± 3.7	min <sup>-1</sup> mM <sup>-1</sup>	-
	2-methyl-1-					
MsIRED <sub>wt</sub>	pyrroline	NADPH	3.56 ± 0.55 mM	89.8 ± 7.3	-	18.05 ± 2.69
	2-methyl-1-					
MsIREDc	pyrroline	NADH	34.06 ± 11.61 mM	161.2 ± 47.5	•	4.94 ± 1.64
	2-methyl-1-		10.57 . 1.10	70.4 . 40.0		44.40.0.40
MSIREDs	pyrroline	NADH	19.57 ± 4.43 mM	78.1 ± 13.2	-	11.42 ± 2.49
	4-				204.4 . 52.0	
EntNfoD	nitropenzoic		$0.141 \pm 0.022 \text{ mM}$	457+00	$324.1 \pm 53.2$	
Entimisewi		NADH	0.141 ± 0.022 milli	45.7 ± 2.5	min mivi ·	-
EntNfcBur	4-nitrobenzyi		*	*	*	
EIIUNISDWI	alconor	NADIT				-
	4- nitrobenzoic				815 7 + 83 9	
EntNfsBs1	acid	NADH	0 051 + 0 0051 mM	416+10	$min^{-1}mM^{-1}$	_
	2-					
	nitrobenzoic				35.1 ± 5.1	
EntNfsB <sub>S1</sub>	acid	NADH	0.81 ± 0.113 mM	28.4 ± 1.3	min <sup>-1</sup> mM <sup>-1</sup>	-
	4-nitrobenzyl				184.5 ± 33.1	
EntNfsB <sub>s2</sub>	alcohol	NADH	1.11 ± 0.19 mM	205.0 ± 11.0	min⁻¹mM⁻¹	-
					19.18 ± 2.46	
CBADHwt	NADP⁺	Isopropanol	55.91 ± 6.58 µM	1072.5 ± 55.17	min⁻¹µM⁻¹	-
					0.31 ± 0.05	
CBADHs	NAD⁺	Isopropanol	934.4 ± 136.2 μM	290.58 ± 23.17	min⁻¹µM⁻¹	-
					8.51 ± 1.81	
TBADHwt	NADP⁺	Isopropanol	56.67 ± 11.63 µM	482.19 ± 25.9	min⁻¹µM⁻¹	-
					105.6 ± 17.1	
TBADH <sub>S1</sub>	NAD⁺	Isopropanol	1.03 ± 0.16 mM	109.0 ± 4.0	min⁻¹mM⁻¹	-
					2.11 ± 0.20	
TBADH <sub>S2</sub>	NAD <sup>+</sup>	Isopropanol	104.4 ± 9.68 μM	220.12 ± 6.17	min <sup>-1</sup> µM <sup>-1</sup>	-
					0.97 ± 0.13	
TBADH <sub>S2</sub>	NADP⁺	Isopropanol	240.7 ± 29.4 μM	234.68 ± 10.57	min⁻¹µM⁻¹	-

		2-methyl-1-			$2.30 \pm 0.36$	
MsIRED <sub>wt</sub>	NADPH	pyrroline	24.67 ± 3.68 µM	56.65 ± 2.9	min⁻¹µM⁻¹	-
		2-methyl-1-			0.64 ± 0.075	
MsIREDc	NADH	pyrroline	23.66 ± 2.5 μM	15.05 ± 0.79	min⁻¹µM⁻¹	-
		2-methyl-1-			1.06 ± 0.1	
MsIREDs	NADH	pyrroline	19.9 ± 1.71 μM	21.10 ± 0.84	min⁻¹µM⁻¹	-
		4-nitrobenzoic			1.56 ± 0.076	
EntNfsBwr	NADH	acid	29.78 ± 1.3 μM	46.48 ± 1.03	min⁻¹µM⁻¹	-
		4-nitrobenzyl			9.71 ± 1.39	
EntNfsBwr	NADH	alcohol	30.65 ± 3.96 µM	297.7 ± 18.23	min⁻¹µM⁻¹	-
		4-nitrobenzoic			1.45 ± 0.10	
EntNfsB <sub>S1</sub>	NADH	acid	28.90 ± 1.87 µM	41.88 ± 1.14	min⁻¹µM⁻¹	-
		2-nitrobenzoic			1.08 ± 0.15	
EntNfsB <sub>S1</sub>	NADH	acid	26.37 ± 3.49 µM	28.57 ± 1.20	min⁻¹µM⁻¹	-
		4-nitrobenzyl			7.59 ± 0.78	
EntNfsB <sub>S2</sub>	NADH	alcohol	28.86 ± 2.73 µM	219.16 ± 9.0	min⁻¹µM⁻¹	-

#### Supplementary Table 3. Plasmids used in this work.

Plasmid	Description	Antibiotic resistance	Source
pUC19	High copy expression vector   (pMB1 origin of replication)   with lacZα – Negative   control for anaerobic growth   complementation   experiments	AmpR	Heap Laboratory (Norrander, Kempe, and Messing, 1983)
pJET1.2	Cloning vector	AmpR	Invitrogen
pET28a	Bacterial expression vector with 6xHis-tag	KanR	Invitrogen
рМАК705	<i>E. coli</i> vector for directed mutagenesis by allele exchange	CmR	Heap Laboratory (Hamilton, <i>et al.</i> , 1989)
pStA0	Start-Stop Assembly Level 0 storage vector	AmpR	Heap Laboratory (Taylor <i>et al</i> ., 2019)
pStA1AB	Start-Stop Assembly Level 1 vector (A and B fusion sites)	TetR	Heap Laboratory (Taylor <i>et al</i> ., 2019)
pStA1BC	Start-Stop Assembly Level 1 vector (B and C fusion sites)	TetR	Heap Laboratory (Taylor <i>et al</i> ., 2019)
pStA1CD	Start-Stop Assembly Level 1 vector (C and D fusion sites)	TetR	Heap Laboratory (Taylor <i>et al</i> ., 2019)
pStA1DE	Start-Stop Assembly Level 1 vector (D and E fusion sites)	TetR	Heap Laboratory (Taylor <i>et al</i> ., 2019)
pStA1EZ	Start-Stop Assembly Level 1 vector (E and Z fusion sites)	TetR	Heap Laboratory (Taylor <i>et al.</i> , 2019)
pGT323	pStA0 containing BBa_J23100 promoter	AmpR	Heap Laboratory (Taylor <i>et al.</i> , 2019)
pGT324	pStA0 containing BBa_J23102 promoter	AmpR	Heap Laboratory (Taylor <i>et al.</i> , 2019)
pGT326	pStA0 containing BBa_J23107 promoter	AmpR	Heap Laboratory (Taylor <i>et al.</i> , 2019)
pGT327	pStA0 containing BBa_J23116 promoter	AmpR	Heap Laboratory (Taylor <i>et al.</i> , 2019)
pGT328	pStA0 containing BBa_J23113 promoter	AmpR	Heap Laboratory (Taylor <i>et al.</i> , 2019)
pGT336	pStA0 containing BBa_J23118 promoter	AmpR	Heap Laboratory (Taylor <i>et al.</i> , 2019)
pGT330	pStA0 containing RBSc13	AmpR	Heap Laboratory (Taylor <i>et al.</i> , 2019)
pGT331	pStA0 containing RBSc33	AmpR	Heap Laboratory (Taylor <i>et al</i> ., 2019)
pGT332	pStA0 containing RBSc44	AmpR	Heap Laboratory (Taylor <i>et al.</i> , 2019)
pGT333	pStA0 containing RBSc58	AmpR	Heap Laboratory (Taylor <i>et al.</i> , 2019)
pGT334	pStA0 containing RBSc36	AmpR	Heap Laboratory (Taylor <i>et al.</i> , 2019)

pGT335	pStA0 containing RBSc42	AmpR	Heap Laboratory (Taylor <i>et</i>
pGT337	pStA0 containing Terminator 1 (L3S2P55)	AmpR	Heap Laboratory (Taylor <i>et al.</i> , 2019)
pGT338	pStA0 containing Terminator 2 (L3S2P21)	AmpR	Heap Laboratory (Taylor et al., 2019)
pGT339	pStA0 containing Terminator 3 (ECK120033737)	AmpR	Heap Laboratory (Taylor <i>et al.</i> , 2019)
pGT340	pStA0 containing Terminator 4 (ECK1200196000)	AmpR	Heap Laboratory (Taylor et al., 2019)
pStA212	Start-Stop Assembly Level 2 vector (fusion sites 1 and 2) – <b>Negative control</b> for isopropanol pathway	KanR	Heap Laboratory (Taylor <i>et</i> al., 2019)
pLS1	pUC19 containing sequence encoding ADHE of <i>E. coli</i> BW25113 (AIN31697.1) – <b>Positive control</b> for anaerobic growth experiments	AmpR	This work
pLS2	pUC19 containing sequence encoding BDHA of <i>Bacillus</i> <i>subtilis</i> 168 (CAB12443.1)	AmpR	This work
pLS3_1	pJET1.2 storage plasmid containing sequence encoding BUDC of <i>Klebsiella</i> <i>pneumoniae</i> (AAC78679.1)	AmpR	This work
pLS3	pUC19 containing sequence encoding BUDC of <i>Klebsiella</i> <i>pneumoniae</i> (AAC78679.1)	AmpR	This work
pLS6	pUC19 containing sequence encoding CBADH of <i>Clostridium beijerinckii</i> (AAA23199.2)	AmpR	This work
pLS10	Library equivalent to pLS6 with saturation mutagenesis of amino acid positions 198, 199, 200 and 218 of CBADH (CBADH <sub>Lib</sub> )	AmpR	This work
pLS10_3	Plasmid from library pLS10 encoding variant CBADH <sub>s</sub> (G198D, S199Y, R200R, Y218P)	AmpR	This work
pLS12_1	pJET1.2 storage plasmid containing sequence encoding TADH of <i>Thermus</i> sp. ATN-1 (ACD50896.1)	AmpR	This work

pLS12	pUC19 containing sequence		
	encoding TADH of Thermus	AmpR	This work
	sp. ATN-1 (ACD50896.1)		
pLS39	pMAK705 with 500 bp		
	upstream and 500 bp		<b>_</b>
	downstream of sthA from E.	CmR	I his work
	coli		
pLS40	pMAK705 with 500 bp		
	upstream and 500 bp		<b>-</b>
	downstream of pntA from E.	CmR	I his work
	coli		
pLS46	pStA0 containing atoB gene	A	The second
	of <i>E. coli</i> (AAC75284.1)	AmpR	I his work
pLS47	pStA0 containing atoD gene	A	This is a
	of <i>E. coli</i> (AAC75281.1)	Атрк	I NIS WORK
pLS48	pStA0 containing atoA gene		
	of <i>E. coli</i> (AAC75282.1)	Атрк	I NIS WORK
pLS49	pStA0 containing adc gene of		
	Clostridium acetobutylicum	AmpR	This work
	(AAA63761.1)		
pLS50	pStA0 containing cbadh gene		
	of Clostridium beijerinckii	AmpR	This work
	(AAA23199.2)		
pLS53	pStA1AB containing full		
	transcription unit coding for		
	acetyl-CoA acetyltransferase	TetR	This work
	from E. coli (atoB) (library of	Telly	
	six promoters and six RBS,		
	Terminator 1)		
pLS54	pStA1BC containing full		
	transcription unit coding for		
	acetate CoA-transferase		
	subunit alpha from E. coli	TetR	This work
	(atoD) (library of six		
	promoters and six RBS,		
	Terminator 2)		
pLS55	pStA1CD containing full		
	transcription unit coding for		
		TUD	<b>T</b> 1.1
	(stat) (library of sign	IEIK	I NIS WORK
	(aloA) (library of six		
	Torminator 2)		
nl 956	nStA1DE containing full		
proso	transcription unit coding for		
	from Clostridium	TetR	This work
	acetobutylicum (adc) (library	Tour	
	of six promoters and six RBS		
	Terminator 4)		
	,		

pLS57	pStA1EZ containing full		
	transcription unit coding for		
	NADP-dependent		
	isopropanol dehydrogenase		
	from Clostridium beijerinckii	TetR	This work
	(cbadh) (library of six		
	promoters and six RBS.		
	Terminator 1)		
nl \$60	nStA212 containing		
P	combinatorial isopropanol	KanR	This work
	pathway library MP	T CONT C	
pLS60_1	Plasmid from library MPLin		
P====	(nl S60) encoding		
	isopropanol pathway variant	KanR	This work
	MP <sub>64</sub>		
nl S60_2	Dathway from library MD		
p2000_2			
	isopropapol pathway variant	KanR	This work
	WIFS2		
pL303	piviAK705 with 500 bp		
	upstream and 500 bp	CmR	This work
	downstream of IanA from E.		
	COII		
pLS67	pJE11.2 storage plasmid		
	containing sequence		
	encoding TBADH of	AmpR	This work
	Thermoanaerobacter brockii		
	(CAA46053.1)		
pLS69	pUC19 containing sequence		
	encoding TBADH of	AmpR	This work
	Thermoanaerobacter brockii		
	(CAA46053.1)		
pLS73	Library equivalent to pLS69		
	with saturation mutagenesis		
	of amino acid positions 198,	AmpR	This work
	199, 200 and 218 of TBADH		
	(TBADH <sub>Lib</sub> )		
pLS73_1	Plasmid from library pLS73		
	encoding variant TBADHs2		
	(G198H, S199R, R200A,	AmpR	This work
	Y218M and a 153 bp	, unpre	
	insertion between residues		
	241 and 242)		
pLS73_2	Plasmid from library pLS73		
	encoding variant TBADHs1	AmnR	This work
	(G198S, S199K, R200P,		
	Y218V)		
pLS90	pET28a containing sequence		
	encoding TBADH <sub>S2</sub> with N-	KanR	This work
	terminal 6xHis-tag		

pLS91	pET28a containing sequence		
	encoding TBADH <sub>S1</sub> with N-	KanR	This work
	terminal 6xHis-tag		
pLS97	pET28a containing sequence		
	encoding wild-type TBADH	KanR	This work
	with N-terminal 6xHis-tag		
pLS98	pFT28a containing sequence		
P	encoding CBADHs with N-	KanR	This work
	terminal 6xHis-tag		
nl \$99	pET28a containing gene		
pross	encoding wild-type CBADH	KanR	This work
	with N-terminal 6xHis-tag		
nl \$120	n IET1 2 storago plasmid		
pE3129			
	containing sequence	Arra D	This words
		Ашрк	
	Myxococcus stipitatus		
1.0.400	(AGC43099.1)		
pLS130	pUC19 containing sequence		
	encoding MSIRED of	AmpR	This work
	Myxococcus stipitatus		
	(AGC43099.1)		
pLS131	pUC19 containing sequence		
	encoding MsIRED <sub>c</sub> variant of		
	Myxococcus stipitatus	AmpR	This work
	MSIRED (N32E, R33Y,		
	T34E, K37R, L67I, T71V)		
pLS133	Library equivalent to pLS131		
	with saturation mutagenesis		
	of amino acid positions 32,	AmpR	I his work
	33, 34 and 37 of MSIRED		
	(MSIRED <sub>Lib</sub> )		
pLS133_1	Plasmid from library pLS133		I his work
	encoding variant MsIREDs	AmpR	
	(N32E, R33V, T34R, K37R)		
pLS161	pUC19 containing sequence		I his work
	encoding wild-type MSIRED	AmpR	
1.0.100	with C-terminal 6xHis-tag		-
pLS162	pUC19 containing sequence		I his work
	encoding MSIRED <sub>C</sub> with C-	AmpR	
	terminal 6xHis-tag		
pLS164	pUC19 containing sequence		I his work
	encoding MSIREDs with C-	AmpR	
	terminal 6xHis-tag		
pLS168_1	pJE11.2 storage plasmid		
	containing sequence		
	encoding EntNfsB of	AmpR	
	Enterobacter cloacae		
	(AAA62801.1)		
pLS168	pUC19 containing sequence	AmpR	This work
	encoding EntNfsB of	r.	

	Enterobacter cloacae (AAA62801.1)		
pLS169	Library equivalent to pLS168 with saturation mutagenesis of amino acid positions 40, 41, 68 and 124 of EntNfsB (EntNfsB <sub>Lib</sub> )	AmpR	This work
pLS169_1	Plasmid from library pLS133 encoding variant EntNfsB <sub>S1</sub> (S40A, T41I, Y68Y, F124A)	AmpR	This work
pLS169_3	Plasmid from library pLS133 encoding variant EntNfsB <sub>S2</sub> (S40S, T41L, Y68L, F124L)	AmpR	This work
pLS180	pUC19 containing sequence encoding EntNfsB with C- terminal 6xHis-tag	AmpR	This work
pLS181	pUC19 containing sequence encoding EntNfsB <sub>S1</sub> with C- terminal 6xHis-tag	AmpR	This work
pLS182	pUC19 containing sequence encoding EntNfsB <sub>S2</sub> with C- terminal 6xHis-tag	AmpR	This work

Supplementary Table 4. Oligonucleotides used in this work. Restriction sites are highlighted in green.

Purpose	Template	Oligo ID	Description	Sequence (5' – 3')
Amplification	pUC19	oligoLS315	Fw - Bbsl	TCTCTGAAGACCCTAAGGATCCCCGGGTACC
of pUC19 for				
Golden Gate				
Assembly				
Amplification	pUC19	oligoLS314	Rv - Bbsl	TCTCT <mark>GAAGAC</mark> TCCATGTGTTCGTACCTCCTGC
of pUC19 for				ATG
Golden Gate				
Assembly				
Construction	adhE of E. coli	oligoLS19	Fw - Sphl	CCGTTC <mark>GCATGC</mark> AGGAGGTACGAACACATGGC
of pLS1	BW25113			TGTTACTAATGT
Construction	adhE of E. coli	oligoLS20	Rv - BamHI	GCTGAA <mark>GGATCC</mark> TTAAGCGGATTTTTTCG
of pLS1	BW25113			
Verification	adhE of E. coli	oligoLS1	Fw - internal	CCTGTGGTGTTCTGTCTG
of adhE KO	BW25113			
Verification	adhE of E. coli	oligoLS2	Rv - internal	TAGATTTCGGAATACCCA
of adhE KO	BW25113			
Verification	adhE of E. coli	oligoLS3	Fw - external	GGCGAAAAGCGATGCTG
of adhE KO	BW25113			
Verification	adhE of E. coli	oligoLS4	Rv - external	CGGTGGGAAGGTGTTCTGC
of adhE KO	BW25113			
Verification	ldhA of E. coli	oligoLS5	Fw - internal	GCCGCCCGGTGCTGGAAG
of IdhA KO	BW25113			
Verification	ldhA of E. coli	oligoLS6	Rv - internal	GGCGACGGAATACGTCAT
of <i>ldhA</i> KO	BW25113			
Verification	ldhA of E. coli	oligoLS7	Fw - external	GAAGGTTGCGCCTACACT
of IdhA KO	BW25113			
Verification	ldhA of E. coli	oligoLS8	Rv - external	CACCAAAGCTGATTTCTG
of <i>ldhA</i> KO	BW25113			
Construction	Sequence encoding	oligoLS23	Fw - Sphl	CCGTTC <mark>GCATGC</mark> AGGAGGTACGAACACATGAA
of pLS2	BDHA of Bacillus			GGCAGCAAGATG
	subtilis 168			
Construction	Sequence encoding	oligoLS24	Rv - BamHl	GCTGAA <mark>GGATCC</mark> TTAGTTAGGTCTAACAAGGAT
of pLS2	BDHA of Bacillus			TTTGACT
	subtilis 168			
Construction	Sequence encoding	oligoLS87	Fw - Sphl	GTTC <mark>GCATGC</mark> ATTCGGATCTATACAGATAAGGA
of pLS6	CBADH of Clostridium			GAAAGAGATGAAAGGCTTTGCCATGCTGGG
	beijerinckii			
Construction	Sequence encoding	oligoLS88	Rv - BamHI	CTTCCAT <mark>GGATCC</mark> TCACTATTAGAGGATAACTA
of pLS6	CBADH of Clostridium			CGGCC
	beijerinckii			
Construction	Sequence encoding	oligoLS112	Rv	CTTGGCGGCCTCAACGCAAATAGGNNNNNNN
of pLS10	CBADH of Clostridium			NGACACCAATAATCCGACCTGC
library	beijerinckii			
(CBADH <sub>Lib</sub> )				

Construction	Sequence encoding	oligoLS113	Fw	TTCTACGGCGCGACCGACATTCTGAATNNNAAA
of pLS10	CBADH of Clostridium			AATGGCCATATTGTGGAC
library	beijerinckii			
(CBADH <sub>Lib</sub> )				
Construction	500 bp upstream and	oligoLS208	Fw - BamHI	TTCAGCGGATCCAATGTATCTGCATGAAGCACA
of pLS39	downstream of <i>sthA</i>	J. J		GACCCACCAGTTACTGG
Construction	500 bp upstream and	oligoLS212	Rv	AACAGGTAAGCCCTACCATGTAAAACTTTATCG
of pLS39	downstream of sthA	-		AAATGGCCATCCATTCTTGCGCGG
Construction	500 bp upstream and	oligoLS213	Fw	GCCATTTCGATAAAGTTTTACATGGTAGGGCTT
of pLS39	downstream of sthA			ACCTGTTCTTATACATAAAAGCAACAGAATGG
Construction	500 bp upstream and	oligoLS209	Rv - HindIII	TTCAGCAAGCTTCATTAAACCGCTCTCATCAAC
of pLS39	downstream of sthA			CATGGTCAGACCCAGTTCG
Verification	sthA of E. coli	oligoLS194	Fw - internal	GATGGAACAAAATTTTCAGCGTGCC
of sthA KO	BW25113			
Verification	sthA of E. coli	oligoLS193	Rv - internal	ATAGTAATAGGTTCCGGCCC
of sthA KO	BW25113			
Verification	sthA of E. coli	oligoLS195	Fw - external	CAGGCAATGGGTTTCTGTTTTG
of sthA KO	BW25113			
Verification	sthA of E. coli	oligoLS196	Rv - external	CGAACTGGGTCTGACCATGGTTGATGAGAGCG
of sthA KO	BW25113			GTTTAATG
Construction	500 bp upstream and	oligoLS216	Fw - BamHI	TTCAGC <mark>GGATCC</mark> GAAACGACCAGAGCCGCCAG
of pLS40	downstream of pntA			GTTCA
Construction	500 bp upstream and	oligoLS217	Rv	CCGATGGAAGGGAATATCATGTAAGGGGTAAC
of pLS40	downstream of pntA			ATATGTCTGGAGGATTAGTTACAGCTGCATACA
				TTGTTGCCGC
Construction	500 bp upstream and	oligoLS219	Fw	CCAGACATATGTTACCCCTTACATGATATTCCC
of pLS40	downstream of pntA			TTCCATCGGTTTTATTGATG
Construction	500 bp upstream and	oligoLS218	Rv - HindIII	TTCAGCAAGCTTCAGGAGGGTGTTCTTAAGCTT
of pLS40	downstream of pntA			CATAAAAATAATCCTTCGCCTTGCGCAAA
Verification	pntA of E. coli	oligoLS223	Fw - internal	GTGCTCCGACAACAATAATCC
of <i>pntA</i> KO	BW25113			
of <i>pntA</i> KO Verification	BW25113 pntA of E. coli	oligoLS224	Rv - internal	TGATGGTGATTGGTGCGGGTG
of <i>pnt</i> A KO Verification of <i>pnt</i> A KO	BW25113 <i>pntA</i> of <i>E. coli</i> BW25113	oligoLS224	Rv - internal	TGATGGTGATTGGTGCGGGTG
of <i>pntA</i> KO Verification of <i>pntA</i> KO Verification	BW25113 pntA of E. coli BW25113 pntA of E. coli	oligoLS224 oligoLS216	Rv - internal Fw - external	TGATGGTGATTGGTGCGGGTG GAAACGACCAGAGCCGCCAGGTTCA
of pntA KO Verification of pntA KO Verification of pntA KO	BW25113 <i>pntA</i> of <i>E. coli</i> BW25113 <i>pntA</i> of <i>E. coli</i> BW25113	oligoLS224 oligoLS216	Rv - internal Fw - external	TGATGGTGATTGGTGCGGGTG GAAACGACCAGAGCCGCCAGGTTCA
of pntA KO Verification of pntA KO Verification of pntA KO Verification	BW25113 pntA of E. coli BW25113 pntA of E. coli BW25113 pntA of E. coli	oligoLS224 oligoLS216 oligoLS221	Rv - internal Fw - external Rv - external	TGATGGTGATTGGTGCGGGTG GAAACGACCAGAGCCGCCAGGTTCA TTTGCGCAAGGCGAAGGATTATTTTTATGAAGC
of pntA KO Verification of pntA KO Verification of pntA KO Verification of pntA KO	BW25113 pntA of E. coli BW25113 pntA of E. coli BW25113 pntA of E. coli BW25113	oligoLS224 oligoLS216 oligoLS221	Rv - internal Fw - external Rv - external	TGATGGTGATTGGTGCGGGTG GAAACGACCAGAGCCGCCAGGTTCA TTTGCGCAAGGCGAAGGATTATTTTATGAAGC
of pntA KO Verification of pntA KO Verification of pntA KO Verification of pntA KO Construction	BW25113 pntA of E. coli BW25113 pntA of E. coli BW25113 pntA of E. coli BW25113 atoB of E. coli	oligoLS224 oligoLS216 oligoLS221 oligoLS230	Rv - internal Fw - external Rv - external Fw - <i>Bsal</i>	TGATGGTGATTGGTGCGGGTG   GAAACGACCAGAGCCGCCAGGTTCA   TTTGCGCAAGGCGAAGGATTATTTTTATGAAGC   AAGGGGTTGGTCTCCATGAAAA
of pntA KO Verification of pntA KO Verification of pntA KO Verification of pntA KO Construction of pLS46	BW25113 pntA of E. coli BW25113 pntA of E. coli BW25113 pntA of E. coli BW25113 atoB of E. coli BW25113	oligoLS224 oligoLS216 oligoLS221 oligoLS230	Rv - internal Fw - external Rv - external Fw - <i>Bsal</i>	TGATGGTGATTGGTGCGGGTG   GAAACGACCAGAGCCGCCAGGTTCA   TTTGCGCAAGGCGAAGGATTATTTTTATGAAGC   AAGGGGTTGGTCTCATGTGCTCTTCGATGAAAA   ATTGTGTCATCGTCAGTGCGGTACG
of pntA KO Verification of pntA KO Verification of pntA KO Verification of pntA KO Construction of pLS46 Construction	BW25113 pntA of E. coli BW25113 pntA of E. coli BW25113 pntA of E. coli BW25113 atoB of E. coli BW25113 atoB of E. coli	oligoLS224 oligoLS216 oligoLS221 oligoLS230 oligoLS231	Rv - internal Fw - external Rv - external Fw - <i>Bsal</i> Rv - <i>Bsal</i>	TGATGGTGATTGGTGCGGGTG   GAAACGACCAGAGCCGCCAGGTTCA   TTTGCGCAAGGCGAAGGATTATTTTTATGAAGC   AAGGGGTTGGTCTCATGTGCTCTTCGATGAAAA   ATTGTGTCATCGTCAGTGCGGTACG   AAGGGGTTGGTCTC   AAGGGGTTGGTCTC
of pntA KO Verification of pntA KO Verification of pntA KO Verification of pntA KO Construction of pLS46 Construction of pLS46	BW25113 pntA of E. coli BW25113 pntA of E. coli BW25113 pntA of E. coli BW25113 atoB of E. coli BW25113 atoB of E. coli BW25113	oligoLS224 oligoLS216 oligoLS221 oligoLS230 oligoLS231	Rv - internal Fw - external Rv - external Fw - <i>Bsal</i> Rv - <i>Bsal</i>	TGATGGTGATTGGTGCGGGTG   GAAACGACCAGAGCCGCCAGGTTCA   TTTGCGCAAGGCGAAGGATTATTTTTATGAAGC   AAGGGGTTGGTCTCATGTGCTCTTCGATGAAAA   ATTGTGTCATCGTCATGTGCGGTACG   AAGGGGTTGGTCTCATGTGCTCTTCGATGAAAA   ATTGTGTCATCGTCAGTGCGGTACG   AAGGGGTTGGTCTCATTCATTCATCACCATCGCAATTCCC
of pntA KO Verification of pntA KO Verification of pntA KO Verification of pntA KO Construction of pLS46 Construction of pLS46	BW25113 pntA of E. coli BW25113 pntA of E. coli BW25113 pntA of E. coli BW25113 atoB of E. coli BW25113 atoB of E. coli BW25113 atoB of E. coli BW25113 atoD of E. coli	oligoLS224 oligoLS216 oligoLS221 oligoLS230 oligoLS231 oligoLS234	Rv - internal Fw - external Rv - external Fw - <i>Bsal</i> Rv - <i>Bsal</i> Fw - <i>Bsal</i>	TGATGGTGATTGGTGCGGGTG   GAAACGACCAGAGCCGCCAGGTTCA   TTTGCGCAAGGCGAAGGATTATTTTTATGAAGC   AAGGGGTTGGTCTCATGTGCTCTTCGATGAAAA   ATTGTGTCATCGTCAGTGCGGTACG   AAGGGGTTGGTCTCATTCGATCAATCACCATCGCAATTCCC   AAGGGGTTGGTCTCATCGATGAAAA
of pntA KO Verification of pntA KO Verification of pntA KO Verification of pntA KO Construction of pLS46 Construction of pLS47	BW25113 pntA of E. coli BW25113 pntA of E. coli BW25113 pntA of E. coli BW25113 atoB of E. coli BW25113 atoB of E. coli BW25113 atoD of E. coli BW25113	oligoLS224 oligoLS216 oligoLS221 oligoLS230 oligoLS231 oligoLS234	Rv - internal Fw - external Rv - external Fw - <i>Bsal</i> Rv - <i>Bsal</i> Fw - <i>Bsal</i>	TGATGGTGATTGGTGCGGGTG   GAAACGACCAGAGCCGCCAGGTTCA   TTTGCGCAAGGCGAAGGATTATTTTTATGAAGC   AAGGGGTTGGTCTCATGTGCTCTTCGATGAAAA   ATTGTGTCATCGTCAGTGCGGTACG   AAGGGGTTGGTCTCAGTGCGCTCTTCGATGAAAA   ATTCAACCGTTCAATCACCATCGCAATTCCC   AAGGGGTTGGTCTCATGAGCTCTTCGATGAAAA   ACAAAATTGATGACATTACAAGACG
of pntA KO Verification of pntA KO Verification of pntA KO Verification of pntA KO Construction of pLS46 Construction of pLS47 Construction	BW25113     pntA of E. coli     BW25113     pntA of E. coli     BW25113     pntA of E. coli     BW25113     atoB of E. coli     BW25113     atoB of E. coli     BW25113     atoB of E. coli     BW25113     atoD of E. coli     BW25113     atoD of E. coli     BW25113	oligoLS224 oligoLS216 oligoLS221 oligoLS230 oligoLS231 oligoLS234 oligoLS243	Rv - internal Fw - external Rv - external Fw - Bsal Rv - Bsal Fw - Bsal Rv - Bsal	TGATGGTGATTGGTGCGGGTG   GAAACGACCAGAGCCGCCAGGTTCA   TTTGCGCAAGGCGAAGGATTATTTTTATGAAGC   AAGGGGTTGGTCTCATGTGCTCTTCGATGAAAA   ATTGTGTCATCGTCATGTGCGGTACG   AAGGGGTTGGTCTCATCGTCGTCATCGCAATTCCC   AAGGGGTTGGTCTCATCGATGAAAA   ATTCAACCGTTCATCATCACCATCGCAATTCCC   AAGGGGTTGGTCTCATTGATGACATTACAAGACG   AAGGGGTTGGTCTCTGGTCTTACGCTCTTCATT
of pntA KO Verification of pntA KO Verification of pntA KO Verification of pntA KO Construction of pLS46 Construction of pLS47 Construction of pLS47	BW25113     pntA of E. coli     BW25113     pntA of E. coli     BW25113     pntA of E. coli     BW25113     atoB of E. coli     BW25113     atoB of E. coli     BW25113     atoD of E. coli     BW25113     atoD of E. coli     BW25113     atoD of E. coli     BW25113	oligoLS224 oligoLS216 oligoLS221 oligoLS230 oligoLS231 oligoLS234 oligoLS243	Rv - internal Fw - external Rv - external Fw - Bsal Rv - Bsal Fw - Bsal Rv - Bsal	TGATGGTGATTGGTGCGGGTG   GAAACGACCAGAGCCGCCAGGTTCA   TTTGCGCAAGGCGAAGGATTATTTTTATGAAGC   AAGGGGTTGGTCTC   AAGGGGTTGGTCTG
of pntA KO Verification of pntA KO Verification of pntA KO Verification of pntA KO Construction of pLS46 Construction of pLS47 Construction of pLS47 Construction	BW25113     pntA of E. coli     BW25113     pntA of E. coli     BW25113     pntA of E. coli     BW25113     atoB of E. coli     BW25113     atoB of E. coli     BW25113     atoD of E. coli     BW25113	oligoLS224 oligoLS216 oligoLS221 oligoLS230 oligoLS231 oligoLS234 oligoLS243 oligoLS235	Rv - internal Fw - external Rv - external Fw - Bsal Rv - Bsal Fw - Bsal Rv - Bsal Fw - Bsal	TGATGGTGATTGGTGCGGGTG   GAAACGACCAGAGCCGCCAGGTTCA   TTTGCGCAAGGCGAAGGATTATTTTTATGAAGC   AAGGGGTTGGTCTCATGTGCTCTTCGATGAAAA   ATTGTGTCATCGTCATGTGCTCTTCGATGAAAA   ATTGTGTCATCGTCAGTGCGGTACG   AAGGGGTTGGTCTC
of pntA KO Verification of pntA KO Verification of pntA KO Verification of pntA KO Construction of pLS46 Construction of pLS47 Construction of pLS47 Construction of pLS47	BW25113     pntA of E. coli     BW25113     pntA of E. coli     BW25113     pntA of E. coli     BW25113     atoB of E. coli     BW25113     atoB of E. coli     BW25113     atoD of E. coli     BW25113     atoA of E. coli     BW25113	oligoLS224 oligoLS216 oligoLS221 oligoLS230 oligoLS231 oligoLS234 oligoLS243 oligoLS235	Rv - internal   Fw - external   Rv - external   Fw - Bsal   Fw - Bsal	TGATGGTGATTGGTGCGGGTG   GAAACGACCAGAGCCGCCAGGTTCA   TTTGCGCAAGGCGAAGGATTATTTTTATGAAGC   AAGGGGTTGGTCTCATGTGCTCTTCGATGAAAA   ATTGTGTCATCGTCATGTGCTCTTCGATGAAAA   ATTGTGTCATCGTCATGTGCGGTACG   AAGGGGTTGGTCTC   AAGGGGTTGGTCTC   AGGGGTTGGTCTC   AAGGGGTTGGTCTC   AAGGGGTTGGTCTC
of pntA KO Verification of pntA KO Verification of pntA KO Verification of pntA KO Construction of pLS46 Construction of pLS47 Construction of pLS47 Construction of pLS47 Construction of pLS48 Construction	BW25113     pntA of E. coli     BW25113     pntA of E. coli     BW25113     pntA of E. coli     BW25113     atoB of E. coli     BW25113     atoB of E. coli     BW25113     atoD of E. coli     BW25113     atoD of E. coli     BW25113     atoD of E. coli     BW25113     atoA of E. coli     BW25113     atoA of E. coli     BW25113	oligoLS224 oligoLS216 oligoLS221 oligoLS230 oligoLS231 oligoLS234 oligoLS243 oligoLS243 oligoLS242	Rv - internal Fw - external Rv - external Fw - Bsal Rv - Bsal Fw - Bsal Rv - Bsal Fw - Bsal Fw - Bsal	TGATGGTGATTGGTGCGGGTG   GAAACGACCAGAGCCGCCAGGTTCA   TTTGCGCAAGGCGAAGGATTATTTTTATGAAGC   AAGGGGTTGGTCTC

Construction	adc of Clostridium	oligoLS228	Fw - Bsal	AAGGGGTT <mark>GGTCTC</mark> ATGTGGCTCTTCGATGTTA
of pLS49	acetobutylicum ATCC			AAGGATGAAGTAATTAAACAAATTAGCACG
	824			
Construction	adc of Clostridium	oligoLS229	Rv - Bsal	AAGGGGTT <mark>GGTCTC</mark> TGGTCTTACGCTCTTCATT
of pl S49	acetobutylicum ATCC			ACTTAAGATAATCATATATAACTTCAGCTCTAGG
01 02040	824			
Construction	024	alizat COOO		
Construction	Sequence encoding	oligoLS232	FW - Bsal	AAGGGGTT <mark>GGTCTC</mark> ATGTGGCTCTTCGATGAAA
of pLS50	CBADH of Clostridium			GGCTTTGCCATGCTGGGTATTAAC
	beijerinckii			
Construction	Sequence encoding	oligoLS233	Rv - <i>Bsal</i>	AAGGGGTT <mark>GGTCTC</mark> TGGTCTTACGCTCTTCATT
of pLS50	CBADH of Clostridium			AGAGGATAACTACGGCCTTAATGAGATCTTTAG
	beijerinckii			G
Construction	500 bp upstream and	oligoLS244	Fw - BamHI	TTCAGC <mark>GGATCC</mark> TGTCTGTTTTGCGGTCGCCA
of pLS63	downstream of IdhA	-		G
Construction	500 bn upstream and	oligol S247	Rv	
of pl S63	downstroom of IdhA	oligoeozari		CCCTCCATTCCAC
			-	
Construction	500 bp upstream and	oligoLS246	Fw	CAGGGGGGGGCGGCAAGATTACATAAGACTTTCT
of pLS63	downstream of IdhA			CCAGTGATGTTGAATC
Construction	500 bp upstream and	oligoLS245	Rv - HindIII	TTCAGCAAGCTTCAAGCAGAATCAAGTTCTACC
of pLS63	downstream of IdhA			GTGC
Construction	Sequence encoding	oligoLS258	Rv	AGCGTCGACACAGACTGGNNNNNNNNAACAG
of pLS73	TBADH of			CAATGATACGTCCTGC
library	Thermoanaerobacter			
	brockii			
Construction	Sequence encoding	oligol \$259	Ew/	
Construction	Sequence encounig	OligoL3233	1 00	000000101110000000000000000000000000000
of pl \$73				
of pLS73	TBADH of			CNNNAAGGATGGGCC
of pLS73 library	TBADH of Thermoanaerobacter			CNNNAAGGATGGGCC
of pLS73 library (TBADH <sub>Lib</sub> )	TBADH of Thermoanaerobacter brockii			CNNNAAGGATGGGCC
of pLS73 library (TBADH <sub>Lib</sub> ) Construction	TBADH of Thermoanaerobacter brockii Sequence encoding	oligoLS288	Fw - Ndel	CNNNAAGGATGGGCC GCAGC <mark>CATATG</mark> ATGAAGGGGTTCGCAATGCTG
of pLS73 library (TBADH <sub>Lib</sub> ) Construction of pLS90	TBADH of <i>Thermoanaerobacter</i> <i>brockii</i> Sequence encoding TBADH <sub>S2</sub>	oligoLS288	Fw - Ndel	CNNNAAGGATGGGCC GCAGC <mark>CATATG</mark> ATGAAGGGGTTCGCAATGCTG TCTATTGG
of pLS73 library (TBADH <sub>Lib</sub> ) Construction of pLS90 Construction	TBADH of <i>Thermoanaerobacter</i> <i>brockii</i> Sequence encoding TBADH <sub>S2</sub> Sequence encoding	oligoLS288 oligoLS289	Fw - Ndel Rv - Blpl	CNNNAAGGATGGGCC GCAGC <mark>CATATG</mark> ATGAAGGGGTTCGCAATGCTG TCTATTGG TTATT <mark>GCTCAGC</mark> TTAAGCCAGAATAACCACTGG
of pLS73 library (TBADH <sub>Lib</sub> ) Construction of pLS90 Construction of pLS90	TBADH of Thermoanaerobacter brockii Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub>	oligoLS288 oligoLS289	Fw - Ndel Rv - Blpl	CNNNAAGGATGGGCC GCAGC <mark>CATATG</mark> ATGAAGGGGTTCGCAATGCTG TCTATTGG TTATT <mark>GCTCAGC</mark> TTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG
of pLS73 library (TBADH <sub>Lib</sub> ) Construction of pLS90 Construction of pLS90	TBADH of Thermoanaerobacter brockii Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding	oligoLS288 oligoLS289 oligoLS288	Fw - Ndel Rv - Blpl Fw - Ndel	CNNNAAGGATGGGCC GCAGC <mark>CATATG</mark> ATGAAGGGGTTCGCAATGCTG TCTATTGG TTATT <mark>GCTCAGC</mark> TTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGC <mark>CATATG</mark> ATGAAGGGGTTCGCAATGCTG
of pLS73 library (TBADH <sub>Lib</sub> ) Construction of pLS90 Construction of pLS90	TBADH of Thermoanaerobacter brockii Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub>	oligoLS288 oligoLS289 oligoLS288	Fw - Ndel Rv - Blpl Fw - Ndel	CNNNAAGGATGGGCC GCAGC <mark>CATATG</mark> ATGAAGGGGTTCGCAATGCTG TCTATTGG TTATT <mark>GCTCAGC</mark> TTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGC <mark>CATATG</mark> ATGAAGGGGTTCGCAATGCTG TCTATTGG
of pLS73 library (TBADHLib) Construction of pLS90 Construction of pLS91 Construction	TBADH of Thermoanaerobacter brockii Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub>	oligoLS288 oligoLS289 oligoLS288	Fw - Ndel Rv - Blpl Fw - Ndel	CNNNAAGGATGGGCC GCAGC <mark>CATATG</mark> ATGAAGGGGTTCGCAATGCTG TCTATTGG TTATT <mark>GCTCAGC</mark> TTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGC <mark>CATATG</mark> ATGAAGGGGTTCGCAATGCTG TCTATTGG
of pLS73 library (TBADHLib) Construction of pLS90 Construction of pLS91 Construction of pLS91	TBADH of Thermoanaerobacter brockii Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub>	oligoLS288 oligoLS289 oligoLS288 oligoLS289	Fw - Ndel Rv - Blpl Fw - Ndel Rv - Blpl	CNNNAAGGATGGGCC GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTATTGCTCAGCTTAAGCCAGAATAACCACTGG
of pLS73 library (TBADH <sub>Lib</sub> ) Construction of pLS90 Construction of pLS91 Construction of pLS91	TBADH of Thermoanaerobacter brockii Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub>	oligoLS288 oligoLS289 oligoLS288 oligoLS289	Fw - Ndel Rv - Blpl Fw - Ndel Rv - Blpl	CNNNAAGGATGGGCC GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG
of pLS73 library (TBADH <sub>Lib</sub> ) Construction of pLS90 Construction of pLS91 Construction of pLS91	TBADH of Thermoanaerobacter brockii Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub>	oligoLS288 oligoLS289 oligoLS288 oligoLS289 oligoLS288	Fw - Ndel Rv - Blpl Fw - Ndel Rv - Blpl Fw - Ndel	CNNNAAGGATGGGCC GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGGTTCGCAATGCTG TCTATTGG TTTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG
of pLS73 library (TBADHLib) Construction of pLS90 Construction of pLS91 Construction of pLS91 Construction of pLS91	TBADH of Thermoanaerobacter brockii Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub>	oligoLS288 oligoLS289 oligoLS288 oligoLS289 oligoLS288	Fw - Ndel Rv - Blpl Fw - Ndel Rv - Blpl Fw - Ndel	CNNNAAGGATGGGCC GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG
of pLS73 library (TBADHLib) Construction of pLS90 Construction of pLS91 Construction of pLS91 Construction of pLS91	TBADH of Thermoanaerobacter brockii Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub>	oligoLS288 oligoLS289 oligoLS288 oligoLS289 oligoLS288	Fw - Ndel Rv - Blpl Fw - Ndel Rv - Blpl Fw - Ndel	CNNNAAGGATGGGCC GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG
of pLS73 library (TBADH <sub>Lib</sub> ) Construction of pLS90 Construction of pLS91 Construction of pLS91 Construction of pLS91	TBADH of Thermoanaerobacter brockii Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub>	oligoLS288 oligoLS289 oligoLS288 oligoLS289 oligoLS288	Fw - Ndel Rv - Blpl Fw - Ndel Rv - Blpl Fw - Ndel	CNNNAAGGATGGGCC GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG
of pLS73 library (TBADH <sub>Lib</sub> ) Construction of pLS90 Construction of pLS91 Construction of pLS91 Construction of pLS97	TBADH of Thermoanaerobacter brockii Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH of Thermoanaerobacter brockii Sequence encoding	oligoLS288 oligoLS289 oligoLS289 oligoLS289 oligoLS288 oligoLS289	Fw - Ndel Rv - Blpl Fw - Ndel Rv - Blpl Fw - Ndel Fw - Ndel	CNNNAAGGATGGGCC GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGGTTCGCAATGCTG TCTATTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TCTATTGG
of pLS73 library (TBADHLib) Construction of pLS90 Construction of pLS91 Construction of pLS91 Construction of pLS97 Construction of pLS97	TBADH of Thermoanaerobacter brockii Sequence encoding TBADHs2 Sequence encoding TBADHs2 Sequence encoding TBADHs2 Sequence encoding TBADHs2 Sequence encoding TBADH of Thermoanaerobacter brockii Sequence encoding TBADH of	oligoLS288 oligoLS289 oligoLS288 oligoLS289 oligoLS288 oligoLS289	Fw - Ndel Rv - Blpl Fw - Ndel Rv - Blpl Fw - Ndel Rv - Blpl	CNNNAAGGATGGGCC GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG
of pLS73 library (TBADH <sub>Lib</sub> ) Construction of pLS90 Construction of pLS91 Construction of pLS91 Construction of pLS97 Construction of pLS97	TBADH of Thermoanaerobacter brockii Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH of Thermoanaerobacter brockii Sequence encoding TBADH of Thermoanaerobacter	oligoLS288 oligoLS289 oligoLS288 oligoLS289 oligoLS288 oligoLS289	Fw - Ndel Rv - Blpl Fw - Ndel Rv - Blpl Fw - Ndel Rv - Blpl	CNNNAAGGATGGGCC GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTATTGCTCAGCTTAAGCCAGAATAACCACTGG TCTATTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG
of pLS73 library (TBADH <sub>Lib</sub> ) Construction of pLS90 Construction of pLS91 Construction of pLS91 Construction of pLS97 Construction of pLS97	TBADH of Thermoanaerobacter brockii Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH of Thermoanaerobacter brockii Sequence encoding TBADH of Thermoanaerobacter brockii	oligoLS288 oligoLS289 oligoLS288 oligoLS289 oligoLS288 oligoLS289	Fw - Ndel Rv - Blpl Fw - Ndel Rv - Blpl Fw - Ndel Rv - Blpl	CNNNAAGGATGGGCC GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTTATTGCTCAGCTTAAGCCAGAATAACCACTGG TCTATTGG
of pLS73 library (TBADHLib) Construction of pLS90 Construction of pLS91 Construction of pLS91 Construction of pLS97 Construction of pLS97	TBADH of Thermoanaerobacter brockii Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH of Thermoanaerobacter brockii Sequence encoding TBADH of Thermoanaerobacter brockii	oligoLS288 oligoLS289 oligoLS289 oligoLS289 oligoLS288 oligoLS289	Fw - Ndel Rv - Blpl Fw - Ndel Rv - Blpl Fw - Ndel Rv - Blpl Rv - Blpl	CNNNAAGGATGGGCC GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGGTTCGCAATGCTG TCTATTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG
of pLS73 library (TBADHLib) Construction of pLS90 Construction of pLS91 Construction of pLS91 Construction of pLS97 Construction of pLS97	TBADH of Thermoanaerobacter brockii Sequence encoding TBADHs2 Sequence encoding TBADHs2 Sequence encoding TBADHs2 Sequence encoding TBADHs2 Sequence encoding TBADH of Thermoanaerobacter brockii Sequence encoding TBADH of Thermoanaerobacter brockii Sequence encoding CBADHs2	oligoLS288 oligoLS289 oligoLS288 oligoLS289 oligoLS288 oligoLS289 oligoLS289	Fw - Ndel Rv - Blpl Fw - Ndel Rv - Blpl Fw - Ndel Rv - Blpl Fw - Ndel	CNNNAAGGATGGGCC GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGCTTTGCCATGCTG GCAGCCATATGATGAAAGGCTTTGCCATGCTG GCAGCCATATGATGAAAGGCTTTGCCATGCTG
of pLS73 library (TBADH <sub>Lib</sub> ) Construction of pLS90 Construction of pLS91 Construction of pLS91 Construction of pLS97 Construction of pLS97 Construction of pLS97	TBADH of Thermoanaerobacter brockii Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH of Thermoanaerobacter brockii Sequence encoding TBADH of Thermoanaerobacter brockii Sequence encoding CBADH <sub>S</sub> Sequence encoding CBADH <sub>S</sub>	oligoLS288 oligoLS289 oligoLS288 oligoLS289 oligoLS288 oligoLS289 oligoLS289	Fw - Ndel   Rv - Blpl   Fw - Ndel   Rv - Blpl   Fw - Ndel   Fw - Ndel   Fw - Ndel   Pv - Blpl   Fw - Ndel   Pv - Blpl   Pv - Ndel   Pv - Blpl	CNNNAAGGATGGGCC GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG
of pLS73 library (TBADH <sub>Lib</sub> ) Construction of pLS90 Construction of pLS91 Construction of pLS91 Construction of pLS97 Construction of pLS97 Construction of pLS97	TBADH of Thermoanaerobacter brockii Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH <sub>S2</sub> Sequence encoding TBADH of Thermoanaerobacter brockii Sequence encoding TBADH of Thermoanaerobacter brockii Sequence encoding CBADH <sub>S</sub> Sequence encoding CBADH <sub>S</sub>	oligoLS288 oligoLS289 oligoLS288 oligoLS289 oligoLS288 oligoLS289 oligoLS289 oligoLS294 oligoLS295	Fw - Ndel   Rv - Blpl   Fw - Ndel   Rv - Blpl   Fw - Ndel   Fw - Ndel   Fw - Ndel   Rv - Blpl   Rv - Blpl	CNNNAAGGATGGGCC GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGGGTTCGCAATGCTG TCTATTGG TTTATTGCTCAGCTTAAGCCAGAATAACCACTGG TTTGATAAGGTCCTTTGG GCAGCCATATGATGAAGGCGTTGCCATGCTG GCAGCCATATGATGAAAGGCTTTGCCATGCTG GGTATTAACAAATTAGG TTATTGCTCAGCTTAGAGGATAACTACGGCCTT

Construction	Sequence encoding	oligoLS294	Fw - Ndel	GCAGCCATATCAAAAGGCTTTGCCATGCTG
of pLS99	CBADH of Clostridium			GGTATTAACAAATTAGG
	beijerinckii			
Construction	Sequence encoding	oligoLS295	Rv - Blpl	TTATTGCTCAGCTTAGAGGATAACTACGGCCTT
of pLS99	CBADH of Clostridium	- C		AATGAGATCTTTAGGTTTATCTTTCATGAG
	beiierinckii			
Construction	Sequence encoding	oligol S344	Rv	ACGATAATATCGCTGCGTTTAAC
of pl S131	MsIREDc	enge_corr		
Construction		oligol \$345	Ew	
of pl S131		oligoeooto		
Construction		oligol S342	P <sub>V</sub>	
of pl S121		UIGOE0042		
OI pL3131		aligal 6242	- Eur	
		OligoL3343	ΓW	ATCACCTCCTC
of pLS131	MSIREDC			GATCAGCTGCTG
Construction	Sequence encoding	oligoLS337	Fw	GCTGAGAAGACCGACCACGGTGTGGNNNNNN
of pLS133	MsIRED of			
library	Myxococcus stipitatus			G
(MsIRED <sub>Lib</sub> )				
Construction	Sequence encoding	oligoLS338	Rv	GCTGAGAAGACCGTGGTCGTGTAGCCAGATTG
of pLS133	MsIRED of			CAGGAATGCTTTAATCAGTGCGGAGCCCATAC
library	Myxococcus stipitatus			GGCC
(MsIRED <sub>Lib</sub> )				
Construction	Sequence encoding	oligoLS358	Rv - Bbsl 6xHis-	TCTCTGAAGACTCCTTAGTGGTGGTGGTGGTG
of pLS161	wild-type MsIRED		tag	GTGTTTCAGGAAGCGGGTCAGAATTGCAAAG
	with C-terminal 6xHis-			
	tag			
Construction	Sequence encoding	oligoLS359	Fw - Bbsl	TCTCTGAAGACAACATGAAACCGACCCTGACC
of pLS161	wild-type MsIRED			GTTATTGGC
	with C-terminal 6xHis-			
	tag			
Construction	Sequence encoding	oligoLS358	Rv - Bbsl 6xHis-	TCTCT <mark>GAAGAC</mark> TCCTTAGTGGTGGTGGTGGTG
of pLS162	MsIRED <sub>c</sub> with C-		tag	GTGTTTCAGGAAGCGGGTCAGAATTGCAAAG
	terminal 6xHis-tag			
Construction	Sequence encoding	oligoLS359	Fw- Bbsl	TCTCTGAAGACAACATGAAACCGACCCTGACC
of pLS162	MsIRED <sub>c</sub> with C-	-		GTTATTGGC
	terminal 6xHis-tag			
Construction	Sequence encoding	oligoLS358	Rv - <i>Bbsl</i> 6xHis-	TCTCT <mark>GAAGAC</mark> TCCTTAGTGGTGGTGGTGGTG
of pLS164	MsIREDs with C-	Ŭ	taq	GTGTTTCAGGAAGCGGGTCAGAATTGCAAAG
	terminal 6xHis-tag			
Construction	Sequence encoding	oligol \$359	Fw - Bbsl	
of pl S164	MsIREDs with C-	0go_0000		GTTATTGGC
01 020101	terminal 6xHis-tag			
Construction	Sequence encoding	oligol \$363	Ry - Bhsl	
of pl S169	EntNifeB of	ONGOLOGO	100 - 2031	
library	Enterobactor closeso			GLACIGGETGGGAGTTNINNNNGGACGGGET
				GIACIGC
(EntiviseLib)	Converse encoding	alizat 0200	Europhal	
Construction	Sequence encoding	OligoLS366	FW - BDSI	
of pLS169	EntNfsB of			ACCIGAACGICGG
	Enterobacter cloacae			

library				
(EntNfsB <sub>Lib</sub> )				
Construction	Sequence encoding	oligoLS364	Fw - Bbsl	CTCT <mark>GAAGAC</mark> AGCACCGAGGAAGGAAAAGCGC
of pLS169	EntNfsB of			GCGTGGCGAAGTCCGCTGCGGGCACCNNNGT
library	Enterobacter cloacae			GTTCAACGAACG
(EntNfsB <sub>Lib</sub> )				
Construction	Sequence encoding	oligoLS365	Rv - Bbsl	TCTCT <mark>GAAGAC</mark> ATCCACTGGTCGTCATCTTTCA
of pLS169	EntNfsB of			GATCCACGCGGTGCATGTCGGCNNNGTAGGTG
library	Enterobacter cloacae			CGGCC
(EntNfsB <sub>Lib</sub> )				
Construction	Sequence encoding	oligoLS385	Rv - Bbsl 6xHis-	TCTCT <mark>GAAGAC</mark> TCCTTAGTGGTGGTGGTGGTG
of pLS180	EntNfsB with C-		tag	GTGGCACTCGGTCACAATCGTGCTCAGC
	terminal 6xHis-tag			
Construction	Sequence encoding	oligoLS386	Fw - Bbsl	TCTCT <mark>GAAGAC</mark> AACATGGATATCATTTCTGTCG
of pLS180	EntNfsB with C-			CCCTG
	terminal 6xHis-tag			
Construction	Sequence encoding	oligoLS385	Rv - Bbsl 6xHis-	TCTCT <mark>GAAGAC</mark> TCCTTAGTGGTGGTGGTGGTG
of pLS181	EntNfsBs1 with C-		tag	GTGGCACTCGGTCACAATCGTGCTCAGC
	terminal 6xHis-tag			
Construction	Sequence encoding	oligoLS386	Fw - Bbsl	TCTCTGAAGACAACATGGATATCATTTCTGTCG
of pLS181	EntNfsBs1 with C-			CCCTG
	terminal 6xHis-tag			
Construction	Sequence encoding	oligoLS385	Rv - Bbsl 6xHis-	TCTCT <mark>GAAGAC</mark> TCCTTAGTGGTGGTGGTGGTG
of pLS182	EntNfsBs2 with C-		tag	GTGGCACTCGGTCACAATCGTGCTCAGC
	terminal 6xHis-tag			
Construction	Sequence encoding	oligoLS386	Fw - Bbsl	TCTCT <mark>GAAGAC</mark> AACATGGATATCATTTCTGTCG
of pLS182	EntNfsBs2 with C-			CCCTG
	terminal 6xHis-tag			
Sequencing	pUC19	M13	Rv	CAGGAAACAGCTATGACC
Sequencing	pUC19	M13	Fw	TGTAAAACGACGGCCAGT
Sequencing	pET28a	Т7	Fw	TAATACGACTCACTATAGGG
Sequencing	pLS60	oligoLS275	Fw	CATCCTATGGAACTGCCTCG
Sequencing	pLS60	oligoLS276	Fw	GAAAGTGAATGTCAACGGCG
Sequencing	pLS60	oligoLS277	Fw	CATCGTTGCGACACACTTGGC
Sequencing	pLS60	oligoLS278	Fw	CTGCACCATGCCACTCACTG
Sequencing	pLS60	oligoLS279	Fw	CCGTACATGAAGCTTGGACAGG

**Supplementary Table 5. Synthetic DNA sequences used in this project.** All synthetic genes were codon-adapted suitably for expression in *E. coli* and chemically synthesized (IDT or DNA2.0). Restriction sites are highlighted in green and start and stop codons in bold.

Synthetic DNA	Sequence (5'- 3')	RBS and Restriction sites
Sequence encoding 2,3-butanedio		Sphi (Send) BamHi (Send)
dehydrogenase BUDC of Klebsiella	ATCAGACAGAGAGAGAGTACAATAIGA	
pneumoniae	AAAAAGTCGCACTTGTTACCGGCGC	Synthetic RBS sequence:
	CGGCCAGGGGATTGGTAAAGCTAT	CAATCTTAATCAAATCAGACAGAGA
	CGCCCTTCGTCTGGTGAAGGATGG	GAGTACAAT
	ATTTGCCGTGGCCATTGCCGATTAT	
	AACGACACCACCGCCAAAGCGGTC	
	GCCTCCGAAATCAACCAGGCCGGC	
	GGCCGCGCCATGGCGGTGAAAGTG	
	GATGTCTCCGACCGCGATCAGGTG	
	TTTGCCGCCGTCGAACAGGCGCGC	
	ACCCCGGAGATIGICGATAAAGICI	
	ACAACATCAACGTTAAAGGGGGTGAT	
	CIGGGGCATICAGGCGGCGGICGA	
	GGCCTTTAAGAAAGAGGGTCACGG	
	CGGGAAAATCATCAACGCCTGTTCC	
	CAGGCCGGCCACGTCGGCAACCCG	
	GAGCTGGCGGTATATAGCTCGAGT	
	AAATTCGCCGTACGCGGCTTAACCC	
	AGACCGCCGCTCGCGACCTCGCGC	
	CGCTGGGCATCACAGTCAACGGCT	
	ACTGCCCGGGGATTGTCAAAACGC	
	CAATGTGGGCCGAAATTGACCGCC	
	GCCTGTCCGAGCCGGAAGATGTCG	
	CCGCCIGCGICICCIAICIIGCCAG	
	CCCGGATTCTGATTATATGACCGGT	
	CAGTCATTGCTGATCGACGGCGGG	
	ATGGTGTTTAAC <b>TAA<mark>GGATCC</mark>GCTG</b>	
	AA	
Sequence encoding NAD-dependent	CCGTTC <mark>GCATGC</mark> AGGAGGTACGAA	SphI (5'end) BamHI (3'end)
alcohol dehydrogenase TADH of	CAC <b>ATG</b> AAGGGGTTCGCAATGCTG	
Thermus sp. ATN-1	TCTATTGGAAAAGTTGGCTGGATTG	Shine-Dalgarno RBS:
•	AAAAGGAGAAGCCAGCGCCAGGGC	AGGAGGTACGAACAC
	CTTTCGACGCAATTGTTCGCCCTTT	
	GGCAGTCGCACCTTGCACGTCTGA	
	CATCCACACCGTTTTCGAAGGAGCC	
	ATTGGTGAACGTCATAACATGATCT	
	TGGGACACGAAGCGGTAGGTGAGG	
	TIGTAGAGGTCGGTTCTGAAGTTAA	
	GGACTITAAACCIGGAGACCGCGI	
	GGIGGIGUUGUGATIAUGUUIGA	
	CIGGCGIACTICAGAGGICCAACG	
	IGGATATCACCAACATAGCGGCGG	
	TATGCTGGCGGGTTGGAAGTTCTC	
	CAATGTGAAGGACGGTGTTTTCGGA	
	GAATTCTTCCATGTTAATGACGCCG	
	ACATGAATTTGGCGCACCTTCCGAA	
	GGAGATTCCGTTAGAAGCCGCGGT	
	AATGATCCCCGACATGATGACCACC	
	GGCTTTCATGGAGCGGAGCTGGCG	
	GACATCGAGTTGGGCGCTACCGTG	
	GCTGTACTTGGCATCGGTCCTGTC	
	GGTCTGATGGCGGTGGCAGGGGC	
	CATTECTETTEETCETCCAGTC	
	GGATGGGCCAATIGAGTCACAGATT	
	ATGAACCITACAGAAGGGAAGGGA	
	GIIGAIGCAGCIAIIAIIGCAGGCG	
	GGAATGCGGATATCATGGCGACAG	
	CCGTCAAGATCGTGAAGCCCGGTG	

	GAACTATTGCTAATGTGAATTACTTT	
	GGTGAGGGAGAAGTTTTGCCGGTG	
	CCTCGCCTGGAATGGGGTTGTGGG	
	ATGGCCCACAAAACGATCAAGGGA	
	GGTCTGTGTCCAGGGGGACGTCTG	
	CGCATGGAACGCTTGATTGACCTTG	
	TCTTTTACAAACGTGTGGACCCGAG	
	TAAATTGGTCACACACGTATTCCGT	
	GGCTTTGATAACATTGAAAAGGCGT	
	CTCCCTTAACCAGIGGIIAII	
Sequence encoding NADP-		Sphl (5'end) BamHl (3'end)
dependent isopropanol	GATAAGGAGAAAGAGATGAAAGGC	Spin (Sena) Bannin (Sena)
dehydrogenase CBADH of	TTTGCCATGCTGGGTATTAACAAAT	Synthetic RBS sequence:
Clostridium beijerinckij	TAGGATGGATTGAAAAAGAACGCCC	ATTCGGATCTATACAGATAAGGAGA
· · · · · · · · · · · · · · · · · · ·	CGTCGCGGGTTCCTATGATGCGATT	AAGAGATGAAAGGCTTTGCC
	GTACGACCCTTAGCCGTTTCCCCGT	
	GCACTAGCGATATTCATACAGTATT	
	TGAAGGGGCTCTCGGCGATCGAAA	
	GAATATGATTTTAGGCCATGAAGCC	
	CTACCCCAGATTGGCGCTCTCTGG	
	AGGTTCAAGCTGGTTTTCAACAACA	
	TAGTAATGGTATGTTGGCCGGCTG	
	GAAGTTTTCCAACTTCAAAGATGGA	
	GTATTTGGGGAGTATTTTCATGTGA	
	ACGATGCGGATATGAATTTGGCCAT	
	CCTGCCAAAAGACATGCCCTTGGA	
	GAATGCTGTAATGATCACCGATATG	
	ATGACCACCGGATTTCATGGGGCC	
	GAGIIGGCCGAIAICCAGAIGGGI	
	CTGGGGCCAAATTACGCGGAGCAG	
	GTCGGATTATTGGTGTCGGCAGTC	
	GGCCTATTTGCGTTGAGGCCGCCA	
	AGTTCTACGGCGCGACCGACATTCT	
	GAATTACAAAAATGGCCATATTGTG	
	GACCAGGTAATGAAGCTAACCAATG	
	GGAAAGGCGTGGACCGTGTGATTA	
	TGGCTGGAGGTGGGAGTGAAACAC	
	GATGTGGCATGGCGCACAAGACGA	
	TCAAAGGCGGTTTGTGTCCCCGGAG	
	GCCGTTTACGGGCCGAAATGCTAC	
	GGGATATGGTGGTGTACAACCGTG	
	TGGATTTGTCCAAGCTGGTGACTCA	
	CGTTTATCACGGTTTTGACCATATT	
	GAAGAAGCCTTGCTACTCATGAAAG	
Sequence encoding NADP-		Sphl (5'end) BamHl (3'end)
dependent isopropanol	CACATGAAGGGGTTCGCAATGCTG	
dehydrogenase TBADH of	TCTATTGGAAAAGTTGGCTGGATTG	Shine-Dalgarno RBS:
Thermoanaerobacter brockii	AAAAGGAGAAGCCAGCGCCAGGGC	AGGAGGTACGAACAC
	CTTTCGACGCAATTGTTCGCCCTTT	
	TGGGACACGAAGGGGTAGGTGAGG	
	TTGTAGAGGTCGGTTCTGAAGTTAA	
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	GGCTTTCATGGAGCGGAGCTGGCG	
	GACATCGAGTTGGGCGCTACCGTG	
	GOTGTACTTGGCATCGGTCCTGTC	
	GGTCTGATGGCGGTGGCAGGGGC	
	CAAGTTGCGTGGTGCAGGACGTAT	
	CATTECTETTEETTEETCETCCAETC	
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	TAAATTGGTCACACGCGTATTCCGT	
	GGCTTTGATAACATTGAAAAGGCGT	
	TCATGTTGATGAAGGATAAACCAAA	
	GGACCTTATCAAACCAGTGGTTATT	
	CIGGCIT <b>AA<mark>GGAICC</mark>IICAGC</b>	
Sequence encoding NADP-	CCGTTC <mark>GCATGC</mark> AGGAGGTACGAA	SphI (5'end) BamHI (3'end)
dependent (R)-selective imine		
	TTOOOOTOOOOTATOOOTOOO	China Dalaama DDO
reductase INSIRED of Myxococcus		Shine-Daigarno RBS:
stipitatus	CACTGATTAAAGCATTCCTGCAATC	AGGAGGTACGAACAC
	TGGCTACACGACCACGGTGTGGAA	
	CCGTACCAAAGCCAAAAGCGAACC	
	001000000000000000000000000000000000000	
	GUIGGUAAAAUIGGGUGUAUAIUI	
	GGCTGATACGGTGCGTGACGCCGT	
	TAAACGCAGCGATATTATCGTGGTT	
	AATGTGCTGGATTATGACACCTCTG	
	ATCAGCTGCTGCGCCAAGACGAAG	
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Sequence encoding oxygen-	ATCAGCTGCTGCGCGCAAGACGAAG TGACGCGTGAACTGCGCGCGCAAAC TGCTGGTTCAGCTGACCAGCGGTT CTCCGGCACTGGCTGGCCACCAGGGAACA TGATTATCTGGACGGTGCGAACAGG AAACGTGGGCGCGCCAACATGGCA TTGATTATCTGGACGGTGCGATCAT GGCCACCCCGGATTTTATTGGCCA GGCAGAATGCGCTCTGCTGAACAG TGGTTCCGCGGCCCTGTTCGAAAA ACACCGTGCTGTCCTGAATGTGGCTG GGCGGTGCCACCAGCCATGTCGGC GAAGATGTTGGTCATGCCTCAGCAC TGGACAGCGCCCTGTTCGGAAAA ACACCGTGCTGTCTGATGCGCG GAAGATGTTGGTCATGCCTCAGCAC TGGACAGCGCCCTGTTCGGAACAC CGGCACCCGGCGTGCCACCAGCC GAAGCACTGGCTATTTCTCGCGCA GAAGGCATCCCGCTGGAAAAAACC ACGGCGTTTATCAAACTGACCGAAC CGGTCACCCAGGGTGCCGTTGCAG ATGTCCTGACCGCAGACGCCAGAC GCTGGCAAGTCTGGAAGCTCATAA CGTGGCGTTCCAACACTGCTGGC CCTGTGTGAAGAACGTAATATCCAT CCCGTTATTCGTGAAGCGACCATGTAC TCCGTTATTCGTGAAGCGGTCAAAG CCGGCCACGGTAAAGATGACTTTG CAATTCTGACCCGCTTCCTGAAATA AGGATCCTTCAGC	Bbsl (5'end)
Sequence encoding oxygen- insensitive NAD(P)-dependent	ATCAGCTGCTGCGCGCAAGACGAAG TGACGCGTGAACTGCGCGCGCAAAC TGCTGGTTCAGCTGACCAGCGGTT CTCCGGCACTGGCTGGCCACCAGCGGTT CTCCGGCACTGGCTGGCGACAGG AAACGTGGGCGCGCCAACATGGCA TTGATTATCTGGACGGTGCGGCACAT GGCACCCCGGATTTTATTGGCCA GGCAGAATGCGCTCTGCTGTACAG TGGTTCCGCGGCCCTGTTCGAAAA ACACCGTGCTGTCCTGAATGTGCTG GGCGGTGCCACCAGCCATGTCGGC GAAGATGTTGGTCATGCCTCAGCAC TGGACAGCGCCCTGCTGTTCCAGAAC GTGGGGCACCCTGTTCCGGAACAC GAAGCATCCGCTGCTGTTCCGCAC GAAGCATCCCGCTGGAAAAAACC ACGGCGTTTATCAAACTGACCGAAC CGGTCACCCAGGGTGCCGTTGCAGC ATGTCCTGACCGCAGACGCTCAGCAC GCTGGCAAGTCTGGAAGCCCATGAC GCTGGCAAGTCTGGAACGCCAAAA TCGCCTGACCGCAGACGCTCAGAC GCTGGCAAGTCTGGAAGCTCATAA CGTGGCGTTCCAACACCTGCTGGC CCTGTGTGAAGAACGTAATATCCAT CGCGGCATCCGGCAGACGCTCAAAG CCGGCCACGGTAAAGATGACTTG CAATTCTGACCCGCTGCAGCATTA AGGATCCTTCAGC	Bbs/ (5'end) Bbs/ (3'end)
Sequence encoding oxygen- insensitive NAD(P)-dependent nitroreductase EntNfsB (NfsB) of	ATCAGCTGCTGCGCGCAAGACGAAG TGACGCGTGAACTGCGCGCGCAAAC TGCTGGTTCAGCTGACCAGCGGTT CTCCGGCACTGGCTGACCAGCGGTT CTCCGGCACTGGCTGACCAGG AAACGTGGGCGCGCCAACATGGCA TTGATTATCTGGACGGTGCGATCAT GGCCACCCCGGATTTTATTGGCCA GGCAGAATGCGCTCTGCTGTACAG TGGTTCCGCGGCCCTGTTGAAAA ACACCGTGCTGTCCTGAATGTGCTG GGCGGTGCCACCAGCCATGTCGGC GAAGATGTTGGTCATGCCTCAGCAC TGGACAGCGCCCTGCTGTTCCAGAAC TGGGGCACCCTGCTGTTCCAGCAC GCAGGCACCGCCTGCTGTTCCAGCAC GGACAGCGCCCTGCTGTTCCAGCAC GGACAGCGCCCTGCTGTTCCGCACAC GGACAGCGCCCTGCTGTTCCGCACAC GCAGGCACCCGCTGGCACCCCGCC GCAAGCACTGGCTATTTCTCGCGCA GAAGGCATCCCGCTGGAAAAAACC ACGGCGTTTATCAAACTGACCGAAC CGGTCACCCAGGGTGCCATGCAG ATGTCCTGACCCGTGGAAGCTCAAAA CGTGGCGTTCCAACACCTGCTGGC CCTGTGTGAAGAACGTCATAA CGCGCCACGGTAAGATGACTTTG CAATTCTGACCCGTGAAGATGACTTTG CAATTCTGACCCGTGAAACGCCAACAT CCGTCTTCAGCC CATCTGAACACACTGGATATCCAT TTCTGTCGCCCTGAAACGCCACTCT ACCAAGGCGTTCGACGCAAGCAAA	Bbsl (5'end) Bbsl (3'end)
Sequence encoding oxygen- insensitive NAD(P)-dependent nitroreductase EntNfsB (NfsB) of Enterobacter cloacae	ATCAGCTGCTGCGCGCGAAGACGAAG TGACGCGTGAACTGCGCGCGCAAAC TGCTGGTTCAGCTGACCAGCGGTT CTCCGGCACTGGCTCGTGAACAGG AAACGTGGGCGCGCGCCAACATGGCA TTGATTATCTGGACGGTGCGATCAT GGCCACCCCGGATTTTATTGGCCA GGCAGAATGCGCTCTGCTGTACAG TGGTTCCGCGGCCCTGTTCGAAAA ACACCGTGCTGTCCTGAATGTGCTG GGCGGTGCCACCAGCCATGTCGGCA GAAGATGTTGGTCATGCCTCAGCAC TGGACAGCGCCCTGTTCGAATGTCGGC GAAGCACTGGCACCAGCCATGTCGCAC GAAGCACTGGCTATTTCTCGCGCA GAAGGCATCCCGCTGGAAAAAACC ACGGCGTTTATCAAACTGACCGAAC GGTCACCCAGGTGCCGCTGCAAAA CGGCGTTCCACAGGTGCCGCAGAAC CGGTCACCCAGGTGCCGCTGCAGAA ACGCCTGACCGCGCGTTCAGCAAA CGGCGTTCCACCAGGTGCCAGAC GCTGGCAAGTCTGGAAGCTCATAA CGTGGCGATCCGCAGACGTCAAAA CGCGGGTTTCCACACCTGCTGGC CCTGTGTGAAGAACGTAATATCCAT CGCGGCACGGTAAAGATGACTTTG CAATTCTGACCCGCTTCCTGAAATA AGGATCCTTCAGC	Bbsl (5'end) Bbsl (3'end)
Sequence encoding oxygen- insensitive NAD(P)-dependent nitroreductase EntNfsB (NfsB) of Enterobacter cloacae	ATCAGCTGCTGCGCGCAAGACGAAG TGACGCGTGAACTGCGCGCGCAAAC TGCTGGTTCAGCTGACCAGCGGTT CTCCGGCACTGGCTGGCCACCAGGGAAC TGATTATCTGGACGGTGCGAACAGG AAACGTGGGCGCGCCAACATGGCA TTGATTATCTGGACGGTGCGATCAT GGCCACCCCGGATTTTATTGGCCA GGCAGAATGCGCTCTGCTGAAAG GGCAGCACCGCGGCCCTGTTCGAAAA ACACCGTGCTGTCCTGAATGTGGCTG GGCGGTGCCACCAGCCATGTCGGC GAAGATGTTGGTCATGCCTCAGCAC TGGACAGCGCCCTGTTCGGTACGCT GCAAGCACTGGCTATTTCTCGCGCA GAAGGCATCCCGCTGGAAAAAACC ACGGCGTTTATCAAACTGACCGAAC CGGTCACCCAGGGTGCCGTTGCAG ATGTCCTGACCGCGGTGCCGTTGCAG ATGTCCTGACCGCGGTGCCGTTGCAG ATGTCCTGACCGCGGTGCCGTTGCAG CCTGGCAAGTCTGGAAGCTCATAA CGCGGGTTCCAACACTGGCCAGAC GCTGGCAAGTCTGGAAGCTCATAA CGCGGGTTCCAACACCTGCTGGC CCTGGCAAGTCTGGAAGCGAAAAACC ACGCGTTCCACCACTGCTGCG CCTGGCAAGTCTGGAAGCTCATAA CGCGGCGTTCCAACACCTGCTGGC CCTGGCACGGTAAAGATGACTTTG CAATTCTGACCGCGCTCCTGAAATA <b>AGGATCC</b> TTCAGC	Bbsl (5'end) Bbsl (3'end)
Sequence encoding oxygen- insensitive NAD(P)-dependent nitroreductase EntNfsB (NfsB) of <i>Enterobacter cloacae</i>	ATCAGCTGCTGCGCAAGACGAAG TGACGCGTGAACTGCCCAAGACGAAG TGCTGGTTCAGCTGACCAGCGGCAAAC TGCTGGTTCAGCTGGCCACAGCGGTT CTCCGGCACTGGCTCGTGAACAGG AAACGTGGGCGCGCCCAACATGGCA TTGATTATCTGGACGGTGCGGATCAT GGCACCCCGGATTTTATTGGCCA GGCAGAATGCGCTCTGCTGTACAG TGGTTCCGCGGCCTGTTCGAAAA ACACCGTGCTGTCCTGAATGTGCGC GAAGATGTTGGTCATGCCTCAGCAC TGGACAGCGCCCTGCTGTTCAGAT GTGGGGCACCCTGCTGTTCCAGCAC TGGACAGCGCCCTGCTGTTCCAGCAC TGGACAGCGCCCTGCTGTTCCAGCAC GCAGGCACCCGCTGCTGTTCCGCACAC GCAGGCACCCGCTGCTGTTCCGCACAC GCAGGCACCCGCTGGAAAAAACC ACGGCGTTTATCAAACTGACCGAAC ACGGCGTTCCAACACGCCTGCTGCAG ATGTCCTGACCGCAGACGCTCAGAC GCTGGCAAGTCTGGAAGCTCATAA CGTCGGCGTTCCAACACCTGCTGGC CCTGTGTGAAGAACGTAATATCCAT CCCGTCATCCGGAAGCGCATGTAC CCGGCCACGGTAAAGATGACTTG CAATTCTGACCCGCTGAAACACTACA CCGGCCACGCTGAAACGCCATCTAA CCGGCCACGCTGAAACGCCATCT CCATCTGAAGACGAACGCCATCT ACGAGCGTTCCACCCGCAGACGCCACTCT ACCAAGGCGTTCCACCCGCAGCACCACTCT ACCAAGGCGTTCCACCCGCAGCACCACTCT ACCAAGGCGTTCGACGCAACACACACACC CATCTGACCCCGCGAACGCCACTCT ACCAAGGCGTTCGACGCAACCCCCCTCT ACCAAGGCGTTCGACGCAAGCAAA AAACTGACCCGCGCAACCACCCCCACCTCT ACCAAGCCCACCGCAACCACCCCCACCTC ACCAAGCCCTCGCAACACCCACCCCAACACACCCCCCCCACCCTGCAGCAAAA AAACTGACCCCGCCCAACCCACCCCAACCACCCCACCCAC	Bbsl (5'end) Bbsl (3'end)
Sequence encoding oxygen- insensitive NAD(P)-dependent nitroreductase EntNfsB (NfsB) of Enterobacter cloacae	ATCAGCTGCTGCGCAAGACGAAG TGACGCGTGAACTGCGCGCGCAAAC TGCTGGTTCAGCTGACCAGCGGTT CTCCGGCACTGGCTGGCCACCAGCGGTT CTCCGGCACTGGCTGGCGACAAG AAACGTGGGCGCGCCAACATGGCA TTGATTATCTGGACGGTGCGACAT GGCACCCCGGATTTTATTGGCCA GGCAGAATGCGCTCTGCTGTACAG TGGTTCCGCGGCCCTGTTGAAAA ACACCGTGCTGTCCTGAATGTGCTG GGCGGTGCCACCAGCCATGTCGGC GAAGATGTTGGTCATGCCTCAGCAC TGGACAGCGCCCTGCTGTTCCAGAAA ACACCGTGCTGTCCTGAATGTCGGC GAAGATGTTGGTCATGCCTCAGCAC TGGACAGCGCCCTGCTGTTCCAGAAC GCAGCACTGGCTATTCCGCGCA GAAGGCATCCCGCTGGAAAAAACC ACGGCGTTTATCAAACTGACCGAAC CGGTCACCCAGGTGCCGTGCAGAA ATGTCCTGACCCGTGTTCAGCAAA CGTGGCAAGTCTGGAAGACCCAGAC GCTGGCAAGTCTGGAAGCTCATAA CGTGGCGTTCCAACACCTGCTGGC CCTGTGTGAAGAACGTAATATCCAT CGCGGTATTCCGGAAGCTCATAA CGCGCCCTGACCCGTGAAAGATGACTTTG CAATTCTGACCCGTGAAACGCCATGTAC TCCGTTATTCGACCGCTTCCTGAAATA <b>AGGATCC</b> TTCAGC CATCTGACCCGTGAAACGCCATCT ACCAAGGCGTTCCAACACTGGATACCT TTCTGTCGCCCTGAAACGCCAACAT AAATTCAAACCTGCCGCAGCAAAA AAACTGACCGCGCGCAAGCAAAAACC CCCGTCCAGCACACCTGCTGCAGAAAAACC ACGCCGCCACGCGAAGAAGCGCAAAA AAACTGACCGCTCCAACACCTGCTGCAG CCCGCCCAGCACCACCTCCCACACACCCGCCAGCAAAAACCCACCTGCTGCAGCAAAAACCCAACCTGCCAACACCACCTCT ACCAAGGCGTTCGACGCAACACCACCACCTCT ACCAAGGCGTTCGACGCAAGCAAAAACCCACCTGCCGCAAGCAA	<i>Bbsl</i> (5'end) <i>Bbsl</i> (3'end)
Sequence encoding oxygen- insensitive NAD(P)-dependent nitroreductase EntNfsB (NfsB) of Enterobacter cloacae	ATCAGCTGCTGCGCAAGACGAAG TGACGCGTGAACTGCGCGCGCAAAC TGCTGGTTCAGCTGACCAGCGGTT CTCCGGCACTGGCTCGTGAACAGG AAACGTGGGCGCGCGCAACATGGCA TTGATTATCTGGACGGTGCGATCAT GGCCACCCCGGATTTTATTGGCCA GGCAGAATGCGCTCTGCTGTACAG TGGTTCCGCGGCCCTGTTGAAAA ACACCGTGCTGTCCTGAATGTGCTG GGCGGTGCCACCAGCCATGTCGGC GAAGATGTTGGTCATGCCTCAGCAC TGGACAGCGCCCTGCTGTTCAGAT GTGGGGCACCCTGCTGTTCCAGAC TGGACAGCGCCCTGCTGTTCCAGAC TGGACAGCGCCCTGCTGTTCCAGCAC GAAGATGTTGGTCATGCCTCAGCAC GGAGACTGCGCCCTGCTGTTCCAGAT GTGGGGCACCCTGTTCGGTACGCT GCAAGCACTGGCTATTTCTCGCGCA GAAGCACTGGCTATTTCTCGCGCA GAAGGCATCCCGCTGGAAAAAACC ACGGCGTTTATCAAACTGACCGAAC CGGTCACCCAGGGTGCCGTTGCAG ATGTCCTGACCCGCTGTTCAGCAAAA TCGCCTGACGCAGACGCTCATAA CGTGGCGATCCGCAGACGCTCATAA CGTGGCGAGTTCCAACACCTGCTGGC CCTGTGTGAAGAACGTAATATCCAT CGCGGCATCCGCGCAGACGCTCAGAAG CCGGCCACGGTAAAGATGACTTTG CAATTCTGACCCGCTTCCAGACGTCAAAG CCGGCCACGGTAAAGATGACTTTG CAATTCTGACCCGCTTCCAGACACCTTC CATTCTGACCCGCGCAGCACCACTCT ACCAAGGCGTTCCAACACGCCACTCT ACCAAGGCGTTCCAACACGCCACTCT ACCAAGGCGTTCGAAACGCCACTCT ACCAAGGCGTTCGAAACGCCACTCT ACCAAGGCGTCCAGCACGCCACGC	Bbsl (5'end) Bbsl (3'end)
Sequence encoding oxygen- insensitive NAD(P)-dependent nitroreductase EntNfsB (NfsB) of Enterobacter cloacae	ATCAGCTGCTGCGCGCGAAGACGAAG TGACGCGTGAACTGCGCGCGCAAAC TGCTGGTTCAGCTGACCAGCGGTT CTCCGGCACTGGCTCGTGAACAGG AAACGTGGGCGCGCGCCAACATGGCA TTGATTATCTGGACGGTGCGATCAT GGCCACCCCGGATTTTATTGGCCA GGCAGAATGCGCTCTGCTGAACAG TGGTTCCGCGGCCCTGTTCGAAAA ACACCGTGCTGTCCTGAATGTGGCTG GGCGGTGCCACCAGCCATGTCGGC GAAGATGTTGGTCATGCCTCAGCAC TGGACAGCGCCCTGTTCGGTACGGC GAAGCACTGGCCACCAGCCATGTCGGC GAAGCACTGGCCACCAGCCATGTCGCC GCAGGCACCCTGTTCGGTACGCT GCAAGCACTGGCTATTTCTCGCGCA GAAGGCATCCCGCTGGAAAAAACC ACGGCGTTTATCAAACTGACCGAAC CGGTCACCCAGGGTGCCGTTCAGAA ACGCCTGACCCGCTGGTTCAGCAAA CGGCGTTCCACCGCGGTTCAGCAAA CGGCGTTCCACCGCGGTTCAGCAAA CGGCGTTCCACCCGTGTTCAGCAAAA CGCCTGACCCGCGGAAGCTCATAA CGTGGCGATCCGCCGCTGCAGAC GCTGGCAAGTCTGGAAGCTCATAA CGTGGCGTTCCAACACCTGCTGGC CCTGTGTAAGAACGTAATATCCAT CGCGGCACGTTCAGCAACGTCATAA CCGGCCACGGTAAAGATGACTTTG CAATTCTGACCCGCTTCCTGAAATA AGGATCCTTCAGC CATCTGACCCGCGCAGCCCATGAC TTCTGCCCCGGAAGAAGCGCAAAA AAACTGACCGCGGAAGAAGCGCAAA AAACTGAACCCTGCTGCAGCAC CGTGCCAGCGACCAACCCAGCCAGCAC AAGCCGTCCAGCACCAACTCCCAGC CGTGGCAACTCGGAAGCAAGCGGAA AAAATCAAAACCCTGCTGCAGGCA CGGCCCCGGCAACCAACTCCCAGC CGTGGCACTTCATTGTAGCCAGCAC CGTGGCACTTCATTGTAGCCAGCAC CGTGGCACTTCATTGTAGCCAGCAAC AAAATCAAAACCCTGCTGCAGCAAC CGGGCACCGCGAACAACCCAGCCACCC CGTGGCACTTCATTGTAGCCAGCAC CGAGGAAGGAAAAGCGCGCGCGGCGCG	Bbsl (5'end) Bbsl (3'end)

GTTCAACGAACGCAAAATGCTGGAT	
GCTTCCCACGTGGTGGTGTTCTGC	
GCGAAAACCGCGATGGATGACGCC	
TGGCTGGAGCGCGTCGTGGATCAG	
GAAGAGGCCGATGGCCGTTTCAAC	
ACGCCGGAAGCCAAAGCCGCAAAC	
CATAAGGGCCGCACCTACTTCGCC	
GACATGCACCGCGTGGATCTGAAA	
GATGACGACCAGTGGATGGCGAAG	
CAGGTTTACCTGAACGTCGGCAACT	
TCCTGCTGGGCGTGGGCGCGATGG	
GTCTGGACGCGGTACCAATTGAAG	
GTTTCGACGCCGCTATTCTCGACGA	
AGAGTTTGGCCTGAAAGAGAAAGG	
CTTCACCAGCCTGGTGGTGGTACC	
GGTTGGGCACCACAGCGTGGAAGA	
TTTCAACGCCACGCTGCCGAAATCT	
CGCCTGCCGCTGAGCACGATTGTG	
ACCGAGTGC <b>TAA</b> GGA <mark>GTCTTC</mark> AGA	
GA	

## Supplementary Table 6. Sequence of saturated positions for 10 random variants of the CBADH library and the selected variant.

Enzymo / voriant	Position						
Enzyme / Vanant	198	199	200	218			
WT	GGC	AGT	CGG	TAC			
R1	тсс	CGG	ACC	TTG			
R2	TTA	TTC	TAA	Deletion of 210 bp			
R3	TTC	CGT	ΑΤΑ	CGG			
R4	TCA	GCT	GTA	TAG			
R5	AAT	GTG	ACA	GGG			
R6	СТА	GCG	AAC	GGG			
R7	CAT	стс	CAA	ACC			
R8	AAC	тсс	TCA	GTG			
R9	AAA	CCA	TCA	GTT			
R10	GCC	ссс	AGG	CGT			
CBADHs	GAC	TAT	AGA	CCG			

**Supplementary Table 7. Sequence of saturated positions for 10 random variants of the TBADH library and selected variants.** TBADH<sub>S2</sub> contained a duplication of residues 191 to 241, in addition to substitutions in the targeted residues both in the positions of the original sequence and in the corresponding positions of the duplication. 198', 199', 200' and 218' denote positions in the duplication equivalent to the original residues 198, 199, 200 and 218. These positions only exist in TBADH<sub>S2</sub>.

Enzyme /	Position								
variant	198	199	200	218	198'	199'	200'	218'	
wт	GGT	тст	CGT	ТАТ	-	-	-	-	
R1	СТТ	тсс	CGC	TTA	-	-	-	-	
R2	TGA	CGC	CGA	GTT	-	-	-	-	
R3	стс	тсс	CGC	GTG	-	-	-	-	
R4	AAT	CAA	AAG	AGG	-	-	-	-	
R5	TTA	ACA	CGA	CGC	-	-	-	-	
R6	AGC	ACC	CGA	CGA	-	-	-	-	
R7	AAC	TGA	ACT	GTA	-	-	-	-	
R8	ссс	CAG	AGG	AGA	-	-	-	-	
R9	Insertion 153 bp GCA	TCA	CGT	TAC	-	-	-	-	
R10	GAC	ТАТ	AGA	CCG	-	-	-	-	
TBADH <sub>S1</sub>	ТСА	AAA	CGG	GTA	-	-	-	-	
TBADH <sub>S2</sub>	CAC	CGC	GCC	ATG	GCA	TCA	AAA	TAC	

## Supplementary Table 8. Sequence of saturated positions for 10 random variants of the MsIRED library and the selected variant.

Enzyme /	Position							
variant	32	33	34	37	67	71		
wт	AAC	CGT	ACC	AAA	ATC	ACC		
R1	GGT	GGT	GAT	GGG				
R2	GGT	GGG	GGG	GGG				
R3	GTG	тст	GCT	GGG				
R4	СТТ	CGT	ACT	ттт				
R5	CAT	GAT	GCG	CAG				
R6	TTG	СТТ	ТАТ	GTG				
R7	СТА	ATT	GTT	ACT				
R8	CCG	AAG	ТАТ	TGT				
R9	ATT	GTT	TAG	ATG				
R10	GAG	CGG	GTG	TTG				
MSIREDc	GAA	ТАТ	GAA	CGT	ATT	GTT		
MSIREDs	GAG	GTG	CGG	CGG				

## Supplementary Table 9. Sequence of saturated positions for 10 random variants of the EntNfsB library and the selected variants.

Enzyme / variant	Position						
	40	41	68	124			
WT	AGC	ACC	ТАТ	TTC			
R1	TTA	TTA	GTG	CAA			
R2	AAC	GTA	TAG	ccc			
R3	TCA	GTA	AAT	GTA			
R4	TAC	ΑΤΑ	ССТ	ACC			
R5	GTA	TTA	TGT	TGC			
R6	AGC	TTC	AAG	TTA			
R7	TCC	TTC	ттт	CAA			
R8	CGA	ΑΤΑ	TAG	TGC			
R9	СТА	TAA	TCG	ccc			
R10	CAA	AAC	ATG	CCA			
EntNfsB <sub>S1</sub>	GCA	ATA	ТАТ	GCA			
EntNfsB <sub>s2</sub>	TCA	СТА	СТТ	стс			

Mutant strain	Transformed plasmid/library	Antibiotic	Added external substrate	Generated reduced product
AL	pUC19	Ampicillin	As required for the complementation experiment	-
AL	pLS1	Ampicillin	-	-
AL	pLS2	Ampicillin	15 mM acetoin	2,3-butanediol
AL	pLS3	Ampicillin	15 mM acetoin	2,3-butanediol
AL	pLS6	Ampicillin	15 mM acetone	Isopropanol
AL	pLS10 (library)	Ampicillin	15 mM acetone	Isopropanol
AL	pLS10_3	Ampicillin	15 mM acetone	Isopropanol
AL	pLS12	Ampicillin	10 mM cyclohexanone or 3- methylcyclohexanone	Cyclohexanol or 3- methylcyclohexanol
AL	pLv2	Kanamycin	-	-
AL	pLS60	Kanamycin	-	Isopropanol
ALPS	pLS69	Ampicillin	15 mM acetone	Isopropanol
ALPS	pLS73 (library)	Ampicillin	15 mM acetone	Isopropanol
ALPS	pLS73_1	Ampicillin	15 mM acetone	Isopropanol
ALPS	pLS73_2	Ampicillin	15 mM acetone	Isopropanol
AL	pLS130	Ampicillin	15 mM 2-methyl-1-pyrroline	2-methylpyrrolidine
AL	pLS131	Ampicillin	15 mM 2-methyl-1-pyrroline	2-methylpyrrolidine
AL	pLS133 (library)	Ampicillin	15 mM 2-methyl-1-pyrroline	2-methylpyrrolidine
AL	pLS133_1	Ampicillin	15 mM 2-methyl-1-pyrroline	2-methylpyrrolidine
AL	pLS168	Ampicillin	2.5 mM 4-nitrobenzoic acid	4-aminobenzoic acid
AL	pLS168	Ampicillin	8 mM 2-nitrobenzoic acid	2-aminobenzoic acid
AL	pLS168	Ampicillin	2.5 mM 4-nitrobenzyl alcohol	4-aminobenzyl alcohol
AL	pLS169 (library)	Ampicillin	8 mM 2-nitrobenzoic acid	2-aminobenzoic acid
AL	pLS169_1	Ampicillin	8 mM 2-nitrobenzoic acid	2-aminobenzoic acid
AL	pLS169 (library)	Ampicillin	2.5 mM 4-nitrobenzyl alcohol	4-aminobenzyl alcohol
AL	pLS169_3	Ampicillin	2.5 mM 4-nitrobenzyl alcohol	4-aminobenzyl alcohol

#### Supplementary Table 10. Conditions for anaerobic growth experiments.

Supplementary Table 11. Characteristic <sup>1</sup>H-NMR signals used to quantify each compound of interest. The multiplicity of each signal (s – singlet, d – doublet, t – triplet, q – quartet, m – multiplet) and the number of contributing protons is shown between brackets.

Metabolite	$\boldsymbol{\delta}$ for characteristic signal of the metabolite	
	(ppm)	
Ethanol	1.19 (t,3)	
Lactate	1.32 (d,3)	
Succinate	2.41 (s,4)	
Acetate	1.92 (s,3)	
Formate	8.46 (s,1)	
Acetoin	1.38 (d, 3)	
2,3-butanediol	1.15 (d,6)	
Cyclohexanone	2.40 (t,4)	
Cyclohexanol	1.72 (m, 2)	
3-methylcyclohexanone	1.01 (d,3)	
3-methylcyclohexanol	0.88 (d,3)	
Acetone	2.24 (s,6)	
Isopropanol	1.18 (d,6)	
Propionate	2.19 (q, 2)	
2-methyl-1-pyrroline	2.42 (s,3)	
2-methylpyrrolidine	1.38 (d,3)	
4-nitrobenzoic acid	8.01 (d,2)	
4-aminobenzoic acid	7.73 (d,2)	
2-nitrobenzoic acid	8.11 (d,1)	
2-aminobenzoic acid	7.30 (t,1)	
4-nitrobenzyl alcohol	8.27 (d,2)	
4-aminobenzyl alcohol	7.23 (d,2)	

Enzyme	Substrate	Cofactor	Enzyme concentration	Absorbance (nm)
CBADH <sub>WT</sub>	Isopropanol	1 mM NADP⁺	110 nM	340
CBADHwt	Isopropanol	10 mM NAD⁺	110 nM	340
CBADHs	Isopropanol	1 mM NADP⁺	110 nM	340
CBADHs	Isopropanol	10 mM NAD⁺	110 nM	340
TBADH <sub>WT</sub>	Isopropanol	1.2 mM NADP⁺	110.8 nM	340
TBADHwt	Isopropanol	10 mM NAD⁺	110.8 nM	340
TBADH <sub>S1</sub>	Isopropanol	1.2 mM NADP⁺	110 nM	340
TBADH <sub>S1</sub>	Isopropanol	10 mM NAD⁺	110 nM	340
TBADH <sub>S2</sub>	Isopropanol	2.35 mM NADP⁺	51.55 nM	340
TBADH <sub>S2</sub>	Isopropanol	2.35 mM NAD⁺	51.55 nM	340
MsIREDwt	2-methyl-1-pyrroline	0.25 mM NADPH	1.2 µM	340
MsIREDwt	2-methyl-1-pyrroline	0.25 mM NADH	1.2 μM	340
MsIREDc	2-methyl-1-pyrroline	0.25 mM NADH	1.26 µM	340
MsIREDs	2-methyl-1-pyrroline	0.25 mM NADH	1.25 µM	340
EntNfsBwt	4-nitrobenzoic acid	0.3 mM NADH	60.8 nM	370
EntNfsB <sub>wt</sub>	2-nitrobenzoic acid	0.3 mM NADH	60.8 nM	370
EntNfsBwr	4-nitrobenzyl alcohol	0.3 mM NADH	26.3 nM	370
EntNfsB <sub>s1</sub>	4-nitrobenzoic acid	0.3 mM NADH	45 nM	370
EntNfsB <sub>S1</sub>	2-nitrobenzoic acid	0.3 mM NADH	45 nM	370
EntNfsB <sub>s2</sub>	4-nitrobenzyl alcohol	0.3 mM NADH	26.3 nM	370

#### Supplementary Table 12. Conditions for enzymatic activity assays.

	CBADH <sub>s</sub> variant	TBADH <sub>S1</sub> variant
Beamline	Diamond I03	Diamond I04
PDB code	6SCH	6SDM
Wavelength	0.9762	0.9795
Resolution range	59.36 - 2.199 (2.278 - 2.199)	71.68 - 2.85 (2.952 - 2.85)
Space group	P 1 21 1	P 21 21 21
	75.8115 99.5695 114.089 90	79.1234 123.946 169.24 90
Unit cell	102.768 90	90 90
Total reflections	265743 (16967)	258210 (26258)
Unique reflections	82558 (7335)	39642 (3923)
Multiplicity	3.2 (2.3)	6.5 (6.7)
Completeness (%)	98.45 (87.66)	99.87 (99.62)
Mean I/sigma(I)	7.57 (0.71)	6.92 (1.11)
Wilson B-factor	34.89	69.95
R-merge	0.09525 (1.064)	0.1918 (1.671)
R-meas	0.114 (1.345)	0.2086 (1.811)
R-pim	0.06202 (0.8095)	0.08117 (0.6942)
CC1/2	0.996 (0.345)	0.991 (0.554)
CC*	0.999 (0.716)	0.998 (0.844)
Reflections used in	82766 (7315)	30507 (3013)
refinement	02700 (7313)	59597 (5913)
Reflections used for R-free	4037 (357)	2015 (182)
R-work	0.1639 (0.2548)	0.1746 (0.3201)
R-free	0.2068 (0.2927)	0.2390 (0.3647)
CC(work)	0.970 (0.619)	0.955 (0.750)
CC(free)	0.960 (0.587)	0.940 (0.651)
Number of non-hydrogen	11354	10622
atoms	11001	10022
macromolecules	10666	10618
ligands	332	4
solvent	356	0
Protein residues	1417	1416
RMS(bonds)	0.007	0.009
RMS(angles)	0.93	1.06
Ramachandran favored (%)	95.87	95.10
Ramachandran allowed (%)	4.13	4.69
Ramachandran outliers (%)	0.00	0.21
Rotamer outliers (%)	0.97	0.27
Clashscore	5.11	4.48
Average B-factor	45.12	77.08
macromolecules	44.13	77.04
ligands	78.13	174.49
solvent	43.81	-
Number of TLS groups	18	15

Supplementary Table 13. Data collection parameters and refinement statistics for the crystal structures of CBADHs and TBADHs1.