[Supporing information]

Isolation of Fully Synthetic Promoters for High-Level Gene Expression in *Corynebacterium glutamicum*

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Table SI. List of PCR primers used in this study.

Primer name	Primer sequence (5' to 3')[a]
Ptrc-F	GA**GGTACC**TTGACAATTAATCATCCGGCTC
Ptrc-R	CT**GGATCC**GGTCTGTTTCCTGTGTGAAATT
GFP-F	GA**GGATCC**ATGAGTAAAGGAGAAGAACTTTTCACTGG
GFP-R	GC**GCGGCCGC**TTATTTGTCATCGTCATCTTTATAATCGTCGACCTTGGATAGTTCATC
Synpro-F	GCA**GGTACC**NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNAGGANNNNNNNN**GGATCC**ATGAGTAAAGGAGAAGAACT
Synpro-R	GTGCCATGCCCGAAGGTTATG
XynA-F	TG**GGATCC**ATGAAGCTTTCACACCGCATCGCAGCAATGGCAGCAACCGCAGGCATCACAGTGGCAGCATTCGCAGCACCTGCTTCCGCAGCCGAGAGCACGCTC
XynA-R	CG**CATATG**CTATTAATGATGGTGATGGTGATGGGTGCGGGTCCAGC
M18-F	TG**GGATCC**ATGAAGCTTTCACACCGCATCGCAGCAATGGCAGCAACCGCAGGCATCACAGTGGCAGCATTCGCAGCACCTGCTTCCGCAATGGACATTCAGATGACCC
M18-R	GTG**GCGGCCGC**TTATCACTTATCATCGTCGTCCTTGTAGTCGGAAGACACGGTAACGGAGG
qPCR-F	CACTACTTTCGCGTATGG
qPCR-R	GTGTCTTGTAGTTCCCGT
RACE-RT-R	ATTGTGTGGACAGG
RACE-GSP-R	TAAT**GGCCCCCGAGGCC**TTCACCCTCTCCACTG
RACE-UP-F	ATTA**GGCCCAGCCGGCC**CTAATACGACTCACTATAGGGC
pETGFP-F	GC**CATATG**ATGAGTAAAGGAGAAGAACTTTTCACTGG
pETGFP-R	AT**AAGCTT**TTATTTGTCATCGTCATCTTTATAATCGTCGACCTTGGATAGTTCATC
H30-F	ATA**AGATCT**GGTACCAAAGTAACTTTTCGGTTAAGGTA
H30-R	A**TCTAGA**GGATCCCAATATACTCCTGCCC
H36-F	ATA**AGATCT**GGTACCTCTATCTGGTGCCC
H36-R	A**TCTAGA**GGATCCCATGCTACTCCTACC

[a] Restriction enzyme sites are shown in bold.

[b]Stratagene Cloning System, La Jolla, CA, USA.

Table S2. Summary of FACS screening of synthetic promoter library

	Sort mode	Screen count	Sort count	Sort %
1st round sorting	Purify	87,200,187	212,590	0.41
2nd round sorting	Purify	20,398,827	500,830	5.29
3rd round sorting	Purify	1,978,403	100,416	11.88
4th round sorting	Purify	869,288	50,031	10.76

Table S3. The three different measures (Mean fluorescence value by FACS, Quantification cycle by qRT-PCR, Expression level by western blotting) from the 20 synthetic promoters and *trc* promoter

Promoter	Fluorescence intensity	Quantification cycle	Expression level (densitometric analysis)
trc	36.52	12.09	308913
L10	215.49	13.76	657712
L26	151.38	13.44	580477
L80	179.53	14.12	648108
I9	517.05	11.41	1435788
I12	475.41	11.36	1460456
I15	428.36	14.51	1206490
I16	441.61	12.91	1087530
I29	553.54	11.54	1152219
I51	367.95	12.04	984199
I64	399.17	13.02	1080083
H3	833.37	11.61	1516687
H4	719.20	9.30	1586477
H5	844.20	9.80	1751440
H17	686.80	11.33	1671780
H28	783.62	11.72	1432382
H30	798.95	10.50	1493492
H34	812.41	12.46	1702859
H36	1183.41	8.08	1755012
H43	1033.32	9.10	1650638
H72	727.30	11.03	1491323

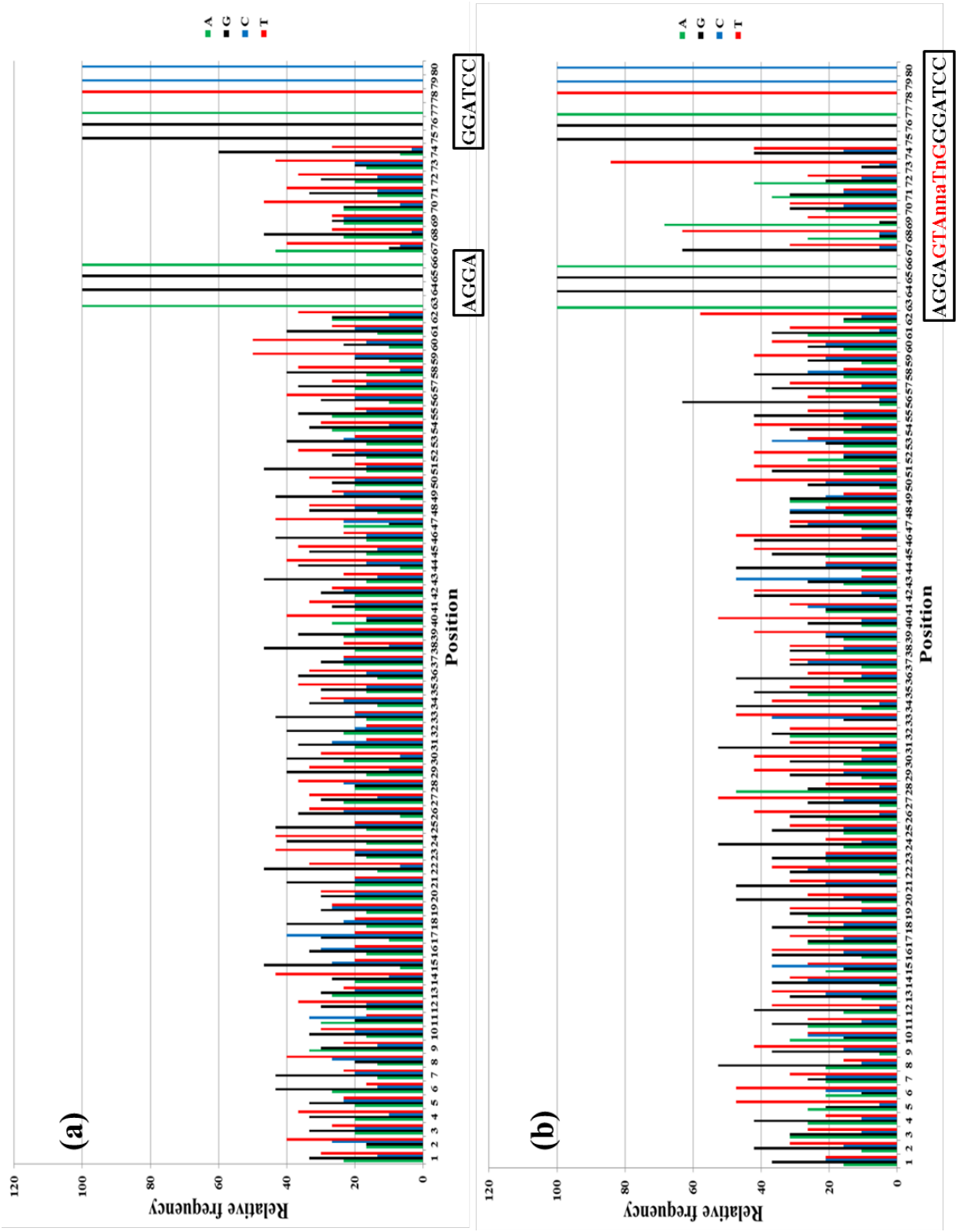


Figure S1. The numerical summary of nucleotides at 80 randomized positions. Nucleotide frequency of (a) 40 random clones in original promoter library and (b) 20 clones isolated by FACS screening. Boxed sequences represent two invariable site - RBS 'AGGA' and BamHI site (GGATCC). In (b), the conserved sequence (GTAnnaTnG) was shown in red-color letters and underlined.

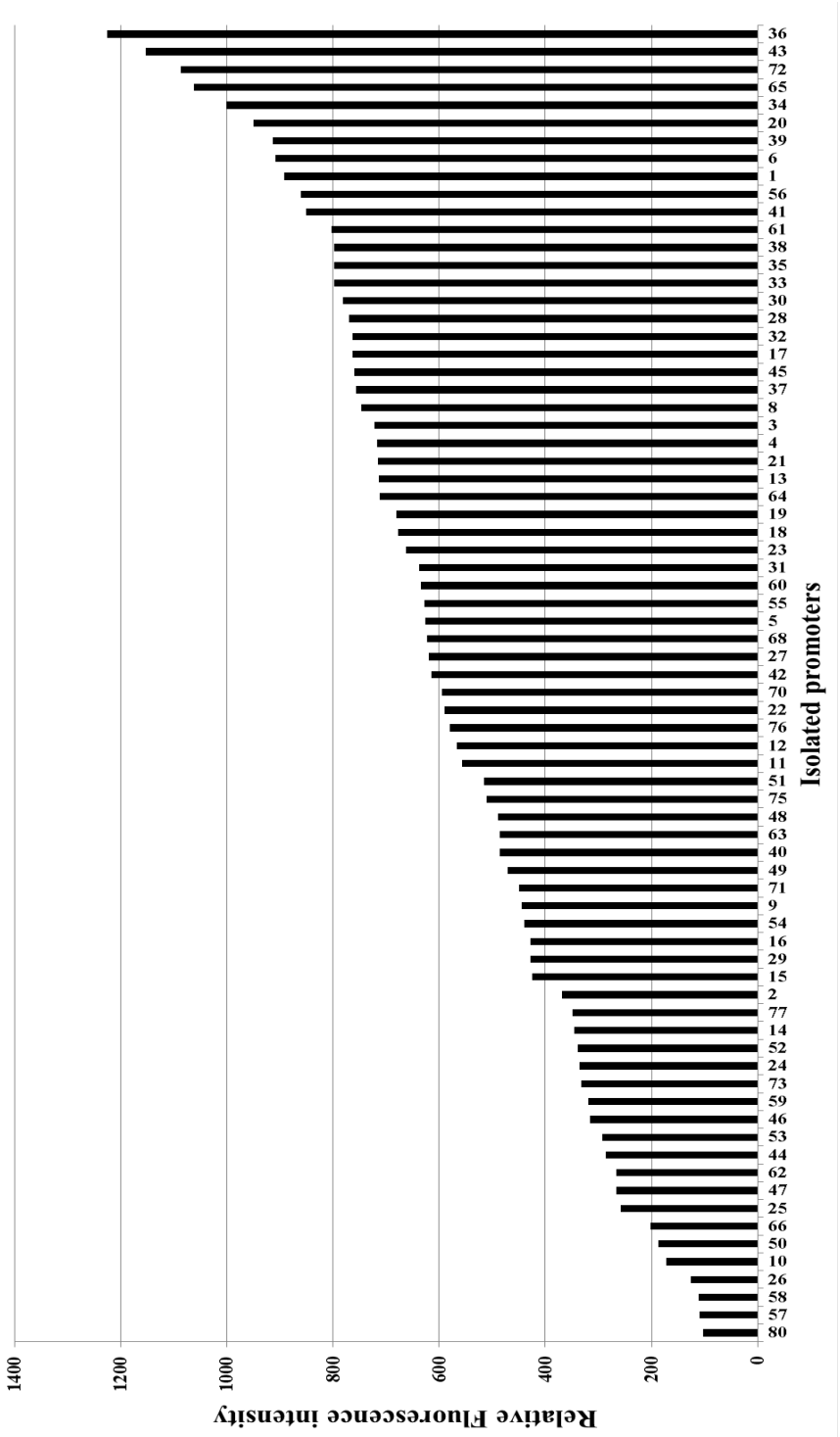
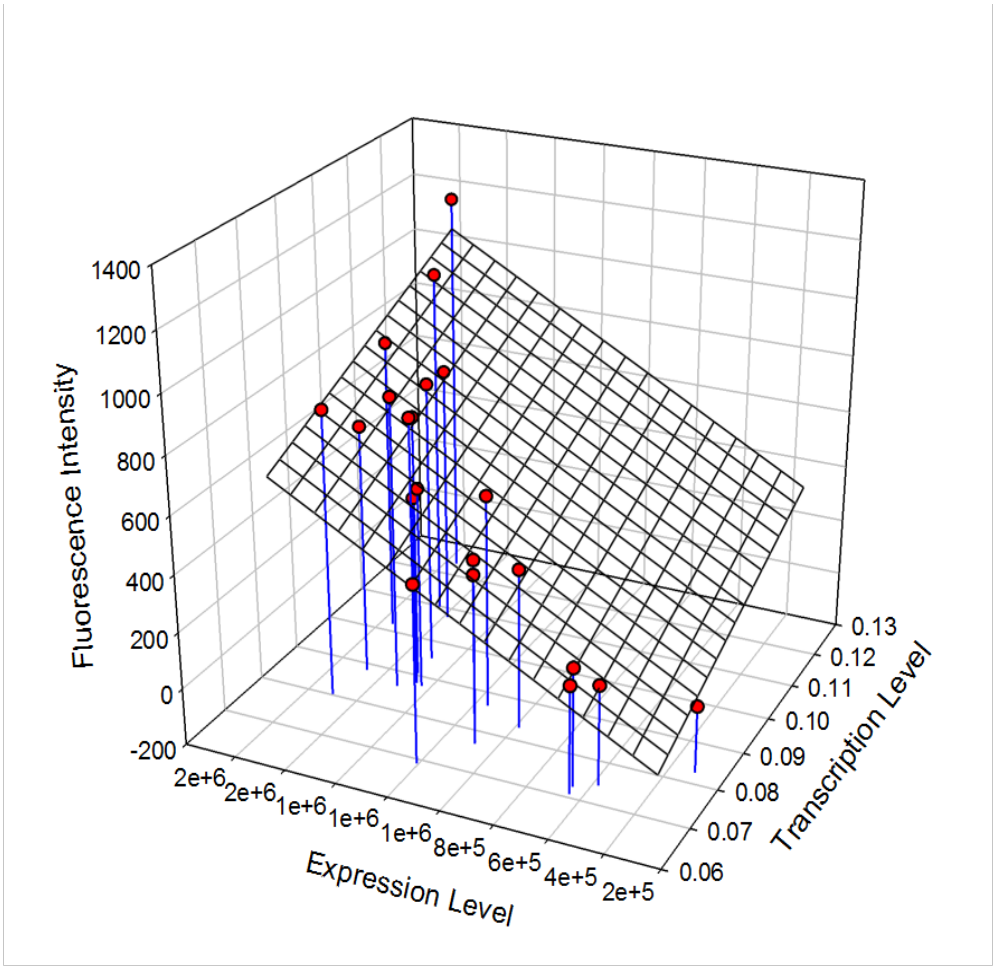


Figure S2. Mean fluorescent intensities of all 80 isolated clones.



$$f = y_0 + ax + by$$

$y_0 = -669.8664\ (P = 0.0003),\qquad a = 7143.5203 (P = 0.0038),\qquad b = 4.9239e^{-4} (P < 0.0001)$

Figure S3. Correlation between GFP expression (x-axis), mRNA transcript level (y-axis) and Mean fluorescent intensity (z-axis). The measured data were shown in Table S3.

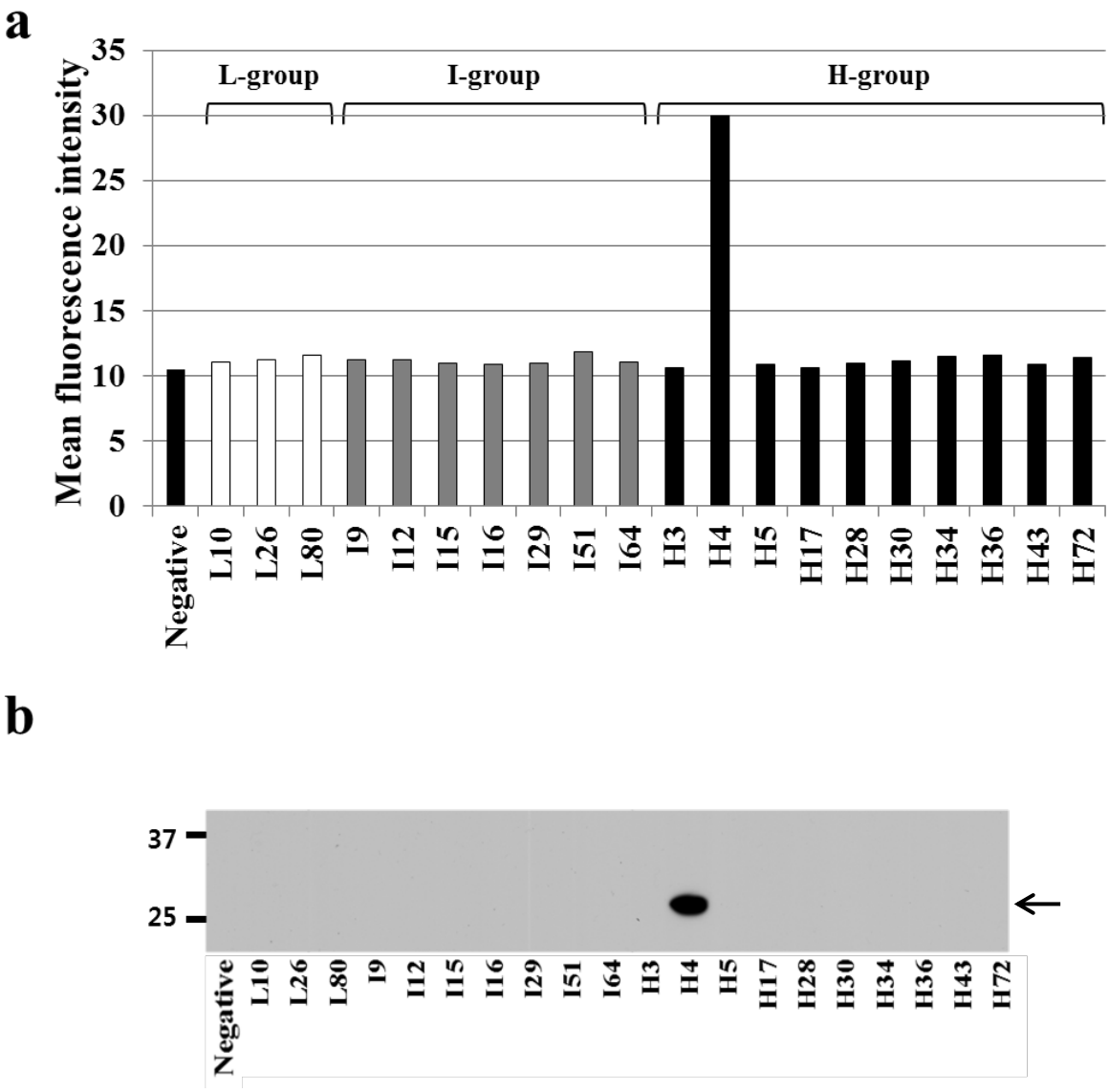


Figure S4. Activity of 20 synthetic promoters in *E. coli*. (a) GFP fluorescence intensities of the synthetic promoters in pCES208 derivatives with GFP in *E. coli* were analyzed by flow cytometry. *E. coli* (pCES208) was used as negative control. (b) The level of GFP expression in each *E. coli* clone was analyzed by western blotting. Arrow indicates GFP (~30 kDa)

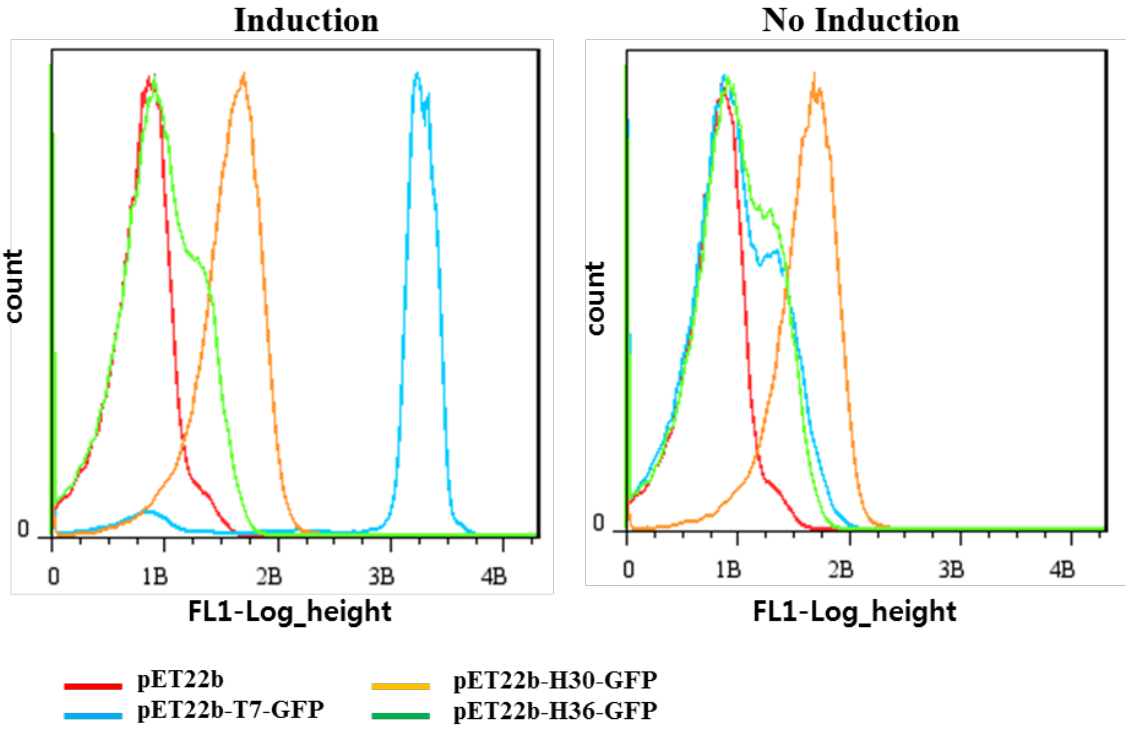


Figure S5. Activity of two synthetic promoters (P_{H30} and P_{H36}) in *E. coli* under IPTG induction (left) and no induction (right). The fluorescent intensities of the *E. coli* harboring pET22b (red), pET22b-T7-GFP (cyan), pET22b-H30-GFP (yellow) and pET22b-H36-GFP (green) were analyzed by flow cytometry.