

Supporting Information

A Fluorescent Split Aptamer for Visualizing RNA–RNA Assembly *In Vivo*

DOI: 10.1021/acssynbio.7b00059

Khalid K. Alam,^{1,2,5} Kwaku D. Tawiah,^{1,2} Matthew F. Lichte,^{1,2} David Porciani^{2,3} & Donald H. Burke^{1,2,3,4,*}

¹Department of Biochemistry, University of Missouri, Columbia, Missouri 65211, United States

²Bond Life Sciences Center, University of Missouri, Columbia, Missouri 65211, United States

³Department of Molecular Microbiology & Immunology, University of Missouri, Columbia, Missouri 65212, United States

⁴Department of Bioengineering, University of Missouri, Columbia, Missouri 65211, United States

⁵Current Address: Department of Chemical & Biological Engineering, Northwestern University, Evanston, Illinois 60208, United States

*Corresponding author, burkedh@missouri.edu

Table S1	2
Figure S1	3
Figure S2	4
Figure S3	5
Figure S4	6
Figure S5	7
Figure S6	8
Figure S7	9
Figure S8	10
Figure S9	11
Figure S10	12
<u>Plasmid DNA Sequences and Annotated Maps</u>	
i. pUC19-T7-SdB-T	13
ii. pUC19-T7-3WJdB-T	14
iii. pUC19-T7-Top-T	15
iv. pUC19-T7-Bottom-T	16
v. pUC19-P70a-3WJdB-T	17
vi. pUC19-P70a-Top-T	18
vii. pUC19-P70a-Bottom-T	19
viii. pUC19-P70a-Top-T~P70a-Bottom-T	20
ix. pUC19-P70a-Top-T~Bottom-T	21
x. pUC19-Split-Broccoli-Toehold-Switch	22
xi. pUC19-Trigger-Bottom	23
xii. pUC19-Top-Toehold-mCherry	24

Table S1. RNA sequences unique to this study.

	RNA Sequence (5' to 3')
<i>SdB</i>	GAGGGAGACGGUCGGGUCCAUCUGAGACGGUCGGGUCCAGAUUUUCGU AUCUGUCGAGUAGAGUGUGGGCUCAGAUGUCGAGUAGAGUGUGGGCUC CCUC
<i>3WJdB</i>	GGACCCACAUACUCUGAUGAUCCGAGACGGUCGGGUCCAGAUUUUCGU AUCUGUCGAGUAGAGUGUGGGCUCGGAUCAUCAUGGCAAGAGAGACGGU CGGGGUCCAGAUUUUCGUAUCUGUCGAGUAGAGUGUGGGCUCUUGCCAU GUGUAUGUGGG
<i>Top</i>	GGAUGAUGGAGACGGUCGGGUCCAGGAUCAUUCAUGGCAAGAGAGACGGU CGGGGUCCAGAUGAUGCAGAU
<i>Bottom</i>	GAUCCGCAUCAUCUGUCGAGUAGAGUGUGGGCUCUUGCCAUGUGUAU GUGGGUCAACCCACAUACUCUGAUGAUCCUGUCGAGUAGAGUGUGGGC UCCAUCAUCC

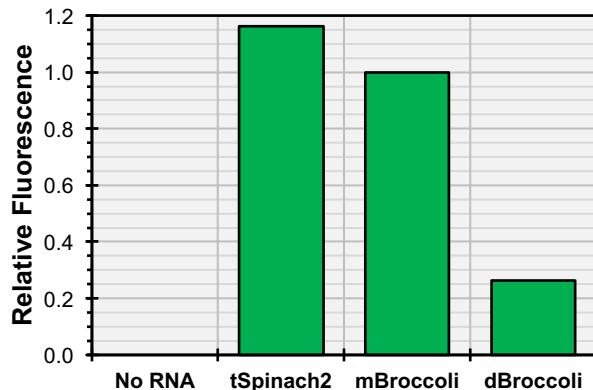


Figure S1. Comparison of *tSpinach2*, *mBroccoli* and *dBroccoli*. The Spinach2 aptamer embedded within a stabilizing tRNA^{Lys}₃ scaffold (*tSpinach2*) and the minimal monomeric Broccoli aptamer (*mBroccoli*) demonstrate similar levels of fluorescence when equimolar amounts of RNA are thermally renatured in buffer containing dye and assayed for fluorescence. In contrast, the minimal dimeric Broccoli aptamer (*dBroccoli*), which is expected to have twice the fluorescence of *mBroccoli*, exhibits about one-quarter the signal of *mBroccoli*, an 8-fold reduction from expected fluorescence activity.

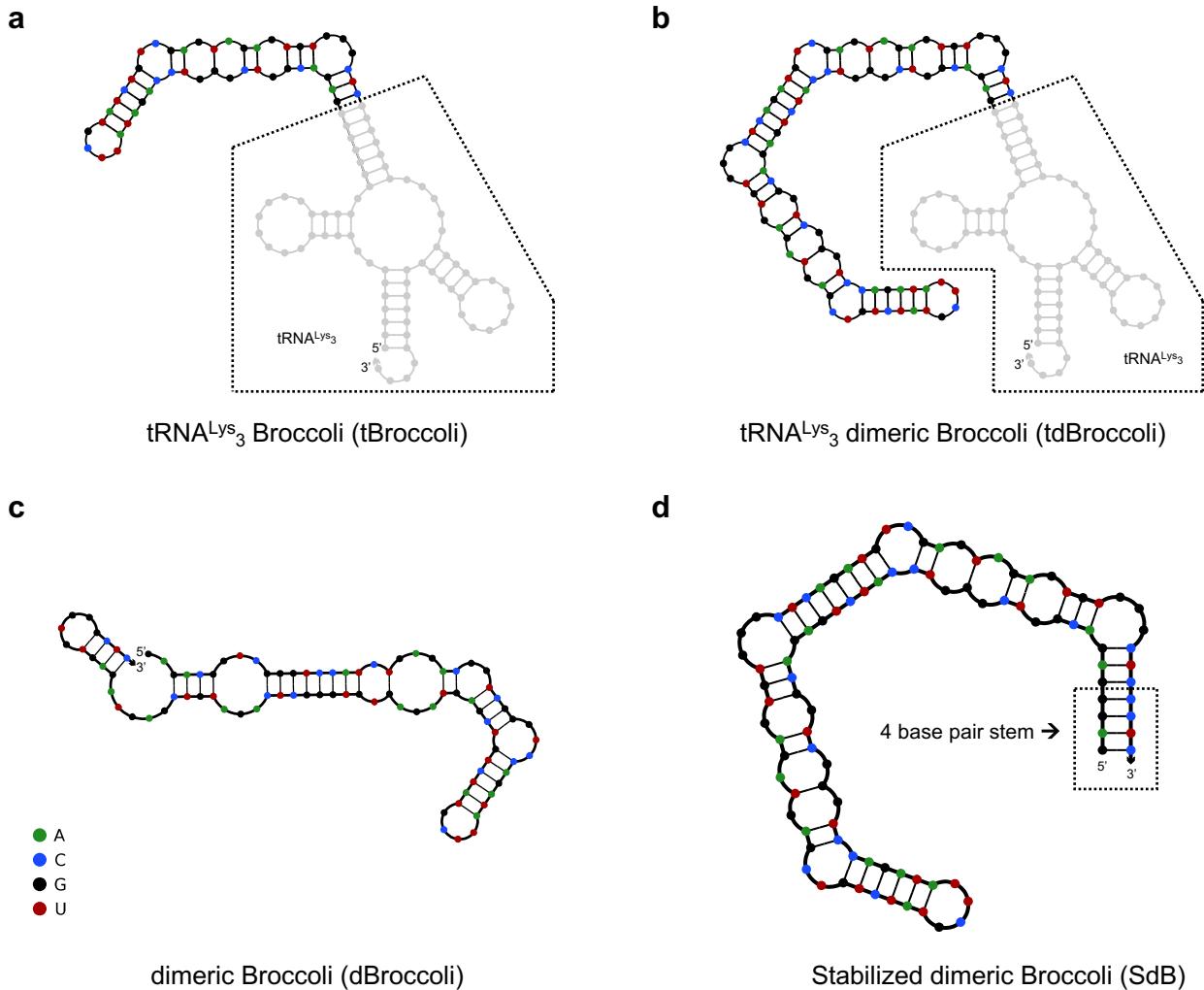


Figure S2. Predicted secondary structures of the Broccoli aptamer in the presence and absence of a tRNA stabilizing scaffold. **(a)** The Broccoli aptamer, when embedded within a tRNA^{Lys}₃ scaffold (*tBroccoli*), is predicted to form the functional fold of the Broccoli aptamer. **(b)** The dimeric Broccoli aptamer (*tdBroccoli*) is similarly stabilized within the tRNA scaffold. **(c)** In the absence of the scaffold, the minimal dimeric Broccoli (*dBroccoli*) is predicted to misfold by forming undesired Watson-Crick base pairs across each monomer that results in a nonfunctional aptamer. **(d)** Stabilized dimeric Broccoli (*SdB*) was designed by appending a 4 base pair terminal stem to the dimeric Broccoli and is predicted to form the functional aptamer fold in the absence of a larger scaffold.

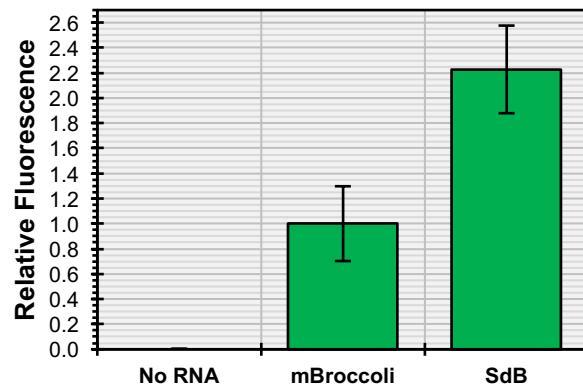


Figure S3. Stabilized dimeric Broccoli (*SdB*) is twice as bright as *mBroccoli*. *SdB* generates more than twice the signal of monomeric Broccoli (*mBroccoli*) when equimolar amounts of *mBroccoli* and *SdB* aptamers are thermally renatured in buffer containing dye and assayed for fluorescence. Mean values are shown ($n \geq 6$) with error bars to indicate standard deviations.

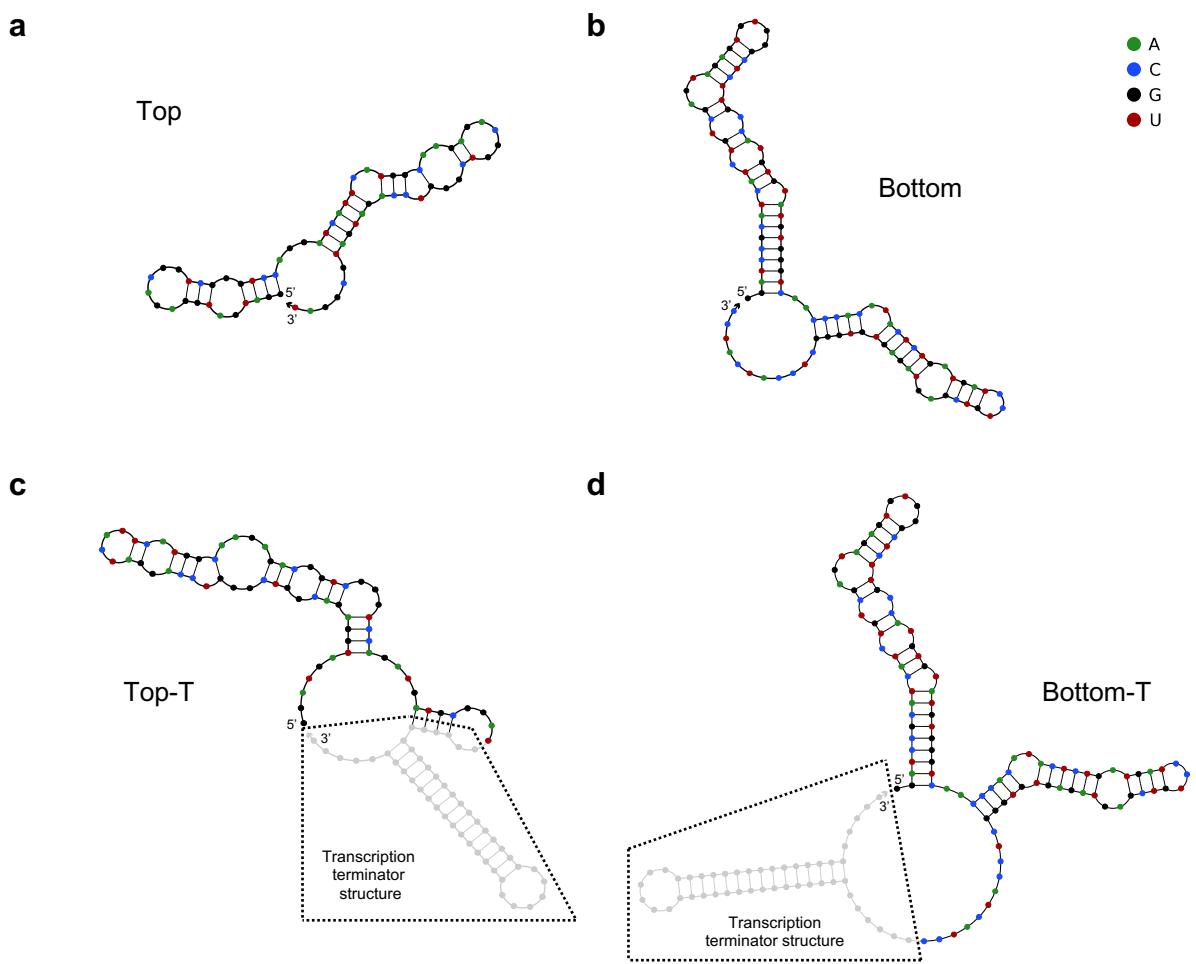


Figure S4. Predicted secondary structures of the Split-Broccoli system components, with and without transcription terminators. RNA corresponding to *Top* (**a**) and *Bottom* (**b**), without transcription terminator structures, and *Top-T* (**c**) and *Bottom-T* (**d**), which contain transcription terminator structures (boxed in lower panels). All four sequences neither contain the full Broccoli aptamer sequence, nor are independently predicted to fold into structures that resemble the secondary structure of the Broccoli aptamer.

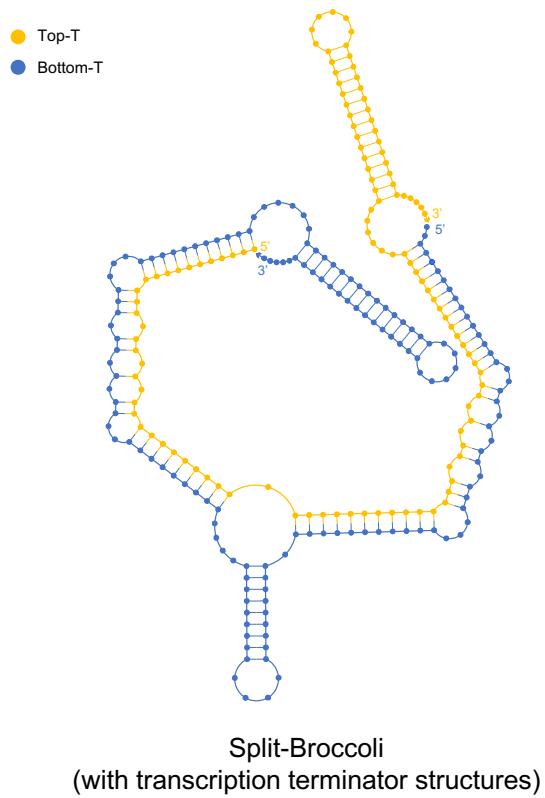


Figure S5. Predicted secondary structure of the hybridized Split-Broccoli system with transcription terminators. *Top-T* (yellow) and *Bottom-T* (blue) are predicted to hybridize into a secondary structure that maintains the expected motifs corresponding to the 3WJ, the Broccoli aptamers and the transcription terminators.

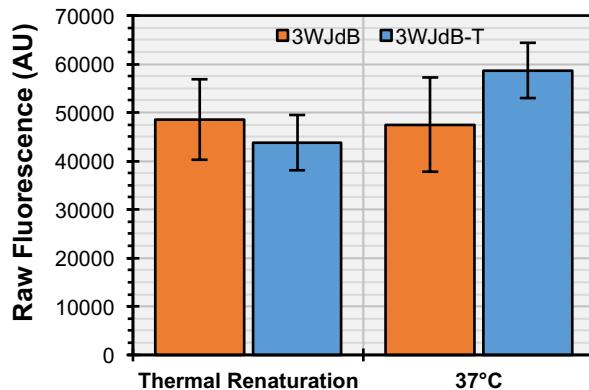


Figure S6. Comparison of raw fluorescence values for *3WJdB* with and without a transcription terminator, and with and without a thermal renaturation step. *3WJdB* without (orange columns) and with a transcription terminator structure (*3WJdB-T*, blue columns) demonstrate similar levels of fluorescence when assembled and measured ($\lambda_{\text{ex}} = 472 \text{ nm}$, $\lambda_{\text{em}} = 507 \text{ nm}$) following thermal renaturation from 90°C to 37°C (left columns) or simple incubation at 37°C (right columns). Mean values are shown ($n = 5$) with error bars to indicate standard deviations.

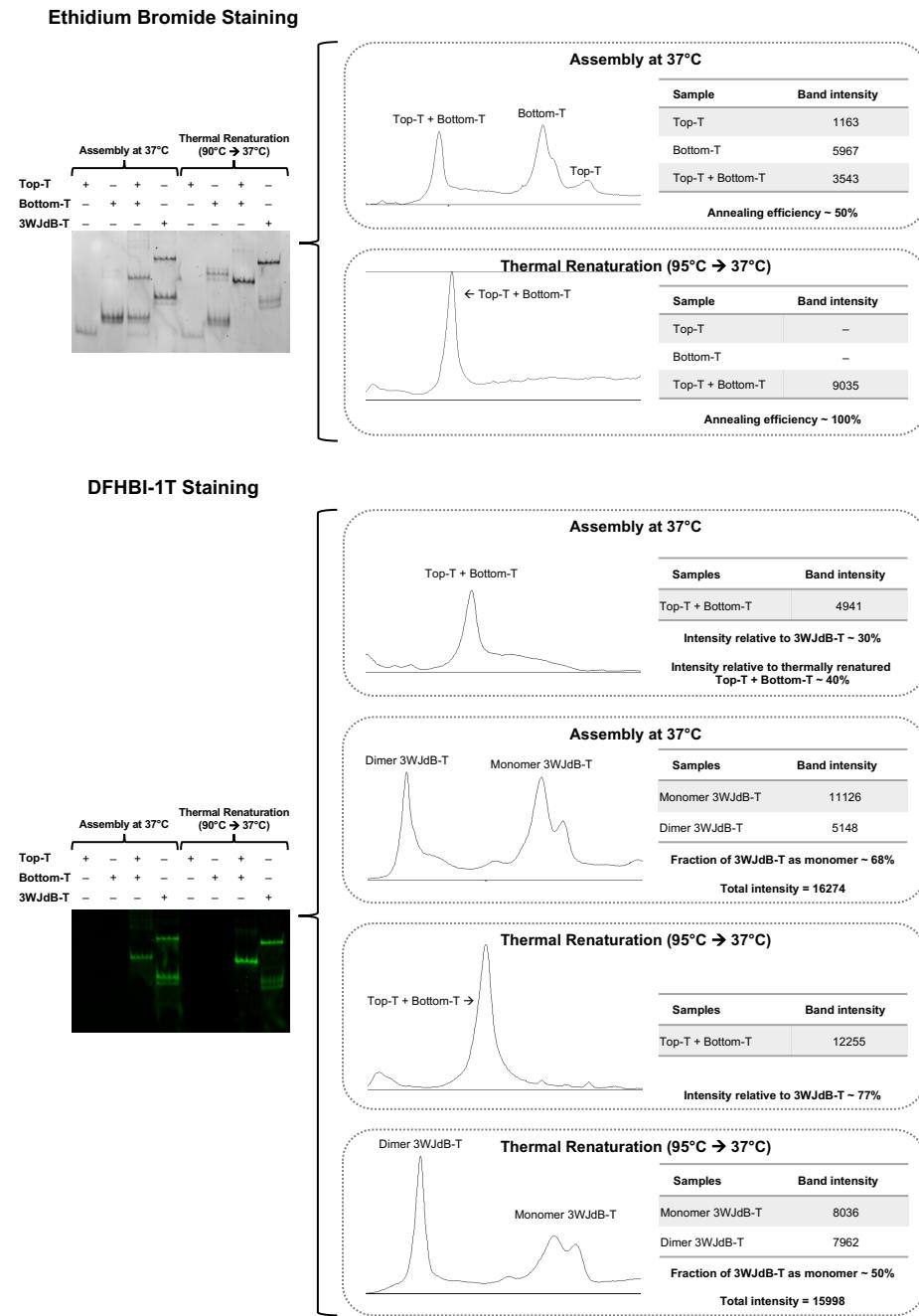


Figure S7. Densitometry analysis of the Split-Broccoli system with transcription terminators. Ethidium bromide staining identifies all molecular species (top), while DFHBI-1T staining (bottom) identifies only the functional, fluorescence-activating species. Thermal renaturation improves both annealing efficiency and fluorescence of the Split-Broccoli system.

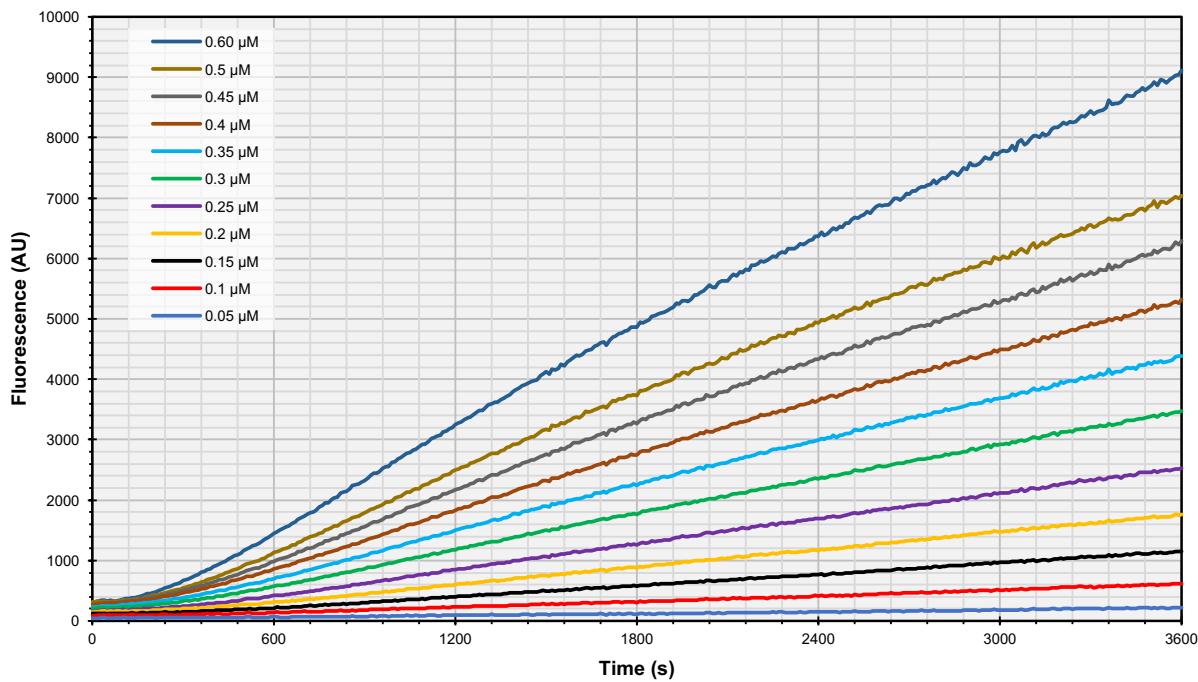


Figure S8. Fluorescence activation kinetics of the Split-Broccoli system, with transcription terminators, at varying concentrations. Functional assembly of equimolar amounts of *Top-T* and *Bottom-T* at 37°C was assayed at a range of concentrations. Rates from the linear region of each concentration replicate were then used to determine the rate of assembly. Mean values ($n = 4$) are shown for each concentration.

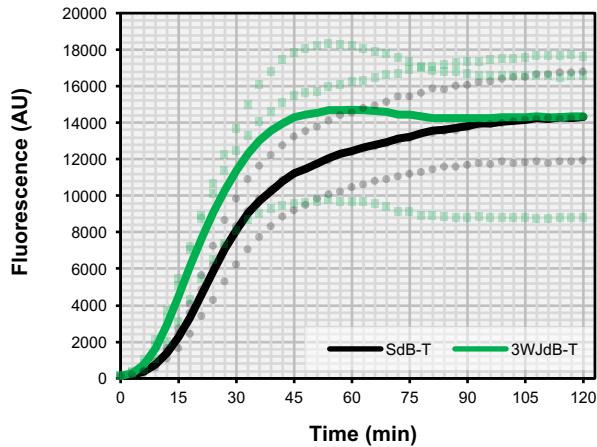


Figure S9. Addition of the 3WJ scaffold does not impede function of dimeric Broccoli when transcribed and assayed for activity *in vitro*. Transcriptional assays of *3WJdB-T* (green lines) and *SdB-T* (black lines) demonstrate that the addition of the 3WJ scaffold present in *3WJdB-T* does not slow the rate of fluorescence activation of dimeric Broccoli when compared to the unscaffolded variant, *SdB-T*. Mean values ($n = 3$) are shown as solid lines and individual replicates are shown as dotted lines.

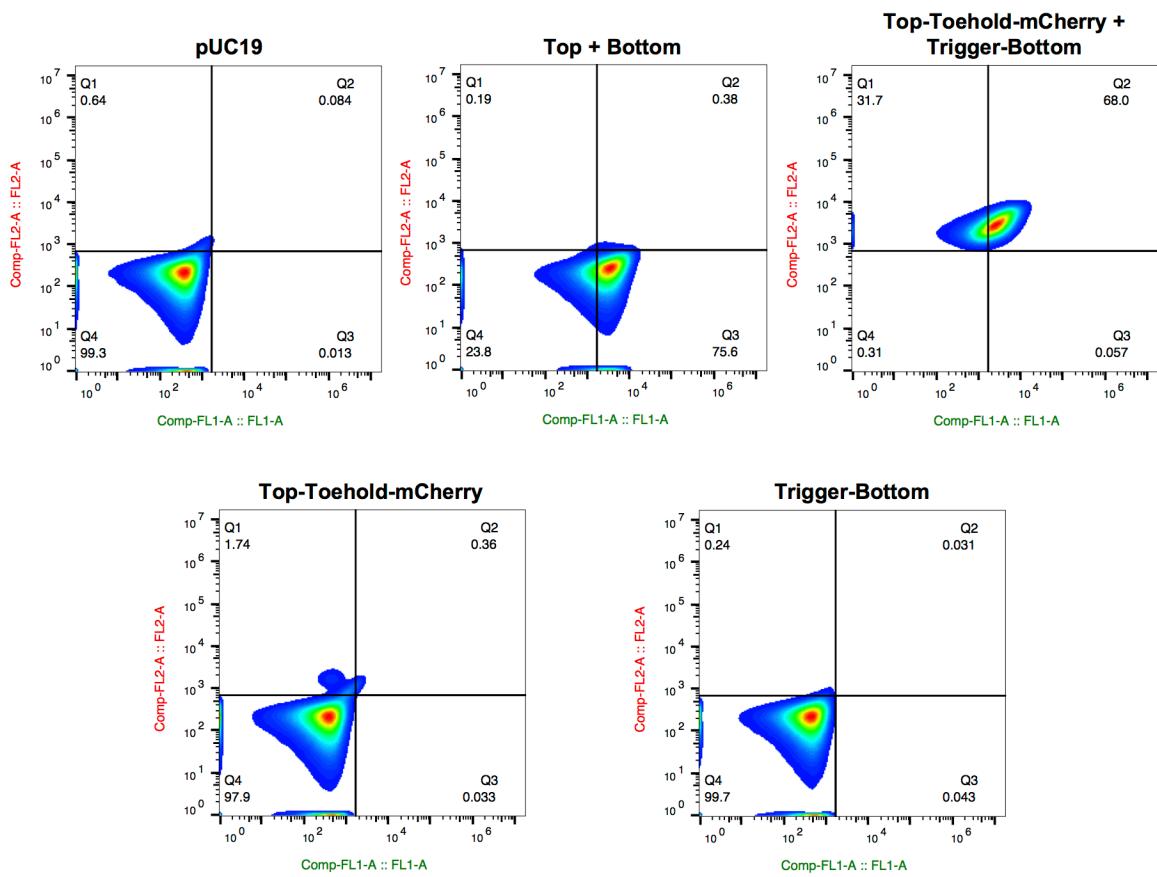
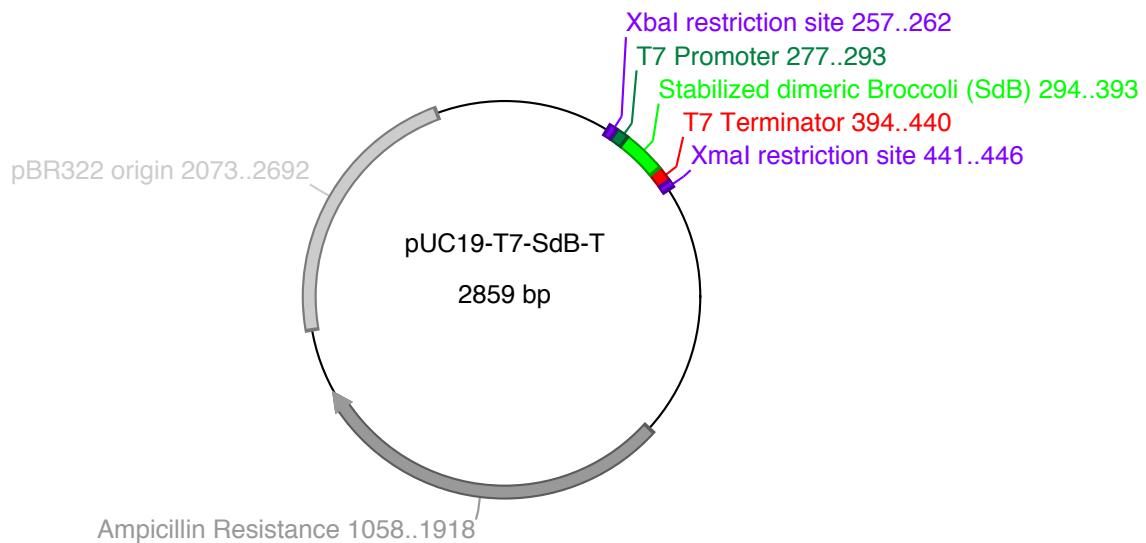
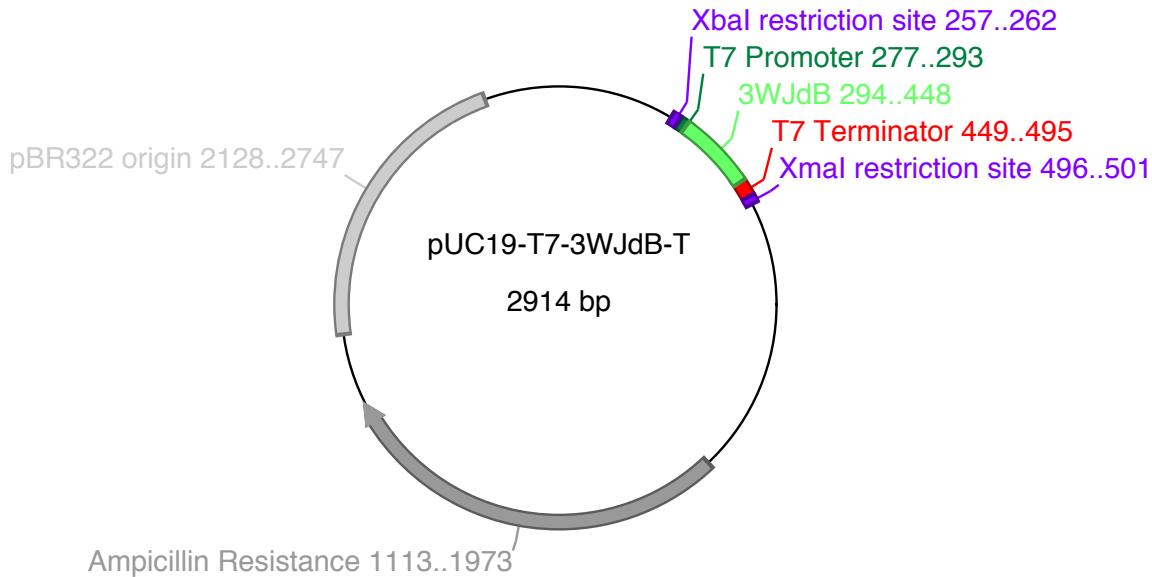


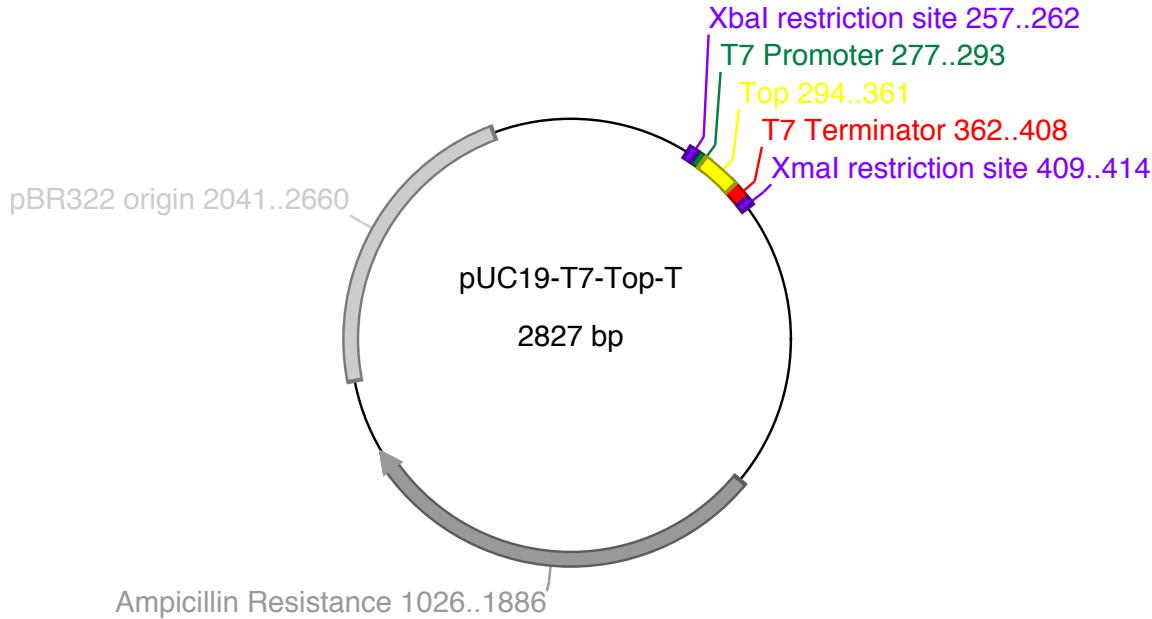
Figure S10. Representative scatter plots from flow cytometric analysis of the Split-Broccoli Toehold Switch demonstrate hybridization and activation of Split-Broccoli and the Toehold switch. *E. coli* containing a plasmid encoding the Split-Broccoli system fused to an RNA toehold switch (*Top-Toehold-mCherry + Trigger-Bottom*, top right) demonstrate a notable shift in both green and red fluorescence (x- and y-axes, respectively), while *E. coli* containing a plasmid encoding the Split-Broccoli system alone (*Top + Bottom*, top middle) only exhibits a shift in green fluorescence. In contrast, *E. coli* transformed with a plasmid encoding either *Top-Toehold-mCherry* (bottom left) or *Trigger-Bottom* alone (bottom right) do not cause a major shift in either red or green cellular fluorescence.



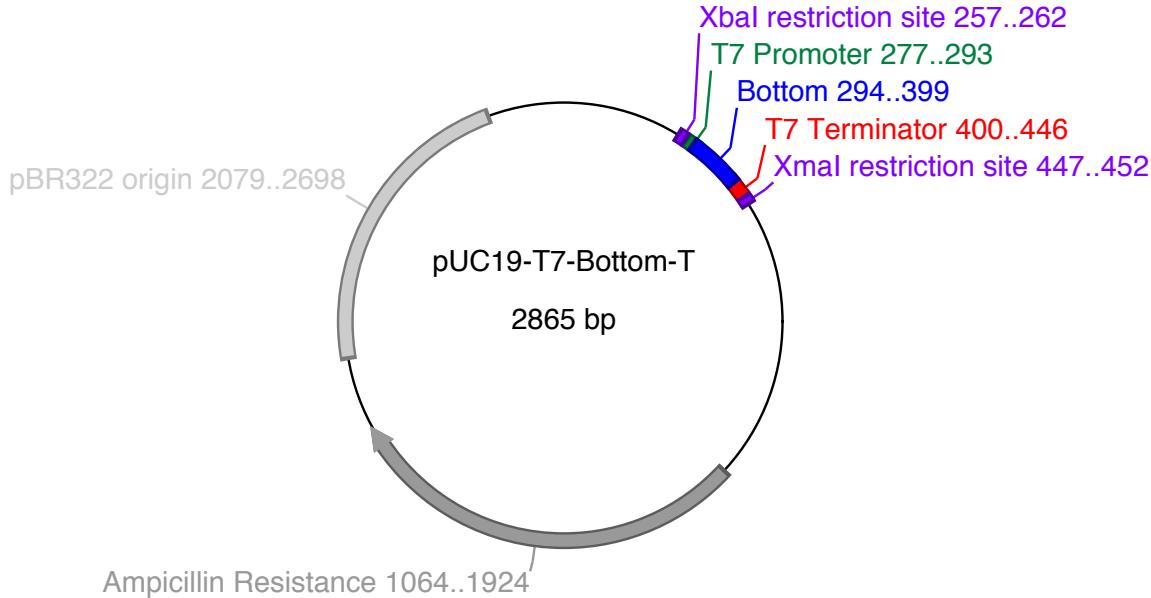
```
>pUC19-T7-SdB-T (Addgene ID 87307)
GCGCCAATACGCAAACCGCCTCTCCCGCGTGGCGATTCAATTAGCAGCTGGCACGACAGGTTCCCGACTGAAAGCGGCAGTGAGCGC
AACGCAATTATGTGAGTTAGCTCACTCATAGCACCCAGGCTTACACTTTATGCTCCGGCTCGATGTGTGGAATTGTGAGCGGATAAC
AATTTCACACAGGAACAGCTATGACCATGATTACGCCAGCTTGCATGCCAGGTGACTCTAGACGATCCCGAAATTTAATACGACTCACTA
TAGAGGGAGACGGTCGGGTCATCTGAGACGGTCGGGTCAGATATTCTGATCTGTCAGTAGAGGTGTCGGCTCAGATGTCAGTAGAGTGTGGGCT
CCCTCTAGCATAACCCCTGGGCCTCTAAACGGGTCTGAGGGTTTTTGCCCCGGTACCGAGCTCGAATTCACTGGCCGTCGTTTACAACGTC
GTGACTGGGAAACCCCTGGCGTTACCAACTTAATGCCCTGCAAGCACATCCCCCTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCG
CCCTCCCAACAGTTGCCAGCCTGAATGGCAATGGCCCTGATGGCGTATTTCCTCCTTACGCATCTGTCGGTATTTCACACCGCAATTGGTC
ACTCTCAGTACAATCTGCTCTGATCCCGCATAGTTAACGCCAGCCCCGACACCCGCCAACACCCGCTGACGCCGCTGACGGGCTTGTCTGCC
CATCCGCTTACAGACAAGCTGTGACCGCTCCGGAGCTGATGTGTCAGAGGTTTCAACGCTCATCACCGAACACGCCGAGACGAAAGGGCCTCGT
GATACGCCATTTTTATAGGTTAATGTCATGATAATAATGGTTCTAGACGTCAGGTGCACTTTCGGGAAATGTGCCGGAACCCCTATTGTT
TTATTTCTAAATACATTCAAATATGATCCGCTCATGAGACAATAACCCGTATAATGCTTCAATAATTGAAAAAGGAAGAGTATGAGTATT
AACATTCCGTGCGCCCTTATCCCTTTTGCAGGCTTTGCCTCTGTTTGCTCACCCAGAACGCTGGTGAAGTAAAGATGCTGAAGA
TCAGTTGGGTGACAGTGGGTTACATGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTCGCCCGAAGAACGTTTCAATGATGAGC
ACTTTAAAGTCTGCTATGTCGGGGTATTACCGTATTGACGCCGGCAAGAGAACCTCGGTCGCCGATACACTATTCTCAGAATGACTTGG
TTGAGTACTCACCAGTCACAGAAAAGCATTACGGGATGCCATGACAGTAAGAGAATTATGCGCTGCCATACCATGAGTGAATAACACTGCC
CAACTTACTCTGACAACGATCGGAGGACCGAAGAGCTAACCGCTTGGCACAAACGGGATCATGTAACTCGCCTTGATCGTTGGGAACCCG
GAGCTGAATGAAGCCATACCAAACGACGAGCGTGAACCCACGATGCCCTAGCAATGGCAACACGTTGCCAAACTATTAACTGGCAACTT
CTCTAGCTTCCCGCAACAATTAAATAGACTGGATGGAGGCGGATAAAGTTCAGGACCACTTCTGCGCTCGGCCCTCCGGCTGGTTATTG
TGATAATCTGGAGCCGGTGAGCGTGGGCTCGCGGTATCATTGCACTGGGCCAGATGGTAAGCCCTCCGTATCGTAGTTATCTACACGACG
GGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTACTCAT
ATATACTTTAGATTGATTAAACTCATTTAAATTAAAGGATCTAGGTGAAGATCCTTTTGATAATCTCATGACCAAATCCCTAACGTGA
GTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTGAGATCCTTTCTGCGCTTAATCTGCTGCTTGCAAAACAAAA
AAACACCCGCTACCAGCGGTTGGTTGCGGATCAAGAGCTACCAACTCTTCCGAAGGTAACTGGCTCAGCAGAGCGCAGATACCAAATA
CTGTTCTCTAGTGTAGCGTACCGCACCACCTCAAGAACTCTGCTAGCACCGCTACATACCTCGCTCGCTAATCCTGTTACCACTGCTGC
TGCCAGTGGCGATAAGTCGCTCTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCCGAGCGCTGGCTGAACGGGGGTTCTG
CACA CAGCCCAGCTGGAGCGAACGACCTACACCGAACTGAGATAACCTACAGCGTAGGCTATGAGAAAGGCCACGCCAGCTCCGAAGGGAGAAAGGCCGACA
GGTATCCGGTAAGCGGCAGGGTCGGAACAGGGAGAGCGCACGAGGGAGCTCCAGGGGAAACGCCCTGGTATCTTATAGTCCTGCGGGTTTCGCCA
CCTCTGACTTGAGCGTCGATTGGTGTGCTCGTCAGGGGGCGGCCATATGAAAACGCCAGCAACGCCCTTTTACGGTTCCCTGGCTTT
TGCTGGCCTTGTCACTGTTCTGCGTTATCCCGTGAATTCTGTTGATAACCGTATTACCGCTTGTAGTGAGCTGATACCCTGCCGCA
GCCGAACGACCGAGCGCAGCGAGCTAGTGAGCGAGGAAGCGGAAGA
```



>pUC19-T7-3WJdB-T (Addgene ID 87308)
 GCGCCAATACGCAAACCGCTCTCCCGCGCTGGCCGATTCAATTAAATGCAGCTGGCACGACAGGTTCCCGACTGGAAAGCGGGCAGTGAGCGC
 AACGCAATTAAATGTGAGTTAGCTCACTTATTAGGCACCCAGGCTTACACTTTATGCTCCGGCTCGTATGTGTGGAATTGTGAGCGGATAAC
 AATTTCACACAGGAAAACAGCTATGACCATGATTACGCCAACGCTTGCGATGCCCTGCAGGTGAC **TCTAGACGATCCCAGAAATTAAATACGACTCACTA**
**TAGGACCCACATACTCTGATGATCCGAGACGGTCGGGTCAGATATTCTGTCAGTAGAGTGTGGGCTCGGATATTCAATGGCAAGAGACGG
 TCGGGTCCAGATATTCTGATCTGTCAGTAGAGTGTGGGCTCTGCCATGTATGTGGG**TAGCATAACCCCTGGGGCCTCTAAACGGGCTTGAG
GGGTTTTTGCCCGGGTACCGAGCTGAATTCACTGGCGCTGTTTACAACGTCGACTGGAAAACCCCTGGCGTTACCAACTTAATGCCCTG
 CACGACATCCCCCTTCGCCAGCTGCCGTAAAGCGAAAGAGGCCGCACCGATGCCCTCCCAACAGTTGCCAGCCTGAATGGCGAATGGCCCT
 GATGCCGTATTTCCTTACGCACTCTGCGGTATTTCACACCCGATATGGTCAGCTCTCAGTACAATCTGCTCTGATGCCCATAGTTAACCCAG
 CCCGACACCCGCCAACACCCGCTGACGCCCTGACGGGCTTGTCTGCTCCGGCATCGCTTACAGACAAGCTGTGACCGCTCCGGGAGCTGCA
 TGTTCAGAGGTTTCACCGTCATCAGGAAACCGCGAGACGAAAGGGCTCGTGTACGCCCTATTTTATAGGTTAATGTCATGATAATAATGGT
 TTCTTAGACGTCAGTGGCACTTTGGGAAATGTCGGGAAACCCCTATTGTTTATTCTAAATCATCAAATATGATTCGCTCATGAGA
 CAATAACCCGTATAATGCTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTCCGTGTCGCCCTTATTCCCTTTTGCGGCATT
 GCCTTCTGTTTGCTCACCGAGAACGCTGGTGAAGTAAAGATGCTGAAGATCAGTGGGTGACAGGGTTACATCGAACTGGATCTCAA
 CACCGTAAGATCCTTGAGAGTTTCGCCCGAAGAACGTTTCAATGATGAGCACTTTAAAGTTCTGCTATGTGGCGCGTATTATCCGTATT
 GACGCCGGCAAGAGCAACTCGGTCGCCATACACTATTCTCAGAGTACTGGTGTACTCACCAGTCACAGAAAAGCATCTACGGATGGCA
 TGACAGTAAGAGAATTATGCACTGCTGCCATAACCATGAGTGTAAACACTGCGGCCACTTACTCTGACAACAGATCGGAGGACCGAAGGAGCTAAC
 CGCTTTTGCAACACATGGGGATCATGTAACCTGCCCTGATCGTGGGAGCTGAAATGAAGGACATACCAAAACGAGCGTGCACACCACG
 ATGCTGTAGCAATGGCAACACGTTGCCAAACTATTAACTGGCAACTACTTACTCTAGCTTCCGGCAACAAATTAAAGACTGGATGGAGGG
 ATAAAGTTGCAAGGACCACTCTGCGCTGCCCTTCCGGCTGGCTGGTTATTGCTGATAAAATCTGGAGCCGGTGAGCGTGGGCTCGCGGTATCAT
 TGCAGCACTGGGGCAGATGTAAGCCCTCCGTATGTTACACGACGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCT
 GAGATAGGTGCCACTGATAAGCATTGTAACTGTCAGACCAAGTTACTCATATAACTTTAGATTAAACTCATTTAAATTAAAA
 GGATCTAGGTGAAGATCCTTTGATAATCTCATGACCAAATCCCTAACGTGAGTTTCGTTCACTGAGCGTCAGACCCCGTAGAAAGATCAA
 AGGATCTCTTGAGATCCTTTTCTGCGCTGAATCTGCTGCTTCAACAAAAAAACCCCGCTACAGCGGTGTTGCTTGCCTGAGAG
 CTACCAACTCTTCCGAAGGTAACTGGCTCAGCAGAGCGCAGATAACCAAAATACTGTTCTCTAGTGTAGCGTAGTTAGGCCACCAACTCAAGA
 ACTCTGTAGCACCGCTACATACCTGCCCTGCTAACTCTGTTACCACTGGCTGCTGCCAGTGGCATAAGCTGTCTACCGGTTGGACTCAAG
 ACGATAGTTACCGATAAGGGCAGCGTGGGCTGAACGGGGGTTGTCGACACAGCCAGCTGGAGCGAACGACCTACACCGAACTGAGATAC
 CTACAGCGTGAGCTATGAGAAAGGCCACGCCGCTCCGAAGGGAGAAAGCGGACAGGTATCCGTAAGCGGAGGGTCCGGAACAGGAGAGCGCACGA
 GGGAGCTTCCAGGGGAAACGCCCTGGTATCTTATAGTCTGTCGGGTTGCCACCTCTGACTTGAGCGTCGATTGGTGTGATGCTCGTACGGGG
 GCGGAGCCTATGAAAAACGCCAGAACGCCGCTTTACGGTTCTGGCCTTTGCTGCCACATGTTCTGCTGTTACCGCTCGGCCAGCGAACGAGCGCAG
 GATTCTGTGGATAACCGTATTACCGCTTGAGTGAACGCTGATACCGCTGCCGCAGCGAACGACCGAGCGCAGTGAGCGAGGAGAGCG
 AAAGA****



>pUC19-T7-Top-T (Addgene ID 87309)
 GCGCCAATACGAAACCGCTCTCCCGCGTGGCGATTCAATTAGCAGCTGGCACGACAGGTTCCGACTGGAAAGCGGGCAGTGAGCGC
 AACCGAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTACACTTTATGCTTCGGCTCGTATGTGTGGAATTGTGAGCGGATAAC
 AATTCACACAGGAAACAGCTATGACCAGTATTACGCCAACGCTTGCATGCCAGGTCAC **TCTAGA**CATCCCGCAGAAATTAAATACGACTCACTA
**TAGGATGATGGAGACGGTCGGGTCCAGGATCATTATGCCAAGAGACGGTCGGGTCCAGATGATGCGGATTAGCATAACCCCTGGGGCTCTAAAC
**GGGTCTTGAGGGGTTTTTCCCCGGGTACCGAGCTGAATTCACTGCCGTCGTTTACAACGTCGTGACTGGAAAACCCCTGGCGTTACCAACTT
 ATCGCCTTGACGACATCCCCCTTCGCAGCTGCCGTAATAGCGAAGAGGCCCCCACCGATGCCCTTCCCAAACAGTTGCGCAGCCTGAATGGCG
 AATGGCGCTGTGCGGATTTCTCTTACGCATCTGCGGATTTCACCCGATATGGCAGCTTCAGTACAATCTGCTCTGATGCCGATA
 GTTAAGCCAGCCCCGACACCCGCAACACCGCTGACCGCCCTGACGGGCTTGTCTGCTCCGGCATCCCTACAGACAAGCTGTGACCGCTCC
 GGGAGCTGCATGTCAGAGGTTTACCGTCATACCGAAACGCGCAGACGAAAGGGCTCGTGTACGCCATTAGGTTAATGTCATGA
 TAATAATGGTTCTTAGACGTCAGGTGGCACTTTGGGAAATGTCGCGGAACCCATTGGTTATTTCTAAATACATTCAAATATGTATCC
 GCTCATGAGACAATAACCTGATAAAATGCTTCAATAATATTGAAAAGAGTATGAGTATTCAACATTCCGTGTCGCCCTATTCCCTTTT
 GCGCATTGCTTCCCTGTTGCTCACCCAGAACGCTGGTAAGGATAAGATGCTGAAGATCAGTTGGGTGACAGAGTGGGTACATCGAAC
 TGGATCTCAACAGCGGTAAGATCCTGAGGTTTCCGGCCGAAGAACGCTTCCAAATGATGAGCAGCTTAAAGTTCTGCTATGTCGGCGGTATT
 ATCCCGTATTGACGCCGGGAAGAGCAACTCGGTGCCGATACACTATTCTCAGAAATGCTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTT
 ACGGATGGCATGACAGTAAGAGAAATTAGCAGTGTGCCATAACCATGACTGATAACACTGCCAACATTACTCTGACAACGATCGGAGGACCGA
 AGGAGCTAACCGCTTTTGACAAACATGGGGATCATGTAACTCGCCCTGATGTTGGAACCGGAGCTGAATGAAGCCATACAAACGAGAGCG
 TGACACCACGATGCCGTAGCAATGCCAACACGTTGCCAAACTATTAACTGGCAACTACTTACTCTAGCTCCGGCAACAATTAAAGACTGG
 ATGGAGCGGATAAAAGTTGCAAGGACACTCTGCCGCTGGCTGGTTATTGCTGATAAAATCTGGAGCGGTGAGCGTGGGTCTC
 GCGTATATTGACGACTGGGCCAGATGGTAAGGCCCTCCGATCTGCTAGTTACTCACGACGGGAGCTCAGGCAACTATGGATGAACGAATAG
 ACAGATCGCTGAGATAGGTGCCCTACTGATTAAGCATTGGTAACTGTCAGACCAAGTTACTCATATACTTGTGATTTAAACTCTGATTT
 TAATTTAAAGATCTAGGTGAAGATCCTTTGATAAATCTCATGACCAAATCCCTAACGTGAGTTCTGCTCCACTGAGCGTCAGACCCGTAG
 AAAAGATCAAAGGATCTCTGAGATCTTTCTGCGCTAATCTGCTGCTGCAAAACAAAAAAACCCGCTACCGCGTGGTTTGCC
 GGATCAAGAGCTACCAACTCTTCCGAAGGTAACTGGCTTCAGCAGCGCAGATAACCAAAACTGTCTCTAGTGTAGCCGTAGTTAGGCCAC
 CACTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCTGTTACCAAGTGGCTGCTGCCAGTGGCATAAGCTGTCTTACCGGGT
 TGGACTCAAGACGATAGTTACCGGATAAGCGCAGCGGCTGGCTGAACGGGGGTTGTCACACAGCCAGCTGGAGCGAACGACCTACCCGA
 ACTGAGATACCTACAGCGTGAAGCTGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGCGGAGCAGGTATCCGTAAGCGGCAGGGTCGGAACAGGA
 GAGCGCACGAGGGAGCTTCCAGGGAAACGCCCTGGTATCTTATAGTCCTGCGGTTTCGCCACCTGACTTGAGCGTCGATTTGTGATGCT
 CGTCAGGGGGGGCGGAGCCTATGGAAAAACGCCAGAACCGCCCTTTACGGTTCTGCCCTTTGCTGCCCTTGTCACTGTTCTTCC
 GTTATCCCCCTGATTCTGTTGAGTAAACCGTATTACCGCTTTGAGTGAACGCTGATACCGCTCCCGCAGCGAACGACCGAGCGAGCTGAGC
 GAGGAAGCGGAAGA****

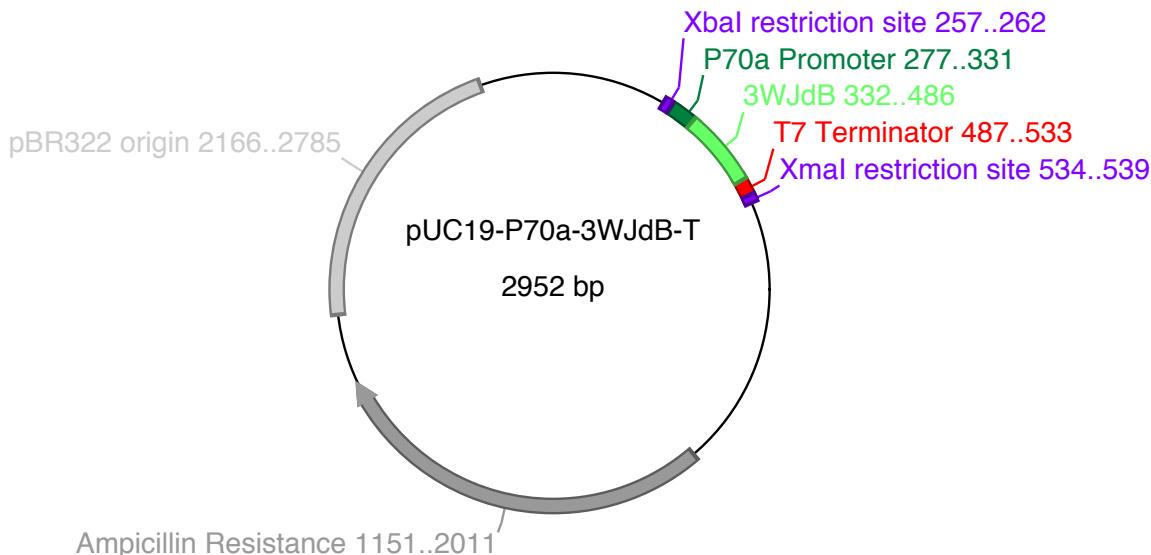


>pUC19-T7-Bottom-T (Addgene ID 87310)

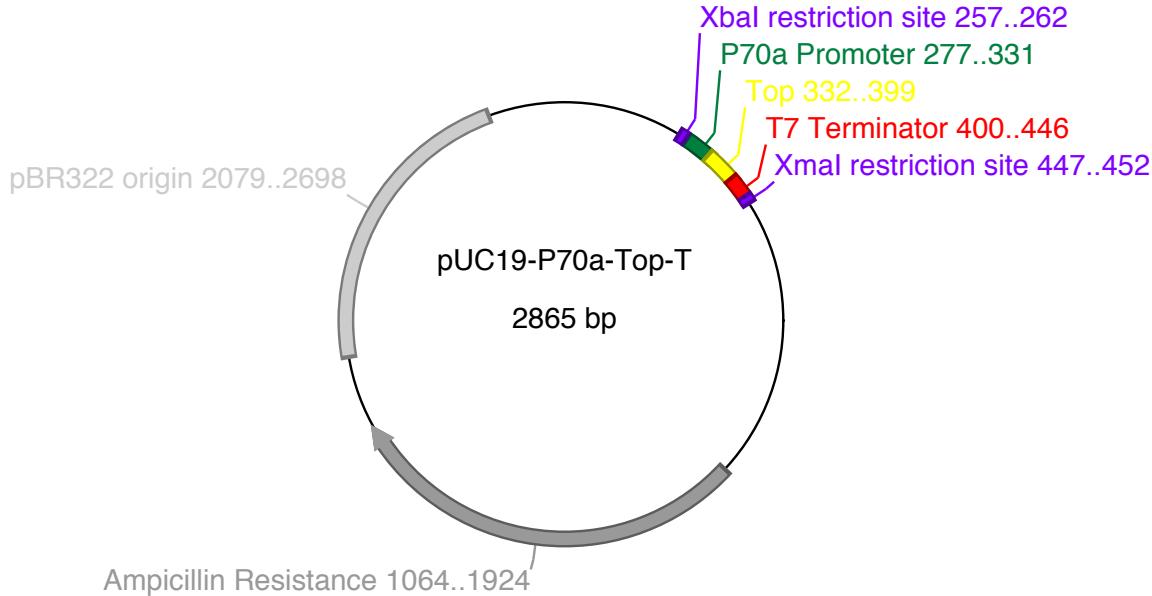
```

GCGCCCAATACGCAAACCGCCTCTCCCGCGCGTGGCCGATTCAATTAGCAGCTGGCACGACAGGTTCCCGACTGGAAAGCGGGCAGTGAGCGC
AACGCAATTAAATGTGAGTTAGCTCACTCATAGGCACCCCAGGCTTACACTTTATGCTTCCGGCTCGTATGTTGAGCTGGAATTGTGAGCGGATAAC
AATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTTGCATGCCCTGCAGGTCAC TCTAGACATCCCAGAATTAATACGACTCACTA
TAGGATCCCGCATCATCTGTCGAGTAGAGTGTGGGCTCTGCCATGTGATGTGGGCAACCCACATACTCTGATGATCCTGTCGAGTAGAGTGTGGG
CTCCCATCATCCTAGCATAACCCCTTGGGGCTCTAAACGGGCTTGGAGGGGTTTTTGCCCAGGTACCGAGCTGAATTCACTGGCCGTGCTTTAC
AACGTCGTGACTGGAAAACCTGGCGTACCCAACCTTAATGCCCTTGCAAGCACATCCCCCTTCGCCAGTGGCATAATAGCGAAGAGGCCGCAC
CGATCGCCCTTCCAAACAGTTCGCGAGCTGAATGGCGATGGCGCTGATGCGGTATTTCTCTACGCATCTGCGGTATTCACACCCGATA
TGGTGCACTCTCAGTACAATCTGCTCTGATGCCGATAGTTAACGCCAGCCCCGACACCCGCAACACCCGCTGACGCCCTGACGGGCTTGTCTGC
TCCCGCATCCGTTACAGACAAGCTGACCCTCGGGAGGCTGATGTCAGGTTTACCGCTCATCCGAAACCGCGAGACAGAAAGGG
CCTCGTATACCGCTATTTTATAGTTATGCTGATGATAATAATGGTTCTTAGACGTAGGTCAGTGGCACTTTCGGGAAATGTGCGGAAACCCCT
ATTGTTTATTTCTAAATACATTCAAAATATGATCCGCTCATGAGACAATAACCCGTATAATGCTTCATAATAATTGAAAAAGGAAGAGTATGA
GTATTCAACATTCCGTCGCCCTTATCCCTTTTGCGGCATTTGCCCTCTGTTGCAACCGAAACGCTGGTAAAGTAAAGATGC
TGAAGATCAGTTGGGTGACAGTGGTTACATGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTCGCCCGAAGAACGTTTCAATG
ATAGACACTTTAAAGTTCTGCTATGTCGCGGTTATATCCGTTATGACGCCGGAAAGAGCAACTCGGTGCGCCGATAACACTATTCTCAGAATG
ACTGGGTTAGACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCACTGTCGCGATAACCATGAGTGATAACAC
TGGGCCAACACTACTTCTGACAACATGGAGGAGCGAGGAGGAGCTAACCGCTTTTGCAACACATGGGGATCATGTAACTGCCCTGATCGTGG
GAACCGGAGCTGAAGCCATACCAACAGCAGCGCTGACACCCGATGCCGTAGCAATGCCAACACTGCGCAAACACTATTAACTGGCGAAC
TACTTACTCTAGCTCCGGCAACAATTAAATAGACTGGATGGAGGGGATAAAAGTGCAGGACCACTTCTGCGCTGGCCCTCCGGCTGGCTGGTT
TATTGCTGATAAAATCTGGAGGCCGGTGGCTGAGCTGGTATCATGAGCACTGGGGCAGATGGTAAGCCCTCCGTTATGTTAGTTAC
ACGACGGGGAGTCAGGCAACTATGGATGAAACGAAATAGACAGATCGTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTT
ACTCATATATACTTTAGATTGATTTAAACTCATTTTAATTTAAAGGATCTAGGTGAAGATCCTTTTGATAATCTCATGACCAAAATCCCTTA
ACGTGAGTTTCGTTCCACTGAGCGTCAGACCCGTTAGAAAAGATCAACAGGATCTCTTGAGAGATCCTTTCTGCGCTAATCTGCTGCTTGCAA
ACAAAAAAACCCCGTACCGCGTGGTTGTTGCCGATCAAGGCTACCAACTCTTCCGAAGGTAACCTGGCTCAGCAGCGCAGATAC
CAAATACTGTTCTAGTGTAGCCGTAGTTAGGCCACACTTCAAGAAACTCTGTAAGCACCGCTACATACTCGCTCTGTAATCCTGTTACCGAT
GGCTGCTGCCAGTGGCGATAAGTCGTTACCGGGTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGCTGAACGGGGGTTCG
TGCACACAGCCCAGCTGGAGCGAAGCAGACTACACCGAAGTGAGATACTACAGCGTGAGCTATGAGAAAGGCCACGCCGCTCCGAAGGGAGAAGG
CGGACAGGTATCCGTAAGCGCAGGGTGGAAACAGGGAGCGCAGCAGGAGGAGCTCCAGGGGAAACGCCGTTATCTTATAGTCTGTCGGTT
TCGCCACCTCTGACTTGAGCGTCGATTTTGATGCTGTCAGGGGGGGAGCTATGGAAAAACGCCAGCAACCGCAGCTTACGTTAC
GCCCTTTGCTGCCCTTTGCTCACATGTTCTTCTGCGTTATCCCTGATTCTGAGGATAACCGTATTACGCCCTTGAGTGAAGCTGATACCGCTC
GCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGA

```



>pUC19-P70a-3WJdB-T (Addgene ID 87311)
GCGCCCAATACGCAAACCGCCTCTCCCGCGCGTGGCGATTCTTAAATGCAGCTGGCACGACAGGTTCCCGACTGGAAAGCGGGCAGTGAGCGC
AACGCAATTAAATGTGAGTTAGCTCACTCATAGGCACCCAGGCTTACACTTTATGCTTCGGCTCGTATGTGTGGAATTGTGAGCGGATAAC
AATTTCACACAGAACAGCTATGACCAGTACGCCAAGCTTGCATGCCCTGCAGGTGAC**TCTAGA**CAGATCCCGCAAA**TGAGCTAACACCGTG**
CGTGTGACAATTTCACCTCTGGCGTATAATGGTGCAG**GGACCCACATACTCTGATGATCCGAGACGGTCGGTCAGATATTGTAATCTGCGA**
**GTAGAGTGTGGGCTCGGATCATTCATGGCAAGAGACGGTCGGTCCAGATATTGTAATCTGTCGAGTAGAGTGTGGGCTCTGCCCAGTGTATGTGG
GTACATACCCCTGGGCTCTAAACGGGTCTTGAGGGTTTTTGCGCCGGGTAACCGAGCTCGGCTGACGGCTCGTGTGCTCTGCTCCGGCATC
CTGGGAAAACCTCTGGCTTACCCAATTAAATCGCCTCTGCAGCACATCCCCCTTCGCCAGCTGGCGTAATCGCAAGAGGCCGACCGATCGCCCT
TCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTGATGCCGTTATTTCTCCTTACGCATCTGTGCCGTTATTCACCCGATATGGCACTC
TCAGTACAATCTGCTGATGCCGATAGTTAACGCCAGCCGACACCCGCCAACACCCGCTGACGCCCTGACGGGCTGTGCTGCCGGCATC
CGCTTACAGACAAGCTGTGACCGCTCCGGAGCTGCTGAGGTTTACCGTCATACCGAAACGCCGAGACGAAAGGGCCTCGTGTGATA
CGCCTATTTTATAGTTAATGTCATGATAATAATGGTTCTAGACGTCAGGTGCACTTTCGGGAAATGTGCGCGGAACCCCTATTGTTAT
TTTCTAAATACATTCAATATGTCGCCATGAGACAATACCCGATAAATGCTTCAATATAATTGAAAAGAGAAGAGTATGAGTATTCAACA
TTCCGTGTCGCCCTATTCCCTTTTGGCGCATTTGCCCTCTGCTTACCCAGAAACGCTGAGAAGTAAAGATGCTGAAGATCAG
TTGGGTGCAAGTGGTTACATGCAACTGGATCTCACAGCGTAAGATCCTGAGAGTTTCGCCCGAAGAACGTTTCAATGATGACCACTT
TTAAAGTTCTGCTATGTCGCGGTATTATCCGTTATGACGCCGGCAAGAGCAACTCGGTGCCGATACACTATTCTCAGAATGACTGGTGA
GTACTCACCAGTCACAGAAAGCATCTACGGATGGCATGACAGTAAGAGAATTATGCACTGCTGCCATAACCATGAGTATAACACTGCCAAC
TTACTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGTTTTCACAACATGGGGATCATGTAACCTGCCCTGATGCTGGGAACCGGAGC
TGAATGAAGCCATACCAAAACGACGAGCGTACACACCGATGCCGTGCAATGCCAACACGTTGCCAAACTATTAAACTGCCGAACTACTACTCT
AGCTTCCGGCAACAATTAAATAGACTGGATGGAGCGGATAAAGTTCAGGACCACTTCGCGCTCGGCCCTCCGGCTGGCTTATTGCTGAT
AAATCTGGAGCCGGTGGGTCTCGGGTATCATGCACTGGGGCCAGATGTTAAGCCTCCCGTATGCTAGTTATCTACACGAGGGGA
GTCAGGCAACTATGGATGAACGAAATAGACAGATCGTGGAGATAGTGGCTCACTGATTAAGCATTGGTAAGTGTAGACCCAAGTTACTCATATAT
ACTTAGATTGATTAAAATTCAATTAAAGGATCTAGGTGAAGATCCTTTGATAATCTCATGACCAAAATCCCTAACGTGAGTTT
TCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTCTTGAGATCCTTTCTGCCGTAATCTGCTGCTGCAAACAAAAAAC
CACCGCTACCGCGTGGTTGTTGCCGATCAAGACTACCAACTCTTTCCGAAGGTAACCTGGCTCAGCAGAGCGCAGATACCAAATACTGT
TCTTCTAGTGAGCGTAGTTAGGCCACCACTTCAGAACACTCTGTAACGCCCTACATACCTCGCTGCTTAATCTGTTACCGAGTGGCTGCTGCC
AGTGGCGATAAGTGTGCTTACCGGGTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGCTGGCTGAACGGGGGGTTCGTGACACAGC
CCAGCTGGAGCGAACGACCTACACCGAACCTGAGATACTACAGCGTAGCTGAGCTATGAGAAAGCGCACGCCACGCTCCGAAGGGAGAAAGCGGAGCTA
TCCGTTAGCGGAGGAGCTGGAGAGCTTCCAGGGGGAAACGCCCTGGTATCTTATAGTCTGCTGGGTTTCGCCACCTC
TGACTTGAGCGTCGATTGGTGTGATGCTCGTCAGGGGGCGGAGCCTATGAAAAAACGCCAGCAACGCCCTTTTACGTTCTGCCCTTGCT
GGCCTTGTGCTACATGTTCTTCTGCCGTTATCCCTGATTCTGTTGAGTACCGCCTTGAGTGTGAGCTGATACCGCTGCCCGAGCCG
AACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGA**

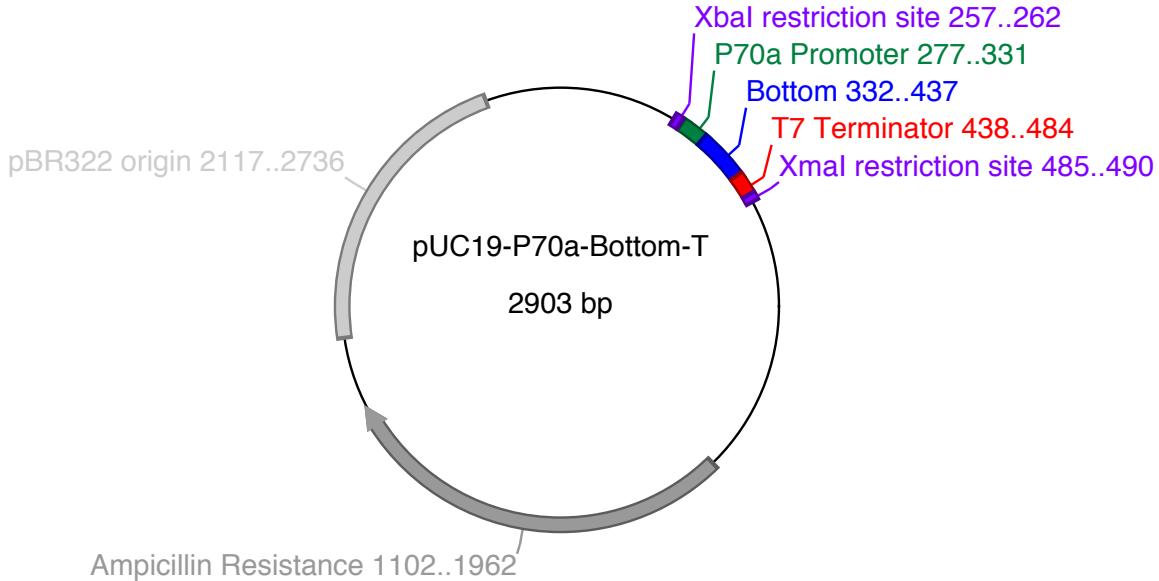


>pUC19-P70a-Top-T (Addgene ID 87312)

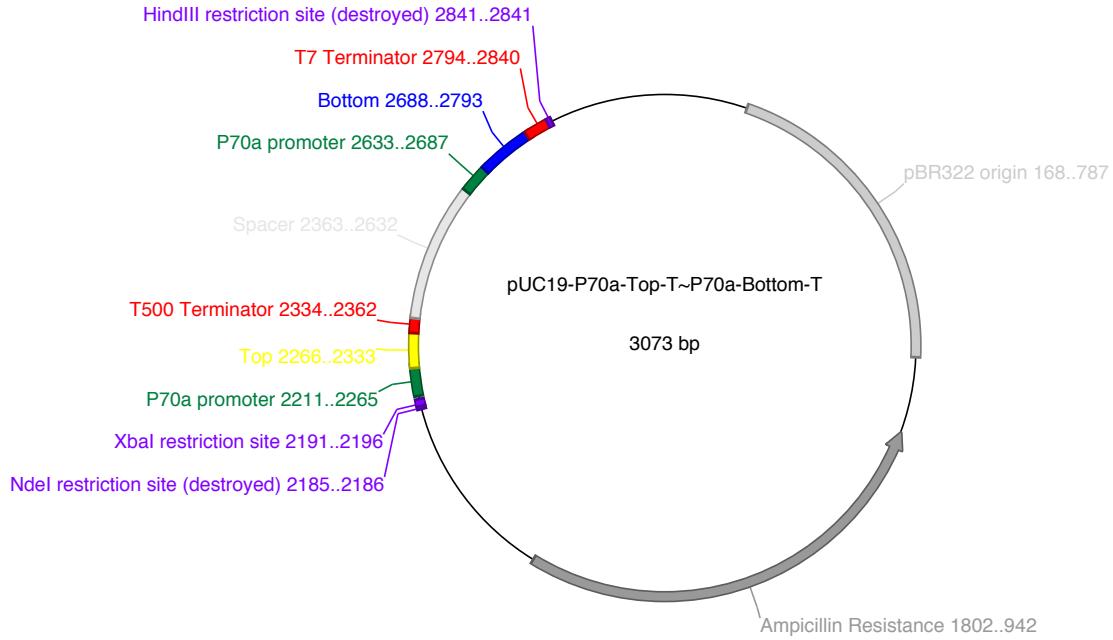
```

GCGCCAATACGCAAACCGCCTCTCCCGCGCGATTCAATTAGCAGCTGGCACGACAGGTTCCCGACTGGAAAGCGGGCAGTGAGCGC
AACGCAATTATGTGAGTTAGCTACTCATAGGCACCCCAGGCTTACACTTTATGCTCCGGCTCGATGTTGTTGGAATTGTGAGCGGATAAC
AATTTCACACAGGAAACAGCTATGACCAGTATTAGCAGCTTGCAGGTCGACTTCTAGACGATCCCGCGAATTGAGCTAACACCGTG
CGTGTGACAATTTCACCTCTGGCGGTGATAATGGTGCAGGATGATGGAGACGGTCGGGTCAGGATCATTGAGCTGGCAAGAGACGGTCGGTCCAG
ATGATGCGGATTAGCATAACCCCTGGGGCTCTAAACGGGCTTGAGGGGTTTTGCCCCGGTACCGAGCTGAATTCACTGGCCGTGCTTAC
AACGTCGTGACTGGAAAACCTGGCGTACCCAACCTTAATGCCCTGAGCACATCCCCCTTCGCCAGTGGCGTAATAGCGAAGAGGCCGAC
CGATGCCCTTCCAAACAGTGCAGCCTGAATGGCAATGGCGCTGATGCGGTATTTCTCTTACCCATCTGCGGTATTCACACCCGATA
TGGTGCACTCTCAGTACAATCTGCTGTGCGCATAGTTAACGCCAGCCCCGACACCCGCAACACCCGCTGACGCCCTGACGGGCTGCTGC
TCCCGCATCCGTTACAGACAAGCTGACCGTCTCGGGAGCTGCGATGTCAGGTTTACCGCTCATCCGAAACCGCGAGACGAAAGGG
CCTCGTACCGCTATTATAGTTAGCTGATGATAATAATGGTTCTTAGACGTCAGGTTGCACTTTGGGGAAATGCGCGGAACCCCT
ATTGTTATTCTAAATACATTCAAAATGTATCCGCTCATGAGACAATAACCTGATAATGCTTCATAATAATTGAAAAAGGAAGAGTATGA
GTATTCAACATTCCGTGTCGCCCTTACCGCTTGTGCGGCTTGTGCTACCCAGAAACGCTGGTAAAGTAAAGATGC
TGAAGATCAGTTGGTGCACGAGTGGTTACATGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTCGCCCGAAGAACGTTTCAATG
ATGAGCACTTTAAAGTCTGCTATGTCGGCGGTTATGCCGTTAGCAGCCGGAAGAGCAACTCGGTGCGCGCATACACTATTCTCAGAATG
ACTGGTGGAGTACTCACCAGTCACAGAAAGCATCTACGGATGGCATGACAGTAAGAGAATTATGCGTGCCTGAGTGTAGAACAC
TGGGCCAACTACTTCTGACAACATGGAGGACCGAGGAGGAGCTAACCGCTTGTGACAACATGGGATCATGTAACTGCCCTGATCGTGG
GAAACGGGAGCTGAAGCCATACAAACGACGAGCTGACACCACGATGCGTAGCAATGGCAACACGTTGCGCAAACATTAAACTGGCGAAC
TACTTACTCTAGCTCCCGGAACAATTAAATAGACTGGATGGAGGGCGATAAAGTGTGAGGACCAACTTGTGCGCTGGCCCTCCGGCTGGCTGGTT
TATTGCTGATAATCTGGAGGCCGGTGGCTCGCGGTATCATGAGCACTGGGGCAGATGGTAAGCCCTCCGTATGTAGTTATCTAC
ACGACGGGAGTCAGGCAACTATGGATGAAACAGACGATCGTGAGATAGGTGCCTACTGATTAAGCATTGGTAACTGTCAGACCAAGTTT
ACTCATATATACTTTAGATTGATTTAAACTCTATTAAATTAAAGGATCTAGGTGAAGATCCTTTTGATAATCTCATGACCAAAATCCCTTA
ACGTGAGTTTCGTTCACTGAGCGTCAGACCCGTTAGAAAAGATCAACAGGATCTCTTGAGAGATCTTTCTGCGCTGAACTCTGCTGCTTGCAA
ACAAAAAAACCCGCTACCGCGGGTTGTTGCGGATCAAGAGCTACCAACTCTTCCGAAGGTAACCTGGCTCAGCAGCGCAGATAC
CAAATACTGTTCTCTAGTGTAGCCGTAGTTAGGCCACACTCAAGAACACTCTGAGCACCGCTACATACCTCGCTCTGTAATCCTGTTACCGAT
GGCTGCTGCCAGTGGCGATAAGTCGTTACCGGGTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGCTGAACGGGGGTTCG
TGCACACAGCCCAGCTGGAGCGAACGACCTACACCGAACTGAGATACTACAGCGTGAGCTATGAGAAAGGCCACGCTCCGAAGGGAGAAGG
CGGACAGGTATCCGTAAGCGCAGGGTGGAACAGGAGAGCGCAGCAGGGAGCTCCAGGGGAAACGCGCTGGTATCTTATAGTCTGCGGGTT
TCGCCACCTCTGACTTGAGCGTCGATTTGTGATGCTCGTCAGGGGGGGAGCCTATGGAAAAACGCCAGCAACCGCCCTTTTACGGTTCTG
GCCCTTGCTGCCCTTGTCACTGTTCTCTGCGTTATCCCTGATTCTGAGTAAACCGTATTACGCCCTTGAGTGAAGCTGATACCGCTC
GCCGAGCCGAACGACCGAGCGCAGCGAGTCAGTGAAGCGAGGAAGCGGAAGA

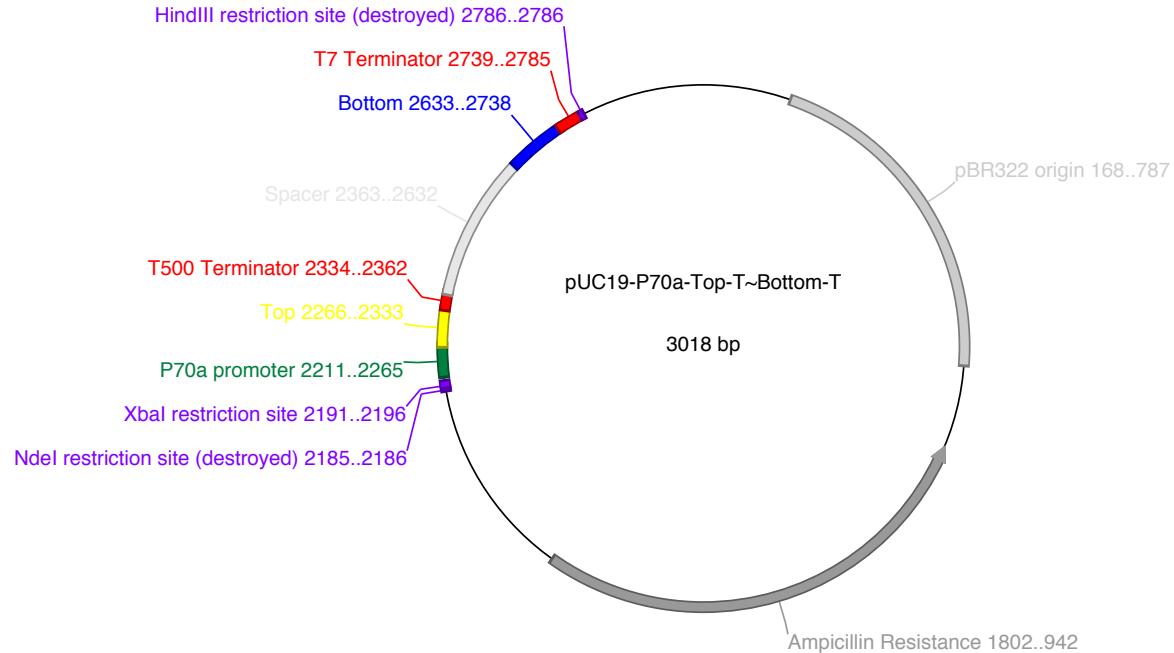
```



>pUC19-P70a-Bottom-T (Addgene ID 87313)
GCGCCAATACGCAAACCGCTCTCCCGCGATTCAATTAGCAGCTGGCACGACAGGTTCCGACTGGAAAGCGGGCAGTGAGCGC
AACGAATTATGTGAGTTAGCTCACTATTAGCACCCAGGCTTACACTTTATGCTTCCGGCTGTATGTGTGGAATTGTGAGCGGATAAC
AATTACACAGAAACAGCTATGACCATGATTACGCCAAGCTGATGCCCTGCAGGTCGAC **TCTAGA**CGATCCCGCGAAAT**TGAGCTAACACCGTG**
CGTGTGACAATTTACCTCTGGCGTATAATGGTTGAGGATCCGCATCATCTGCGAGTAGAGTGTGGGCTCTTGCCTATGTATGTGGGTCAA
CCCACATACTCTGATCTGTCGAGTAGAGTGTGGGCCATCATCTAGCATAACCCCTGGGGCTCTAACACGGGTCTTGGGGTTGC
CCGGGTACCGAGCTGAATTCACTGCCGCTGTTTACACGTCGACTGGGAAACCCCTGGCGTTACCCAACTTAATGCCCTGCAGCACATCCC
CCTTCGCCAGCTGGCGTAATAGCGAAGAGGCCGACCGATGCCCTCCAAACAGTTCGCGAGCCTGAATGGCGAATGCCCTGATGCCGTATT
TTTCCTTACGCATCTGCGGTATTCACACCGCATATGGCACTCTAGTACAATCTGCTGATGCCGATAGTTAACGCCAGCCCCAACACC
GCCAACACCCGCTGACGCCCTGACGGCTTGTCTGCTGCCGGCATCCGTTACAGACAAGCTGTCACCGTCCGGAGCTGCATGTGTCAGAGG
TTTCACCGTCATCACCGAAACGCCGAGACGAAAGGCCCTCGTGTACGCCTATTTTATAGGTTAATGTCATGATAATAATGGTTCTAGACGT
CAGGTGGCACTTTGGGGAAATGTGCGCGGAACCCCTATTGTTTATTCTAAACATTCAAAATGTATCCGCTCATGAGACAATAACCTG
ATAAATGCTTCAATAATTAGAAAAGGAAGAGTATGAGTATTCAACATTCCGTTGCGCTTATTCCCTTTTGGCGCATTTGCCCTCTGTT
TTGCTCACCCAGAACGCTGGTAAAGTAAAGATCTGAAAGATCACTGGGTGACAGTGGGTTACATGAACTGGATCTCAACAGCGGTAAAGA
TCCTTGAGAGTTTCGCCCGAAGAACGTTTCAATGATGAGCACTTTAAAGTTCTGCTATGTGGCGGGTATTATCCGCTATTGACGCCGGCA
AGAGCAACTCGTCGCCGATACACTATTCTCAGAATGACTTGGTGTAGTACTCACCAGTCACAGAAAAGCATTTACGGATGGCATGACAGTAAGA
GAATTATGCACTGCTGCCATAACCATGAGTGATAAACACTGCCGAACTTACTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGTTTTGC
ACAACATGGGAGTCATGTAACCTGCCGCTGATCGTGGGAACCGGAGCTGAATGAGCCATACCAACGACGAGCGTGACACACGATGCCCTGAGC
AATGGCAACACGTTGCGCAAACATTAACTGCGCAACTACTTACTCTAGTCTCCCGCAACAAATTAAAGAGCTGGATGGAGGCGGATAAAGTGC
GGACCACTTCTGCGCTGCCCTTGGCTGGTTATTGCTGATAAAATCTGGAGCGTGGCTCGCGGTATATTGAGCTGACTGG
GGCCAGATGGTAAGCCCTCCGTTCTGAGTAACTGCAACAGGAGTCAGGCAACTATGGATGAAACAAATAGACAGATCGCTGAGATAGGTG
CTCACTGATTAAGCATTGGTAACCTGCAACCAAGTTACTCATATAACTTGTAGATTAAAACCTCATTTAAAGGATCTAGGTG
AAGATCCTTTGATAATCTCATGACAAAATCCCTAACGTGAGTTTCTGTTCACTGAGCGTCAGACCCCTAGAAAAGATCAAAGGATCTTCTT
GAGATCCTTTTCTGCGCTAATCTGCTGTTGCAAAACAAAAAACCCCGTACAGCGGTGGTTGTTGCCGGATCAAGAGCTACCAACTCT
TTTCCGAAGGTAACTGGCTCAGCAGAGCGCAGATAACCAAAACTGTTCTAGTGTAGCCGTAGTTAGGCACCCACTTCAGAAACTCTGAGCA
CCGCCTACATACCTGCTCTGTAATCCTGTTACCGTGGCTGCTGCCAGTGGGAGATAAGTCGTTGCTTACCGGGTGGACTCAAGACGATAGTTAC
CGGATAAGGGCGACGGCTGGCTGAACGGGGGGTCTGTCACACAGCCAGCTGGAGCGAACGACCTACACCGAACACTGAGATAACCTACAGCGTGA
GCTATGAGAAAGCGCCACGCCCTCCGAAGGGAGAAAGCGGAGACAGGTATCCGTAAGCGGAGGGTGGAAACAGGGAGCGCACGAGGGAGCTTCCA
GGGGAAACGCCCTGGTATCTTATAGTCTGTCGGGTTCGCCACCTCTGACTTGAGCGTCGATTTGTGATGCTCGTCAGGGGGCGGAGCCTAT
GGAAAAACGCCAGAACCGGCCCTTTACGGTTCTGGCTTTGCTGGCCTTGTGTCACATGTTCTGCGTTACCGTCAAGGAGGAGCTTCCA
TAACCGTATTACCGCTTGTAGTGAGCTGATACCGCTGCCGAGCCGAACGACCGAGCGCAGCAGTGAGCGAGGAAGCGGAAGA

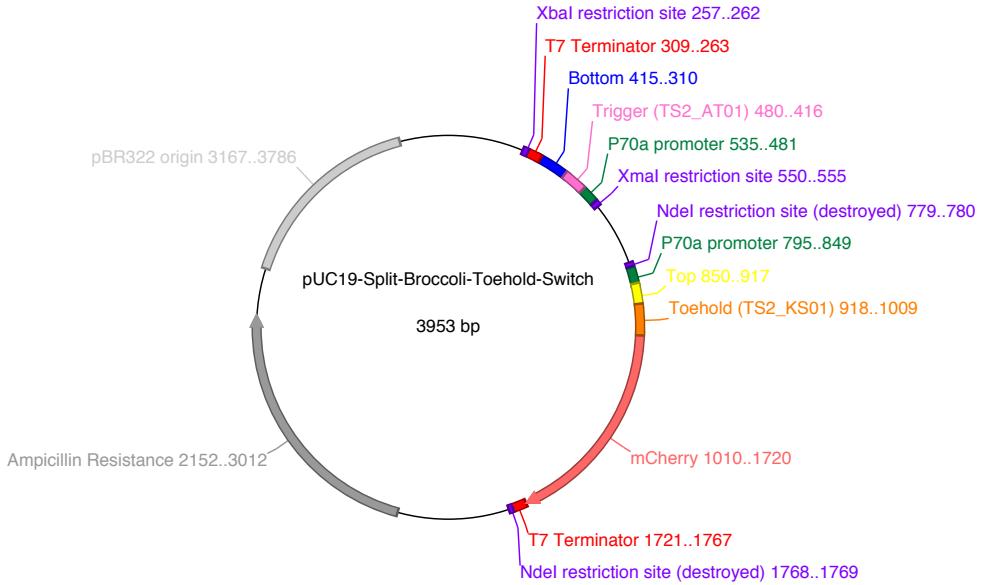


>pUC19-P70a-Top-T~P70a-Bottom-T (Addgene ID 87314)
TCTCCGCTTCCTCGCTCACTGACTCGCTGGCTCGGTGTTGGCTGGCGAGCGGTATCAGCTCACTCAAAGCGGTAAACGGTTATCCACAG
AACAGGGATAACCGAGGAAGAACATGTGAGCAAAGGCCAGCAAAGCGTAAAAGGCCAGGACTAAAGGCCTTGCTGGCTTTCCATAGGCT
CCGCCCTGAGAGCATCACAAATCGACGCTCAAGTCAGAGGTGGCGAACCCGACAGGACTATAAGATAACCAGCGTTCCCCCTGGAAGC
TCCCTCGTGCCTCTCTGTCCGACCTGCCGTTACCGGATACCTGTCGCCCTTCTCCCTCGGAAGCGTGGCGCTTCTCATAGCTCACGCT
GTAGGTATCTCAGTTCGGTAGGTGTTGCTCCAAGCTGGGCTGTGTCACGAACCCCCGTCAGCCGACCGCTGCGCCTATCCGTAACTA
TCGCTTGAGTCCAACCGGTAAAGCACGACTTATGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCCTGCTACA
GAGTTCTGAAGTGGCTCAACTACGGCTACACTAGAAGAACAGTATTGGTATCTGGCTCTGCTGAAGCAGCTACCTTCGGAAAAAGAGTTG
GTAGCTCTGATCCGCAAACAAACACCCTGGTAGCGGTGGTTTTGTTGCAAGCAGCAGATTACGCGAGAAAAAAAGGATCTCAAGAAGA
TCCCTGATCTTCTACGGGTCTACGCTCAGTGGAAACGAAACACTACGTTAAGGGATTGGTATGAGATTATCAAAAGGATCTTACCTAG
ATCTTTAAATTAAAATGAAGTTTAAATCAATCTAAAGTATATAAGTAAACTTGTCTGACAGTACCGAATGCTTAATCAGTGAGGACCTA
TCTAGCGATCTGCTATTCGTTCATCCATAGTGCCTGACTCCCCGTCGTGAGATAACTACGATAACGGGAGGGCTTACCATCTGGCCCAGTGC
TGCAATGATACCGCAGACCCACGCTCACCGCTCCAGATTACGCAATAAACACAGCCAGCCGAAGGGCCGAGCGCAGAAGTGGCCTGCAACT
TTATCCGCTCATCCAGTCATTAATTGGTGCCTGGAGCTAGAGTAAGTAGTCTGCGCAGTTAATAGTTGCGAACGTTGTCATTGCTACAG
GCATCGTGGTGTACGCTCGTGTGGTATGGCTCATTCAGCTCCGGTCCACGATCAAGGCAGTTACATGATCCCCATGTTGCAAAAAA
AGCGGTTAGCTCTCGGTCTCGATCGTCAAAGTAAGTGGCCGAGCTTACACTATGGTATGCGCAGCTGCATAATTCTCTTACT
GTCATGCCATCCGTAAGATGCTTTCTGACTGGTGAAGTCAACAAAGTCATTGAGAATAGTGTATGCCGAGCTGCTCTGGCG
CGTCAATACGGATAATACCGGCCACATAGCAGAACTTAAAGTGTCTCATTTGAAAACGTTCTCGGGCGAAAACCTCAAGGATCTTACC
GCTGTTGAGATCCAGTCAGTGTAAACCACTCGCACCCAACGATCTCAGCATCTTTACTTCACAGCGTTCTGGTGAGAAAAACAGGA
AGGCAAATGCCAAAAAGGAAAGGGCACAGGAATGTTAACTCATACTCTCCCTTTCAATATTATTGAAGCATTACGGGTT
ATTGTCATGAGCGGACATATTGAATGTATTAGAAAATAAACAAATAGGGGTTCCGCGACATTCCCGAAAAGTGCACCTGAGCTA
AGAACCAATTATTCATGACATTAACCTATAAAATAGGCGTATCACGAGGCCCTTCGCTCGCGTTCCGTGATGACGGTGAAACCTCTGA
CACATCGAGCTCCCGGAGACGGTCACAGCTGTGTAAGCGGAGCAGACAAGCCGTCAGGGCGCTCAGGGGGTGTGGGGGTGTC
GGGGCTGGCTTAACATGCGCATCAGAGCAGATTGACTGAGAGTCACATCGTCAAGGATCTAGCAGTAAACCGTGGTGT
GACAATTTACCTCGCGGTGATAATGGTGAGGATGATGGAGACGGTCGGGTCAGGATATTCATGCCAAGAGACGGTGGGTCCAGATGATG
CGGATCAAAGCCCCGAAAGCGGGCTTTTTAAGCTTGCATGCCGAGGTCAGCTACTAGAGGATCCCCGGTACCGAGCTCGAATTCACTGGC
CGTCTTACACGCTGACTGGAAAACCCCTGGCTTACCCAACCTAAATGCCCTGAGCACATCCCCCTTCGCCAGTGGCTAATAGCGAA
GAGGCCCGCACCGATGCCCTCCAAACAGTTGCGCAGCCTGAATGGCAATGGCCCTGATGCCGTATTTCTCCTTACGATCTGCGGTATTT
CACACCGCATATGTGAGCTAACACCGTGCCTGTTGACAATTACCTCTGGCGGTGATAATGGTGTGAGGATCCGCATCATCTGTCGAGTAGTGT
GGCTCTGCCATGTGTATGTGGGTCACCCACATCTGATGATCTGTCAGTAGAGTGTGGCTCCATCATCCTAGCATAACCCCTGGGGCC
TCTAAACGGGCTTGAGGGGTTTTGTGCGTAATCATGGTCAAGCTGGGTGCTTAATGAGTGAACCTACCATTAATTGCGTTGCGCTACTGCCGTTCCAGTCGGGAAAC
AGCCGGAAGCATAAAAGTGTAAAGCCTGGGTGCTTAATGAGTGAACCTACCATTAATTGCGTTGCGCTACTGCCGTTCCAGTCGGGAAAC
CTGCTGCGCAGCTCATTAATGAGTGAACCTGGCAACGCGGGGAGAGCGGTTGCGTATTGGCGC



>pUC19-P70a-Top-T~Bottom-T (Addgene ID 87315)

```
TCTCCGCTCCTCGTCACTGACTCGTGCCTCGCTCGGGTACAGCTCACTCAAAGCGGTAAACGGTTATCCACAG
AATCAGGGGATAACGCAGGAAAAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAGGCCGCTTGCTGGCTTTCCATAGGCT
CCGCCCCCTGACGAGCATCACAAATCGACGCTCAAGTCAGAGTGGCACCAGGACTATAAGATACCAGCGTTCCCCCTGGAAGCT
TCCCTCGCCTCTCGTCCGACCTGCGCTTACCGATACTGTCCGCCTTCTCCCTCGGAAGCGTGGCCTTCTCATAGCTCACGCT
GTAGGTATCTCAGTCGGTAGGTCTCGTCAAGCTGGGCTGTGCAACGAAACCCCCGTCAGCCGACCGCTGCCCTATCCGTAACCTA
TCGGTCTGAGTCCAACCCGTAAGACACGACTTATCGCAGTGGCAGCAGCAGCTGGTAACAGGATTAGCAGAGCGAGGTGTAGGCGGTCTACA
GAGTCTTGAAGTGGCTCAACTACGGCTACATAGAAGAACAGTATTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTCGGAAAAGAGTTG
GTAGCTCTGATCCGCAAACAAACACCAGCTGGTAGCGGTGGTTTTGTTGCAAGCAGCAGATTACCGCAGAAAAAAAGGATCTAAAGAAGA
TCCTTGATCTTCTACGGGTCTGACGCTCAGTGGAAACAACTACGTTAACGGATTGGTATGAGATTATCAAAGGATCTCACCTAG
ATCCTTTAAATTAAAGTTAAATCAATCTAAAGTATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTA
TCTAGCGATCTGCTATTCGTTCATCCATAGTGCCTGACTCCCCGCTGTAGATAACTACGATAACGGAGGGCTTACCATCTGGCCCGAGTGC
TGCATGATACCGCAGACCCACGCTCACCGGCTCCAGATTATCAGAATAAACAGCCAGGGCAGAGGCGCAGAAGTGGCTCTGCAACT
TTATCCGCTCCATCCAGTCTTAAATTGGTGGGGAGCTAGAGTAAAGTGGCTCTGCTGAACGTTACGTTGGCCTTGGCCTTGGCATTGCTCACAG
GCATCGTGGTGTACCGCTCGTGGTGTGGCTTACGCTCCGGTCCACGATCAAGGGAGGTACATGATCCCCCATGTTGCAAAA
AGCGGTTAGCTCTCGGTCTCCGATGTTGTCAGAGTAAGTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTACT
GTCATGCCATCGTAAGATGCTTCTGTGACTGGTAGTACTCAACCAAGTCATCTGAGAATAGTGTATGGCGGACCGAGTTGCTCTGCCGG
CGTCAATACGGATAATACCGGCCACATAGCAGAACTTAAAGTCTCATCTGGAAAACGTTCTCGGGGCAAAACTCTCAAGGATCTTACC
GCTGGTAGATCCAGTCGATGTAACCACTCGTCACCAACTGATCTCAGCATCTTACTTCACAGCGTTCTGGGAGCAAAACAGGA
AGCCAAAATCGCAGAAAAGGGATAAGGGCAGACCGGAATGTTGAATACTCACTCTCCCTTTCAATATTGAAGCATTATCAGGGTT
ATTGTCTCATGAGCGGATACATATTGAATGTTAGAAAATAACAAATAAGGGGTTCCGCGCACATTCCCGGAAAGTGCACCTGAGCTCTA
AGAACCATATTATCATGACATTAACCTATAAAAGTGGCTATCAGGCCCCCTTCGCTCGCGCTTCCGGTATGACGGTGAAACCTCTGA
CACATGCAGCTCCGGAGACGGTACAGCTTGTCTGTAAGCGGATGCCGGAGCAGACAAGCCGTCAGGGCGCTCAGGGGTTGGCGGTGTC
GGGGCTGGCTTAACATATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCAATCGTCTAGAAGATCCCGCAGATTGAGCTAACACCGTGCAGT
GACAATTTCACCTGGCGGTGATAATGGTGCAAGATGAGACGGTGGGTCAGGATCATTCATGGCAAGAGACGGTGGGTCAGATGAT
CGGATCAAAGCCCCGAAAGGGGGCTTTTTTAACTGCTGAGCTGGAAAACCCCTGGCTTACCCAACTTAATGCCCTTGCGAGCACATCCCCTTGCAGCTGAATTCACTGGC
CGTCTTACAACGCTGTGACTGGAAAACCCCTGGCTTACCCAACTTAATGCCCTTGCGAGCACATCCCCTTGCAGCTGGCTGAATACCGAA
GAGGCCCGACCGATGCCCTTCCCAACAGTGGCGCAGCTGAATGCCCTGAGTGGCTTACGCACTGTGCGGTATTTCTCCCTACGCACTGTGCGGTATTT
CACACCGCATATGGATCCGCACTGTGAGTGGAGTGTGGGCTCTGGCATGTGTATGTGGGCAACCCACATACTCTGATGATCTGAG
TAGAGTGTGGGCTCATCATCCAGTCAACCCCTGGGCTCTAAACGGGTTTTTGAGGGCTTGGCTAATCATGGTATGAGCTAACACTCACA
TGTGTGAAATTGTTATCCGCTCACAATTCCACACAACTACGAGCGGAAGCATAAAAGTGTAAAGCCTGGGTCGCTAATGAGTGGCTAAC
TTAATTGCGTTGCGCTACTGCCGTTCCAGTCGGAAACCTGTCGTCAGCTGCATTAAATGAATCGGCCAACCGCAGGGAGAGGGCTTGC
GTATTGGCGC
```

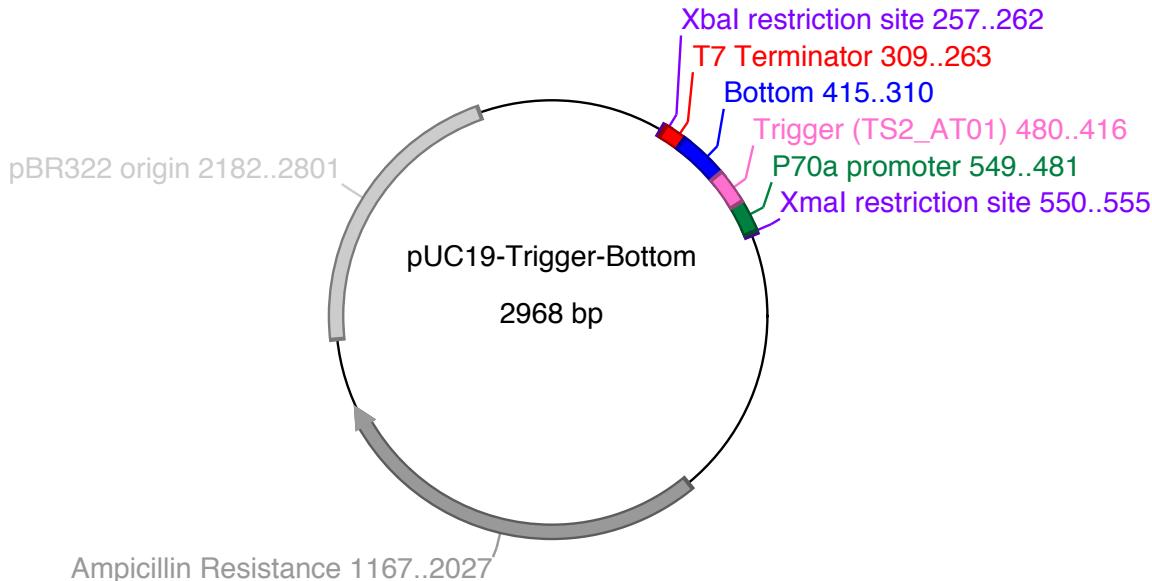


>pUC19-Split-Broccoli-Toehold-Switch (Addgene ID 87316)

```

GCGCCAATACGCAAACCGCCTCTCCCGCGTGGCGATTCAATTAGCAGCTGCACAGAGTTCCCGACTGAAAGCGGGCAGTGAGCGC
AACGCAATTATGTGAGTTAGCTCACTATTAGCACCCAGGCTTACACTTTATGCTCCGGCTCGATGTGTGGAATTGTGAGCGGATAAC
AATTACACAGAACAGCTATGACCATGATTACGCCAGCTTGCGATGCCAGTCAC TCTAGACAAAAAACCCCTCAAGACCGTTAGAGG
CCCCAAGGGGTTATGCTAGGATGATGGAGCCCACACTCTACTCGACAGGATCATCAGACTATGGGGTTGACCCACATACACATGGCAAGACCCAC
ACTCTACTCGACAGATGATGGGATCCCTATCTTATCTTATCTATCTCGTTATCCCTGCTTACTGACTATTGACACAGAATAGTCAGTC TGCAA
CCATTATCACCGCCAGAGTAAAATTGTCAACACGACGGTGTAGCTCAATTCCGGGATGCCCGGGTACCGAGCTGAATTCACTGCCGTCG
TTTACAACGCTGACTGGAAACCCCTGGCTTACCAACTTAATGCCCTTGCAAGCACATCCCCCTTCCGCAAGCTGGCTAATAGCGAAAGAGGC
CCCCACCGATGCCCTTCCAAACAGTTCGCCAGCTGAATGGCGAATGGCGCTGATCTGGTGTATTTCTCTTACGCACTGTGCGGTATTTACAC
CGCAGCATCCCGAATTGAGCTAACACCGTGCCTGACAATTTCGCGGTGATAATGGTGCAGGATGATGGAGACGGTCCGGTCCA
GGATCATTCATGGCAAGAGACGGTCCAGATGATGGGATCCCTGCTTATCTTATCTGTTATCCCTGCATACAGAAACAGAGGAGA
TATGCAATGATAAACGAGAACCTGGCGCAGCGCAAAGATGGTGAAGGGAGGAGATAACATGCCATCATCAAGGAGTTATGCCCTCA
AGGTTCACATGGAGGGCTCCGTGAACGGCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCTACAGGGCACCCAGGCCAACGTGAA
GGTAGCCAAGGGTGGCCCCCTGCCCTGCCCTGGGACATCCTGCCCCCTAGTTCAATGTCAGGGCTCCAAGGCCAACGTGAAGCACCCGCCACATC
CCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAGTGGGAGCCCTGATGAACCTTCAGGGACGGCGCGTGGTACCCAGGACTCCT
CCCTGCAAGACGGCAGGTTACATACAAGGTGAAGCTGGCGCCACCAACTTCCCTCCGACGGCCCTGAATGCAAGAAAGACTATGGCTGGGA
GGCCTCTCCCGAGGGATGTACCCCGAGGACGGCGCCTGAAGGGGAGATAACAGGGCTGAAGCTGAAGGACGGCCACTACGACGCTGAG
GTCAAGACCACTACAAGGCCAAGAACCCCTGCACTCCCCGGCGCTACACGTCACATCAAGTTGGACATCACCTCCACAACGAGGACTACA
CCATCGTGGAACACTACGAACCGCCAGGGCGCCACTCCACCGGGCATGGACGACTGTACAAGTAATAGCATAACCCCTGGGCTCTAAA
CGGGTCTTGAGGGTTTTGTGGTCACTCTCAGTACAATCTGCTGATGCCGATAGTTAACGCCAGCCCCGACACCCGCAACACCCGCTGACG
CGCCCTGACGGGCTGTCTGCTCCCGCATCCGCTTACAGACAAGCTGTGACCGCTCTCCGGAGCTGATGTGTCAGAGGTTTCAACGCTCATCACC
GAAACGCGCAGACGAAAGGGCCTGTGATACGCCATTTTTATAGGTTATGTGATGATAATAATGGTTCTAGACGTCAGGTGGCACTTTCTGG
GGAATGTGCCGGAACCCCATTGTTTATTTCTAAATACATTCAAAATGATGATCCGCTCATGAGACAATACCCGATAATAGCTTCAATAAT
ATTGAAAGAGAGTATGAGTATTCAACATTCCGTTCTTCCGGCATTTGCGGCTATTGCGCTTCTGCTCAGGGTTCACCCAGAAC
GCTGGTGAAGAGTAAAGATGCTGAAGATCAGTGGTGCAGGTGGGTTACATCGAAGCTGATCTCAACAGGGTAAGATCTTGAGAGTTTCGC
CCCGAAGAACGTTTCCAATGATGAGCACCTTTAAAGTCTGCTATGCGCGGTATTACCGCTATTGCGCTTACAGGGCAGGAAAGAGCAACTGGCTGCC
GCATACACTATTCTCAGAATGACTGGTGAGTACTCACAGTCACAGAAAGCATCTACGGATGGCATGACAGTAAGAGAATTATGCACTGCTGC
CATAACCATGAGTATAACACTGCGCCAACCTACTTGACAAACGATCGGAGGACGAAGGAGCTAACGCTTTTGACAAACATGGGGATCAT
GTAACCTGCCCTGATCGTGGGAACGGAGCTGAATGAAGCCATACCAACGACGAGCGTGCACACCACGATGCTGTAGCAATGGCAACACGTTGC
GCAAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCGGCAACAAATTAAATAGACTGGATGGAGGCGATAAAGTGCAGGACCAACTTCTGCC
GGCCCTTCCGGCTGGTTATTGCTGATAAAATCTGGAGGCCGGTGAAGCTGGGTCTCGCGGTATTACGGACTGGGAGATGGTAAGGCC
TCCCTATCGTAGTTATCTACAGCGAGGAGTCAGGCAACTATGGATGACAAAGTAATAGACGATCTGCTGAGATAGGTGCTACTGATTAAGCATT
GGTAACCTGCACTGAGGAGTTACTCATATACTTTAGATTGATTAAAACCTTACATTAAAGGATCTAGGTGAAGATCCTTTTGATCAA
TCTCATGACCAAAATCCCTAACGTTGAGTTCTGCTTCACTGAGCGTCAAGACCCCTAGAAAAGATCAAAGGATCTTCTGAGATCCTTTCTG
CGCGTAACTCTGCTGCTGCAACAAAAAACACCACCGTACCGCGGTGGTTGCTGCGGATCAAGAGCTACCAACTCTTCCGAAGGTAACG
GCTTCAGCAGAGCGCAGATAACAAATACTGTTCTCTAGTGTAGCCAGTTAGGCCACCTCAAGAAACTCTGCTAGCACCCCTACACCTCGC
TCTGCTAATCTGTTACCGAGTGGCTGCGATAAGTGTGCTTACCGGTTGGACTCAAGACGATAAGTTACCGGATAAGGCCAGCGG
TCGGGCTGAACGGGGGGTTCGTGACACAGGCCAGCTGGAGCGAAGCAGCTACCGAAGCTGAGGATACCTACAGCGTGAAGCTATGAGAAAGGCCA
CGCTTCCCGAAGGGAGAAAGGGAGACAGGTATCCGGTAAGCGGCAAGGGTGGGAACAGGAGAGCGCAGGAGGAGCTTCCAGGGAAACGCC
TCTTATAGTCTGCGGTTTCGCCCCCTCTGACTTGAGCGTCAAGTTGTGATGCTGTCAGGGGGCGGAGCTATGAAACACGCCAGCAAC
GGGGCTTTTACGGTCTGGCCTTGGCTCACATGTTCTGCTGTTACCCCTGATTCGTTGAGGATAACCGTATTACCGCCT
TTGAGTGAGCTGATAACCGCTCGCCGAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAAGCGGAAGAAGCGGAAGA

```

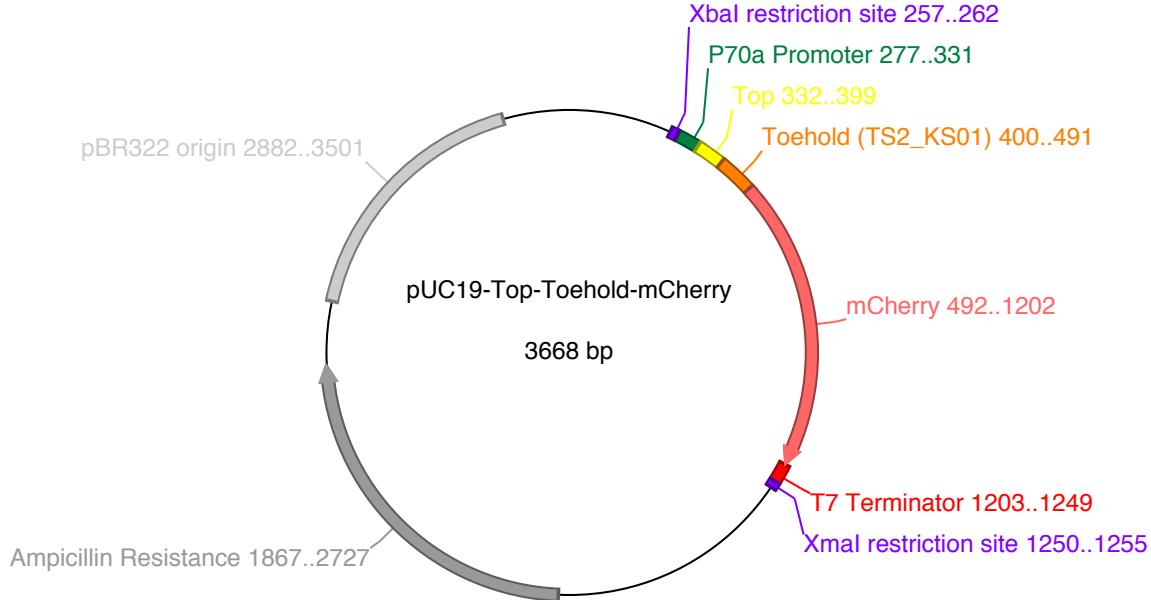


>pUC19-Trigger-Bottom (Addgene ID 87317)

```

GCGCCAATACGCAAACCGCTCTCCCGCGCTTGGCGATTCTAAATGCAGCTGGCACGACAGGTTCCGACTGGAAAGCGGGCAGTGAGCGC
AACGCAATTATGTGAGTTAGCTCACTTAGGCACCCAGGCTTACACTTTATGCTTCCGGCTGTATGTTGAGGGATAAC
AATTTCACACAGGAAACAGCTATGACCATGATTGCCAAGCTGAGCTGCAGGTGAC TCTAGA CAAAAAACCCCTCAAGACCCGTTAGAGG
CCCCAAGGGGTTATGCTAGGATGATGGAGCCCACACTACTCGACAGGATCATCAGAGTATGTTGAGGTTGACCCACATACACATGGCAAGAGCCCAC
ACTCTACTCGACAGATGTCGGATCCATCTTATCTATCTCGTTATCCCGTACTGACTATTGACAGAATAGTCAGTCCC TGCAA
CCATTATCACGCCAGAGGTAAGGTTAACTGTCACACAGCACGGTGTAGCTCAATTCCGGGATGCCCGGGTACCGAGCTGAATTCACTGCCGTG
TTTACAACGTCGTGACTGGAAAACCTGGCTTACCAACTTAATGCCCTTGCAAGCACATCCCCCTTCGCCAGCTGGCTAATAGCGAAGAGGC
CCGCACCGATGCCCTTCCAAACAGTTGCCAGCCTGAATGGCAATGGCCTGATGCCGTATTCCTCCTACGCATGTCGGTATTCACAC
CGCATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCCATAGTTAACGCCAGCCGCCAACACCGCTGACGCCCTGACGGCTT
GTCGCTCCGCATCCGCTACAGACAAGCTGTGACCGTCTCCGGAGCTGATGTCAGAGGTTTACCGTCATACCGAAACGCCGAGACG
AAAGGGCTCGTGTACGCCATTTTTATAGGTTATGTCATGATAATAGGTTCTTAGACGTCAGGGTACCTTGGGAAATGTCGCGGAA
ACCCATTGTTGTTAACTTCTAAATACATCCTAAATATGTCATGCCCTCATGAGACAATAACCCGTATAATGCTTCATAATATTGAAAAAGAAGA
GTATGAGTATTCAACATTCCGTGTCGCCCTTATTCCCTTTGCGGATTTGCCCTCTGTTGCTACCCAGAACCGCTGGTAAAGTAAA
AGATGCTGAAGATCAGTGGGTGACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAGATCCTGAGAGTTTCCGCCGAAGAACGTTT
CCAATGATGAGCACTTTAAAGTTGCTATGTCGGCGGTATTATCCGTATTGACGCCGGCAAGAGCAACTCGGTGCCGATACACTATTCTC
AGAATGACTTGGTTGAGTACTCACAGAAAGCATCTTACCGATGGCATGACAGTAAGAGAATTATGCACTGCTGCCATAACCAGTGA
TAACACTGCCGAAACTTACTCTGACAAACGATGGAGGACCGAAGGAGCTAACCGTTTGCACAAACATGGGGATCATGTAACTCGCCTGAT
CGTGGGAACCGGAGCTGAAGCCATACCAACGACGAGCGTGAACCCACGATGCCCTGATGCAATGCCAACCGTGGCCTGCAACTATTAG
GCGAACACTTACTCTAGCTCCCGCAACAAATTATAGACTGGATGGAGGCGGATAAAAGTGCAGGACACTCTGCGCTGCCCTCCGGCTGG
CTGGTTATTGCTGATAAAACTCTGAGCCGGTGGCTCGCGGTATCTGCGCAACTGGGGCAGATGGTAAGCCCTCCGTATGCTAGTT
ATCTACACGACGGGAGTCAGGCAACTATGGATGACGAAATAGACAGATGCTGAGATAGGTGCCACTGATTAAGCATGGTAACTGTCAGACC
AAGTTACTCATATATACTTAGATTAAAATCTTAAATTAAAAGATCTAGGTGAAGATCCTTTGATAATCTCATGACCAAAT
CCCTAACGTGAGTTCTGTCGACTGAGCGTCAGACCCGTAGAAAGATCAAAGGATCTTGAGATCCTTTCTGCGCTAATCTGCTGC
TTGCAACAAACAAACACCACCGCTACCGAGCGTGGTTGTTGCCGATCAAGAGCTACCAACTCTGAGACCCGCTACATACCTGCTCTGCTAATCCTGTT
AGATACCAAATACTGTTCTAGTGTAGCGTAGTTAGGCCACCCACTCAAGAAGCTACCGTACAGACGATAGTTACCGGATAAGGGCAGGGTGGGCTGAACGGGG
ACCACTGGCTGCTGCCAGTGGCATAACTCTGTTACCGGGTTGGAACGACTCACCGAAGTACAGCGTGAAGCTATGAGAAAGCGCCACGCTCCGAAGGGA
GGTTGCGCACAGCCCAGCTGGAGCGAACGACCTACACCGAAGTACAGCGTGAAGCTATGAGAAAGCGCCACGCTCCGAAGGGAACCGCTGGTATCTTATAGTCTGT
GAAAGGGCGGACAGGTATCCGTAAGCGGCAAGGGTGGAACAGGAGAGCGCACGAGGGAGCTTCAAGGGGAAACGCCCTGGTATCTTATAGTCTGT
CGGGTTTCCGCACTCTGACTTGAGCGTCGATTGTTGATGCTCGTCAAGGGGGCGGAGCTATGAAAGAACGCCAGCAACGCCCTTTTACGG
TTCTGGCCTTTGCTGCCCTTGTACATGTTCTCTGCTGATTCTGCTGAGTACCGTGGATAACCGTATTACCGCCTTGAGTGAAGCTGATA
CCGCTGCCGAGCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGA

```



>pUC19-Top-Toehold-mCherry (Addgene ID 87318)
 GCGCCAATACGCAACCGCTCTCCCGCGTTGGCGATTCAATTAGCAGCTGGCACGACAGGTTCCGACTGGAAAGCGGGCAGTGAGCGC
 AACGCAATTATGTGAGTTAGCTCACTATTAGCACCCCAGGCTTACACTTTATGCTTCGGCTCGATGTGTGGAATTGTGAGCGGATAAC
 AATTCACACAGAACAGCTATGACCATGATTACGCCAAGCTTGATGCCAGGTCAC **TCTAGA** CGATCCCGCAGATTGAGCTAACACCGTG
 CGTGTGACAATTTCACCTCTGGCGTGTATAATGGT~~CCA~~**GGATGATGGAGACGGT** CGGGTCCAGGATCATTATGGCAAGAGACGGTGGGTCCAG
**ATGATGCGGATCCCCTGCTTATCTATCTATCTGTTATCCCTGATACAGAAAACAGGAGGATATGCAATGATAAAACGGAAACCTGGCGGCAGCG
 CAAAGAGTGGTAGCAAGGGCAGGGAGGATAACATGGCCATCATCAAGGGAGTTCATGGCCTTCAGGTTCACATGGAGGGCTCGTAACGGCCACG
 AGTCGGAGATCGAGGGCGAGGGCGAGGGCGAGGGCCGGCCCTACAGGGCACCCAGGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTG
 GGACATCCCTGCCCCCTCAGTCATGTCAGGCTCAAAGCCTACGTGAAGCACCAGGCCACATCCCCGACTACTTGAAGCTGCTTCCCCAGGGC
 TTCAAGTGGGAGCGCGTGTAGAACTCGAGGACGGCGGGTGGTACCCGTCAGGACTCCTCCCTGCAAGACGGCGAGTCATCTACAAGGTGA
 AGCTGCGCGGACCAACTCCCTCGACGGCCCCGTAATGCAAGAAGACTATGGGCTGGGAGGCTCTCCGAGCGGATGTACCCGAGGACGG
 CGCCGTGAAGGGCGAGATCAAGCAGAGGCTGAAGGACGGCGGACTACAGACGCTGAGGTCAAGACCACCTACAAGCCAAGAACGCCGTG
 CAACGCGCCGGCGTACAACGTCACATCAAGTGGACATCACCTCCACAACGAGGACTACACCATCTGGAAACAGTACAGCAGCGCCAGGGC
 GCCACTCACCAGGGCGATGGACGCTGTACAAGTAAAGCATAACCCCTTGGGCTCTAAACGGGTTGGGCTTGGGGTTTTTGCCCCGGTACCGA
 GCTCGAATTACTGGCGCTGTTTACACGTCGTGACTGGAAACCCCTGGCGTACCCAACTAATGCCCTGCGACACATCCCCCTTCCGAG
 CTGGCGTAATAGCGAAGAGGCCGACCGATGCCCTCCAAACAGTGGCGCAGCCTGAATGGCGAATGCCCTGATGCCGTATGCCGTATTTCTCTTACG
 CATCTGTGCGGTATTCACACCGCATATGGTCACTCTCAGTACAATCTGCTCTGATGCCCATAGTTAACGCCAGCCCCGACACCGCCAACACCCG
 CTGACGCCCTGACGGCTGTCTGCCGACCGCTTACAGACAAGCTGTGACCGTCTCCGGAGCTGCATGTCAGAGGTTTACCGTC
 ATCACCGAACCGCGAGACGAAAGGGCTCGTGTACCGCTATTGTTATAGGTTAATGTCATGATAATAATGTTCTAGACGTCAGGGCACT
 TTTGGGAAATGCGCGAACCCCTATTGTTATTTCTAAACATTCAATGTCATGCGCTCATGAGACAATAACCTGATAATGCTTC
 AATAATATTGAAAAGGAAGAGTATGAGTATTCAACATTCCGTGTCGCCCTTATCCCTTTGCGGATTTCGCTTCTGTCACCC
 AGAACCGCTGGTAAAGTAAAAGATCTGAGATCACTGGGTGACAGTGGGTTACATGCAACTGGATCTCAACAGCGTAAGATCCTGAGAGT
 TTTGCCCGAAGAACGTTTCAATGATGAGCACTTTAAAGTTCTGCTATGTCAGGCGGTATTATCCGTTATTGACGCCGGCAAGAGCACTCG
 GTGCCGCAACTATTCTCAGAATGACTGGTGAGTACTCACCAGTCAGAAAAGCATCTACGGATGGCATGACAGTAAGAGAATTATGAG
 TGCTGCCATAACCATGAGTGTAAACACTGGGCCACTACTTCTGACAACGATCGGAGGACGAAGGAGCTAACGCTTTTGACACAACATGGGG
 GATCATGTAACCGCTTGATGTTGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTACACCGATGCCTGAGCAATGCCA
 CGTGGCGAACATTAAACTGGCAACTACTACTGCTTCCGGCAACAATTAAAGACTGGATGGAGGCGGATAAGTGCAGGACCACTTCT
 GCGCTCGGCCCTCCGGCTGGTTATGCTGATAATCTGGAGCCGGTGGCTGGTGGCTCGCGTATCTGGCAGCAGTGGGCCAGATGGT
 AAGCATTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAAGCAAATAGACAGATCGTGGAGATAGGTGCGCTACTGATTA
 AGCATTGGTAACGTGACCCAAGTTACTCATATATACATTAGTTAGTTAGTTAAACTCATTTAAAGGATCTAGGTGAAGATCTT
 TGATAATCTCATGACCAAAATCCCTAACGTGAGTTCTGTTCACTGAGCGTCAGACCCGTTAGAAAAGATCAAAGGATCTTCTGAGATCTT
 TTTCTGCGTAATCTGCTGTTGCAAACAAAAAACACCGCTACAGCGGTGGTTGTTGCCGATCAAGAGCTACCAACTCTTCCGAGG
 TAATGGCTTCAGCAGAGCGCAGATAACAAATACTGTTCTACTGTTAGTGTAGCCGTAGTTAGGCCACCACTTCAGAAGACTCTGTCAGCAGCGCTACATA
 CCTCGCTCTGCTAACTCTGTTACCACTGGCTGGCGATAAGTCGTGCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCG
 CAGCGGTGGCGTAACGGGGGGTCTGACAGGCCAGCTGGAGCGAACGCCAGTACACCGAAACTGAGATACCTACAGCGTGGAGCTGAGAAAA
 GCGCCACGCTTCCGAAGGGAGAAAGGCCGACAGGTACCCGTAAGGCCAGGGTGGAAACAGGAGAGGCCACCGGGAGCTTCCAGGGGAAACGCC
 CTGGTATCTTATAGTCTGCGGTTGCCACCTCTGACTTGAGCGTCGATTGATGCTGTCAGGGGGCGGAGCTATGGAAAACCGC
 AGCAACCGGGCTTTTACGGTCTGGCCTTGTGGCTACATGTTCTGCTTACGTTCTGCTGAGCTGAGCGAGTCAGTGAGCGAGGAAGCGGAAGA
 CCCTTGAGTGAAGCTGATACCCTGCCGAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGA**