Supplementary Materials

Quorum Sensing Systems Engineering for Enhanced *iso*-Butylamine Production in *Escherichia coli*

Mingxiong Liu [1], Yang Li [1], Pingru Yu [1], Hongxin Fu [1,2,3,*] and Jufang Wang [1,2,3,*]

[1] School of Biology and Biological Engineering, South China University of Technology, Guangzhou 510006, China; 1787858593@qq.com (M.L.); 1239098031@qq.com (Y.L.); 34215251@qq.com (P.Y.)
[2] Guangdong Provincial Key Laboratory of Fermentation and Enzyme Engineering, South China University of Technology, Guangzhou 510006, China
[3] State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 5100640, China

* Corresponding author. E-mail: hongxinfu@scut.edu.cn (H.F.); jufwang@scut.edu.cn (J.W.)

Supplementary Tables

Table S1. The plasmids used in this study.

Plasmids	Description	Source
pBAD33	P_{araBAD}, Cm^R, f1 ori	Lab storage
pTrcHisA	P_{trc}, Amp^R, ColE1 ori	Lab storage
pRR	pBAD33, Cm^R, P_{luxR}-*mCherry*	This study
pRI	pBAD33, Cm^R, P_{luxI}-*mCherry*	This study
pSM1	pTrcHisA, Amp^R, P_{T5}-*luxR*-P_{T5}-*luxI* (ES114)	This study
pSM2	pTrcHisA, Amp^R, P_{T5}-*luxR*-P_{T5}-*luxI* (JM11)	This study
pSM3	pTrcHisA, Amp^R, P_{LS}-*luxR*-P_{LS}-*luxI* (JM11)	This study
pSM4	pTrcHisA, Amp^R, P_{J23118}-*luxR*-P_{J23118}-*luxI* (JM11)	This study
pSM5	pTrcHisA, Amp^R, P_{LS}-*luxR*-P_{T5}-*luxI* (JM11)	This study
pSM6	pTrcHisA, Amp^R, P_{LS}-*luxR*-P_{J23118}-*luxI* (JM11)	This study
pSM7	pTrcHisA, Amp^R, P_{T5}-*luxR*-P_{LS}-*luxI* (JM11)	This study
pSM8	pTrcHisA, Amp^R, P_{T5}-*luxR*-P_{J23118}-*luxI* (JM11)	This study
pSM9	pTrcHisA, Amp^R, P_{J23118}-*luxR*-P_{LS}-*luxI* (JM11)	This study
pSM10	pTrcHisA, Amp^R, P_{J23118}-*luxR*-P_{T5}-*luxI* (JM11)	This study
pLuxR	pTrcHisA, Amp^R, P_{J23118}-*luxR* (JM11)	This study
pRM1	pTrcHisA, Amp^R, P_{J23118}-*luxR* (V36E, H89L, P97L)-P_{T5}-*luxI* (JM11)	This study
pRM2	pTrcHisA, Amp^R, P_{J23118}-*luxR* (C38Y, Y47C, I76M)-P_{T5}-*luxI* (JM11)	This study
pRM3	pTrcHisA, Amp^R, P_{J23118}-*luxR* (E112G)-P_{T5}-*luxI* (JM11)	This study
pRM4	pTrcHisA, Amp^R, P_{J23118}-*luxR* (S30P, Y84H)-P_{T5}-*luxI* (JM11)	This study
pRM5	pTrcHisA, Amp^R, P_{J23118}-*luxR* (N88D)-P_{T5}-*luxI* (JM11)	This study
pIB01	pTrcHisA, Amp^R, P_{trc}-*ilvBN*-P_{trc}-*ilvCD*-P_{trc}-*ilvE-vlmD*	Lab storage
pLRP	pBAD33, Cm^R, P_{araBAD}-*lrp*	This study
pAS	pBAD33, Cm^R, P_{araBAD}-as*ilvA*-P_{araBAD}-as*panB*- P_{araBAD}-as*leuA*	This study
pIB02	pTrcHisA, Amp^R, P_{J23119}-*ilvB-ilvN*-P_{trc}-*ilvC-ilvD*-P_{trc}-*ilvE-vlmD*	This study
pIB03	pTrcHisA, Amp^R, P_{trc}-*ilvB-ilvN*-P_{trc}-*ilvC-ilvD*-P_{J23119}-*ilvE-vlmD*	This study
pIB04	pTrcHisA, Amp^R, P_{const_trc}-*ilvB-ilvN*-P_{const_trc}-*ilvC-ilvD*-P_{const_trc}-*ilvE-vlmD*	This study
pIBQS	pBAD33, Cm^R, P_{luxI}-*ilvB-ilvN*-P_{luxI}-*ilvC-ilvD*-P_{luxI}-*ilvE-vlmD*	This study
pIBQS2	pBAD33, Cm^R, P_{luxI}-*ilvB-ilvN*-P_{luxI}-*ilvC-ilvD*-P_{luxI}-*ilvE-vlmD*-P_{luxI}-asgltA	This study
pNPTS	pTrcHisA, Amp^R, P_{LS}-*luxR*-P_{LS}-*luxI*-P_{J23118}-*glf-glk* (JM11)	This study

Table S2. The strains used in this study.

Strains	Description	Source
E. coli DH5α	For plasmid construction	Tsingke
E. coli MG1655	For the host	Lab storage
QRR	MG1655 harboring pRR, Cm^R	This study
QRI	MG1655 harboring pRI, Cm^R	This study
QMR1	MG1655 harboring pRR and pSM1, Cm^R, Amp^R	This study
QMI1	MG1655 harboring pRI and pSM1, Cm^R, Amp^R	This study
QMI2	MG1655 harboring pRI and pSM2, Cm^R, Amp^R	This study
QMI3	MG1655 harboring pRI and pSM3, Cm^R, Amp^R	This study
QMI4	MG1655 harboring pRI and pSM4, Cm^R, Amp^R	This study
QMI5	MG1655 harboring pRI and pSM5, Cm^R, Amp^R	This study
QMI6	MG1655 harboring pRI and pSM6, Cm^R, Amp^R	This study
QMI7	MG1655 harboring pRI and pSM7, Cm^R, Amp^R	This study
QMI8	MG1655 harboring pRI and pSM8, Cm^R, Amp^R	This study
QMI9	MG1655 harboring pRI and pSM9, Cm^R, Amp^R	This study
QMI10	MG1655 harboring pRI and pSM10, Cm^R, Amp^R	This study
RM1	MG1655 harboring pRI and pRM1, Cm^R, Amp^R	This study
RM2	MG1655 harboring pRI and pRM2, Cm^R, Amp^R	This study
RM3	MG1655 harboring pRI and pRM3, Cm^R, Amp^R	This study
RM4	MG1655 harboring pRI and pRM4, Cm^R, Amp^R	This study
RM5	MG1655 harboring pRI and pRM5, Cm^R, Amp^R	This study
IB00	MG1655 Δ*ldhA* Δ*adhE* Δ*poxB* Δ*pta*	Lab storage
IB00B	MG1655 Δ*ldhA* Δ*adhE* Δ*poxB* Δ*pta* Δ*ptsGHI*	Lab storage
IB01	IB00 harboring pIB01, Amp^R	This study
IB02	IB01 harboring pLRP, Cm^R, Amp^R	This study
IB03	IB01 harboring pAS, Cm^R, Amp^R	This study
IB04	IB00 harboring pIB02, Amp^R	This study
IB05	IB00 harboring pIB03, Amp^R	This study
IB06	IB00 harboring pIB04, Amp^R	This study
IB12	IB00 harboring pIBQS and pSM1, Cm^R, Amp^R	This study
IB13	IB00 harboring pIBQS and pSM2, Cm^R, Amp^R	This study
IB21	IB00 harboring pIBQS and pSM3, Cm^R, Amp^R	This study
IB22	IB00 harboring pIBQS and pSM9, Cm^R, Amp^R	This study
IB23	IB00 harboring pIBQS and pSM4, Cm^R, Amp^R	This study
IB31	IB00 harboring pIBQS and pRM4, Cm^R, Amp^R	This study
IB32	IB00 harboring pIBQS and pRM5, Cm^R, Amp^R	This study
IB33	IB00 harboring pIBQS and pSM10, Cm^R, Amp^R	This study
IB21A	IB00 harboring pIBQS2 and pSM3, Cm^R, Amp^R	This study
IB21B	IB00B harboring pIBQS and pNPTS, Cm^R, Amp^R	This study

Table S3. The primers used in this study.

Primers	**Sequence (5'-3')**
P_{T5}-R	TATCCGCTCAGGAAGCAAATAAATTTTTTATGAGTCGACCGGTCTAGACAGC
P_{T5}-F	ATTTGCTTCCTGAGCGGATAACAATTATAATATTTCACACAGGAAACAGC
ESLuxR-F	GATCGATGGGGATCCGAGCTCGGATGAACATTAAAAACATTAAC
ESLuxR-R	ATCCCTGCGGCGTCCATTTGTTAGTTTTTCAGATACGGGCAG
ESLuxI-F	TTCCGAAGGTAACTGGCTTCTCATGATTAAAAAAAGCGATTTTC
ESLuxI-R	CAAAACAGCCAAGCTTCGAATTCTTAGTTGCTCACCGCTTTGCG
JMLuxR-F	CGATGGGGATCCGAGCTCGGATGATATATAACACGCAAAAC
JMLuxR-R	ATCCCTGCGGCGTCCATTTGTTAATTTTTAAAGTATGGGCAATC
JMLuxI-F	TTCCGAAGGTAACTGGCTTCGGAGAAAGGTACCATGACTATAATG
JMLuxI-R	ACAGCCAAGCTTCGAATTCTAGCACGCGTTTACGCTG
P_{luxI}-F	TTCTGTAACAAAGCGGGACCACCCATCTCTTTATCCTTAC
P_{luxI}-R	CCTCGCCCTTGCTCACCATGGTACCTTTCTCCTCTTTA
P_{luxR}-F	TCTGTAACAAAGCGGGACCGGTACCTTTCTCCTCTTTAA
P_{luxR}-R	CCTCGCCCTTGCTCACCATACCCATCTCTTTATCCTTAC
MLuxR-F	GGTAAGGATAAAGAGATGGG
MLuxR-R	TGAATAGGGAAACTAAACCCAGTGATAAGACC
DLuxI-F	TTGCCCATACTTTAAAAATTAAGAATTCGAAGCTTGGCTG
DLuxI-R	TTAATTTTTAAAGTATGGGCAATCAATTGC
ilvB-ilvN-F	CATTAAAGAGGAGAAAGGTACCATGGCAAGTTCGGGCACA
ilvB-ilvN-R	CAGGTCGACTCAAGGGGATCCTTACTGAAAAAACACCGCG
ilvC-ilvD-F	TTAAAGAGGAGAAAGGTACCATGGCTAACTACTTCAATACAC
ilvC-ilvD-R	CAGGTCGACTCAAGGGGATCCTTAACCCCCCAGTTTCGAT
ilvE-vlmD-F	TTAAAGAGGAGAAAGGTACCATGACCACGAAGAAAGCTG
ilvE-vlmD-R	AGGTCGACTCAAGGGGATCCTTAACTACCACCACCATCTTC

Table S4. Amino acid sequences of heterologous genes.

Gene	Sequence
luxI (from *V. fisheri* ES114)	MTIMIKKSDFLAIPSEEYKGILSLRYQVFKQRLEWDLVVENNLESDEYDN SNAEYIYACDDTENVSGCWRLLPTTGDYMLKSVFPELLGQQSAPKDPNI VELSRFAVGKNSSKINNSASEITMKLFEAIYKHAVSQGITEYVTVTSTAIER FLKRIKVPCHRIGDKEIHVLGDTKSVVLSMPINEQFKKAVLNAANDENYA LAA
luxR (from *V. fisheri* ES114)	MKNINADDTYRIINKIKACRSNNDINQCLSDMTKMVHCEYYLLAIIYPHS MVKSDISILDNYPKKWRQYYDDANLIKYDPIVDYSNSNHSPINWNIFENN AVNKKSPNVIKEAKTSGLITGFSFPIHTANNGFGMLSFAHSEKDNYIDSLF LHACMNIPLIVPSLVDNYRKINIANNKSNNDLTKREKECLAWACEGKSSW DISKILGCSERTVTFHLTNAQMKLNTTNRCQSISKAILTGAIDCPYFKN*
luxI (from *V. fisheri* JM11)	MIKKSDFLGIPSEEYRGILSLRYQVFKRRLEWDLVSEDNLESDEYDNSNA EYIYACDDAEEVNGCWRLLPTTGDYMLKTVFPELLGDQVAPRDPNIVEL SRFAVGKNSSKINNSASEITMKLFQAIYKHAVSQGITEYVTVTSIAIERFLK RIKVPCHRIGDKEIHLLGNTRSVVLSMPINDQFRKAVSN
luxR (from *V. fisheri* JM11)	MNIKNINANEKIIDKIKTCNNNKDINQCLSEIAKIIHCEYYLFAIIYPHSIIKP DVSIIDNYPEKWRKYYDDAGLLEYDPVVDYSKSHHSPINWNVFEKKTIK KESPNVIKEAQESGLITGFSFPIHTASNGFGMLSFAHSDKDIYTDSLFLHAS TNVPLMLPSLVDNYQKINTTRKKSDSILTKREKECLAWASEGKSTWDISKI LGCSERTVTFHLTNTQMKLNTTNRCQSISKAILTGAINCPYLKN
vlmD	MSTSSASSGPDLPFGPEDTPWQKAFSRLRAVDGVPRVTAPSSDPREVYMD IPEIPFSKVQIPPDGMDEQQYAEAESLFRRYVDAQTRNFAGYQVTSDLDY QHLSHYLNRHLNNVGDPYESSSYTLNSKVLERAVLDYFASLWNAKWPH DASDPETYWGYVLTMGSSEGNLYGLWNARDYLSGKLLRRQHREAGGD KASVVYTQALRHEGQSPHAYEPVAFFSQDTHYSLTKAVRVLGIDTFHSIGS SRYPDENPLGPGTPWPTEVPSVDGAIDVDKLASLVRFFASKGYPILVSLNY GSTFKGAYDDVPAVAQAVRDICTEYGLDRRRVYHDRSKDSDFDERSGFWI HIDAALGAGYAPYLQMARDAGMVEEAPPVFDFRLPEVHSLTMSGHKWM GTPWACGVYMTRTGLQMTPPKSSEYIGAADTTFAGSRNGFSSLLLWDYL SRHSYDDLVRLAADCDRLAGYAHDRLLTLQDKLGMDLWVARSPQSLTV RFRQPCADIVRKYSLSCETVYEDNEQRTYVHLYAVPHLTRELVDELVRDL RQPGAFTNAGALEGEAWAGVIDALGRPDPDGTYAGALSAPASGPRSEDG GGS

Supplementary Figures

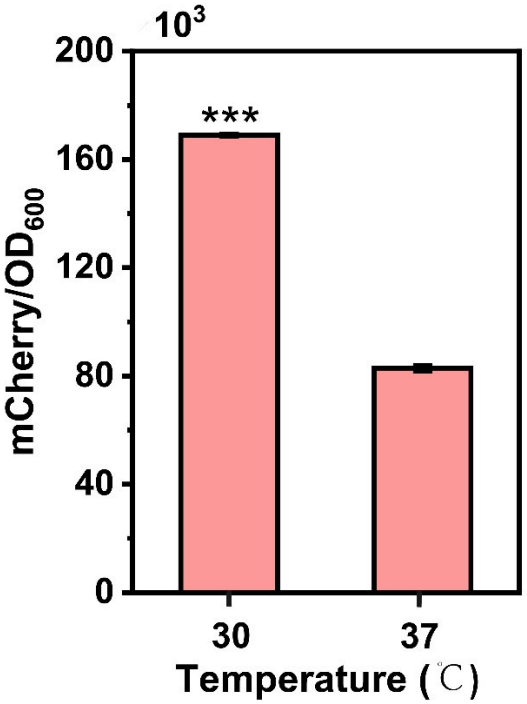

Figure S1. The dynamic range of the engineered strain QMI2 at different temperatures $^{***}p < 0.001$.

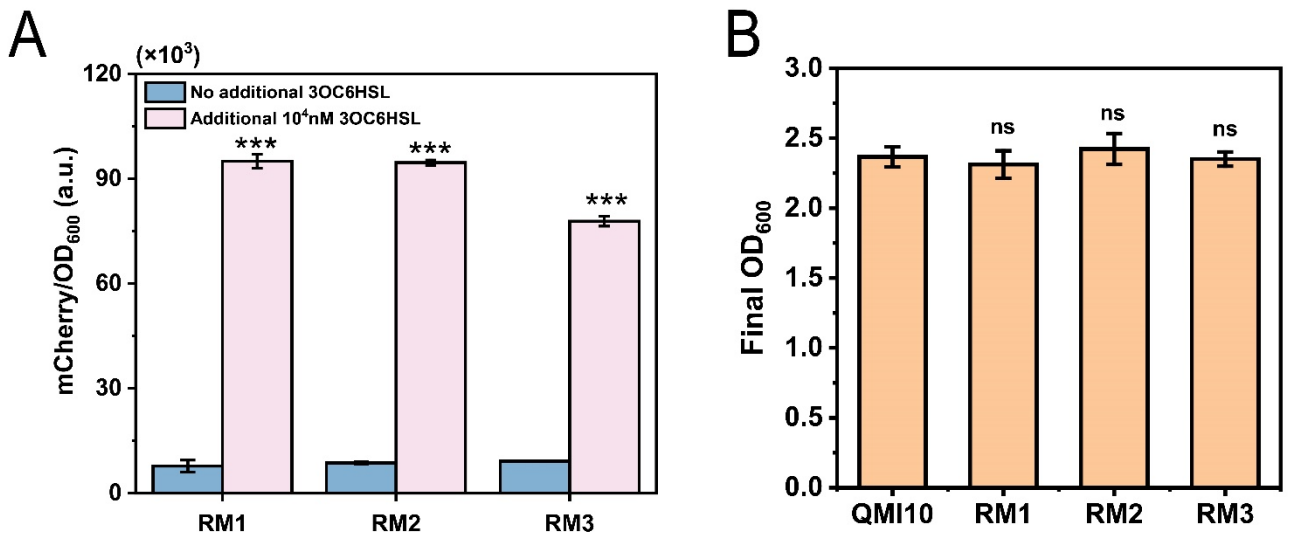

Figure S2. The characterization of LuxR mutants failing to self-induce. **(A)** The normalized mCherry fluorescence. 10,000 nM of exogenous 3OC6HSL was added to verify the activity of mutants RM1, RM2 and RM3 in LB medium, and the control group was cultivated without additional 3OC6HSL for the comparison. **(B)** The final OD_{600} during cultivation. $^{***}p < 0.001$.

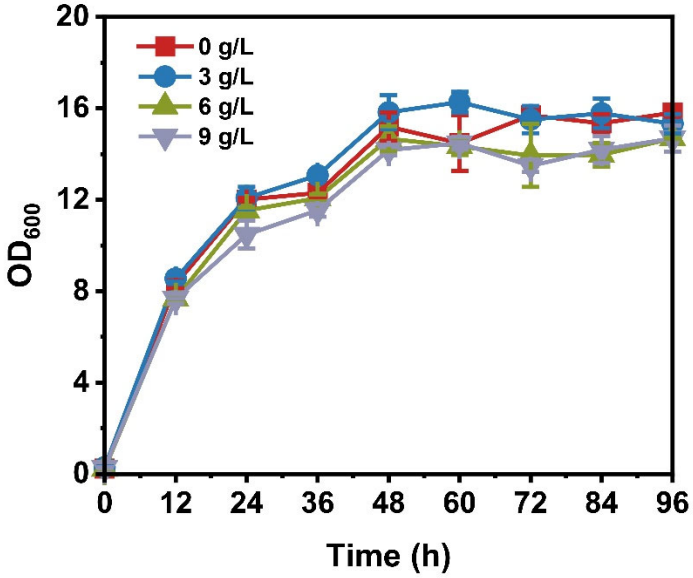

Figure S3. Growth profiles of strain IB01 with exogenous pyruvate addition. The concentration of exogenously added pyruvate ranged from 3 to 6 g/L. The control group was cultivated without addition for comparison.

A

QS system	Threshold Rate	Dynamic Range Rate	Resulted Strain
QMI1	Low	Medium	IB12
QMI2	Low	High	IB13
QMI3	Medium	Low	IB21
QMI9	Medium	Medium	IB22
QMI4	Medium	High	IB23
RM4	High	Low	IB31
RM5	High	Medium	IB32
QMI10	High	High	IB33

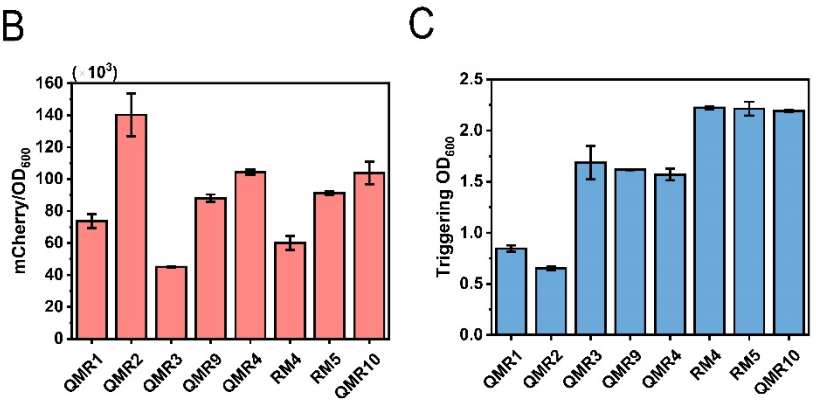

Figure S4. The characterization of selected QS systems for the regulation of *iso*-butylamine synthesis. **(A)** Description of the selected QS systems. The rates were evaluated based on the characterized values. Rating standards for threshold (triggering OD_{600}): Low (OD_{600} ~ 0.7), Medium (OD_{600} ~ 1.7), High (OD_{600} ~ 2.1). Rating standards for dynamic range: Low (< 40,000 a.u.), Medium (40,000 - 80,000 a.u.), High (> 80,000 a.u.). Resulted strain stands for the *iso*-butylamine-producer with the corresponding QS system. **(B, C)** The comparison of dynamic ranges **(B)** and Triggering OD_{600} **(C)** of selected QS systems characterized in NM2 medium and LB medium.

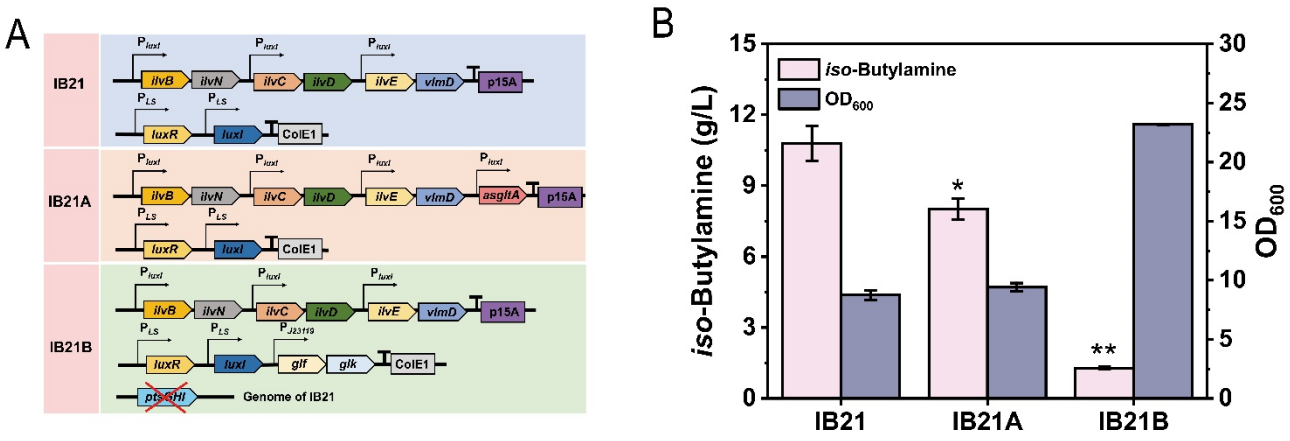

Figure S5. Effects of increasing pyruvate supply by downregulating TCA cycle or reconstructing non-PTS glucose transportation system on the titer. **(A)** Schematic diagram of recombinant plasmids. **(B)** *iso*-Butylamine synthesis in engineered strains. $^{**}p < 0.01$, $^{*}p < 0.05$.