Supporting Information for

Orthogonality and burdens of heterologous AND gate gene circuits in *E. coli*

Qijun Liu[1,2,3], Jörg Schumacher[4], Xinyi Wan[1,2], Chunbo Lou[5] and Baojun Wang[1,2]*

[1]School of Biological Sciences, University of Edinburgh, Edinburgh, EH9 3FF, UK
[2]Centre for Synthetic and Systems Biology, University of Edinburgh, Edinburgh, EH9 3JR, UK
[3]Department of Chemistry and Biology, National University of Defense Technology, China
[4]Department of Life Sciences, Imperial College London, London, SW7 2AZ, UK
[5]CAS Key Laboratory of Microbial Physiological and Metabolic Engineering, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, China

*Corresponding author. Email: baojun.wang@ed.ac.uk

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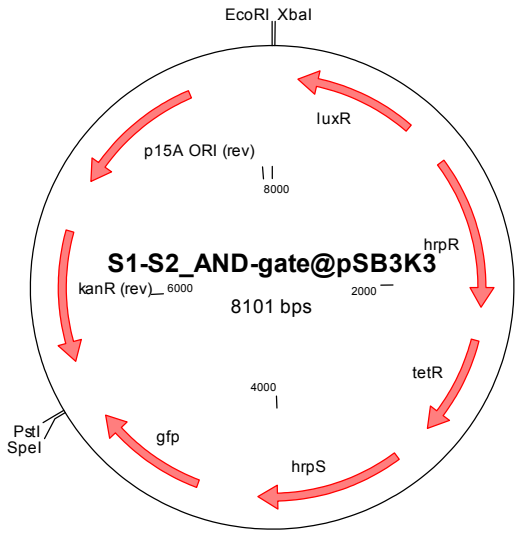
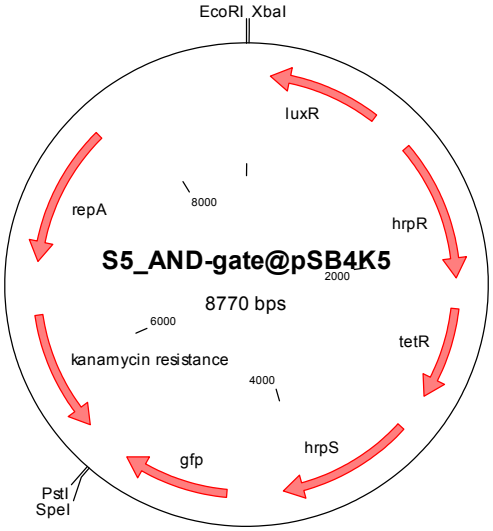
1. Plasmid maps showing the gene circuit constructs used in this study

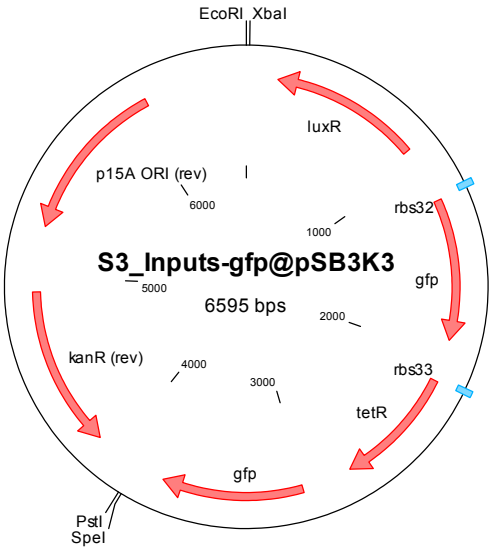
A *pSB3K3* carrying the AND gate



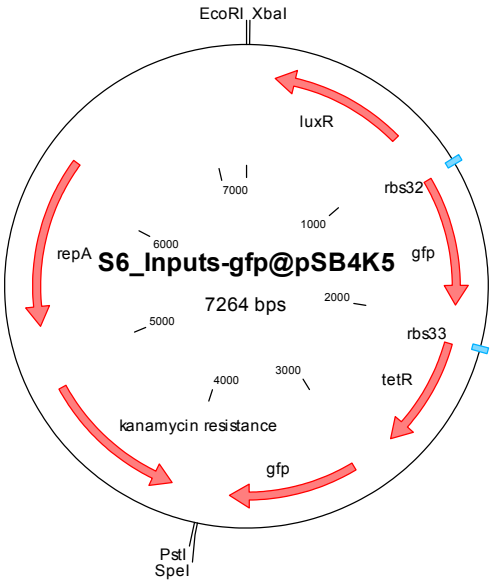
B *pSB4K5* carrying the AND gate



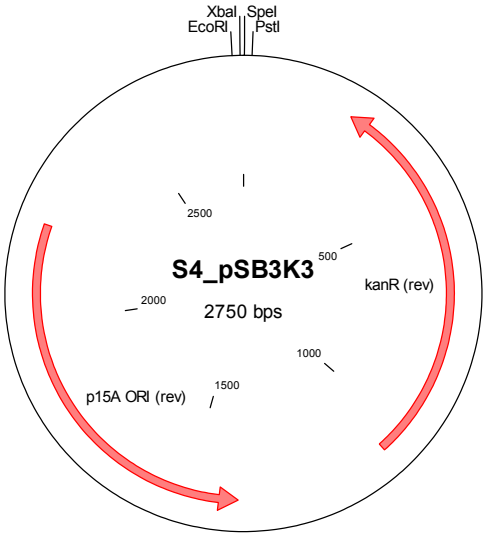
C *pSB3K3* carrying the Inputs-*gfp*



D *pSB4K5* carrying the Inputs-*gfp*



E *pSB3K3* plasmid backbone



F *pSB4K5* plasmid backbone

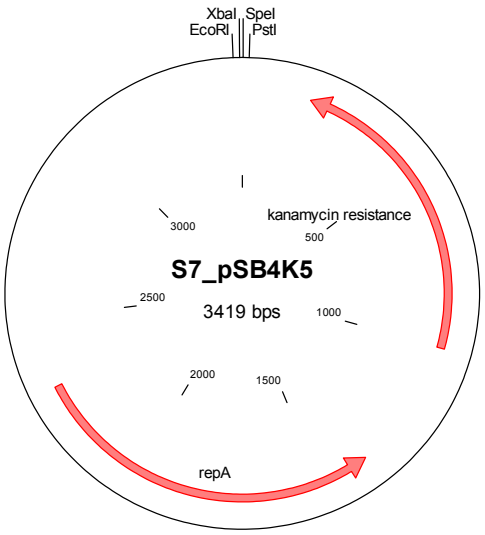


Figure S1. **Plasmid maps showing the gene circuit constructs used in this study**. (A) The AND gate circuit (*J115-rbs32luxR-*P_{lux2}*-rbs32hrpR-B15-J114-rbs30tetR-B15-*P_{tet2}*-rbs33St-hrpL-rbs30gfp-B15*) in the medium copy number *pSB3K3* plasmid (Samples 1 &2). (B) The AND gate circuit in the low copy number *pSB4K5* plasmid (Sample 5). (C) The promoter-inputs circuit (*J115-rbs32luxR-*P_{lux2}*-rbs32gfp-B15-J114-rbs30tetR-B15-*P_{tet2}*-rbs33gfp-B15*) in *pSB3K3* plasmid (Sample 3). (D) The promoter-inputs circuit in *pSB4K5* plasmid (Sample 6). (E) The empty *pSB3K3* plasmid (Sample 4). (F) The empty *pSB4K5* plasmid (Sample 7). Maps were generated in Clone Manager 7.1. Related to Figure 1.

2. Cell growth measurement and the growth curve model fitting

Cell growth was monitored by measuring the sample cell density (OD_{600} readings) periodically (around 30 min). The data were recorded as shown in the below table.

Table S1. Cell growth density raw data (OD_{600} readings)

Time (min)	S1-1	S1-2	S1-3	S2-1	S2-2	S2-3	S3-1	S3-2	S3-3	S4-1
0	0.021	0.018	0.024	0.018	0.019	0.021	0.024	0.019	0.021	0.016
65	0.033	0.037	0.041	0.035	0.038	0.034	0.030	0.037	0.032	0.064
95	0.054	0.055	0.06	0.057	0.059	0.065	0.056	0.062	0.057	0.097
125	0.093	0.086	0.087	0.092	0.091	0.098	0.083	0.089	0.088	0.182
155	0.148	0.151	0.152	0.154	0.162	0.153	0.138	0.139	0.144	0.322
185	0.259	0.258	0.263	0.272	0.278	0.275	0.243	0.247	0.251	0.608
230	0.551	0.560	0.555	0.586	0.595	0.589	0.527	0.528	0.508	1.165
250	0.840	0.852	0.840	0.890	0.906	0.881	0.801	0.779	0.754	1.666
275	1.261	1.228	1.139	1.368	1.361	1.384	1.226	1.163	1.188	2.010
305	1.620	1.654	1.642	1.834	1.868	1.850	1.584	1.606	1.596	2.316
335	2.010	2.05	2.044	2.306	2.290	2.312	1.894	1.912	1.908	2.700

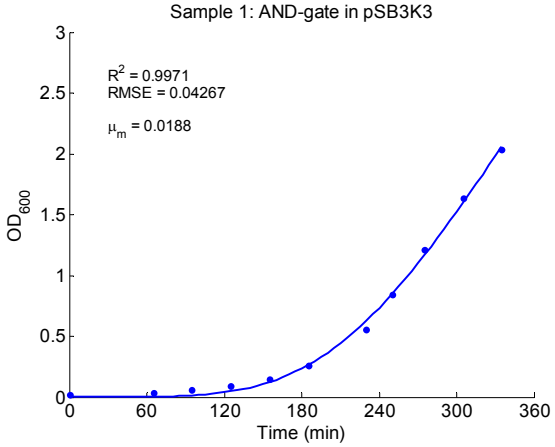
continued columns

S4-2	S4-3	S5-1	S5-2	S5-3	S6-1	S6-2	S6-3	S7-1	S7-2	S7-3
0.019	0.018	0.021	0.018	0.024	0.016	0.024	0.020	0.015	0.020	0.018
0.068	0.065	0.041	0.042	0.047	0.040	0.038	0.043	0.072	0.073	0.070
0.098	0.103	0.071	0.078	0.076	0.070	0.073	0.078	0.097	0.106	0.101
0.178	0.179	0.131	0.126	0.127	0.107	0.113	0.114	0.176	0.180	0.182
0.315	0.312	0.220	0.228	0.230	0.210	0.197	0.192	0.331	0.337	0.328
0.615	0.589	0.428	0.435	0.426	0.366	0.132	0.369	0.664	0.650	0.654
1.116	1.004	0.950	0.965	0.956	0.830	0.836	0.797	1.223	1.184	1.181
1.651	1.582	1.327	1.315	1.308	1.185	1.165	1.098	1.694	1.659	1.627
1.973	2.002	1.862	1.856	1.792	1.702	1.712	1.720	2.014	2.024	1.968
2.306	2.292	2.120	2.144	2.136	2.050	2.066	2.056	2.398	2.406	2.384
2.724	2.702	2.550	2.564	2.546	2.406	2.446	2.420	2.854	2.822	2.834

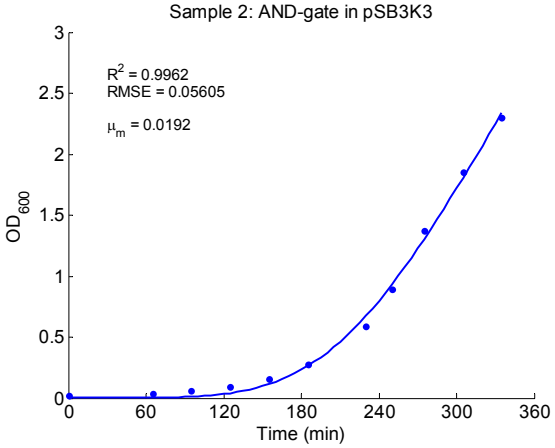
S1-S7 indicates sample type labels. Measurement are three repeats for each sample type.

The cell growth data (Table S1) above were used to plot growth curves as shown in Figure 2A. The nonlinear least square fitting function (cftool) in Matlab (MathWorks R2014a) was applied to fit the experimental data to parameterize the Gompertz model for cell growth[1] (see Methods section for detail). Figure S2 shows the model fitting performance for each sample cell growth data.

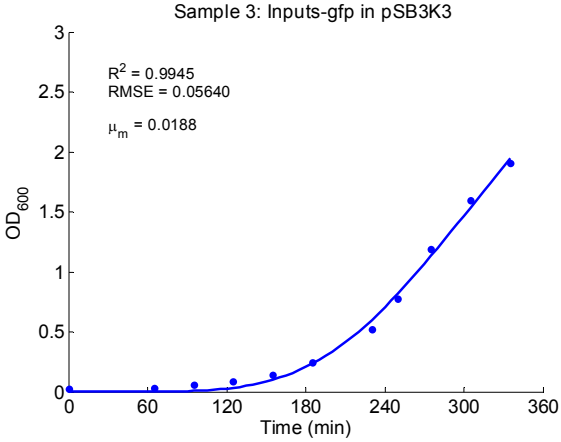
A


Sample 1: AND-gate in pSB3K3
R2 = 0.9971
RMSE = 0.04267
μm = 0.0188
OD600
Time (min)

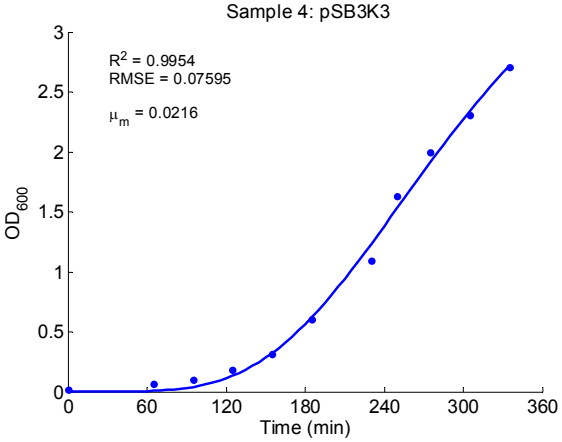
B


Sample 2: AND-gate in pSB3K3
R2 = 0.9962
RMSE = 0.05605
μm = 0.0192
OD600
Time (min)

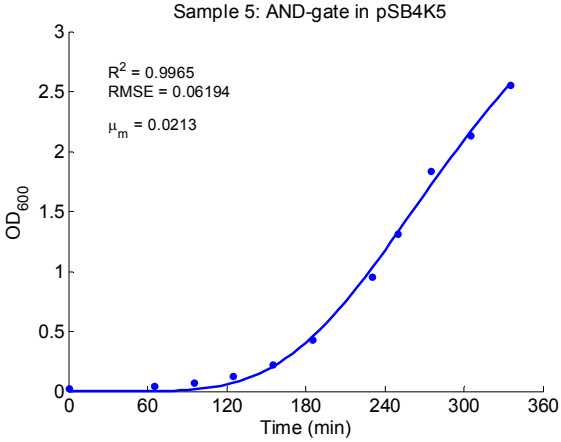
C


Sample 3: Inputs-gfp in pSB3K3
R2 = 0.9945
RMSE = 0.05640
μm = 0.0188
OD600
Time (min)

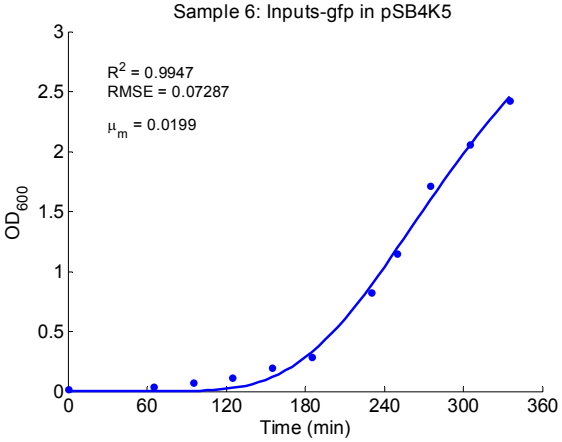
D


Sample 4: pSB3K3
R2 = 0.9954
RMSE = 0.07595
μm = 0.0216
OD600
Time (min)

E


Sample 5: AND-gate in pSB4K5
R2 = 0.9965
RMSE = 0.06194
μm = 0.0213
OD600
Time (min)

F


Sample 6: Inputs-gfp in pSB4K5
R2 = 0.9947
RMSE = 0.07287
μm = 0.0199
OD600
Time (min)

G

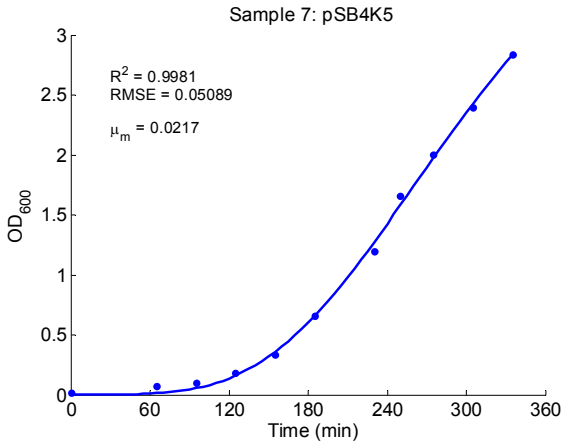


Figure S2. Growth curve model fitting results for all samples in the study. The displayed include the coefficient of determination (R^2), root mean squared error (RMSE) and value of μ_m for each fitting.

3. Gene expression analysis

3.1 Gene expression calculation

To obtain the expression level for each gene, we mapped the reads in RNA-Seq sequencing datasets to the genome of *E. coli* K-12 substrain MG1655 (NCBI accession number NC_00913) and then counted the number of reads mapped to each gene according to their location in the chromosome. The reads were then normalized according to the cognate gene length to obtain the relative expression level for each gene (RPKM value). The distribution of the expression levels of all genes across all seven samples, shown in Figure S3, seems to follow an expected normal distribution.

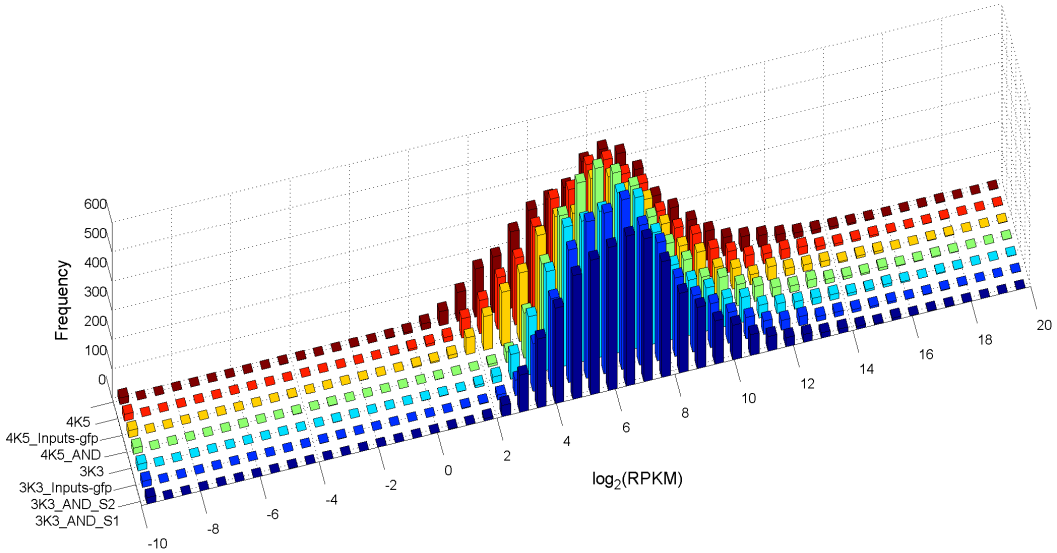


Figure S3. Distribution of transcriptome expression profiles of all samples.

To increase accuracy, under the assumption of normal distribution, we treated genes with the expression values that are out of the typical range of μ ± 3σ as exceptions and thus did not take them into account for subsequent statistical comparison analysis. Here, we filtered out those genes due to their expression levels are either too high or too low, as listed in Table S2.

Table S2. List of genes with too high or too low expression levels and their RPKM values

Samples	S1	S2	S3	S4	S5	S6	S7
Means	6.4747	6.5724	6.5059	6.5862	6.2619	6.2717	6.1154
Std.	2.4243	2.3076	2.3905	2.3015	2.5717	2.5756	2.6779
Too low expressed genes	*insX 0.0386* *yagB 0* *yagA 0* *yagE 0* *yagF 0* *yagG 0* *yagH 0* *yagI 0* *argF 0* *rfbD 0*	*insX 0.0826* *yagB 0* *yagA 0* *yagE 0* *yagF 0* *yagG 0* *yagH 0* *yagI 0* *argF 0.1305* *rfbD 0*	*insX 0* *yagB 0* *yagA 0* *yagE 0* *yagF 0* *yagG 0* *yagH 0* *yagI 0* *argF 0* *rfbD 0*	*insX 0.0414* *yagB 0* *yagA 0* *yagE 0* *yagF 0* *yagG 0* *yagH 0* *yagI 0* *argF 0* *rfbD 0*	*insX 0.0496* *yagB 0* *yagA 0* *yagE 0* *yagF 0* *yagG 0* *yagH 0* *yagI 0* *argF 0* *ykgS 0*	*insX 0* *yagB 0* *yagA 0* *yagE 0* *yagF 0* *yagG 0* *yagH 0* *yagI 0* *argF 0* *rfbD 0*	*insX 0.053* *yagB 0* *yagA 0* *yagE 0* *yagF 0* *yagG 0* *yagH 0* *yagI 0* *argF 0.083* *rfbD 0*

	rfbB 0 *wcaN 0* *wcaM 0* *wcaL 0* *wcaK 0* *wzxC 0* *wcaJ 0* *cpsG 0* *cpsB 0* *wcaI 0* *wcaH 0* *wcaG 0* *gmd 0*	*rfbB 0* *wcaN 0* *wcaM 0* *wcaL 0* *wcaK 0* *wzxC 0* *wcaJ 0* *cpsG 0* *cpsB 0* *wcaI 0* *wcaH 0* *wcaG 0* *gmd 0*	*rfbB 0* *wcaN 0* *wcaM 0* *wcaL 0* *wcaK 0* *wzxC 0* *wcaJ 0* *cpsG 0* *cpsB 0* *wcaI 0* *wcaH 0* *wcaG 0* *gmd 0.132*	*rfbB 0* *wcaN 0* *wcaM 0* *wcaL 0* *wcaK 0* *wzxC 0* *wcaJ 0* *cpsG 0.096* *cpsB 0* *wcaI 0* *wcaH 0* *wcaG 0* *gmd 0*	*ybfI 0* *safA 0.305* *rfbD 0* *rfbB 0* *wcaN 0* *wcaM 0* *wcaL 0* *wcaK 0* *wzxC 0* *wcaJ 0* *cpsG 0* *cpsB 0* *wcaI 0* *wcaH 0* *wcaG 0* *gmd 0*	*rfbB 0* *wcaN 0* *wcaM 0* *wcaL 0* *wcaK 0* *wzxC 0* *wcaJ 0* *cpsG 0* *cpsB 0* *wcaI 0* *wcaH 0* *wcaG 0* *gmd 0*	*rfbB 0* *wcaN 0* *wcaM 0* *wcaL 0* *wcaK 0* *wzxC 0* *wcaJ 0* *cpsG 0* *cpsB 0* *wcaI 0* *wcaH 0* *wcaG 0* *gmd 0* *wcaF 0* *yfdM 0.310*
Too high expressed genes	*mcaS 7.76E5* *cyaR 1.99E5* *gcvB 9.29E5* *ssrS 1.75E5*	*mcaS 9.56E5* *cyaR 2.44E5* *gcvB 1.09E5* *ssrS 1.84E5*	*cyaR 2.11E5* *gcvB 1.54E5* *ssrS 1.89E5*	*mcaS 7.65E5* *cyaR 2.06E5* *gcvB 1.02E5* *ssrS 1.60E5*	*cyaR 2.05E5* *ssrS 2.71E5*	*nmpC 1.24E5* *mcaS 9.44E5* *cyaR 2.18E5* *ryfD 4.83E4* *gcvB 7.12E4* *ssrS 2.44E5* *rbsD 1.55E4*	*nmpC 1.14E5* *mcaS 1.02E6* *cyaR 2.14E5* *ryfD 4.75E4* *gcvB 3.59E4* *ssrS 2.07E5*

3.2 Identicality of the RNA-Seq biological duplicate samples

To verify the repeatability and quality of the RNA-Seq in this study, we have produced biological duplicate for the AND-gate in *pSB3K3* condition, i.e. Sample 1 and Sample 2. Figure S4 shows that the correlation of gene expression in the two replicate samples is significantly high (R^2 = 0.9788), indicating the RNA-Seq performed have excellent reproducibility and is of high credibility. This is also reflected in the uniform mapped reads profiles of the plasmid hosted genes from the two biological duplicate samples (S1 and S2) as shown in Figure S5A-B.

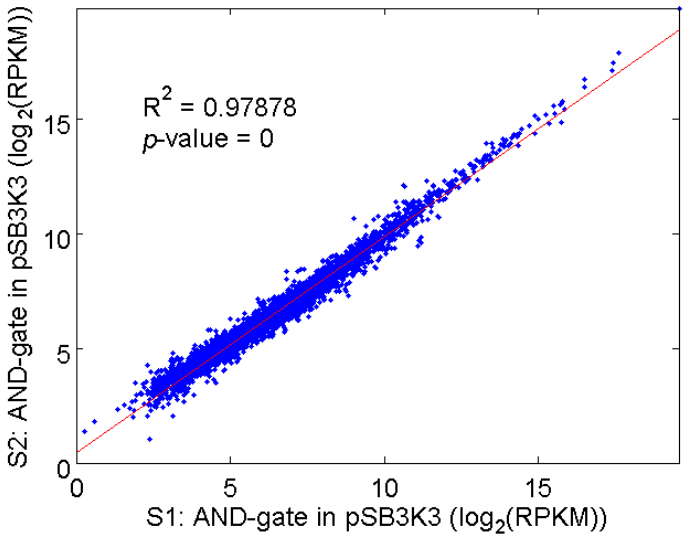
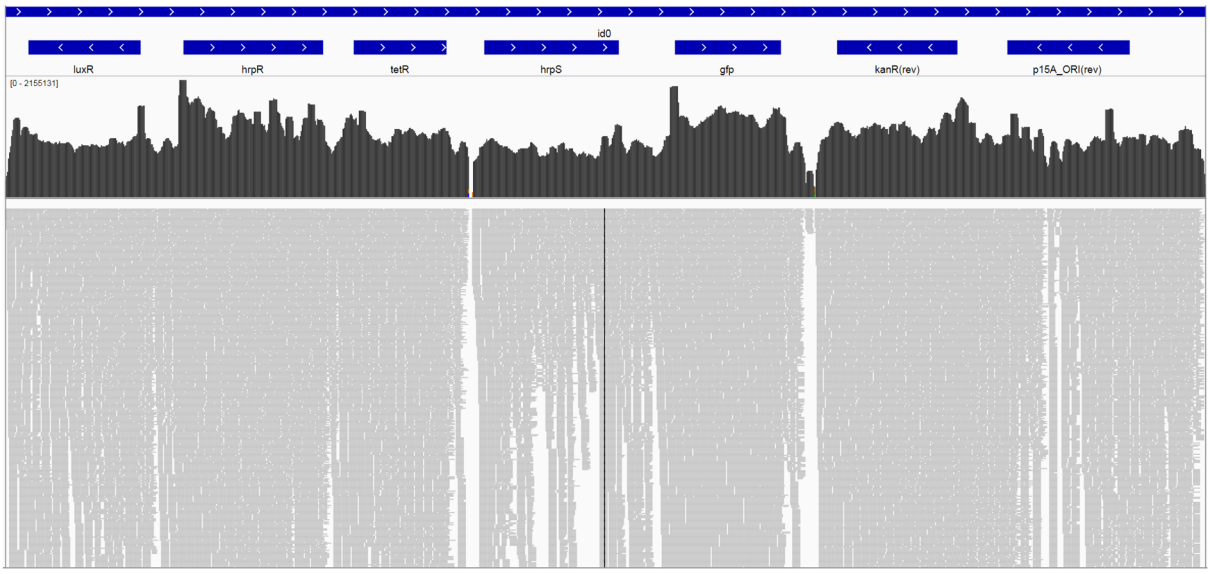
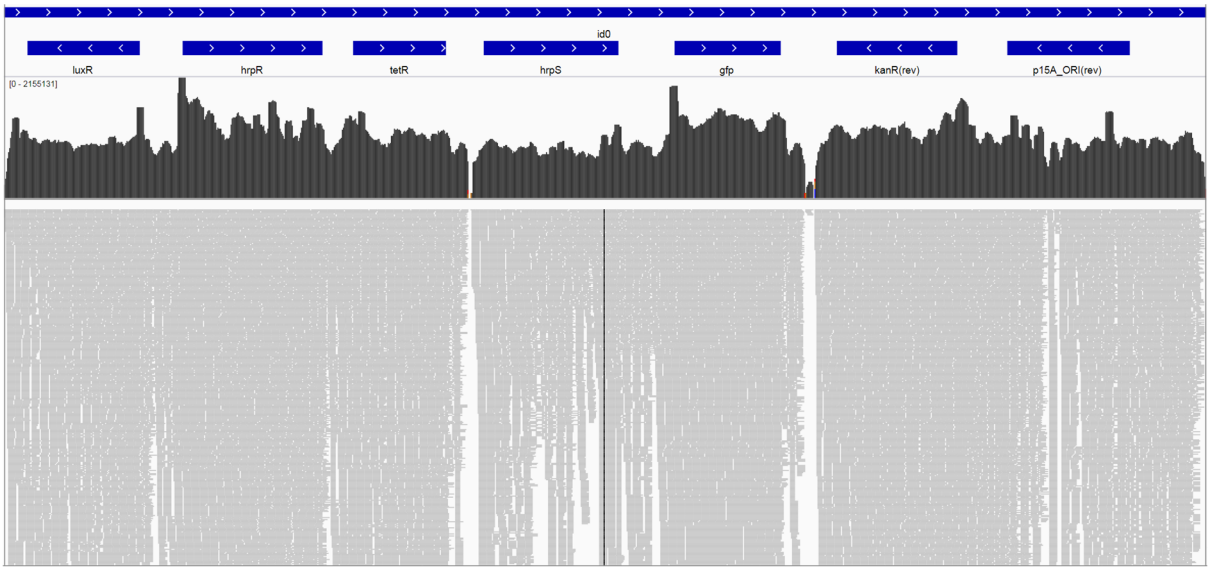


Figure S4. Correlation of gene expression of the biological duplicate samples (S1 vs S2).

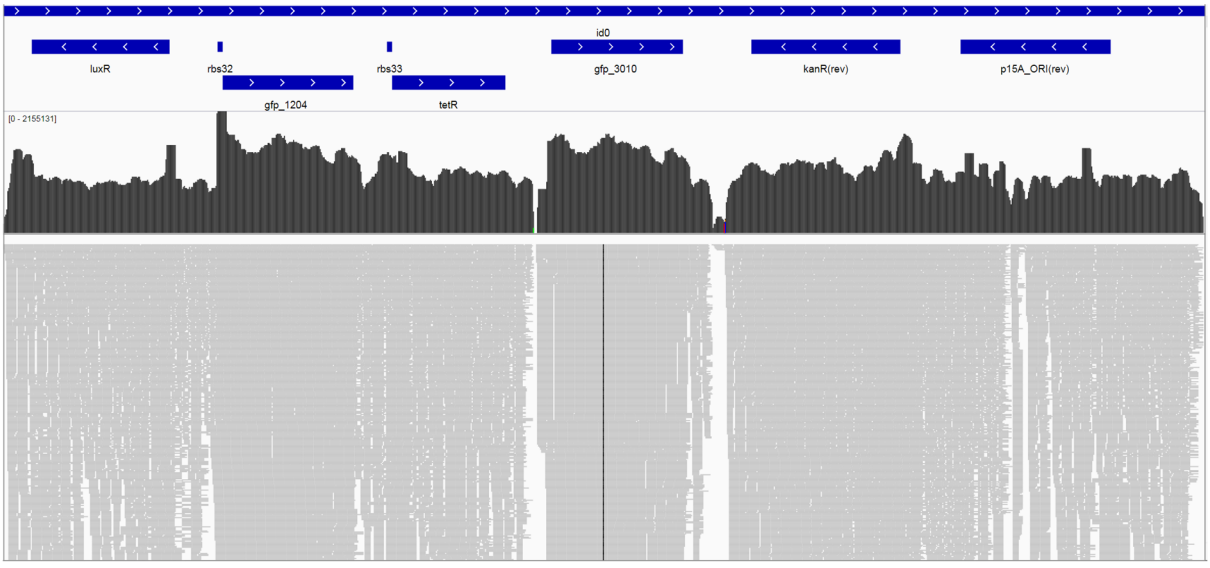
A S1: AND-gate in pSB3K3



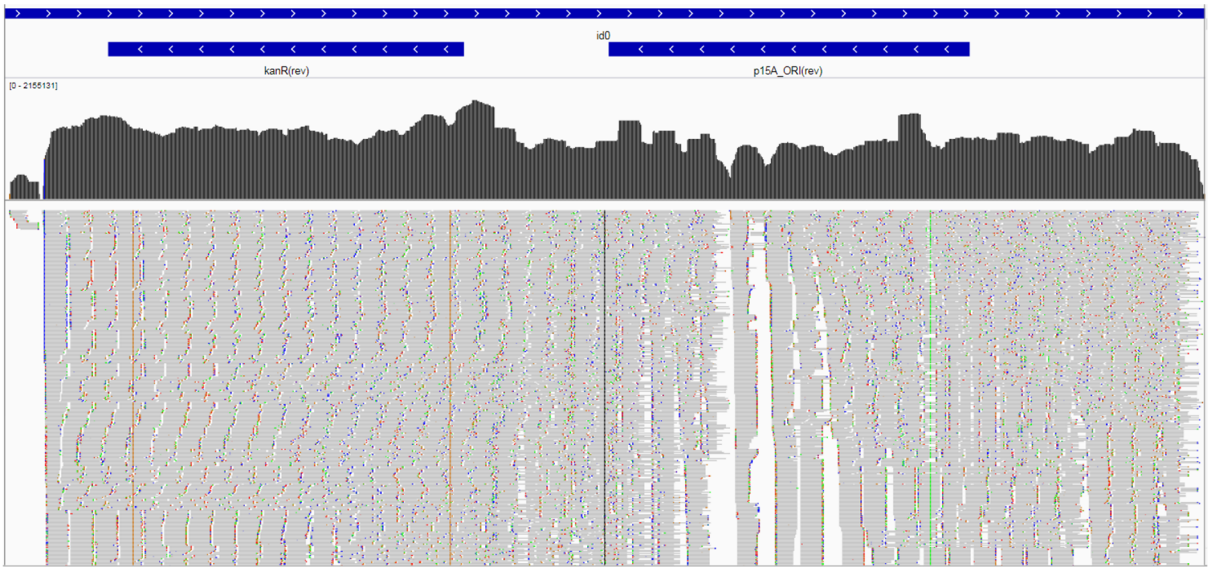
B S2: AND-gate in pSB3K3



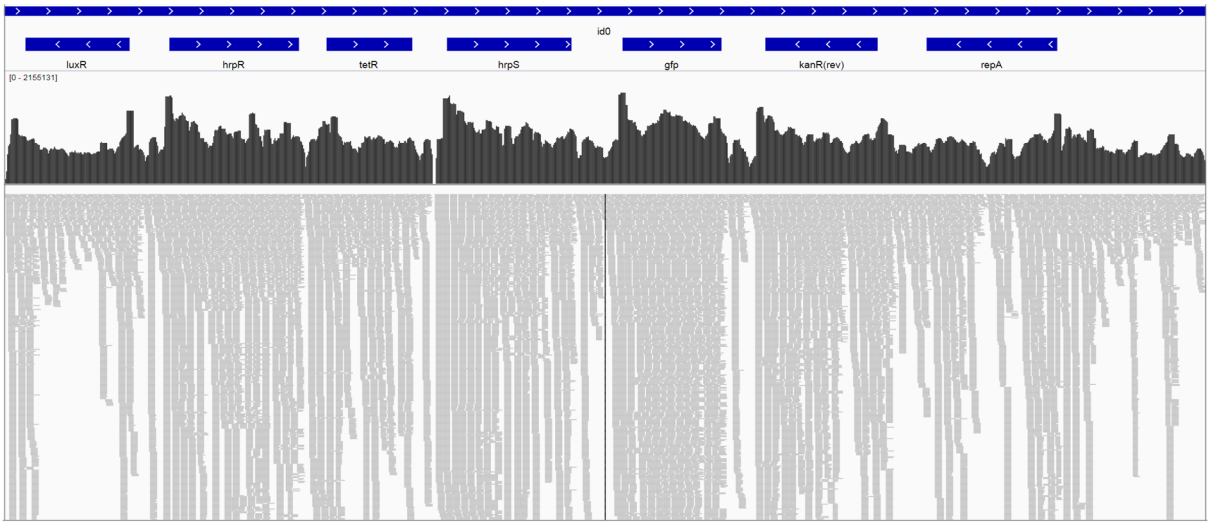
C S3: Inputs-gfp in pSB3K3



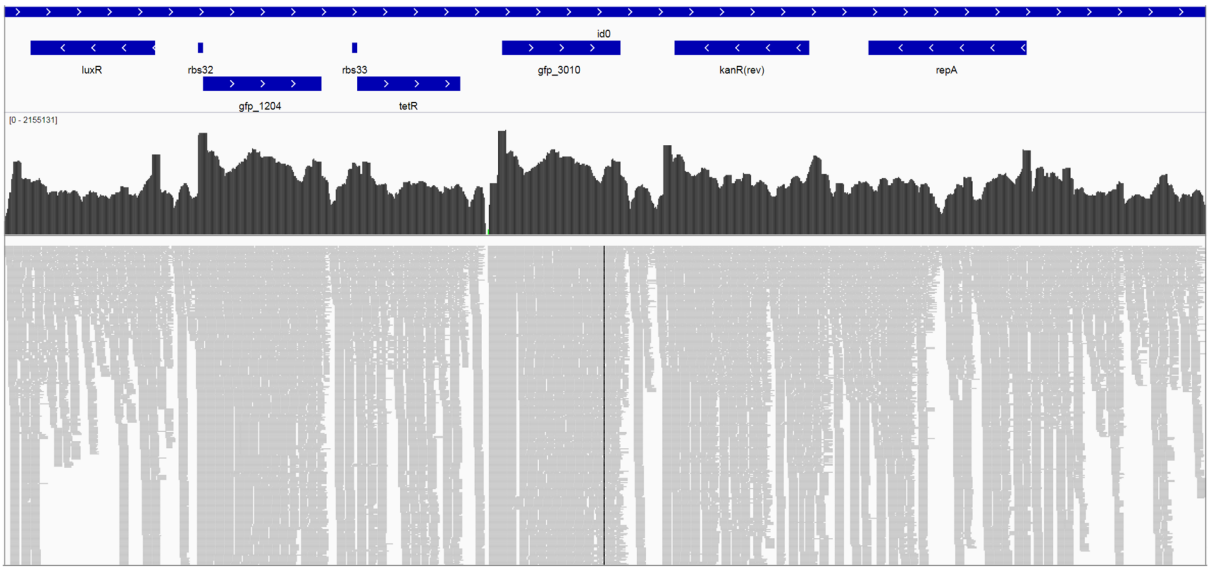
D S4: pSB3K3



E S5: AND-gate in pSB4K5



F S6: Inputs-gfp in pSB4K5



G S7: pSB4K5

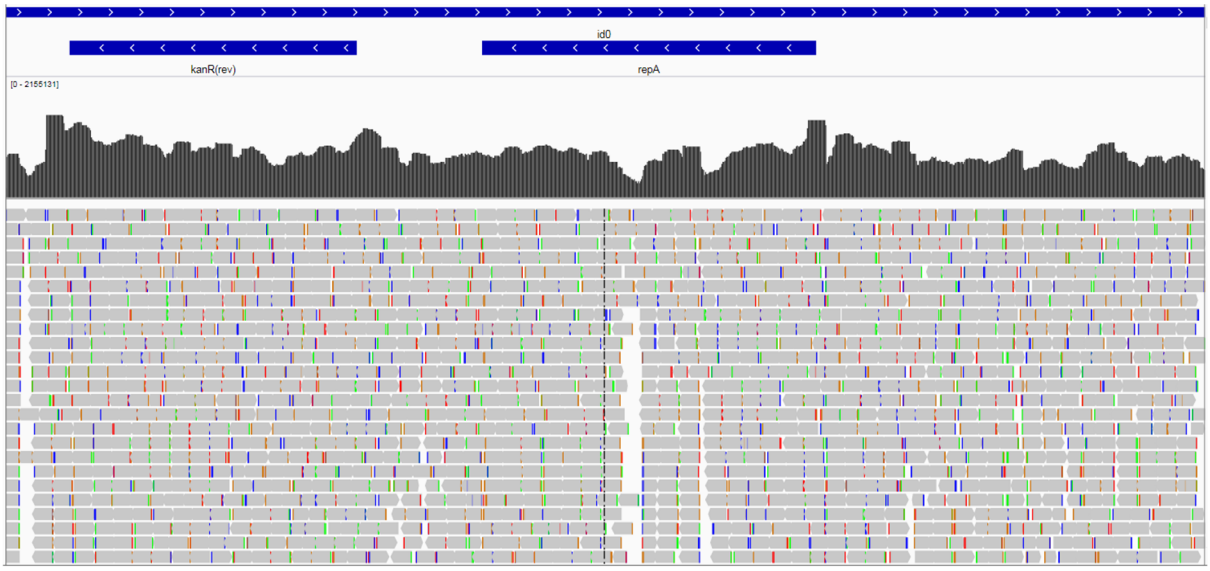


Figure S5. Full mapped transcription profiles and RNA-Seq reads of all the genes in the circuit-hosting plasmids under different conditions for all seven samples S1-S7 (A-G). Read mapping were visualized using the Integrative Genomics Viewer tool (IGV)[2].

3.3 Expression of other genes in the circuit-hosting plasmids

Figure S6 shows the transcription profiles of the antibiotic resistance and origin of replication control genes in the circuit-hosting plasmids under different circuit conditions. Clearly the expression levels of the antibiotics resistance gene (*kan*^{R}) and copy control related genes (*p15A* for *pSB3K3*, *repA* for *pSB4K5*) in the samples containing medium-copy number plasmid (S1-S4) are significantly higher (3-5 times) than those in samples (S5-S7) containing the low-copy number plasmid. The copy number of medium-copy *pSB3K3* is around 3~4 times that of the low-copy *pSB4K5*, consistent with the expression ratios (RPKM values) of *p15A* and *repA* in the two plasmids across all samples (Figure S6).

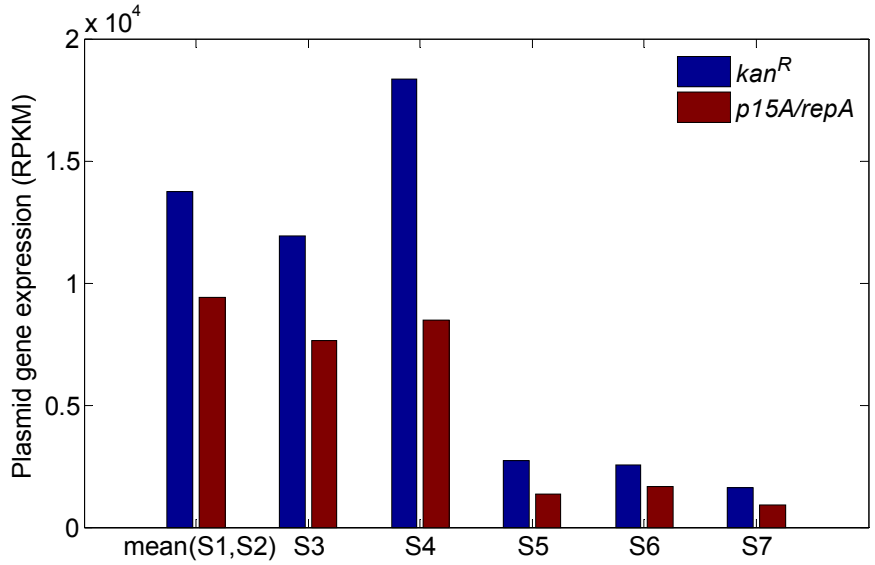


Figure S6. Transcription profiles of the antibiotic resistance and origin of replication control genes in the circuit-hosting plasmids under different conditions.

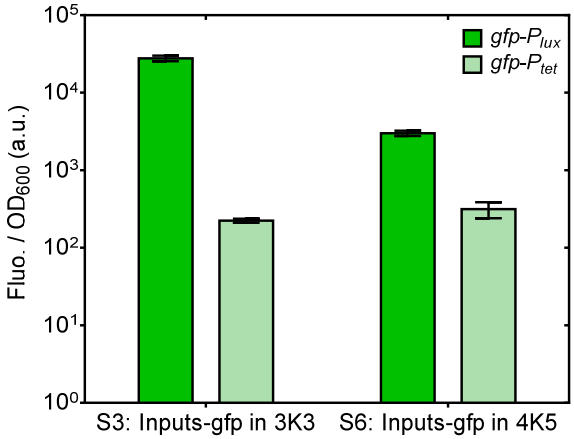


Figure S7. GFP reporter expression under the two inducible promoters in the Inputs-*gfp* circuit hosted in the two plasmids pSB3K3 and pSB4K5. Green bars are the condition when sample cultures were induced with 100 nM AHL, and light green bars are the condition when sample cultures were induced with 20 ng/ml aTc. Cells were grown in M9-glycerol media at 37 °C and assayed 4 hr after induction using a fluorescent microplate reader. Error bars, s.d. (n = 3). a.u., arbitrary units.

3.4 Identification of differentially expressed genes

To minimize potential false positives, two parallel methods were used to find and cross-validate differentially expressed genes between compared conditions.

The first method used is the combined 2-fold expression change detection and χ^2-test. Differentially expressed genes were determined when both the expression levels (RPKM values) between compared conditions having more than 2-fold difference and the p-value < 0.05 from the χ^2-test. The results are listed in the file named *list_of_Chi-Test_DEGs_s2.xls*.

For the second method, the software edgeR[3] was used. edgeR identifies the DEGs from statistical test, hence suitable not only for the samples with biological duplication but also for the case without repeats. The biological duplicate samples are used to calculate the dispersion of gene expression levels, which is then used in the normalization of all other gene expression. Since duplicate is available for one circuit condition, as suggested by edgeR, we used the duplicate samples to calculate the dispersion value in the experiment which was subsequently adopted for all other paired comparison analysis to screen out the DEGs. Here, we calculated the dispersion value 0.0252 from the two duplicate samples (S1 and S2: AND-gate in pSB3K3). The results are listed in the file named *list_of_edgeR_DEGs_s3.xls*.

We then integrated the results from the above two methods to obtain the cross-validated intersection set of identified DEGs (Table 3), as listed in the file *final_DEGs*.xls.

4. Functional analysis of the identified differentially expressed genes

The online tool DAVID[4, 5] was used for the functional enrichment analysis among the identified overlapped differentially expressed genes (Table 3). Gene functions were retrieved from the GO biological process and KEGG pathway databases. The results (Table S4 and S5) show that a few specific biological processes were affected by the heterologous genetic circuits as well as by the copy number variation of their hosting plasmid(s).

Table S3. Function annotations of DEGs in comparisons between circuit-hosting plasmids of different copy number

Functional Catalogues	C5: S1/2 vs S5 (129 genes)	C6: S3 vs S6 (273 genes)	C7: S4 vs S7(627 genes)	
Transport (GO_BP)	ion transport (18) electron transport chain (9)	ion transport (38) electron transport chain (13) organic acid transport (8) carbohydrate transport (14)	ion transport (46) electron transport chain (18) organic acid transport (10) carbohydrate transport (24) phosphonate transport (5)	
Transport (KEGG Pathways)	ABC transporters (13)	ABC transporters (31) Phosphotransferase system (PTS) (3)	ABC transporters (29) Phosphotransferase system (PTS) (8)	
Membrane (SP_PIR_KEYWORDS)	membrane (47)	membrane (95)	membrane (187)	
Metabolic process (in GO_BP)	amine biosynthetic process (9) catechol metabolic process (4) phenol metabolic process (4) diol metabolic process (4) cofactor biosynthetic process (6) nitrogen compound biosynthetic process (12) energy derivation by oxidation of organic compounds (9) tryptophan biosynthetic process(4)	amine biosynthetic process (13) catechol metabolic process (5) phenol metabolic process (5) diol metabolic process (5) cofactor biosynthetic process (9) nitrogen compound biosynthetic process (20) energy derivation by oxidation of organic compounds (20) cellular respiration (18) generation of precursor metabolites and energy (25) organic acid biosynthetic process (14)	amine biosynthetic process (18) catechol metabolic process (5) phenol metabolic process (5) diol metabolic process (5) cofactor biosynthetic process (11) nitrogen compound biosynthetic process (28) energy derivation by oxidation of organic compounds (24) cellular respiration (21) generation of precursor metabolites and energy (31) organic acid biosynthetic process (17) cellular amino acid derivative metabolic process (9)	organophosphate metabolic process (9) alditol metabolic process (7) polyol metabolic process (7) glycerol metabolic process (5) colanic acid biosynthetic process (6) polysaccharide metabolic process (22) carbohydrate biosynthetic process (21) nucleobase metabolic process (7) phospholipid metabolic process (7) phosphorus metabolic process (17) lipid biosynthetic process (16) lipopolysaccharide metabolic process (13) indolalkylamine biosynthetic process (4) fatty acid metabolic process (11) oxidation reduction (50)
metabolic process (KEGG Pathways)	biosynthesis of siderophore group nonribosomal peptides (4) Purine metabolism (3) Nitrogen metabolism (3) Phenylalanine, tyrosine and tryptophan biosynthesis (3)	biosynthesis of siderophore group nonribosomal peptides (5) Purine metabolism (6) Glycerophospholipid metabolism (5) Fructose and mannose metabolism(4) Nitrogen metabolism (6) Citrate cycle (TCA cycle) (3) Glyoxylate and dicarboxylate metabolism (4) Valine, leucine and isoleucine biosynthesis (3) Alanine, aspartate and glutamate metabolism (3)	biosynthesis of siderophore group nonribosomal peptides (5) Purine metabolism (9) Glycerophospholipid metabolism (5) Fructose and mannose metabolism (6) Pentose and glucuronate interconversions (6)	Galactose metabolism (7) Propanoate metabolism (5) Benzoate degradation via CoA ligation (4) Butanoate metabolism (3)
Regulation (GO_BP)	regulation of transcription (7) regulation of RNA metabolic process (5)	regulation of transcription (10) regulation of RNA metabolic process (9)	regulation of transcription (54) regulation of RNA metabolic process (46)	cell division (6)
Signal (KEGG Pathways)	Two-component system (8)	Two-component system (6)	Two-component system (26)	
Others (GO_BP)	protein complex assembly (7) viral infectious cycle (3)	protein complex assembly (7) Bacterial chemotaxis (3)	protein complex assembly (10) Bacterial chemotaxis (3)	response to abiotic stimulus (9) Flagellar assembly (5), cell adhesion (12),

Table S4. Function annotations of DEGs in comparisons between different circuit compositions

Copy number	AND-gate vs. Inputs-*gfp* (mid-copy C2=#25, low-copy C8=#8)	AND-gate vs. empty plasmid (mid-copy C4=#41, low-copy C10=#42)	Inputs-*gfp* vs. empty plasmid (mid-copy C3=#46, low-copy C9=#62)	Function annotation sources
Mid-copy	tryptophan biosynthetic process (3) carboxylic acid biosynthetic process (5) nitrogen compound biosynthetic process (5) oxidation reduction (5) cellular amino acid biosynthetic process (5) indole derivative biosynthetic process (3) indolalkylamine biosynthetic process (3) aromatic amino acid family biosynthetic process (3) heterocycle biosynthetic process (5) dicarboxylic acid metabolic process (3) generation of precursor metabolites and energy(4)	tryptophan biosynthetic process (4) carboxylic acid biosynthetic process (13) nitrogen compound biosynthetic process (13) oxidation reduction (4) cellular amino acid biosynthetic process (13) indole derivative biosynthetic process (4) indolalkylamine biosynthetic process (4) aromatic amino acid family biosynthetic process (4) heterocycle biosynthetic process (4) dicarboxylic acid metabolic process (4) chorismate metabolic process (4) ion transport (5) biogenic amine biosynthetic process (4) sulfur metabolic process (10) sulfate assimilation (5) serine family amino acid biosynthetic process (5) cysteine biosynthetic process (4) sulfate transport (4) inorganic anion transport (4)	tryptophan biosynthetic process (3) carboxylic acid biosynthetic process (11) nitrogen compound biosynthetic process (15) oxidation reduction (6) cellular amino acid biosynthetic process (10) indole derivative biosynthetic process (3) indolalkylamine biosynthetic process (3) aromatic amino acid family biosynthetic process (3) heterocycle biosynthetic process (4) dicarboxylic acid metabolic process (3) generation of precursor metabolites and energy (5) chorismate metabolic process (3) ion transport (4) organic acid biosynthetic process (11) nucleotide biosynthetic process (6) glutamine family amino acid metabolic process (4) purine nucleotide biosynthetic process (3) ribonucleoside monophosphate biosynthetic process (3) nucleoside monophosphate biosynthetic process (3) cellular amino acid derivative biosynthetic process (3) energy derivation by oxidation of organic compounds (5) anaerobic respiration (3)	GO Biological Processes
	Two-component system (3) Phenylalanine, tyrosine and tryptophan biosynthesis (3)	Two-component system (3) Alanine, aspartate and glutamate metabolism (3) ABC transporters (6) Sulfur metabolism (6) Selenoamino acid metabolism (3) Phenylalanine, tyrosine and tryptophan biosynthesis (3)	Two-component system (4) Alanine, aspartate and glutamate metabolism (5) ABC transporters (5) Purine metabolism (5) Pyrimidine metabolism (3)	KEGG pathways
Low-copy	fatty acid oxidation (2)	metal ion transport (12) enterobactin biosynthetic process (4) siderophore biosynthetic process from catechol (4) nonribosomal peptide biosynthetic process (4) phenol metabolic process (4) diol metabolic process (4) cofactor biosynthetic process (4) fatty acid oxidation (3) aromatic amino acid family biosynthetic process (3) nitrogen compound biosynthetic process (4) carboxylic acid biosynthetic process (3)	ion transport (5) metal ion transport (4) aromatic amino acid family biosynthetic process (4) tryptophan biosynthetic process (3) indole derivative biosynthetic process (3) indolalkylamine biosynthetic process (3) dicarboxylic acid metabolic process (4) nitrogen compound biosynthetic process (6) carboxylic acid biosynthetic process (5) amine biosynthetic process (5) regulation of transcription (7)	GO Biological Processes
	Two-component system (3)	Two-component system (10) Biosynthesis of siderophore group nonribosomal peptides (4)	Two-component system (10)	KEGG pathways

5. List of host genes of specific functional categories analyzed in this study

In this study we studied the effect of the imported genetic circuits on the host cell, including the change of expression levels of resource related genes, transcription regulatory genes, housekeeping genes and essential genes.

5.1 Resource related genes

These include the DNA polymerases (Table S5), RNA polymerases (Table S6), transcription termination factors (Table S7), other transcription related genes (Table S8), ribosome and tRNA genes (see the file tRNA_related_genes.xls) and translation related genes (see the file translation_related_genes.xls).

Table S5. List of DNA polymerases genes (retrieved from NCBI database)

Symbol	Alias	Description	Start position	End position	Orientation
polA	b3863, ECK3855, JW3835, *resA*	fused DNA polymerase I 5'->3' polymerase/3'->5' exonuclease/5'->3' exonuclease	4046966	4049752	plus
dinB	b0231, ECK0232, JW0221, *dinP*	DNA polymerase IV	250898	251953	plus
dnaX	b0470, ECK0464, JW0459, *dnaZ*	DNA polymerase III/DNA elongation factor III, tau and gamma subunits	492092	494023	plus
umuD	b1183, ECK1171, JW1172	DNA polymerase V, subunit D	1230767	1231186	plus
dnaN	b3701, ECK3693, JW3678	DNA polymerase III, beta subunit	3881221	3882321	minus
umuC	b1184, ECK1172, JW1173, *uvm*	DNA polymerase V, subunit C	1231186	1232454	plus
dnaE	b0184, ECK0183, JW0179, *polC*, *sdgC*	DNA polymerase III alpha subunit	205126	208608	plus
polB	b0060, ECK0061, JW0059, *dinA*	DNA polymerase II	63429	65780	minus
dnaQ	b0215, ECK0215, JW0205, *mutD*	DNA polymerase III epsilon subunit	236067	236798	plus
holA	b0640, ECK0633, JW0635	DNA polymerase III, delta subunit	670574	671605	minus
holB	b1099, ECK1085, JW1085	DNA polymerase III, delta prime subunit	1155762	1156766	plus
holE	b1842, ECK1843, JW1831	DNA polymerase III, theta subunit	1925108	1925338	plus
holC	b4259, ECK4252, JW4216	DNA polymerase III, chi subunit	4483837	4484280	minus
holD	b4372, ECK4363, JW4334	DNA polymerase III, psi subunit	4607803	4608216	plus
dnaC	b4361, ECK4351, JW4325, *dnaD*	DNA biosynthesis protein	4600238	4600975	minus
dnaA	b3702, ECK3694, JW3679, *hsm-2*	chromosomal replication initiator protein DnaA, DNA-binding transcriptional dual regulator	3882326	3883729	minus
radA	b4389, ECK4381, JW4352, *sms*	DNA repair protein	4625912	4627294	plus

Table S6. List of RNA polymerases genes (retrieved from NCBI database)

Symbol	Alias	Description	Start position	End position	Orientation
rpoA	b3295, ECK3282, JW3257, *pez, phs, sez*	RNA polymerase, alpha subunit	3440039	3441028	minus
rpoB	b3987, ECK3978, JW3950, *ftsR, groN*, *mbrD?, nitB, rif, ron, sdgB, stl, stv, tabD, tabG*	RNA polymerase, beta subunit	4181244	4185272	plus

rpoC	b3988, ECK3979, JW3951, *tabB*	RNA polymerase, beta prime subunit	4185349	4189572	plus
rpoZ	b3649, ECK3639, JW3624, *spoS*	RNA polymerase, omega subunit	3822105	3822380	plus
rpoD	b3067, ECK3057, JW3039, alt	RNA polymerase, sigma 70 (sigma D) factor	3213046	3214887	plus
rpoE	b2573, ECK2571, JW2557, *sigE*	RNA polymerase, sigma 24 (sigma E) factor	2709436	2710011	minus
rpoH	b3461, ECK3445, JW3426, *fam*, *hin*, htpR	RNA polymerase, sigma 32 (sigma H) factor	3599928	3600782	minus
rpoN	b3202, ECK3191, JW3169, *glnF*, *ntrA*	RNA polymerase, sigma 54 (sigma N) factor	3344716	3346149	plus
rpoS	b2741, ECK2736, JW5437, *abrD, appR, csi2, dpeB, katF, nur, otsX, sigS*	RNA polymerase, sigma S (sigma 38) factor	2866558	2867550	minus
fecI	b4293, ECK4283, JW4253	KpLE2 phage-like element; RNA polymerase, sigma 19 factor	4517713	4518234	minus
fliA	b1922, ECK1921, JW1907, *flaD, rpoF*	RNA polymerase, sigma 28 (sigma F) factor	2001069	2001788	minus
rseA	b2572, ECK2570, JW2556, *mclA*, *yfiJ*	anti-sigma factor	2708753	2709403	minus
rseB	b2571, ECK2569, JW2555	anti-sigma E factor, binds RseA	2707798	2708754	minus
flgM	b1071, ECK1056, JW1058, *mviS*	anti-sigma factor for FliA (sigma 28)	1129835	1130128	minus
rapA	b0059, ECK0060, JW0058, *hepA, yabA*	RNA polymerase remodeling/recycling factor ATPase; RNA polymerase-associated, ATP-dependent RNA translocase	60357	63263	minus

Table S7. List of transcription termination factor genes (retrieved from NCBI database)

Symbol	**Aliases**	**Description**	**Start position**	**End position**	**Orientation**
rho	b3783, ECK3775, JW3756, *hdf*, *nitA*, *nusD*, *psuA*, *rnsC*, *sbaA*, *sun*, *tabC*, *tsu*	transcription termination factor	3966416	3967675	plus
greA	b3181, ECK3170, JW3148	transcript cleavage factor	3328238	3328714	minus
greB	b3406, ECK3393, JW3369	transcript cleavage factor	3536811	3537287	plus
nusA	b3169, ECK3158, JW3138	transcription termination/antitermination L factor	3316038	3317525	minus
nusB	b0416, ECK0410, JW0406, *groNB*, *ssaD*, *ssyB*	transcription antitermination protein	435137	435556	plus
nusG	b3982, ECK3973, JW3945	transcription termination factor	4177742	4178287	plus

Table S8. List of other transcription related genes (retrieved from NCBI database)

Symbol	Alias	Description	Start position	End position	Orientation
ruvA	b1861, ECK1862, JW1850	component of RuvABC resolvasome, regulatory subunit	1945364	1945975	minus
ruvB	b1860, ECK1861, JW1849	ATP-dependent DNA helicase, component of RuvABC resolvasome	1944345	1945355	minus
ruvC	b1863, ECK1864, JW1852	component of RuvABC resolvasome, endonuclease	1946854	1947375	minus
rep	b3778, ECK3770, JW5604, *dasC*, *mbrA*, *mmrA*	DNA helicase and single-stranded DNA-dependent ATPase	3960676	3962697	plus
uvrD	b3813, ECK3808, JW3786, *dar*-2, *dda*, *mutU*, *pdeB*, *rad*, *recL*, *srjC*, *uvr502*, *uvrE*	DNA-dependent ATPase I and helicase II	3997982	4000144	plus
dnaB	b4052, ECK4044, JW4012, *groP*, *grpA*, *grpD*	replicative DNA helicase	4264314	4265729	plus
pcnB	b0143, ECK0142, JW5808	poly(A) polymerase	157729	159126	minus
mfd	b1114, ECK1100, JW1100	transcription-repair coupling factor	1170517	1173963	minus
rsd	b3995, ECK3987, JW3959, *yjaE*	stationary phase protein, binds sigma 70 RNA polymerase subunit	4196331	4196807	minus
mfd	b1114, ECK1100, JW1100	transcription-repair coupling factor	1170518	1173964	minus

5.2 The expression of transcription factors

We also studied the expression levels of transcription factors as downloaded from database RegulonDB[6] (Version 8.0). There are 162 transcription factors in total as listed in file regulonDB_TFs.xls and their expression levels among all samples are shown in Figure 4D.

5.3 The expression of housekeeping genes

The expression levels of housekeeping genes are generally constant in various conditions. Here, we studied the expression of 39 housekeeping genes (Table S9) as reported before in the reference[7].

Table S9. List of housekeeping genes

Symbol	description	Symbol	description
mdoG	Glucan biosynthesis protein G	*tolB*	Periplasmic protein
dapA	Dihydrodipicolinate synthase	*rnc*	RNase III
crp	DNA-binding transcriptional dual regulator	*ntpA*	Dihydroneopterin triphosphate pyrophosphatase
hslV	Peptidase component of the HslUV protease	*yabB*	Conserved protein, MraZ family
mrdB	Cell wall shape-determining protein	*lolA*	Chaperone for lipoproteins
fucU	L-Fucose mutarotase	*yggD*	Predicted DNA-binding transcriptional regulator
yjgP	LPS transport (lptF)	*pnp*	Polynucleotide phosphorylase/polyadenylase
yigC	3-Octaprenyl-4-hydroxybenzoate decarboxylase	*xerC*	Site-specific tyrosine recombinase

gor	Glutathione oxidoreductase	*rfaF*	ADP-heptose:LPS heptosyltransferase II
hflB	ATP-dependent metalloprotease	*yigP*	Conserved protein, SCP2 family
yqiB	Predicted dehydrogenase	*gyrB*	DNA gyrase, subunit B
murG	N-Acetylglucosaminyl transferase	*nrdR*	Conserved protein
yrbG	Predicted calcium/sodium:proton antiporter	*hemD*	Uroporphyrinogen III synthase
yejK	Nucleotide associated protein	*pheT*	Phenylalanine tRNA synthetase, beta subunit
yfgA	Cytoskeletal protein required for MreB assembly	*frr*	Ribosome recycling factor
hflX	Putative GTPase HflX	*holC*	DNA polymerase III, chi subunit
cls	Cardiolipin synthase 1	*xerD*	Site-specific tyrosine recombinase
nagC	DNA-binding transcriptional dual regulator, repressor of N-acetylglucosamine	*yheS*	Fused predicted transporter subunits of ABC superfamily: ATP-binding components
spoT	Bifunctional (p)ppGpp synthetase II/guanosine-3,5-bis pyrophosphate 3-pyrophosphohydrolase	*sun*	16S rRNA m(5)C967 methyltransferase,S-adenosyl-L-methionine-dependent
yrbB	ABC transporter maintaining OM lipid asymmetry,cytoplasmic STAS component		

5.4 The expression of essential genes in the host

Essential genes used in this study are referred from the database DEG[8]. These genes are listed in the file named essential_genes_collect.xls, available in the supplementary excel file (Supplementary_file_ list_of_grouped_host_genes_s1).

6. List of genetic part sequences used in the study

Table S10. List of genetic parts, circuit and plasmid backbone sequences used in this study (promoters are in red, RBSs are in italic and bold, protein coding sequences are in brown and terminators are in bold)

Part name	Type and source	DNA sequence (5'– 3')
P_{J114}-*rbs30-tetR-B0015-*P_{tet2}	Inducible promoter with TetR receptor (de novo synthesized)[9]	TTTATGGCTAGCTCAGTCCTAGGTACAATGCTAGCTACTAGAG***ATTAAAGAGGAGAAA***TACCATATGTCCAGATTAGATAAAAGTAAAGTGATTAACAGCGCATTAGAGCTGCTTAATGAGGTCGGAATCGAAGGTTTAACAACCCGTAAACTCGCCCAGAAGCTAGGTGTAGAGCAGCCTACATTGTATTGGCATGTAAAAAATAAGCGGGCTTTGCTCGACGCCTTAGCCATTGAGATGTTAGATAGGCACCATACTCACTTTTGCCCTTTAGAAGGGGAAAGCTGGCAAGATTTTTTACGTAATAACGCTAAAAGTTTTAGATGTGCTTTACTAAGTCATCGCGATGGAGCAAAAGTACATTTAGGTACACGGCCTACAGAAAAACAGTATGAAACTCTCGAAAATCAATTAGCCTTTTTATGCCAACAAGGTTTTTCACTAGAGAATGCATTATATGCACTCAGCGCTGTGGGGCATTTTACTTTAGGTTGCGTATTGGAAGATCAAGAGCATCAAGTCGCTAAAGAAGAAAGGGAAACACCTACTACTGATAGTATGCCGCCATTATTACGACAAGCTATCGAATTATTTGATCACCAAGGTGCAGAGCCAGCCTTCTTATTCGGCCTTGAATTGATCATTTGCGGATTAGAAAAACAACTTAAATGTGAAAGTGGGTCCTAATAATACTAGAG**CCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATA**TACTAGAGTTTTCAGCAGGACGCACTGACCTCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGATACTGAGCACATAT
P_{J115}-*rbs32-luxR-B0015-*P_{lux2}	Inducible promoter with LuxR receptor (de novo synthesized)[9]	TTTATAGCTAGCTCAGCCCTTGGTACAATGCTAGCTACTAGAG***TCACACAGGAAAG***TACTAGATGAAAAACATAAATGCCGACGACACATACAGAATAATTAATAAAATTAAAGCTTGTAGAAGCAATAATGATATTAATCAATGCTTATCTGATATGACTAAAATGGTACATTGTGAATATTATTTACTCGCGATCATTTATCCTCATTCTATGGTTAAATCTGATATTTCAATCCTAGATAATTACCCTAAAAAATGGAGGCAATATTATGATGACGCTAATTTAATAAAATATGATCCTATAGTAGATTATTCTAACTCCAATCATTCACCAATTAATTGGAATATATTTGAAAACAATGCTGTAAATAAAAAATCTCCAAATGTAATTAAAGAAGCGAAAACATCAGGTCTTATCACTGGGTTTAGTTTCCCTATTCATACGGCTAACAATGGCTTCGGAATGCTTAGTTTTGCACATTCAGAAAAAGACAACTATATAGATAGTTTATTTTTACATGCGTGTATGAACATACCATTAATTGTTCCTTCTCTAGTTGATAATTATCGAAAAATAAATATAGCAAATAATAAATCAAACAACGATTTAACCAAAAGAGAAAAAGAATGTTTAGCGTGGGCATGCGAAGGAAAAAGCTCTTGGGATATTTCAAAAATATTAGGTTGCAGTGAGCGTACTGTCACTTTCCATTTAACCAATGCGCAAATGAAACTCAATACAACAAACCGCTGCCAAAGTATTTCTAAAGCAATTTTAACAGGAGCAATTGATTGCCCATACTTTAAAAATTAATAACACTGATAGTGCTAGTGTAGATCACTACTAGAG**CCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATA**TACTAGAGACCTGTAGGATCGTACAGGTTTACGCAAGAAAATGGTTTGTTACTTTCGAATAAA

hrpR	Gene[10, 11]	ATGAGTACAGGCATCGATAAGGACGTCCGAGAGTGTTGGGGCGTAACTGCATT ATCAGCGGGTCATCAAATTGCAATGAATAGCGCGTTTCTGGATATGGACTTGC TGTTGTGCGGGGAAACCGGCACCGGCAAGGACACACTGGCCAACCGCATTCAC GAGTTGTCCAGCAGGTCGGGACCCTTTGTGGGCATGAACTGCGCCGCCATTCC CGAGTCGCTGGCAGAGAGCCAGTTATTCGGTGTGGTCAACGGTGCATTCACCG GCGTATGCCGGGCTCGCGAGGGCTACATAGAGGCCTCCAGTGGTGGCACCTTG TACCTGGATGAAATCGACAGCATGCCGTTGAGCCTGCAAGCCAAACTGCTGCG TGTGTTGGAGAGTCGAGGTATCGAGCGTCTGGGCTCGACCGAATTTATCCCGG TGGATCTGCGGATCATTGCCTCGGCCCAGCGGCCACTGGATGAACTGGTGGAA CAAGGACTTTTCCGTCGCGACCTGTTTTTTCGGCTCAACGTGCTGACGCTTCA CTTGCCAGCCTTGCGCAAACGTCGTGAACAGATCCTGCCATTGTTCGACCAGT TCACCCAGGGTATCGCTGCCGAGTTCGGACGTCCCGCTCCTGCGCTGGACAGC GGGCGTGTGCAGCTGCTGCTCAGCCACGACTGGCCGGGCAACATCCGCGAATT GAAGTCTGCGGCCAAGCGCTTCGTACTCGGCTTCCCCTTGCTGGGCGCCGACC CTGTGGAAGCGCTTGACCCTGCCACGGGGCTGCGCACGCAAATGCGCATCATC GAGAAAATGCTCATCCAGGATGCCTTGAAGCGGCACAGGCACAATTTCGACGC GGTGCTTCAGGAGTTGGAGTTGCCAAGACGCACCCTGTATCACCGCATGAAGG AACTGGGAGTTGCAGCGCCGATCGCTGCGACGGCCGGGGTCTAATAA
hrpS	Gene[10, 11]	ATGAGTCTTGATGAAAGGTTTGAGGATGATCTGGACGAGGAGCGGGTTCCGAA TCTGGGGATAGTTGCCGAAAGTATTTCGCAACTGGGTATCGACGTGCTGCTAT CGGGTGAGACCGGCACGGGCAAAGACACGATTGCCCGACGGATTCATGAGATG TCAGGCCGCAAAGGGCGCCTGGTGGCGATGAATTGCGCGGCCATTCCGGAGTC CCTCGCCGAGAGCGAGTTATTCGGCGTGGTCAGCGGTGCCTACACCGGCGCTG ATCGCTCCAGAGTCGGTTATGTCGAAGCGGCGCAGGGCGGCACGCTGTACCTG GATGAGATCGATAGCATGCCGCTGAGCCTGCAAGCCAAATTGCTGAGGGTGCT GGAAACCCGAGCGCTTGAACGGCTGGGTTCGACGTCGACGATCAAGCTGGATA TCTGCGTGATCGCCTCCGCCCAATGCTCGCTGGACGACGCCGTCGAGCGGGGG CAGTTTCGTCGCGATCTGTATTTTCGCCTGAACGTCCTGACACTCAAGCTTCC TCCGCTACGTAACCAGTCTGATCGCATAGTTCCCCTGTTCACACGTTTTACGG CCGCCGCCGCGAGGGAGCTCGGTGTTCCCGTTCCCGATGTTTGCCCACTGCTG CACAAAGTGCTGCTGGGCCACGACTGGCCCGGCAATATCCGTGAGCTCAAGGC GGCAGCCAAACGCCATGTGCTGGGTTTCCCCTTGCTGGGCGCCGAGCCGCAGG GCGAAGAGCACTTGGCCTGTGGGCTCAAATCGCAATTGCGAGTGATCGAAAAA GCCCTGATTCAGGAGTCGCTCAAGCGCCACGACAATTGTGTGGATTCGGTAAG CCTGGAACTGGACGTGCCACGCCGTACGCTCTATCGACGCATCAAAGAATTGC AGATCTAATAA
hrpL	Promoter[10, 11]	GCCGGATTATGTCCGCTGAGTGGGTCACGGTCCCGGATCAGTTCCCTTGCGAA GCTGACCGATGTTTTTGTGCCAAAAGCTGTTGTGGCAAAAAACGGTTTGCGCA AAGTTTTGTATTACAAAGAATTTCACATTTTAAAATATCTTTATAAATCAATC AGTTATTTCTATTTTTAAGCTGGCATGGTTATCGCTATAGGGCTTGTAC

gfp	Gene[12]	ATGCGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATT AGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTG ATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTA CCTGTTCCATGGCCAACACTTGTCACTACTTTCGGTTATGGTGTTCAATGCTT TGCGAGATACCCAGATCATATGAAACAGCATGACTTTTTCAAGAGTGCCATGC CCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTAC AAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGA GTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGG AATACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAAT GGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCA ACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTT TACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAAC GAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTAC ACATGGCATGGATGAACTATACAAATAATAA
rbs30	RBS[10]	TCTAGAG***ATTAAAGAGGAGAAA***TACTAG**ATG**
rbs32	RBS[10]	TCTAGAG***TCACACAGGAAAG***TACTAG**ATG**
rbs33	RBS[10]	TCTAGAG***TCACACAGGAC***TACTAG**ATG**
J114	Promoter[13]	TTTATGGCTAGCTCAGTCCTAGGTACAATGCTAGC
J115	Promoter[13]	TTTATAGCTAGCTCAGCCCTTGGTACAATGCTAGC
B0015 (B15)	Terminator[14]	**CCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTT ATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTC GGGTGGGCCTTTCTGCGTTTATA**

pSB3K3	Plasmid backbone[15, 16]	TACTAGTAGCGGCCGCTGCAGTCCGGCAAAAAAACGGGCAAGGTGTCACCACCCTGCCCT TTTTCTTTAAAACCGAAAAGATTACTTCGCGTTATGCAGGCTTCCTCGCTCACTGACTCC CTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATCTCC AGTCCCGTCAAGTCAGCGTAATGCTCTGCCAGTGTTACAACCAATTAACCAATTCTGATT AGAAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATAC CATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATA GGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTA TTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTC AATCCGGTGAGAATGGCAAAAGCTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGC CATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTCATTCGTGATTGCC CCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAAT GCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATT CTTCTAATACCTGGAATGCTGTTTTCCCGGGGATCGCAGTGGTGAGTAACCATGCATCAT CAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTA GTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACA ACTCTGGCGCATCGGGCTTCCCATACAATCGATAGATTGTCGCACCTGATTGCCCGACAT TATCGCGAGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCC TCGAGCAAGACGTTTCCCGTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGT AAGCAGACAGTTTTATTGTTCATGATGATATATTTTTATCTTGTGCAATGTAACATCAGA GATTTTGAGACACAACGTGGCTTTGTTGAATAAATCGAACTTTTGCTGAGTTGAAGGATC AGATCACGCATCTTCCCGACAACGCAGACCGTTCCGTGGCAAAGCAAAAGTTCAAAATCA CCAACTGGTCCACCTACAACAAAGCTCTCATCAACCGTGGCTCCCTCACTTTCTGGCTGC ATGATGGGGCGATTCAGGCCTGGTATGAGTCAGCAACACCTTCTTCACGAGGCAGACCTC AGCGCTAGCGGAGTGTATACTGGCTTACTATGTTGGCACTGATGAGGGTGTCAGTGAAGT GCTTCATGTGGCAGGAGAAAAAAGGCTGCACCGGTGCGTCAGCAGAATATGTGATACAGC ATATATTCCGCTTCCTCGCTCACTGACTCGCTACGCTCGGTCGTTCGACTGCGGCGAGCC GAAATGGCTTACGAACGGGGCGGAGATTTCCTGGAAGATGCCAGGAAGATACTTAACAGC GAAGTGAGAGGGCCGCGGCAAAGCCGTTTTTCCATAGGCTCCGCCCCCCTGACAAGCATC ACGAAATCTGACGCTCAAATCAGTGGTGGCGAAACCCGACAGGACTATAAAGATACCAGC CGTTTCCCCTGGCGGCTCCCTCGTGCGCTCTCCTGTTCCTGCCTTTCGGTTTACCGGTGT CATTCCGCTGTTATGGCCGCGTTTGTCTCATTCCACGCCTGACACTCAGTTCCGGGTAGC CAGTTCGCTCCAAGCTGGACTGTATGCACGAACCCCCCGTTCAGTCCGACCGCTGCGCCT TATCCGGTAACTATCGTCTTGAGTCCAACCCGGAAAGACATGCAAAAGCACCACTGGCAC CAGCCACTGGTAATTGATTTAGAGGAGTTAGTCTTGAAGTCATGCGCCGGTTAAGGCTAA ACTGAAAGGACAAGTTTTGGTGACTGCGCTCCTCCAAGCCAGTTACCTCGGTTCAAAGAC TTGGTAGCTCAGAGAACCTTCGAAAAACCGCCCTGCAAGGCGGTTTTTTCGTTTTCAGAC CAAGAGATTACGCGCAGACCAAAACGATCTCAAGAAGATCATCTTATTAAGGGGTCTGAC GCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATC TTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAC TAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGT CTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAC GGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCA GATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACT TTATCCGCCTCCATCCAGTCTATTCCATGGTGCCACCTGACGTCTAAGAAACCATTATTA TCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCAGAATTTCAGATAAAAAAAAT CCTTAGCTTTCGCTAAGGATGATTTCTGGAATTCGCGGCCGCTTCTAGAG

pSB4K5	Plasmid backbone[16, 17]	TACTAGTAGCGGCCGCTGCAGGAGTCACTAAGGGTTAGTTAGTTAGATTAGCAGAAAGTC AAAAGCCTCCGACCGGAGGCTTTTGACTAAAACTTCCCTTGGGGTTATCATTGGGGCTCA CTCAAAGGCGGTAATCAGATAAAAAAAATCCTTAGCTTTCGCTAAGGATGATTTCTGCTA GTATTATTAGAAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATT ATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCA GTTCCAAAGAATGGCAAGGTCCTGGTAACGGTCTGCGATTCCGACCCGTCCAACATCAAT ACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGT GACGACTGAATCCGGTGAGAATGGCAAGAGCTTGTGCATTTCTTTCCAGACTTGTTCAAC AGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTCATGCC TGATTGCGCCTGAGCAAGACGAAATACACGATCGCTGTTAAAAGGACAATTACAAACAGC AATCGAATGTAACCGGCGCAGGAACACGGCCAGCGCATCAACAATATTTTCACCTGAATC AGGATATTCTTCTAATACCTGGAAGGCTGTTTTCCCAGGAATCGCGGTGGTGAGTAACCA CGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGGAGAGGCATAAACTCCGTCAC CCAGTTGAGACGGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTT CAGAAACAACTCTGGCGCATCGGGCTTCCCATACAAGCGATAGATTGTCGCACCTGATTC CCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCGTCCATGTTGGAGTTTAA GCGCGGACGGGAGCAAGACGTTTCCCGTTGAATATGGCTCATAACACCCCTTGTATTACT GTTTATGTAAGCAGACAGTTTTATTGTTCATGATGATATATTTTTATCTTGTGCAATGTA ACATCAGAGATTTTGAGACACAACGTGGCTTTGTTGAATAAATCGAACTTTTGCTGAGTT GAAGGATCAGCTCTAGTAGTTACATTGTCGATCTGTTCATGGTGAACAGCTTTGAATGCA CCAAAAACTCGTAAAAGCTCTGATGTATCTATCTTTTTTACACCGTTTTCATCTGTGCAT ATGGACAGTTTTCCCTTTGATATGTAACGGTGAACAGTTGTTCTACTTTTGTTTGTTAGT CTTGATGCTTCACTGATAGATACAAGAGCCATAAGAACCTCAGATCCTTCCGTATTTAGC CAGTATGTTCTCTAGTGTGGTTCGTTGTTTTTGCGTGAGCCATGAGAACGAACCATTGAC ATCATACTTACTTTGCATGTCACTCAAAAATTTTGCCTCAAAACTGGTGAGCTGAATTTT TGCAGTTAAAGCATCGTGTAGTGTTTTTCTTAGTCCGTTATGTAGGTAGGAATCTGATGT AATGGTTGTTGGTATTTTGTCACCATTCATTTTTATCTGGTTGTTCTCAAGTTCGGTTAC GAGATCCATTTGTCTATCTAGTTCAACTTGGAAAATCAACGTATCAGTCGGGCGGCCTCG CTTATCAACCACCAATTTCATATTGCTGTAAGTGTTTAAATCTTTACTTATTGGTTTCAA AACCCATTGGTTAAGCCTTTTAAACTCATGGTAGTTATTTTCAAGCATTAACATGAACTT AAATTCATCAAGGCTAATCTCTATATTTGCCTTGTGAGTTTTCTTTTGTGTTAGTTCTTT TAATAACCACTCATAAATCCTCATAGAGTATTTGTTTTCAAAAGACTTAACATGTTCCAG ATTATATTTTATGAATTTTTTTAACTGGAAAAGATAAGGCAATATCTCTTCACTAAAAAG TAATTCTAATTTTTCGCTTGAGAACTTGGCATAGTTTGTCCACTGGAAAATCTCAAAGCG TTTAACCAAAGGATTCCTGATTTCCACAGTTCTCGTCATCAGCTCTCTGGTTGCTTTAGC TAATACACCATAAGCATTTTCCCTACTGATGTTCATCATCTGAGCGTATTGGTTATAAGT GAACGATACCGTCCGTTCTTTCCTTGTAGGGTTTTCAATCGTGGGGTTGAGTAGTGCCAC ACAGCATAAAATTAGCTTGGTTTCATGCTCCGTTAAGTCATAGCGACTAATCGCTAGTTC ATTTGCTTTGAAAACAACTAATTCAGACATACATCTCAATTGGTCTAGGTGATTTTAATC ACTATACCAATTGAGATGGGCTAGTCAATGATAATTACATGTCCTTTTCCTTTGAGTTGT GGGTATCTGTAAATTCTGCTAGACCTTTGCTGGAAAACTTGTAAATTCTGCTAGACCCTC TGTAAATTCCGCTAGACCTTTGTGTGTTTTTTTTGTTTATATTCAAGTGGTTATAATTTA TAGAATAAAGAAAGAATAAAAAAAGATAAAAAGAATAGATCCCAGCCCTGTGTATAACTC ACTACTTTAGTCAGTTCCGCAGTATTACAAAAGGATGTCGCAAACGCTGTTTGCTCCTCT ACAAAACAGACCTTAAAACCCTAAAGGCTTAAGTAGCACCCTCGCAAGCTCGGGCAAATC GCTGAATATTCCTTTTGTCTCCGACCATCAGGCACCTGAGTCGCTGTCTTTTTCGTGACA TTCAGTTCGCTGCGCTCACGGCTCTGGCAGTGAATGGGGGTAAATGGCACTACAGGCGCC TTTTATGGATTCATGCAAGGAAACTACCCATAATACAAGAAAAGCCCGTCACGGGCTTCT CAGGGCGTTTTATGGCGGGTCTGCTATGTGGTGCTATCTGACTTTTTGCTGTTCAGCAGT TCCTGCCCTCTGATTTTCCAGTCTGACCACTTCGGATTATCCCGTGACAGGTCATTCAGA CTGGCTAATGCACCCAGTAAGGCAGCGGTATCATCAACAGGCTTACCCGTCTTACTGTCC CTAGTGCTTGGATTCTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAA TCCAGATGGAGTTCTGAGGTCATTACTGGATCTATCAACAGGAGTCCAAGCGAGCTCGTA AACTTGGTCTGACAGCTCTAGCTCCGGCAAAAAAACGGGCAAGGTGTCACCACCCTGCCC TTTTTCTTTAAAACCGAAAAGATTACTTCGCGTTTGCCACCTGACGTCTAAGAAAAGGAA TATTCAGCAATTTGCCCGTGCCGAAGAAAGGCCCACCCGTGAAGGTGAGCCAGTGAGTTC ATTGCTACGTAATTAGTTAGTTAGCCCTTAGTGACTCGAATTCGCGGCCGCTTCTAGAG

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