nature research

https://doi.org/10.1038/s41589-020-0639-1

Supplementary information

Optogenetic control of the *lac* operon for bacterial chemical and protein production

In the format provided by the authors and unedited

Supplementary Information for:

Optogenetic control of the *lac* operon for bacterial chemical and protein production

Makoto A. Lalwani[1], Samantha S. Ip[1*], César Carrasco-López[1*], Catherine Day[3], Evan M. Zhao[1], Hinako Kawabe[1], & José L. Avalos[1,2,3†]

1. Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ 08544, USA
2. The Andlinger Center for Energy and the Environment, Princeton University, Princeton, NJ 08544, USA
3. Department of Molecular Biology. Princeton University, Princeton, NJ 08544, USA

* These authors contributed to this work equally.

† Lead contact and correspondence: javalos@princeton.edu

Supplementary Table 1: Plasmids used in this study

Plasmid	Description	Origin of replication	Resistance marker	Additional information	Source
pDusk	P_{lacIQ}_YF1-FixJ, P_{FixK2}_MCS	ColE1	KanR	Template plasmid for dark-inducible gene expression	Ohlendorf et al.[1]
pDawn	P_{lacIQ}_YF1-FixJ, P_{FixK2}_*cI-ssrA*-(LVA), P_R_MCS	ColE1	KanR	Template plasmid for blue light-inducible gene expression	Ohlendorf et al.[1]
pKD4	FRT_*KanR*_FRT	R6K γ	AmpR, KanR	Used as a template for gene deletions	Datsenko and Wanner[2]
pKD46	*araC*, P_{araBAD}_*Gam_Beta_Exo*	pSC101 (temp. sensitive)	AmpR	Used to induce gene deletion	Datsenko and Wanner[2]
pCP20	*FLP*$^+$, λ cI857$^+$, λ P_R	pSC101 (temp. sensitive)	AmpR	Used for antibiotic marker recycling	Cherepanov and Wackernagel[3]

pET28a	P_{lacIQ}_*lacI*, P_{T7}_MCS-6xHis	ColE1	KanR	Contains constitutively expressed *lacI*; used for IPTG-inducible controls	Millipore Sigma
pMevT	P_{lac}_*atoB_HMGS_tHMGR*	p15A	CamR	Template plasmid for mevalonate production	Martin *et al*.[4]
pSA65	P_{L_lacO1}_*Llkivd_LladhA*	ColE1	AmpR	Template plasmid for isobutanol production	Atsumi *et al*.[5]
pSA69	P_{L_lacO1}_*BsalsS_ilvCD*	p15A	KanR	Template plasmid for isobutanol production	Atsumi *et al*.[6]
pCri-8b	P_{T7}_YFP-6xHis, P_{lacIQ}_*lacI*	ColE1	KanR	YFP expressed from P_{T7} promoter with constitutively expressed *lacI*	Goulas *et al*.[7]
pMAL207	Empty vector	pSC101	AmpR	Used for negative control to subtract autofluorescence	This study
pMAL288	P_{lacIQ}_*YF1-FixJ*, P_{FixK2}_*cI-ssrA*-(LVA), P_{R}_*LacI*	ColE1	KanR	pDawn driving *lacI*	This study
pMAL292	$P_{T5\text{-}lacO}$_sfGFP	pSC101	AmpR	LacI-controlled sfGFP reporter plasmid using $P_{T5\text{-}lacO}$ promoter	This study

pMAL294	P_{lacIQ}_*YF1-FixJ*, P_{FixK2}_*cI-ssrA*-(LVA), P_R_*LacI-ssrA*-(LAA)	ColE1	KanR	OptoLAC1 circuit (pDawn driving *lacI-ssrA*-(LAA))	This study
pMAL296	P_{lacIQ}_*YF1-FixJ*, P_{FixK2}_*cI-ssrA*-(LVA), P_R_*LacI-ssrA*-(AAV)	ColE1	KanR	OptoLAC2 circuit (pDawn driving *lacI-ssrA*-(AAV))	This study
pMAL300	P_{lacIQ}_*YF1-FixJ*, P_{FixK2}_*cI-ssrA*-(LVA), P_R_*LacI-ssrA*-(ASV)	ColE1	KanR	pDawn driving *lacI-ssrA*-(ASV)	This study
pMAL301	P_{BBa_J23100}_sfGFP	pSC101	AmpR	Used for positive control expressing sfGFP constitutively to check for photobleaching of sfGFP	This study
pMAL302	P_{lacUV5}_sfGFP	pSC101	AmpR	LacI-controlled sfGFP reporter plasmid using P_{lacUV5} promoter	This study
pMAL303	P_{trc}_sfGFP	pSC101	AmpR	LacI-controlled sfGFP reporter plasmid using P_{trc} promoter	This study

pMAL441	P_{FixK2}_sfGFP	pSC101	AmpR	sfGFP reporter plasmid using P_{FixK2} promoter	This study
pMAL442	P_{FixK2_lacO}_sfGFP	pSC101	AmpR	sfGFP reporter plasmid using P_{FixK2_lacO} promoter	This study
pMAL487	P_{lac}_*atoB_HMGS_tHMGR*	p15A	CamR	Mevalonate biosynthesis pathway without constitutive *lacI*	This study
pMAL534	P_{L_lacO1}_*Llkivd_LladhA*, P_{L_lacO1}_*BsalsS_ilvCD*	p15A	AmpR	Isobutanol biosynthesis pathway without constitutive *lacI*	This study
pMAL630	P_{lacIQ}_*YF1-FixJ*, P_{FixK2_lacO}_*cI-ssrA*-(LVA), P_R_*LacI-ssrA*-(AAV)	ColE1	KanR	OptoLAC3 circuit (pDawn driving *lacI-ssrA*-(AAV) with P_{FixK2_lacO} driving *cI-ssrA*-(LVA))	This study
pMAL658	P_{lacIQ}_*YF1-FixJ*, P_{FixK2}_*cI-ssrA*-(LVA), P_R_*LacI-ssrA*-(LAA)	p15A	CamR	OptoLAC1B circuit (pDawn driving *lacI-ssrA*-(LAA) with p15A origin and chloramphenicol resistance)	This study

pMAL659	P_{lacIQ}_*YF1-FixJ*, P_{FixK2}_*cI-ssrA*-(LVA), P_R_*LacI-ssrA*-(AAV)	p15A	CamR	OptoLAC2B circuit (pDawn driving *lacI-ssrA*-(AAV) with p15A origin and chloramphenicol resistance)	This study
pMAL887	P_{T7}_FdeR-6xHis	ColE1	KanR	FdeR expressed from P_{T7} promoter without constitutively expressed *lacI*	This study
pMAL897	$P_{T5\text{-}lacO}$_sfGFP-*ssrA*-(AAV)	pSC101	AmpR	Unstable sfGFP reporter plasmid	This study
pC9	P_{T7}_FdeR-6xHis, P_{lacIQ}_*lacI*	ColE1	KanR	FdeR expressed from P_{T7} promoter with constitutively expressed *lacI*	This study
pC85	P_{T7}_YFP-6xHis	ColE1	KanR	YFP expressed from P_{T7} promoter without constitutively expressed *lacI*	This study

Supplementary Table 2: Bacterial strains used in this study

Strain	Genotype	Plasmid(s)	Additional information	Source
MG1655	str. K F- λ- rph-1	None	*E. coli* K strain	Blattner *et al.*[8]

BL21 DE3	str. B F– ompT gal dcm lon hsdSB(rB–mB–) λ(DE3 [lacI lacUV5-T7p07 ind1 sam7 nin5]) [malB+]K-12(λS)	None	*E. coli* B strain containing λ prophage	Jeong *et al.*[9]
Rosetta 2	BL21 DE3	pRARE2	BL21 DE3 strain harboring pRARE2 plasmid for rare codon translation	Novagen
MG1655 ΔlacI::KanR	F- λ- rph-1 Δ*lacI*::FRT-KanR-FRT	None	MG1655 containing *lacI* deletion	Orman and Brynildsen[10]
EMAL52	MG1655 Δ*lacI*::FRT	None	OptoMG: Base K strain for chemical production	This study
EMAL57	MG1655 Δ*lacI*::FRT	pMAL288, pMAL292	OptoMG containing pDawn_*lacI* and dark-inducible sfGFP driven by $P_{T5\text{-}lacO}$	This study

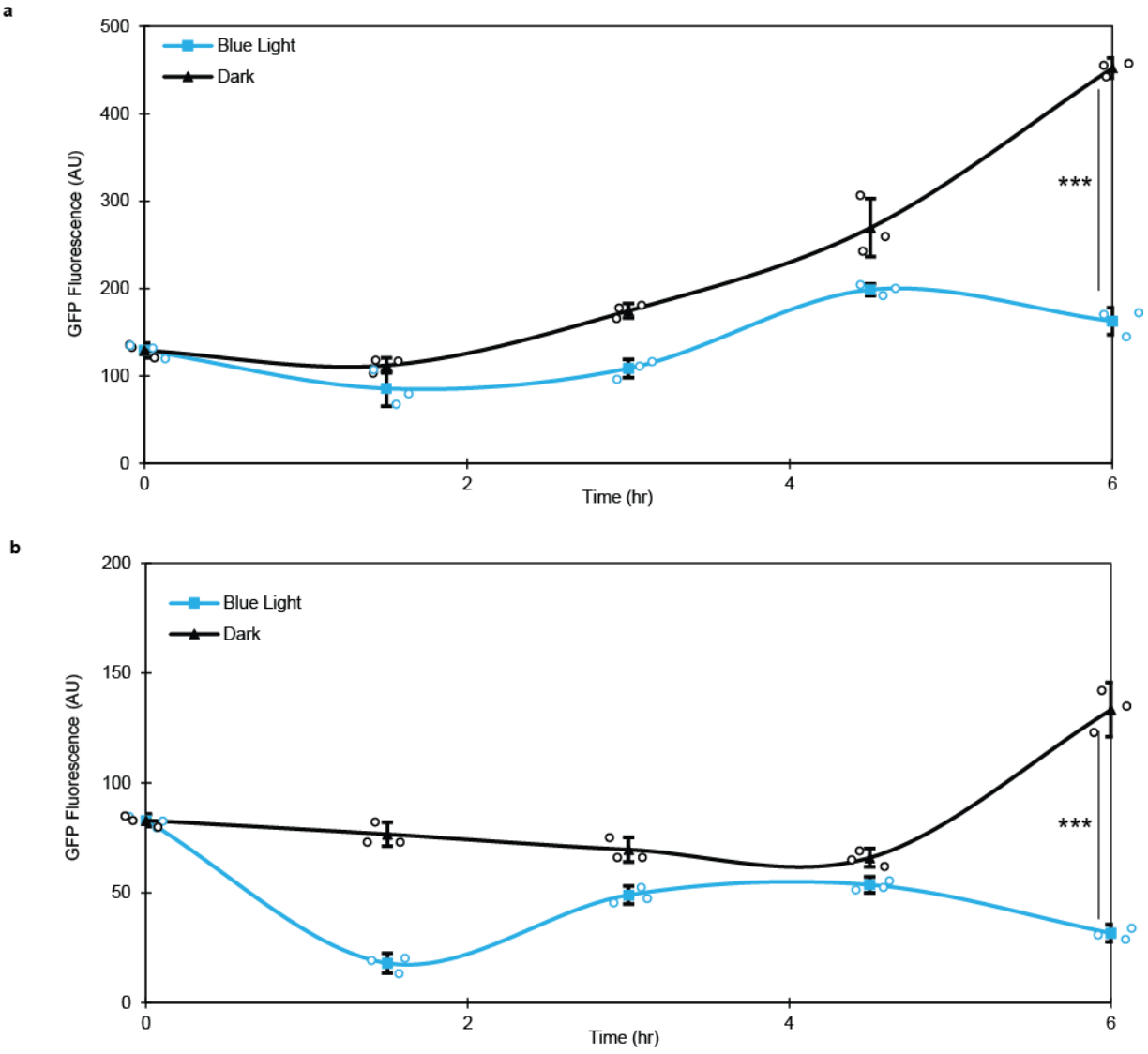
EMAL68	MG1655 Δ*lacI*::FRT	pMAL294, pMAL292	OptoMG containing OptoLAC1 and dark-inducible sfGFP driven by $P_{T5\text{-}lacO}$	This study
EMAL69	MG1655 Δ*lacI*::FRT	pMAL296, pMAL292	OptoMG containing OptoLAC2 and dark-inducible sfGFP driven by $P_{T5\text{-}lacO}$	This study
EMAL71	MG1655 Δ*lacI*::FRT	pMAL300, pMAL292	OptoMG containing pDawn_*lacI-ssrA*-(ASV) and dark-inducible sfGFP driven by $P_{T5\text{-}lacO}$	This study
EMAL77	MG1655 Δ*lacI*::FRT	pET28a, pMAL292	OptoMG containing IPTG-inducible sfGFP	This study
EMAL135	MG1655 Δ*lacI*::FRT	pET28a, pMAL487	OptoMG containing IPTG-inducible mevalonate production pathway	This study
EMAL152	MG1655 Δ*lacI*::FRT	pDusk, pMAL441	OptoMG containing YF1-FixJ and dark-inducible sfGFP driven by P_{FixK2}	This study
EMAL153	MG1655 Δ*lacI*::FRT	pDusk, pMAL442	OptoMG containing YF1-FixJ and dark-inducible sfGFP driven by P_{FixK2_lacO}	This study
EMAL199	MG1655 Δ*lacI*::FRT	pMAL294, pMAL534	OptoMG containing OptoLAC1 and dark-inducible isobutanol production pathway	This study

EMAL200	MG1655 Δ*lacI*::FRT	pMAL296, pMAL534	OptoMG containing OptoLAC2 and dark-inducible isobutanol production pathway	This study
EMAL201	MG1655 Δ*lacI*::FRT	pET28a, pMAL534	OptoMG containing IPTG-inducible isobutanol production pathway	This study
EMAL208	MG1655 Δ*lacI*::FRT	pMAL294, pMAL487	OptoMG containing OptoLAC1 and dark-inducible mevalonate production pathway	This study
EMAL209	MG1655 Δ*lacI*::FRT	pMAL296, pMAL487	OptoMG containing OptoLAC2 and dark-inducible mevalonate production pathway	This study
EMAL224	BL21 DE3 Δ*lacI*::FRT	None	Precursor to EMAL255: BL21 DE3 with single deletion of *lacI*	This study
EMAL229	MG1655 Δ*lacI*::FRT	pMAL630, pMAL207	Negative control OptoMG strain for subtraction of autofluorescence	This study
EMAL230	MG1655 Δ*lacI*::FRT	pMAL630, pMAL292	OptoMG containing OptoLAC3 and dark-inducible sfGFP driven by $P_{T5\text{-}lacO}$	This study
EMAL231	MG1655 Δ*lacI*::FRT	pMAL630, pMAL301	Positive control OptoMG strain containing sfGFP driven constitutively by P_{BBa_J23100}	This study

EMAL235	MG1655 Δ*lacI*::FRT	pMAL630, pMAL487	OptoMG containing OptoLAC3 and dark-inducible mevalonate production pathway	This study
EMAL239	MG1655 Δ*lacI*::FRT	pMAL630, pMAL534	OptoMG containing OptoLAC3 and dark-inducible isobutanol production pathway	This study
EMAL255	BL21 DE3 Δ*lacI* Δ*lacI* (DE3)::SpecR	None	OptoBL: Base B strain for chemical production	This study
EMAL267	MG1655 Δ*lacI*::FRT	pMAL294, pMAL302	OptoMG containing OptoLAC1 and dark-inducible sfGFP driven by P_{lacUV5}	This study
EMAL268	MG1655 Δ*lacI*::FRT	pMAL294, pMAL303	OptoMG containing OptoLAC1 and dark-inducible sfGFP driven by P_{trc}	This study
EMAL276	BL21 DE3	pRARE2, pCri-8b	IPTG-inducible Rosetta 2 strain for YFP driven by P_{T7}	This study
EMAL283	BL21 DE3	pCri-8b	BL21 DE3 strain with IPTG-inducible YFP production driven by P_{T7}	This study
EMAL284	BL21 DE3 Δ*lacI* Δ*lacI* (DE3)::SpecR	pC85, pMAL658	OptoBL containing OptoLAC1B and dark-inducible YFP driven by P_{T7}	This study

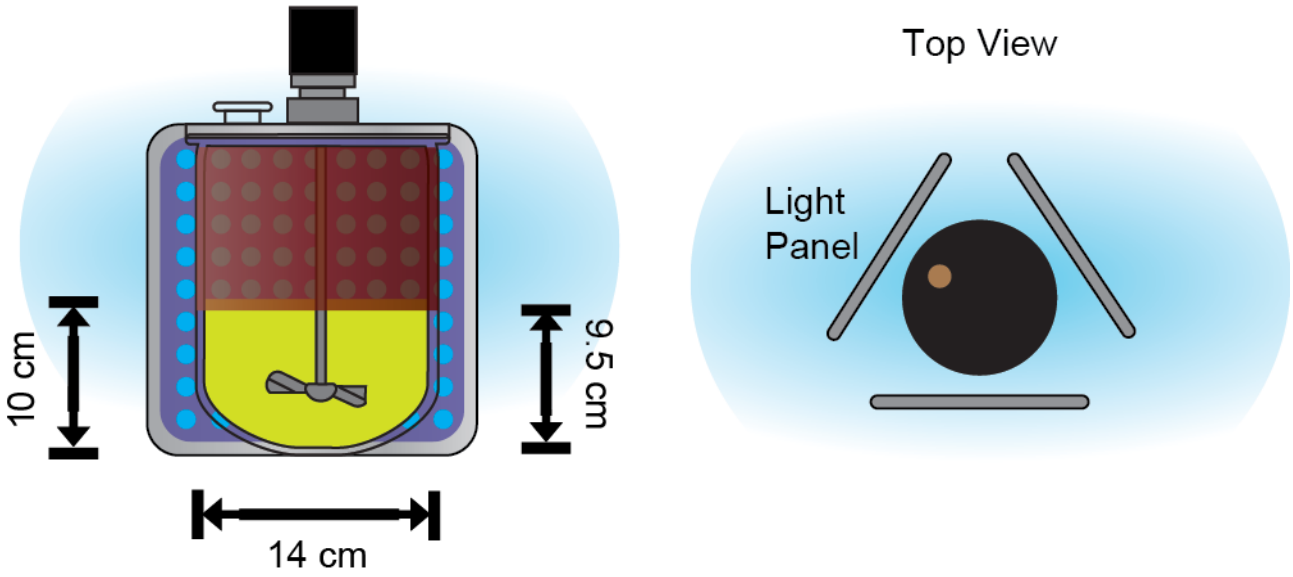
EMAL329	BL21 DE3	pC9	BL21 DE3 strain with IPTG-inducible FdeR production driven by P_{T7}	This study
EMAL335	BL21 DE3 Δ*lacI* Δ*lacI* (DE3)::SpecR	pMAL658, pMAL887	OptoBL containing OptoLAC1B and dark-inducible FdeR driven by P_{T7}	This study
EMAL336	BL21 DE3 Δ*lacI* Δ*lacI* (DE3)::SpecR	pMAL659, pMAL887	OptoBL containing OptoLAC2B and dark-inducible FdeR driven by P_{T7}	This study
EMAL343	MG1655 Δ*lacI*::FRT	pMAL294, pMAL897	OptoMG containing OptoLAC1 and dark-inducible sfGFP-*ssrA*-(AAV) controlled by $P_{T5\text{-}lacO}$	This study
EMAL344	MG1655 Δ*lacI*::FRT	pMAL296, pMAL897	OptoMG containing OptoLAC2 and dark-inducible sfGFP-*ssrA*-(AAV) controlled by $P_{T5\text{-}lacO}$	This study
EMAL345	MG1655 Δ*lacI*::FRT	pMAL630, pMAL897	OptoMG containing OptoLAC3 and dark-inducible sfGFP-*ssrA*-(AAV) controlled by $P_{T5\text{-}lacO}$	This study

Supplementary Figures:



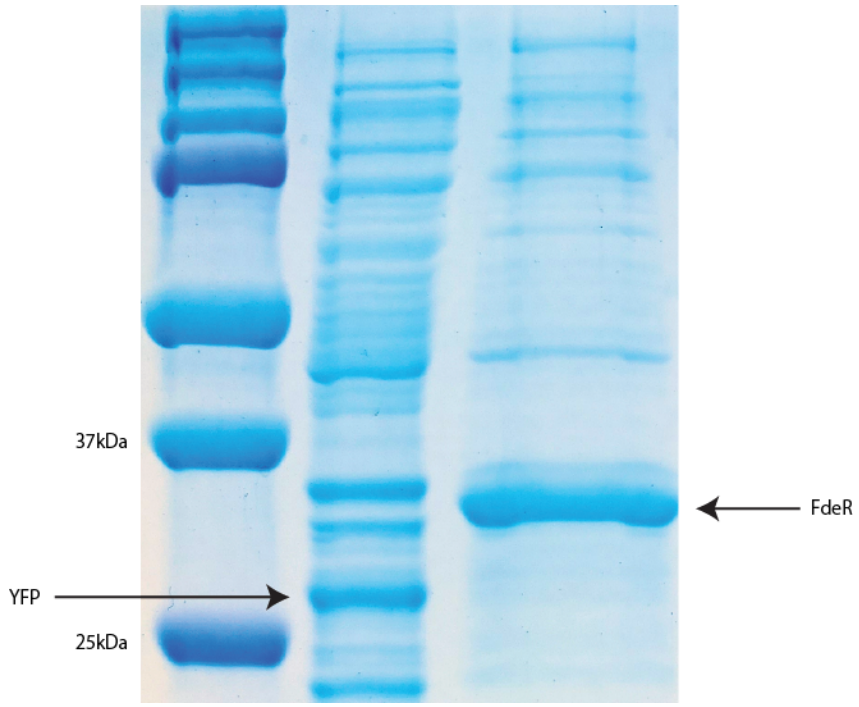
Supplementary Fig. 1: ON-OFF kinetics of OptoLAC2 and OptoLAC3 circuits.

a,**b**, Time course of expression of destabilized GFP (GFP- SsrA-AAV) from $P_{T5\text{-}lacO}$ controlled by **a**, OptoLAC2 (EMAL344), or **b**, OptoLAC3 (EMAL345). For **b**, P = 0.000935; For **c**, P = 0.000505. ***$P < 0.001$. Statistics are derived using a two-sided *t*-test. All data shown as median values of 10,000 single-cell flow cytometry events. Error bars represent standard deviation for n = 3 biologically independent samples. Data are representative of n = 3 independent experiments.



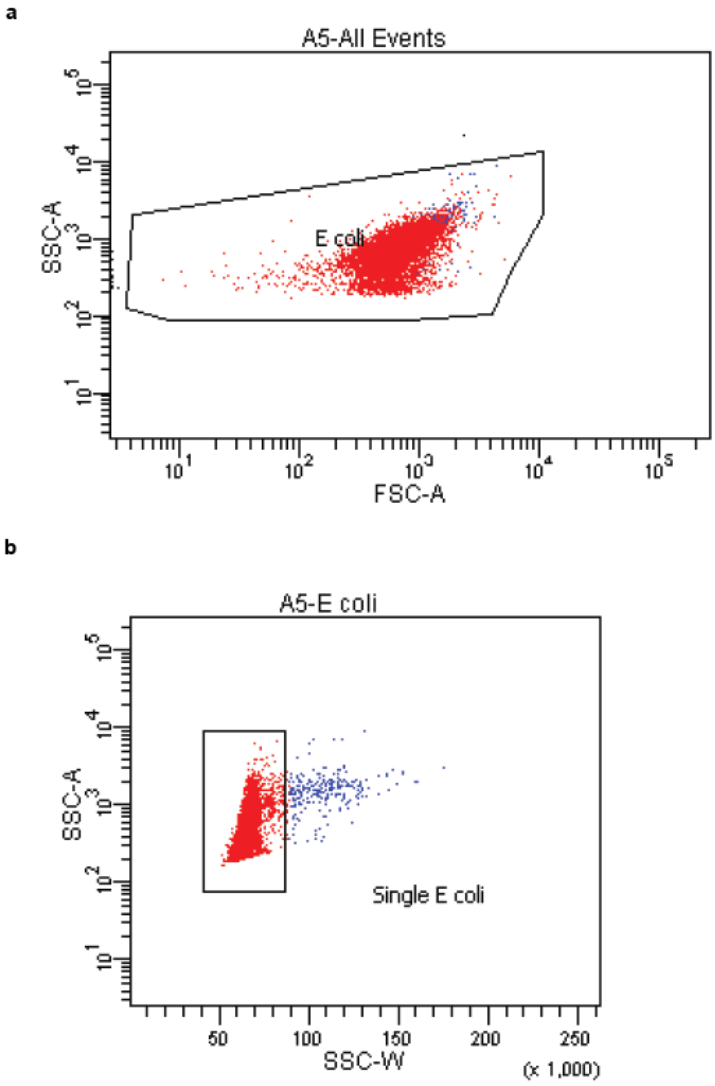
Supplementary Fig. 2: Bioreactor illumination setup.

Side- and top-view of a light-controlled 2-L bioreactor for mevalonate production, with three light panels arranged triangularly ~20 cm from the glass vessel wall such that the light intensity at the vessel surface is between 80-110 µmol/m^2/s. Elevating the heating blanket (red) allows exposure of ~76% of the fermentation bulk surface to light.



Supplementary Fig. 3: Recombinant protein production using OptoLAC circuits under optogenetically uninduced conditions plus IPTG.

Production of recombinant YFP using OptoLAC1B (EMAL284) and FdeR using OptoLAC2B (EMAL336) under simultaneous blue light and IPTG induction. Samples were resolved via SDS-PAGE (12% polyacrylamide). Data are representative of n = 2 independent experiments.



Supplementary Fig. 4: Representative gating settings for flow cytometry analysis.

a, Total *E. coli* population was visualized and gated using forward and side scatter. **b**, Single-cell *E. coli* events were isolated for analysis using a gate of side scatter area vs. width.

Supplementary Notes

Supplementary Note 1: Sequence of P_{FixK2}:

ACGCCCGTGATCCTGATCACCGGCTATCCGGACGAAAACATCTCGACCCGGGCCGC

CGAGGCCGGCGTAAAAGACGTGGTTTTGAAGCCGCTTCTCGACGAAAACCTGCTCA

AGCGTATCCGCCGCGCCATCCAGGACCGGCCTCGGGCATGACCTACGGGGTTCTAC

GTAAGGCACCCCCCTTAAGATATCGCTCGAAATTTTCGAACCTCCCGATACCGCGTA

CCAATGCGTCATCACAACGGAGTACTAGTAAAGAGGAGAAA

Red highlight indicates RBS[11]

Supplementary Note 2: Sequence of P_{FixK2_lacO}:

ACGCCCGTGATCCTGATCACCGGCTATCCGGACGAAAACATCTCGACCCGGGCCGC

CGAGGCCGGCGTAAAAGACGTGGTTTTGAAGCCGCTTCTCGACGAAAACCTGCTCA

AGCGTATCCGCCGCGCCATCCAGGACCGGCCTCGGGCATGACCTACGGGGTTCTAC

GTAAGGCACCCCCCTTAAGATATCGCTCGAAATTTTCGAACCTCCCGATACCGCGTA

CCAATGCGTCATCACAACGGAG

TTGTGAGCGGATAACAAACTAGTAAAGAGGAGAAA

Red highlight indicates RBS[11]; yellow highlight indicates *lacO* sequence.

Supplementary Note 3: OptoLAC1 performance in different media

The decreased sensitivity of OptoLAC1 in M9 medium (Fig. 2c) may be due to stress response in minimal media[12], which could lead to higher rates of LacI degradation and FixJ phosphorylation,

as proteolysis and increased phosphorylation of two-component systems are associated with this response[13,14].

Supplementary Note 4: Analysis of ON-OFF kinetics

The ON to OFF kinetics (dark to light) is determined by the rates of cI degradation (or mitotic dilution), *lacI* accumulation, and GFP degradation (or mitotic dilution). While using a destabilized GFP helps reduce this last time delay, it also reduces the amount of GFP accumulation during the ON phase of the experiment, which prevents us from measuring the off kinetics of the weaker OptoLAC2 and OptoLAC3 circuits in the short timescales of these batch fermentations (Supplementary Fig. 1). Nevertheless, the strongest OptoLAC1 achieves enough GFP accumulation to measure the OFF kinetics in short 9-hour fermentations, demonstrating the potential of these circuits for reversibility.

Supplementary Note 5: Effects of different OptoLAC circuits on chemical production

Our optogenetic circuits match or exceed the production levels achieved with IPTG induction. Despite the initial delay, OptoLAC circuits eventually match or surpass the levels of GFP expression (Fig. 2d) and recombinant protein production (Fig. 5a,b) obtained with IPTG. Additionally, OptoLAC circuits can outperform IPTG induction for mevalonate and isobutanol production (Figs. 3,4). While previous studies achieve higher titers for these chemicals by deleting competing pathways and optimizing fermentation conditions[6,15,16], the strains in this study only contain plasmids with biosynthetic pathways, which is enough to demonstrate the functionality of our circuits and, because the control strains are equally engineered, also provide a fair comparison with IPTG induction. Our results suggest that, at least in some conditions, cI-

mediated repression of *lacI* by OptoLAC circuits is more effective at allowing gene expression from *lacO*-containing promoters than IPTG is at sustaining full inhibition of LacI.

Our suite of OptoLAC circuits provides flexibility to control engineered metabolic pathways. OptoLAC1 and OptoLAC2 are the best at producing mevalonate (6.4 ± 0.2 and 6.1 ± 0.2 g/L, respectively) at relatively low optimal ρ_s values (0.17 and 0.23, respectively). Given mevalonate's low toxicity, inducing at low OD_{600} would be expected to improve production. In contrast, OptoLAC1 and OptoLAC3 are the best at producing isobutanol (2.5 ± 0.1 and 2.4 ± 0.2 g/L, respectively), but at substantially different ρ_s values (1.00 and 0.24, respectively), which reflects the ability of our circuits to adapt to the relatively higher toxicity of isobutanol. In the case of OptoLAC1, which is stronger but also leakier than OptoLAC3, maximum titers are achieved when inducing at higher OD_{600}, possibly due to the accumulation of toxic product during the growth phase. On the other hand, OptoLAC3 is not as strong as OptoLAC1 but is better at keeping isobutanol biosynthesis tightly repressed, which may explain why maximum isobutanol titers are achieved when inducing at lower OD_{600}. This highlights the benefit of having a flexible suite of OptoLAC circuits with different strengths, sensitivities, and folds of induction, which could make each of them more suitable for different metabolic pathways or biotechnological applications.

Supplementary Note 6: Overcoming light penetration

OptoLAC circuits are designed to be inducible by darkness to preempt potential challenges of light penetration. This feature might have played a role in our successful scale-up experiments in

a 2-L bioreactor. However, our circuits are also remarkably sensitive to even the lowest light intensity (5 μmol/m^2/s) we could attain (Extended Data Fig. 5). In addition, all optimal ρ_s values found for chemical or protein production are low (OD_{600} < 1.5) (Figs. 3,4,5), which makes light penetration less challenging. Thus, inducing gene expression with darkness, the high sensitivity displayed by our OptoLAC circuits, and availability of several photobioreactor designs[17,18], bode well for overcoming light penetration hurdles in future scaling up efforts.

Supplementary Note 7: Sequence of OptoLAC1:

TCAATCGTTGAGCATGCCGGCGCGCATCGCGAGGCGAACCAGCTCCGAAAGGCTGTTGGCCTGCATCTTGGTCA

TGACGTTGGCCCGATACACCTCGATGGTGCGCGGGCTGATGTCGTACTCGCGGGCGATCAGCTTGTTGGAAAG

GCCGGCGATCAGCCCTTCCATGACCTGGCGCTCCCTGGGGCTCAACGAGGCGACGCGGGCGGCGATATCCTGC

GCGACGGCCTCGCTCTTGGCGGCCGGCTCGGCCTGGCGGATCGCCGATTCGATCATGGCGGTGAGGCGGTCGT

CCTCGAAAGGCTTTTCCAGAAAGTCCACCGCCCCTAACTTCATCGCCTCGACCGCGAGCGGCACGTCGCCGTGA

CCGGTCATGATGAGGATCGGAAAGGGGCTTTGCTGCGCCTTCATCCGCTTCAACAGCTCGATGCCGTCAAGGCC

CGGCATGCGCACGTCGGAGACGACGCAGCCGAAGGAGAGACCCGGCAGGGCGTCGAGAAAGGCTTGCGCGTC

GTCAAACAGCGTGACGCCGAAGCCGGCAGAATCCAGCAGGAAATTCAGCGAATCCCGCATCGCCGCGTCGTCG

TCGATGACGTAGATATGTCCCTTGGTCGTCATGCTAGACCTCCTATCATCTCGTCGGCTGCCGGGAGGGTGAAG

CGGAAGGTCGCCCCGCCCGATGCGTTGCTCTCGGCCCACATGCGCCCGCCGTGAGCTTCGATGATCGAGCGGC

TGATGGACAGTCCCACGCCCATGCCGGTGTCCTTGGTGGTGAAGAAAGTCTGAAACAGGTTCGGAATGACGTCG

TCCTGGAAACCGCTGCCGGTGTCGGACACTTCGACCTCGATCATGTCGTCGGCGGCGGGGGTGTTGGTGACGA

CGAGCTCGCGTCGCTGCGACTGAGCCATCGCTTCCAGCGCGTTGCGGAACAGGTTGACCAGGACCTGCTGGAT

CTGCACCCGGTCGGCGAGAACGAGATCGGCGCCCGGATCGAGACTGAAGCGGAGCTGCACGTTCTGCTCGCGC

GCGCCGGCAAGCCCGAGCGCGCCGGCCTCCTCGATCAGCTTGGAGAGACTCTCGACCCGCTTCTCCGATTCGCC

GCGGGCAACGAAGTCGCGCAGGCGCCGGATGATCTGGCCGGCGCGCAGCGCCTGCTCGGCGGCGCGGTCCAG

GGCGCTTTCGACCTTCGGTGTGTTCGGATCACTGCTGCCGGCAAGCAGCCGCCGCGAGCCCTTCATGTAGTTGC

TGATCGCCGCCAGCGGCTGGTTGAGCTCGTGCGCGAGCGCGGACGCCATTTCGCCCATGGCGCTCAGCCTGGA

GACGTGGACGAGCTCGGATTGCAGTTCCTGGAGGCGCGCCTGGGTCTGCTGGTGCTCGGTGATATCATTCTGAA

TACCGACAAAATACGTTTTATCCTCTATTTCCATTGGATCAATATTTAATTCATTCCAGAACATCGTTCCGTCTTT

TTTGTAGTTTTGGATCTGAACGGTGACCGGTTCTTTATTTTGTAAAGCGGTTCTGATGTTGTCCACTTCTGCAGG

ATCTGTGTGTTTCCCCTGTAAGAAGCGACAGTTCTTTCCTAAAATTTCCTCGGTCTCGTAGCCGGTCATTTGAAC

AAAGCCTTGATTTACGTAGACAATAGGATTATCTTCAAGTGCGGGATCTGTAATTACCACACCGACTCGCACGTG

ATCAAGTGCTTTTTTGATGACTTCCAGCTGTCCTGGTATCCCAAATGATTGAAAACTAGCCACATTCACCACCCT

CAATTGACTCTCTTCCGGGCGCTATCATGCCATACCGCGAAAGGTTTTGCACCATTCGATGGTGTCCGGGATCT

CGACGCTCTCCCTTATGCGACTCCTGCATTAGGAAGCAGCCCAGTAGTAGGTTGAGGCCGTTGAGCACCGCCGC

CGCAAGGAATGGTGCATGCAAGGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACCATACCCACG

CCGAAACAAGCGCTCATGAGCCCGAAGTGGCGAGCCCGATCTTCCCCATCGGTGATGTCGGCGATATAGGCGC

CAGCAACCGCACCTGTGGCGCCGGTGATGCCGGCCACGATGCGTCCGGCGTAGAGGATCGAGATCTACGCCCG

TGATCCTGATCACCGGCTATCCGGACGAAAACATCTCGACCCGGGCCGCCGAGGCCGGCGTAAAAGACGTGGT

TTTGAAGCCGCTTCTCGACGAAAACCTGCTCAAGCGTATCCGCCGCGCCATCCAGGACCGGCCTCGGGCATGAC

CTACGGGGTTCTACGTAAGGCACCCCCCTTAAGATATCGCTCGAAATTTTCGAACCTCCCGATACCGCGTACCA

ATGCGTCATCACAACGGAGTACTAGTAAAGAGGAGAAATACTAGATGAGCACAAAAAAGAAACCATTAACACAA

GAGCAGCTTGAGGACGCACGTCGCCTTAAAGCAATTTATGAAAAAAAGAAAAATGAACTTGGCTTATCCCAGGA

ATCTGTCGCAGACAAGATGGGGATGGGGCAGTCAGGCGTTGGTGCTTTATTTAATGGCATCAATGCATTAAATG

CTTATAACGCCGCATTGCTTGCAAAAATTCTCAAAGTTAGCGTTGAAGAATTTAGCCCTTCAATCGCCAGAGAAA

TCTACGAGATGTATGAAGCGGTTAGTATGCAGCCGTCACTTAGAAGTGAGTATGAGTACCCTGTTTTTTCTCATG

TTCAGGCAGGGATGTTCTCACCTGAGCTTAGAACCTTTACCAAAGGTGATGCGGAGAGATGGGTAAGCACAACC

AAAAAAGCCAGTGATTCTGCATTCTGGCTTGAGGTTGAAGGTAATTCCATGACCGCACCAACAGGCTCCAAGCC

GAGCTTTCCTGACGGAATGTTAATTCTCGTTGACCCTGAGCAGGCTGTTGAGCCAGGTGATTTCTGCATAGCCA

GACTTGGGGGTGATGAGTTTACCTTCAAGAAACTGATCAGGGATAGCGGTCAGGTGTTTTTACAACCACTAAAC

CCACAGTACCCAATGATCCCATGCAATGAGAGTTGTTCCGTTGTGGGGAAAGTTATCGCTAGTCAGTGGCCTGA

AGAGACGTTTGGCGCTGCAAACGACGAAAACTACGCTTTAGTAGCTTAATAACGCTGATAGTGCTAGTGTAGAT

CGCTACTAGAGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTT

GTCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATATACTAGAGTA

ACACCGTGCGTGTTGACTATTTTACCTCTGGCGGTGATAATGGTTGCTCTAGAAATAATTTTGTTTAACTTTAAG

AAGGAGAGCTAGCATGAAACCAGTAACGTTATACGATGTCGCAGAGTATGCCGGTGTCTCTTATCAGACCGTTT

CCCGCGTGGTGAACCAGGCCAGCCACGTTTCTGCGAAAACGCGGGAAAAAGTGGAAGCGGCGATGGCGGAGCT

GAATTACATTCCCAACCGCGTGGCACAACAACTGGCGGGCAAACAGTCGTTGCTGATTGGCGTTGCCACCTCCA

GTCTGGCCCTGCACGCGCCGTCGCAAATTGTCGCGGCGATTAAATCTCGCGCCGATCAACTGGGTGCCAGCGTG

GTGGTGTCGATGGTAGAACGAAGCGGCGTCGAAGCCTGTAAAGCGGCGGTGCACAATCTTCTCGCGCAACGCG

TCAGTGGGCTGATCATTAACTATCCGCTGGATGACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTT

CCGGCGTTATTTCTTGATGTCTCTGACCAGACACCCATCAACAGTATTATTTTCTCCCATGAAGACGGTACGCGA

CTGGGCGTGGAGCATCTGGTCGCATTGGGTCACCAGCAAATCGCGCTGTTAGCGGGCCCATTAAGTTCTGTCTC

GGCGCGTCTGCGTCTGGCTGGCTGGCATAAATATCTCACTCGCAATCAAATTCAGCCGATAGCGGAACGGGAAG

GCGACTGGAGTGCCATGTCCGGTTTTCAACAAACCATGCAAATGCTGAATGAGGGCATCGTTCCCACTGCGATG

CTGGTTGCCAACGATCAGATGGCGCTGGGCGCAATGCGCGCCATTACCGAGTCCGGGCTGCGCGTTGGTGCGG

ATATCTCGGTAGTGGGATACGACGATACCGAAGACAGCTCATGTTATATCCCGCCGTTAACCACCATCAAACAG

GATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCA

ATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGC

GCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGGCAGCAAACGACG

AAAACTACGCTTTAGCAGCTTAATGAGCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAA

GGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGA

GGGGTTTTTG

Green highlight indicates YF1-FixJ; Light blue indicates P_{lacIQ} promoter; yellow highlight indicates P_{FixK2} promoter; red highlight indicates cI-LVA; grey highlight indicates T_{B0015} terminator; pink highlight indicates P_R promoter; purple highlight indicates *lacI_ssrA*-(LAA); brown highlight indicates T_{T7} terminator.

Supplementary Note 8: Sequence of OptoLAC2:

TCAATCGTTGAGCATGCCGGCGCGCATCGCGAGGCGAACCAGCTCCGAAAGGCTGTTGGCCTGCATCTTGGTCA

TGACGTTGGCCCGATACACCTCGATGGTGCGCGGGCTGATGTCGTACTCGCGGGCGATCAGCTTGTTGGAAAG

GCCGGCGATCAGCCCTTCCATGACCTGGCGCTCCCTGGGGCTCAACGAGGCGACGCGGGCGGCGATATCCTGC

GCGACGGCCTCGCTCTTGGCGGCCGGCTCGGCCTGGCGGATCGCCGATTCGATCATGGCGGTGAGGCGGTCGT

CCTCGAAAGGCTTTTCCAGAAAGTCCACCGCCCCTAACTTCATCGCCTCGACCGCGAGCGGCACGTCGCCGTGA

CCGGTCATGATGAGGATCGGAAAGGGGCTTTGCTGCGCCTTCATCCGCTTCAACAGCTCGATGCCGTCAAGGCC

CGGCATGCGCACGTCGGAGACGACGCAGCCGAAGGAGAGACCCGGCAGGGCGTCGAGAAAGGCTTGCGCGTC

GTCAAACAGCGTGACGCCGAAGCCGGCAGAATCCAGCAGGAAATTCAGCGAATCCCGCATCGCCGCGTCGTCG

TCGATGACGTAGATATGTCCCTTGGTCGTCATGCTAGACCTCCTATCATCTCGTCGGCTGCCGGGAGGGTGAAG

CGGAAGGTCGCCCCGCCCGATGCGTTGCTCTCGGCCCACATGCGCCCGCCGTGAGCTTCGATGATCGAGCGGC

TGATGGACAGTCCCACGCCCATGCCGGTGTCCTTGGTGGTGAAGAAAGTCTGAAACAGGTTCGGAATGACGTCG

TCCTGGAAACCGCTGCCGGTGTCGGACACTTCGACCTCGATCATGTCGTCGGCGGCGGGGGTGTTGGTGACGA

CGAGCTCGCGTCGCTGCGACTGAGCCATCGCTTCCAGCGCGTTGCGGAACAGGTTGACCAGGACCTGCTGGAT

CTGCACCCGGTCGGCGAGAACGAGATCGGCGCCCGGATCGAGACTGAAGCGGAGCTGCACGTTCTGCTCGCGC

GCGCCGGCAAGCCCGAGCGCGCCGGCCTCCTCGATCAGCTTGGAGAGACTCTCGACCCGCTTCTCCGATTCGCC

GCGGGCAACGAAGTCGCGCAGGCGCCGGATGATCTGGCCGGCGCGCAGCGCCTGCTCGGCGGCGCGGTCCAG

GGCGCTTTCGACCTTCGGTGTGTTCGGATCACTGCTGCCGGCAAGCAGCCGCCGCGAGCCCTTCATGTAGTTGC

TGATCGCCGCCAGCGGCTGGTTGAGCTCGTGCGCGAGCGCGGACGCCATTTCGCCCATGGCGCTCAGCCTGGA

GACGTGGACGAGCTCGGATTGCAGTTCCTGGAGGCGCGCCTGGGTCTGCTGGTGCTCGGTGATATCATTCTGAA

TACCGACAAAATACGTTTTATCCTCTATTTCCATTGGATCAATATTTAATTCATTCCAGAACATCGTTCCGTCTTT

TTTGTAGTTTTGGATCTGAACGGTGACCGGTTCTTTATTTTGTAAAGCGGTTCTGATGTTGTCCACTTCTGCAGG

ATCTGTGTGTTTCCCCTGTAAGAAGCGACAGTTCTTTCCTAAAATTTCCTCGGTCTCGTAGCCGGTCATTTGAAC

AAAGCCTTGATTTACGTAGACAATAGGATTATCTTCAAGTGCGGGATCTGTAATTACCACACCGACTCGCACGTG

ATCAAGTGCTTTTTTGATGACTTCCAGCTGTCCTGGTATCCCAAATGATTGAAAACTAGCCACATTCACCACCCT

CAATTGACTCTCTTCCGGGCGCTATCATGCCATACCGCGAAAGGTTTTGCACCATTCGATGGTGTCCGGGATCT

CGACGCTCTCCCTTATGCGACTCCTGCATTAGGAAGCAGCCCAGTAGTAGGTTGAGGCCGTTGAGCACCGCCGC

CGCAAGGAATGGTGCATGCAAGGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACCATACCCACG

CCGAAACAAGCGCTCATGAGCCCGAAGTGGCGAGCCCGATCTTCCCCATCGGTGATGTCGGCGATATAGGCGC

CAGCAACCGCACCTGTGGCGCCGGTGATGCCGGCCACGATGCGTCCGGCGTAGAGGATCGAGATCTACGCCCG

TGATCCTGATCACCGGCTATCCGGACGAAAACATCTCGACCCGGGCCGCCGAGGCCGGCGTAAAAGACGTGGT

TTTGAAGCCGCTTCTCGACGAAAACCTGCTCAAGCGTATCCGCCGCGCCATCCAGGACCGGCCTCGGGCATGAC

CTACGGGGTTCTACGTAAGGCACCCCCCTTAAGATATCGCTCGAAATTTTCGAACCTCCCGATACCGCGTACCA

ATGCGTCATCACAACGGAGTACTAGTAAAGAGGAGAAATACTAGATGAGCACAAAAAAGAAACCATTAACACAA

GAGCAGCTTGAGGACGCACGTCGCCTTAAAGCAATTTATGAAAAAAAGAAAAATGAACTTGGCTTATCCCAGGA

ATCTGTCGCAGACAAGATGGGGATGGGGCAGTCAGGCGTTGGTGCTTTATTTAATGGCATCAATGCATTAAATG

CTTATAACGCCGCATTGCTTGCAAAAATTCTCAAAGTTAGCGTTGAAGAATTTAGCCCTTCAATCGCCAGAGAAA

TCTACGAGATGTATGAAGCGGTTAGTATGCAGCCGTCACTTAGAAGTGAGTATGAGTACCCTGTTTTTTCTCATG

TTCAGGCAGGGATGTTCTCACCTGAGCTTAGAACCTTTACCAAAGGTGATGCGGAGAGATGGGTAAGCACAACC

AAAAAAGCCAGTGATTCTGCATTCTGGCTTGAGGTTGAAGGTAATTCCATGACCGCACCAACAGGCTCCAAGCC

GAGCTTTCCTGACGGAATGTTAATTCTCGTTGACCCTGAGCAGGCTGTTGAGCCAGGTGATTTCTGCATAGCCA

GACTTGGGGGTGATGAGTTTACCTTCAAGAAACTGATCAGGGATAGCGGTCAGGTGTTTTTACAACCACTAAAC

CCACAGTACCCAATGATCCCATGCAATGAGAGTTGTTCCGTTGTGGGGAAAGTTATCGCTAGTCAGTGGCCTGA

AGAGACGTTTGGCGCTGCAAACGACGAAAACTACGCTTTAGTAGCTTAATAACGCTGATAGTGCTAGTGTAGAT

CGCTACTAGAGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTT

GTCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATATACTAGAGTA

ACACCGTGCGTGTTGACTATTTTACCTCTGGCGGTGATAATGGTTGCTCTAGAAATAATTTTGTTTAACTTTAAG

AAGGAGAGCTAGCATGAAACCAGTAACGTTATACGATGTCGCAGAGTATGCCGGTGTCTCTTATCAGACCGTTT

CCCGCGTGGTGAACCAGGCCAGCCACGTTTCTGCGAAAACGCGGGAAAAAGTGGAAGCGGCGATGGCGGAGCT

GAATTACATTCCCAACCGCGTGGCACAACAACTGGCGGGCAAACAGTCGTTGCTGATTGGCGTTGCCACCTCCA

GTCTGGCCCTGCACGCGCCGTCGCAAATTGTCGCGGCGATTAAATCTCGCGCCGATCAACTGGGTGCCAGCGTG

GTGGTGTCGATGGTAGAACGAAGCGGCGTCGAAGCCTGTAAAGCGGCGGTGCACAATCTTCTCGCGCAACGCG

TCAGTGGGCTGATCATTAACTATCCGCTGGATGACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTT

CCGGCGTTATTTCTTGATGTCTCTGACCAGACACCCATCAACAGTATTATTTTCTCCCATGAAGACGGTACGCGA

CTGGGCGTGGAGCATCTGGTCGCATTGGGTCACCAGCAAATCGCGCTGTTAGCGGGCCCATTAAGTTCTGTCTC

GGCGCGTCTGCGTCTGGCTGGCTGGCATAAATATCTCACTCGCAATCAAATTCAGCCGATAGCGGAACGGGAAG

GCGACTGGAGTGCCATGTCCGGTTTTCAACAAACCATGCAAATGCTGAATGAGGGCATCGTTCCCACTGCGATG

CTGGTTGCCAACGATCAGATGGCGCTGGGCGCAATGCGCGCCATTACCGAGTCCGGGCTGCGCGTTGGTGCGG

ATATCTCGGTAGTGGGATACGACGATACCGAAGACAGCTCATGTTATATCCCGCCGTTAACCACCATCAAACAG

GATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCA

ATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGC

GCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGGCAGCAAACGACG

AAAACTACGCTGCAGCAGTTTAATGAGCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAA

GGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGA

GGGGTTTTTTG

Green highlight indicates YF1-FixJ; Light blue indicates P_{lacIQ} promoter; yellow highlight indicates P_{FixK2} promoter; red highlight indicates cI-LVA; grey highlight indicates T_{B0015} terminator; pink highlight indicates P_R promoter; purple highlight indicates *lacI_ssrA*-(AAV); brown highlight indicates T_{T7} terminator.

Supplementary Note 9: Sequence of OptoLAC3:

GACACCATCGAATGGTGCAAAACCTTTCGCGGTATGGCATGATAGCGCCCGGAAGAGAGTCAATTGAGGGTGGT

GAATGTGGCTAGTTTTCAATCATTTGGGATACCAGGACAGCTGGAAGTCATCAAAAAAGCACTTGATCACGTGC

GAGTCGGTGTGGTAATTACAGATCCCGCACTTGAAGATAATCCTATTGTCTACGTAAATCAAGGCTTTGTTCAAA

TGACCGGCTACGAGACCGAGGAAATTTTAGGAAAGAACTGTCGCTTCTTACAGGGGAAACACACAGATCCTGCA

GAAGTGGACAACATCAGAACCGCTTTACAAAATAAAGAACCGGTCACCGTTCAGATCCAAAACTACAAAAAAGA

CGGAACGATGTTCTGGAATGAATTAAATATTGATCCAATGGAAATAGAGGATAAAACGTATTTTGTCGGAATTCA

GAATGATATCACCGAGCACCAGCAGACCCAGGCGCGCCTCCAGGAACTGCAATCCGAGCTCGTCCACGTCTCCA

GGCTGAGCGCCATGGGCGAAATGGCGTCCGCGCTCGCGCACGAGCTCAACCAGCCGCTGGCGGCGATCAGCAA

CTACATGAAGGGCTCGCGGCGGCTGCTTGCCGGCAGCAGTGATCCGAACACACCGAAGGTCGAAAGCGCCCTG

GACCGCGCCGCCGAGCAGGCGCTGCGCGCCGGCCAGATCATCCGGCGCCTGCGCGACTTCGTTGCCCGCGGCG

AATCGGAGAAGCGGGTCGAGAGTCTCTCCAAGCTGATCGAGGAGGCCGGCGCGCTCGGGCTTGCCGGCGCGCG

CGAGCAGAACGTGCAGCTCCGCTTCAGTCTCGATCCGGGCGCCGATCTCGTTCTCGCCGACCGGGTGCAGATCC

AGCAGGTCCTGGTCAACCTGTTCCGCAACGCGCTGGAAGCGATGGCTCAGTCGCAGCGACGCGAGCTCGTCGT

CACCAACACCCCCGCCGCCGACGACATGATCGAGGTCGAAGTGTCCGACACCGGCAGCGGTTTCCAGGACGAC

GTCATTCCGAACCTGTTTCAGACTTTCTTCACCACCAAGGACACCGGCATGGGCGTGGGACTGTCCATCAGCCG

CTCGATCATCGAAGCTCACGGCGGGCGCATGTGGGCCGAGAGCAACGCATCGGGCGGGGCGACCTTCCGCTTC

ACCCTCCCGGCAGCCGACGAGATGATAGGAGGTCTAGCATGACGACCAAGGGACATATCTACGTCATCGACGAC

GACGCGGCGATGCGGGATTCGCTGAATTTCCTGCTGGATTCTGCCGGCTTCGGCGTCACGCTGTTTGACGACGC

GCAAGCCTTTCTCGACGCCCTGCCGGGTCTCTCCTTCGGCTGCGTCGTCTCCGACGTGCGCATGCCGGGCCTTG

ACGGCATCGAGCTGTTGAAGCGGATGAAGGCGCAGCAAAGCCCCTTTCCGATCCTCATCATGACCGGTCACGGC

GACGTGCCGCTCGCGGTCGAGGCGATGAAGTTAGGGGCGGTGGACTTTCTGGAAAAGCCTTTCGAGGACGACC

GCCTCACCGCCATGATCGAATCGGCGATCCGCCAGGCCGAGCCGGCCGCCAAGAGCGAGGCCGTCGCGCAGGA

TATCGCCGCCCGCGTCGCCTCGTTGAGCCCCAGGGAGCGCCAGGTCATGGAAGGGCTGATCGCCGGCCTTTCC

AACAAGCTGATCGCCCGCGAGTACGACATCAGCCCGCGCACCATCGAGGTGTATCGGGCCAACGTCATGACCAA

GATGCAGGCCAACAGCCTTTCGGAGCTGGTTCGCCTCGCGATGCGCGCCGGCATGCTCAACGATTGACAATTGA

TGTAAGTTAGCTCACTCATTAGGCACCGGGATCTCGACCGATGCCCTTGAGAGCCTTCAACCCAGTCTGTACGC

GGCCGCGTCCGGCGTAGAGGATCGAGATCTACGCCCGTGATCCTGATCACCGGCTATCCGGACGAAAACATCTC

GACCCGGGCCGCCGAGGCCGGCGTAAAAGACGTGGTTTTGAAGCCGCTTCTCGACGAAAACCTGCTCAAGCGT

ATCCGCCGCGCCATCCAGGACCGGCCTCGGGCATGACCTACGGGGTTCTACGTAAGGCACCCCCCTTAAGATAT

CGCTCGAAATTTTCGAACCTCCCGATACCGCGTACCAATGCGTCATCACAACGGAGTTGTGAGCGGATAACAAA

CTAGTAAAGAGGAGAAATACTAGATGAGCACAAAAAAGAAACCATTAACACAAGAGCAGCTTGAGGACGCACGT

CGCCTTAAAGCAATTTATGAAAAAAAGAAAAATGAACTTGGCTTATCCCAGGAATCTGTCGCAGACAAGATGGG

GATGGGGCAGTCAGGCGTTGGTGCTTTATTTAATGGCATCAATGCATTAAATGCTTATAACGCCGCATTGCTTGC

AAAAATTCTCAAAGTTAGCGTTGAAGAATTTAGCCCTTCAATCGCCAGAGAAATCTACGAGATGTATGAAGCGG

TTAGTATGCAGCCGTCACTTAGAAGTGAGTATGAGTACCCTGTTTTTTCTCATGTTCAGGCAGGGATGTTCTCAC

CTGAGCTTAGAACCTTTACCAAAGGTGATGCGGAGAGATGGGTAAGCACAACCAAAAAAGCCAGTGATTCTGCA

TTCTGGCTTGAGGTTGAAGGTAATTCCATGACCGCACCAACAGGCTCCAAGCCGAGCTTTCCTGACGGAATGTT

AATTCTCGTTGACCCTGAGCAGGCTGTTGAGCCAGGTGATTTCTGCATAGCCAGACTTGGGGGTGATGAGTTTA

CCTTCAAGAAACTGATCAGGGATAGCGGTCAGGTGTTTTTACAACCACTAAACCCACAGTACCCAATGATCCCAT

GCAATGAGAGTTGTTCCGTTGTGGGGAAAGTTATCGCTAGTCAGTGGCCTGAAGAGACGTTTGGCGCTGCAAAC

GACGAAAACTACGCTTTAGTAGCTTAATAACGCTGATAGTGCTAGTGTAGATCGCTACTAGAGCCAGGCATCAA

ATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAG

AGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATATACTAGAGTAACACCGTGCGTGTTGACTATTT

TACCTCTGGCGGTGATAATGGTTGCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGAGCTAGCATGAAACCA

GTAACGTTATACGATGTCGCAGAGTATGCCGGTGTCTCTTATCAGACCGTTTCCCGCGTGGTGAACCAGGCCAG

CCACGTTTCTGCGAAAACGCGGGAAAAAGTGGAAGCGGCGATGGCGGAGCTGAATTACATTCCCAACCGCGTG

GCACAACAACTGGCGGGCAAACAGTCGTTGCTGATTGGCGTTGCCACCTCCAGTCTGGCCCTGCACGCGCCGTC

GCAAATTGTCGCGGCGATTAAATCTCGCGCCGATCAACTGGGTGCCAGCGTGGTGGTGTCGATGGTAGAACGAA

GCGGCGTCGAAGCCTGTAAAGCGGCGGTGCACAATCTTCTCGCGCAACGCGTCAGTGGGCTGATCATTAACTAT

CCGCTGGATGACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTTCCGGCGTTATTTCTTGATGTCTCT

GACCAGACACCCATCAACAGTATTATTTTCTCCCATGAAGACGGTACGCGACTGGGCGTGGAGCATCTGGTCGC

ATTGGGTCACCAGCAAATCGCGCTGTTAGCGGGCCCATTAAGTTCTGTCTCGGCGCGTCTGCGTCTGGCTGGCT

GGCATAAATATCTCACTCGCAATCAAATTCAGCCGATAGCGGAACGGGAAGGCGACTGGAGTGCCATGTCCGGT

TTTCAACAAACCATGCAAATGCTGAATGAGGGCATCGTTCCCACTGCGATGCTGGTTGCCAACGATCAGATGGC

GCTGGGCGCAATGCGCGCCATTACCGAGTCCGGGCTGCGCGTTGGTGCGGATATCTCGGTAGTGGGATACGAC

GATACCGAAGACAGCTCATGTTATATCCCGCCGTTAACCACCATCAAACAGGATTTTCGCCTGCTGGGGCAAAC

CAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGG

TGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAG

CTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGGCAGCAAACGACGAAAACTACGCTGCAGCAGTTTAAT

GAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCAC

CGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG

Green highlight indicates YF1-FixJ; Light blue indicates P_{lacIQ} promoter; yellow highlight indicates P_{FixK2} promoter; red highlight indicates cI-LVA; grey highlight indicates T_{B0015} terminator; pink highlight indicates P_R promoter; purple highlight indicates *lacI_ssrA*-(AAV); brown highlight indicates T_{T7} terminator.

Supplementary References

1. Ohlendorf, R., Vidavski, R. R., Eldar, A., Moffat, K. & Möglich, A. From dusk till dawn: One-plasmid systems for light-regulated gene expression. *J. Mol. Biol.* **416**, 534–542 (2012).

2. Datsenko, K. A. & Wanner, B. L. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. *Proc. Natl. Acad. Sci.* **97**, 6640–6645 (2000).

3. Cherepanov, P. P. & Wackernagel, W. Gene disruption in Escherichia coli: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. *Gene* **158**, 9–14 (1995).

4. Martin, V. J. J., Pitera, D. J., Withers, S. T., Newman, J. D. & Keasling, J. D. Engineering a mevalonate pathway in Escherichia coli for production of terpenoids. *Nat. Biotechnol.* **21**, 796–802 (2003).

5. Atsumi, S. *et al.* Engineering the isobutanol biosynthetic pathway in Escherichia coli by comparison of three aldehyde reductase/alcohol dehydrogenase genes. *Appl. Microbiol. Biotechnol.* **85**, 651–657 (2010).

6. Atsumi, S., Hanai, T. & Liao, J. C. Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. *Nature* **451**, 86–89 (2008).

7. Goulas, T. *et al.* The pCri system: A vector collection for recombinant protein expression and purification. *PLoS One* **9**, (2014).

8. Blattner, F. R. *et al.* The complete genome sequence of Escherichia coli K-12. *Science (80-.).* **277**, 1453–1462 (1997).

9. Jeong, H. *et al.* Genome Sequences of Escherichia coli B strains REL606 and BL21(DE3). *J. Mol. Biol.* **394**, 644–652 (2009).

10. Orman, M. A. & Brynildsen, M. P. Dormancy Is Not Necessary or Sufficient for Bacterial Persistence. *Antimicrob. Agents Chemother.* **57**, 3230–3239 (2013).

11. Elowitz, M. B. & Leibler, S. A synthetic oscillatory network repressilator. *Nature* **403**, 335–338 (2000).

12. Tao, H., Bausch, C., Richmond, C., Blattner, F. R. & Conway, T. Functional genomics: Expression analysis of Escherichia coli growing on minimal and rich media. *J. Bacteriol.* **181**, 6425–6440 (1999).

13. Marles-Wright, J. & Lewis, R. J. Stress responses of bacteria. *Curr. Opin. Struct. Biol.* **17**, 755–760 (2007).

14. Gottesman, S. Trouble is coming: Signaling pathways that regulate general stress responses in bacteria. *J. Biol. Chem.* **294**, 11685–11700 (2019).

15. Baez, A., Cho, K. M. & Liao, J. C. High-flux isobutanol production using engineered Escherichia coli: A bioreactor study with in situ product removal. *Appl. Microbiol. Biotechnol.* **90**, 1681–1690 (2011).

16. Xiong, M., Schneiderman, D. K., Bates, F. S., Hillmyer, M. A. & Zhang, K. Scalable production of mechanically tunable block polymers from sugar. *Proc. Natl. Acad. Sci. U.*

S. A. **111**, 8357–8362 (2014).

17. Kunjapur, A. M. & Eldridge, R. B. Photobioreactor design for commercial biofuel production from microalgae. *Ind. Eng. Chem. Res.* **49**, 3516–3526 (2010).

18. Chen, C. Y., Yeh, K. L., Aisyah, R., Lee, D. J. & Chang, J. S. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. *Bioresour. Technol.* **102**, 71–81 (2011).