

Supplementary

Table S1. Strains and plasmids used in this study.

Plasmid or strain	Relevant properties or genotype	Source
Plasmids		
pETM6-eGFP	pETM6 vector carrying reporter gene eGFP, vector is used to carry the T7 promoter library, 5'UTR library, T7 terminator library and the cis-repressing RNA	[1]
pCDM4	Vector backbone used to carry the trans-activating RNA library	[1]
Strains		
<i>E. coli</i> NEB5 α	<i>fhuA2</i> Δ (<i>argF-lacZ</i>) <i>U169 phoA glnV44</i> Φ 80 Δ (<i>lacZ</i>) <i>M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17</i>	New England Biolabs
<i>E. coli</i> BL21*(DE3)	<i>F ompT hsdS_B (r_B, m_B) galdcmrne131 (DE3)</i>	Invitrogen

Table S2. Primers and synthetic oligoes used in this study

No	Primer	Nucleotide sequence (5' >3')
1	T7lacO_F	ATACNNCTCACTATAGGAAT NGTNNNCGGNNNACNATTC CTAGA AATAATTTGTTA
2	T7lacO_R	ATTCCTATAGTGAGNNGTAT TAAATTTNNGATCNNNNNAT CCTAGG CTACGCCGACGCA
3	Rbslib_F	TTCACACAGGAAACCGAGGAGTC TNNNNNGAGANNNNNA CATATG GTGAGCAAGGGCGAG
4	Rbslib_R	GACTCCTCGGTTTCTGTGTGAA ANNNNNCTNNGNNT TCTAGA GGGAATTGTTATCC
5	T7ter_F	CTTGGGGCCTCTAAACGGGT CTNNNNGGTTNNNT GCTAGC GAAAGGAGGAGTCGACTAT
6	T7ter_R	ACCCGTTTAGAGGCCCAAG GGGNNNTGCNNNNTG ACTAGT AGACGAGTCCATGTGCTGG
7	cisRNA1_F	agcggataacaattcccctctaga AAttGAtAttGtGAttAtGtGAagAttACTACAAGAACAGAGGAGAtAggCACAtat gtgag caaggcgaggag
8	cisRNA1_R	ctcctgccttgctcaccataTGTGccTaTCTCCTCTGTTCTGTAGTaaTctTCaCaTaaTCaCaaTaTCaaTTtctagagggaatt ggtatccgct
9	TranAlib_R	ctgtatgtcaaatgacacacata aNNNNNacataNNNNNa ctagaccctatagtgagt
10	TranAlib_F	tatgtgtgcatttgacatacag tggNNNNNtccNNNNgt ggtagctcagctcacta

Supplementary Figures

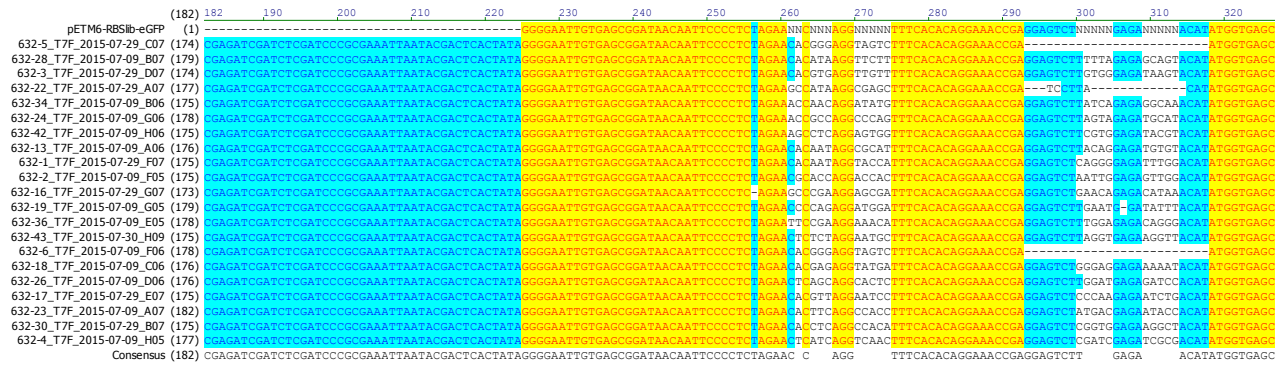


Figure S1. Representative sequencing results for 5'UTR library constructed in this study.

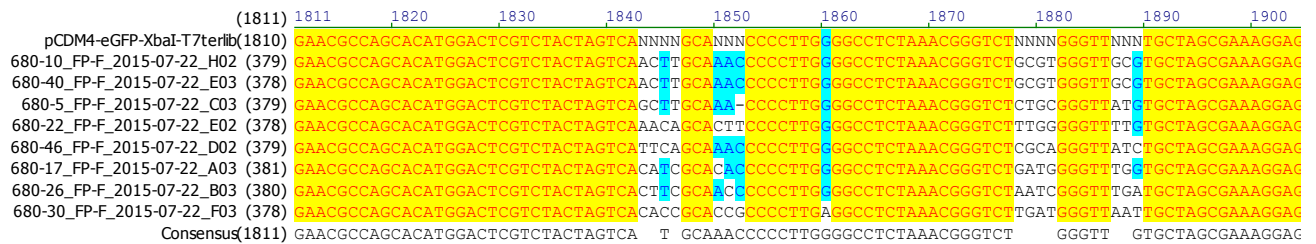


Figure S2. Representative sequencing results for T7 terminator library constructed in this study.

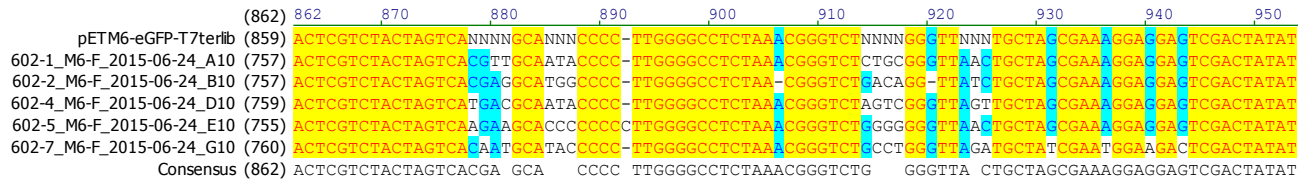


Figure S3. Representative sequencing results for T7 terminator library constructed in this study.

	(951)	951	960	970	980	990	1000	1010	1020	1030	1040
pCDM4-transA1-XbaI	(951)	TAATACGACTCACTATAGGGGCTAGAT	GTGAT	TATGTGAAGA	TTATGTGTGTCATTTGACATACAGTGG	CATAG	TCCGTAT	GTGGTACCC			
572a_T7_2015-04-14_A04	(199)	TAATACGACTCACTATAGGGGCTAGAT	TCCAG	TATGTAAGAG	TTATGTGTGTCATTTGACATACAGTGG	TTAAG	TCCAAAT	GTGGTACCC			
572c_T7_2015-04-14_C04	(200)	TAATACGACTCACTATAGGGGCTAGAT	TTTC	TATGTTGAAAT	TTATGTGTGTCATTTGACATACAGTGG	CTATA	TCC	TGGTGGTACCC			
572d_T7_2015-04-14_D04	(199)	TAATACGACTCACTATAGGGGCTAGAT	AATGG	TATGTTTTGA	TTATGTGTGTCATTTGACATACAGTGG	TAAAG	TCCAAAT	GTGGTACCC			
573a_T7_2015-04-14_E04	(199)	TAATACGACTCACTATAGGGGCTAGAT	TGGCA	TATGTGATAG	TTATGTGTGTCATTTGACATACAGTGG	GGGT	TCC	TAGGGTGGTACCC			
573c_T7_2015-04-14_G04	(200)	TAATACGACTCACTATAGGGGCTAGAT	GAAC	TATGTACCCC	TTATGTGTGTCATTTGACATACAGTGG	TGTT	TCC	AAAGTGGTACCC			
573d_T7_2015-04-14_H04	(200)	TAATACGACTCACTATAGGGGCTAGAT	CGCA	TATGTCCTCAG	TTATGTGTGTCATTTGACATACAGTGG	GGG	TCC	AAATGTGGTACCC			
572b_T7_2015-04-14_B04	(200)	TAATACGACTCACTATAGGGGCTAGAT	GCAT	TATGTTAACG	TTATGTGTGTCATTTGACATACAGTGG	TAGAG	TCC	AAATGTGGTACCC			
573b_T7_2015-04-14_F04	(201)	TAATACGACTCACTATAGGGGCTAGAT	CGGT	TATGTCGATA	TTATGTGTGTCATTTGACATACAGTGG	AACCT	TCC	AAATGTGGTACCC			
Consensus	(951)	TAATACGACTCACTATAGGGGCTAGAT	CTATGT	TTATGTGTGTCATTTGACATACAGTGG			GTCCAAT	GTGGTACCC			

Figure S4. Representative sequencing results for trans-activating RNA library No 1-8 constructed in this study.

	(942)	942	950	960	970	980	990	1000	1010	1020	1030
pCDM4-transA1-XbaI	(942)	CCGCGAAATTAATACGACTCACTATAGGGGCTAGAT	GTGAT	TATGTGA	GATTATGTGTGTCATTTGACATACAGTGG	CATAG	TCCGTAT	GTGGTAC			
TAlib21_T7_2015-04-17_E01	(193)	CCGCGAAATTAATACGACTCACTATAGGGGCTAGAT	ATCCAT	TATGCTT	GATTATGTGTGTCATTTGACATACAGTGG	CGACT	TCC	AAATGTGGTAC			
TAlib10_T7_2015-04-17_B01	(192)	CCGCGAAATTAATACGACTCACTATAGGGGCTAGAT	AGACA	TATGTC	ATTATGTGTGTCATTTGACATACAGTGG	AGGAA	TCC	AAATGTGGTAC			
TAlib11_T7_2015-04-17_C01	(194)	CCGCGAAATTAATACGACTCACTATAGGGGCTAGAT	ACAGC	TATGTAC	TTTATGTGTGTCATTTGACATACAGTGG	CCGTC	TCC	AAAGTGGTAC			
TAlib13_T7_2015-04-17_E01	(193)	CCGCGAAATTAATACGACTCACTATAGGGGCTAGAT	CAACG	TAT	TGAATTTATGTGTGTCATTTGACATACAGTGG	TTT	TCC	AAATGTGGTAC			
TAlib22_T7_2015-04-17_F02	(196)	CCGCGAAATTAATACGACTCACTATAGGGGCTAGAT	TCATT	TATG	GGCGCTTATGTGTGTCATTTGACATACAGTGG	TTCTC	TCC	AAATGTGGTAC			
TAlib12_T7_2015-04-17_D01	(193)	CCGCGAAATTAATACGACTCACTATAGGGGCTAGAT	CAGCA	TATG	CGTATGTGTGTCATTTGACATACAGTGG	AGATC	TCC	AAATGTGGTAC			
TAlib16_T7_2015-04-17_H01	(192)	CCGCGAAATTAATACGACTCACTATAGGGGCTAGAT	CACGA	TATG	TCCCGCTTATGTGTGTCATTTGACATACAGTGG	GGCTA	TCC	AAATGTGGTAC			
TAlib18_T7_2015-04-17_B02	(191)	CCGCGAAATTAATACGACTCACTATAGGGGCTAGAT	GCTGT	TATG	TCCCTTATGTGTGTCATTTGACATACAGTGG	GTCTG	TCC	AAAGTGGTAC			
TAlib19_T7_2015-04-17_C02	(192)	CCGCGAAATTAATACGACTCACTATAGGGGCTAGAT	GAGGC	TATG	TCCGATTTATGTGTGTCATTTGACATACAGTGG	TGTCAG	TCC	AAATGTGGTAC			
TAlib23_T7_2015-04-17_G02	(193)	CCGCGAAATTAATACGACTCACTATAGGGGCTAGAT	CGCCT	TATG	TAAAGCCTTATGTGTGTCATTTGACATACAGTGG	ACAAA	TCC	AAAGTGGTAC			
TAlib15_T7_2015-04-17_G01	(194)	CCGCGAAATTAATACGACTCACTATAGGGGCTAGAT	CTAAC	TATG	TCCAGTTATGTGTGTCATTTGACATACAGTGG	AATA	TCC	AAATGTGGTAC			
TAlib20_T7_2015-04-17_D02	(194)	CCGCGAAATTAATACGACTCACTATAGGGGCTAGAT	GAAAA	TATG	TATAATTTATGTGTGTCATTTGACATACAGTGG	GGTGT	TCC	AAATGTGGTAC			
Consensus	(942)	CCGCGAAATTAATACGACTCACTATAGGGGCTAGAT	TATGT	A	TTATGTGTGTCATTTGACATACAGTGG	TCC	AAATGTGGTAC				

Figure S5. Representative sequencing results for trans-activating RNA library No 9-23 constructed in this study.

	(954)	954	960	970	980	990	1000	1010	1020	1030	1040
pCDM4-transA1-XbaI	(953)	ATACGACTCACTATAGGGGCTAGAT	GTG	TATGT	GAAAGA	TTATGTGTGTCATTTGACATACAGTGG	CATAG	TCCGTAT	GTGGTACCC	TCGAGT	
TAlib30_T7_2015-04-19_G08	(203)	ATACGACTCACTATAGGGGCTAGAT	CG	TATGT	TATATT	TTATGTGTGTCATTTGACATACAGTGG	TCTCT	TCC	CAAG	GTGGTACCC	TCGAGT
TAlib33_T7_2015-04-19_E02	(204)	ATACGACTCACTATAGGGGCTAGAT	CTACC	TATGT	GTTTT	TTATGTGTGTCATTTGACATACAGTGG	TCGAT	TCC	CGGG	GTGGTACCC	TCGAGT
TAlib32_T7_2015-04-19_A09	(205)	ATACGACTCACTATAGGGGCTAGAT	CAGGG	TATGT	GTTC	TTATGTGTGTCATTTGACATACAGTGG	TTTTT	TCC	TTGT	GTGGTACCC	TCGAGT
TAlib29_T7_2015-04-19_F08	(206)	ATACGACTCACTATAGGGGCTAGAT	AA	TTATGT	GCAAC	TTATGTGTGTCATTTGACATACAGTGG	TTTG	TCC	CCTT	GTGGTACCC	TCGAGT
TAlib24_T7_2015-04-19_A08	(207)	ATACGACTCACTATAGGGGCTAGAT	GGT	TATGT	CAGAT	TTATGTGTGTCATTTGACATACAGTGG	TGCG	TCC	TCTC	GTGGTACCC	TCGAGT
TAlib38_T7_2015-04-19_G09	(205)	ATACGACTCACTATAGGGGCTAGAT	GT	TATGT	TAAAC	TTATGTGTGTCATTTGACATACAGTGG	TACCG	TCC	CGGG	GTGGTACCC	TCGAGT
TAlib35_T7_2015-04-19_D09	(204)	ATACGACTCACTATAGGGGCTAGAT	TGT	TATGT	TCTCA	TTATGTGTGTCATTTGACATACAGTGG	GCCCA	TCC	AAAT	GTGGTACCC	TCGAGT
TAlib37_T7_2015-04-19_F09	(202)	ATACGACTCACTATAGGGGCTAGAT	TAT	TATGT	TCTCC	TTATGTGTGTCATTTGACATACAGTGG	GGG	TCC	ATCC	GTGGTACCC	TCGAGT
TAlib26_T7_2015-04-19_C08	(205)	ATACGACTCACTATAGGGGCTAGAT	GAC	TATGT	TATAA	TTATGTGTGTCATTTGACATACAGTGG	GACAA	TCC	TGCA	GTGGTACCC	TCGAGT
TAlib34_T7_2015-04-19_C09	(203)	ATACGACTCACTATAGGGGCTAGAT	GAT	TATGT	TAAAGA	TTATGTGTGTCATTTGACATACAGTGG	GATTC	TCC	TGTA	GTGGTACCC	TCGAGT
TAlib25_T7_2015-04-19_B08	(208)	ATACGACTCACTATAGGGGCTAGAT	AAAGG	TATGT	TCCGT	TTATGTGTGTCATTTGACATACAGTGG	GATTC	TCC	TGAA	GTGGTACCC	TCGAGT
TAlib28_T7_2015-04-19_E08	(207)	ATACGACTCACTATAGGGGCTAGAT	CC	TATGT	CAGCC	TTATGTGTGTCATTTGACATACAGTGG	TAGT	TCC	GAAG	GTGGTACCC	TCGAGT
TAlib36_T7_2015-04-19_E09	(206)	ATACGACTCACTATAGGGGCTAGAT	CAAGT	TATGT	TTCCC	TTATGTGTGTCATTTGACATACAGTGG	CAGGC	TCC	CGTG	GTGGTACCC	TCGAGT
TAlib31_T7_2015-04-19_H08	(207)	ATACGACTCACTATAGGGGCTAGAT	ACAG	TATGT	TAAAGT	TTATGTGTGTCATTTGACATACAGTGG	GAGAT	TCC	GGCC	GTGGTACCC	TCGAGT
TAlib27_T7_2015-04-19_D08	(207)	ATACGACTCACTATAGGGGCTAGAT	CA	TATGT	GGTTG	TTATGTGTGTCATTTGACATACAGTGG	CCATG	TCC	TTGT	GTGGTACCC	TCGAGT
TAlib39_T7_2015-04-19_H09	(203)	ATACGACTCACTATAGGGGCTAGAT	AGAGT	TATGT	TCCGG	TTATGTGTGTCATTTGACATACAGTGG	CCATG	TCC	GTTT	GTGGTACCC	TCGAGT
Consensus	(954)	ATACGACTCACTATAGGGGCTAGAT	TA	TATGT	TTATGTGTGTCATTTGACATACAGTGG	TCC	AAATGTGGTAC				

Figure S6. Representative sequencing results for trans-activating RNA library No 24-39 constructed in this study.

	(946)	946	960	970	980	990	1000	1010	1020	1030	1040
pCDM4-transA1 (946)	GAAATTAATACGACTCACTATAGGGGCTAGAT	GTGAT	TATGT	GAAGA	TTATGTGTGTCATT	GACATACAGTGG	CATAG	TCCGTAT	GTGGTACCCCTCGAGT		
TAlib43_T7_2015-04-21_D10 (197)	GAAATTAATACGACTCACTATAGGGGCTAGAT	ACTAG	TATGT	CACAA	TTATGTGTGTCATT	GACATACAGTGG	TCCAT	TCCCTCC	GTGGTACCCCTCGAGT		
TAlib47_T7_2015-04-21_H10 (193)	GAAATTAATACGACTCACTATAGGGGCTAGAT	GTAT	TATGT	GACAG	TTATGTGTGTCATT	GACATACAGTGG	GGCT	TCCAACCC	GTGGTACCCCTCGAGT		
TAlib40_T7_2015-04-21_A10 (196)	GAAATTAATACGACTCACTATAGGGGCTAGAT	AAAGC	TATGT	ACAT	TTATGTGTGTCATT	GACATACAGTGG	CCTA	TCCGAAT	GTGGTACCCCTCGAGT		
TAlib41_T7_2015-04-21_B10 (195)	GAAATTAATACGACTCACTATAGGGGCTAGAT	TGAGA	TATGT	ACCG	TTATGTGTGTCATT	GACATACAGTGG	TTGA	TCCGCTC	GTGGTACCCCTCGAGT		
TAlib42_T7_2015-04-21_C10 (195)	GAAATTAATACGACTCACTATAGGGGCTAGAT	TGAC	TATGT	TCT	TTATGTGTGTCATT	GACATACAGTGG	TAGA	TCCGGGA	GTGGTACCCCTCGAGT		
TAlib44_T7_2015-04-21_E10 (195)	GAAATTAATACGACTCACTATAGGGGCTAGAT	ACCC	TATGT	TATG	TTATGTGTGTCATT	GACATACAGTGG	AGCG	TCCGTCAG	GTGGTACCCCTCGAGT		
TAlib45_T7_2015-04-21_F10 (195)	GAAATTAATACGACTCACTATAGGGGCTAGAT	CAC	TATGT	GAC	TTATGTGTGTCATT	GACATACAGTGG	ACAG	TCCATAG	GTGGTACCCCTCGAGT		
TAlib46_T7_2015-04-21_G10 (195)	GAAATTAATACGACTCACTATAGGGGCTAGAT	CGAGA	TATGT	CATAA	TTATGTGTGTCATT	GACATACAGTGG	TGAT	TCCGTGT	GTGGTACCCCTCGAGT		
Consensus (946)	GAAATTAATACGACTCACTATAGGGGCTAGAT	CA	TATGT	C	TTTATGTGTGTCATT	TGACATACAGTGGT	TCC		GTGGTACCCCTCGAGT		

Figure S7. Representative sequencing results for trans-activating RNA library No 40-47 constructed in this study.

References

1. Xu, P., et al., *ePathBrick: A Synthetic Biology Platform for Engineering Metabolic Pathways in E. coli*. ACS Synth. Biol., 2012. 1(7): p. 256-266.