

# Fine-tuning the expression of glycolate biosynthetic pathway in *Escherichia coli* using synthetic promoters

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**Table S1 Strains and plasmids used in this study**

<b>Name</b>	<b>Relevant genotype</b>	<b>Reference</b>
<b>Strains</b>		
JM109	For plasmid construction	Invitrogen
MG1655	<i>E. coli</i> K12 wild type, for expressing genes	Invitrogen
Mgly4	MG1655(DE3) $\Delta$ ldhA $\Delta$ glcB $\Delta$ aceB $\Delta$ aldA	[14]
Mgly43	Mgly4 carrying pJNU-3	This study
Mgly434	Mgly4 carrying pJNU-3 and pJNU-4	[14]
Mgly45	Mgly4 carrying pTrc-aceA-ycdw	This study
Mgly454	Mgly4 carrying pTrc-aceA-ycdw and pJNU-4	This study
Mgly4T	Mgly4 carrying pGly-2	This study
Mgly4T4	Mgly4 carrying pGly-2 and pJNU-4	This study
MTet	MG1655 carrying pTet-sfGFP, Amp <sup>R</sup>	This study
ML3153	MG1655 carrying pL3153-sfGFP, Amp <sup>R</sup>	This study
ML3429	MG1655 carrying pL3429-sfGFP, Amp <sup>R</sup>	This study
ML1562	MG1655 carrying pL1562-sfGFP, Amp <sup>R</sup>	This study
ML3262	MG1655 carrying pL3262-sfGFP, Amp <sup>R</sup>	This study
ML2353	MG1655 carrying pL2353-sfGFP, Amp <sup>R</sup>	This study
ML3090	MG1655 carrying pL3090-sfGFP, Amp <sup>R</sup>	This study
ML2917	MG1655 carrying pL2917-sfGFP, Amp <sup>R</sup>	This study
ML3149	MG1655 carrying pL3149-sfGFP, Amp <sup>R</sup>	This study
ML3195	MG1655 carrying pL3195-sfGFP, Amp <sup>R</sup>	This study
ML2538	MG1655 carrying pL2538-sfGFP, Amp <sup>R</sup>	This study
ML1993	MG1655 carrying pL1993-sfGFP, Amp <sup>R</sup>	This study

Mrep1(Mgly4T3429)	Mgly4 carrying pRep1, PL3429 promoter	This study
Mrep2	Mgly4 carrying pRep2, PL3429 promoter	This study
Mrep3	Mgly4 carrying pRep3, PL3429 promoter	This study
Mgly4T3153	Mgly4 carrying pGly3153	This study
Mgly4T3429	Mgly4 carrying pGly3429	This study
Mgly4T1562	Mgly4 carrying pGly1562	This study
Mgly4T3262	Mgly4 carrying pGly3262	This study
Mgly4T2353	Mgly4 carrying pGly2353	This study
Mgly4T3090	Mgly4 carrying pGly3090	This study
Mgly4T2917	Mgly4 carrying pGly2917	This study
Mgly4T3149	Mgly4 carrying pGly3149	This study
Mgly4T3195	Mgly4 carrying pGly3195	This study
Mgly4T2538	Mgly4 carrying pGly2538	This study
Mgly4T1993	Mgly4 carrying pGly1993	This study
<b>Plasmids</b>		
pGly-2	pHHD01K harboring ycdW-aceA-aceK, p15 ori, tetR, Ptet, Kan <sup>R</sup>	[27]
pJNU-3	pTrc99a harboring ycdw-aceA-aceK from pGly-2, Amp <sup>R</sup>	[14]
pJNU-4	pCDFDuet-1 harboring gltA from <i>E. coli</i> , Strep <sup>R</sup>	[14]
pTrc-aceA-ycdw	pTrc99a harboring aceA-ycdw from <i>E. coli</i> , Amp <sup>R</sup>	[28]
pL0-sfGFP	pTrc99a harboring the sfGFP from pJKR-H, no trc promoter, Amp <sup>R</sup>	[25]
pTet-sfGFP	pL0-sfGFP harboring Tet promoter, Amp <sup>R</sup>	This study
pL3429-sfGFP	pL0-sfGFP harboring PL3429 promoter, Amp <sup>R</sup>	[25]
pL3429-sfGFP	pL0-sfGFP harboring PL3429 promoter, Amp <sup>R</sup>	[25]

pL3429-sfGFP	pL0-sfGFP harboring PL3429 promoter, Amp <sup>R</sup>	[25]
pL3429-sfGFP	pL0-sfGFP harboring PL3429 promoter, Amp <sup>R</sup>	[25]
pL3429-sfGFP	pL0-sfGFP harboring PL3429 promoter, Amp <sup>R</sup>	[25]
pL3429-sfGFP	pL0-sfGFP harboring PL3429 promoter, Amp <sup>R</sup>	[25]
pL3429-sfGFP	pL0-sfGFP harboring PL3429 promoter, Amp <sup>R</sup>	[25]
pRep1	pGly-2 harboring PL3429 promoter replace Tet promoter, no TetR, Kan <sup>R</sup>	This study
pRep2	pGly-2 harboring PL3429 promoter replace Tet promoter, no TetR, no RBS, Kan <sup>R</sup>	This study
pRep3	pGly-2 harboring PL3429 promoter replace Tet promoter, Kan <sup>R</sup>	This study
pGly3153	pRep1 harboring PL3153 replace PL3429 promoter, Kan <sup>R</sup>	This study
pGly1562	pRep1 harboring PL1562 replace PL3429 promoter, Kan <sup>R</sup>	This study
pGly3262	pRep1 harboring PL3262 replace PL3429 promoter, Kan <sup>R</sup>	This study
pGly2353	pRep1 harboring PL2353 replace PL3429 promoter, Kan <sup>R</sup>	This study
pGly3090	pRep1 harboring PL3090 replace PL3429 promoter, Kan <sup>R</sup>	This study
pGly2917	pRep1 harboring PL2917 replace PL3429 promoter, Kan <sup>R</sup>	This study
pGly3149	pRep1 harboring PL3149 replace PL3429 promoter, Kan <sup>R</sup>	This study
pGly3195	pRep1 harboring PL3195 replace PL3429 promoter, Kan <sup>R</sup>	This study
pGly2538	pRep1 harboring PL2538 replace PL3429 promoter, Kan <sup>R</sup>	This study
pGly1993	pRep1 harboring PL1993 replace PL3429 promoter, Kan <sup>R</sup>	This study

**Table S2 Primers used in this study**

<b>Primer</b>	<b>Sequence (5' to 3')</b>
pRep1 F	GAATTGTGAGCGGATACAATTTACGCAGGAAACAAAC CGAGCACCTAGGTTTCTCCATAC
pRep1 R	CACACACTATACGAGCCGGATGATTAATTGTCAACTCGA GGATAAGCTGTCAAACATGAG
pRep2 F	GAATTGTGAGCGGATACAATTTACGCAGGAAACAAAC CATGGATATCATCTTTTATCAC
pRep2 R	CACACACTATACGAGCCGGATGATTAATTGTCAACTCGA GGATAAGCTGTCAAACATGAG
pRep3 F	GAATTGTGAGCGGATACAATTTACGCAGGAAACAAAC CGAGCACCTAGGTTTCTCCATAC
pRep3 R	CACACACTATACGAGCCGGATGATTAATTGTCAATTTCT CCTCTTTGCTAGCGTGAAGAC
pGly3429 F	GAATTGTGAGCGGATACAATTTACGCAGGAAACAAAC CGAATTCCAACGCTTTTCGGG
pGly3429 R	CACACACTATACGAGCCGGATGATTAATTGTCAACTCGA GGATAAGCTGTCAAACATGA
pGly3153 F	GAATTGTGAGCAGATAACAATTTACACAGGAAACAGA CCGAATTCCAACGCTTTTCGGGAGTCAGTATG
pGly3153 R	CACACATTTACGAGCCGGATGATTAATTGTCAACTCGAG GATAAGCTGTCAAACATGAGCAGATCCTCTACG
pGly1562 F	GAATTGTGGGCGGATAAACTTTACACAGGAAACAGA CCGAATTCCAACGCTTTTCGGGAGTC
pGly1562 R	CGCATACTATACGAGCCGGATGATTAATTGTCAACTCGA

	GGATAAGCTGTCAAACATGAGCAG
pGly3262 F	GAACTGTGGGCGGATACAATTTACACAGGAAACAGAC CGAATTCCAACGCTTTTCGGG
pGly3262 R	CACACATTATACGAGCCGGATGATTAATTGTCAACTCGA GGATAAGCTGTCAAACATGAG
pGly2353 F	GAATTGTGGGCGGATAACAATTTACACAGGAAACAGA CCGAATTCCAACGCTTTTCGGGAGTC
pGly2353 R	CACACATTATACTAGCCGGATGATTAATTGTCAACTCGA GGATAAGCTGTCAAACATGAGCAG
pGly3090 F	GAATTGTGGGCGGATAGCATTTTACACAGGAAACAGAC CGAATTCCAACGCTTTTCGG
pGly3090 R	CACGCATTATACGAGCCGGATGATTAATTGTCAACTCGA GGATAAGCTGTCAAACATGA
pGly2917 F	GAATTGTGAGTGGATAACAATTTACACAGGAAACAGA CCGAATTCCAACGCTTTTCGG
pGly2917 R	CACACATTATACGGGCCGGATGATTAATTGTCAACTCGA GGATAAGCTGTCAAACATGA
pGly3149 F	GAATCGTGAGCGGATACAATTTACACAGGAAACAGAC CGAATTCCAACGCTTTTCGGGAGTCAGTATG
pGly3149 R	CACACATTATACGAGCCGGATGATTAATTGTCAACTCGA GGATAAGCTGTCAAACATGAGCAGATCCTCTACG
pGly3195 F	GAATTGCGGGCGGATAACAATTTACACAGGAAACAGA CCGAATTCCAACGCTTTTCGGGAGTCAGTATG
pGly3195 R	CACACATTATAGAGCCGGATGATTAATTGTCAACTCGAG GATAAGCTGTCAAACATGAGCAGATCCTCTACG

pGly2538 F	GAATTGTAAGCGGATAACAATTTACACAGGAAAAAGA CCGAATTCCAACGCTTTTCGG
pGly2538 R	CACACATTATACGAGCCGGATGATTAATTGTCAACTCGA GGATAAGCTGTCAAACATGA
pGly1993 F	GAATTGTGAGTGGATAACAATTTACACAGGAAACAGA CGGAATTCCAACGCTTTTCGG
pGly1993 R	CACACATTATACGAGCCGGATGATTAATTGTCAACTCGA GGATAAGCTGTCAAACATGA
veri-pGly3429 F	CGTATAGTGTGTGGAATTGTGAGCGG
veri-pGly3153F	CGTAAATGTGTGGAATTGTGAG
veri-pGly1562 F	CGTATAGTATGCGGAATTGTGGGC
veri-pGly3262 F	CGTATAATGTGTGGAAGTGTGGGCGGATAC
veri-pGly2353 F	GCTAGTATAATGTGTGGAATTGTGGG
veri-pGly3090 F	CGTATAATGCGTGGAATTGTG
veri-pGly2917 F	CGTATAATGTGTGGAATTGTGAG
veri-pGly3149 F	CGTATAATGTGTGGAATCGTGAG
veri-pGly3195 F	CTATAATGTGTGGAATTGCGGG
veri-pGly2538 F	CGTATAATGTGTGGAATTGTAAG
veri-pGly1993 F	AATGTGTGGAATTGTGAGTGGATAAC
veri-ycdW R	GTTCTTCCCGTCCGGCAAAG
q16s F	TCTTGACATCCACAGAACTT
q16s R	TAACCCAACATTTACAACA
qycdw F	TACCCGTCCCGCTGAAGCTG
qycdw R	ACTTGCCCGCAGACCCTCTC
qaceA F	CGATGCTGATGCGGCGGATC

qaceA R	ACGGCTGATCGCTTGCTCAATG
qaceK F	AAGACGAACTTGCCAGCGAACC
qaceK R	GAACAGGTCGGCGTGCATCTC

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**Table S3 Synthetic promoters sequence used in this study**

Promoter	Sequence (5' to 3')
PL3153	TTGACAATTAATCATCCGGCTCGTAAATGTGTGGAATTG TGAGCAGATAACAATTTACACAGGAAACAGACC
PL3429	TTGACAATTAATCATCCGGCTCGTATAGTGTGTGGAATT GTGAGCGGATAACAATTTACGCAGGAAACAAACC
PL1562	TTGACAATTAATCATCCGGCTCGTATAGTATGCGGAATT GTGGGCGGATAACACTTTCACACAGGAAACAGACC
PL3262	TTGACAATTAATCATCCGGCTCGTATAATGTGTGGAATT GTGGGCGGATAACAATTTACACAGGAAACAGACC
PL2353	TTGACAATTAATCATCCGGCTAGTATAATGTGTGGAATT GTGGGCGGATAACAATTTACACAGGAAACAGACC
PL3090	TTGACAATTAATCATCCGGCTCGTATAATGCGTGGAATT GTGGGCGGATAGCATTTTACACAGGAAACAGACC
PL2917	TTGACAATTAATCATCCGGCCCGTATAATGTGTGGAATT GTGAGTGGATAACAATTTACACAGGAAACAGACC
PL3149	TTGACAATTAATCATCCGGCTCGTATAATGTGTGGAATC GTGAGCGGATAACAATTTACACAGGAAACAGACC
PL3195	TTGACAATTAATCATCCGGCTCTATAATGTGTGGAATTG CGGGCGGATAACAATTTACACAGGAAACAGACC
PL2538	TTGACAATTAATCATCCGGCTCGTATAATGTGTGGAATT GTAAGCGGATAACAATTTACACAGGAAAAAGACC
PL1993	TTGACAATTAATCATCCGGCTCGTATAATGTGTGGAATT GTGAGTGGATAACAATTTACACAGGAAACAGACG

**Table S4 The transcription levels of *aceK*, *aceA* and *ycdW* genes in Mgly4T and Mrep1(Mgly4T3429).**

Strains	<i>ycdW</i>	<i>aceA</i>	<i>aceK</i>
Mgly4T	0.86	2.98	3.2
Mrep1	0.72	3.17	5

Note: strain Mgly4 was used as the control.

**Table S5 The transcription levels of *aceK*, *aceA* and *ycdW* genes in different strains.**

Strains	<i>ycdW</i>	<i>aceA</i>	<i>aceK</i>
Mgly4T3153	1.38	0.82	0.77
Mrep1(Mgly4T3429)	0.72	3.17	5
Mgly4T1562	0.26	2.78	3.79
Mgly4T3262	0.31	2.77	5.5
Mgly4T2353	1.71	4.44	5.62
Mgly4T3090	0.61	2.26	3.23
Mgly4T2917	0.27	2.47	3.87
Mgly4T3149	2.59	2.55	0.11
Mgly4T3195	2.33	0.55	1.12
Mgly4T2538	1.08	1.39	2.49
Mgly4T1993	0.7	2.3	3.72

Note: strain Mgly4 was used as the control.

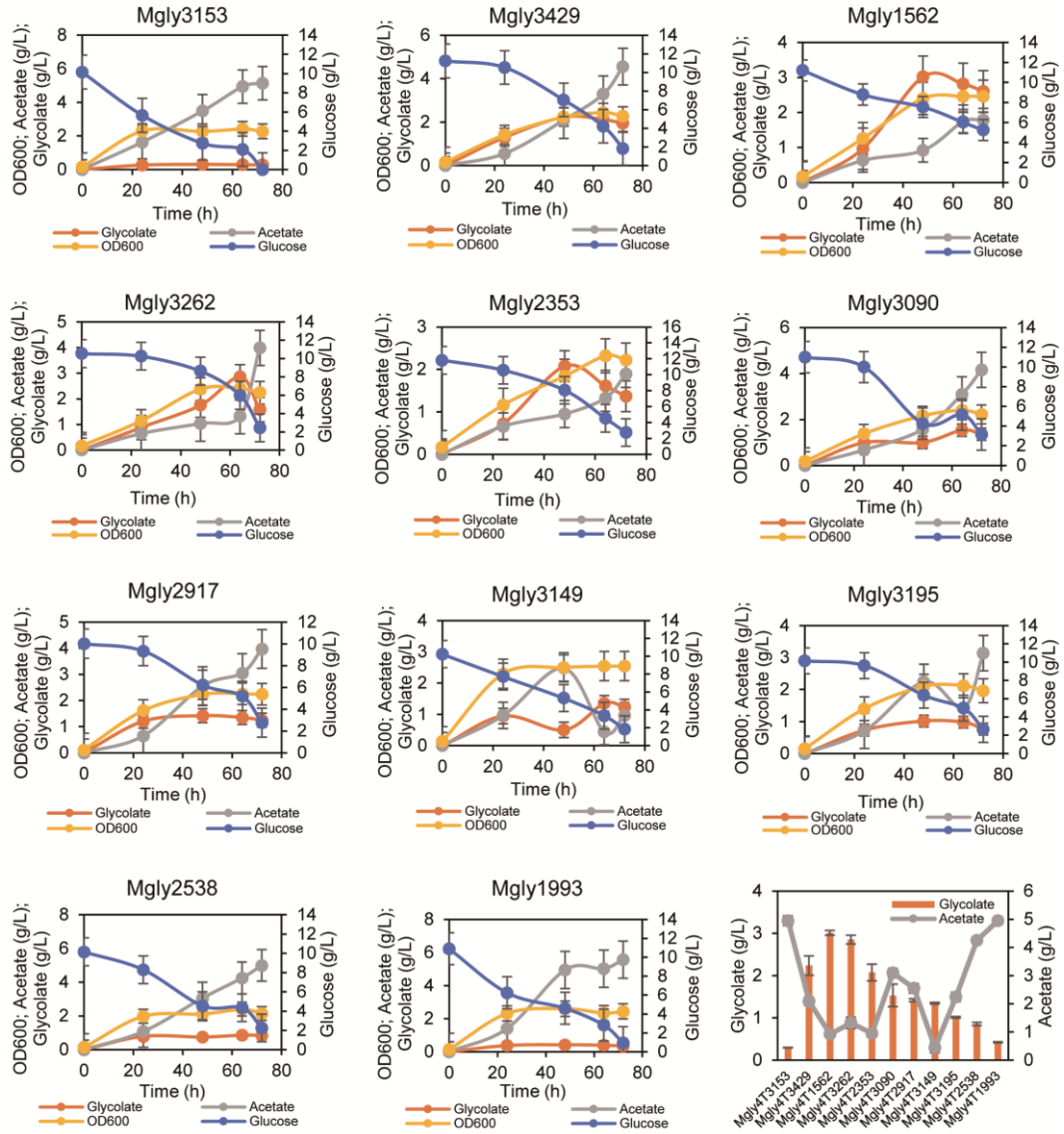


Figure S1 Cultivation process of strain *Mgly4T*3153, *Mgly4T*3429, *Mgly4T*1562, *Mgly4T*3262, *Mgly4T*2353, *Mgly4T*3090, *Mgly4T*2917, *Mgly4T*3149, *Mgly4T*3195, *Mgly4T*2538, *Mgly4T*1993 and comparison of the highest glycolate and acetate titer.