

A portable regulatory RNA array design enables tunable and complex regulation across diverse bacteria

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Supplementary Table 1. All plasmids used in this study. CmR: chloramphenicol resistance gene, AmpR: ampicillin resistance gene, SpcR: spectinomycin resistance gene, KanR: kanamycin resistance gene, p15A: origin of replication, ColE1: origin of replication, CDF: CloDF origin of replication, pBBR1: broad-host range origin of replication, pSP6: SP6 promoter, TrnB: terminator, t500: terminator, RBS: ribosome binding site, Csy4: Csy4 ribonuclease, csy4hp: csy4 cleavage hairpin, shcsyhp: strong hairpin csy4 cleavage hairpin, PlmJ: ribozyme.

Plasmid	Description	Plasmid Architect	Figure
pBL176	STAR positive	J23119-Target10-RBS-GFP-TrnB-J23119-STAR10-t500-pBBR1-KanR	Fig1
pBL177	STAR negative	J23119-Target10-RBS-GFP-TrnB-pBBR1-KanR	Fig1
pBL301	STAR negative+Csy4	J23119-Target10-RBS-GFP-TrnB-t500-J23101-Csy4-Terminator-pBBR1-KanR	Fig1/Fig4/FigS9
pBL302	STAR positive+Csy4	J23119-Target10-RBS-GFP-TrnB-J23119-STAR10-t500-J23101-Csy4-Terminator-pBBR1-KanR	Fig1/FigS9
pBL115	SP6 RNAP	AraC-pBAD-RBS-SP6 RNAP-TrnB-SpcR-CDF	Fig1/FigS1
pBL068	SP6 Target-GFP	pSP6-Target6-RBS-GFP-TrnB-CmR-p15A	Fig1
pBL071	SP6 STAR	pSP6-STAR6-t500-ColE1-AmpR	Fig1
pJEC102	Control	ColE1-AmpR	Fig1/FigS1/FigS5
pJEC250	Target50-GFP	J23119-Target50-RBS-GFP-TrnB-CmR-p15A	Fig2/FigS3
pBLM443	PlmJ_STAR50x4	AraC_pBAD-PlmJ_STAR50x4-t500-ColE1-AmpR	Fig2
pBLM444	PlmJ_STAR50x1	AraC_pBAD-PlmJ_STAR50x1-t500-ColE1-AmpR	Fig2
pBL111	Csy4	J23101-Csy4-CDF-SpcR	Fig2/FigS5
pBL451	csy4hp_STAR50x4	J23119-Target50-RBS-GFP-TrnB-AraC_pBAD-csy4hp_STAR50x4-CmR-p15A	Fig2
pBL452	csy4hp_STAR50x1	J23119-Target50-RBS-GFP-TrnB-AraC_pBAD-csy4hp_STAR50x1-CmR-p15A	Fig2
pBL170	shcsy4hp_STAR50x4	J23119-Target50-RBS-GFP-TrnB-AraC_pBAD-shcsy4hp_STAR50x4-CmR-p15A	Fig2
pBL171	shcsy4hp_STAR50x1	J23119-Target50-RBS-GFP-TrnB-AraC_pBAD-shcsy4hp_STAR50x1-CmR-p15A	Fig2

pBL161	shcsy4hp_STAR10x4	J23119-Target10-RBS-GFP-TrnB-AraC_pBAD-shcsy4hp_STAR10x4-CmR-p15A	Fig2
pBL162	shcsy4hp_STAR10x1	J23119-Target10-RBS-GFP-TrnB-AraC_pBAD-shcsy4hp_STAR10x1-CmR-p15A	Fig2
pJEC216	Target10-GFP	J23119-Target10-RBS-GFP-TrnB-CmR-p15A	Fig2/Fig4/ FigS3/ FigS5
pBL303	STAR10x1 (pBAD)	J23119-Target10-RBS-GFP-TrnB-AraC_pBAD-shcsy4hp_STAR10x1-J23101-Csy4-Terminator-pBBR1-KanR	Fig3/FigS7/ FigS8/ FigS9
pBL304	STAR10x2 (pBAD)	J23119-Target10-RBS-GFP-TrnB-AraC_pBAD-shcsy4hp_STAR10x2-J23101-Csy4-Terminator-pBBR1-KanR	Fig3/FigS7/ FigS8/ FigS9
pBL305	STAR10x4 (pBAD)	J23119-Target10-RBS-GFP-TrnB-AraC_pBAD-shcsy4hp_STAR10x4-J23101-Csy4-Terminator-pBBR1-KanR	Fig3/FigS7/ FigS8/ FigS9
pBL306	STAR10x6 (pBAD)	J23119-Target10-RBS-GFP-TrnB-AraC_pBAD-shcsy4hp_STAR10x6-J23101-Csy4-Terminator-pBBR1-KanR	Fig3/FigS7/ FigS8/ FigS9
pBL307	STAR10x8 (pBAD)	J23119-Target10-RBS-GFP-TrnB-AraC_pBAD-shcsy4hp_STAR10x8-J23101-Csy4-Terminator-pBBR1-KanR	Fig3/FigS7/ FigS8/ FigS9/ FigS10
pBL401	STAR10x1 (pCymR)	J23119-Target10-RBS-GFP-TrnB-CymR_pCymR-shcsy4hp_STAR10x1-J23101-Csy4-Terminator-pBBR1-KanR	Fig3/FigS7/ FigS8/ FigS9
pBL402	STAR10x2 (pCymR)	J23119-Target10-RBS-GFP-TrnB-CymR_pCymR-shcsy4hp_STAR10x2-J23101-Csy4-Terminator-pBBR1-KanR	Fig3/FigS7/ FigS8/ FigS9
pBL403	STAR10x4 (pCymR)	J23119-Target10-RBS-GFP-TrnB-CymR_pCymR-shcsy4hp_STAR10x4-J23101-Csy4-Terminator-pBBR1-KanR	Fig3/FigS7/ FigS8/ FigS9
pBL404	STAR10x6 (pCymR)	J23119-Target10-RBS-GFP-TrnB-CymR_pCymR-shcsy4hp_STAR10x6-J23101-Csy4-Terminator-pBBR1-KanR	Fig3/FigS7/ FigS8/ FigS9
pBL405	STAR10x8 (pCymR)	J23119-Target10-RBS-GFP-TrnB-CymR_pCymR-shcsy4hp_STAR10x8-J23101-Csy4-Terminator-pBBR1-KanR	Fig3/FigS7/ FigS8/ FigS9/ FigS10

pBL512	pBAD-GFP	AraC_pBAD-RBS-GFP-TrnB-pBBR1-KanR	Fig3/FigS6
pBL513	pCymR-GFP	CymR_pCymR-RBS-GFP-TrnB-pBBR1-KanR	Fig3/FigS6
pBLM366	Target50-STAR10x1	J23119-Target50-shCsy4_STAR10x1-t500-RSF1030-SpcR	Fig4
pBLM367	Target50-STAR10x4	J23119-Target50-shCsy4_STAR10x4-t500-RSF1030-SpcR	Fig4
pJEC251	STAR50	J23119-STAR50-t500-ColE1-AmpR	Fig4
pBL501	Target10-GFP+Target50-STAR10x1	J23119-Target10-RBS-GFP-TrnB-J23119-Target50-shCsy4_STAR10x1-t500-J23101-Csy4-Terminator-pBBR1-KanR	Fig4
pBL502	Target10-GFP+Target50-STAR10x4	J23119-Target10-RBS-GFP-TrnB-J23119-Target50-shCsy4_STAR10x4-t500-J23101-Csy4-Terminator-pBBR1-KanR	Fig4
pBL504	Target10-GFP+Target50-STAR10x1+STAR50	J23119-Target10-RBS-GFP-TrnB-J23119-Target50-shCsy4_STAR10x1-t500-J23119-STAR50-t500-J23101-Csy4-Terminator-pBBR1-KanR	Fig4
pBL505	Target10-GFP+Target50-STAR10x4+STAR50	J23119-Target10-RBS-GFP-TrnB-J23119-Target50-shCsy4_STAR10x4-t500-J23119-STAR50-t500-J23101-Csy4-Terminator-pBBR1-KanR	Fig4
pBL506	Target10-GFP+Target50-mRFP+STAR10x1+STAR50x1	J23119-Target10-RBS-GFP-TrnB-J23119-RBS-RFP-t500-AraC_pBAD-shcsy4hp_STAR10x1-shcsy4hp_STAR50x1-t500-J23101-Csy4-Terminator-pBBR1-KanR	Fig4/FigS13
pBL507	Target10-GFP+Target50-mRFP+STAR10x1+STAR50x2	J23119-Target10-RBS-GFP-TrnB-J23119-RBS-RFP-t500-AraC_pBAD-shcsy4hp_STAR10x1-shcsy4hp_STAR50x2-t500-J23101-Csy4-Terminator-pBBR1-KanR	Fig4/FigS13
pBL508	Target10-GFP+Target50-mRFP+STAR10x2+STAR50x1	J23119-Target10-RBS-GFP-TrnB-J23119-RBS-RFP-t500-AraC_pBAD-shcsy4hp_STAR10x2-shcsy4hp_STAR50x1-t500-J23101-Csy4-Terminator-pBBR1-KanR	Fig4/FigS13
pBL509	Target10-GFP+Target50-mRFP+STAR10x2+STAR50x2	J23119-Target10-RBS-GFP-TrnB-J23119-RBS-RFP-t500-AraC_pBAD-shcsy4hp_STAR10x2-shcsy4hp_STAR50x2-t500-J23101-Csy4-Terminator-pBBR1-KanR	Fig4/FigS13

pBL059	SP6 RNAP	pTet-RBS-luxR-Terminator-pLuxR-RBS-SP6 RNAP-TrnB-SpcR-CDF	FigS1
pBL062	SP6 promoter- Target28-GFP	pSP6-Target28(AD1.S7)-RBS-sfGFP-TrnB-CmR- p15A	FigS1
pJEC154	STAR28(12nt)	J23119-STAR28(12nt)-t500-ColE1-AmpR	FigS1
pJEC163	STAR28(17nt)	J23119-STAR28(17nt)-t500-ColE1-AmpR	FigS1
pJEC164	STAR28(27nt)	J23119-STAR28(27nt)-t500-ColE1-AmpR	FigS1
pJEC165	STAR28(37nt)	J23119-STAR28(37nt)-t500-ColE1-AmpR	FigS1
pJEC166	STAR28(47nt)	J23119-STAR28(47nt)-t500-ColE1-AmpR	FigS1
pJEC167	STAR28(57nt)	J23119-STAR28(57nt)-t500-ColE1-AmpR	FigS1
pBL116	pSP6(wt)- Target10-GFP	pSP6-Target10-RBS-GFP-TrnB-CmR-p15A	FigS1
pBL120	pSP6(wt)-STAR10	pSP6-STAR10-t500-ColE1-AmpR	FigS1
pBL144	pSP6(90p)- STAR10	pSP6(90p)-STAR10-t500-ColE1-AmpR	FigS1
pBL145	pSP6(50p)- STAR10	pSP6(50p)-STAR10-t500-ColE1-AmpR	FigS1
pBL146	pSP6(20p)- STAR10	pSP6(20p)-STAR10-t500-ColE1-AmpR	FigS1
pBL117	pSP6-Target28- GFP	pSP6-Target28-RBS-GFP-TrnB-CmR-p15A	FigS1
pBL118	pSP6-Target50- GFP	pSP6-Target50-RBS-GFP-TrnB-CmR-p15A	FigS1
pBL121	pSP6-STAR28	pSP6-STAR28-t500-ColE1-AmpR	FigS1
pBL122	pSP6-STAR50	pSP6-STAR50-t500-ColE1-AmpR	FigS1
pBLM476	STAR10	J23119-STAR10-ColE1-AmpR	FigS5
pBLM335	csy4hp-STAR10	J23119-csy4hp-STAR10-ColE1-AmpR	FigS5
pBLM477	shcsy4hp-STAR10	J23119-shcsy4hp-STAR10-ColE1-AmpR	FigS5
pJEC101	Control	p15A-CmR	FigS5
pJEC103	Control	CDF-SpcR	FigS5

Supplementary Table 2. Example DNA plasmid sequence.

Name	DNA sequence
Regulatory RNA array plasmid STAR10x8 (pBL307) J23119- Target10- RBS-GFP- TrmB-AraC- pBAD- (shcsy4hp_S TAR10)x8- Csy4-pBBR1- KanR	GAATTCTAAAGATCTTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGT CTCATCTCATTTCGCTCTCACATTTCTACACCTTTATCTTGCGGGGAATGT ATACAGTTCATGTATATATTCCCGCTTTTTTTTTTGGATCTAGGAGGAAG GATCTATGAGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTT GTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCCGTGGAGA GGGTGAAGGTGATGCTACAAACGGAAAACCTACCCCTTAAATTTATTTGCA CTACTGGAAAACCTACCTGTTCCGTGGCCAACACTTGTCACTACTCTGACC TATGGTGTTCAATGCTTTTCCCGTTATCCGGATCACATGAAACGGCATGA CTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACTATAT CTTTCAAAGATGACGGGACCTACAAGACGCGTGCTGAAGTCAAGTTTGA AGGTGATACCCTTGTTAATCGTATCGAGTTAAAGGGTATTGATTTTAAAG AAGATGGAAACATTCTTGGACACAACTCGAGTACAACCTTAACTCACAC AATGTATACATCACGGCAGACAAACAAAAGAATGGAATCAAAGCTAACTT CAAAATTCGCCACAACGTTGAAGATGGTTCCGTTCAACTAGCAGACCATT ATCAACAAAATACTCCAATTGGCGATGGCCCTGTCTTTTACCAGACAAC CATTACCTGTCGACACAATCTGTCCTTTCGAAAGATCCCAACGAAAAGCG TGACCACATGGTCCTTCTTGAGTTTGTAAGTGTGCTGCTGGGATTACACATG GCATGGATGAGCTCTACAAATAAGGATCTGAAGCTTGGGCCCCGAACAAA AACTCATCTCAGAAGAGGATCTGAATAGCGCCGTCGACCATCATCATCAT CATCATTGAGTTTAAACGGTCTCCAGCTTGGCTGTTTTGGCGGATGAGA GAAGATTTTCAGCCTGATACAGATTAAATCAGAACGCAGAAGCGGTCTG ATAAAACAGAATTTGCCTGGCGGCAGTAGCGCGGTGGTCCCACCTGACC CCATGCCGAACCTCAGAAGTGAAACGCCGTAGCGCCGATGGTAGTGTGG GGTCTCCCCATGCGAGAGTAGGGAATGCCAGGCATCAAATAAAACGAA AGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTGCGTG AACTGGATCCCCAATTATTGAAGGCCGCTAACGCGGCCCTTTTTTTGTTTC TGGTCTGCCTTAATCAATGACTAAGAATTCGCGGCCGCTTCTAGAGCGT CTCACTTCGGATCCGCTGCAGGCTTCTCAAGTCAAAAGCCTCCGGTCGG AGGTTTTTGACTTTCTGCTATGGAGGTCAGGTATGATTTTATGACAACTT GACGGCTACATCATTCACTTTTTCTTCACAACCGGCACGGAACCTCGCTC GGGCTGGCCCCGGTGCATTTTTTAAATACCCGCGAGAAATAGAGTTGAT CGTCAAAACCAACATTGCGACCGACGGTGGCGATAGGCATCCGGGTGG TGCTCAAAAGCAGCTTCGCCTGGCTGATACGTTGGTCCTCGCGCCAGCT TAAGACGCTAATCCCTAACTGCTGGCGGAAAAGATGTGACAGACGCGAC GGCGACAAGCAAACATGCTGTGCGACGCTGGCGATATCAAAATTGCTGT CTGCCAGGTGATCGCTGATGTACTGACAAGCCTCGCGTACCCGATTATC CATCGGTGGATGGAGCGACTCGTTAATCGCTTCCATGCGCCGCAGTAAC AATTGCTCAAGCAGATTTATCGCCAGCAGCTCCGAATAGCGCCCTTCCC CTTGCCCGGCGTTAATGATTTGCCCAAACAGGTCGCTGAAATGCGGCTG GTGCGCTTCATCCGGGCGAAAGAACCCCGTATTGGCAAATATTGACGGC

CAGTTAAGCCATT CATGCCAGTAGGCGCGCGGACGAAAAGTAAACCCACT
GGTGATAACCATTCGCGAGCCTCCGGATGACGACCGTAGTGATGAATCTC
TCCTGGCGGGAACAGCAAAATATCACCCGGTCGGCAAACAAATTCTCGT
CCCTGATTTTTTACCACCCCCTGACCGCGAATGGTGAGATTGAGAATATA
ACCTTTCATTCCCAGCGGTCTGGTCGATAAAAAAATCGAGATAACCGTTG
GCCTCAATCGGCGTTAAACCCGCCACCAGATGGGCATTAAACGAGTATC
CCGGCAGCAGGGGATCATTTTGCCTTCAGCCATACTTTTTCATACTCCC
GCCATTGAGAGAAGAAACCAATTGTCCATATTGCATCAGACATTGCCGTC
ACTGCGTCTTTTACTGGCTCTTCTCGCTAACCAAACCGGTAACCCCGCTT
ATTAAGCATTCTGTAACAAAGCGGGACCAAAGCCATGACAAAAACGC
GTAACAAAAGTGTCTATAATCACGGCAGAAAAGTCCACATTGATTATTG
CACGGCGTCACACTTTGCTATGCCATAACATTTTTATCCATAAGATTAGC
GGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATACC
CGTTTTTTTGGGCTAGCCGACCTGTGGACGGATAAAGTCAGTTGTGAAA
TAAACGCGGCCATAGGCCGCGTAGTTCAGTCCGTATAGGCAGCTAAGA
AAATGTGAACTGTATACATTCCCCGCAAGATAAAGGTGTAGAAATGTGA
GAGCGAAATGAGATGAGCGTTTTGTGAAATAAACGCGGCCATAGGCCGC
GTAGTTCAGTCCGTATAGGCAGCTAAGAAAAATGTGAACTGTATACATT
CCCCGCAAGATAAAGGTGTAGAAATGTGAGAGCGAAATGAGATGAGTTC
CTTGTGAAATAAACGCGGCCATAGGCCGCGTAGTTCAGTCCGTATAGG
CAGCTAAGAAAAATGTGAACTGTATACATTCCCCGCAAGATAAAGGTGTA
GAAATGTGAGAGCGAAATGAGATGAGTCATTTGTGAAATAAACGCGGCC
ATAGGCCGCGTAGTTCAGTCCGTATAGGCAGCTAAGAAAAATGTGAAC
TGTATACATTCCCCGCAAGATAAAGGTGTAGAAATGTGAGAGCGAAATG
AGATGAGAAATGTTGTGAAATAAACGCGGCCATAGGCCGCGTAGTTCAGT
GCCGTATAGGCAGCTAAGAAAAATGTGAACTGTATACATTCCCCGCAAG
ATAAAGGTGTAGAAATGTGAGAGCGAAATGAGATGAGCGTTTTGTGAAAT
AAACGCGGCCATAGGCCGCGTAGTTCAGTCCGTATAGGCAGCTAAGAA
AAATGTGAACTGTATACATTCCCCGCAAGATAAAGGTGTAGAAATGTGAG
AGCGAAATGAGATGAGTTCCTTGTGAAATAAACGCGGCCATAGGCCGCG
TAGTTCAGTCCGTATAGGCAGCTAAGAAAAATGTGAACTGTATACATT
CCCCGCAAGATAAAGGTGTAGAAATGTGAGAGCGAAATGAGATGAGTCAT
TTGTGAAATAAACGCGGCCATAGGCCGCGTAGTTCAGTCCGTATAGGC
AGCTAAGAAAAATGTGAACTGTATACATTCCCCGCAAGATAAAGGTGTAG
AAATGTGAGAGCGAAATGAGATGAGTGGCCAAAGCCCGCCGAAAGGCG
GGCTTTTTTTTTGCTGTTCTTGAGACGTACTAGTAGGCCGCTGCAGTTAAT
CACTGATTAACTCGGTACCAAATTCAGAAAAAGAGGCCTCCCGAAAGGG
GGGCCTTTTTTTCGTTTTGGTCCCTCGAGTCTAGACTGCAGTTGATCGGC
GTTAATATTTTGTAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTT
TTAACCAATAGGCCGACTGCGATGAGTGGCAGGGCGGGGCGTAATTTTT
TTAAGGCAGTTATTGGTGCCCTTAAACGCGTTCCTTACAGCTAGCTCAGT
CCTAGGTATTATGCTAGCAGATCTAAAGAGGAGAAAGGATCTATGGACC
ACTACCTCGACATTGCTTGCACCGGACCCGGAATTTCCCCCGGCGCA

ACTCATGAGCGTGCTCTTCGGCAAGCTCCACCAGGCCCTGGTGGCACA
GGGCGGGGACAGGATCGGCGTGAGCTTCCCCGACCTCGACGAAAGCC
GCTCCCGGCTGGGCGAGCGCCTGCGCATTTCATGCCTCGGCGGACGAC
CTTCGTGCCCTGCTCGCCCGGCCCTGGCTGGAAGGGTTGCGGGACCAT
CTGCAATTCGGAGAACCGGCAGTCGTGCCTCACCCCACACCGTACCGT
CAGGTCAGTCGGGTTTCAGGCGAAAAGCAATCCGGAACGCCTGCGGGCGG
CGGCTCATGCGCCGGCACGATCTGAGTGAGGAGGAGGCTCGGAAACGC
ATTCCCGATACGGTCGCGAGAGCCTTGACCTGCCCTTCGTACGCTAC
GCAGCCAGAGCACCGGACAGCACTTCCGTCTCTTCATCCGCCACGGGC
CGTTGCAGGTGACGGCAGAGGAAGGAGGATTACCTGTTACGGGTTGA
GCAAAGGAGGTTTCGTTCCTGGTTCTGATAACTCGAGTAAGGATCTCC
AGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTT
TTATCTGTTGTTTGTGCGGTGAACCTACTCGAGTCTAGACTGCAGCTGAAA
CTGGTGCTACGCCTGAATAAGTGATAATAAGCGGATGAATGGCAGAAAT
TCGAAAGCAAATTCGACCCGGTCGTGCGTTTCAGGGCAGGGTCGTTAAAT
AGCCGCTTATGTCTATTGCTGGTTTACCGGTTTATTGACTACCGGAAGCA
GTGTGACCGTGTGCTTCTCAAATGCCTGAGGCCAGTTTGCTCAGGCTCT
CCCCGTGGAGGTAATAATTGACGATATGATCATTATTCTGCCTCCCAGA
GCCTGATAAAAACGGTGAATCCGTTAGCGAGGTGCCGCCGGCTTCCATT
CAGGTCGAGGTGGCCCGGCTCCATGCACCGCGACGCAACGCGGGGAG
GCAGACAAGGTATAGGGCGGGCAGGCGGCTACAGCCGATAGTCTGGAA
CAGCGCACTTACGGGTTGCTGCGCAACCCAAGTGCTACCGGCGCGGCA
GCGTGACCCGTGTGCGCGGCTCCAACGGCTCGCCATCGTCCAGAAAAC
ACGGCTCATCGGGCATCGGCAGGCGCTGCTGCCCGCGCCGTTCCCAT
CCTCCGTTTCGGTCAAGGCTGGCAGGTCTGGTTCCATGCCCGGAATGC
CGGGCTGGCTGGGCGGCTCCTCGCCGGGGCCGGTCGGTAGTTGCTGC
TCGCCCGGATACAGGGTCGGGATGCGGCGCAGGTGCGCATGCCCAAC
AGCGATTCTGCCTGGTCGTCGTGATCAACCACCACGGCGGCACTGAACA
CCGACAGGCGCAACTGGTCGCGGGGCTGGCCCCACGCCACGCGGTCA
TTGACCACGTAGGCCGACACGGTGCCGGGGCCGTTGAGCTTCACGACG
GAGATCCAGCGCTCGGCCACCAAGTCCTTGACTGCGTATTGGACCGTCC
GCAAAGAACGTCCGATGAGCTTGAAAGTGCTTCTGGCTGACCACCAC
GGCGTTCTGGTGGCCATCTGCGCCACGAGGTGATGCAGCAGCATTGC
CGCCGTGGGTTTCTCGCAATAAGCCCGGCCACGCCTCATGCGCTTT
GCGTTCCGTTTGACCCAGTGACCGGGCTTGTTCTTGGCTTGAATGCCG
ATTTCTCTGGAATGCGTGCCATGCTTATCTCCATGCGGTAGGGTGCCG
CACGGTTGCGGCACCATGCGCAATCAGCTGCAACTTTTCGGCAGCGCG
ACAACAATTATGCGTTGCGTAAAAGTGGCAGTCAATTACAGATTTTCTTTA
ACCTACGCAATGAGCTATTGCGGGGGGTGCCGCAATGAGCTGTTGCGT
ACCCCCCTTTTTTAAGTTGTTGATTTTTTAAGTCTTTCGCATTTCCGCCAT
ATCTAGTTCTTTGGTGCCCAAGAAGGGCACCCCTGCGGGGTTCCCCCA
CGCCTTCGGCGCGGCTCCCCCTCCGGCAAAAAGTGGCCCTCCGGGG
CTTGTTGATCGACTGCGCGGCCTTCGGCCTTGCCCAAGGTGGCGCTGC

CCCCTTGGAACCCCCGCACTCGCCGCCGTGAGGCTCGGGGGGCAGGC
GGGCGGGCTTCGCCTTCGACTGCCCCCACTCGCATAGGCTTGGGTCTG
TCCAGGCGCGTCAAGGCCAAGCCGCTGCGCGGTCTGCTGCGCGAGCCT
GACCCGCCTTCCACTTGGTGTCCAACCGGCAAGCGAAGCGCGCAGGCC
GCAGGCCGGAGGCTTTTCCCCAGAGAAAATTAAAAAAATTGATGGGGCA
AGGCCGCAGGCCGCGCAGTTGGAGCCGGTGGGTATGTGGTCTGAAGGC
TGGGTAGCCGGTGGGCAATCCCTGTGGTCAAGCTCGTGGGCAGGCCGA
GCCTGTCCATCAGCTTGTCCAGCAGGGTTGTCCACGGGCGGAGCGAAG
CGAGCCAGCCGGTGGCCGCTCGCGGCCATCGTCCACATATCCACGGGC
TGGCAAGGGAGCGCAGCGACCGCGCAGGGCGAAGCCCGGAGAGCAAG
CCCGTAGGGCGCCGCAAGCCGCCGTAGGCGGTACGACTTTGCGAAGCA
AAGTCTAGTGAGTATACTCAAGCATTGAGTGGCCCCGCCGGAGGCACCG
CCTTGCGCTGCCCCCGTCGAGCCGTTGGACACCAAAGGGAGGGGCA
GGCATGGCGGCATACGCGATCATGCGATGCAAGAAGCTGGCGAAAATG
GGCAACGTGGCGGCCAGTCTCAAGCACGCCTACCGCGAGCGCGAGAC
GCCCAACGCTGACGCCAGCAGGACGCCAGAGAACGAGCACTGGGCGG
CCAGCAGCACCGATGAAGCGATGGGCCGACTGCGCGAGTTGCTGCCAG
AGAAGCGGCGCAAGGACGCTGTGTTGGCGGTGAGTACGTCATGACGG
CCAGCCCGGAATGGTGGAAGTCGGCCAGCCAAGAACAGCAGGCGGGC
TTCTTCGAGAAGGCGCACAAAGTGGCTGGCGGACAAGTACGGGGCGGAT
CGCATCGTGACGGCCAGCATCCACCGTGACGAAACCAGCCCGCACATG
ACCGCGTTCTGTGGTGGCGCTGACGCAGGACGGCAGGCTGTGCGCCAA
GGAGTTCATCGGCAACAAAGCGCAGATGACCCGCGACCAGACCAGTT
TGCGGCCGCTGTGGCCGATCTAGGGCTGCAACGGGGCATCGAGGGCA
GCAAGGCACGTCACACGCGCATTCAAGCGTTCTACGAGGGCCCTGGAGC
GGCCACCAGTGGGCCACGTCACCATCAGCCCGCAAGCGGTCTGAGCCAC
GCGCCTATGCACCGCAGGGATTGGCCGAAAAGCTGGGAATCTCAAAGC
GCGTTGAGACGCCGGAAGCCGTGGCCGACCGGCTGACAAAAGCGGTT
GGCAGGGGTATGAGCCTGCCCTACAGGCCGCGCAGGAGCGCGTGAG
ATGCGCAAGAAGGCCGATCAAGCCCAAGAGACGGCCCGAGACCTTCGG
GAGCGCCTGAAGCCCGTTCTGGACGCCCTGGGGCCGTTGAATCGGGAT
ATGCAGGCCAAGGCCGCGCGATCATCAAGGCCGTGGGCGAAAAGCTG
CTGACGGAACAGCGGGAAGTCCAGCGCCAGAAACAGGCCCAGCGCCA
GCAGGAACGCGGGCGCGCACATTTCCCCGAAAAGTGCCACCTGGGATG
AATGTCAGCTACTGGGCTATCTGGACAAGGGAAAACGCAAGCGCAAAGA
GAAAGCAGGTAGCTTGCAGTGGGCTTACATGGCGATAGCTAGACTGGG
CGGTTTATGGACAGCAAGCGAACCGBAATTGCCAGCTGGGGCGCCCT
CTGGTAAGGTTGGGAAGCCCTGCAAAGTAACTGGATGGCTTTCTTGCC
GCCAAGGATCTGATGGCGCAGGGGATCAAGATCTGATCAAGAGACAGG
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CATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGG
GGTTCGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGAGATTT
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CGGGACGCCGGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAG
TTCTTCGCCCACCCCCATGGGCAAATATTATACGCAAGGCGACAAGGTG
CTGATGCCGCTGGCGATTGAGGTTTCATCATGCCGTTTGTGATGGCTTCC
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GGCAGGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAA
TTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCCTAGATTTTCAGTG
CAATTTATCTCTTCAAATGTAGCACCTGAAGTCAGCCCCATACGATATAA
GTTGTAATTCTCATGTTTGACAGCTTATCATCGATAAGCTTCCGATGGCG
CGCCGAGAGGCTTTACACTTTATGCTTCCGGCT

Supplementary Table 3. SP6 promoter sequences used in this study.

Promoter	Sequence (5' to 3')
pSP6_wt	ATTTAGGTGACACTATAGGG
pSP6_90	ATTTAGGTGCCACTATAGGG
pSP6_50	ATTTAGGTGACACTATGGGG
pSP6_20	ATTTAGGTGACATTATAGGG

Supplementary Table 4. Golden Gate assembly primers and overhangs. An example of the 8-copy RNA array assembly. BsmBI site: **GAGACC**, BsaI site: **GGTCTC**, Overhang: **ctcg**. Table corresponds to the naming conventions shown in Supplementary Figure 2. We note that some of the parts indicated in the table below (e.g., spacer, connectors) are not included in Supplementary Figure 2 for simplicity. The top section of the table indicates how to construct a 4-copy STAR array modular cloning (MoClo) part for position 3b. Position 4 can be used to create another 4-copy STAR array MoClo part using the same overhang logic indicated in the top section of the table. If constructing a multiplex array (Figure 4D) the STAR identity in positions 3b and 4 can be varied.

Assembly parts	Sequence (5' to 3')
Level 1 => Level 2 (Position 3b)	
Backbone	- CGTCTC aa GAGACC - backbone - GGTCTC gt GAGACC - GFP dropout -
PCR products 1	gt CGTCTC actcgctcag - Insulator+STAR - cgttt GAGACC tg
PCR products 2	gt CGTCTC acggtt - Insulator+STAR - ttctt GAGACC tg
PCR products 3	gt CGTCTC attcc - Insulator+STAR - tcatt GAGACC tg
PCR products 4	gt CGTCTC atcat - Insulator+STAR - aatgagagt GAGACC tg
Level 2 => Level 3	
Position 1	GGTCTC gacgg - Connector* - ctcca GAGACC
Position 2	GGTCTC gcttc - Promoter - cgcaa GAGACC
Position 3a	GGTCTC gcgca - Spacer/Target - tcaga GAGACC
Position 3b	GGTCTC gtcag - Inserts - aatga GAGACC
Position 4	GGTCTC gaatg - Inserts - tggca GAGACC
Position 5	GGTCTC gtggc - Terminator - gctga GAGACC
Position 6	GGTCTC ggctg - Connector - taca GAGACC
Position 7	GGTCTC gtaca - Resistance & Origin of replication - accga GAGACC

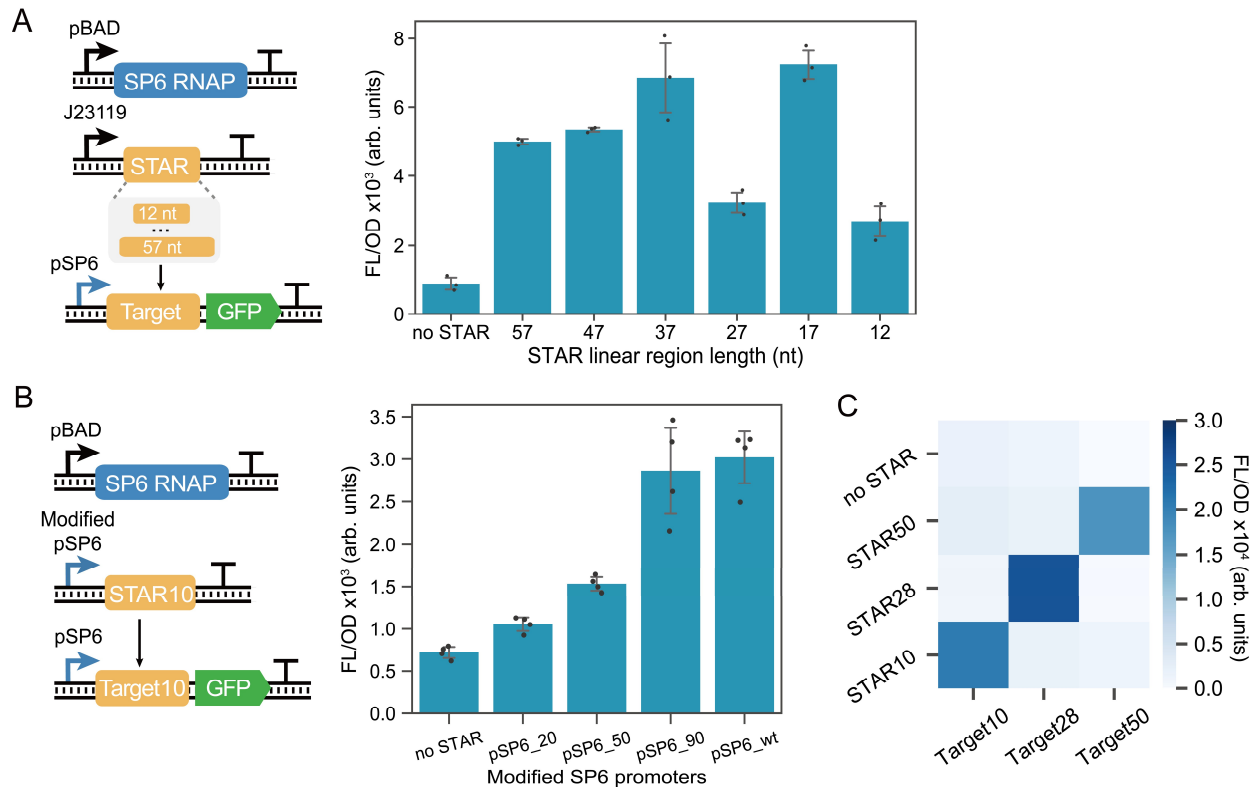
*Connectors contain BsmBI sites allowing further assembly

Supplementary Table 5. Strains, growth conditions, and electroporation settings used in this study.

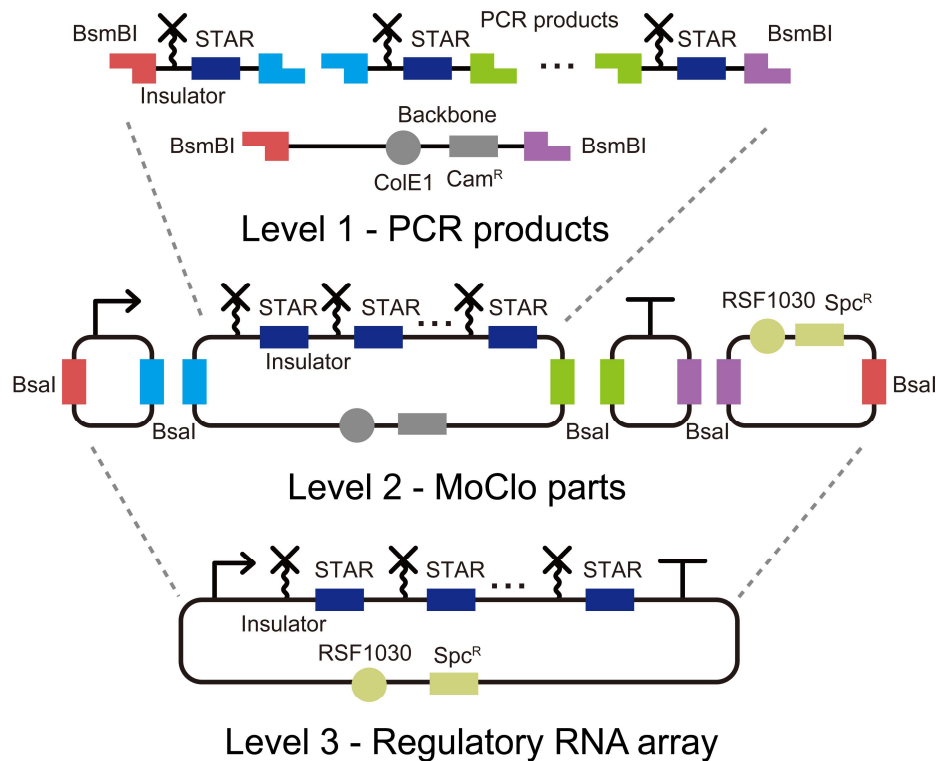
Species	Strain name	Culture media	Culture temperature (°C)	Kanamycin conc. (µg/mL)	Electroporation setting (kV)
<i>Escherichia coli</i>	NEB Turbo (cloning strain)	LB	37	100	-
<i>Escherichia coli</i>	TG1	LB	37	100	-
<i>Shewanella oneidensis</i>	MR-1	LB	30	50	1.2
<i>Pseudomonas fluorescens</i>	A506	LB	30	50	2.5
<i>Pseudomonas putida</i>	F1	LB	30	50	1.25
<i>Pseudomonas stutzeri</i>	JM300	LB	30	50	2.5
<i>Vibrio natriegens</i>	Vmax	LB3	37	200	0.7

Supplementary Table 6. Regulatory RNA array insulator sequences.

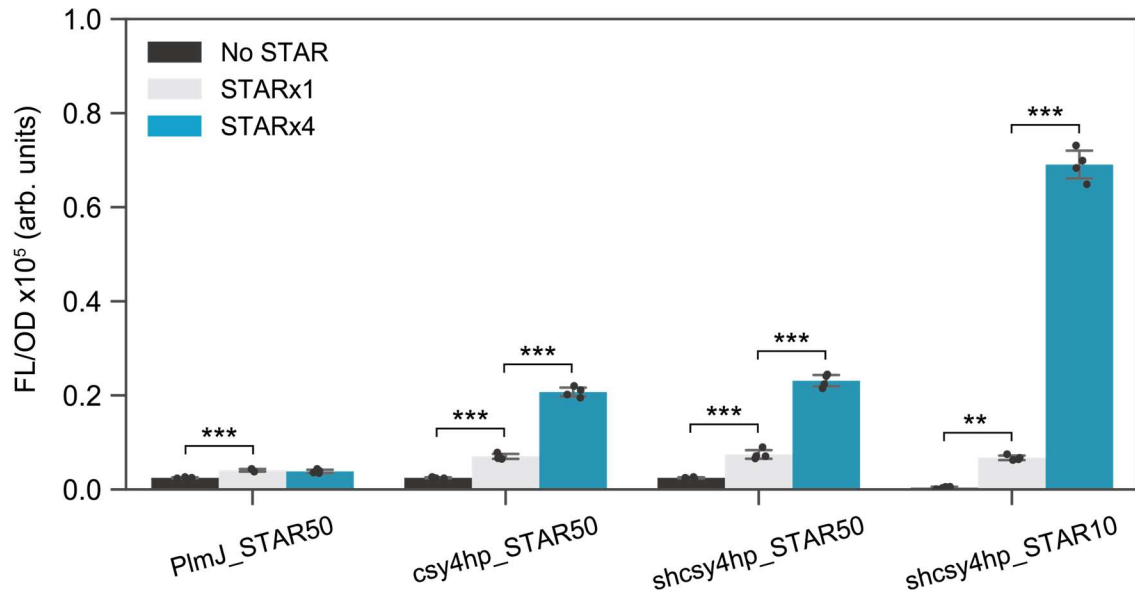
Insulator	Sequence (5' to 3')
PlmJ	AGUCAUAAGUCUGGGCUAAGCCCACUGAUGAGUCGCUGAAAUGCGACG AAACUUAUGA
csy4hp	GUUCACUGCCGUAUAGGCAGCUAAGAAA
shcsy4hp	UUGUGAAAUAAACGCGGCCAUAGGCCGCGUAGUUCACUGCCGUAUAGG CAGCUAAGAAA
PlmJ- SATR50x4 (Example sequence used for NUPACK simulation)	AGUCAUAAGUCUGGGCUAAGCCCACUGAUGAGUCGCUGAAAUGCGACG AAACUUAUGAUGAACUGUAUACAUUCCCCGCAAAGUGCCUAUCUGUCG UCGUGUUAUCUUUAUGUUUCUGGCGUUAGUCAUAAGUCUGGGCUAAG CCCACUGAUGAGUCGCUGAAAUGCGACGAAACUUAUGAUGAACUGUAU ACAUUCCCCGCAAAGUGCCUAUCUGUCGUCGUGUUAUCUUUAUGUUUC UGGUUCCAGUCAUAAGUCUGGGCUAAGCCCACUGAUGAGUCGCUGAAA UGCGACGAAACUUAUGAUGAACUGUAUACAUUCCCCGCAAAGUGCCUA UCUGUCGUCGUGUUAUCUUUAUGUUUCUGGUCAUAGUCAUAAGUCUG GGCUAAGCCCACUGAUGAGUCGCUGAAAUGCGACGAAACUUAUGAUGA ACUGUAUACAUUCCCCGCAAAGUGCCUAUCUGUCGUCGUGUUAUCUUU AUGUUUCUGG



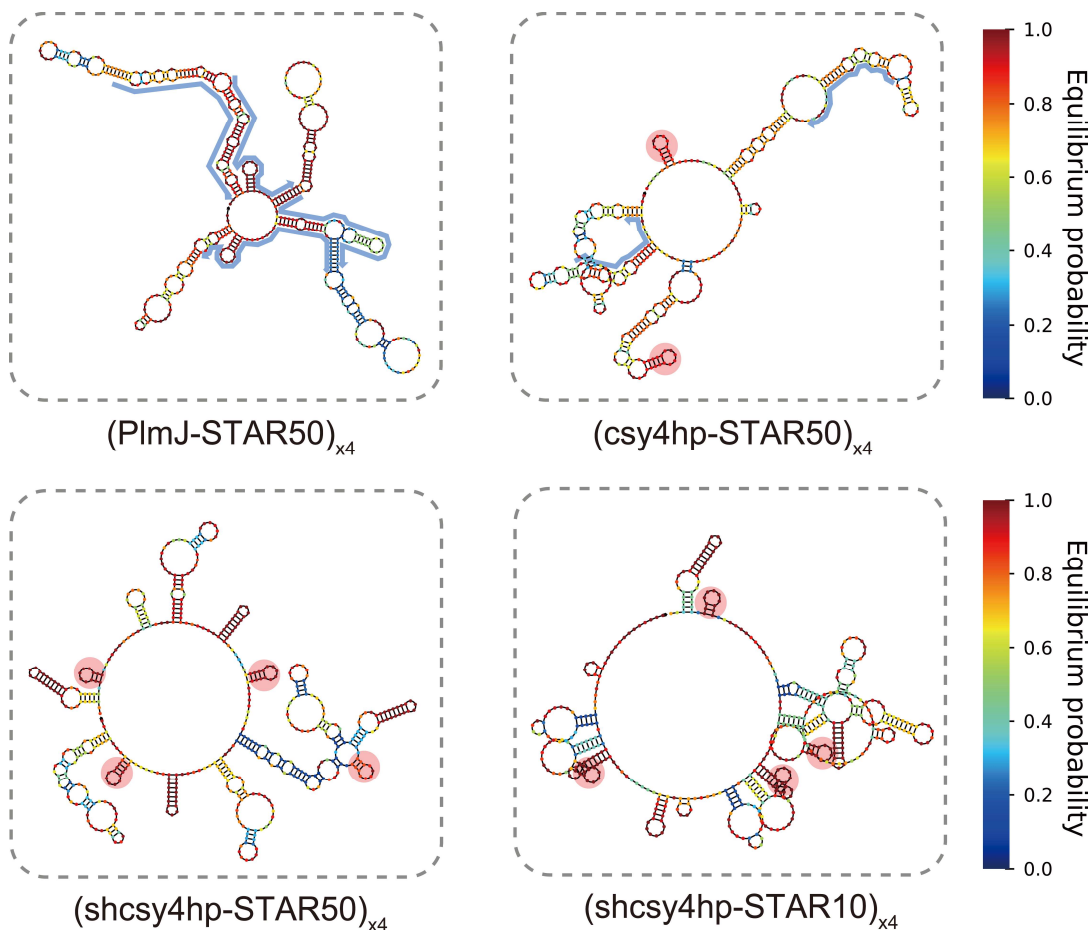
Supplementary Fig. 1. Characterization of a STAR system that uses the SP6 RNA polymerase. (A) Investigating the impact of length on the SP6 STAR system. STAR systems with different length linear regions (12 to 57 nucleotides [nt]) are used to activate a target RNA-controlled GFP that is transcribed using an SP6 promoter. The left panel shows a schematic of genetic circuitry and the right panel shows fluorescent characterization in *E. coli* cells transformed with corresponding plasmids. The results show a length of ~40 nt to be optimal which is comparable to prior STAR characterization results using the native *E. coli* RNAP¹. Bars show mean values and error bars represent s.d. of $n = 3$ biological replicates shown as points. (B) Investigating the tunability of SP6-controlled STARs. STAR systems transcribed using only SP6 RNAP. The left panel shows a schematic of genetic circuitry and the right panel shows fluorescent characterization in *E. coli* cells transformed with corresponding plasmids. Variable strength SP6 promoters² are used to produce the STAR and a constant SP6 promoter is used to produce the target RNA. Bars show mean values and error bars represent s.d. of $n = 4$ biological replicates shown as points. (C) Investigating the orthogonality of SP6 STARs. Fluorescent characterization of cells transformed with different combinations of plasmids encoding cognate and non-cognate STAR and target RNA pairs. Cognate pairs are across the diagonal and the no STAR control is in the top row. Heatmap shows the mean values of $n = 4$ biological replicates.



Supplementary Fig. 2. A modular cloning (MoClo) approach for regulatory RNA arrays. The regulatory RNA array is constructed through a 3-level modular cloning approach. PCR products are made with primers containing the recognition site for type 2 restriction enzyme BsmBI. The copy number of STARs can be altered by adjusting the number of PCR fragments with different overhangs. The PCR products are then assembled as MoClo part plasmids containing BsaI sites for long-term storage. Next, the MoClo parts are further assembled as the target plasmid with full regulatory RNA array cassette. We note that some of the parts indicated in Supplementary Table 4 (e.g., spacer, connectors) are not included in this for simplicity.

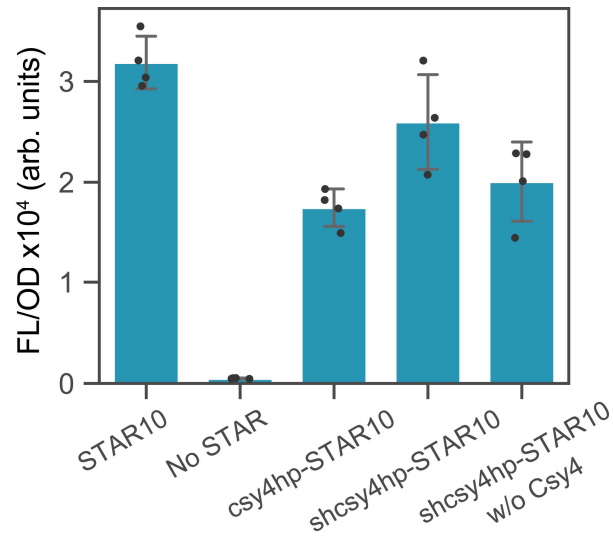


Supplementary Fig. 3. Insulator screening for regulatory RNA arrays. Fluorescent characterization of regulatory RNA arrays using PlmJ, csy4 hairpin, strong-hairpin csy4 hairpin as insulators. STAR50 and STAR10 arrays are tested with either x1, x4 or no copies. Bars show mean values and error bars represent s.d. of $n = 4$ biological replicates shown as points. One-way ANOVA with post-hoc Tukey test were used and the significance between groups are marked by asterisks indicating $p < .05$ (*), $p < .01$ (**), $p < .001$ (***).

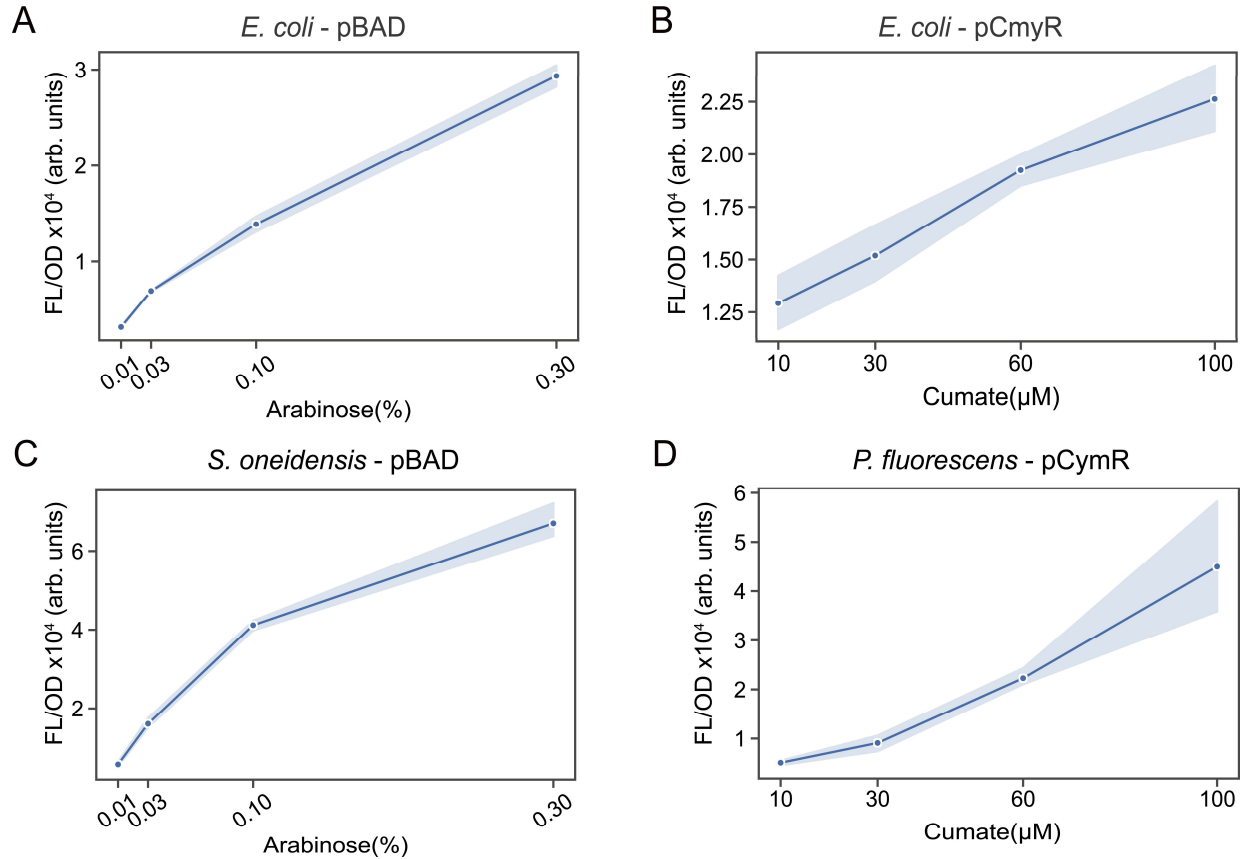


Supplementary Fig. 4. NUPACK analysis of regulatory RNA arrays with different insulators.

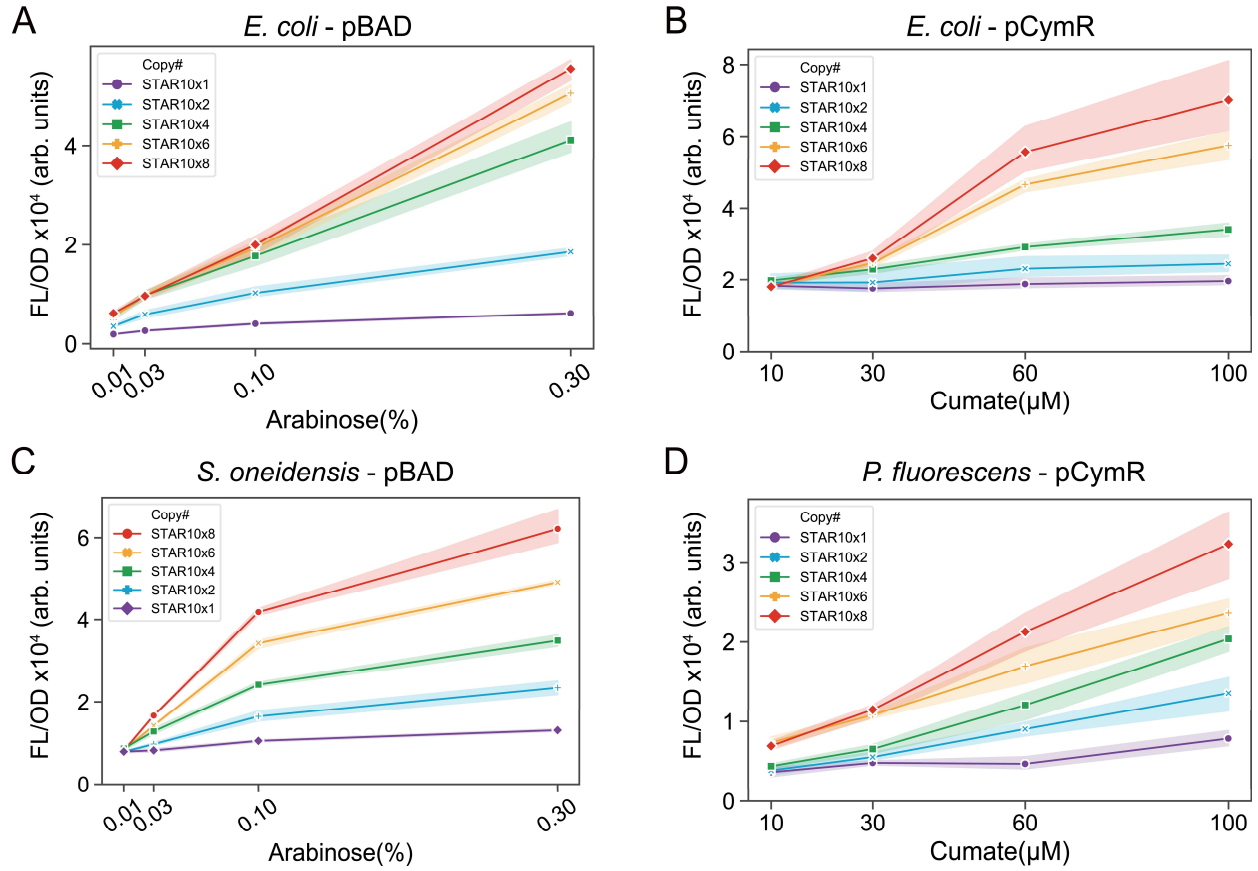
Secondary structure predictions of 4-copy STAR arrays insulated with ribozyme PlmJ, ribonuclease site *csy4* (*csy4hp*), and a strong-hairpin *csy4* (*shcsy4hp*). Predictions use the online version of NUPACK³. The color of each nucleotide represents the equilibrium probability for each nucleotide to be in that state (e.g., paired/unpaired). Correctly folded insulator structures are labeled with red shading and insulator sequences that do not form the expected structure are indicated with blue shading.



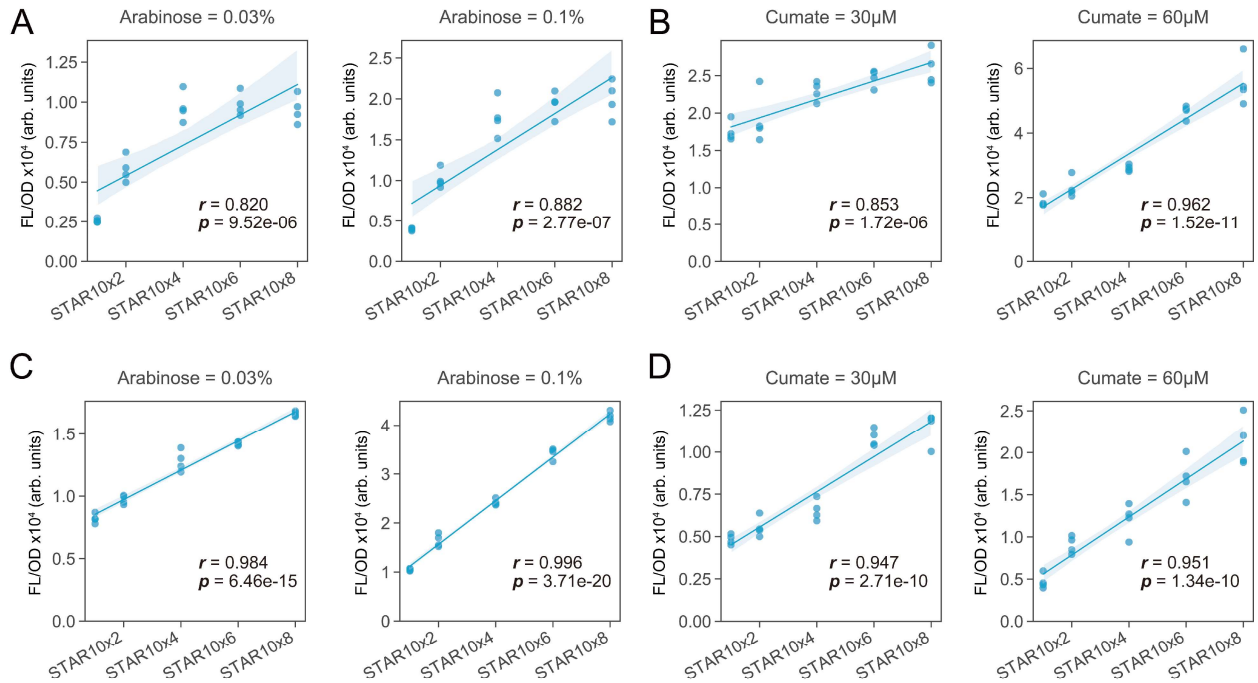
Supplementary Fig. 5. Impact of insulators on STAR activation. Fluorescent characterization of a 3-plasmid system containing STAR, Target-GFP, and Csy4 endonuclease. From left to right: STAR10 without insulator, no STAR, STAR10 with csy4hp, STAR10 with shcsy4hp, and STAR10 with shcsy4hp but without Csy4 endonuclease. Bars show mean values and error bars represent s.d. of $n = 4$ biological replicates shown as points.



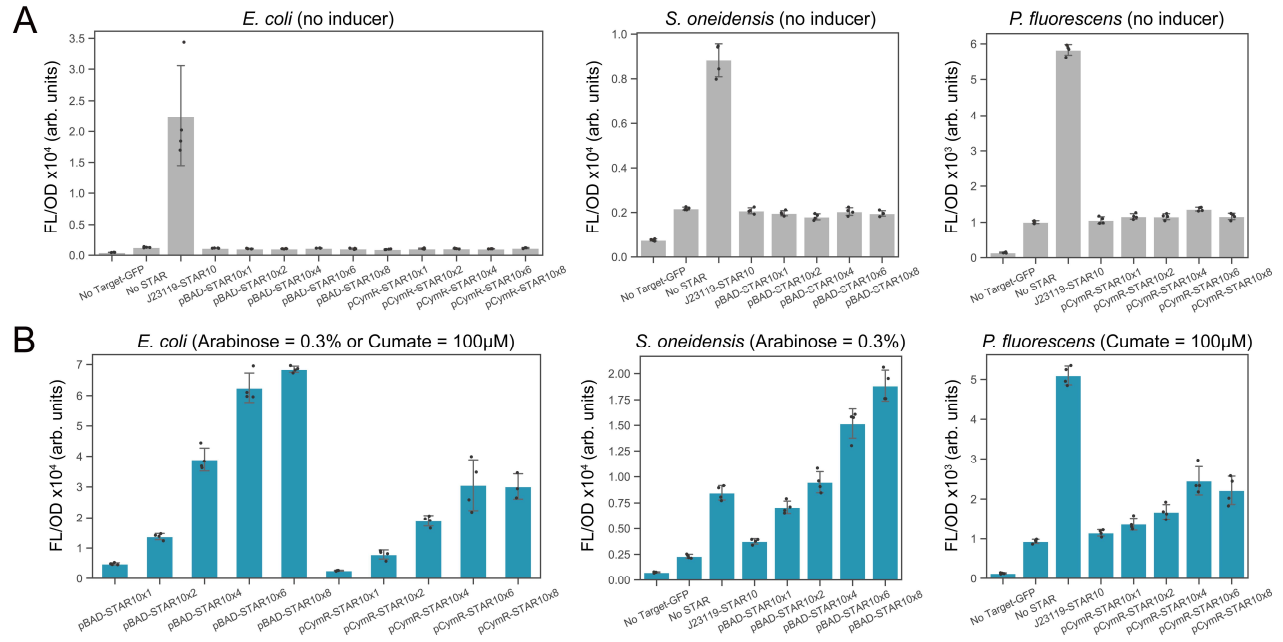
Supplementary Fig. 6. Calibration curves of inducible promoters to relate promoter strength to transcriptional output in different bacterial species. Fluorescent characterization of cells transformed with plasmids encoding a GFP driven by either pCymR or pBAD under different concentrations of inducer. Graphs show data from (A) pBAD in *E. coli*, (B) pCymR in *E. coli*, (C) pBAD in *S. oneidensis*, and (D) pCymR in *P. fluorescens*. Each point shows the mean value and the shade represents s.d. of $n = 4$ biological replicates.



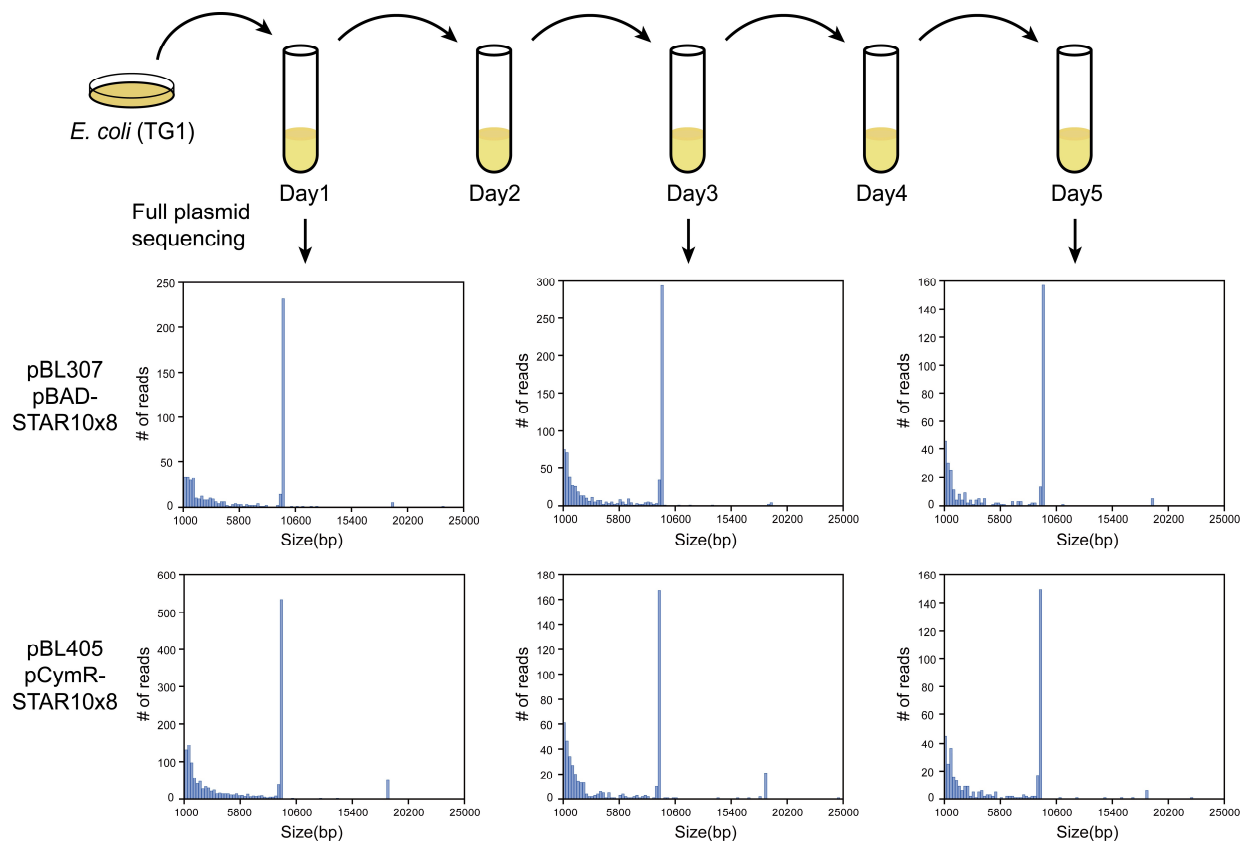
Supplementary Fig. 7. The induction curves of the regulatory RNA arrays before the promoter activity normalization. Fluorescent characterization of cells transformed with plasmids encoding regulatory RNA arrays with 1, 2, 4, 6, and 8 copies of STARs driven by either pCymR or pBAD under different concentrations of inducer. Graphs show data from (A) pBAD in *E. coli*, (B) pCymR in *E. coli*, (C) pBAD in *S. oneidensis*, and (D) pCmyR in *P. fluorescens*. Each point shows the mean value and the shade represents s.d. of $n = 4$ biological replicates.



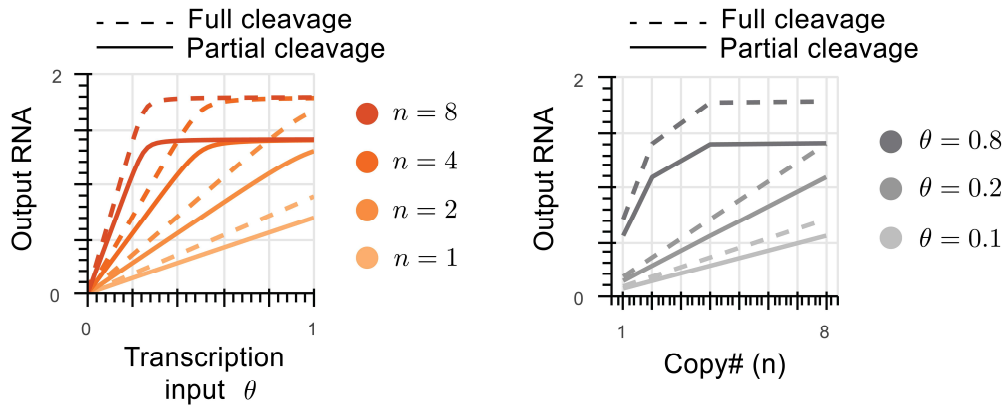
Supplementary Fig. 8. Relation between STAR copy and fluorescent output across different transcription input levels. The correlation between the STAR copy number in the RNA array and the corresponding fluorescent output with different inducer concentrations. Graphs show data from (A) pBAD in *E. coli*, (B) pCymR in *E. coli*, (C) pBAD in *S. oneidensis*, and (D) pCmyR in *P. fluorescens*. Pearson correlation coefficient and p-value were calculated and shown as r and p .



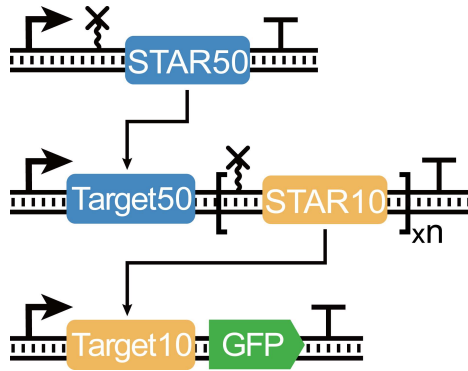
Supplementary Fig. 9. Characterization of leakiness in regulatory RNA arrays using different STAR copies. (A) The full set of inducible regulatory RNA arrays were tested without induction together with no target-GFP control, no STAR control, and constitutive STAR control in *E. coli*, *S. oneidensis*, and *P. fluorescens*. (B) A parallel test was done with 0.3% arabinose or 100 μM cumate as positive control. Bars show mean values and error bars represent s.d. of $n = 4$ biological replicates shown as points.



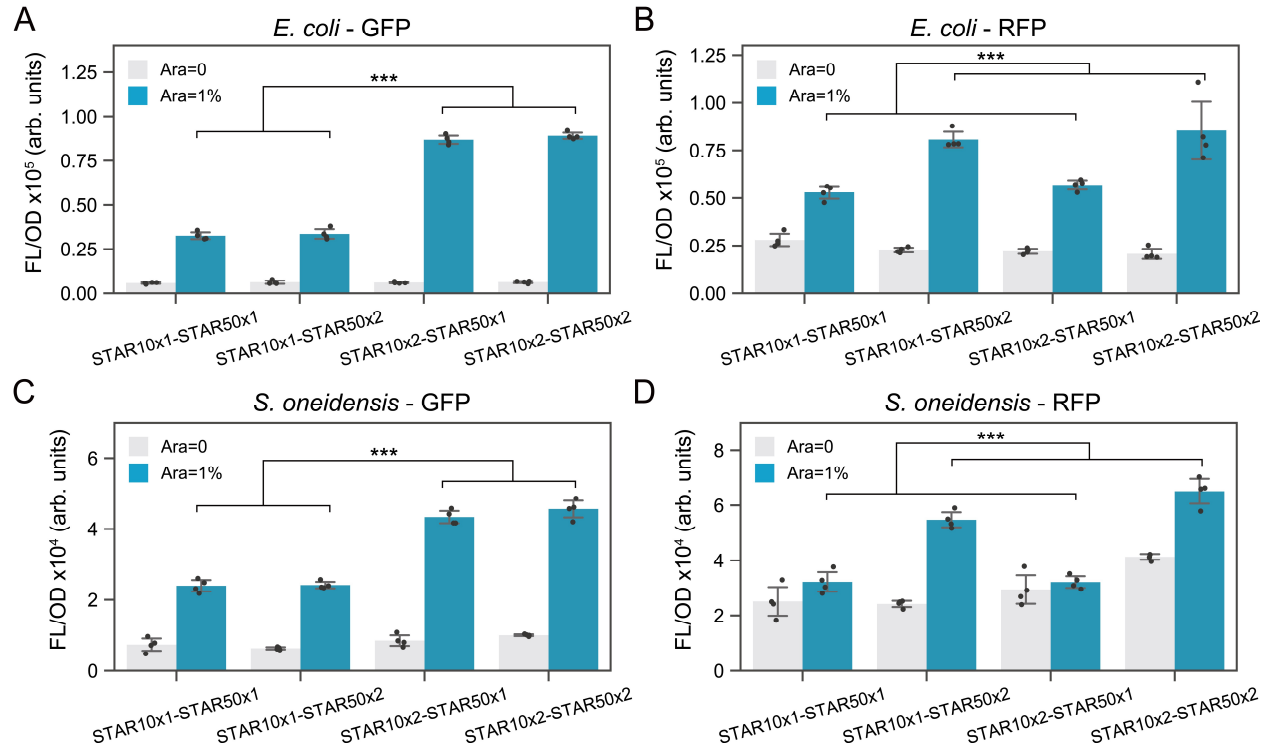
Supplementary Fig. 10. Genetic stability test of regulatory RNA array. Plasmids with pBAD-STAR10x8 and pCymR-STAR10x8 were transformed into *E. coli* (TG1) and cultured overnight on LB plate with kanamycin. Single colonies were picked and inoculated in the culture tubes containing 5 mL LB with kanamycin at 37 °C. The bacteria were successively inoculated (1:1000 dilution) and cultured over 5 days. The plasmids were extracted through mini-prep and sent for full plasmid sequencing (plasmidsaurus) on day 1, 3, and 5. The size distributions of plasmid sequencing reads are shown in the bar plots. This analysis showed comparable sequencing results across the days tested and no mutations in RNA arrays were observed. The sequencing data is included in Supplementary Data 2 file.



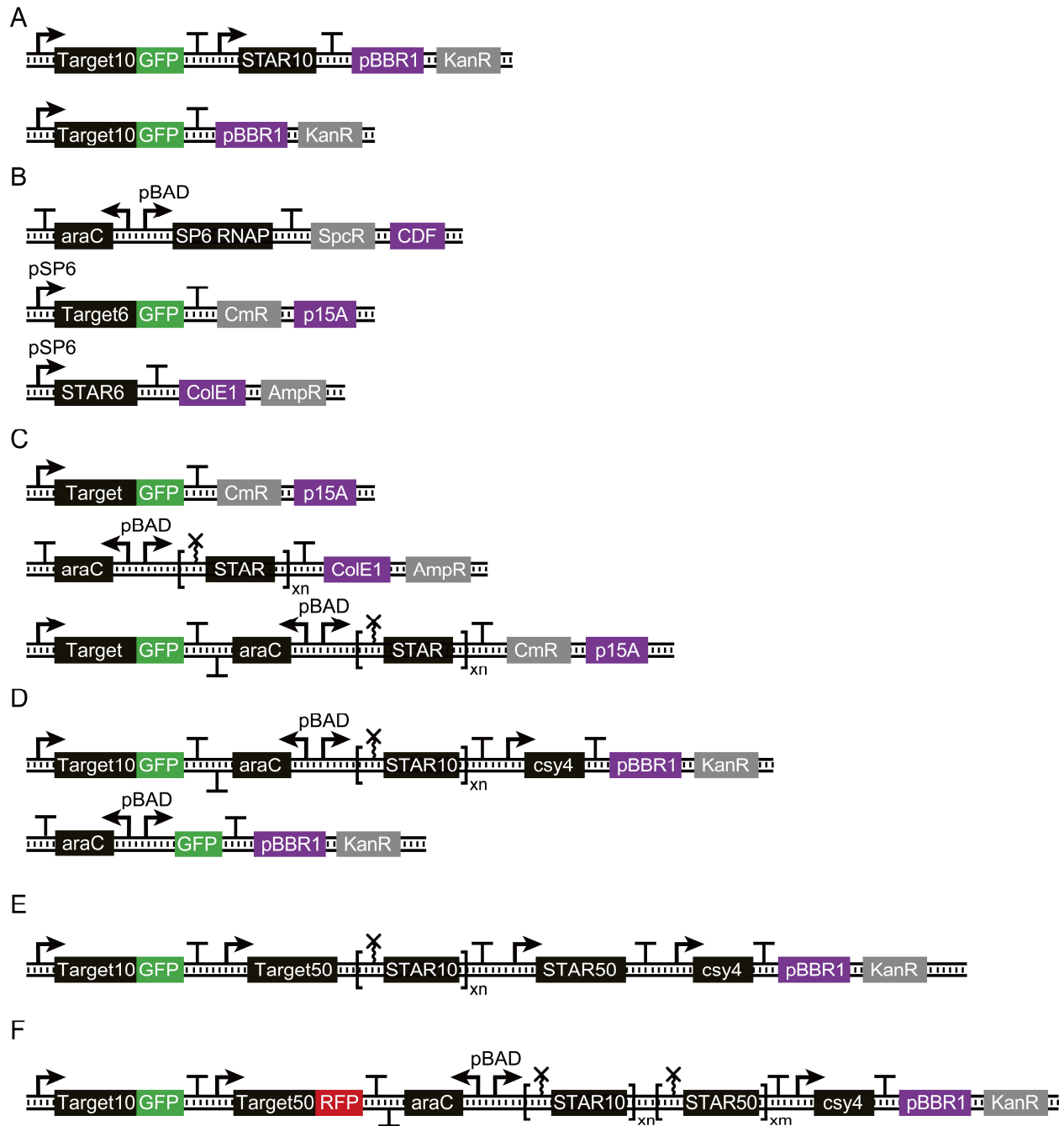
Supplementary Fig. 11. Model predictions of regulatory RNA arrays. (Left panel) The input-output behavior between transcription input and output RNA for multiple STAR from 1 to 8 copies. The dotted line indicates full cleavage and the solid line indicates partial cleavage of the RNA array. (Right panel) The relationship between number of repeats and output for three different transcriptional inputs from $\theta = 0.1$ to 0.8.



Supplementary Fig. 12. The schematic of an RNA activation-activation cascade. The RNA cascade is built with an orthogonal pair STAR10 and STAR50. STAR50 is constitutively transcribed and activates the transcription of the regulatory RNA array composed of variable copies of STAR10. STAR10 production leads to the activation of Target10, which in turn activates the production of GFP.



Supplementary Fig. 13. Fluorescent characterization of multiplex RNA arrays. Fluorescent characterization of the multiplex RNA arrays with the 2x2 matrix of 4 possible STAR10 and STAR50 combinations. The graphs show uninduced and induced (arabinose=1%) data from (A) GFP characterization in *E. coli*, (B) RFP characterization in *E. coli*, (C) GFP characterization in *S. oneidensis*, (D) RFP characterization in *S. oneidensis*. Bars show mean values and error bars represent s.d. of $n = 4$ biological replicates shown as points. Two-way ANOVA (STAR10x1/x2 and STAR50x1/x2 as two categorical variables) with post-hoc Tukey test were used and the significance between groups are marked by asterisks indicating $p < .05$ (*), $p < .01$ (**), $p < .001$ (***).

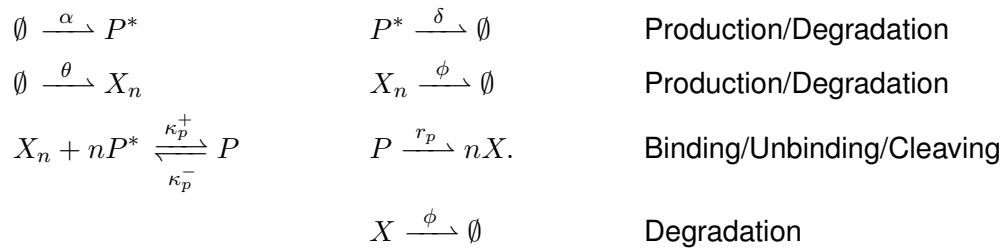


Supplementary Fig. 14. The schematics of representative plasmids used in this study. The example plasmid set used in (A) STAR for Gram-negative RNAP, (B) SP6 RNAP-driven STAR, (C) regulatory RNA array insulation screening, (D) regulatory RNA array and standard inducible GFP on broad-host range plasmid, (E) single-plasmid RNA cascade, (F) multiplex regulatory RNA array.

Supplementary Note 1. Mathematical model of regulatory RNA arrays.

The model consists of two main process: (1) Csy4 processing of the RNA array and (2) the regulation of small transcription activating RNA (STAR) system. For the rest of this note, we will use capital letter to indicate chemical species, and lower case to denote the corresponding concentration. For example, species X has concentration x .

Modeling Csy4 cleaving. First, we define P^* to describe the unbound molecular specie Csy4, P for bound Csy4 to RNA array, and X_n for the full RNA array with n repeats. We consider a production rate constant α and θ for P^* and X_n respectively. A degradation rate constant δ and ϕ for P^* and X_n . n unbound proteins P^* can bind/unbinding to a single RNA array X_n and form a complex P with a rate constant k_p^+ and k_p^- . Finally, the state P can cleave and produce n copies of STAR X at a rate constant r_p , which decays at a rate constant ϕ . We summarize the reactions below:



As a result, we can write down the Ordinary Differential Equations (ODEs) by using the law of mass action:

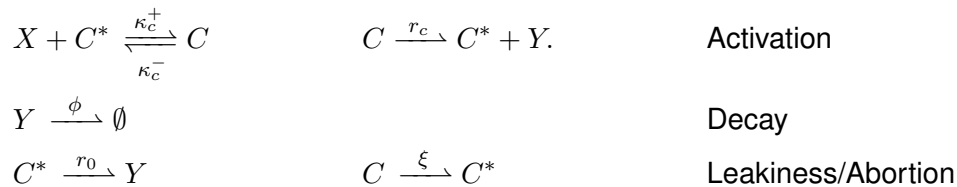
$$\dot{p}^* = \alpha - \delta p^* - n * k_p^+ x_n p^* + n k_p^- p \quad (1)$$

$$\dot{x}_n = \theta - \phi x_n - k_p^+ x_n p^* + k_p^- p \quad (2)$$

$$\dot{p} = k_p^+ x_n p^* - k_p^- p - r_p p - \delta p \quad (3)$$

$$\dot{x} = n r_p p - \phi x \quad (4)$$

Modeling STAR system. A cleaved STAR X can bind to the target RNA complex C^* in which transcription is blocked and form a complex C to activate transcription, with a binding and unbinding rate k_c^+ , and k_c^- . These interactions will modify the model for x , equation (4). The activated complex C then transcribes the output Y at the rate of r_c . Also, we consider leakiness when the blocked transcription complex C^* can produce an output species Y at a rate constant r_0 . Finally, abortion can be incorporated when the activate transcription complex C become C^* at a rate constant ξ . We summarize the chemical reactions below:



Following law mass of action, we can find the ODEs:

$$\dot{x} = n r_p p - \phi x - \kappa_c^+ x c^* + \kappa_c^- c \quad (5)$$

$$\dot{c} = \kappa_c^+ x c^* - \kappa_c^- c - r_c c - \xi c \quad (6)$$

$$\dot{y} = r_0 c^* + r_c c - \delta y \quad (7)$$

with a mass conservation of the total amount of DNA complex constant, $c + c^* = c^{tot}$.

Numerical Simulations. Below we list the parameters used in the model for simulations.

Parameter	Value	Annotation	Other studies
ϕ, δ (1/s)	2.7×10^{-4}	degradation	$10^{-4} - 10^{-3}$ [4]
θ (M/s)	2.7×10^{-10}	transcription	$2.8 \times 10^{-11} - 2.8 \times 10^{-8}$ [5,6]
α (M/s)	5.4×10^{-10}	transcription	$2.8 \times 10^{-11} - 2.8 \times 10^{-8}$ [5,6]
k_p^+, k_c^+ (/M/s)	2.7×10^4	binding	$10^4 - 10^6$ [7,8]
k_p^-, k_c^- (1/s)	2.7×10^{-4}	unbinding	
r_p, r_c (1/s)	2.7×10^{-3}	complex transcription	$0.05 - 0.2$ [9]*
r_0 (1/s)	2.7×10^{-4}	leakiness	
c^{tot} (nM)	1000	total DNA	
ξ (1/s)	0	abortion rate	

* Estimated from the average pause-free velocity of RNAP at saturating concentrations of nucleotide triphosphates (NTP) for an RNA length of 200 nt.

Supplementary References

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