

Supplementary Table S1 All strains used in this study

Strain ID	Relevant genotype	Parental strain	Origin
Modular assembly			
WT	<i>A. pullulans</i> CTCC M2012223		X. Zou, Y. P. Zhou and S. T. Yang, <i>Biotechnol. Bioeng.</i> , 2013, 110, 2105-2113
EGG 01	WT-OE::(<i>gpdAp-adh-trapCt</i>)	WT	This study
EGG 02	WT-OE::(<i>gpdAp-acs-trapCt</i>)	WT	This study
EGG 03	WT-OE::(<i>gpdAp-adh</i> -"gggs"- <i>acs-trapCt</i>)	WT	This study
EGG 04	WT-OE::(<i>gpdAp-icl-trapCt</i>)	WT	This study
EGG 05	WT-OE::(<i>gpdAp-mls-trapCt</i>)	WT	This study
EGG 06	WT-OE::(<i>gpdAp-icl</i> -"gggs"- <i>mls-trapCt</i>)	WT	This study
EGG 07	WT-OE::(<i>gpdAp-cat8-trapCt</i>)+(<i>gpdAp-icl</i> -"gggs"- <i>mls-trapCt</i>)	EGG 06	This study
EGG 09	WT-OE::(<i>gpdAp-adh</i> -"gggs"- <i>acs-trapCt</i>)+(<i>gpdAp-cat8-trapCt</i>)+(<i>gpdAp-icl</i> -"gggs"- <i>mls-trapCt</i>)	EGG 07	This study
EGG 10	WT-OE::(<i>gpdAp-adh</i> -"gggs"- <i>acs-trapCt</i>)+(<i>gpdAp-cat8-trapCt</i>)+(<i>gpdAp-icl</i> -"gggs"- <i>mls-trapCt</i>)+(<i>gpdAp-pck-trapCt</i>)	EGG 09	This study
EGG 11	WT-OE::(<i>Asp. PGKp-cat8-CYC1t</i>)+(<i>gpdAp-icl</i> -"gggs"- <i>mls-trapCt</i>)	EGG 07	This study
EGG 12	WT-OE::(<i>Asp. FBAP-cat8-CYC1t</i>)+(<i>gpdAp-icl</i> -"gggs"- <i>mls-trapCt</i>)	EGG 07	This study
EGG 13	WT-OE::(<i>Asp. PGIp-cat8-CYC1t</i>)+(<i>gpdAp-icl</i> -"gggs"- <i>mls-trapCt</i>)	EGG 07	This study
EGG 14	WT-OE::(<i>Rt. FBAP-cat8-CYC1t</i>)+(<i>gpdAp-icl</i> -"gggs"- <i>mls-trapCt</i>)	EGG 07	This study
EGG 15	WT-OE::(<i>Rt. PGIp-cat8-CYC1t</i>)+(<i>gpdAp-icl</i> -"gggs"- <i>mls-trapCt</i>)	EGG 07	This study
Promoter engineering			
EGG 16	WT-OE::(<i>Asp. PGKp-cat8-CYC1t</i>)+(<i>gpdAp-icl</i> -"gggs"- <i>mls-trapCt</i>)+(<i>Asp. FBAP-adh</i> -"gggs"- <i>acs-CYC1t</i>)	EGG 11	This study
EGG 17	WT-OE::(<i>Asp. PGKp-cat8-CYC1t</i>)+(<i>gpdAp-icl</i> -"gggs"- <i>mls-trapCt</i>)+(<i>Rt. FBAP-adh</i> -"gggs"- <i>acs-CYC1t</i>)	EGG 11	This study

EGG 11N1	WT-OE:: <i>(Asp. PGKp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Asp. PGKp-adh-"gggs"-acs-CYC1t)</i>	EGG 11	This study
EGG 11N3	WT-OE:: <i>(Asp. PGKp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Asp. PGIp-adh-"gggs"-acs-CYC1t)</i>	EGG 11	This study
EGG 11N5	WT-OE:: <i>(Asp. PGKp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Rt. PGIp-adh-"gggs"-acs-CYC1t)</i>	EGG 11	This study
EGG 18	WT-OE:: <i>(Asp. FBAp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Asp. PGIp-adh-"gggs"-acs-CYC1t)</i>	EGG 12	This study
EGG 12N1	WT-OE:: <i>(Asp. FBAp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Asp. PGKp-adh-"gggs"-acs-CYC1t)</i>	EGG 12	This study
EGG 12N2	WT-OE:: <i>(Asp. FBAp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Asp. FBAp-adh-"gggs"-acs-CYC1t)</i>	EGG 12	This study
EGG 12N4	WT-OE:: <i>(Asp. FBAp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Rt. FBAp-adh-"gggs"-acs-CYC1t)</i>	EGG 12	This study
EGG 12N5	WT-OE:: <i>(Asp. FBAp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Rt. PGIp-adh-"gggs"-acs-CYC1t)</i>	EGG 12	This study
EGG 19	WT-OE:: <i>(Asp. PGIp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Asp. PGIp-adh-"gggs"-acs-CYC1t)</i>	EGG 13	This study
EGG 13N1	WT-OE:: <i>(Asp. PGIp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Asp. PGKp-adh-"gggs"-acs-CYC1t)</i>	EGG 13	This study
EGG 13N2	WT-OE:: <i>(Asp. PGIp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Asp. FBAp-adh-"gggs"-acs-CYC1t)</i>	EGG 13	This study
EGG 13N4	WT-OE:: <i>(Asp. PGIp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Rt. FBAp-adh-"gggs"-acs-CYC1t)</i>	EGG 13	This study
EGG 13N5	WT-OE:: <i>(Asp. PGIp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Rt. PGIp-adh-"gggs"-acs-CYC1t)</i>	EGG 13	This study
EGG 20	WT-OE:: <i>(Rt. FBAp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Asp. PGIp-adh-"gggs"-acs-CYC1t)</i>	EGG 14	This study
EGG 21	WT-OE:: <i>(Rt. FBAp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Asp. PGKp-adh-"gggs"-acs-CYC1t)</i>	EGG 14	This study
EGG 22	WT-OE:: <i>(Rt. FBAp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Asp. PGIp-adh-"gggs"-acs-CYC1t)</i>	EGG 14	This study
EGG 23	WT-OE:: <i>(Rt. FBAp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Rt. PGIp-adh-"gggs"-acs-CYC1t)</i>	EGG 14	This study
EGG 14N2	WT-OE:: <i>(Rt. FBAp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Asp. FBAp-adh-"gggs"-acs-CYC1t)</i>	EGG 14	This study
EGG 14N4	WT-OE:: <i>(Rt. FBAp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Rt. FBAp-adh-"gggs"-acs-CYC1t)</i>	EGG 14	This study
EGG 24	WT-OE:: <i>(Rt. PGIp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Asp. PGIp-adh-"gggs"-acs-CYC1t)</i>	EGG 15	This study
EGG 25	WT-OE:: <i>(Rt. PGIp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Rt. PGIp-adh-"gggs"-acs-CYC1t)</i>	EGG 15	This study
EGG 15N1	WT-OE:: <i>(Rt. PGIp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Asp. PGKp-adh-"gggs"-acs-CYC1t)</i>	EGG 15	This study
EGG 15N2	WT-OE:: <i>(Rt. PGIp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Asp. FBAp-adh-"gggs"-acs-CYC1t)</i>	EGG 15	This study
EGG 15N4	WT-OE:: <i>(Rt. PGIp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Rt. FBAp-adh-"gggs"-acs-CYC1t)</i>	EGG 15	This study
EGG 26	WT-OE:: <i>(Rt. FBAp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Rt. PGIp-adh-"gggs"-acs-CYC1t)+(Asp. PGKp-pck-CYC1t)</i>	EGG 23	This study
EGG 28	WT-OE:: <i>(Rt. FBAp-cat8-CYC1t)+(gpdAp-icl-"gggs"-mls-trapCt)+(Rt. PGIp-adh-"gggs"-acs-CYC1t)+(Asp. FBAp-pck-CYC1t)</i>	EGG 23	This study

EGG 29	WT-OE::(<i>Rt. FBAP-cat8-CYC1t</i>)+(gpdAp-icl-"gggs"-mls-trapCt)+(Rt. PGIp-adh-"gggs"-acs-CYC1t)+(Asp. PGIp-pck-CYC1t)	EGG 23	This study
EGG 30	WT-OE::(<i>Rt. FBAP-cat8-CYC1t</i>)+(gpdAp-icl-"gggs"-mls-trapCt)+(Rt. PGIp-adh-"gggs"-acs-CYC1t)+(Rt. FBAP-pck-CYC1t)	EGG 23	This study
EGG 35	WT-OE::(<i>Rt. FBAP-cat8-CYC1t</i>)+(gpdAp-icl-"gggs"-mls-trapCt)+(Rt. PGIp-adh-"gggs"-acs-CYC1t)+(Rt. PGIp-pck-CYC1t)	EGG 23	This study
Adaptive evolution of 1%~3%(v/v) ethanol			
EGG 36	14 droplet in adaptive evolution	EGG 35	This study
EGG 37	17 droplet in adaptive evolution	EGG 35	This study
EGG 38	19 droplet in adaptive evolution	EGG 35	This study
EGG 39	21 droplet in adaptive evolution	EGG 35	This study
EGG 40	23 droplet in adaptive evolution	EGG 35	This study
EGG 41	26 droplet in adaptive evolution	EGG 35	This study
EGG 42	27 droplet in adaptive evolution	EGG 35	This study
EGG 43	28 droplet in adaptive evolution	EGG 35	This study
EGG 44	41 droplet in adaptive evolution	EGG 35	This study
EGG 45	42 droplet in adaptive evolution	EGG 35	This study
EGG 46	50 droplet in adaptive evolution	EGG 35	This study
Adaptive evolution of 1%~5%(v/v) ethanol			
EGG 47	2 droplet in adaptive evolution	EGG 36	This study
EGG 48	29 droplet in adaptive evolution	EGG 36	This study
EGG 49	32 droplet in adaptive evolution	EGG 36	This study
EGG 50	34 droplet in adaptive evolution	EGG 36	This study
EGG 51	35 droplet in adaptive evolution	EGG 36	This study
EGG 52	36 droplet in adaptive evolution	EGG 36	This study
EGG 53	38 droplet in adaptive evolution	EGG 36	This study
EGG 54	39 droplet in adaptive evolution	EGG 36	This study
EGG 55	40 droplet in adaptive evolution	EGG 36	This study
EGG 56	44 droplet in adaptive evolution	EGG 36	This study
