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Multilevel Gene Regulation using Switchable Transcription Terminator and Toehold Switch in *Escherichia coli*

Supplementary Information

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Detailed Experimental Protocols

Flow Cytometry Analysis

Cells were grown in shaking liquid culture (LB media with proper antibiotics) at 37 °C overnight. Following this, the culture was diluted 1/50-fold (1× M9 media with proper antibiotics) and IPTG (1 mM) was treated immediately. Induction was done at 37 °C for 4 hours. After the induction, OD600 value was measured using BioTek Synergy H1 plate reader (BioTek, Winooski, VT, USA), and for cell number normalization, each sample was diluted using 1× phosphate-buffered saline (PBS) according to the OD600 values (to 0.1~0.2). Final FACS sample volume was 100 μL in a 96-well black plate (SPL Life Science, Pocheon, Korea), and the number of replicates was three for each condition. Flow cytometry measurements were performed using a CytoFLEX Flow Cytometer (Beckman Coulter, Pasadena, CA, USA) and 15,000 cell events were recorded for each measurement. Cell populations were gated according to their forward scatter and side scatter distribution, and sample flow rate was set by 40 μL/min. GFP fluorescence was calculated by geometric mean value, including populations of approximately 80% or more of the total events. Acquired data were analyzed using CytExpert software (Beckman Coulter, Pasadena, CA, USA).

Total RNA Extraction for Quantitative PCR

Cells were grown in shaking liquid culture (LB media with proper antibiotics) at 37 °C overnight. Following this, the culture was diluted 1/50-fold (1× M9 media with proper antibiotics) and IPTG (1 mM) was treated immediately. Induction was done at 37 °C for 4 hours. (All subsequent steps were done in DNase, RNase free condition.) For each experiment condition, 1.2 mL of cells were collected from three triplicated wells (400 μL, each). Cells were pelleted by centrifugation at 13,000 rpm for 3 min. The supernatant was removed, and the remaining pellet was resuspended in 1 mL of RiboEx (GeneAll, Seoul, Korea) reagent. Samples were stored at −80 °C overnight and defrosted on ice. After short vortexing, the samples were incubated at room temperature during 5 min for homogenization. 200 μL of chloroform (Daejung Chemicals & Metals, Siheung, Korea) was added, and the samples were mixed by short vortexing and incubated at room temperature for 3 min. Following incubation, the samples were centrifuged for 15 min at 12,000 rpm at 4 °C, and 400 μL of the top aqueous layer was added into 500 μL of isopropanol (Sigma-Aldrich, Munich, Germany). After gentle inverting, samples were incubated at room temperature for 10 min and centrifuged for 15 min at 13,000 rpm at 4 °C. The isopropanol was carefully removed from RNA pellets, washed in 1 mL of 70% ethanol (EtOH), and tubes were centrifuged for another 2 min at 13,000 rpm at 4 °C to remove left ethanol perfectly. Pellets were air-dried for 5 min and resuspended in 20 μL of RNase free pure water (Enzynomics, Daejeon, Korea) for 20 min.

Normalization of Total RNA, Reverse Transcription and qPCR Measurements

To enable comparison between different samples, each total RNA sample was normalized to the same concentration. Initial sample concentration and purity were measured using BioTek Synergy H1 plate reader, and each sample was diluted to 250 ng/μL in RNase free pure water. One microliter of this total RNA (250 ng), GoScript™ Reverse Transcription Mix (Promega, Madison, WI, USA) 1 rxn, and GFP specific reverse transcription primer (Table S8) 2 μM were used for cDNA synthesis, and the total mix was incubated at 25 °C 5 min, 42 °C 60 min, 70 °C 15 min 1 cycle, and cooled on ice for 10 min. For reference gene (16S rRNA), the same amount of total RNA (250 ng) and GoScript™ Reverse Transcription Mix with random primers were used for cDNA synthesis. Following cooldown, each cDNA sample was diluted 1/30 fold using DNase free pure water. Four microliter of this cDNA, 5 μL of ORA™ qPCR Green ROX L Mix (2×) (highQu, Kraichtal, Germany), and 1 μL of GFP or 16S rRNA specific qPCR primers (forward/ reverse each 0.5 μL, 250 nM) (Table S8) were mixed for quantitative PCR. A Stratagene Mx3000P (Agilent Technologies, Santa Clara, CA, USA) was used for qPCR data collection using the following thermal program: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. All of the measurements were followed by melting curve analysis. A Non-Skirted Clear 96 Well PCR plates (Neptune, San Diego, CA, USA) and a Flat PCR Cap (Kirgen, Shanghai, China) were used for all measurements. Ct values were analyzed using MxPRO software (Agilent Technologies, Santa Clara, CA, USA).

Plate-reader Analysis for TetR

Cells were grown in shaking liquid culture (LB media with proper antibiotics) at 37 °C overnight. Following this, the culture was diluted 1/100 fold (LB media with proper antibiotics). After 80 min of recovery, IPTG (1 mM) and aTc (0 ng or 40 ng/mL) were treated and induction was done at 37 °C for 3 hour 30 mins. Following the induction, OD600 value and GFP fluorescence (excitation: 479 nm, emission: 520 nm) were measured using BioTek Synergy H1 plate reader. The number of replicates was three for each condition. GFP fluorescence data were normalized by OD600 values (GFP/OD600).

Supplementary Tables

Supplementary Table S1. Plasmids Used in This Study. Abbreviations are as follows: T7term = T7 terminator, T500term = T500 terminator, AmpR = ampicillin resistance gene, SpecR = spectinomycin resistance gene, KanR = kanamycin resistance gene, CmR = chloramphenicol resistance gene.

Name.	Plasmid architecture	Figure
	Decoy	
pET_Decoy	pT7–decoy–T7term–AmpR–pBR322 origin	2, 3, 4, 4, S2, S3, S4
pCDF_Decoy	pT7–decoy–T7term–SpecR–CloDF13 origin	6, S4, S5
	SWT, STAR, THS	
T500_SWT	pJ23119–T500_SWT–linker A–GFPmut3b–T7term–KanR–ColA origin	2, 3, 4, S1, S2
BBa_SWT	pJ23119–BBa_SWT–linker A–GFPmut3b–T7term–KanR–ColA origin	3, S1
STAR_target_WT	pJ23119–STAR_target_WT–linker A–GFPmut3b–T7term–KanR–ColA origin	3, S1
STAR_target_1	pJ23119–STAR_target_1–linker A–GFPmut3b–T7term–KanR–ColA origin	3
THS_A	pT7–THS_A–linker A–GFPmut3b–T7term–KanR–ColA origin	5, S3, S4
THS_B	pT7–THS_B–linker A–GFPmut3b–T7term–KanR–ColA origin	5, S3, S4
THS_C	pT7–THS_C–linker A–GFPmut3b–T7term–KanR–ColA origin	3
T500_SWT_P1V1	pJ23119–T500_SWT_P1V1–linker A–GFPmut3b–T7term–KanR–ColA origin	4

T500_SWT_P1V2	pJ23119–T500_SWT_P1V2–linker A–GFPmut3b–T7term–KanR–ColA origin	4
T500_SWT_P1V3	pJ23119–T500_SWT_P1V3–linker A–GFPmut3b–T7term–KanR–ColA origin	4
T500_SWT_P2V3	pJ23119–T500_SWT_P2V3–linker A–GFPmut3b–T7term–KanR–ColA origin	4
T500_SWT_P3V3	pJ23119–T500_SWT_P3V3–linker A–GFPmut3b–T7term–KanR–ColA origin	4
T500_SWT_THS_A	pJ23119–T500_SWT–linker B–THS_A–linker A–GFPmut3b–T7term–KanR–ColA origin	5, S3, S4
T500_SWT_THS_B	pJ23119–T500_SWT–linker C–THS_B–linker A–GFPmut3b–T7term–KanR–ColA origin	5, S3, S4
Koeppl's_AND-gate	pJ23119–Target_6–Toehold_3–GFPmut3b–T7term–KanR–ColA origin	5, S4
T500_SWT_THS_A_mCherry	pJ23119–T500_SWT–linker B–THS_A–linker A–mCherry–T7term–CmR–p15A origin	S5
T500_SWT_THS_B_mCherry	pJ23119–T500_SWT–linker C–THS_B–linker A–mCherry–T7term–CmR–p15A origin	6
T500_SWT_2	pJ23119–T500_SWT_2–linker A–GFPmut3b–T7term–KanR–ColA origin	S2
T500_SWT_3	pJ23119–T500_SWT_3–linker A–GFPmut3b–T7term–KanR–ColA origin	S2
TetO_GFP	pT7–TetO–GFPmut3b–T7term–KanR–ColA origin	7
TetR_negative	pT7–TetR(No RBS)–T7term–SpecR–CloDF13 origin	7
J23119_T500_SWT_TetR	pJ23119–T500_SWT–linker A–TetR–T7term–SpecR–CloDF13 origin	7
pLlacO_AD1_TetR	pLlacO–STAR_target_WT–linker A–TetR–T7term–SpecR–CloDF13 origin	7
pLlacO_THS_A_TetR	pLlacO–THS_A–linker A–TetR–T7term–SpecR–CloDF13 origin	7
Trigger		
T500_SWT_trigger	pT7–T500_SWT_trigger–T7term–AmpR–pBR322 origin	2, 3, 4, 7, S2, S3, S4
BBa_SWT_trigger	pT7–BBa_SWT_trigger–T7term–AmpR–pBR322 origin	3
STAR_target_WT_trigger	pT7–STAR_target_WT_trigger–T7term–AmpR–pBR322 origin	3, 7
STAR_target_1_trigger	pT7–STAR_target_1_trigger–T7term–AmpR–pBR322 origin	3
THS_A_trigger	pT7–THS_A_trigger–T7term–AmpR–pBR322 origin	5, 7, S3
THS_B_trigger	pT7–THS_B_trigger–T7term–AmpR–pBR322 origin	5, S3
THS_C_trigger	pT7–THS_C_trigger–T7term–AmpR–pBR322 origin	3
T500_SWT_P1V1_trigger	pT7–T500_SWT_P1V1_trigger–T7term–AmpR–pBR322 origin	4
T500_SWT_P2V1_trigger	pT7–T500_SWT_P2V1_trigger–T7term–AmpR–pBR322 origin	4
Koeppl's_STAR	pT7–STAR_6–T500term–SpecR–CloDF13 origin	5, S4
Koeppl's_trigger	pT7–Trigger_3–T500term–AmpR–pBR322 origin	5, S4
THS_B_trigger_pCDF	pT7–THS_B_trigger–T7term–SpecR–CloDF13 origin	6, S5
T500_SWT_trigger_THS_A_trigger	pT7–T500_SWT_trigger–T7term–pT7–THS_A_trigger–T7term–AmpR–pBR322 origin	5, 6, S5
T500_SWT_trigger_Decoy	pT7–T500_SWT_trigger–T7term–pT7–decoy–T7term–AmpR–pBR322 origin	5, 6, S5
Decoy_THS_A_trigger	pT7–decoy–T7term–pT7–THS_A_trigger–T7term–AmpR–pBR322 origin	5, 6, S5
Decoy_Decoy	pT7–decoy–T7term–pT7–decoy–T7term–AmpR–pBR322 origin	5, 6, S5
T500_SWT_trigger_THS_B_trigger	pT7–T500_SWT_trigger–T7term–pT7–THS_B_trigger–T7term–AmpR–pBR322 origin	5
Decoy_THS_B_trigger	pT7–decoy–T7term–pT7–THS_B_trigger–T7term–AmpR–pBR322 origin	5
T500_SWT_2_trigger	pT7–T500_SWT_2_trigger–T7term–AmpR–pBR322 origin	S2
T500_SWT_3_trigger	pT7–T500_SWT_3_trigger–T7term–AmpR–pBR322 origin	S2

Supplementary Table S2. Examples of DNA Plasmid Sequences.

Name (architecture)	Sequence
pET_Decoy (pT7–decoy–T7term–(Bla Promoter)–AmpR–pBR322 origin–backbone)	TAATACGACTCACTATAGGGTCTCACGCCCTCAGCTGGGCGTGA-GATGAGCCTCGTCTCCAGATGACGAGGCAACGTAGGATCTGACTGATCCTACTATTAGCATAA CCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCG-GATATCCCGCAAGAGGCCCGGCAGTACCGGCATAACCAAGCCTATGCCTACAGCATCCAGGG TGACGGTGCCGAGGATGACGATGAGCGCATTGTTAGATTTCATACAC-GGTGCCTGACTGCGTTAGCAATTTAACTGTGATAAACTACCGCATTAAAGCTTATCGATGATA AGCTGTCAAACATGAGAATTCTTGAAGACGAAAGGGCCTCGTGATACGCC-TATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGG AAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAA-TATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGA GTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTT-GCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGG GTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAG-TTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTA TTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACAC-TATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATG ACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACAC-TGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAA CATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAA-GCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAA ACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTG-GATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTAT TGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCAC-TGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACT ATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGG-TAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTA AAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAG-TTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTT TTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTT-GCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACC AAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCAC-CGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGT GTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAAC-GGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTAC AGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGG-TAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTA TCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTT-GTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGT TCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTG-GATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCG CAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTTAC-GCATCTGTGCGGTATTTCACACCGCATATATGGTGCACTCTCAGTACAATCTGCTCTGATGCC GCATAGTTAAGCCAGTATACACTCCGCTATCGCTAC-GTGACTGGGTCATGGCTGCGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTT GTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTG-CATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGAGGCAGCTGCGGTAAAGCTCATC AGCGTGGTCGTGAAGCGATTCACAGATGTCTGCCTGTTCATCCGCGTCCAGCTCGTTGAG-TTTCTCCAGAAGCGTTAATGTCTGGCTTCTGATAAAGCGGGCCATGTTAAGGGCGGTTTTTTCC TGTTTGGTCACTGATGCCTCCGTGTAAGGGGGATTTCTGTTCATGGGGGTAATGATAC-CGATGAAACGAGAGAGGATGCTCACGATACGGGTTACTGATGATGAACATGCCCGGTTACTG GAACGTTGTGAGGGTAAACAACTGGCGGTATGGATGCGGCGGGACCAGAGAAAAATCAC-TCAGGGTCAATGCCAGCGCTTCGTTAATACAGATGTAGGTGTTCCACAGGGTAGCCAGCAGC ATCCTGCGATGCAGATCCGGAACATAATGGTG-CAGGGCGCTGACTTCCGCGTTTCCAGACTTTACGAAACACGGAAACCGAAGACCATTCATGTT GTTGCTCAGGTCGCAGACGTTTTGCAGCAGCAGTCGCTTCACGTTCGCTCGCG-TATCGGTGATTCATTCTGCTAACCAGTAAGGCAACCCCGCCAGCCTAGCCGGGTCCTCAACGA CAGGAGCACGATCATGCGCACCCGTGGCCAGGACCCAACGCTGCCCGA-GATGCGCCGCGTGCGGCTGCTGGAGATGGCGGACGCGATGGATATGTTCTGCCAAGGGTTGG

TTTGCGCATTCACAGTTCTCCGCAAGAATTGATTGGCTCCAATTCTTGGAG-
TGGTGAATCCGTTAGCGAGGTGCCGCCGGCTTCCATTCAGGTCGAGGTGGCCCGGCTCCATGC
ACCGCGACGCAACGCGGGGAGGCAGACAAGGTATAGGGCGGCGCC-
TACAATCCATGCCAACCCGTTCCATGTGCTCGCCGAGGCGGCATAAATCGCCGTGACGATCAG
CGGTCCAGTGATCGAAGTTAGGCTGGTAAGAGCCGCGAGCGATCCTTGAA-
GCTGTCCCTGATGGTCGTCATCTACCTGCCTGGACAGCATGGCCTGCAACGCGGGCATCCCGA
TGCCGCCGGAAGCGAGAAGAATCATAATGGGGAAGGCCATCCAGCCTCGCGTCGCGAAC-
GCCAGCAAGACGTAGCCCAGCGCGTCGGCCGCCATGCCGGCGATAATGGCCTGCTTCTCGCC
GAAACGTTTGGTGGCGGGACCAGTGACGAAGGCTTGAGCGAGGGCGTGCAAGATTCCGAA-
TACCGCAAGCGACAGGCCGATCATCGTCGCGCTCCAGCGAAAGCGGTCCTCGCCGAAAATGA
CCCAGAGCGCTGCCGGCACCTGTCCTACGAGTTGCATGATAAAGAAGACAG-
TCATAAGTGCGGCGACGATAGTCATGCCCCGCGCCCACCGGAAGGAGCTGACTGGGTTGAAG
GCTCTCAAGGGCATCGGTCGAGATCCCGGTGCCTAATGAGTGAGCTAACTTACATTAATT-
GCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATC
GGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCCAGGGTGGTTTTTCTTTTCAC-
CAGTGAGACGGGCAACAGCTGATTGCCCTTCACCGCCTGGCCCTGAGAGAGTTGCAGCAAGC
GGTCCACGCTGGTTTGCCCCAGCAGGCGAAAATCCTGTTTGATGGTGGTTAACGGCGGGATA-
TAACATGAGCTGTCTTCGGTATCGTCGTATCCCACTACCGAGATATCCGCACCAACGCGCAGC
CCGGACTCGGTAATGGCGCGCATTGCGCCCAGCGCCATCTGATCGTTGGCAACCAGCATCG-
CAGTGGGAACGATGCCCTCATTCAGCATTTGCATGGTTTGTTGAAAACCGGACATGGCACTCC
AGTCGCCTTCCCGTTCCGCTATCGGCTGAATTTGATTGCGAGTGAGA-
TATTTATGCCAGCCAGCCAGACGCAGACGCGCCGAGACAGAACTTAATGGGCCCGCTAACAG
CGCGATTTGCTGGTGACCCAATGCGACCAGATGCTCCACGCCCAGTCGCGTAC-
CGTCTTCATGGGAGAAAATAATACTGTTGATGGGTGTCTGGTCAGAGACATCAAGAAATAAC
GCCGGAACATTAGTGCAGGCAGCTTCCACAGCAATGGCATCCTGGTCATCCAGCGGA-
TAGTTAATGATCAGCCCACTGACGCGTTGCGCGAGAAGATTGTGCACCGCCGCTTTACAGGCT
TCGACGCCGCTTCGTTCTACCATCGACACCACCACGCTGGCACCCAGTTGATCGGCGCGA-
GATTTAATCGCCGCGACAATTTGCGACGGCGCGTGCAGGGCCAGACTGGAGGTGGCAACGCC
AATCAGCAACGACTGTTTGCCCGCCAGTTGTTGTGCCACGCGGTTGG-
GAATGTAATTCAGCTCCGCCATCGCCGCTTCCACTTTTTCCCGCGTTTTCGCAGAAACGTGGCT
GGCCTGGTTCACCACGCGGGAAACGGTCTGATAAGAGACACCGGCATACTCTGCGACATCG-
TATAACGTTACTGGTTTCACATTCACCACCCTGAATTGACTCTCTTCCGGGCGCTATCATGCCA
TACCGCGAAAGGTTTTGCGCCATTCGATGGTGTCCGGGATCTCGACGCTCTCCCTTATGAAC-
GTGTACGGGCTATCTGGCTTTCGTTGCGC

T500_SWT (pJ23119–T500_SWT–linker A–(RBS)–GFPmut3b–T7term–KanR–(Bla Promoter)–ColA origin–backbone)

TTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTCCATCTTACCTTTGCATCTC-
TATCGTTCTCATCTCATCCTAAAGCCCGCCGAAAGGCGGGCTTTTTTTTAACCTGGCGGCAGC
GCAAAAGAGAGGAGACAGAGATGCGTAAAGGAGAAGAACTTTTCACTGGAGTT-
GTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAG
GGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAAC-
TACCTGTTCCGTGGCCAACACTTGTCACTACTTTCGGTTATGGTGTTCAATGCTTTGCGAGATA
CCCAGATCACATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTACGTACAG-
GAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGA
AGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAA-
GATGGAAACATTCTTGGACACAAATTGGAATACAACTATAACTCACACAATGTATACATCATG
GCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAA-
GATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCGATTGGCGATGGCCCTGTC
CTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAA-
GAGAGACCACATGGTCCTTCTTGAGTTTGTAACCGCTGCTGGGATTACACATGGCATGGATGA
ACTATACAAAAGGCCTGCAGCAAACGACGAAAACTACGCTGCATCAGTTTAATAAGA-
TAAAC-
CAGAGCGGCACGGCAAGCAGAGTATACGAGATTCGGTAGCCACCGCTGAGCAATAACTAGCA
TAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAACCTCAGGCATTTGA-
GAAGCACACGGTCACACTGCTTCCGGTAGTCAATAAACCGGTAAACCAGCAATAGACATAAG
CGGCTATTTAACGACCCTGCCCTGAACCGACGACAAGCTGACGACCGGGTCTCCGCAAGTGG-
CACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATG
TATCCGCTCATGAATTAATTCTTAGAAAAACTCATCGAGCATCAAATGAAACTG-
CAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGG
AGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGAT-
TCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAG
TGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGG-
CAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAA

	ATCACTCGCATCAACCAAACCGTTATTCATTCGTGATTGCGCCTGAGCGAGACGAAATAC- GCGGTCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTG CCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCTTCTAATACCTG- GAATGCTGTTTTCCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAA AATGCTTGATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGAC- CATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACTCTGGCGC ATCGGGCTTCCCATACAATCGATAGATTGTCGCAC- CTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAAT TTAATCGCGGCCTAGAGCAAGACGTTTCCCGTTGAATATGGCTCATACTCTTCCTTTTTCAA- TATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGA AAAATAAACAAATAGGCATGCTAGCGCAGAAACGTCCTAGAAGATGCCAGGAGGA- TACTTAG- CAGAGAGACAATAAGGCCGGAGCGAAGCCGTTTTTCCATAGGCTCCGCCCCCCTGACGAACA TCACGAAATCTGACGCTCAAATCAGTGGTGGCGAAACCCGACAGGACTATAAAGATAC- CAGGCGTTTCCCCCTGATGGCTCCCTCTTGCGCTCTCCTGTTCCCGTCCTGCGGCGTCCGTGTT GTGGTGGAGGCTTTACCCAAATCACCACGTCCCGTTCCGTGTAGACAGTTCGCTCCAA- GCTGGGCTGTGTGCAAGAACCCCCCGTTCAGCCCGACTGCTGCGCCTTATCCGGTAACTATCA TCTTGAGTCCAACCCGGAAAGACACGACAAAACGCCACTGGCAGCAGCCATTGGTAACTGA- GAATTAGTGGATTTAGATATCGAGAGTCTTGAAGTGGTGGCCTAACAGAGGCTACACTGAAA GGACAGTATTTGGTATCTGCGCTCCACTAAAGCCAGTTACCAGGTTAAGCAG- TTCCCCAACTGACTTAACCTTCGATCAAACCGCCTCCCCAGGCGGTTTTTTCGTTTACAGAGCA GGAGATTACGACGATCGTAAAAGGATCTCAAGAAGATCCTTTACGGATTCCCGACAC- CATCACTCTAGATTTCAGTGCAATTTATCTCTTCAAATGTAGCACCTGAAGTCAGCCCCATAC GATATAAGTTGTAATTCTCATGTTAGTCATGCCCCGCGCCCACCG- GAAGGAGCTGACTGGGTTGAAGGCTCTCAAGGGCATCGGTCGAGATCCCGGTGCCTAATGAG TGAGCTAACTTACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAAC- CTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGG GCGCCAGGGTGGTTTTTCTTTTCACCAGTGAGACGGGCAACAGCTGATTGCCCTTCAC- CGCCTGGCCCTGAGAGAGTTGCAGCAAGCGGTCCACGCTGGTTTGCCCCAGCAGGCGAAAAT CCTGTTTGATGGTGGTTAACGGCGGGATATAACATGAGCTGTCTTCGGTATCGTCG- TATCCCACTACCGAGATGTCCGCACCAACGCGCAGCCCGGACTCGGTAATGGCGCGCATTGC GCCCAGCGCCATCTGATCGTTGGCAACCAGCATCGCAGTGGGAACGATGCCCTCATTCAG- CATTTGCATGGTTTGTTGAAAACCGGACATGGCACTCCAGTCGCCTTCCCGTTCCGCTATCGG CTGAATTTGATTGCGAGTGAGATATTTATGCCAGCCAGCCAGACGCAGACGCGCCGAGA- CAGAACTTAATGGGCCCGCTAACAGCGCGATTTGCTGGTGACCCAATGCGACCAGATGCTCC ACGCCCAGTCGCGTACCGTCTTCATGGGAGAAAATAATACTGTTGATGGGTGTCTGGTCAGA- GACATCAAGAAATAACGCCGGAACATTAGTGCAGGCAGCTTCCACAGCAATGGCATCCTGGT CATCCAGCGGATAGTTAATGATCAGCCCACTGACGCGTTGCGCGAGAAGATTGTGCAC- CGCCGCTTTACAGGCTTCGACGCCGCTTCGTTCTACCATCGACACCACCACGCTGGCACCCAG TTGATCGGCGCGAGATTTAATCGCCGCGACAATTTGCGACGGCGCGTGCAGGGCCAGACTG- GAGGTGGCAACGCCAATCAGCAACGACTGTTTGCCCGCCAGTTGTTGTGCCACGCGGTTGGG AATGTAATTCAGCTCCGCCATCGCCGCTTCCACTTTTTCCCGCGTTTTCGCAGAAAC- GTGGCTGGCCTGGTTCACCACGCGGGAAACGGTCTGATAAGAGACACCGGCATACTCTGCGA CATCGTATAACGTTACTGGTTTCACATTCACCACCCTGAATTGACTCTCTTCCGGGCGC- TATCATGCCATACCGCGAAAGGTTTTGCGCCATTCGATGGTGTCCGGGATCTCGACGCTCTCC CTTATGAAGTCTAACGCTGCTCTGGGCTAACTGTCGCGC
T500_SWT_THS_A (pJ23119–T500_SWT–linker B–THS_A(RBS)–linker A–GFPmut3b–T7term–KanR–(Bla Promoter)–ColA origin–backbone)	TTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTCCATCTTACCTTTGCATCTC- TATCGTTCTCATCTCATCCTAAAGCCCGCCGAAAGGCGGGCTTTTTTTTCTCTTCTATCCTCCT AACTCTGAGTAAGATAATGAAGGTAGGTATGTTAAACTTTAGAACAGAGGAGATAAA- GATGAACATACCTACGAACCTGGCGGCAGCGCAAAAGATGCGTAAAGGAGAAGAACTTTTCA CTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGG- CACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAA ATTTATTTGCACTACTGGAAAACTACCTGTTCCGTGGCCAACACTTGTCAC- TACTTTCGGTTATGGTGTTCAATGCTTTGCGAGATACCCAGATCACATGAAACAGCATGACTT TTTCAAGAGTGCCATGCCCGAAGGTTACGTACAGGAAAGAACTATATTTTTCAAAGATGAC- GGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGA GTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATACAAC- TATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTT CAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAA- TACTCCGATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCC CTTTCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAAC- CGCTGCTGGGATTACACATGGCATGGATGAACTATACAAAAGGCCTGCAGCAAACGACGAAA

	ACTACGCTGCATCAGTTTAATAAGATAAACCAGAGCGGCACGGCAAGCAGAGTATACGA-GAT- TCGGTAGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGG GGTTTTTTGCTGAAACCTCAGGCATTTGAGAAGCACACGGTCACACTGCTTCCGGTAGTCAA- TAAACCGGTAAACCAGCAATAGACATAAGCGGCTATTTAACGACCCTGCCCTGAACCGACGA CAAGCTGACGACCGGGTCTCCGCAAGTGGCACTTTTCGGGGAAATGTGCGCG- GAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAATTAATTCTT AGAAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATAC- CATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGA TGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAAC- CTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTG AATCCGGTGAGAATGGCAAAAGTTTATGCATTTCTTTCCAGACTT- GTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTCA TTCGTGATTGCGCCTGAGCGAGACGAAATAC- GCGGTCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTG CCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCTTCTAATACCTG- GAATGCTGTTTTCCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAA AATGCTTGATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGAC- CATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACTCTGGCGC ATCGGGCTTCCCATACAATCGATAGATTGTCGCAC- CTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAAT TTAATCGCGGCCTAGAGCAAGACGTTTCCCGTTGAATATGGCTCATACTCTTCCTTTTTCAA- TATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGA AAAATAAACAAATAGGCATGCTAGCGCAGAAACGTCCTAGAAGATGCCAGGAGGA-TACTTAG- CAGAGAGACAATAAGGCCGGAGCGAAGCCGTTTTTCCATAGGCTCCGCCCCCCTGACGAACA TCACGAAATCTGACGCTCAAATCAGTGGTGGCGAAACCCGACAGGACTATAAAGATAC- CAGGCGTTTCCCCCTGATGGCTCCCTCTTGCGCTCTCCTGTTCCCGTCCTGCGGCGTCCGTGTT GTGGTGGAGGCTTTACCCAAATCACCACGTCCCGTTCCGTGTAGACAGTTCGCTCCAA- GCTGGGCTGTGTGCAAGAACCCCCCGTTCAGCCCGACTGCTGCGCCTTATCCGGTAACTATCA TCTTGAGTCCAACCCGGAAAGACACGACAAAACGCCACTGGCAGCAGCCATTGGTAACTGA- GAATTAGTGGATTTAGATATCGAGAGTCTTGAAGTGGTGGCCTAACAGAGGCTACACTGAAA GGACAGTATTTGGTATCTGCGCTCCACTAAAGCCAGTTACCAGGTTAAGCAG- TTCCCCAACTGACTTAACCTTCGATCAAACCGCCTCCCCAGGCGGTTTTTTCGTTTACAGAGCA GGAGATTACGACGATCGTAAAAGGATCTCAAGAAGATCCTTTACGGATTCCCGACAC- CATCACTCTAGATTTCAGTGCAATTTATCTCTTCAAATGTAGCACCTGAAGTCAGCCCCATAC GATATAAGTTGTAATTCTCATGTTAGTCATGCCCCGCGCCCACCG- GAAGGAGCTGACTGGGTTGAAGGCTCTCAAGGGCATCGGTCGAGATCCCGGTGCCTAATGAG TGAGCTAACTTACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAAC- CTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGG GCGCCAGGGTGGTTTTTCTTTTCACCAGTGAGACGGGCAACAGCTGATTGCCCTTCAC- CGCCTGGCCCTGAGAGAGTTGCAGCAAGCGGTCCACGCTGGTTTGCCCCAGCAGGCGAAAAT CCTGTTTGATGGTGGTTAACGGCGGGATATAACATGAGCTGTCTTCGGTATCGTCG- TATCCCACTACCGAGATGTCCGCACCAACGCGCAGCCCGGACTCGGTAATGGCGCGCATTGC GCCCAGCGCCATCTGATCGTTGGCAACCAGCATCGCAGTGGGAACGATGCCCTCATTCAG- CATTTGCATGGTTTGTTGAAAACCGGACATGGCACTCCAGTCGCCTTCCCGTTCCGCTATCGG CTGAATTTGATTGCGAGTGAGATATTTATGCCAGCCAGCCAGACGCAGACGCGCCGAGA- CAGAACTTAATGGGCCCGCTAACAGCGCGATTTGCTGGTGACCCAATGCGACCAGATGCTCC ACGCCCAGTCGCGTACCGTCTTCATGGGAGAAAATAATACTGTTGATGGGTGTCTGGTCAGA- GACATCAAGAAATAACGCCGGAACATTAGTGCAGGCAGCTTCCACAGCAATGGCATCCTGGT CATCCAGCGGATAGTTAATGATCAGCCCACTGACGCGTTGCGCGAGAAGATTGTGCAC- CGCCGCTTTACAGGCTTCGACGCCGCTTCGTTCTACCATCGACACCACCACGCTGGCACCCAG TTGATCGGCGCGAGATTTAATCGCCGCGACAATTTGCGACGGCGCGTGCAGGGCCAGACTG- GAGGTGGCAACGCCAATCAGCAACGACTGTTTGCCCGCCAGTTGTTGTGCCACGCGGTTGGG AATGTAATTCAGCTCCGCCATCGCCGCTTCCACTTTTTCCCGCGTTTTCGCAGAAAC- GTGGCTGGCCTGGTTCACCACGCGGGAAACGGTCTGATAAGAGACACCGGCATACTCTGCGA CATCGTATAACGTTACTGGTTTCACATTCACCACCCTGAATTGACTCTCTTCCGGGCGC- TATCATGCCATACCGCGAAAGGTTTTGCGCCATTCGATGGTGTCCGGGATCTCGACGCTCTCC CTTATGAAGTCTAACGCTGCTCTGGGCTAACTGTCGCGC
TetO_GFP (pT7–TetO–(Weiss RBS)–GFPmut3b–T7term–	TAATACGACTCACTATAGGTCTATCATTGATAGGGTTTATTAAAGAGGAGAAATTAA-GCATGCGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTG

KanR–(Bla Promoter)–ColA origin–backbone)	ATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCGTGGCCAACACTTGTCACTACTTTCGGTTATGGTGTTCAATGCTTTGCGAGATACCCAGATCACATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTACGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCGATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACCGCTGCTGGGATTACACATGGCATGGATGAACTATACAAAAGGCCTGCAGCAAACGACGAAAACTACGCTGCATCAGTTTAATAAGATAAACCAGAGCGGCACGGCAAGCAGAGTATACGAGATTCGGTAGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAACCTCAGGCATTTGAGAAGCACACGGTCACACTGCTTCCGGTAGTCAATAAACCGGTAAACCAGCAATAGACATAAGCGGCTATTTAACGACCCTGCCCTGAACCGACGACAAGCTGACGACCGGGTCTCCGCAAGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAATTAATTCTTAGAAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTCATTCGTGATTGCGCCTGAGCGAGACGAAATACGCGGTCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTCCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAATCGATAGATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTAGAGCAAGACGTTTCCCGTTGAATATGGCTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTTGAATGTATTTAGAAAAATAAACAAATAGGCATGCTAGCGCAGAAACGTCCTAGAAGATGCCAGGAGGATACTTAGCAGAGAGACAATAAGGCCGGAGCGAAGCCGTTTTTCCATAGGCTCCGCCCCCCTGACGAACATCACGAAATCTGACGCTCAAATCAGTGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGATGGCTCCCTCTTGCGCTCTCCTGTTCCGTCCTGCGGCGTCCGTGTTGTGGTGGAGGCTTTACCCAAATCACCACGTCCCGTTCCGTGTAGACAGTTCGCTCCAAGCTGGGCTGTGTGCAAGAACCCCCCGTTCAGCCCGACTGCTGCGCCTTATCCGGTAACTATCATCTTGAGTCCAACCCGGAAAGACACGACAAAACGCCACTGGCAGCAGCCATTGGTAACTGAGAATTAGTGGATTTAGATATCGAGAGTCTTGAAGTGGTGGCCTAACAGAGGCTACACTGAAAGGACAGTATTTGGTATCTGCGCTCCACTAAAGCCAGTTACCAGGTTAAGCAGTTCCCCAACTGACTTAACCTTCGATCAAACCGCCTCCCCAGGCGGTTTTTTCGTTTACAGAGCAGGAGATTACGACGATCGTAAAAGGATCTCAAGAAGATCCTTTACGGATTCCCGACACCATCACTCTAGATTTCAGTGCAATTTATCTCTTCAAATGTAGCACCTGAAGTCAGCCCCATACGATATAAGTTGTAATTCTCATGTTAGTCATGCCCCGCGCCCACCGGAAGGAGCTGACTGGGTTGAAGGCTCTCAAGGGCATCGGTCGAGATCCCGGTGCCTAATGAGTGAGCTAACTTACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCCAGGGTGGTTTTTCTTTTCACCAGTGAGACGGGCAACAGCTGATTGCCCTTCACCGCCTGGCCCTGAGAGAGTTGCAGCAAGCGGTCCACGCTGGTTTGCCCCAGCAGGCGAAAATCCTGTTTGATGGTGGTTAACGGCGGGATATAACATGAGCTGTCTTCGGTATCGTCGTATCCCACTACCGAGATGTCCGCACCAACGCGCAGCCCGGACTCGGTAATGGCGCGCATTGCGCCCAGCGCCATCTGATCGTTGGCAACCAGCATCGCAGTGGGAACGATGCCCTCATTCAGCATTTGCATGGTTTGTTGAAAACCGGACATGGCACTCCAGTCGCCTTCCCGTTCCGCTATCGGCTGAATTTGATTGCGAGTGAGATATTTATGCCAGCCAGCCAGACGCAGACGCGCCGAGACAGAACTTAATGGGCCCGCTAACAGCGCGATTTGCTGGTGACCCAATGCGACCAGATGCTCCACGCCCAGTCGCGTACCGTCTTCATGGGAGAAAATAATACTGTTGATGGGTGTCTGGTCAGAGACATCAAGAAATAACGCCGGAACATTAGTGCAGGCAGCTTCCACAGCAATGGCATCCTGGTCATCCAGCGGATAGTTAATGATCAGCCCACTGACGCGTTGCGCGAGAAGATTGTGCACCGCCGCTTTACAGGCT

	TCGACGCCGCTTCGTTCTACCATCGACACCACCACGCTGGCACCCAGTTGATCGGCGCGA- GATTTAATCGCCGCGACAATTTGCGACGGCGCGTGCAGGGCCAGACTGGAGGTGGCAACGCC AATCAGCAACGACTGTTTGCCCGCCAGTTGTTGTGCCACGCGGTTGG- GAATGTAATTCAGCTCCGCCATCGCCGCTTCCACTTTTTCCCGCGTTTTCGCAGAAACGTGGCT GGCCTGGTTCACCACGCGGGAAACGGTCTGATAAGAGACACCGGCATACTCTGCGACATCG- TATAACGTTACTGGTTTCACATTCACCACCCTGAATTGACTCTCTTCCGGGCGCTATCATGCCA TACCGCGAAAGGTTTTGCGCCATTCGATGGTGTCCGGGATCTCGAC- GCTCTCCCTTATGAAGTCTAACGCTGCTCTGGGCTAACTGTCGCGC
J23119_T500_SWT_TetR (pJ23119–T500_SWT–linker A–(RBS)–TetR–T7term–SpecR–(Bla Promoter)–CloDF13 origin–backbone)	TTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTCCATCTTACCTTTGCATCTC- TATCGTTCTCATCTCATCCTAAAGCCCGCCGAAAGGCGGGCTTTTTTTTAACCTGGCGGCAGC GCAAAAGAGAGGAGACAGAGATGTCTAGATTAGATAAAAGTAAAGTGATTAACAGCGCATT- AGAGCTGCTTAATGAGGTCGGAATCGAAGGTTTAACAACCCGTAAACTCGCCCAGAAGCTAG GTGTAGAGCAGCCTACATTGTATTGGCATGTAAAAAATAAGCGGGCTTTGCTCGAC- GCCTTAGCCATTGAGATGTTAGATAGGCACCATACTCACTTTTGCCCTTTAGAAGGGGAAAGC TGGCAAGATTTTTTACGTAATAACGCTAAAAGTTTTAGATGTGCTTTACT- AAGTCATCGCGATGGAGCAAAAGTACATTTAGGTACACGGCCTACAGAAAAACAGTATGAAA CTCTCGAAAATCAATTAGCCTTTTTATGCCAACAAGGTTTTTCACTAGAGAATGCATTA- TATGCACTCAGCGCTGTGGGGCATTTTACTTTAGGTTGCGTATTGGAAGATCAAGAGCATCAA GTCGCTAAAGAAGAAAGGGAAACACCTACTACTGATAGTATGCCGCCATTATTACGACAAGC- TATCGAATTATTTGATCACCAAGGTGCAGAGCCAGCCTTCTTATTCGGCCTTGAATTGATCATA TGCGGATTAGAAAAACAACTTAAATGTGAAAGTGGGTCTAGGCCTGCAGCAAACGAC- GAAAACTACGCTGCATCAGTTTAATAAGATAAACCAGAGCGGCACGGCAAGCAGAGTATACG AGATTCGGTAGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAAC- GGGTCTTGAGGGGTTTTTTGCTGAAACCTCAGGCATTTGAGAAGCACACGGTCACACTGCTTC CGGTAGTCAATAAACCGGTAAACCAGCAATAGACATAAGCGGCTATTTAAC- GACCCTGCCCTGAACCGACGACCGGGTCATCGTGGCCGGATCTTGCGGCCCCTCGGCTTGAAC GAATTGTTAGACATTATTTGCCGACTACCTTGGTGATCTCGCCTTTCACGTAGTG- GACAAATTCTTCCAACTGATCTGCGCGCGAGGCCAAGCGATCTTCTTCTTGTCCAAGATAAGC CTGTCTAGCTTCAAGTATGACGGGCTGATACTGGGCCGGCAGGCGCTCCATTGCCCAGTCGG- CAGCGACATCCTTCGGCGCGATTTTGCCGGTTACTGCGCTGTACCAAATGCGGGACAACGTAA GCACTACATTTCGCTCATCGCCAGCCCAGTCGGGCGGCGAGTTCCA- TAGCGTTAAGGTTTCATTTAGCGCCTCAAATAGATCCTGTTCAGGAACCGGATCAAAGAGTTC CTCCGCCGCTGGACCTACCAAGGCAACGCTATGTTCTCTTGCTTTTGTCAGCAAGA- TAGCCAGATCAATGTCGATCGTGGCTGGCTCGAAGATACCTGCAAGAATGTCATTGCGCTGCC ATTCTCCAAATTGCAGTTCGCGCTTAGCTGGATAACGCCACGGAATGATGTCGTCGTG- CACAACAATGGTGACTTCTACAGCGCGGAGAATCTCGCTCTCTCCAGGGGAAGCCGAAGTTTC CAAAAGGTCGTTGATCAAAGCTCGCCGCGTTGTTTCATCAAGCCTTACGGTCACCGTAAC- CAGCAAATCAATATCACTGTGTGGCTTCAGGCCGCCATCCACTGCGGAGCCGTACAAATGTAC GGCCAGCAACGTCGGTTCGAGATGGCGCTCGATGACGCCAACTACCTCTGATAGTTGAG- TCGATACTTCGGCGATCACCGCTTCCCTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTAT CAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAA- TAGCTAGCTCACTCGGTCGCTACGCTCCGGGCGTGAGACTGCGGCGGGCGCTGCGGACACAT ACAAAGTTACCCACAGATTCCGTGGATAAGCAGGGGACTAACATGTGAGGCAAAACAG- CAGGGCCGCGCCGGTGGCGTTTTTCCATAGGCTCCGCCCTCCTGCCAGAGTTCACATAAACAG ACGCTTTTCCGGTGCATCTGTGGGAGCCGTGAGGCTCAACCATGAATCTGACAGTAC- GGGCGAAACCCGACAGGACTTAAAGATCCCCACCGTTTCCGGCGGGTCGCTCCCTCTTGCGCT CTCCTGTTCCGACCCTGCCGTTTACCGGATACCTGTTCCGCCTTTCTCCCTTACGG- GAAGTGTGGCGCTTTCTCATAGCTCACACACTGGTATCTCGGCTCGGTGTAGGTCGTTCGCTC CAAGCTGGGCTGTAAGCAAGAACTCCCCGTTCAGCCCGACTGCTGCGCCTTATCCGG- TAACTGTTCACTTGAGTCCAACCCGGAAAAGCACGGTAAAACGCCACTGGCAGCAGCCATTG GTAACTGGGAGTTCGCAGAGGATTTGTTTAGCTAAACACGCGGTTGCTCTT- GAAGTGTGCGCCAAAGTCCGGCTACACTGGAAGGACAGATTTGGTTGCTGTGCTCTGCGAAA GCCAGTTACCACGGTTAAGCAGTTCCCCAACTGACTTAACCTTCGATCAAACCAC- CTCCCCAGGTGGTTTTTTCGTTTACAGGGCAAAAGATTACGCGCAGAAAAAAAGGATCTCAAG AAGATCCTTTGATCTTTTCTACTGAACCGCTCTAGATTTCAGTG- CAATTTATCTCTTCAAATGTAGCACCTGAAGTCAGCCCCATACGATATAAGTTGTAATTCTCAT GTTAGTCATGCCCCGCGCCCACCGGAAGGAGCTGACTGGGTTGAAGGCTCTCAAGGG- CATCGGTCGAGATCCCGGTGCCTAATGAGTGAGCTAACTTACATTAATTGCGTTGCGCTCACT GCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAAC- GCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCCAGGGTGGTTTTTCTTTTCACCAGTGAGACG GGCAACAGCTGATTGCCCTTCACCGCCTGGCCCTGAGAGAGTTGCAGCAAGCGGTCCAC- GCTGGTTTGCCCCAGCAGGCGAAAATCCTGTTTGATGGTGGTTAACGGCGGGATATAACATGA

	GCTGTCTTCGGTATCGTCGTATCCCACTACCGAGATGTCCGCACCAACGCGCAGCCCG- GACTCGGTAATGGCGCGCATTGCGCCCAGCGCCATCTGATCGTTGGCAACCAGCATCGCAGTG GGAACGATGCCCTCATTCAGCATTTGCATGGTTTGTTGAAAACCGGACATGGCACTCCAG- TCGCCTTCCCGTTCCGCTATCGGCTGAATTTGATTGCGAGTGAGATATTTATGCCAGCCAGCC AGACGCAGACGCGCCGAGACAGAACTTAATGGGCCCGCTAACAGCGCGATTT- GCTGGTGACCCAATGCGACCAGATGCTCCACGCCCAGTCGCGTACCGTCTTCATGGGAGAAA ATAATACTGTTGATGGGTGTCTGGTCAGAGACATCAAGAAATAACGCCGGAACATTAGTG- CAGGCAGCTTCCACAGCAATGGCATCCTGGTCATCCAGCGGATAGTTAATGATCAGCCCACTG ACGCGTTGCGCGAGAAGATTGTGCACCGCCGCTTTACAGGCTTCGACGCCGCTTCGTTCTAC- CATCGACACCACCACGCTGGCACCCAGTTGATCGGCGCGAGATTTAATCGCCGCGACAATTTG CGACGGCGCGTGCAGGGCCAGACTGGAGGTGGCAACGCCAATCAGCAACGACTGTTT- GCCCGCCAGTTGTTGTGCCACGCGGTTGGGAATGTAATTCAGCTCCGCCATCGCCGCTTCCAC TTTTTCCCGCGTTTTCGCAGAAACGTGGCTGGCCTGGTTCACCACGCGGGAAACGGTCTGA- TAAGAGACACCGGCATACTCTGCGACATCGTATAACGTTACTGGTTTCACATTCACCACCCTG AATTGACTCTCTTCCGGGCGCTATCATGCCATACCGCGAAAGGTTTT- GCGCCATTCGATGGTGTCCGGGATCTCGACGCTCTCCCTTATGAGACTCAGCAAGATTCGTGT AGTGGCGAGTGCGC
T500_SWT_trigger (pT7–T500_SWT_trigger–T7term–(Bla Promoter)–AmpR–pBR322 origin–backbone)	TAATACGACTCACTATAGGGATACACATAGAATCATGTGTATGGCGGGCTTTAGGATGA- GATGAGAACGATAGAGATGCAAAGGTAAGATGGTAGCATAACCCCTTGGGGCCTCTAAACGG GTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGATATCCCGCAAGAGGCCCGG- CAGTACCGGCATAACCAAGCCTATGCCTACAGCATCCAGGGTGACGGTGCCGAGGATGACGA TGAGCGCATTGTTAGATTTCATACACGGTGCCTGACTGCGTTAGCAATTTAACTGTGA- TAAACTACCGCATTAAAGCTTATCGATGATAAGCTGTCAAACATGAGAATTCTTGAAGACGAA AGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGAC- GTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACAT TCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAA- TATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGG CATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAA- GATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGA GAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGC- TATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACT ATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGG- CATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACT TACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGG- GATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGA GCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAAC- TACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGAC CACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTG- GAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCC CGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGA- CAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCA TATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAA- GATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCA GACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCG- TAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAG AGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAA- TACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTAC ATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGA- TAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGG GCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGA- GA- TACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTA TCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAAC- GCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGAT GCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTAC- GGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTG GATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCG- CAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGCATC TGTGCGGTATTTCACACCGCATATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCG- CATAGTTAAGCCAGTATACACTCCGCTATCGCTACGTGACTGGGTCATGGCTGCGCCCCGACA CCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGA- CAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGC

	GCGAGGCAGCTGCGGTAAAGCTCATCAGCGTGGTCGTGAAGCGAT- TCACAGATGTCTGCCTGTTCATCCGCGTCCAGCTCGTTGAGTTTCTCCAGAAGCGTTAATGTCT GGCTTCTGATAAAGCGGGCCATGTTAAGGGCGGTTTTTTCCTGTTTGGTCAC- TGATGCCTCCGTGTAAGGGGGATTTCTGTTCATGGGGGTAATGATACCGATGAAACGAGAGA GGATGCTCACGATACGGGTTACTGATGATGAACATGCCCGGTTACTGGAACGTTGTGAGGG- TAAACAACTGGCGGTATGGATGCGGCGGGACCAGAGAAAAATCACTCAGGGTCAATGCCAGC GCTTCGTTAATACAGATGTAGGTGTTCCACAGGGTAGCCAGCAG- CATCCTGCGATGCAGATCCGGAACATAATGGTGCAGGGCGCTGACTTCCGCGTTTCCAGACTT TACGAAACACGGAAACCGAAGACCATTCATGTTGTTGCTCAGGTCGCAGACGTTTTGCAG- CAGCAGTCGCTTCACGTTCGCTCGCGTATCGGTGATTCATTCTGCTAACCAGTAAGGCAACCC CGCCAGCCTAGCCGGGTCCTCAACGACAGGAGCACGATCATGCGCACCCGTGGCCAG- GACCCAACGCTGCCCGAGATGCGCCGCGTGCGGCTGCTGGAGATGGCGGACGCGATGGATAT GTTCTGCCAAGGGTTGGTTTGCGCATTCACAGTTCTCCGCAAGAATT- GATTGGCTCCAATTCTTGGAGTGGTGAATCCGTTAGCGAGGTGCCGCCGGCTTCCATTCAGGT CGAGGTGGCCCGGCTCCATGCACCGCGACGCAACGCGGGGAGGCAGA- CAAGGTATAGGGCGGCGCCTACAATCCATGCCAACCCGTTCCATGTGCTCGCCGAGGCGGCA TAAATCGCCGTGACGATCAGCGGTCCAGTGATCGAAGTTAGGCTGGTAA- GAGCCGCGAGCGATCCTTGAAGCTGTCCCTGATGGTCGTCATCTACCTGCCTGGACAGCATGG CCTGCAACGCGGGCATCCCGATGCCGCCGGAAGCGAGAAGAATCATAATGGG- GAAGGCCATCCAGCCTCGCGTCGCGAACGCCAGCAAGACGTAGCCCAGCGCGTCGGCCGCCA TGCCGGCGATAATGGCCTGCTTCTCGCCGAAACGTTTGGTGGCGGGACCAGTGAC- GAAGGCTTGAGCGAGGGCGTGCAAGATTCCGAATACCGCAAGCGACAGGCCGATCATCGTCG CGCTCCAGCGAAAGCGGTCCTCGCCGAAAATGACCCAGAGCGCTGCCGGCACCTGTCCTAC- GAGTTGCATGATAAAGAAGACAGTCATAAGTGCGGCGACGATAGTCATGCCCCGCGCCCACC GGAAGGAGCTGACTGGGTTGAAGGCTCTCAAGGGCATCGGTCGA- GATCCCGGTGCCTAATGAGTGAGCTAACTTACATTAATTGCGTTGCGCTCACTGCCCGCTTTCC AGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGA- GAGGCGGTTTGCGTATTGGGCGCCAGGGTGGTTTTTCTTTTCACCAGTGAGACGGGCAACAGC TGATTGCCCTTCACCGCCTGGCCCTGAGAGAGTTGCAGCAAGCGGTCCACGCTGGTTT- GCCCCAGCAGGCGAAAATCCTGTTTGATGGTGGTTAACGGCGGGATATAACATGAGCTGTCTT CGGTATCGTCGTATCCCACTACCGAGATATCCGCACCAACGCGCAGCCCGGACTCGG- TAATGGCGCGCATTGCGCCCAGCGCCATCTGATCGTTGGCAACCAGCATCGCAGTGGGAACG ATGCCCTCATTCAGCATTTGCATGGTTTGTTGAAAACCGGACATGGCACTCCAG- TCGCCTTCCCGTTCCGCTATCGGCTGAATTTGATTGCGAGTGAGATATTTATGCCAGCCAGCC AGACGCAGACGCGCCGAGACAGAACTTAATGGGCCCGCTAACAGCGCGATTT- GCTGGTGACCCAATGCGACCAGATGCTCCACGCCCAGTCGCGTACCGTCTTCATGGGAGAAA ATAATACTGTTGATGGGTGTCTGGTCAGAGACATCAAGAAATAACGCCGGAACATTAGTG- CAGGCAGCTTCCACAGCAATGGCATCCTGGTCATCCAGCGGATAGTTAATGATCAGCCCACTG ACGCGTTGCGCGAGAAGATTGTGCACCGCCGCTTTACAGGCTTCGACGCCGCTTCGTTCTAC- CATCGACACCACCACGCTGGCACCCAGTTGATCGGCGCGAGATTTAATCGCCGCGACAATTTG CGACGGCGCGTGCAGGGCCAGACTGGAGGTGGCAACGCCAATCAGCAACGACTGTTT- GCCCGCCAGTTGTTGTGCCACGCGGTTGGGAATGTAATTCAGCTCCGCCATCGCCGCTTCCAC TTTTTCCCGCGTTTTCGCAGAAACGTGGCTGGCCTGGTTCACCACGCGGGAAACGGTCTGA- TAAGAGACACCGGCATACTCTGCGACATCGTATAACGTTACTGGTTTCACATTCACCACCCTG AATTGACTCTCTTCCGGGCGCTATCATGCCATACCGCGAAAGGTTTT- GCGCCATTCGATGGTGTCCGGGATCTCGACGCTCTCCCTTATGAACGTGTACGGGCTATCTGG CTTTCGTTGCGC
T500_SWT_trigger_THS_A_trigger (pT7–T500_SWT_trigger–T7term–pT7–THS_A_trigger–T7term–(Bla Promoter)–AmpR–pBR322 origin–backbone)	TAATACGACTCACTATAGGGATACACATAGAATCATGTGTATGGCGGGCTTTAGGATGA- GATGAGAACGATAGAGATGCAAAGGTAAGATGGTAGCATAACCCCTTGGGGCCTCTAAACGG GTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGATATCCCGCAAGAGGCCCGG- CAGTACCGGCATAACCAAGCCTATGCCTACAGCATCCAGGGTGACGGTGCCGAGGATGACGA TGAGCGCATTGTTAGATTTCATACACGGTGCCTGACTGCGTTAGCCGTGTACGGGC- TATCTGGCTTTCGTTGCGCTAATACGACTCACTATAGGGCTCGATCACTAATCTGATCGAGAC GAACATACCTACCTTCATTATCTTACTTGTTAGCATAACCCCTTGGGGCCTCTAAAC- GGGTCTTGAGGGGTTTTTTG CTGAAAGGAGGAACTATATCCGGATATCCCGCAA- GAGGCCCGGCAGTACCGGCATAACCAA- GCCTATGCCTACAGCATCCAGGGTGACGGTGCCGAGGATGACGATGAGCGCATTGTTAGATTT CATACACGGTGCCTGACTGCGTTAGCAATTTAACTGTGATAAACTACCGCATTAAA- GCTTATCGATGATAAGCTGTCAAACATGAGAATTCTTGAAGACGAAAGGGCCTCGTGATACG CCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCAC- TTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTAT

CCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAG-
TATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTC
ACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAG-
TGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAAC
GTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGAC-
GCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCA
CCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCA-
TAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAG
CTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCG-
GAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAAC
AACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAA-
TAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCT
GGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCAC-
TGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACT
ATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGG-
TAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTA
AAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAG-
TTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTT
TTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTT-
GCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACC
AAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCAC-
CGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGT
GTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAAC-
GGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTAC
AGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGG-
TAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTA
TCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTT-
GTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGT
TCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTG-
GATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCG
CAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTTAC-
GCATCTGTGCGGTATTTCACACCGCATATATGGTGCACTCTCAGTACAATCTGCTCTGATGCC
GCATAGTTAAGCCAGTATACACTCCGCTATCGCTAC-
GTGACTGGGTCATGGCTGCGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTT
GTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTG-
CATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGAGGCAGCTGCGGTAAAGCTCATC
AGCGTGGTCGTGAAGCGATTCACAGATGTCTGCCTGTTCATCCGCGTCCAGCTCGTTGAG-
TTTCTCCAGAAGCGTTAATGTCTGGCTTCTGATAAAGCGGGCCATGTTAAGGGCGGTTTTTTCC
TGTTTGGTCACTGATGCCTCCGTGTAAGGGGGATTTCTGTTCATGGGGGTAATGATAC-
CGATGAAACGAGAGAGGATGCTCACGATACGGGTTACTGATGATGAACATGCCCGGTTACTG
GAACGTTGTGAGGGTAAACAACTGGCGGTATGGATGCGGCGGGACCAGAGAAAAATCAC-
TCAGGGTCAATGCCAGCGCTTCGTTAATACAGATGTAGGTGTTCCACAGGGTAGCCAGCAGC
ATCCTGCGATGCAGATCCGGAACATAATGGTG-
CAGGGCGCTGACTTCCGCGTTTCCAGACTTTACGAAACACGGAAACCGAAGACCATTCATGTT
GTTGCTCAGGTCGCAGACGTTTTGCAGCAGCAGTCGCTTCACGTTCGCTCGCG-
TATCGGTGATTCATTCTGCTAACCAGTAAGGCAACCCCGCCAGCCTAGCCGGGTCCTCAACGA
CAGGAGCACGATCATGCGCACCCGTGGCCAGGACCCAACGCTGCCCGA-
GATGCGCCGCGTGCGGCTGCTGGAGATGGCGGACGCGATGGATATGTTCTGCCAAGGGTTGG
TTTGCGCATTCACAGTTCTCCGCAAGAATTGATTGGCTCCAATTCTTGGAG-
TGGTGAATCCGTTAGCGAGGTGCCGCCGGCTTCCATTCAGGTCGAGGTGGCCCGGCTCCATGC
ACCGCGACGCAACGCGGGGAGGCAGACAAGGTATAGGGCGGCGCC-
TACAATCCATGCCAACCCGTTCCATGTGCTCGCCGAGGCGGCATAAATCGCCGTGACGATCAG
CGGTCCAGTGATCGAAGTTAGGCTGGTAAGAGCCGCGAGCGATCCTTGAA-
GCTGTCCCTGATGGTCGTCATCTACCTGCCTGGACAGCATGGCCTGCAACGCGGGCATCCCGA
TGCCGCCGGAAGCGAGAAGAATCATAATGGGGAAGGCCATCCAGCCTCGCGTCGCGAAC-
GCCAGCAAGACGTAGCCCAGCGCGTCGGCCGCCATGCCGGCGATAATGGCCTGCTTCTCGCC
GAAACGTTTGGTGGCGGGACCAGTGACGAAGGCTTGAGCGAGGGCGTGCAAGATTCCGAA-
TACCGCAAGCGACAGGCCGATCATCGTCGCGCTCCAGCGAAAGCGGTCCTCGCCGAAAATGA
CCCAGAGCGCTGCCGGCACCTGTCCTACGAGTTGCATGATAAAGAAGACAG-
TCATAAGTGCGGCGACGATAGTCATGCCCCGCGCCCACCGGAAGGAGCTGACTGGGTTGAAG
GCTCTCAAGGGCATCGGTCGAGATCCCGGTGCCTAATGAGTGAGCTAACTTACATTAATT-
GCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATC

GGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCCAGGGTGGTTTTTCTTTTCAC- CAGTGAGACGGGCAACAGCTGATTGCCCTTCACCGCCTGGCCCTGAGAGAGTTGCAGCAAGC GGTCCACGCTGGTTTGCCCCAGCAGGCGAAAATCCTGTTTGATGGTGGTTAACGGCGGGATA- TAACATGAGCTGTCTTCGGTATCGTCGTATCCCACTACCGAGATATCCGCACCAACGCGCAGC CCGGACTCGGTAATGGCGCGCATTGCGCCCAGCGCCATCTGATCGTTGGCAACCAGCATCG- CAGTGGGAACGATGCCCTCATTCAGCATTTGCATGGTTTGTTGAAAACCGGACATGGCACTCC AGTCGCCTTCCCGTTCCGCTATCGGCTGAATTTGATTGCGAGTGAGA- TATTTATGCCAGCCAGCCAGACGCAGACGCGCCGAGACAGAACTTAATGGGCCCGCTAACAG CGCGATTTGCTGGTGACCCAATGCGACCAGATGCTCCACGCCCAGTCGCGTAC- CGTCTTCATGGGAGAAAATAATACTGTTGATGGGTGTCTGGTCAGAGACATCAAGAAATAAC GCCGGAACATTAGTGCAGGCAGCTTCCACAGCAATGGCATCCTGGTCATCCAGCGGA- TAGTTAATGATCAGCCCACTGACGCGTTGCGCGAGAAGATTGTGCACCGCCGCTTTACAGGCT TCGACGCCGCTTCGTTCTACCATCGACACCACCACGCTGGCACCCAGTTGATCGGCGCGA- GATTTAATCGCCGCGACAATTTGCGACGGCGCGTGCAGGGCCAGACTGGAGGTGGCAACGCC AATCAGCAACGACTGTTTGCCCGCCAGTTGTTGTGCCACGCGGTTGG- GAATGTAATTCAGCTCCGCCATCGCCGCTTCCACTTTTTCCCGCGTTTTCGCAGAAACGTGGCT GGCCTGGTTCACCACGCGGGAAACGGTCTGATAAGAGACACCGGCATACTCTGCGACATCG- TATAACGTTACTGGTTTCACATTCACCACCCTGAATTGACTCTCTTCCGGGCGCTATCATGCCA TACCGCGAAAGGTTTTGCGCCATTCGATGGTGTCCGGGATCTCGACGCTCTCCCTTATGAAC- GTGTACGGGCTATCTGGCTTTCGTTGCGC

Supplementary Table S3. Promoter Sequences Used in This Study. Plasmid sequences can be constructed by replacing the yellow region in the example plasmids in Table S2 with the yellow region indicated here.

Name	Sequence
pT7	TAATACGACTCACTATAGG
pJ23119	TTGACAGCTAGCTCAGTCCTAGGTATAA- TACTAGT
pLlacO	ATAAATGTGAGCGGATAACATTGACATT- GTGAGCGGATAACAAGATACTGAG- CACGG

Supplementary Table S4. Insert Sequences Used in This Study. Plasmid sequences can be constructed by replacing the grey region in the example plasmids in Table S2 with the grey region indicated here.

Name	Sequence
decoy	TCTCACGCCCTCAGCTGGGCGTGAGATGAGCCTCGTCTCCAGATGACGAGGCAAC- GTAGGATCTGACTGATCCTACTAT
T500_SWT	CCATCTTACCTTTGCATCTCTATCGTTCTCATCTCATCCTAAA- GCCCGCCGAAAGGCGGGCTTTTTTTT
BBa_SWT	CCATCTTACCTTTGCATCTCTATCGTTCTCATCTCATCCTTTGTTCAGAAC- GCTCGGTCTTGCACACCGGGCGTTTTTTTTTT
STAR_target_WT	AGTTTTTACAGTGAATTGTTTTAATTAGTTGTATAAATGTTGGAGCAGCGGG- GAATGTATACAGTTCATGTATATATTCCCCGCTTTTTTTTT
STAR_target_1	CCATCTTACCTTTGCATCTCTATCGTTCTCATCTCATCCTGCGGGGAATGTATA- CAGTTCATGTATATATTCCCCGCTTTTTTTTT
THS_A	GAGTAAGATAATGAAGGTAGGTATGTTAAACTTTAGAACAGAGGAGATAAA- GATGAACATACCTACG
THS_B	GACTGATTTGAATACACTGCTTCGTTCAAGATTCAGAACAGAGGAGATGAA- TATGGAACGAAGCAGA
THS_C	GATTGAATATGATAGAAGTTTAGTAGTAGACAATAGAACAGAGGAGA- TATTGATGACTACTAAACT
T500_SWT_P1V1	CCATCTTACCTTTGCATCTC- TATCGTTCTCATCTCATCCTAAACCCCGCCGAAAGGCGGGGTTTTTTTT
T500_SWT_P1V2	CCATCTTACCTTTGCATCTCTATCGTTCTCATCTCATCCTAAA- GCCCGCCGAAAGGCGGGTTTTTTTTT
T500_SWT_P1V3	CCATCTTACCTTTGCATCTC- TATCGTTCTCATCTCATCCTAAAACCCGCCGAAAGGCGGGTTTTTTTTT
T500_SWT_P2V3	CCATCTTACCTTTGCATCTC- TATCGTTCTCATCTCATCCTAAAGTCCGCCGAAAGGCGGACTTTTTTTT

T500_SWT_P3V3	CCATCTTACCTTTGCATCTCTATCGTTCTCATCTCATCCTAAA-GCTCGCCGAAAGGCGAGCTTTTTTTT
Target_6	CCAGTCATCAAGTCAGTCCAGTCAAAGTTTCCGTCGTTCAGCGGGGAATGTATA-CAGTTCATGTATATATTCCCCGCTTTTTTTTT
Toehold_3	GGGATCTATTACTACTTACCATTGTCTTGCTCTATACAGAAACAGAGGAGATA-TAGAATGAGACAATGG
T500_SWT_2	CCTCCATCTCCATATTCTTATCTCATTATCATCTCACTTTAAA-GCCCGCCGAAAGGCGGGCTTTTTTTT
T500_SWT_3	CCTCTATCCTATCTATCTGCCTGTCCTGTCCTGTGTCTTTAAA-GCCCGCCGAAAGGCGGGCTTTTTTTT

Supplementary Table S5. Trigger Sequences Used in This Study. Plasmid sequences can be constructed by replacing the red region in the example plasmids in Table S2 with the red region indicated here.

Name	Sequence
T500_SWT_trigger	GATACACATAGAATCATGTGTATGGCGGGCTTTAGGATGAGATGAGAACGATA-GAGATGCAAAGGTAAGATGG
BBa_SWT_trigger	GATACACATAGAATCATGTGTATGTGCAAGAC-CGAGCGTTCTGAACAAAGGATGAGATGAGAACGATAGAGATGCAAAGG
STAR_target_WT_trigger	TGAACTGTATACATTCCCCGCTGCTCCAACATTTATA-CAACTAATTAAAACAATTCACTGTAAAAACT
STAR_target_1_trigger	TGAACTGTATACATTCCCCGCAGGATGAGATGAGAACGATAGA-GATGCAAAGGTAAGATGG
THS_A_trigger	GCTCGATCACTAATCTGATCGAGACGAACATACCTACCTTCATTATCTTACTTGT
THS_B_trigger	GAGTTGCGGCACGGACCGCAACTATAGAACGAAGCAGTGTATTCAAATCAGTTAG
THS_C_trigger	GATACACATAGAATCATGTGTATAACACTACTAAACTTCTATCATATTCAATCAC
T500_SWT_P1V1_trigger	GATACACATAGAATCATGTGTATGGCGGGGTTTAGGATGAGATGAGAACGATA-GAGATGCAAAGGTAAGATGG
T500_SWT_P2V1_trigger	GATACACATAGAATCATGTGTATGGCGGCCTTTAGGATGAGATGAGAACGATA-GAGATGCAAAGGTAAGATGG
STAR_6	TGAACTGTATACATTCCCCGCTGAACGACGGAAACTTTGACTGGACTGACTT-GATGACTGG
Trigger_3	GGGTGATGGGACATTCCGATGTCCCATCAATAAGAGCAAGACAATGGTAAGTAG-TAATAGATAAG
T500_SWT_2_trigger	GATACACATAGAATCATGTGTATGGCGGGCTTTAAAGTGAGATGATAATGAGA-TAAGAATATGGAGATGGAGG
T500_SWT_3_trigger	GATACACATAGAATCATGTGTATGGCGGGCTTTAAAGACACAGGACAG-GACAGGCAGATAGATAGGATAGAGG

Supplementary Table S6. Terminator Sequences Used in This Study. Plasmid sequences can be constructed by replacing the pink region in the example plasmids in Table S2 with the pink region indicated here.

Name	Sequence
T7term	TAGCATAACCCCTTGGGGCCTCTAAAC-GGGTCTTGAGGGGTTTTTTG
T500term	CAAA-GCCCGCCGAAAGGCGGGCTTTTTTTT

Supplementary Table S7. Other Accessary Sequences Used in This Study. Accessary sequences used for constructing plasmids are indicated here.

Name	Sequence
RBS	AGAGGAGA
WeissRBS	ATTAAAGAGGAGAAATTAAGC
linker A	AACCTGGCGGCAGCGCAAAAG
linker B	CTCTTCTATCCTCCTAACTCT
linker C	CACCTAACTACGACCCAACCT
GFPmut3b	ATGCGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATT-AGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGC

	AACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTAC- CTGTTCCGTGGCCAACACTTGTCACTACTTTCGGTTATGGTGTTCAATGCTTTGCGAGA TACCCAGATCACATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTAC- GTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAA GTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGAT- TTTAAA- GAAGATGGAAACATTCTTGGACACAAATTGGAATACAACTATAACTCACACAATGTA TACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGA- CACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCG ATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTAC- CTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGAGAGACCACATGGTC CTTCTTGAGTTTGTAACCGCTGCTGGGATTACACATGGCATGGATGAACTATA- CAAAAGGCCTGCAGCAAACGACGAAAACTACGCTGCATCAGTTTAATAA
mCherry	ATGCGTAAAGTGAGCAAGGGCGAAGAAGATAACATGGCCATCATCAAGGAG- TTCATGCGCTTCAAGGTTCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATC GAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAA- GCTGAAGGTGAC- CAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGC TCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAA- GCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGG CGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAG- TTCATCTACAAGGTGAA- GCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGG CTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCGCTGAAGGGCGA- GATCAA- GCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCT ACAAGGCCAAGAAGCCCGTGCAACTGCCCGGCGCGTACAACGTCAACATCAAGTT- GGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGA GGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTA
TetO	TCTATCATTGATAGGGTTT
TetR	ATGTCTAGATTAGATAAAAGTAAAGTGATTAACAGCGCATT- AGAGCTGCTTAATGAGGTCG- GAATCGAAGGTTTAACAACCCGTAAACTCGCCCAGAAGCTAGGTGTAGAGCAGCCTA CATTGTATTGGCATGTAAAAAATAAGCGGGCTTTGCTCGACGCCTTAGCCATTGA- GATGTTA- GATAGGCACCATACTCACTTTTGCCCTTTAGAAGGGGAAAGCTGGCAAGATTTTTTAC GTAATAACGCTAAAAGTTTTAGATGTGCTTTACTAAGTCATCGCGATGGAG- CAAAAGTACATTTAGGTACACGGCCTACAGAAAAACAGTATGAAACTCTCGAAAATC AATTAGCCTTTTTATGCCAACAAGGTTTTTCACTAGAGAATGCATTATATGCAC- TCAGCGCTGTGGGGCATTTTACTTTAGGTTGCGTATTGGAAGATCAAGAGCATCAAGT CGCTAAAGAAGAAAGGGAAACACCTACTACTGATAGTATGCCGCCATTATTAC- GACAAGC- TATCGAATTATTTGATCACCAAGGTGCAGAGCCAGCCTTCTTATTCGGCCTTGAATTG ATCATATGCGGATTAGAAAAACAACTTAAATGTGAAAGTGGGTCT

Supplementary Table S8. Primers Used for Reverse Transcription and qPCR.

Primer Name	Sequence (5' to 3')
RT_GFP	TTACAAACTCAAGAAGGACC
qPCR_GFP_F	CACTGGAGTTGTCCCAATTCT
qPCR_GFP_R	TCCGTATGTTGCATCACCTTC
qPCR_16S_F	TGGTAGTCCACGCCGTAAAC
qPCR_16S_R	TTTAACCTTGCGGCCGTACT

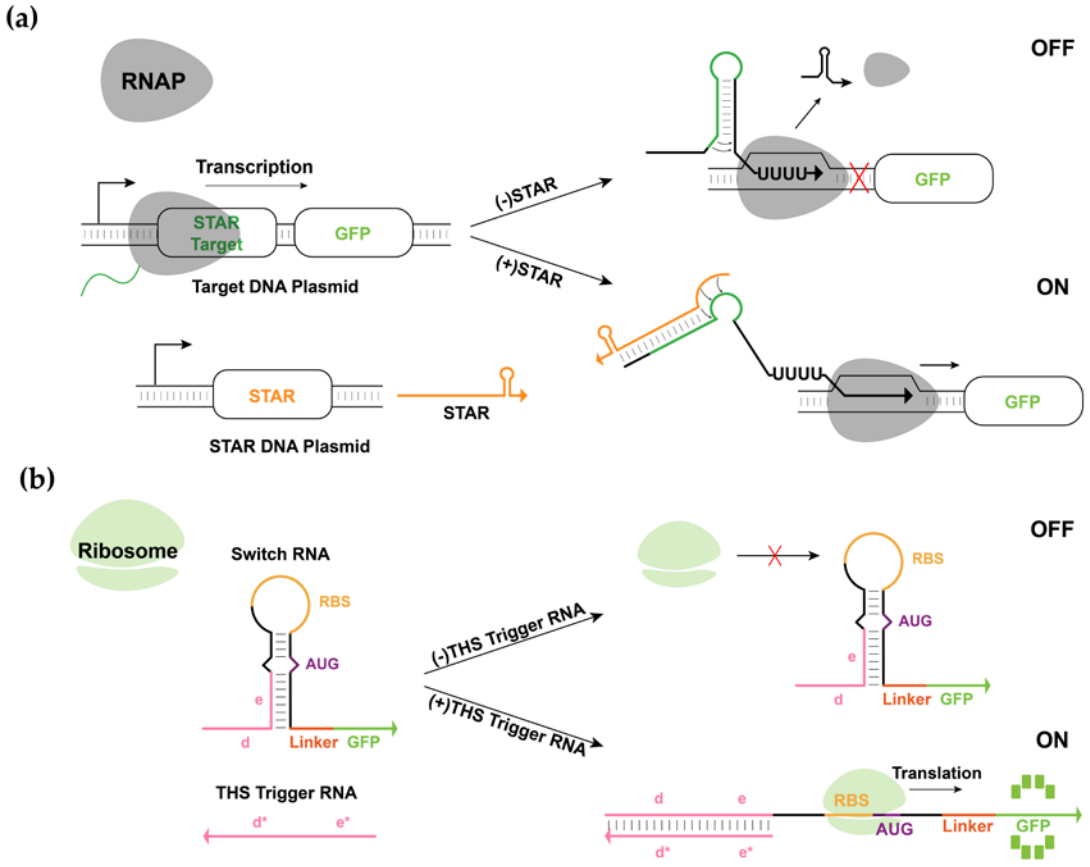
Supplementary Table S9. RT-qPCR Raw Data.

GFP Raw Ct	Trigger	Decoy
BBa K864600	13.50 ± 0.07	15.77 ± 0.09
T500	13.31 ± 0.32	20.79 ± 0.09
STAR target WT	10.97 ± 0.15	15.62 ± 0.17
THS C	12.99 ± 0.23	14.06 ± 0.16
GFP control	10.13 ± 0.27	29.59 ± 0.96

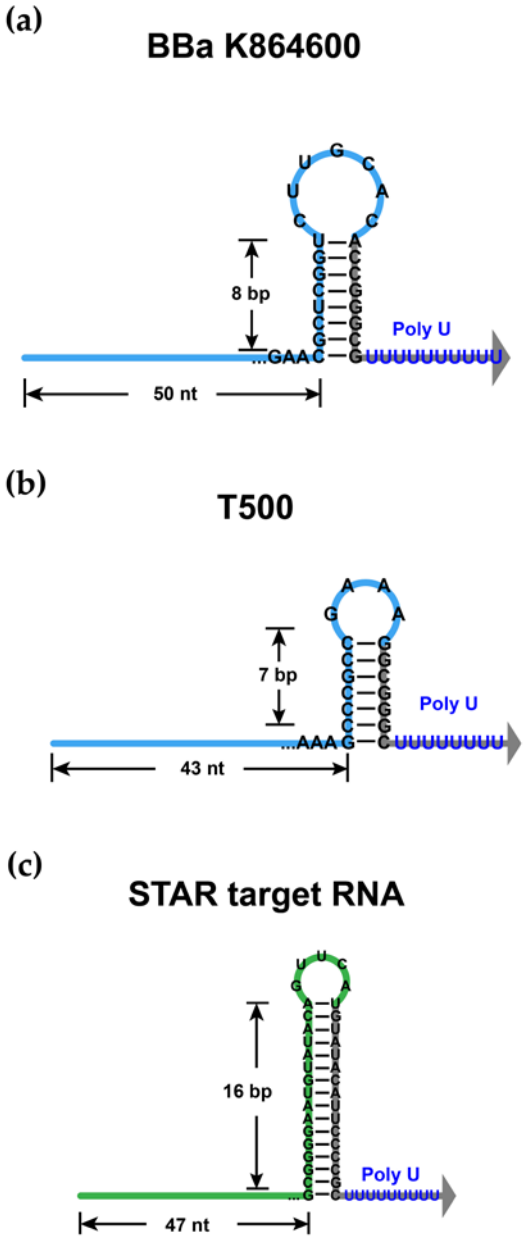
16S rRNA Raw Ct	Trigger	Decoy
BBa K864600	7.45 ± 0.02	7.84 ± 0.05
T500	7.62 ± 0.14	7.76 ± 0.15
STAR target WT	11.68 ± 0.15	11.60 ± 0.09
THS C	11.43 ± 0.06	11.64 ± 0.05
GFP control	11.44 ± 0.10	11.29 ± 0.20

	ΔΔCt
BBa K864600	1.88 ± 0.13
T500	7.34 ± 0.39
STAR target WT	4.73 ± 0.29
THS C	0.86 ± 0.29
GFP control	19.61 ± 1.02

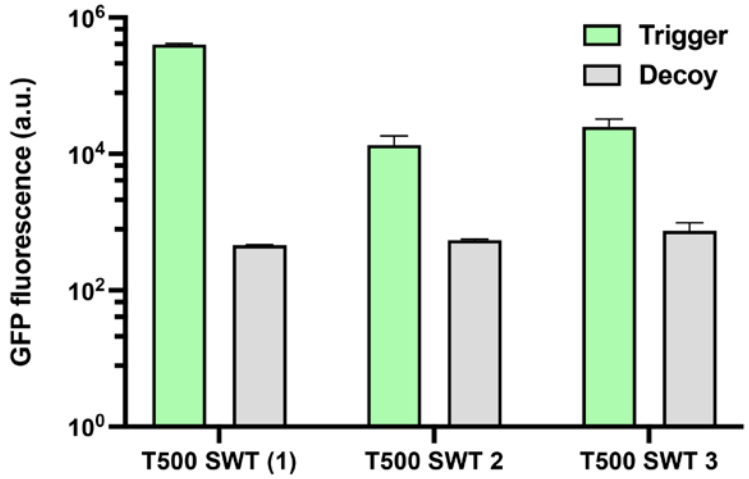
Supplementary Figures



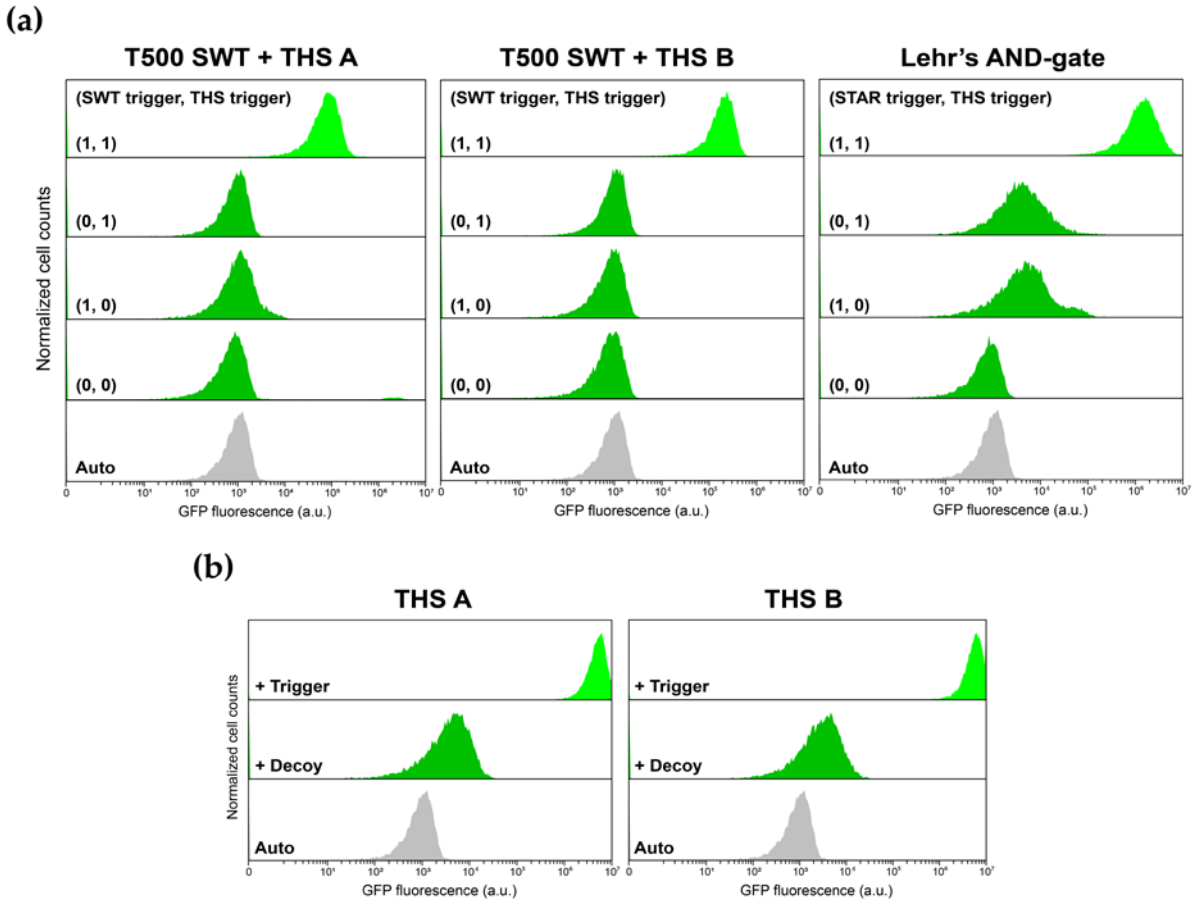
Supplementary Figure S1. De-novo-designed Synthetic RNA Regulators, STAR and THS. (**a**) Schematic of the STAR mechanism. The target RNA folds into a rho-independent transcription terminator that causes RNA polymerase to halt transcription. When STAR binds to the target RNA, terminator formation is prevented, allowing transcription elongation of the gene. (**b**) Schematic of the toehold switch mechanism. The ribosomal binding site (RBS) and start codon (AUG) of the switch RNA are exposed only when the cognate trigger RNA disrupts the secondary structure.



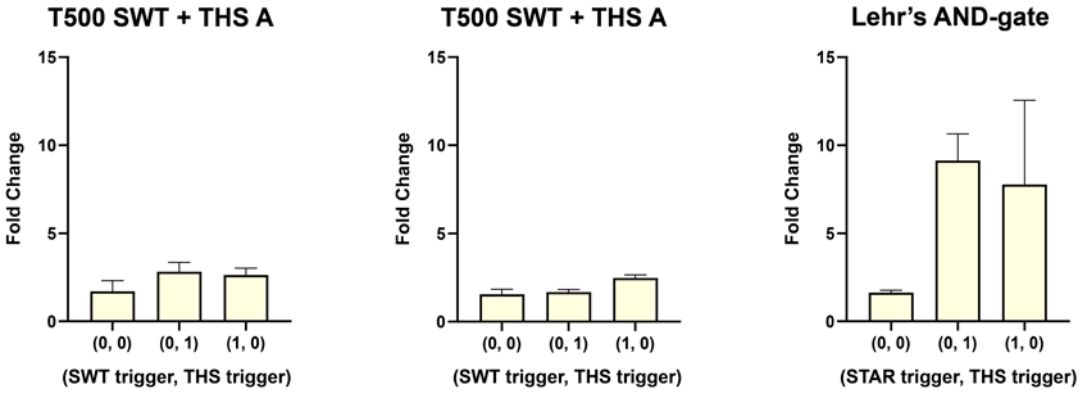
Supplementary Figure S2. Switchable Terminator Structure and Sequences. Structures and stem-loop sequences of switchable terminators (SWTs); (**a**) Natural terminator BBa K864600, (**b**) synthetic terminator T500, (c) STAR target RNA. For the BBa K864600, the stem length is 1 base pair longer and the loop size is 3 nt longer than the T500. The poly-U tract length of BBa K864600 and T500 is 10 nt and 8 nt, respectively. Sequences of toehold region were omitted. RNA secondary structure was confirmed by NUPACK [20].



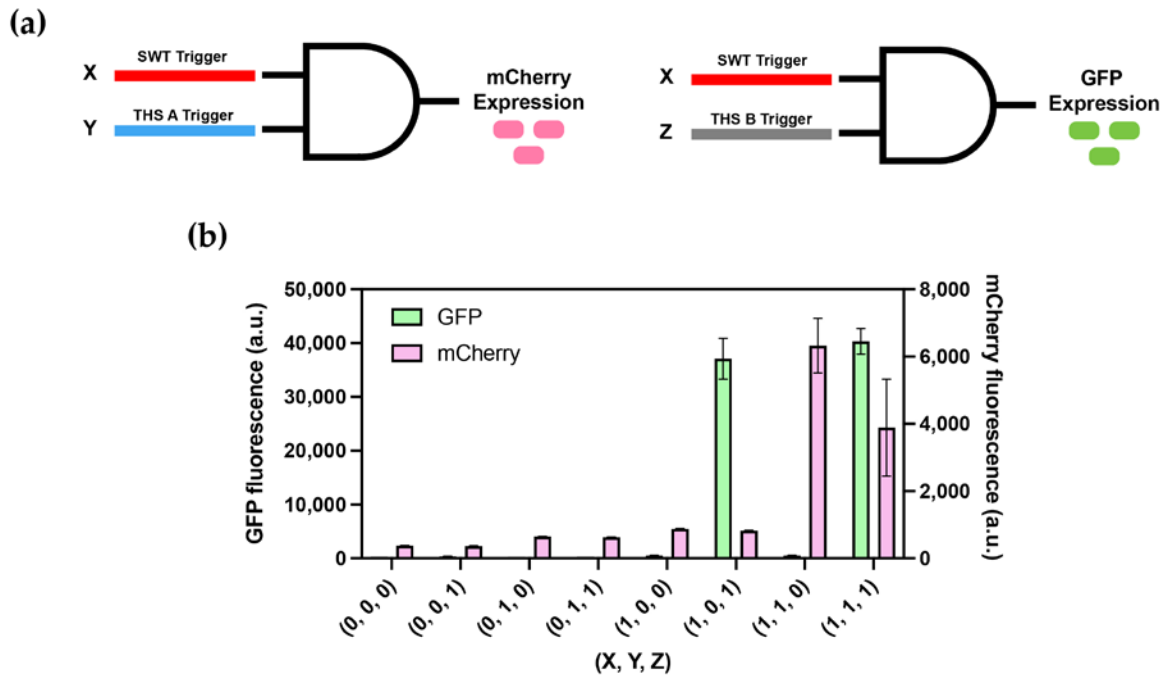
Supplementary Figure S3. Fluorescence Characterization of T500 SWT Toehold Variants. The GFP fluorescence of T500 SWT toehold variants were measured in the presence of trigger RNA or decoy RNA. Among the toehold sequences of STAR AD1 Target [25], the top three sequences with the highest fold change were implemented in front of the T500 synthetic terminator. There was no significant difference at the leakage level of each variant, and T500 SWT (1) showed the largest expression. T500 SWT (1) is the same construct with T500 SWT. Relative errors for the GFP fluorescence are from the s.d. of three biological replicates.



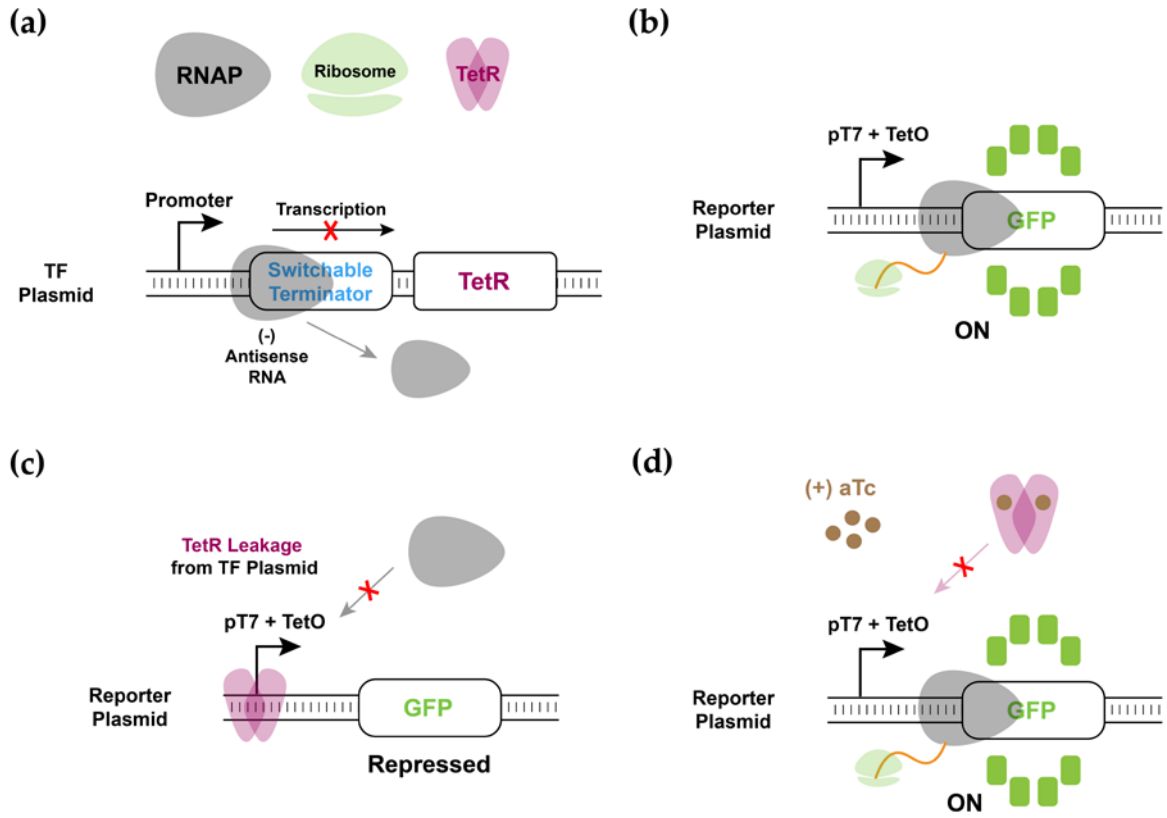
Supplementary Figure S4. GFP Fluorescence Histograms of Multilevel Regulatory Systems and Single THS. (**a**) The GFP fluorescence histograms according to the presence or absence of SWT trigger and THS trigger for the multilevel regulatory systems and Lehr's AND-gate. In the multilevel regulatory system, T500 SWT is followed by THS A or THS B. In the OFF state, the multilevel regulatory systems showed very little leakage level compared to Lehr's AND-gate. (**b**) The GFP fluorescence histograms according to the presence or absence of cognate THS trigger for the single THS system. Compared to the multilevel regulatory system, single THS showed higher leakage level in the OFF state.



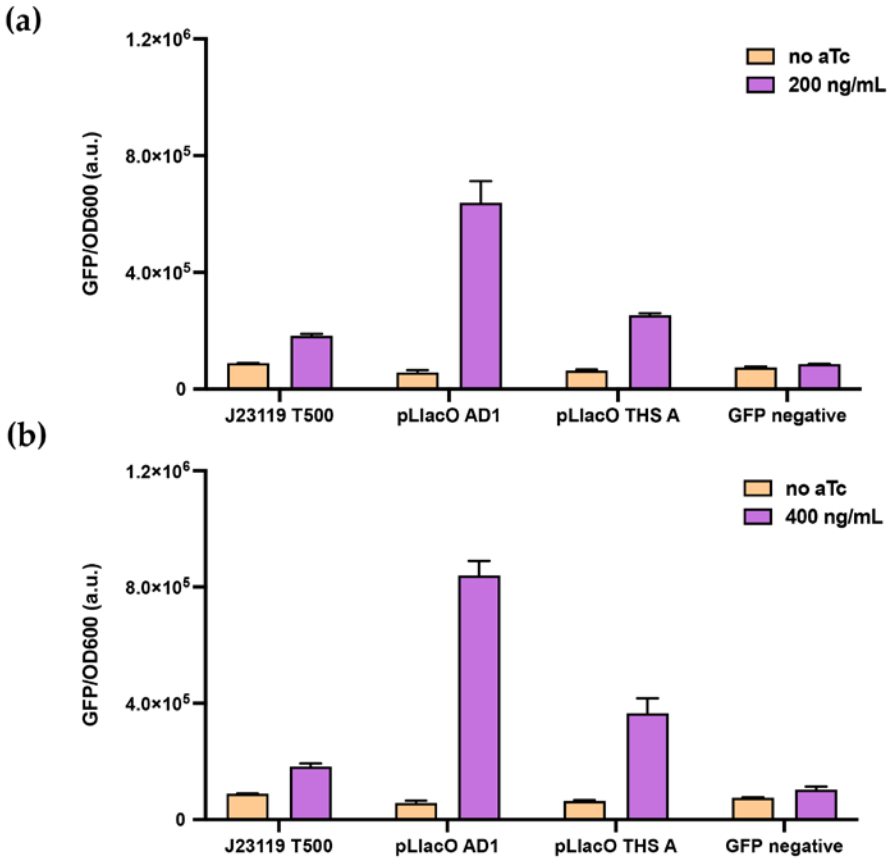
Supplementary Figure S5. Logical FALSE States of Multilevel Regulatory Systems. Fold change values were computed by autofluorescence (from Figure S4a) for the logical FALSE states of the multilevel regulatory systems. T500 SWT + THS A and T500 SWT + THS B had little leakage in all three FALSE states. In the absence of both trigger RNAs, Lehr's AND-gate had little leakage, but higher leakage levels were observed in the presence of either the STAR trigger or the THS trigger. Relative errors for fold change value of each conditions are from the s.d. of three biological replicates.



Supplementary Figure S6. Multiplexed Gene Expression Control with Exchanged Reporter Protein. (**a**) Design schematics of multiplexing implementation. mCherry is expressed under the control of the T500 SWT trigger and THS A trigger, and GFP is expressed under the control of the T500 SWT trigger and THS B trigger. (**b**) Measurement of fluorescent protein outputs via flow cytometry for all input combinations. Each reporter protein was expressed in the presence of an appropriate combination of cognate triggers as expected. Relative errors for mCherry and GFP fluorescence are from the s.d. of three biological replicates.



Supplementary Figure S7. Detailed Schematics of Multi-layered Circuits. Detailed description of the TetR leakage detection system in Figure 7b. (a) TF plasmid encodes SWT and TetR. In the absence of trigger RNA, TetR expression is repressed. (b) GFP is encoded in reporter plasmid, and fluorescence can be measured when IPTG is treated. (**c**) The pT7(TetO) in the reporter plasmid can be blocked by leaky TetR from TF plasmid. In this case, the GFP expression will be repressed. (**d**) If aTc is treated, TetR is released from pT7(TetO) and GFP expression can be restored again.



Supplementary Figure S8. Characterization of Multi-layered Circuits at High aTc Concentrations. (**a, b**) T500 SWT, STAR target WT, or THS A was attached upstream of TetR to regulate its expression. GFP fluorescence outputs were measured in the presence of trigger RNA. aTc was treated at different concentrations: 200 ng/mL (**a**) and 400 ng/mL (**b**). GFP negative did not contain RBS upstream of the GFP gene. Relative errors for GFP fluorescence are from the s.d. of three biological replicates.