Supporting Information

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

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Table S1. RNA sequences unique to this study.

	RNA Sequence (5' to 3')
SdB	GAGGGAGACGGUCGGGUCCAUCUGAGACGGUCGGGUCCAGAUAUUCGUAUCUGUCGAGUAGAGUGUGGGCUCAGAUGUCGAGUAGAGUGUGGGCUCCCCUC
3WJdB	GGACCCACAUACUCUGAUGAUCCGAGACGGUCGGGUCCAGAUAUUCGUAUCUGUCGAGUAGAGUGUGGGCUCGGAUCAUUCAUGGCAAGAGACGGUCGGGUCCAGAUAUUCGUAUCUGUCGAGUAGAGUGUGGGCUCUUGCCAUGUGUAUGUGGG
Top	GGAUGAUGGAGACGGUCGGGUCCAGGAUCAUUCAUGGCAAGAGACGGUCGGGUCCAGAUGAUGCGGAU
Bottom	GGAUCCGCAUCAUCUGUCGAGUAGAGUGUGGGCUCUUGCCAUGUGUAUGUGGGUCAACCCACAUACUCUGAUGAUCCUGUCGAGUAGAGUGUGGGCUCCAUCAUCC

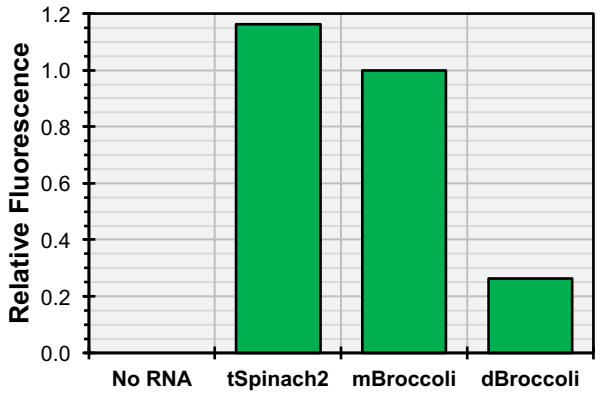


Figure S1. Comparison of *tSpinach2*, *mBroccoli* and *dBroccoli*. The Spinach2 aptamer embedded within a stabilizing $tRNA^{Lys}_3$ scaffold (*tSpinach2*) and the minimal monomeric Broccoli aptamer (*mBroccoli*) demonstrate similar levels of fluorescence when equimolar amounts of RNA are thermally renatured 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.

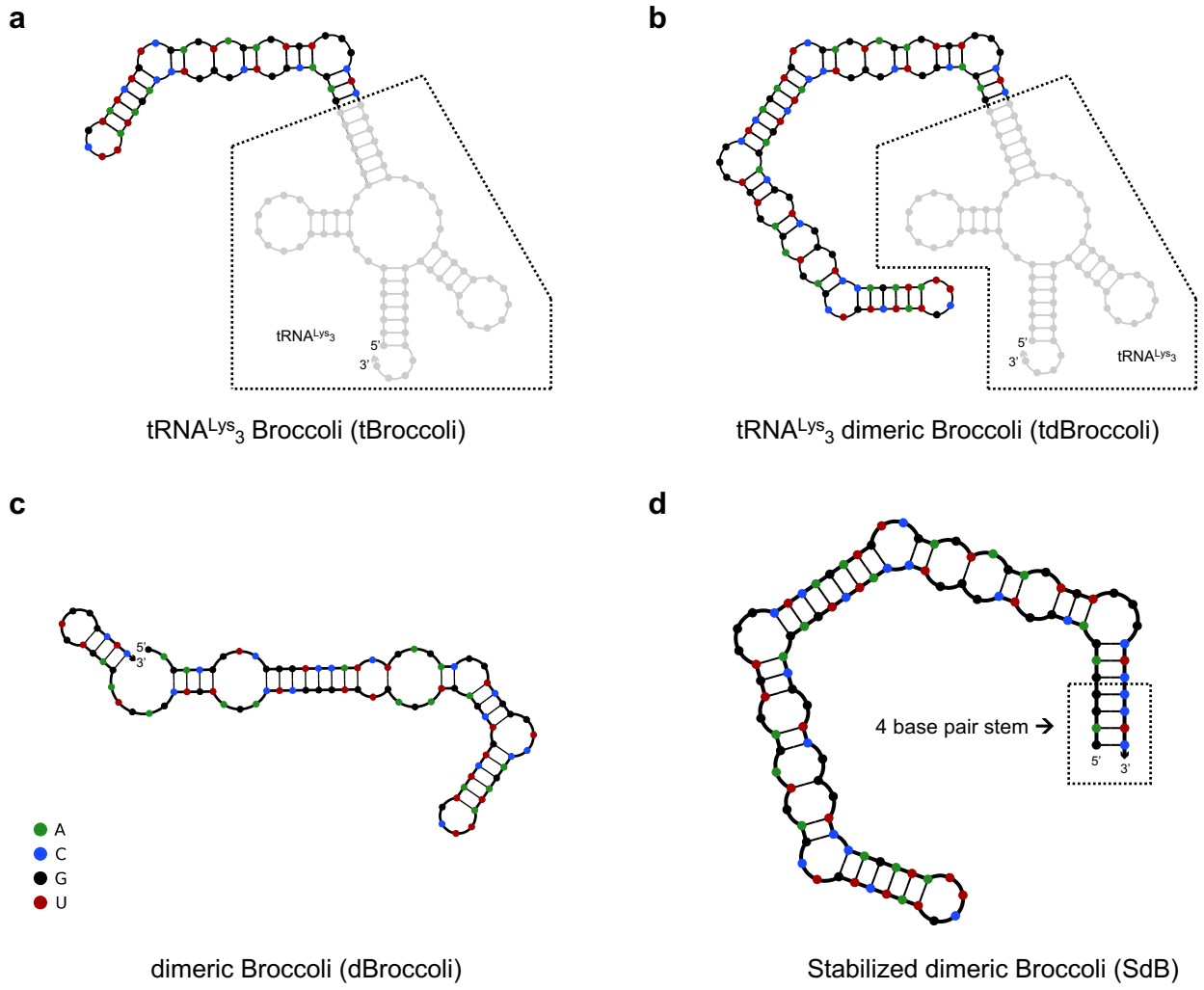


Figure S2. Predicted secondary structures of the Broccoli aptamer in the presence and absence of a tRNA stabilizing scaffold. (**a**) The Broccoli aptamer, when embedded within a $tRNA^{Lys}_3$ scaffold (*tBroccoli*), is predicted to form the functional fold of the Broccoli aptamer. (**b**) The dimeric 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.

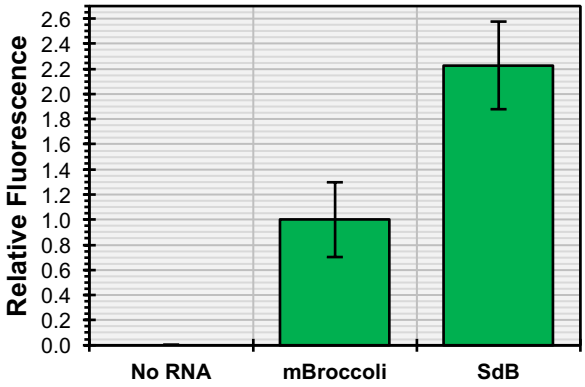


Figure S3. Stabilized dimeric Broccoli (*SdB*) is twice as bright as *mBroccoli*. *SdB* generates more than twice the signal of monomeric Broccoli (*mBroccoli*) when equimolar amounts of *mBroccoli* and *SdB* aptamers are thermally renatured in buffer containing dye and assayed for fluorescence. Mean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

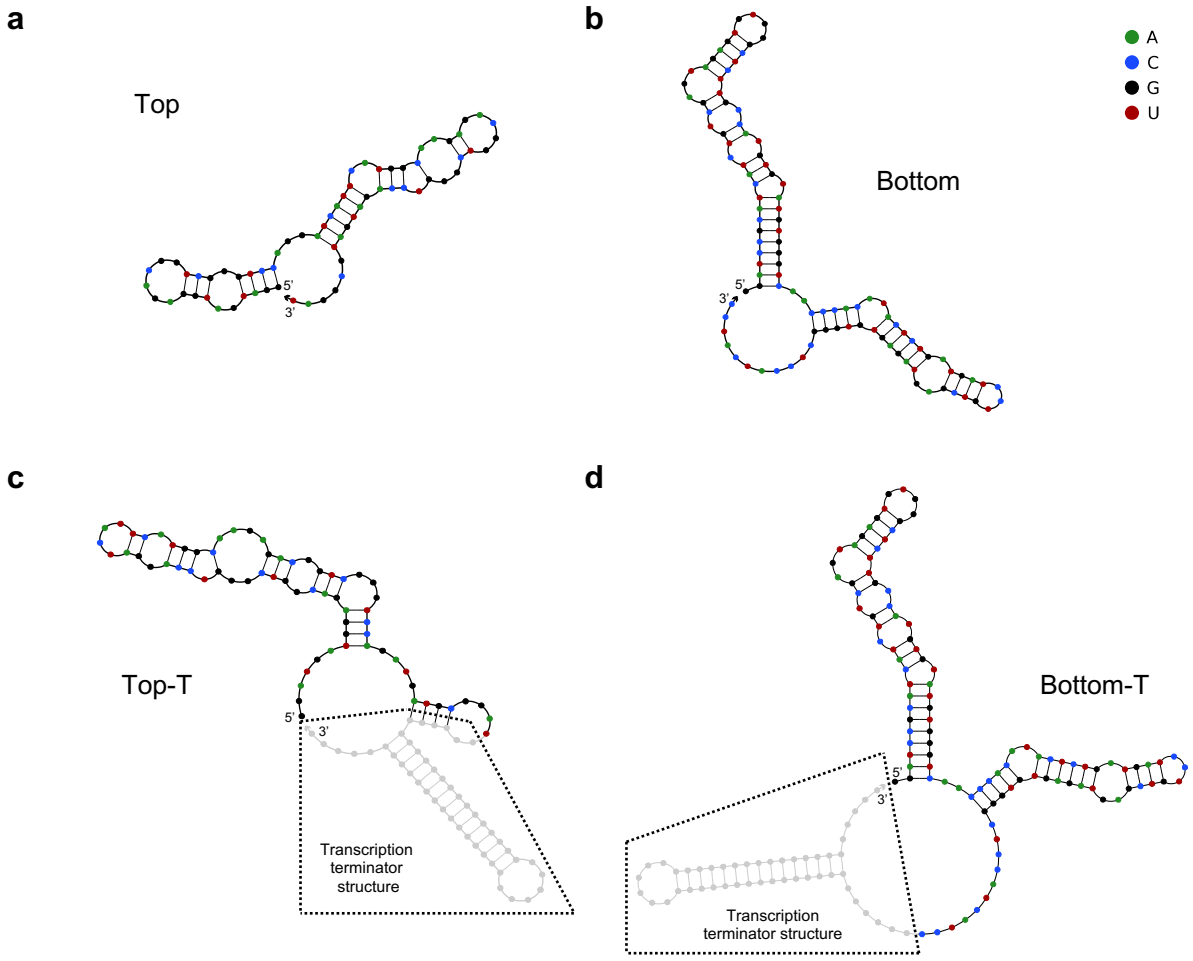


Figure S4. Predicted secondary structures of the Split-Broccoli system components, with and without transcription terminators. RNA corresponding to *Top* (**a**) and *Bottom* (**b**), without transcription terminator structures, and *Top-T* (**c**) and *Bottom-T* (**d**), which contain transcription 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.

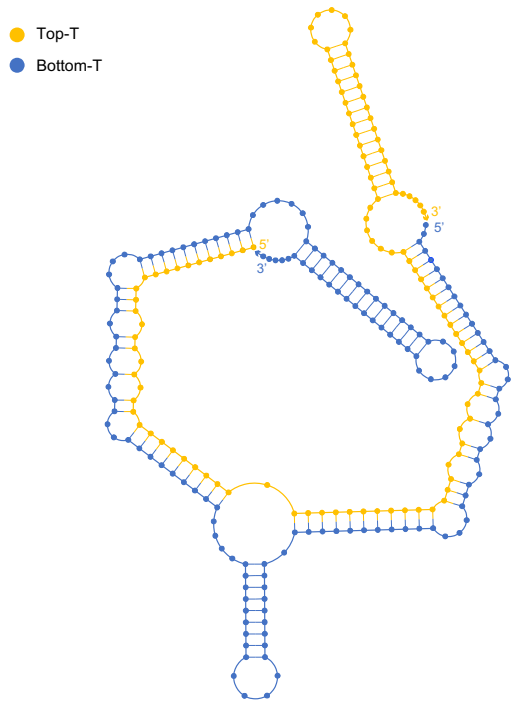


Figure S5. Predicted secondary structure of the hybridized Split-Broccoli system with transcription terminators. *Top-T* (yellow) and *Bottom-T* (blue) are predicted to hybridize into a secondary structure that maintains the expected motifs corresponding to the 3WJ, the Broccoli aptamers and the transcription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

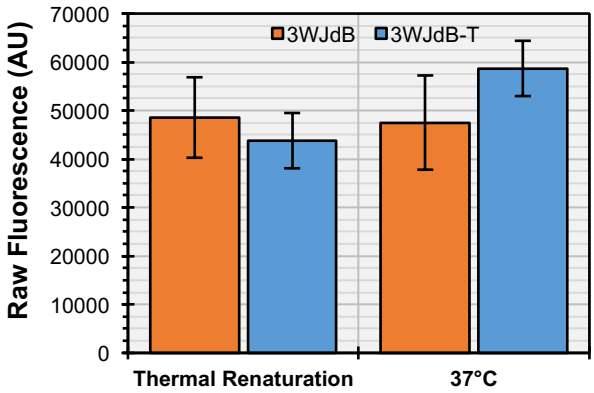


Figure S6. Comparison of raw fluorescence values for *3WJdB* with and without a transcription terminator, and with and without a thermal renaturation step. *3WJdB* without (orange columns) and with a transcription terminator structure (*3WJdB-T*, blue columns) demonstrate similar levels of fluorescence when assembled and measured (λ_{ex} = 472 nm, λ_{em} = 507 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.

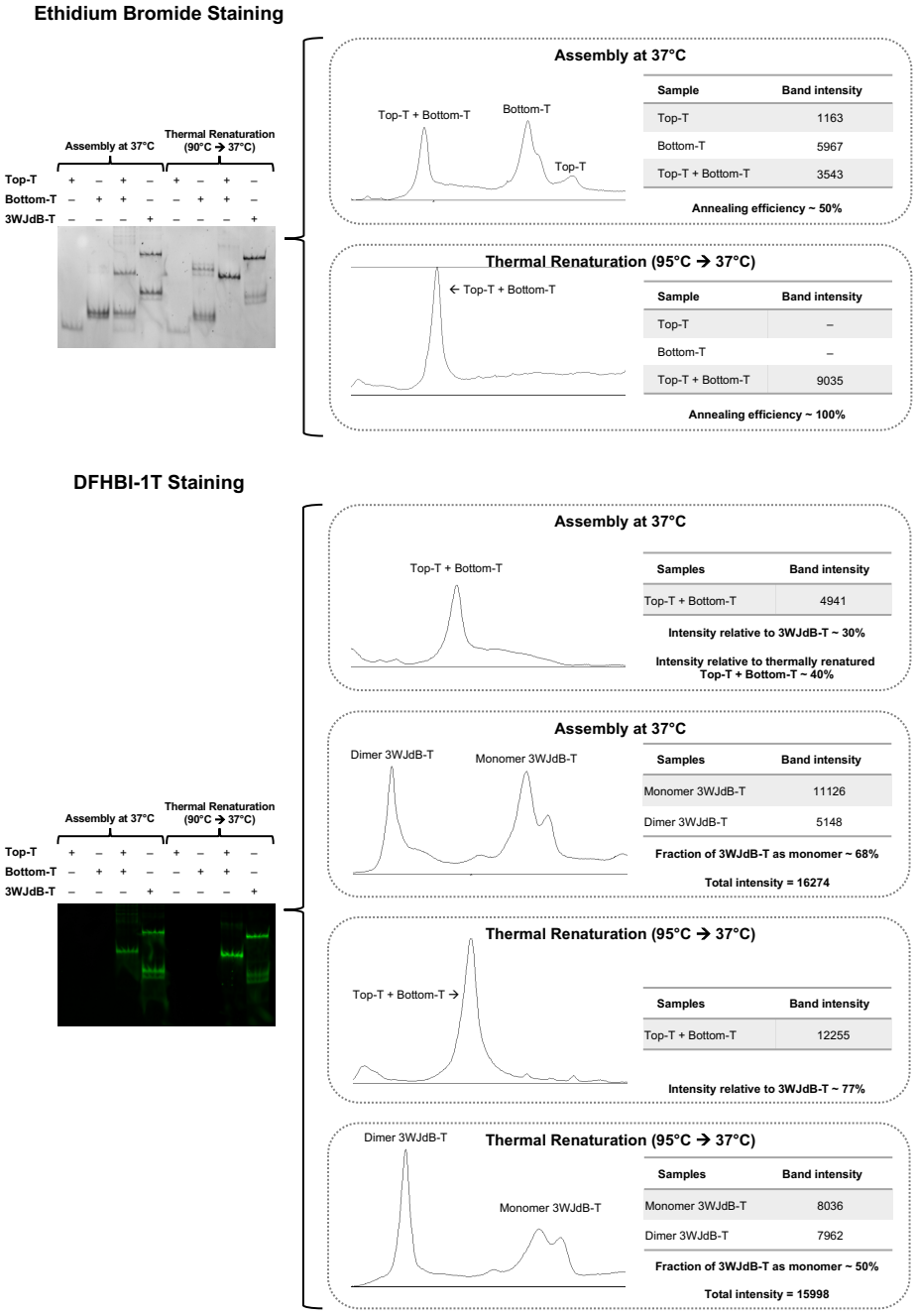


Figure S7. Densitometry analysis of the Split-Broccoli system with transcription terminators. Ethidium bromide staining identifies all molecular species (top), while DFHBI-1T staining (bottom) identifies only the functional, fluorescence-activating species. Thermal renaturation improves both annealing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

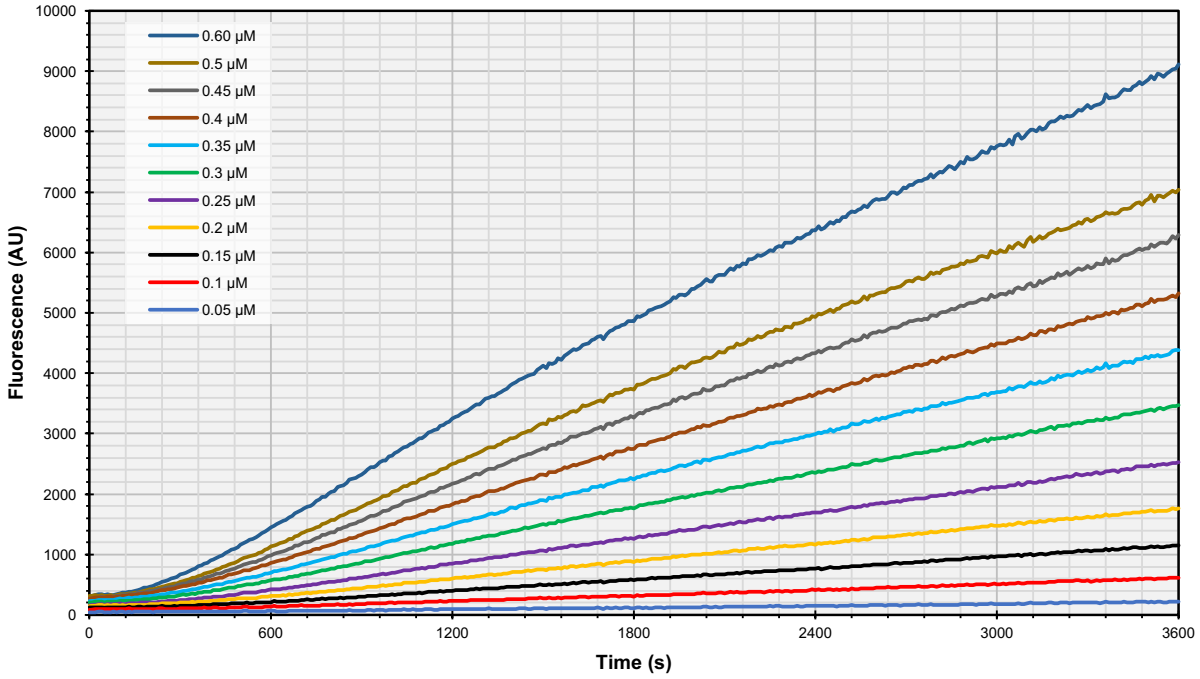


Figure S8. Fluorescence activation kinetics of the Split-Broccoli system, with transcription terminators, at varying concentrations. Functional assembly of equimolar amounts of *Top-T* and *Bottom-T* at 37°C was assayed at a range of concentrations. Rates from the linear region of each concentration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

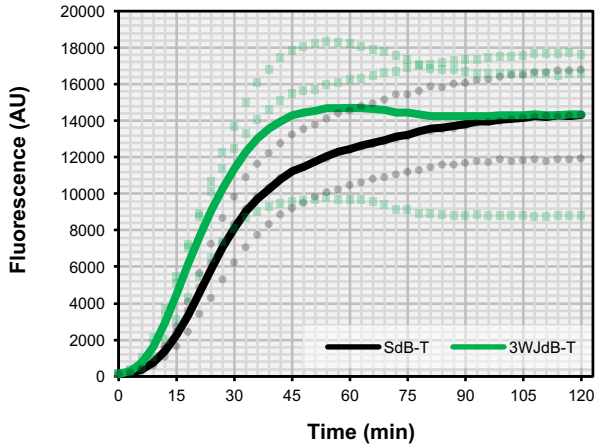


Figure S9. Addition of the 3WJ scaffold does not impede function of dimeric Broccoli when transcribed and assayed for activity *in vitro*. Transcriptional assays of *3WJdB-T* (green lines) and *SdB-T* (black lines) demonstrate that the addition of the 3WJ scaffold present in *3WJdB-T* does not slow the 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.

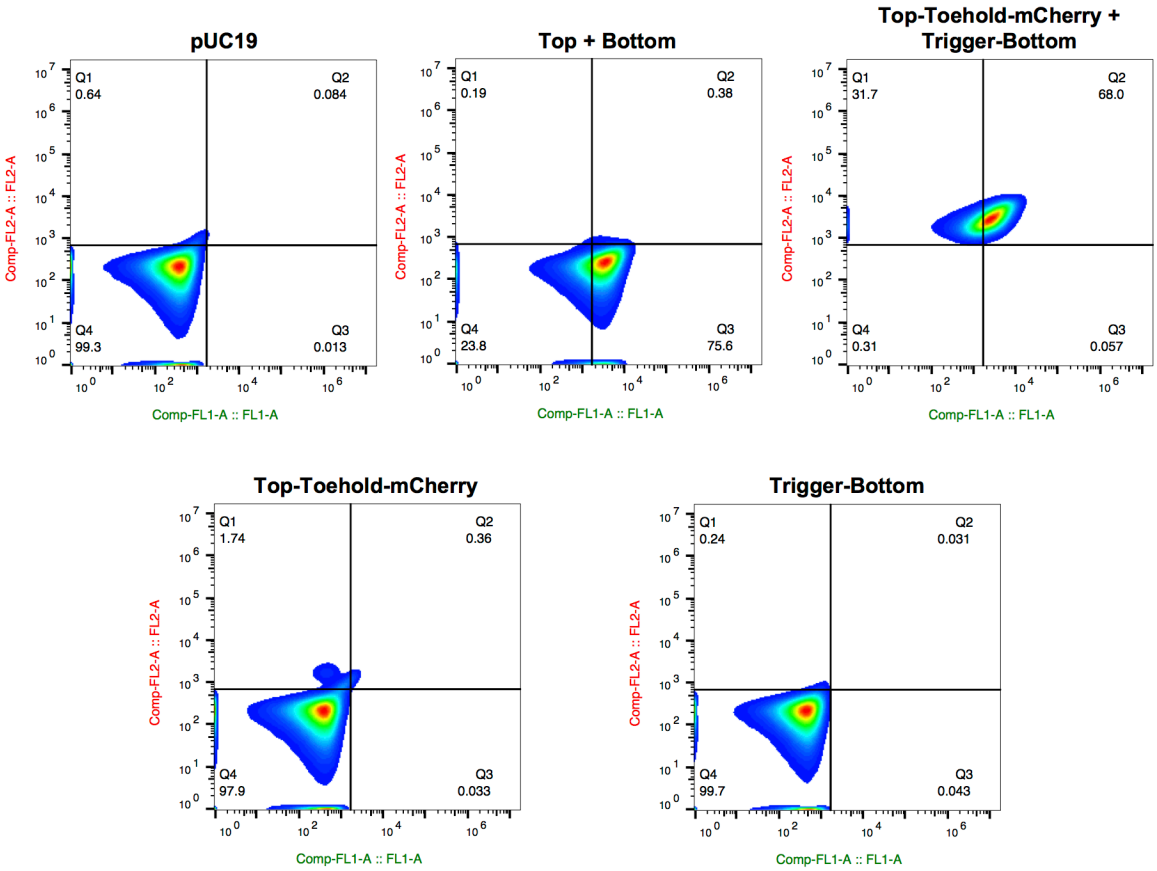
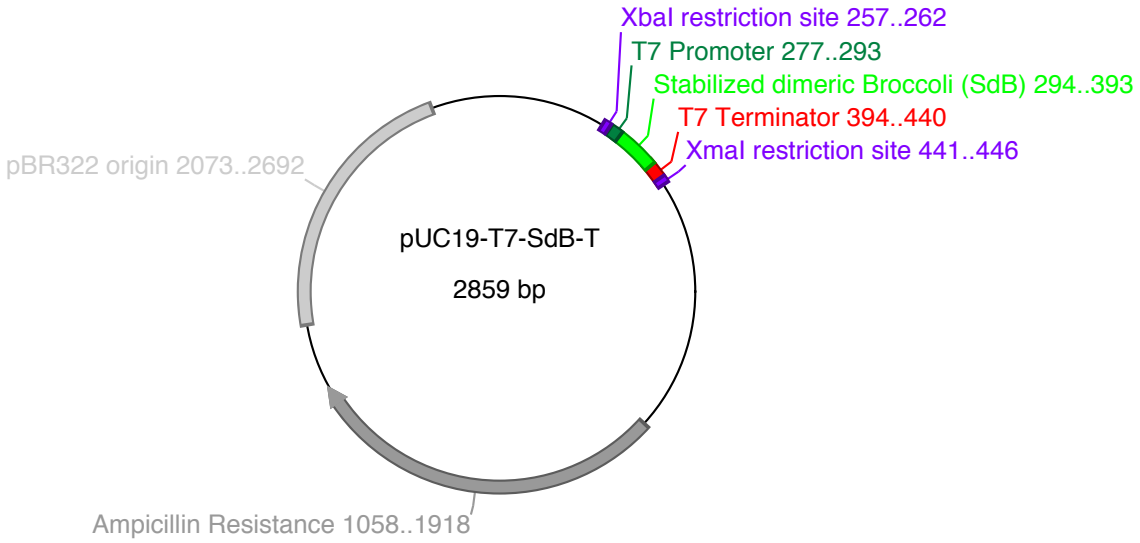


Figure S10. Representative scatter plots from flow cytometric analysis of the Split-Broccoli Toehold Switch demonstrate hybridization and activation of Split-Broccoli and the Toehold switch. *E. coli* containing a plasmid encoding the Split-Broccoli system fused to an RNA toehold switch (*Top-Toehold-mCherry* + *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.



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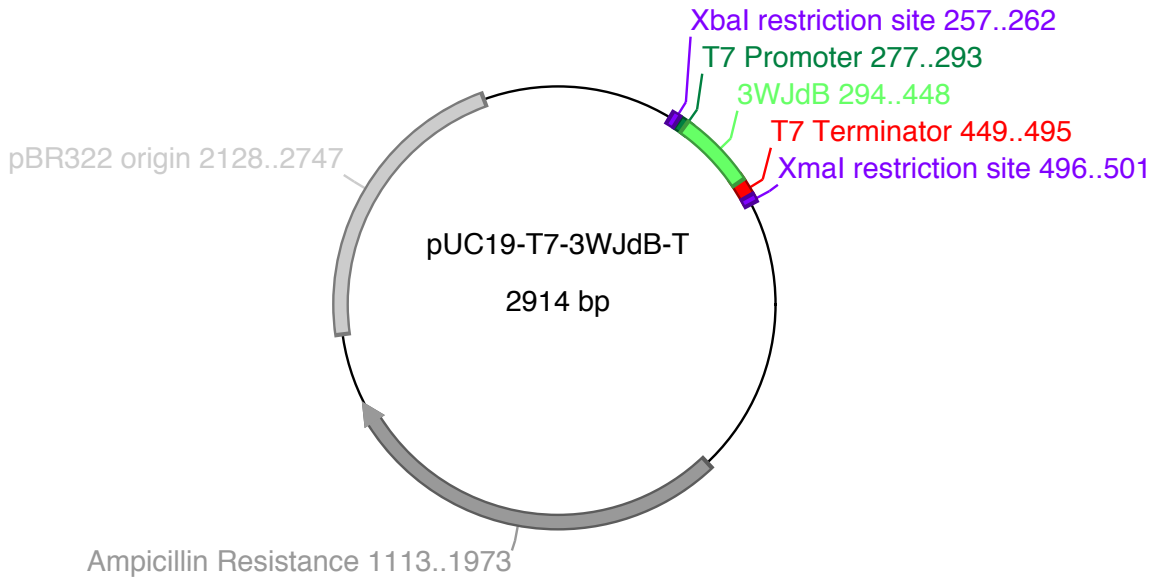

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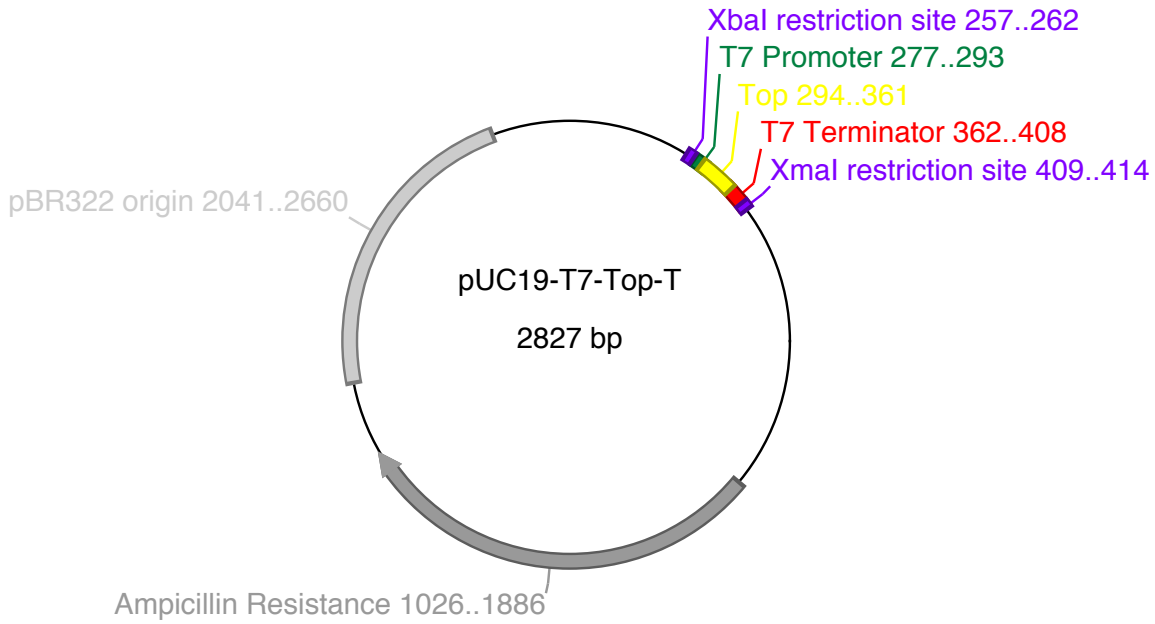

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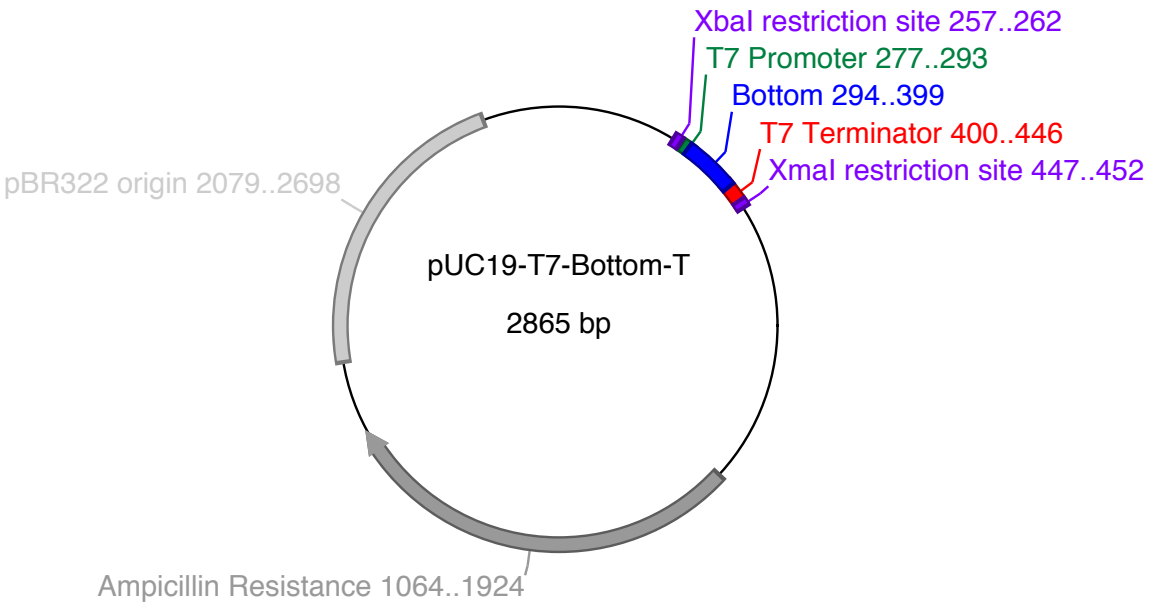

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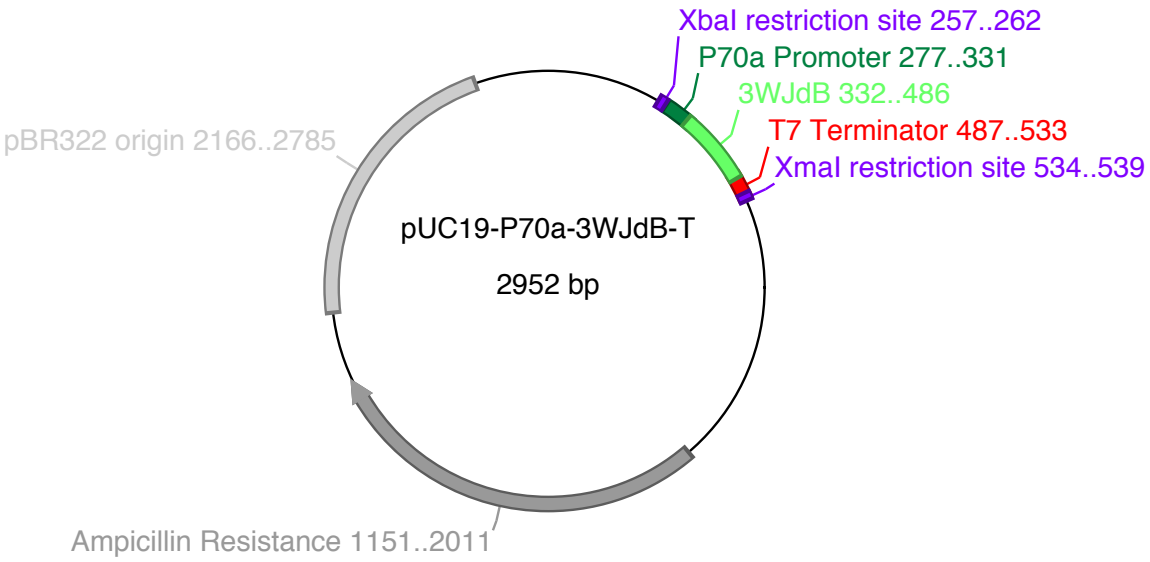

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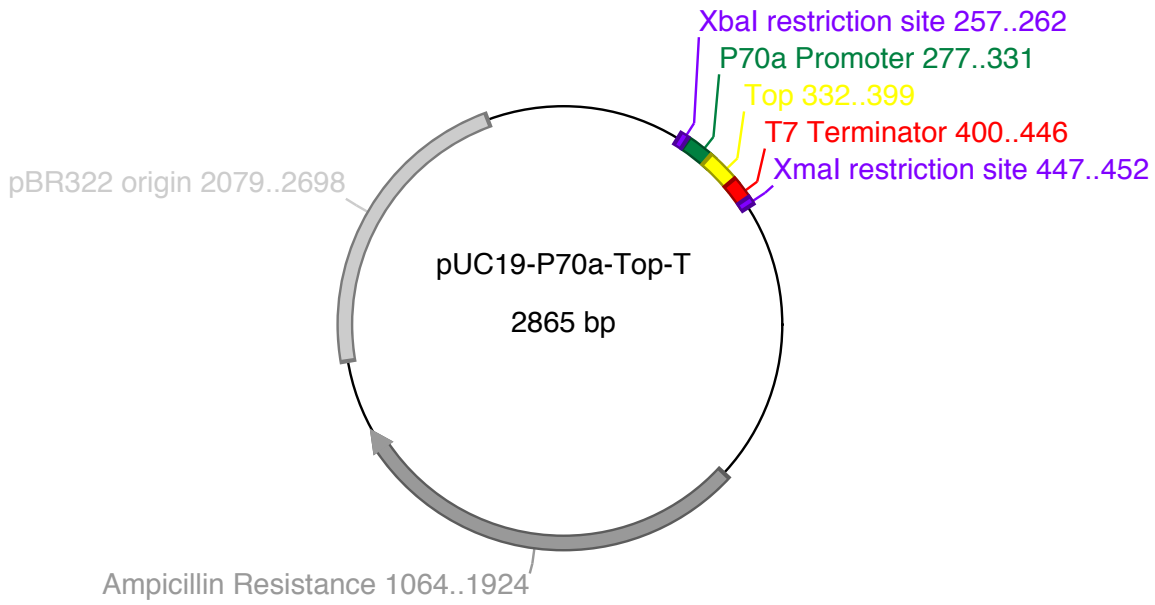

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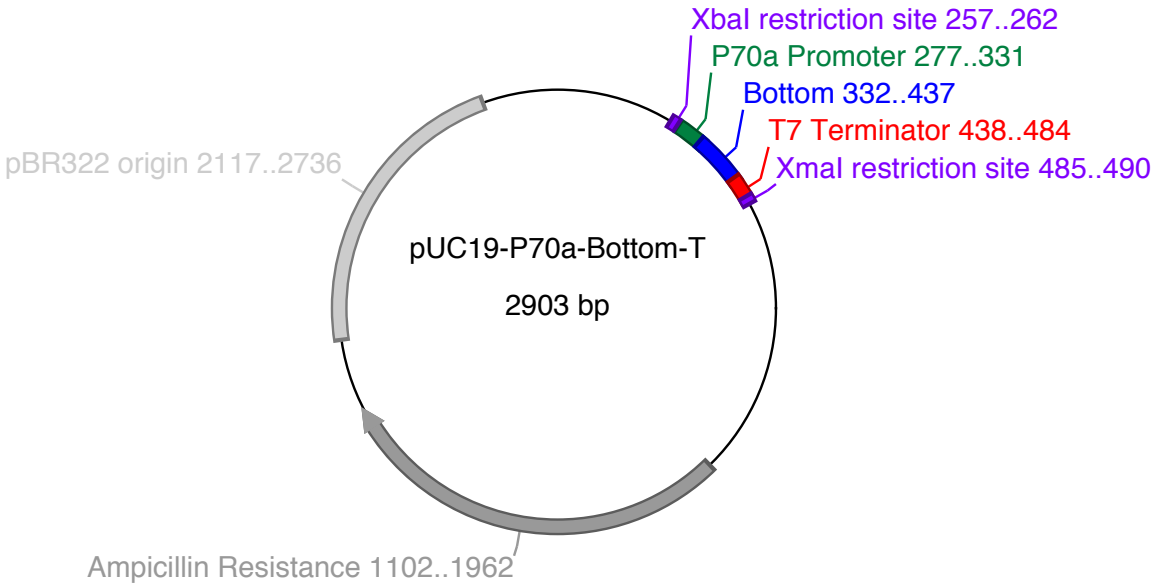

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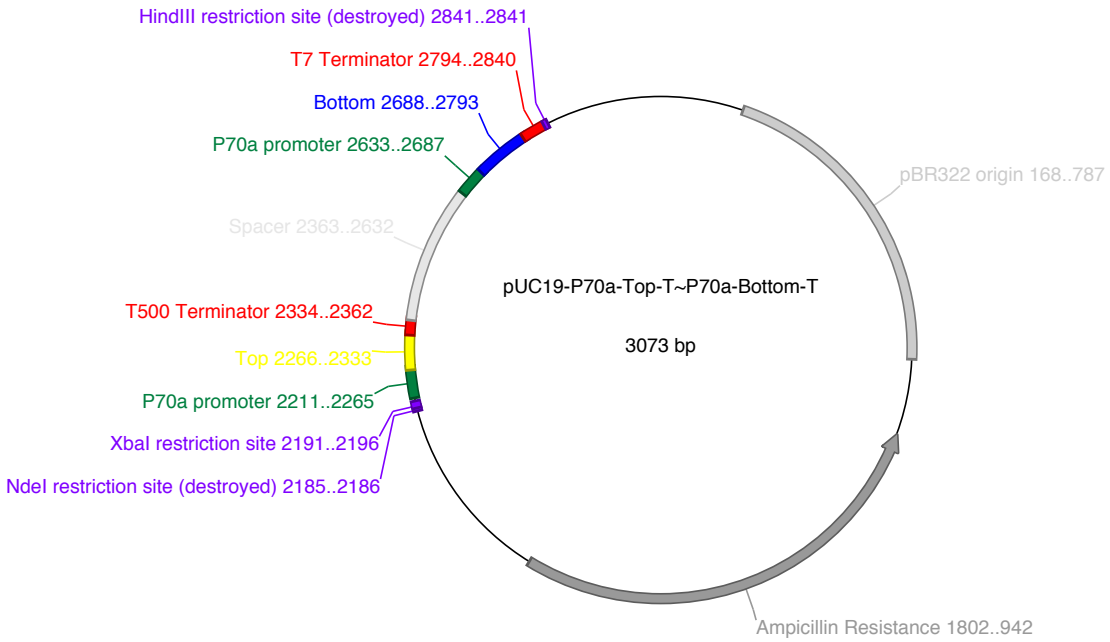

>pUC19-P70a-Top-T~P70a-Bottom-T (Addgene ID 87314)

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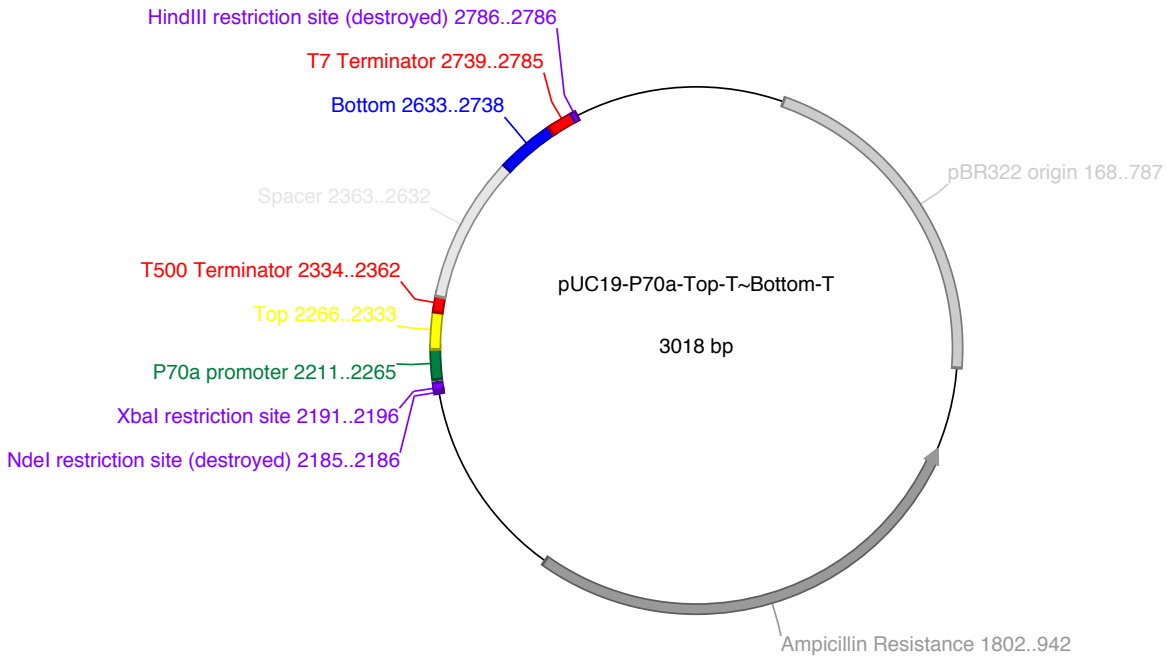

>pUC19-P70a-Top-T~Bottom-T (Addgene ID 87315)

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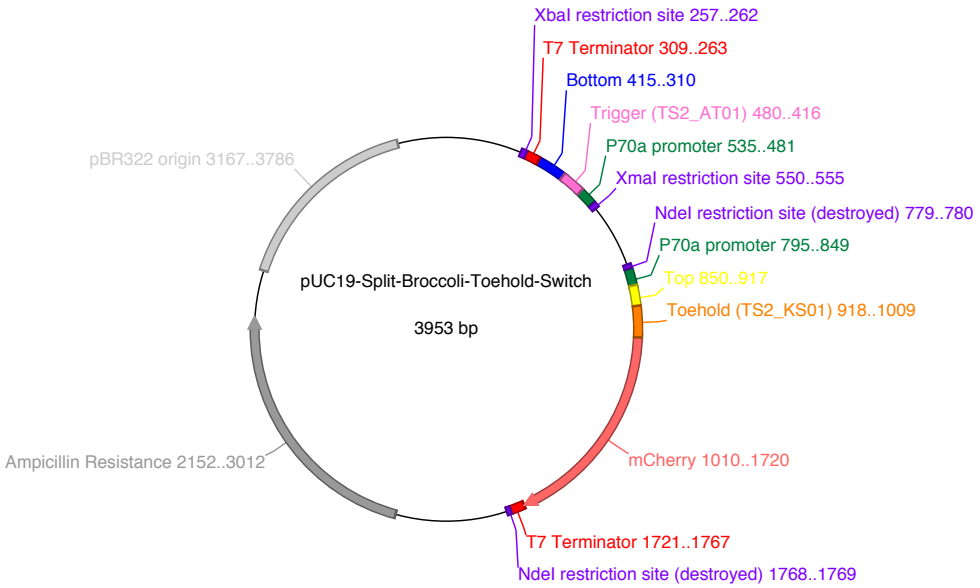

>pUC19-Split-Broccoli-Toehold-Switch (Addgene ID 87316)
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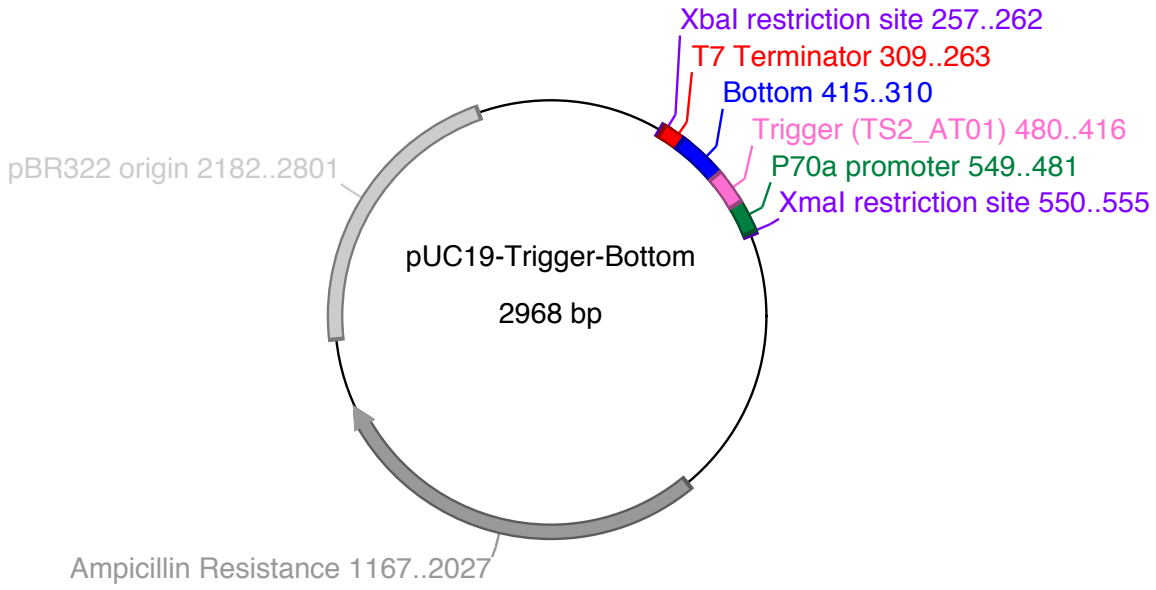

>pUC19-Trigger-Bottom (Addgene ID 87317)
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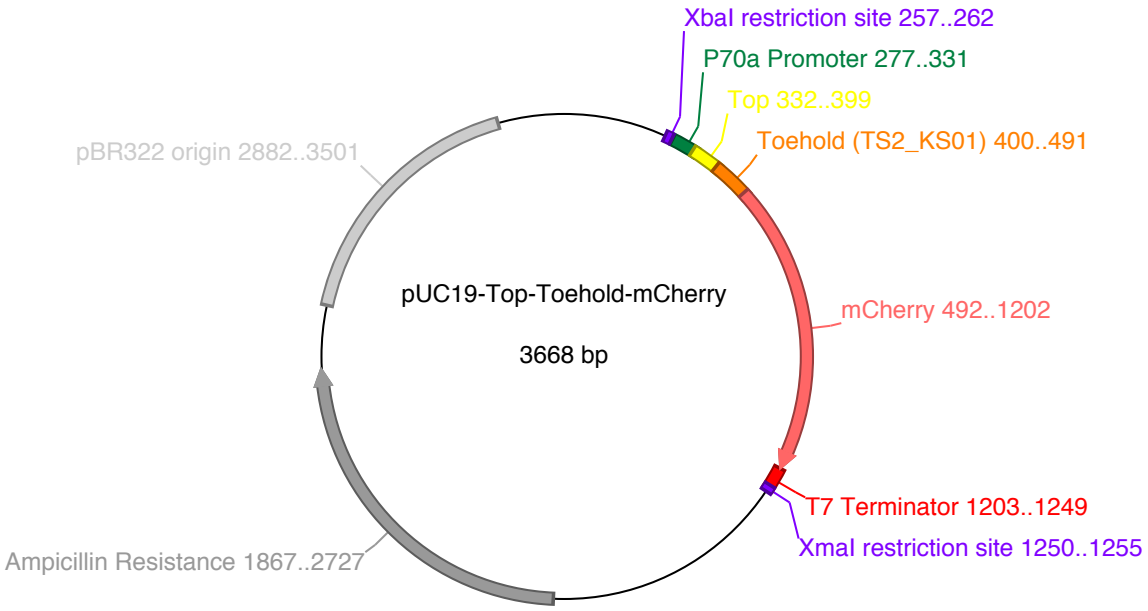

>pUC19-Top-Toehold-mCherry (Addgene ID 87318)

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