Supplementary Information

Reprogrammed tracrRNAs enable repurposing RNAs as crRNAs and detecting RNAs

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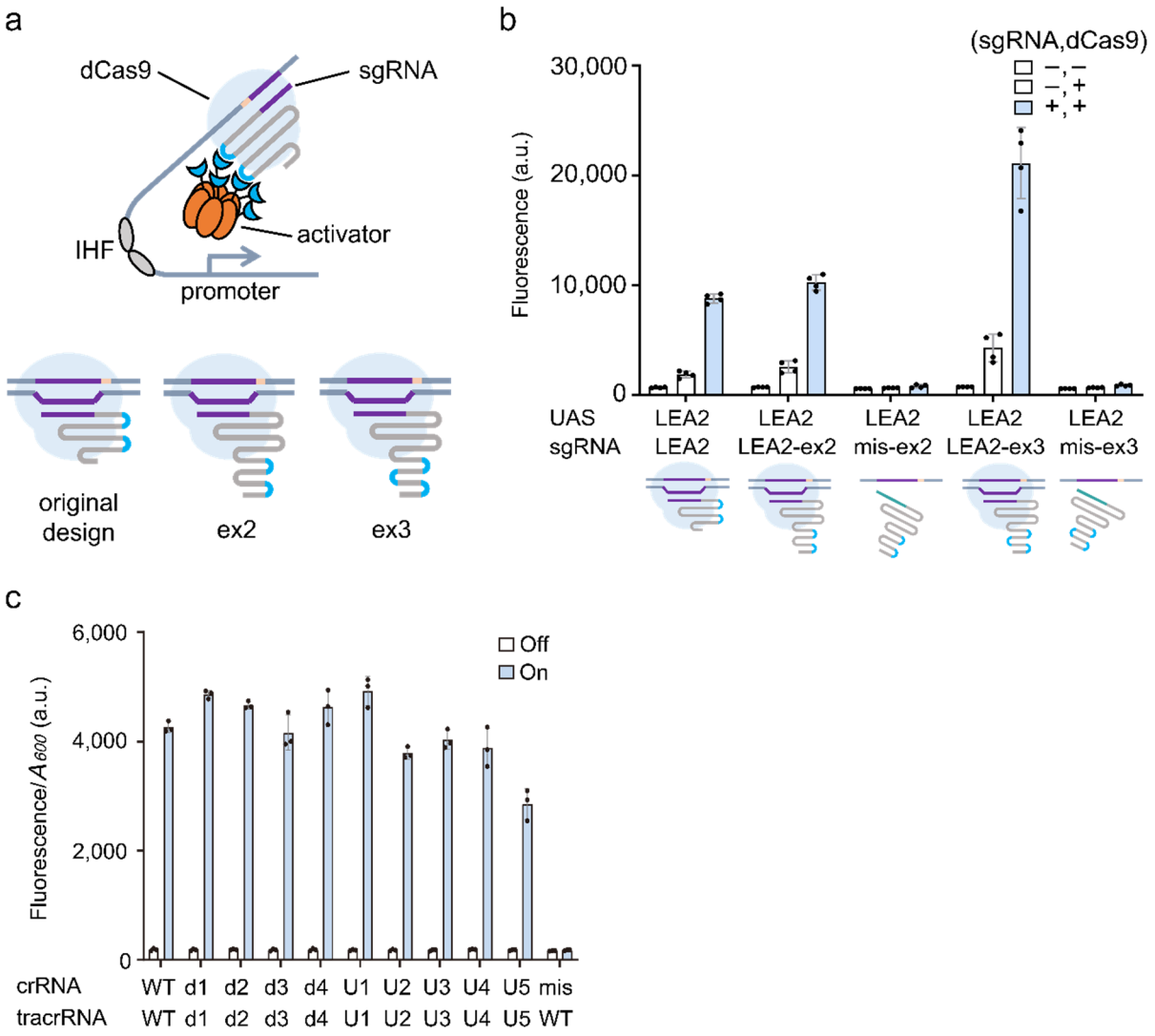
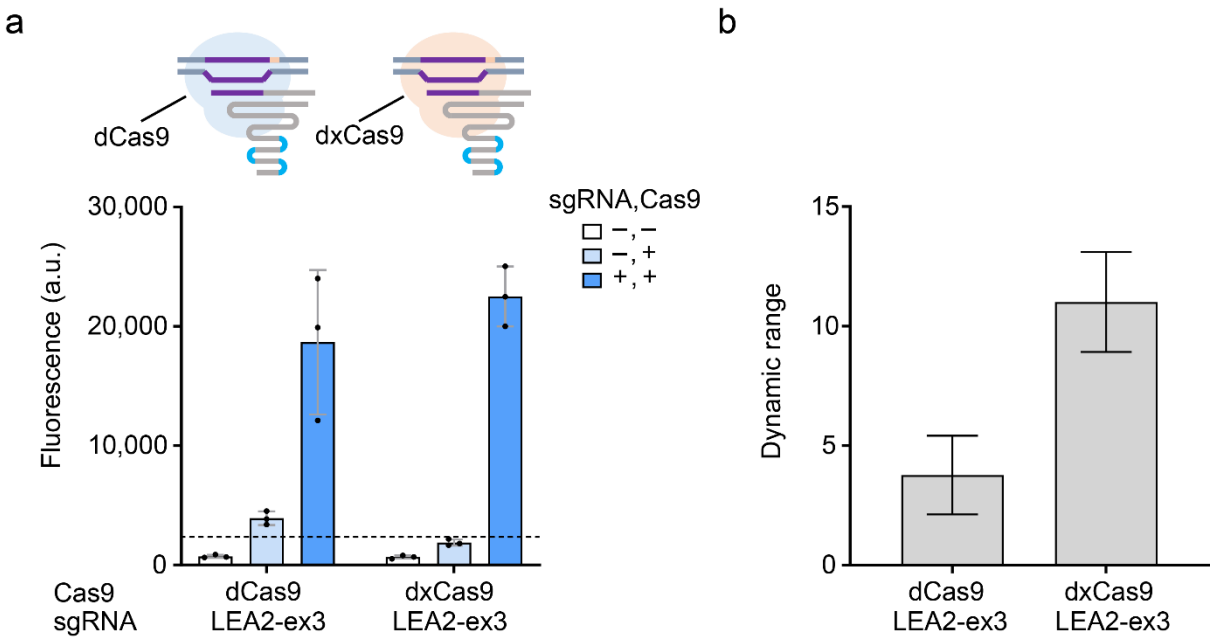
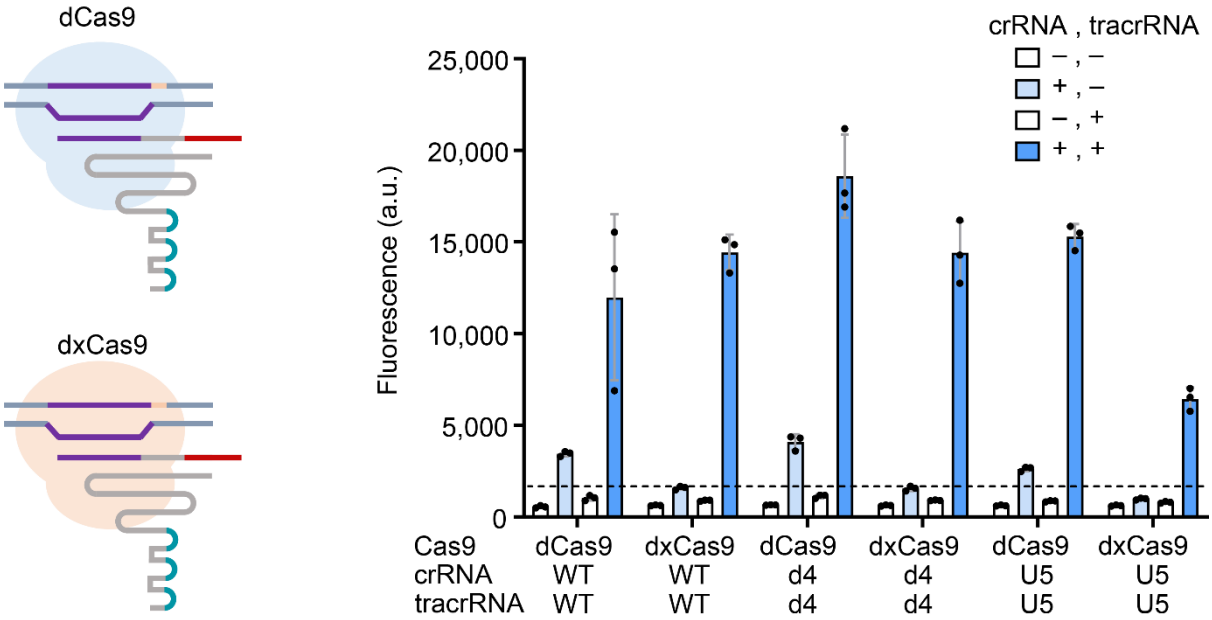


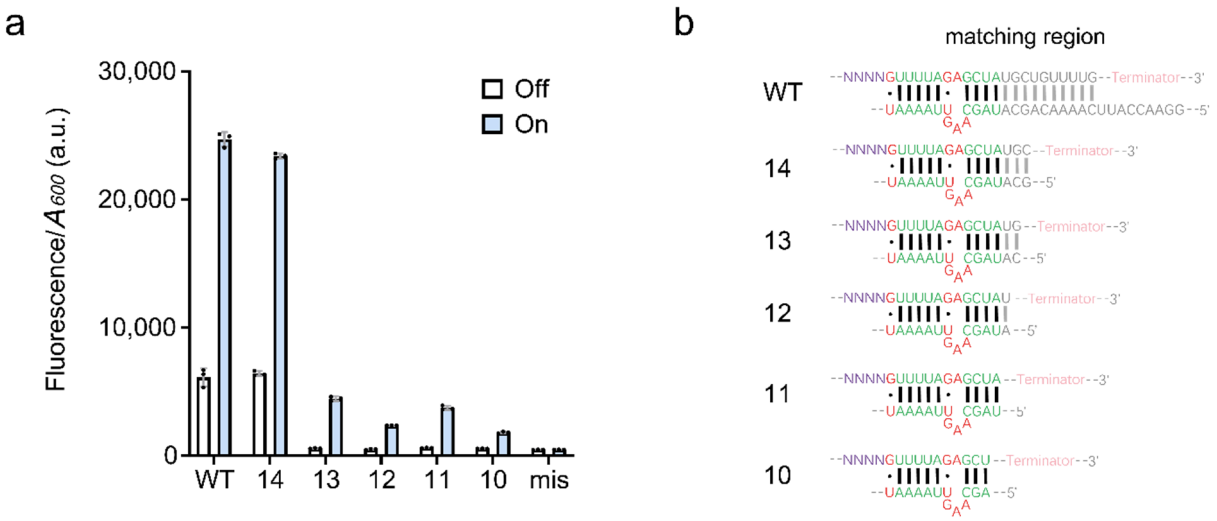
Supplementary Figure 1. CRISPRa function with tail-fused aptamers and CRISPRa output from reprogrammed crRNA-tracrRNA pairs (a) Schematic of the CRISPRa devices showing three different designs of sgRNA. The light blue U shape line segments in the sgRNA scaffold indicate BoxB RNA aptamers. **(b)** CRISPRa function with three different kinds of sgRNA design. The random sequence LEA2 was used and UAS and sgRNA spacer. For each of the two new designs, a sgRNA with the same sgRNA scaffold but with different spacer (random sequence LEB3) was employed as mismatched control. dCas9 expression was controlled by P_{tet} promoter, and activator expression was driven by a constitutive promoter J23106 (Anderson promoter). A P_{lux2} promoter was used to transcribe sgRNA. 2.5 ng mL^{-1} aTc and 1.6 μM AHL were used for dCas9 and sgRNA induction respectively. Error bars, s.d. (n = 4). **(c)** The CRISPRa function of the various crRNA-tracrRNA pairs in the library shown in **Figure 1e**. The label 'mis' means the WT crRNA has a mismatched spacer (LEB3) with the target UAS (LEA2). All the other crRNAs in this test have spacer LEA2, and a corresponding σ^{54}-dependent promoter with UAS LEA2 was used for the reporter. Expression of dCas9 and activator expression was driven by the P_{tet} and constitutive promoter J23106. A P_{lux2} promoter was used to transcribe sgRNA. No inducer was added for the OFF state. 2.5 ng mL^{-1} aTc, 1.6 μM AHL, and 0.08 mM arabinose were used for dCas9, crRNA, tracrRNA induction (ON state), respectively. Error bars, s.d. (n = 3); a.u., arbitrary units.



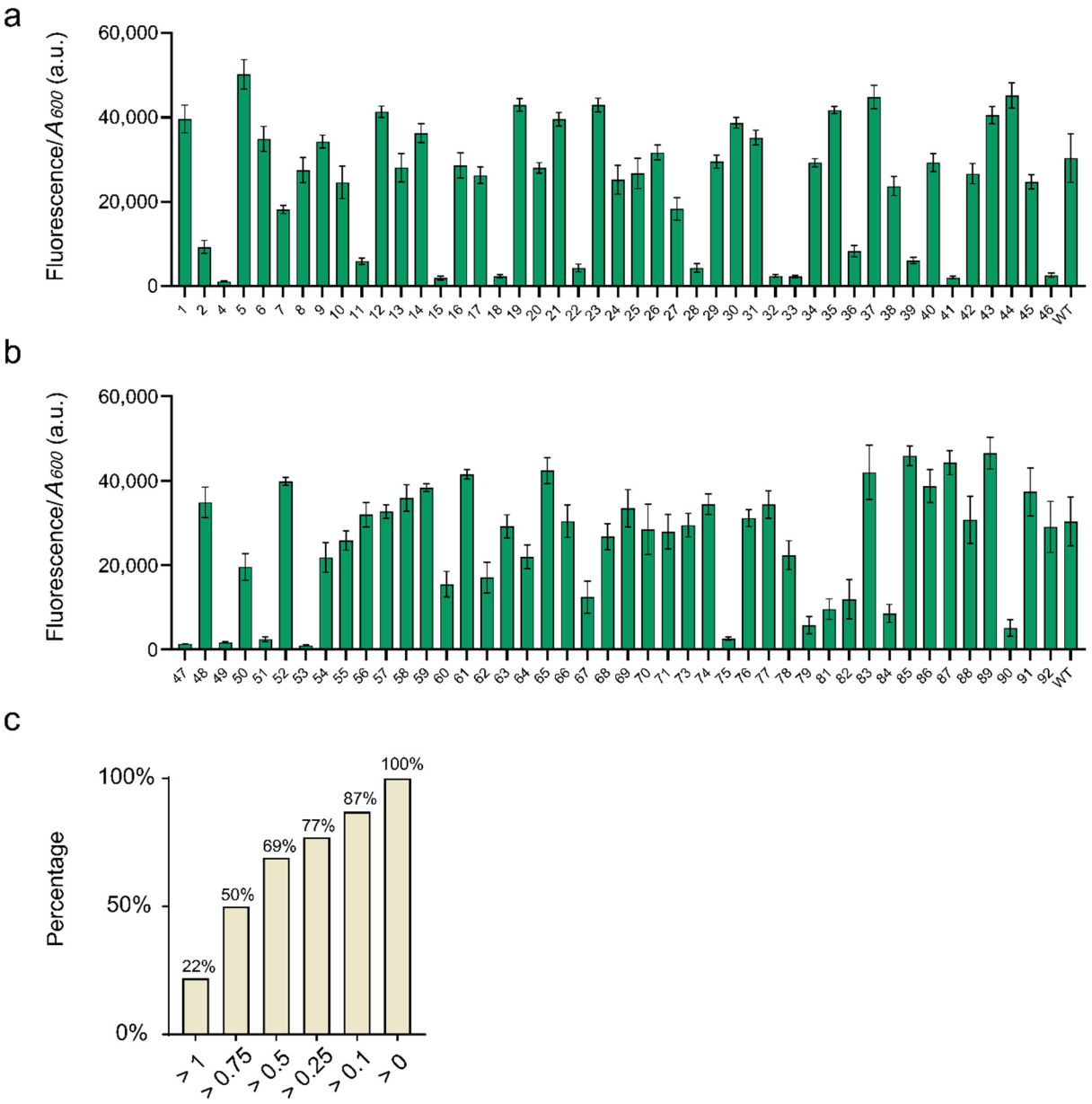
Supplementary Figure 2. sgRNA tuning-based output dynamic range can be improved by employing dxCas9 instead of dCas9. (a) A sgRNA with three BoxB aptamers added to its 3' end tail (LEA2-ex3) was used for CRISPRa. The UAS of promoter and spacer of sgRNA had the LEA2 sequence. P_{tet} controlled dCas9 or dxCas9 generator, and P_{rhaB} drove activator expression, P_{lux2} drove sgRNA transcription. Inducer concentrations: 2.5 ng mL^{-1} aTc, 0.4 mM rhamnose, and 1.6 μM AHL. The plus and minus signs represent the presence or absence of inducers, respectively. **(b)** The sgRNA tuning based dynamic range was calculated from the two sets of data with dCas9 or dxCas9 in the left bar chart. Error bars, s.d. (n = 3); a.u., arbitrary units.



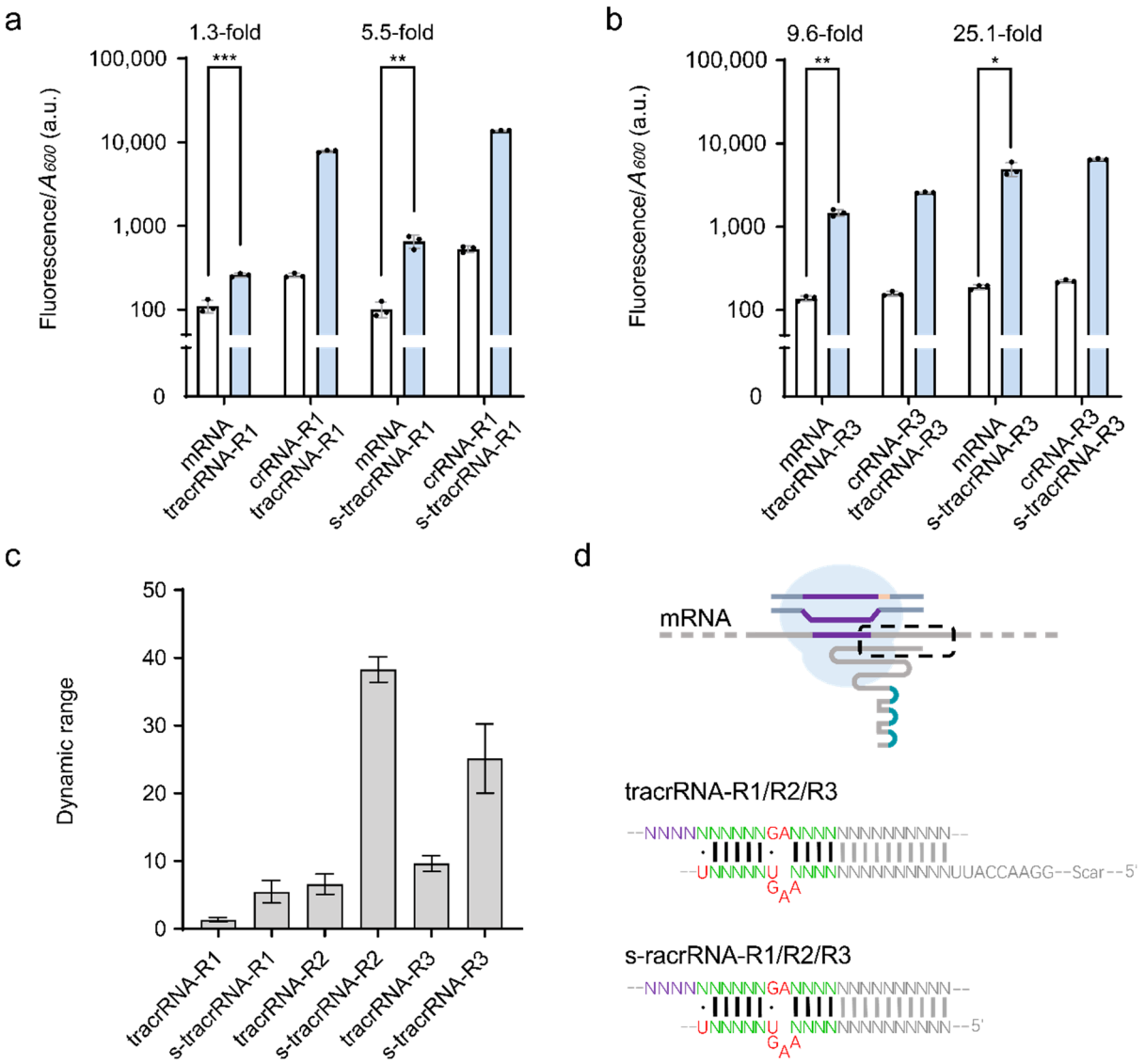
Supplementary Figure 3. Optimizing asymmetry sensitivity to inputs of crRNA-tracrRNA mediated CRISPRa by utilizing dxCas9. The cartoon on the left shows the two CRISPR complexes used in the experiment. The purple line segment represents DNA and its complementary paired spacer sequence of crRNA. The red line indicates the terminator sequence on crRNA. The blue U shaped segment indicates BoxB aptamers at the tail of tracrRNA. The bar chart on the right shows the output of three CRISPRa devices with different crRNA-tracrRNA matching sequences (WT, d4, U5). Random sequence LEA2 was used for UAS and spacer in these circuits. Expression of dCas9 or dxCas9 was driven by P_{tet}, and expression of activator was driven by P_{rhaB}. crRNA and tracrRNA transcription was driven by P_{lux2} and $P_{BAD,}$ respectively. Inducer concentrations: 2.5 ng mL^{-1} aTc, 0.4 mM rhamnose, 1.6 μM AHL, and 0.08 mM arabinose. The plus and minus signs represent the presence or absence of inducers. Error bars, s.d. (n = 3); a.u., arbitrary units.



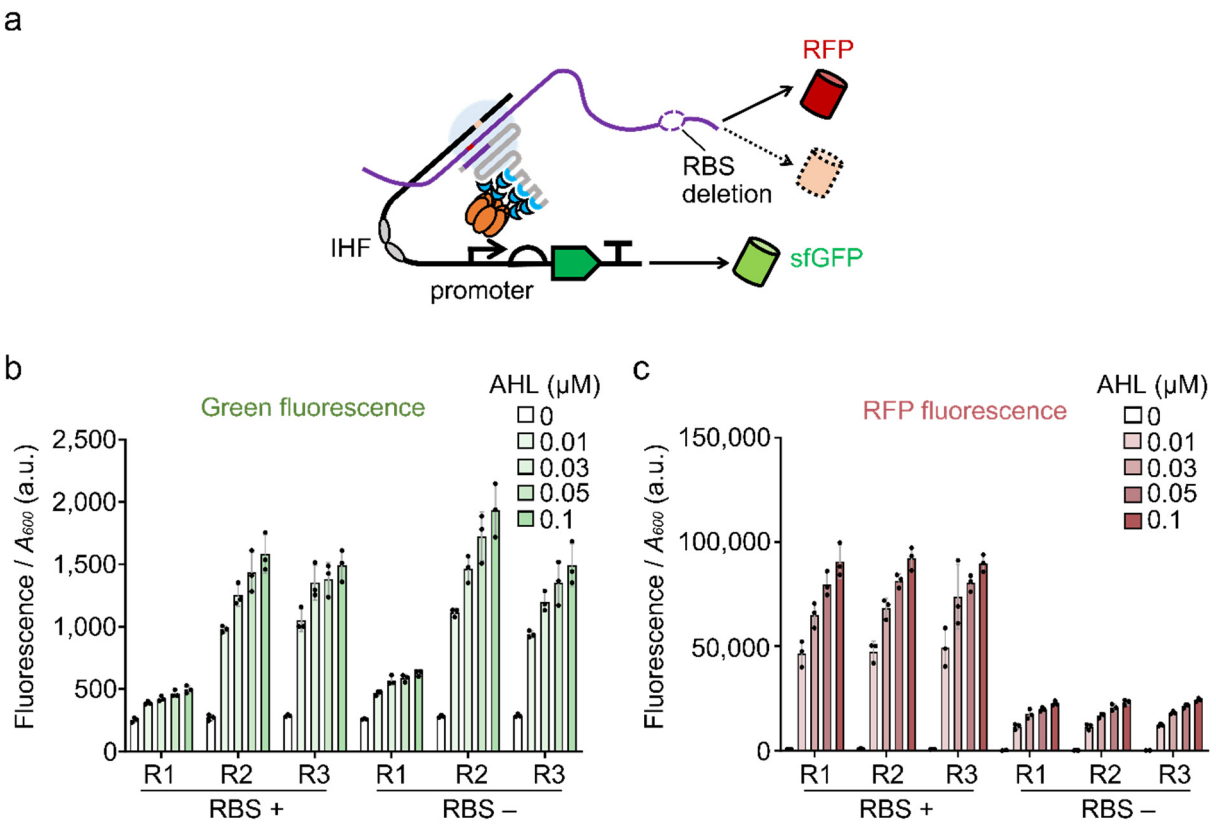
Supplementary Figure 4. Minimal length of the hybridizing sequences required for functional crRNA-tracrRNA pair. (a) CRISPRa output from different lengths of the hybridizing region. The bar chart shows the results from crRNA-tracrRNA pairs with 10 –14 bp length of the matching region. The dCas9 was expressed by P_{tet} promoter with 2.5 ng mL^{-1} aTc. The activator, crRNA and tracrRNA are all driven by the constitutive promoter J23106. The label 'mis' means the WT crRNA has a mismatched spacer (LEB3) with the target UAS (LEA2). All the other crRNAs in this test have spacer LEA2, and a corresponding σ^{54}-dependent promoter with UAS LEA2 was used for the reporter. The data of crRNA-tracrRNA pairs WT, 14 –12 bp, and mis is equal to that in **Figure 2a**. Error bars, s.d. (*n* = 3); a.u., arbitrary units. **(b)** The structure and sequences of the crRNA-tracrRNA pairs we employed in this experiment.



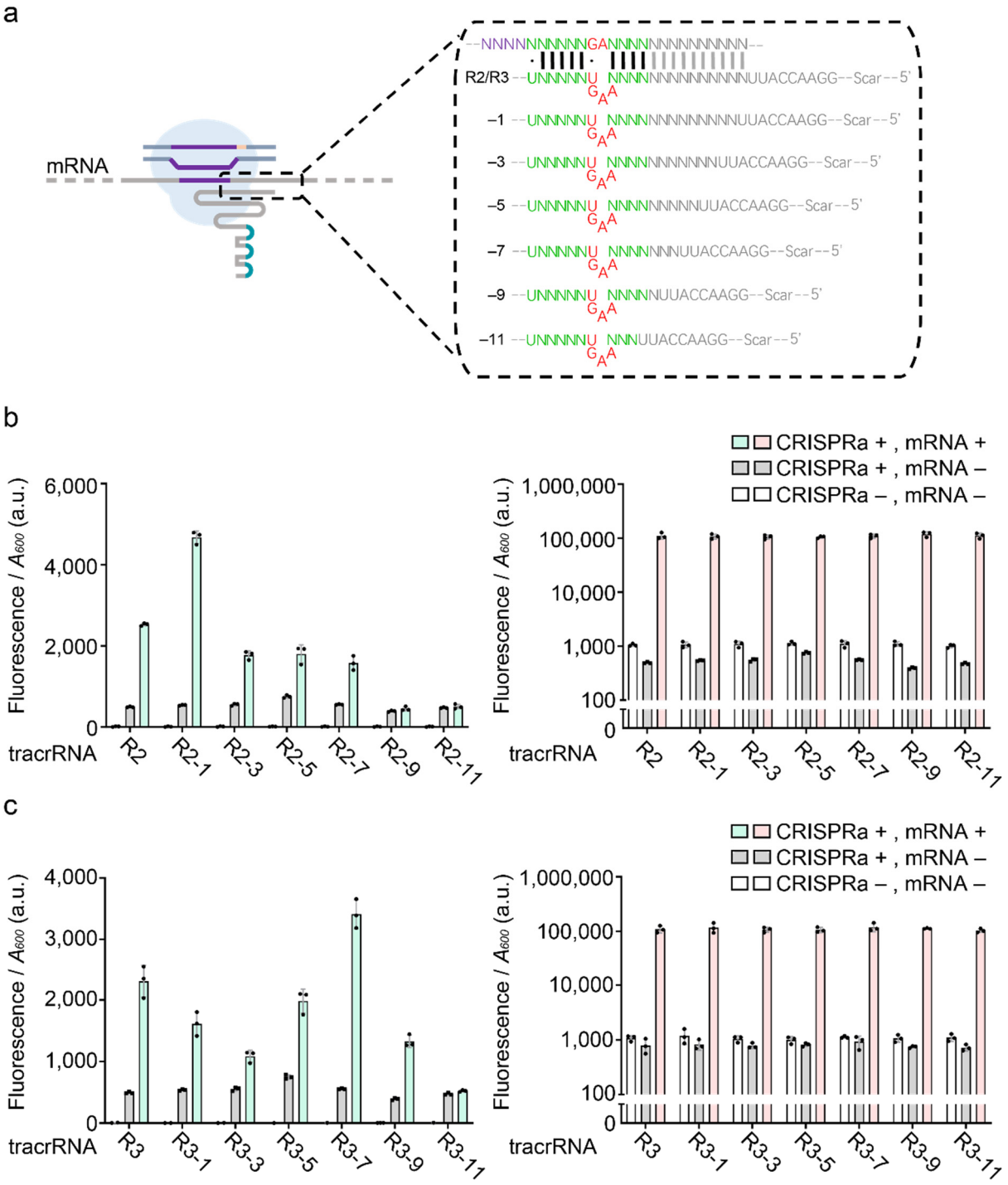
Supplementary Figure 5. CRISPRa output from the paired crRNA-tracrRNA randomized-sequence library. (a) The CRISPRa output from strains 1 – 46 and a positive control strain with the WT crRNA-tracrRNA matching region sequence. The dCas9 generator was controlled by P_{tet}. The crRNA and tracrRNA transcription and activator expression was driven by Promoter J23106. 2.5 ng mL^{-1} aTc was used in this test. Error bars, s.d. (n = 3); a.u., arbitrary units. **(b)** The CRISPRa output from strains 47 – 92 and a positive control strain with the WT crRNA-tracrRNA matching region sequence. The induction condition is the same as in **a**. Error bars, s.d. (*n* = 3); a.u., arbitrary units. **(c)** The percentage of the candidates whose output level is higher than a certain value. The abscissa defines the output range for each group, the values are the different multipliers for output from the positive control strain. The corresponding output lower limit is the product of these multipliers and the output value from the positive control strain.



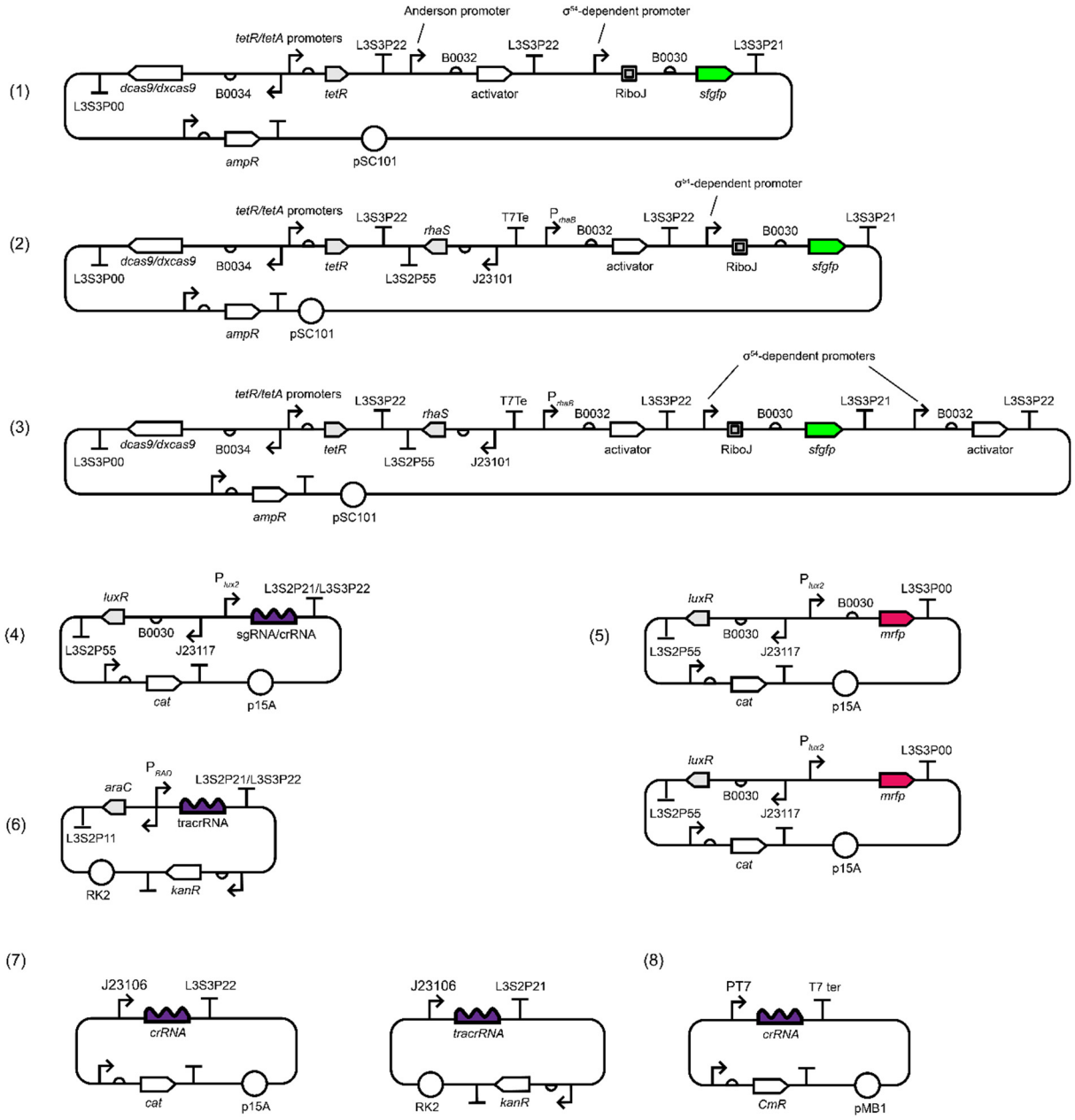
Supplementary Figure 6. Engineered tracrRNA repurposes mRNA into crRNA. (a) 5' end truncation of tracrRNA improves the mRNA-mediated CRISPR function. The mRNA fragment with the same sequences as that in the mRNA target site R1 was expressed as controls. For the bar of mRNA + tracrRNA-R1, the data is the same as in **Figure 3b**. Expression of dCas9 and activator was driven by the P_{tet} and P_{rhaB} promoters, respectively. The mRNA of RFP and the tracrRNA were transcribed from P_{lux2} and $P_{BAD,}$ respectively. Inducer concentrations: 2.5 ng mL^{-1} aTc, 0.2 mM rhamnose, and 0.08 mM arabinose. 0.1 μM AHL was used for mRNA expression. Statistical difference was determined by a two-tailed *t* test: mRNA + s-tracrRNA-R1, $p = 0.0014$, t = 7.904. Error bars, s.d. ($n = 3$) **(b)** The content is the same as in **a**, except that the RNA target is R3. Statistical difference was determined by a Welch's *t* test: mRNA + s-tracrRNA-R3, $p = 0.0121$, t = 8.994. Error bars, s.d. ($n = 3$). **(c)** The dynamic range of different mRNA-mediated CRISPRa devices. All the induction condition is the same as in **a**. Error bars, s.d. (n=3). **(d)** Schematic diagram shows the structures of the hybridizing regions for the original tracrRNA version and the 5' end truncated tracrRNA. a.u., arbitrary units.



Supplementary Figure 7. Test of ribosome binding site (RBS) deletion in the RFP mRNA hijacking experiment. (a) Schematic showing the circuit design for the RBS deletion experiment. The oval purple dotted line represents the RBS, and the red cylindrical structure drawn by the dotted line indicates that the translation of red fluorescent protein is greatly reduced after the RBS is deleted. **(b)** The green fluorescence output from the mRNA mediated CRISPRa with or without an RBS on the mRNA of RFP. Expression of dCas9 and activator was driven by P_{tet} and P_{rhaB} promoters. mRNA and tracrRNA transcription were driven by P_{lux2} and $P_{BAD,}$ respectively. Inducer concentrations: 2.5 ng mL^{-1} aTc, 0.2 mM rhamnose, and 0.08 mM arabinose. AHL gradian (0, 0.01, 0.03, 0.05, 0.1 μM) was used for mRNA induction. **(c)** The red fluorescence output from the mRNA mediated CRISPRa with or without an RBS on the mRNA of RFP. The data was from the same experiment in **b**. Error bars, s.d. (n = 3). a.u., arbitrary units.



Supplementary Figure 8. Length of the crRNA-tracrRNA hybridizing region affects the CRISPRa function. (a) Schematic showing the design of the library, including truncated tracrRNA anti-coding regions. In this test, tracrRNA-R2 and tracrRNA-R3 were employed and coupled with mRNA of RFP for CRISPRa. The initial pairing length is 21 bp. **(b)** The result of the anti-coding region truncation test on tracrRNA-R2. Green fluorescence detection results on the left and red fluorescence detection results on the right. Expression of dCas9 generator and activator were driven by P_{tet} and P_{rhaB}. mRNA and tracrRNA transcription were driven by P_{lux2} and $P_{BAD,}$ respectively. Inducer concentrations: 2.5 ng mL^{-1} aTc, 1.6 μM AHL, 0.4 mM rhamnose, and 0.08 mM arabinose. CRISPR + or CRISPR – indicates that all the inducers were added or not except AHL (for mRNA induction). **(c)** The result of the anti-coding region truncation test on tracrRNA-R3. Green fluorescence detection results are on the left, and red fluorescence detection results are on the right. All the conditions were the same as that in **b.** Error bars, s.d. (n = 3); a.u., arbitrary units.



Supplementary Figure 9. Representative plasmid maps for key circuit constructs used in this study. (1) The plasmid map of pLY54. pLY54 was used in the experiments in **Figure 1,2**, and also was used in tests of **Supplementary Figure 1−5**. **(2)** The plasmid map of pLY162−pLY164, and pLY167-pLY169, pLY255, pLY256. pLY162−pLY164 were used in the RFP mRNA hijacking experiment (**Figure 3**, **Supplementary Figure 6,7,8**); pLY167, pLY168, pLY255, pLY256 are reporter circuits for the hijacking of endogenous mRNA of the arsenic-related gene cluster in *E. coli* (**Figure 4**); pLY169 is the CRISPRa circuit with dxCas9, which was used in experiments of **Supplementary Figure 2,3**. **(3)** The plasmid map of pLY165 and pLY166, they are positive feedback circuits used in **Figure 3**. **(4)** The plasmid map of pLY76, pLY170−pLY184, pLY187−pLY189, pLY218−pLY231 and pLY246. They are all the crRNA generators in this study. **(5)** The plasmid map of pLY185 and pLY186. They are two mRFP generators with or without RBS, which were used in **Supplementary Figure 7**. **(6)** The plasmid map of pLY190−pLY217, pLY232−pLY239, pLY251−pLY254. They are all the tracrRNA generators in this study. **(7)** The plasmid map of pLY241−pLY250, and the 180 crRNA/tracrRNA generators in our library **(Supplementary Table 1)**. **(8)** The plasmid map of plY240, which is a CONAN circuit **(Figure 5)**.

Supplementary tables

Supplementary Table 1: Sequences from the library of randomized hybridizing region of the paired crRNA-tracrRNA

Name	Sequence in hybridized region (crRNA 5' – 3')	Sequence in hybridized region (tracrRNA 5' – 3')
c(+)	GUUUUAGAGCUAUGC	GCAUAGCAAGUUAAAAU
c1	AGCAGGGAACAGACU	AGUCUGUAAGUCCUGCU
c2	GAUCGGUAAAAGUUA	UAACUUUAAGUCCGAUU
c4	AAAUGUAUUUAAAGA	UCUUUAAAAGUACAUUU
c5	AUUCACAUGAAUCCU	AGGAUUCAAGUGUGAAU
c6	UACGUACCGGGUGAA	UUCACCCAAGUUACGUU
c7	CAGGCUCGGAUGUCC	GGACAUCAAGUAGCCUU
c8	UUCAACCAGCCCUUG	CAAGGGCAAGUGUUGAU
c9	AGAAUCCUCGCUCAG	CUGAGCGAAGUGAUUCU
c10	GCCUGGAACACCUAU	AUAGGUGAAGUCCAGGU
c11	CCCUGAUACCCUGUU	AACAGGGAAGUUCAGGU
c12	GUUCGCGCACGCGCG	CGCGCGUAAGUGCGAAU
c13	CACCUCCGAGCGCUG	CAGCGCUAAGUGAGGUU
c14	GGUCUCACCCUUCAA	UUGAAGGAAGUGAGACU
c15	AAAGAAAUUUCUGAU	AUCAGAAAAGUUUCUUU
c16	UCGCGUUUUGUAACU	AGUUACAAAGUACGCGU
c17	GACUCAUGGGUAUUU	AAAUACCAAGUUGAGUU
c18	CAAAUGUCGUAUUUG	CAAAUACAAGUCAUUUU
c19	GGCCUCGAAUCCAUG	CAUGGAUAAGUGAGGCU
c20	GACCCAUAUGGCGGG	CCCGCCAAAGUUGGGUU
c21	CCAGUGUCUCCCAAC	GUUGGGAAAGUCACUGU
c22	UCGAGAUGACUUUCU	AGAAAGUAAGUUCUCGU
c23	GGAGAAAUAGUUGCG	CGCAACUAAGUUUCUCU
c24	CCAUAGAUGAUCAUU	AAUGAUCAAGUCUAUGU
c25	UCCAACAAUCCACAC	GUGUGGAAAGUGUUGGU

Name	Sequence in hybridized region (crRNA 5’ – 3’)	Sequence in hybridized region (tracrRNA 5’ – 3’)
c26	ACUAGUUUCCCCAAG	CUUGGGGAAGUACUAGU
c27	CGACAAAAGAGUUAA	UUAACUCAAGUUUGUCU
c28	GCUGGGGCAGCCCUA	UAGGGCUAAGUCCCAGU
c29	CGAGAUGUGUUGGUU	AACCAACAAGUAUCUCU
c30	CCAGUAAGACACAGC	GCUGUGUAAGUUACUGU
c31	ACGGUCCACUCUUUU	AAAAGAGAAGUGACCGU
c32	GCGCUAAAAUAGCUG	CAGCUAUAAGUUAGCGU
c33	CGAUUUGAGGCUGAG	CUCAGCCAAGUAAAUCU
c34	GUGUUAAAAUCCUUG	CAAGGAUAAGUUAACAU
c35	AAAUUGGUCUUAACC	GGUUAAGAAGUCAAUUU
c36	UAGGAGCUUGACAGA	UCUGUCAAAGUCUCCUU
c37	CUGCCGUGUGUCAGU	ACUGACAAAGUCGGCAU
c38	UUCAGGCGCCUCUGC	GCAGAGGAAGUCCUGAU
c39	AGCCGGACCACCGAG	CUCGGUGAAGUCCGGCU
c40	UUGGAAGUCCUCCGG	CCGGAGGAAGUUUCCAU
c41	GUCUAAUCGACCUAG	CUAGGUCAAGUUUAGAU
c42	UUCGGGUUCAGCGCA	UGCGCUGAAGUCCCGAU
c43	UACUCCUUUACGAAA	UUUCGUAAAGUGGAGUU
c44	CAUUGAGCCGUUGCC	GGCAACGAAGUUCAAUU
c45	ACCUGGUGCUGAUCA	UGAUCAGAAGUCCAGGU
c46	UAGACCCAGGCCGGC	GCCGGCCAAGUGGUCUU
c47	UACUUUGGAAAUAUA	UAUAUUUAAGUAAAGUU
c48	AUUAUUGCCGACACA	UGUGUCGAAGUAAUAAU
c49	GCUGUUUAGAGGCUG	CAGCCUCAAGUAACAGU
c50	CGAGUGUCGACCCUG	CAGGGUCAAGUCACUCU
c51	GUAAUGAUCCGUAAU	AUUACGGAAGUCAUUAU
c52	CUUAUCCGGCAACGC	GCGUUGCAAGUGAUAAU
c53	UCCGCGACCGGCGGG	CCCGCCGAAGUCGCGGU
c54	UCGAACGAGCAGAUG	CAUCUGCAAGUGUUCGU

Name	Sequence in hybridized region (crRNA 5’ – 3’)	Sequence in hybridized region (tracrRNA 5’ – 3’)
c55	AGUCAAGACGGAUAA	UUAUCCGAAGUUUGACU
c56	UGUAGGUCACGGAGU	ACUCCGUAAGUCCUACU
c57	AGUACGAUGAGCGGG	CCCGCUCAAGUCGUACU
c58	UAUCUCUAGGAGGGU	ACCCUCCAAGUGAGAUU
c59	CAUAUCCCGGUCCCA	UGGGACCAAGUGAUAUU
c60	AUCCGAGCGGCGGCC	GGCCGCCAAGUUCGGAU
c61	GUCCGUAUACUCCUG	CAGGAGUAAGUACGGAU
c62	CGUCUGCACCGUUGA	UCAACGGAAGUCAGACU
c63	UUGACGGCAUGUAGG	CCUACAUAAGUCGUCAU
c64	UCUAGGCACCCCUAC	GUAGGGGAAGUCCUAGU
c65	GCAUAUCCCUUAAGC	GCUUAAGAAGUAUAUGU
c66	UUAUACAGGGAGUAC	GUACUCCAAGUGUAUAU
c67	CCCACCCGAAUCAUC	GAUGAUUAAGUGGUGGU
c68	ACCCUGCACGACGUU	AACGUCGAAGUCAGGGU
c69	AAGGUCUGACGCCGG	CCGGCGUAAGUGACCUU
c70	UAUGAGACACACGCA	UGCGUGUAAGUCUCAUU
c71	CCUUGGCGUCUGCUU	AAGCAGAAAGUCCAAGU
c73	CAUGAGGUCGAUAUC	GAUAUCGAAGUCUCAUU
c74	AAUCGGGAGCUGCCU	AGGCAGCAAGUCCGAUU
c75	ACUUUGGUACAGUGA	UCACUGUAAGUCAAAGU
c76	GUCAGUCUGCUGCGC	GCGCAGCAAGUACUGAU
c77	AUAGAUGUCCUGCGG	CCGCAGGAAGUAUCUAU
c78	GAAUCACAAGAGGCG	CGCCUCUAAGUUGAUUU
c79	AACCAUAGAAACAUU	AAUGUUUAAGUAUGGUU
c81	UCUCAGAGAACCUGA	UCAGGUUAAGUCUGAGU
c82	GUUAACCCCUUCUAA	UUAGAAGAAGUGUUAAU
c83	UUUCACGAUAGUCUU	AAGACUAAAGUGUGAAU
c84	GCACACCCGGUCGAA	UUCGACCAAGUGUGUGU
c85	AAGUGAGAUUCCGGU	ACCGGAAAAGUUCACUU

Name	Sequence in hybridized region (crRNA 5’ – 3’)	Sequence in hybridized region (tracrRNA 5’ – 3’)
c86	GACCGAUCAGCCCUG	CAGGGCUAAGUUCGGUU
c87	CAGUGUUUGCAUCGC	GCGAUGCAAGUACACUU
c88	CGCAGAUCCAUGUAC	GUACAUGAAGUUCUGCU
c89	GGUAGGAAGGCAUCC	GGAUGCCAAGUCCUACU
c90	CCGUCGCGAGCCUAU	AUAGGCUAAGUCGACGU
c91	GCAUUCGAGUGGCAA	UUGCCACAAGUGAAUGU
c92	UAGUCAACUAGGGGU	ACCCCUAAAGUUGACUU

Supplementary Table 2: Synthetic RNAs used in CONAN sensor assays

Name	Sequence in hybridized region
SARS-CoV-II RNA 1	GCCACUUCUGCUGCUCUUCAACCUGAAGAAGAGCAAGAAGAAGAUUGG
tracrRNA CoV1	GGAACCAUUAAAUCUUCUUCUUGCAAGUUUCUUUAAGGCUAGUCCGUUAUCAACUUG AAAAAGUGGCACCGAGUCGGUGCUUUUUUU
SARS-CoV-II RNA 2	CAACUUGUAUGAUGUGUUACAAACGUAAUAGAGCAACAAGAGUCGAAUGU
tracrRNA CoV2	GGAACCAUUUCGACUCUUGUUGCAAGUUAUUAUAAGGCUAGUCCGUUAUCAACUUG AAAAAGUGGCACCGAGUCGGUGCUUUUUUU

Supplementary Table 3: Plasmids used in this study

'dCas9' in this table refers 'dCas9 generator'

'dxCas9' in this table refers 'dxCas9 generator'

'Reporter' in this table refers 'Reporter with sfGFP::ASV tag'

Notes:

- Scars between the parts coming from construction are not shown.
- Circuits in the randomized sequence library are not included in this table.

Name	Structure	Vector	Reference
pLY54	dCas9-J23106-PSPFΔHTH::λN22plus-P_{pspA}-LEA2B2-Reporter	pSB4A3*	Previous study(*1*)
pLY162	dCas9-P_{rhaB}-PSPFΔHTH::λN22plus-P_{pspA}-R1-Reporter	pSB4A3*	This study
pLY163	dCas9-P_{rhaB}-PSPFΔHTH::λN22plus-P_{pspA}-R2-Reporter	pSB4A3*	This study
pLY164	dCas9-P_{rhaB}-PSPFΔHTH::λN22plus-P_{pspA}-R3-Reporter	pSB4A3*	This study
pLY165	dCas9-P_{rhaB}-PSPFΔHTH::λN22plus-P_{pspA}-R2-Reporter-P_{pspA}-R2-Reporter	pSB4A3*	This study
pLY166	dCas9-P_{rhaB}-PSPFΔHTH::λN22plus-P_{pspA}-R3-Reporter-P_{pspA}-R3-Reporter	pSB4A3*	This study
pLY167	dCas9-P_{rhaB}-PSPFΔHTH::λN22plus-P_{pspA}-Ar1-Reporter	pSB4A3*	This study
pLY168	dCas9-P_{rhaB}-PSPFΔHTH::λN22plus-P_{pspA}-Ar2-Reporter	pSB4A3*	This study
pLY169	dxCas9-P_{rhaB}-PSPFΔHTH::λN22plus-P_{pspA}-LEA2B2-Reporter	pSB4A3*	This study
pLY76	P_{lux2}-sgRNA-LEA2	p15AC	Previous study(*1*)
pLY170	P_{lux2}-sgRNA-LEA2-ex2	p15AC	This study
pLY171	P_{lux2}-sgRNA-LEA2-ex3	p15AC	This study
pLY172	P_{lux2}-sgRNA-LEB3-ex2	p15AC	This study
pLY173	P_{lux2}-sgRNA-LEB3-ex3	p15AC	This study
pLY174	P_{lux2}-crRNA-LEA2-WT	p15AC	This study
pLY176	P_{lux2}-crRNA-LEA2-d1	p15AC	This study

Name	Structure	Vector	Reference
pLY177	P_{lux2}-crRNA-LEA2-d2	p15AC	This study
pLY178	P_{lux2}-crRNA-LEA2-d3	p15AC	This study
pLY179	P_{lux2}-crRNA-LEA2-d4	p15AC	This study
pLY180	P_{lux2}-crRNA-LEA2-U1	p15AC	This study
pLY181	P_{lux2}-crRNA-LEA2-U2	p15AC	This study
pLY182	P_{lux2}-crRNA-LEA2-U3	p15AC	This study
pLY183	P_{lux2}-crRNA-LEA2-U4	p15AC	This study
pLY184	P_{lux2}-crRNA-LEA2-U5	p15AC	This study
pLY185	P_{lux2}-mRFP	p15AC	This study
pLY186	P_{lux2}-mRFP-ΔRBS	p15AC	This study
pLY187	P_{lux2}-crRNA-LEA2-U5A	p15AC	This study
pLY188	P_{lux2}-crRNA-LEA2-U5C	p15AC	This study
pLY189	P_{lux2}-crRNA-LEA2-U5T	p15AC	This study
pLY190	P_{BAD}-tracrRNA-WT	pSEVA221 (JX560327)(*2*)	This study
pLY192	P_{BAD}-tracrRNA-d1	pSEVA221 (JX560327)	This study
pLY193	P_{BAD}-tracrRNA-d2	pSEVA221 (JX560327)	This study
pLY194	P_{BAD}-tracrRNA-d3	pSEVA221 (JX560327)	This study
pLY195	P_{BAD}-tracrRNA-d4	pSEVA221 (JX560327)	This study
pLY196	P_{BAD}-tracrRNA-U1	pSEVA221 (JX560327)	This study
pLY197	P_{BAD}-tracrRNA-U2	pSEVA221 (JX560327)	This study
pLY198	P_{BAD}-tracrRNA-U3	pSEVA221 (JX560327)	This study
pLY199	P_{BAD}-tracrRNA-U4	pSEVA221	This study

Name	Structure	Vector	Reference
		(JX560327)	
pLY200	P_{BAD}-tracrRNA-U5	pSEVA221 (JX560327)	This study
pLY201	P_{BAD}-tracrRNA-R1	pSEVA221 (JX560327)	This study
pLY202	P_{BAD}-tracrRNA-R2	pSEVA221 (JX560327)	This study
pLY203	P_{BAD}-tracrRNA-R3	pSEVA221 (JX560327)	This study
pLY204	P_{BAD}-tracrRNA-Ar1	pSEVA221 (JX560327)	This study
pLY205	P_{BAD}-tracrRNA-Ar2	pSEVA221 (JX560327)	This study
pLY206	P_{BAD}-tracrRNA-R2L1	pSEVA221 (JX560327)	This study
pLY207	P_{BAD}-tracrRNA-R2L3	pSEVA221 (JX560327)	This study
pLY208	P_{BAD}-tracrRNA-R2L5	pSEVA221 (JX560327)	This study
pLY209	P_{BAD}-tracrRNA-R2L7	pSEVA221 (JX560327)	This study
pLY210	P_{BAD}-tracrRNA-R2L9	pSEVA221 (JX560327)	This study
pLY211	P_{BAD}-tracrRNA-R2L11	pSEVA221 (JX560327)	This study
pLY212	P_{BAD}-tracrRNA-R3L1	pSEVA221 (JX560327)	This study
pLY213	P_{BAD}-tracrRNA-R3L3	pSEVA221 (JX560327)	This study
pLY214	P_{BAD}-tracrRNA-R3L5	pSEVA221	This study

Name	Structure	Vector	Reference
		(JX560327)	
pLY215	P_{BAD}-tracrRNA-R3L7	pSEVA221 (JX560327)	This study
pLY216	P_{BAD}-tracrRNA-R3L9	pSEVA221 (JX560327)	This study
pLY217	P_{BAD}-tracrRNA-R3L11	pSEVA221 (JX560327)	This study
pLY218	P_{lux2}-crRNA-R1	p15AC	This study
pLY219	P_{lux2}-crRNA-R2	p15AC	This study
pLY220	P_{lux2}-crRNA-R3	p15AC	This study
pLY221	P_{lux2}-mRNA-Ar	p15AC	This study
pLY222	P_{lux2}-crRNA-Ar1	p15AC	This study
pLY223	P_{lux2}-crRNA-Ar2	p15AC	This study
pLY224	P_{lux2}-crRNA-Ar3	p15AC	This study
pLY225	P_{lux2}-crRNA-Ar4	p15AC	This study
pLY226	P_{lux2}-s-crRNA-LEB3	p15AC	This study
pLY227	P_{lux2}-s-crRNA-LEA2-WT	p15AC	This study
pLY228	P_{lux2}-s-crRNA-LEA2-C6	p15AC	This study
pLY229	P_{lux2}-s-crRNA-LEA2-C9	p15AC	This study
pLY230	P_{lux2}-s-crRNA-LEA2-C48	p15AC	This study
pLY231	P_{lux2}-s-crRNA-LEA2-C74	p15AC	This study
pLY232	P_{BAD}-s-tracrRNA- WT	pSEVA221 (JX560327)	This study
pLY233	P_{BAD}-s-tracrRNA- C6	pSEVA221 (JX560327)	This study
pLY234	P_{BAD}-s-tracrRNA- C9	pSEVA221 (JX560327)	This study
pLY235	P_{BAD}-s-tracrRNA- C48	pSEVA221 (JX560327)	This study
pLY236	P_{BAD}-s-tracrRNA- C74	pSEVA221	This study

Name	Structure	Vector	Reference
		(JX560327)	
pLY237	P_{BAD}-s-tracrRNA- R1	pSEVA221 (JX560327)	This study
pLY238	P_{BAD}-s-tracrRNA- R2	pSEVA221 (JX560327)	This study
pLY239	P_{BAD}-s-tracrRNA- R3	pSEVA221 (JX560327)	This study
pLY240	P_{T7}-CONAN	pSB1C3**	This study
pLY241	J23106-s-crRNA-13	p15AC	This study
pLY242	J23106-s-crRNA-12	p15AC	This study
pLY243	J23106-s-crRNA-11	p15AC	This study
pLY244	J23106-s-crRNA-10	p15AC	This study
pLY247	J23106-s-tracrRNA-13	pSEVA221 (JX560327)	This study
pLY248	J23106-s-tracrRNA-12	pSEVA221 (JX560327)	This study
pLY249	J23106-s-tracrRNA-11	pSEVA221 (JX560327)	This study
pLY250	J23106-s-tracrRNA-10	pSEVA221 (JX560327)	This study
pLY251	P_{BAD}-s-tracrRNA- Ar2	pSEVA221 (JX560327)	This study
pLY245	P_{BAD}-tracrRNA-ESI	pSEVA221 (JX560327)	This study
pLY246	P_{lux2}-crRNA-LEB3-WT	p15AC	This study
pLY252	P_{BAD}-tracrRNA-Ar3	pSEVA221 (JX560327)	This study
pLY253	P_{BAD}-tracrRNA-Ar4	pSEVA221 (JX560327)	This study
pLY254	P_{BAD}-tracrRNA-WT-21T	pSEVA221	This study

Name	Structure	Vector	Reference
		(JX560327)	
pLY255	dCas9- P_{rhaB}-PSPFΔHTH::λN22plus-P_{pspA}-Ar3-Reporter	pSB4A3*	This study
pLY256	dCas9- P_{rhaB}-PSPFΔHTH::λN22plus-P_{pspA}-Ar4-Reporter	pSB4A3*	This study
pLY257	J23106-crRNA-ESI	p15AC	This study
pLY258	J23106-tracrRNA-ESI	pSEVA221 (JX560327)	This study

*http://parts.igem.org/Part:pSB4A3 (There are a few point mutations)

**http://parts.igem.org/Part:pSB1C3

Supplementary Table 4: Key primers and oligos used in this study

Name	Sequence	Purpose
23-gRNA-F1	CTAGAAGTTATTATATAGTTCGGTCGTTTGAGAGCTAGAAATA GCAAGTTCAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTG GCACCGAGTCG	For sgRNA-LEA2-ex2/ex3 synthesis
23-gRNA-R1	GCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGC CTTATTTGAACTTGCTATTTCTAGCTCTCAAACGACCGAACTA TATAATAACTT	For sgRNA-LEA2-ex2/ex3 synthesis
exBoxB-F2	GTGCGGGCCCTGAAGAAGGGCCCTAGCAAGTTCAAATAAGGCT AGTCCGTTAT	For sgRNA-LEA2-ex2 synthesis
exBoxB-R2	GTTGATAACGGACTAGCCTTATTTGAACTTGCTAGGGCCCTTC TTCAGGGCCC	For sgRNA-LEA2-ex2 synthesis
exBoxB-R3	GCGGCCGCTACTAGTAAAAAAAAGCACCGACTCGGTGCCACTT GGGCCCTTCTTCAGGGCCCAA	For sgRNA-LEA2-ex2 synthesis
exBoxB-F3	CAACTTGGGCCCTGAAGAAGGGCCCAAGTGGCACCGAGTCGGT GCTTTTTTTTACTAGTAGCGGCCGCTGCA	For sgRNA-LEA2-ex2 synthesis
ex3BoxB-F2	GTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAA GGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGTA GCGGCCGCTGCA	For sgRNA-LEA2-ex3 synthesis
ex3BoxB-R2	GCGGCCGCTACTAGTAAAAAAAAGGGCCCTTCTTCAGGGCCCT GCGGGCCCTTCTTCAGGGCCCTAGCCTTGGGCCCTTCTTCAGG GCCC	For sgRNA-LEA2-ex3 synthesis
R2L1-F	CTAGAGGAACCATTCTCGGAGGAGGCCAAGTAGCGTTAAGGCT AGTC	For tracrRNA-R2L1 synthesis
R2L1-R	AACGGACTAGCCTTAACGCTACTTGGCCTCCTCCGAGAATGGT TCCT	For tracrRNA-R2L1 synthesis
R2L3-F	CTAGAGGAACCATTCGGAGGAGGCCAAGTAGCGTTAAGGCTAG TC	For tracrRNA-R2L3 synthesis
R2L3-R	AACGGACTAGCCTTAACGCTACTTGGCCTCCTCCGAATGGTTC CT	For tracrRNA-R2L3 synthesis
R2L5-F	CTAGAGGAACCATTGAGGAGGCCAAGTAGCGTTAAGGCTAGTC	For tracrRNA-R2L5 synthesis
R2L5-R	AACGGACTAGCCTTAACGCTACTTGGCCTCCTCAATGGTTCCT	For tracrRNA-R2L5 synthesis
R2L7-F	CTAGAGGAACCATTGGAGGCCAAGTAGCGTTAAGGCTAGTC	For tracrRNA-R2L7 synthesis
R2L7-R	AACGGACTAGCCTTAACGCTACTTGGCCTCCAATGGTTCCT	For tracrRNA-R2L7 synthesis
R2L9-F	CTAGAGGAACCATTAGGCCAAGTAGCGTTAAGGCTAGTC	For tracrRNA-R2L9 synthesis
R2L9-R	AACGGACTAGCCTTAACGCTACTTGGCCTAATGGTTCCT	For tracrRNA-R2L9 synthesis
R2L11-F	CTAGAGGAACCATTGCCAAGTAGCGTTAAGGCTAGTC	For tracrRNA-R2L11 synthesis
R2L11-R	AACGGACTAGCCTTAACGCTACTTGGCAATGGTTCCT	For tracrRNA-R2L11 synthesis
R0L1-F	CTAGAGGAACCATTCTCGGAGGAGGCCAAGTCCAGCTAAGGCT AGTC	For tracrRNA-R3L1 synthesis
R0L1-R	AACGGACTAGCCTTAGCTGGACTTGGCCTCCTCCGAGAATGGT TCCT	For tracrRNA-R3L1 synthesis
R0L3-F	CTAGAGGAACCATTCGGAGGAGGCCAAGTCCAGCTAAGGCTAG TC	For tracrRNA-R3L3 synthesis
R0L3-R	AACGGACTAGCCTTAGCTGGACTTGGCCTCCTCCGAATGGTTC CT	For tracrRNA-R3L3 synthesis
R0L5-F	CTAGAGGAACCATTGAGGAGGCCAAGTCCAGCTAAGGCTAGTC	For tracrRNA-R3L5 synthesis
R0L5-R	AACGGACTAGCCTTAGCTGGACTTGGCCTCCTCAATGGTTCCT	For tracrRNA-R3L5 synthesis
R0L7-F	CTAGAGGAACCATTGGAGGCCAAGTCCAGCTAAGGCTAGTC	For tracrRNA-R3L7 synthesis
R0L7-R	AACGGACTAGCCTTAGCTGGACTTGGCCTCCAATGGTTCCT	For tracrRNA-R3L7 synthesis
R0L9-F	CTAGAGGAACCATTAGGCCAAGTCCAGCTAAGGCTAGTC	For tracrRNA-R3L9 synthesis
R0L9-R	AACGGACTAGCCTTAGCTGGACTTGGCCTAATGGTTCCT	For tracrRNA-R3L9 synthesis
R0L11-F	CTAGAGGAACCATTGCCAAGTCCAGCTAAGGCTAGTC	For tracrRNA-R3L11 synthesis
R0L11-R	AACGGACTAGCCTTAGCTGGACTTGGCAATGGTTCCT	For tracrRNA-R3L11 synthesis
Ar1F	CTAGAGGAACCATTAGCTGCAATGGCGCAAGTACCGGTAAGGC TAGTC	For tracrRNA-Ar1 synthesis
Ar1R	AACGGACTAGCCTTACCGGTACTTGCGCCATTGCAGCTAATGG TTCCT	For tracrRNA-Ar1 synthesis
Ar2F	CTAGAGGAACCATTAATGGTAATGTTGCAAGTATATCTAAGGC TAGTC	For tracrRNA-Ar2 synthesis
Ar2R	AACGGACTAGCCTTAGATATACTTGCAACATTACCATTAATGG TTCCT	For tracrRNA-Ar2 synthesis

Name	Sequence	Purpose
SpeI-wt-u5d3T-cr-R	CGCTACTAGTACAAAACAGCAATCGTCATTTTAGACCGAACTATATAATAACTTCTAGTTTTATTCG	For crRNA-LEA2-U5T synthesis
SpeI-wt-u5d3C-cr-R	CGCTACTAGTACAAAACAGCAATCGTCATTTTGGACCGAACTATATAATAACTTCTAGTTTTATTCG	For crRNA-LEA2-U5C synthesis
SpeI-wt-u5d3A-cr-R	CGCTACTAGTACAAAACAGCAATCGTCATTTTTGACCGAACTATATAATAACTTCTAGTTTTATTCG	For crRNA-LEA2-U5A synthesis
XbaI-wt-tracr-F	CGCTTCTAGAGGAACCATTCAAAACAGCATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG	For tracrRNA-WT synthesis
SpeI-wt-cr-R	CGCTACTAGTACAAAACAGCATAGCTCTAAAACGACCGAACTATATAATAACTTCTAGTTTTATTCG	For crRNA-LEA2-WT synthesis
XbaI-wt-d1-tracr-F	CGCTTCTAGAGGAACCATTCAAAACAGCAAAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG	For tracrRNA-d1 synthesis
SpeI-wt-d1-cr-R	CGCTACTAGTACAAAACAGCAAAGCTCTAAAACGACCGAACTATATAATAACTTCTAGTTTTATTCG	For crRNA-LEA2-d1 synthesis
XbaI-wt-d2-tracr-F	CGCTTCTAGAGGAACCATTCAAAACAGCAATGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG	For tracrRNA-d2 synthesis
SpeI-wt-d2-cr-R	CGCTACTAGTACAAAACAGCAATGCTCTAAAACGACCGAACTATATAATAACTTCTAGTTTTATTCG	For crRNA-LEA2-d2 synthesis
SpeI-wt-d3-cr-R	CGCTACTAGTACAAAACAGCAATCCTCTAAAACGACCGAACTATATAATAACTTCTAGTTTTATTCG	For crRNA-LEA2-d3 synthesis
XbaI-wt-d3-tracr-F	CGCTTCTAGAGGAACCATTCAAAACAGCAATCCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG	For tracrRNA-d3 synthesis
SpeI-wt-d4-cr-R	CGCTACTAGTACAAAACAGCAATCGTCTAAAACGACCGAACTATATAATAACTTCTAGTTTTATTCG	For crRNA-LEA2-d4 synthesis
XbaI-wt-d4-tracr-F	CGCTTCTAGAGGAACCATTCAAAACAGCAATCGAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG	For tracrRNA-d4 synthesis
SpeI-wt-d4U1-cr-R	CGCTACTAGTACAAAACAGCAATCGTCAAAAACGACCGAACTATATAATAACTTCTAGTTTTATTCG	For crRNA-LEA2-U1 synthesis
XbaI-wt-d4U1-tracr-F	CGCTTCTAGAGGAACCATTCAAAACAGCAATCGAAGTAAAAATAAGGCTAGTCCGTTATCAACTTG	For tracrRNA-U1 synthesis
SpeI-wt-d4U2-cr-R	CGCTACTAGTACAAAACAGCAATCGTCATAAACGACCGAACTATATAATAACTTCTAGTTTTATTCG	For crRNA-LEA2-U2 synthesis
XbaI-wt-d4U2-tracr-F	CGCTTCTAGAGGAACCATTCAAAACAGCAATCGAAGTATAAATAAGGCTAGTCCGTTATCAACTTG	For tracrRNA-U2 synthesis
SpeI-wt-d4U3-cr-R	CGCTACTAGTACAAAACAGCAATCGTCATTAACGACCGAACTATATAATAACTTCTAGTTTTATTCG	For crRNA-LEA2-U3 synthesis
XbaI-wt-d4U3-tracr-F	CGCTTCTAGAGGAACCATTCAAAACAGCAATCGAAGTATTAATAAGGCTAGTCCGTTATCAACTTG	For tracrRNA-U3 synthesis
SpeI-wt-d4U4-cr-R	CGCTACTAGTACAAAACAGCAATCGTCATTTACGACCGAACTATATAATAACTTCTAGTTTTATTCG	For crRNA-LEA2-U4 synthesis
XbaI-wt-d4U4-tracr-F	CGCTTCTAGAGGAACCATTCAAAACAGCAATCGAAGTATTTATAAGGCTAGTCCGTTATCAACTTG	For tracrRNA-U4 synthesis
SpeI-wt-d4U5-cr-R	CGCTACTAGTACAAAACAGCAATCGTCATTTTCGACCGAACTATATAATAACTTCTAGTTTTATTCG	For crRNA-LEA2-U5 synthesis
XbaI-wt-d4U5-tracr-F	CGCTTCTAGAGGAACCATTCAAAACAGCAATCGAAGTATTTTTAAGGCTAGTCCGTTATCAACTTG	For tracrRNA-U5 synthesis
XbaI-R1-PspA	CGCTTCTAGAGCCCAGGACTCCTCACTTCAGCGGTTAGTGTAATTCGCTAACTCATCCTGGC	For P_{pspA}-R1 synthesis
XbaI-R2-PspA	CGCTTCTAGAGTAATGCAGAAGAAGACCATGCGGTTAGTGTAATTCGCTAACTCATCCTGGC	For P_{pspA}-R2 synthesis
XbaI-R3-PspA	CGCTTCTAGAGTGAAGGACGGCGGCCACTACCGGTTAGTGTAATTCGCTAACTCATCCTGGC	For P_{pspA}-R3 synthesis
XbaI-R1-tra	CGCTTCTAGAGGAACCATTCCTTGTAGATGAACAAGTGCCGTTAAGGCTAGTCCGTTATCAACTTG	For tracrRNA-R1 synthesis
XbaI-R2-tra	CGCTTCTAGAGGAACCATTGCTCGGAGGAGGCCAAGTCCAGCTAAGGCTAGTCCGTTATCAACTTG	For tracrRNA-R2 synthesis
XbaI-R3-tra	CGCTTCTAGAGGAACCATTAGGTGGTCTTGACCAAGTAGCGTTAAGGCTAGTCCGTTATCAACTTG	For tracrRNA-R3 synthesis
XP- L3S3P22-F	CTAGAGCCAATTATTGAAGGCCGCTAACGCGGCCTTTTTTTGTTTCTGGTCTCCCTACTAGTAGCGGCCGCTGCA	For terminator L3S3P22 synthesis
XP- L3S3P22-R	GCGGCCGCTACTAGTAGGGAGACCAGAAACAAAAAAAGGCCGCGTTAGCGGCCTTCAATAATTGGCT	For terminator L3S3P22 synthesis
XP-L3S2P21-F	CTAGAGCTCGGTACCAAATTCCAGAAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCCTACTAGTAGCGGCCGCTGCA	For terminator L3S2P21 synthesis
XP-L3S2P21-R	GCGGCCGCTACTAGTAGGACCAAAACGAAAAAAGGCCCCCCTTTCGGGAGGCCTCTTTTCTGGAATTTGGTACCGAGCT	For terminator L3S2P21 synthesis
PS1 (*2*)	AGGGCGGCGGATTTGTCC	For sequencing
PS2 (*2*)	GCGGCAACCGAGCGTTC	For sequencing

Name	Sequence	Purpose
VF2*	TGCCACCTGACGTCTAAGAA	For sequencing
VR**	ATTACCGCCTTTGAGTGAGC	For sequencing
CF2	GTTCGTAAGCCATTTCCGCTCGCCGCAGTC	For sequencing
CR	AACGGTCTGGTTATAGGTACATTGAGCAAC	For sequencing

* http://parts.igem.org/wiki/index.php?title=Part:BBa_G00100

** http://parts.igem.org/wiki/index.php?title=Part:BBa_G00101

Supplementary Table 5: Sequences for inducible promoters used in this study

Name	Type and source	Parts	DNA sequence (5'– 3')
P_{BAD}	Inducible promoter Composite Module (This study)	Reverse Terminator L3S2P11	CCTAGTTGTCTTCATGCATGAAGACAAAATTAATACTAGAGGGACCAAAA CGAAAAAAGACGCTCGAAAGCGTCTCTTTTCTGGAATTTGGTACCGAGCT CTAGTA
		Reverse *araC* CDS	TTATGACAACTTGACGGCTACATCATTCACTTTTTCTTCACAACCGGCAC GGAACTCGCTCGGGCTGGCCCCGGTGCATTTTTTAAATACCCGCGAGAAA TAGAGTTGATCGTCAAAACCAACATTGCGACCGACGGTGGCGATAGGCAT CCGGGTGGTGCTCAAAAGCAGCTTCGCCTGGCTGATACGTTGGTCCTCGC GCCAGCTTAAGACGCTAATCCCTAACTGCTGGCGGAAAAGATGTGACAGA CGCGACGGCGACAAGCAAACATGCTGTGCGACGCTGGCGATATCAAAATT GCTGTCTGCCAGGTGATCGCTGATGTACTGACAAGCCTCGCGTACCCGAT TATCCATCGGTGGATGGAGCGACTCGTTAATCGCTTCCATGCGCCGCAGT AACAATTGCTCAAGCAGATTTATCGCCAGCAGCTCCGAATAGCGCCCTTC CCCTTGCCCGGCGTTAATGATTTGCCCAAACAGGTCGCTGAAATGCGGCT GGTGCGCTTCATCCGGGCGAAAGAACCCCGTATTGGCAAATATTGACGGC CAGTTAAGCCATTCATGCCAGTAGGCGCGCGGACGAAAGTAAACCCACTG GTGATACCATTCGCGAGCCTCCGGATGACGACCGTAGTGATGAATCTCTC CTGGCGGGAACAGCAAAATATCACCCGGTCGGCAAACAAATTCTCGTCCC TGATTTTTCACCACCCCCTGACCGCGAATGGTGAGATTGAGAATATAACC TTTCATTCCCAGCGGTCGGTCGATAAAAAAATCGAGATAACCGTTGGCCT CAATCGGCGTTAAACCCGCCACCAGATGGGCATTAAACGAGTATCCCGGC AGCAGGGGATCATTTTGCGCTTCAGCCAT
		P_{BAD}	ACTTTTCATACTCCCGCCATTCAGAGAAGAAACCAATTGTCCATATTGCA TCAGACATTGCCGTCACTGCGTCTTTTACTGGCTCTTCTCGCTAACCAAA CCGGTAACCCCGCTTATTAAAAGCATTCTGTAACAAAGCGGGACCAAAGC CATGACAAAAACGCGTAACAAAAGTGTCTATAATCACGGCAGAAAAGTCC ACATTGATTATTTGCACGGCGTCACACTTTGCTATGCCATAGCATTTTTA TCCATAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCAACTCTCTAC TGTTTCTCCATA (TTS)
P_{lux2}	Inducible promoter Composite Module (This study)	Reverse Terminator L3S2P55	CCTAGTTGTCTTCATGCATGAAGACAAAATTAATACTAGAGGGACCAAAA CGAAAAAAGACGCTTTTCAGCGTCTTATTGTTCGTCTTTGGTACCGAGCT CTAGTAGTGATCTACACTAGCACTATCAGTG
		Reverse *luxR* CDS	TTATTAATTTTTAAAGTATGGGCAATCAATTGCTCCTGTTAAAATTGCTT TAGAAATACTTTGGCAGCGGTTTGTTGTATTGAGTTTCATTTGCGCATTG GTTAAATGGAAAGTGACAGTACGCTCACTGCAACCTAATATTTTTGAAAT ATCCCAAGAGCTTTTTCCTTCGCATGCCCACGCTAAACATTCTTTTTCTC TTTTGGTTAAATCGTTGTTTGATTTATTATTTGCTATATTTATTTTTCGA TAATTATCAACTAGAGAAGGAACAATTAATGGTATGTTCATACACGCATG TAAAAATAAACTATCTATATAGTTGTCTTTTTCTGAATGTGCAAAACTAA GCATTCCGAAGCCATTGTTAGCCGTATGAATAGGGAAACTAAACCCAGTG ATAAGACCTGATGTTTTCGCTTCTTTAATTACATTTGGAGATTTTTTATT TACAGCATTGTTTTCAAATATATTCCAATTAATTGGTGAATGATTGGAGT TAGAATAATCTACTATAGGATCATATTTTATTAAATTAGCGTCATCATAA TATTGCCTCCATTTTTTAGGGTAATTATCTAGGATTGAAATATCAGATTT AACCATAGAATGAGGATAAATGATCGCGAGTAAATAATATTCACAATGTA CCATTTTAGTCATATCAGATAAGCATTGATTAATATCATTATTGCTTCTA CAAGCTTTAATTTTATTAATTATTCTGTATGTGTCGTCGGCATTTATGTT TTTCAT
		Reverse RBS B0030	CTAGTATTTCTCCTCTTTAATCTCTAGTA (Scar/Spacer)
		Reverse J23117	GCTAGCACAATCCCTAGGACTGAGCTAGCTGTCAATCACACT (Scar/Spacer)
		T7Te Terminator	GGCTCACCTTCGGGTGGGCCTTTCTGCG

Name	Type and source	Parts	DNA sequence (5'– 3')
		P_{lux2}	TTTATATACTAGAGACCTGTAGGATCGTACAGGTTTACGCAAGAAAATGG TTTGTTACTTTCGAATAAAA (TTS, Scar/Spacer)
P_{rhaB}	Inducible promoter Composite Module (This study)	Reverse Terminator L3S2P55	CCTAGTTGTCTTCATGCATGAAGACAAAATTAATACTAGAGGGACCAAAA CGAAAAAAGACGCTTTTCAGCGTCTTATTGTTCGTCTTTGGTACCGAGCT AGTA
		Reverse *rhaS* CDS	TTATTGCAGAAAGCCATCCCGTCCCTGGCGAATATCACGCGGTGACCAGT TAAACTCTCGGCGAAAAAGCGTCGAAAAGTGGTTACTGTCGCTGAATCCA CAGCGATAGGCGATGTCAGTAACGCTGGCCTCGCTGTGGCGTAGCAGATG TCGGGCTTTCATCAGTCGCAGGCGGTTCAGGTATCGCTGAGGCGTCAGTC CCGTTTGCTGCTTAAGCTGCCGATGTAGCGTACGCAGTGAAAGAGAAAAT TGATCCGCCACGGCATCCCAATTCACCTCATCGGCAAAATGGTCCTCCAG CCAGGCCAGAAGCAAGTTGAGACGTGATGCGCTGTTTTCCAGGTTCTCCT GCAAACTGCTTTTACGCAGCAAGAGCAGTAATTGCATAAACAAGATCTCG CGACTGGCGGTCGAGGGTAAATCATTTTCCCCTTCCTGCTGTTCCATCTG TGCAACCAGCTGTCGCACCTGCTGCAATACGCTGTGGTTAACGCGCCAGT GAGACGGATACTGCCCATCCAGCTCTTGTGGCAGCAACTGATTCAGCCCG GCGAGAAACTGAAATCGATCCGGCGAGCGATACAGCACATTGGTCAGACA CAGATTATCGGTATGTTCATACAGATGCCGATCATGATCGCGTACGAAAC AGACCGTGCCACCGGTGATGGTATAGGGCTGCCCATTAAACACATGAATA CCCGTGCCATGTTCGACAATCACAATTTCATGAAAATCATGATGATGTTC AGGAAAATCCGCCTGCGGGAGCCGGGGTTCTATCGCCACGGACGCGTTAC CGGACGGAAAAAAATCCACACTATGTAATACGGTCAT
		Reverse J23101-RBS	TTGGGCTCCCTCTAGTAGCTAGCATAATACCTAGGACTGAGCTAGCTGTA AACTCTAGTATCACACT (Scar/Spacer)
		T7Te Terminator	GGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATATACTAGAGAGACCTTT ACGCCGCTGGAGCAGGAATGCGGTGAGCATCACAT (Scar/Spacer)
		P_{rhaB}	CACCACAATTCAGCAAATTGTGAACATCATCACGTTCATCTTTCCCTGGT TGCCAATGGCCCATTTTCCTGTCAGTAACGAGAAGGTCGCGAATTGAGGC GCTTTTTAGACTGGTCGTAA (TTS)

Supplementary Table 6: Sequences for dCas9/dxCas9 generator

Name	Type and source	Parts	DNA sequence (5'-3')
dCas9 generator	Composite Module (This study)	BioBrick Prefix	GAATTCGCGGCCGCTTCTAGAG
		Reverse Terminator L3S3P00	GGGAGACCAGAAACAAAAAAAGGGGAGCGGTTTCCCGCTCCCCTTCAATAATTGG
		Reverse Standardized *dCas9* CDS	CTCTAGTATTAGTCACCTCCTAGCTGACTCAAATCAATGCGTGTTTCATAAAG ACCAGTGATGGATTGATGGATAAGAGTGGCATCTAAAACTTCTTTTGTAGACG TATATCGTTTACGATCAATTGTTGTATCAAAATATTTAAAAGCAGCGGGAGCT CCAAGATTCGTCAACGTAAATAAATGAATAATATTTTCTGCTTGTTCACGTAT TGGTTTGTCTCTATGTTTGTTATATGCACTAAGAACTTTATCTAAATTGGCAT CTGCTAAAATAACACGCTTAGAAAATTCACTGATTTGCTCAATAATCTCATCT AAATAATGCTTATGCTGCTCCACAAACAATTGTTTTTGTTCGTTATCTTCTGG ACTACCCTTCAACTTTTCATAATGACTAGCTAAATATAAAAAATTCACATATT TGCTTGGCAGAGCCAGCTCATTTCCTTTTTGTAATTCTCCGGCACTAGCCAGC ATCCGTTTACGACCGTTTTCTAACTCAAAAAGACTATATTTAGGTAGTTTAAT GATTAAGTCTTTTTTAACTTCCTTATATCCTTTAGCTTCTAAAAAGTCAATCG GATTTTTTTCAAAGGAACTTCTTTCCATAATTGTGATCCCTAGTAACTCTTTA ACGGATTTTAACTTCTTCGATTTCCCTTTTTCCACCTTAGCAACCACTAGGAC TGAATAAGCTACCGTTGGACTATCAAAACCACCATATTTTTTTGGATCCCAGT CTTTTTTACGAGCAATAAGCTTGTCCGAATTTCTTTTTGGTAAAATTGACTCC TTGGAGAATCCGCCTGTCTGTACTTCTGTTTTCTTGACAATATTGACTTGGGG CATGGACAATACTTTGCGCACTGTGGCAAAATCTCGCCCTTTATCCCAGACAA TTTCTCCAGTTTCCCCATTAGTTTCGATTAGAGGGCGTTTGCGAATCTCTCCA TTTGCAAGTGTAATTTCTGTTTTGAAGAAGTTCATGATATTAGAGTAAAAGAA ATATTTTGCGGTTGCTTTGCCTATTTCTTGCTCAGACTTAGCAATCATTTTAC GAACATCATAAACTTTATAATCACCATAGACAAACTCCGATTCAAGTTTTGGA TATTTCTTAATCAAAGCAGTTCCAACGACGGCATTTAGATACGCATCATGGGC ATGATGGTAATTGTTAATCTCACGTACTTTATAGAATTGGAAATCTTTTCGGA AGTCAGAAACTAATTTAGATTTTAAGGTAATCACTTTAACCTCTCGAATAAGT TTATCATTTTCATCGTATTTAGTATTCATGCGACTATCCAAAATTTGTGCCAC ATGCTTAGTGATTTGGCGAGTTTCAACCAATTGGCGTTTGATAAAACCAGCTT TATCAAGTTCACTCAAACCTCCACGTTCAGCTTTCGTTAAATTATCAAACTTA CGTTGAGTGATTAACTTGGCGTTTAGAAGTTGTCTCCAATAGTTTTTCATCTT TTTGACTACTTCTTCACTTGGAACGTTATCCGATTTACCACGATTTTTATCAG AACGCGTTAAGACCTTATTGTCTATTGAATCGTCTTTAAGGAAACTTTGTGGA ACAATGGCATCGACATCATAATCACTTAAACGATTAATATCTAATTCTTGGTC CACATACATGTCTCTTCCATTTTGGAGATAATAGAGATAGAGCTTTTCATTTT GCAATTGAGTATTTTCAACAGGATGCTCTTTAAGAATCTGACTTCCTAATTCT TTGATACCTTCTTCGATTCGTTTCATACGCTCTCGCGAATTTTTCTGGCCCTT TTGAGTTGTCTGATTTTCACGTGCCATTTCAATAACGATATTTTCTGGCTTAT GCCGCCCCATTACTTTGACCAATTCATCAACAACTTTTACAGTCTGTAAAATA CCTTTTTTAATAGCAGGGCTACCAGCTAAATTTGCAATATGTTCATGTAAACT ATCGCCTTGTCCAGACACTTGTGCTTTTTGAATGTCTTCTTTAAATGTCAAAC TATCATCATGGATCAGCTGCATAAAATTGCGATTGGCAAAACCATCTGATTTC AAAAAATCTAATATTGTTTTGCCAGATTGCTTATCCCTAATACCATTAATCAA TTTTCGAGACAAACGTCCCCAACCAGTATAACGGCGACGTTTAAGCTGTTTCA TCACCTTATCATCAAAGAGGTGAGCATATGTTTTAAGTCTTTCCTCAATCATC TCCCTATCTTCAAATAAGGTCAATGTTAAAACAATATCCTCTAAGATATCTTC ATTTTCTTCATTATCCAAAAAATCTTTATCTTTAATAATTTTTAGCAAATCAT GGTAGGTACCTAATGAAGCATTAAATCTATCTTCAACTCCTGAAATTTCAACA CTATCAAAACATTCTATTTTTTTGAAATAATCTTCTTTTAATTGCTTAACGGT TACTTTTCGATTTGTTTTGAAGAGTAAATCAACAATGGCTTTCTTCTGTTCAC CTGAAAGAAATGCTGGTTTTCGCATTCCTTCAGTAACATATTTGACCTTTGTC AATTCGTTATAAACCGTAAAATACTCATAAAGCAAACTATGTTTTGGTAGTAC TTTTTCATTTGGAAGATTTTTATCAAAGTTTGTCATGCGTTCAATAAATGATT GAGCTGAAGCACCTTTATCGACAACTTCTTCAAAATTCCATGGGGTAATTGTT TCTTCAGACTTCCGAGTCATCCATGCAAAACGACTATTGCCACGCGCCAATGG ACCAACATAATAAGGAATACGAAAAGTCAAGATTTTTTCAATCTTCTCACGAT TGTCTTTTAAAAATGGATAAAAGTCTTCTTGTCTTCTCAAAATAGCATGCAGC TCACCCAAGTGAATTTGATGGGGAATAGAGCCGTTGTCAAAGGTCCGTTGCTT GCGCAGCAAATCTTCACGATTTAGTTTCACCAATAATTCCTCAGTACCATCCA TTTTTTCTAAAATTGGTTTGATAAATTTATAAAATTCTTCTTGGCTAGCTCCC

Name	Type and source	Parts	DNA sequence (5'-3')
			CCATCAATATAACCTGCATATCCGTTTTTTGATTGATCAAAAAAGATTTCTTT ATACTTTTCTGGAAGTTGTTGTCGAACTAAAGCTTTTAAAAGAGTCAAGTCTT GATGATGTTCATCGTAGCGTTTAATCATTGAAGCTGATAGGGGAGCCTTAGTT ATTTCAGTATTTACTCTTAGGATATCTGAAAGTAAAATAGCATCTGATAAATT CTTAGCTGCCAAAAACAAATCAGCATATTGATCTCCAATTTGCGCCAATAAAT TATCTAAATCATCATCGTAAGTATCTTTTGAAAGCTGTAATTTAGCATCTTCT GCCAAATCAAAATTTGATTTAAAATTAGGGGTCAAACCCAATGACAAAGCAAT GAGATTCCCAAATAAGCCATTTTTCTTCTCACCGGGGAGCTGAGCAATGAGAT TTTCTAATCGTCTTGATTTACTCAATCGTGCAGAAAGAATCGCTTTAGCATCT ACTCCACTTGCGTTAATAGGGTTTTCTTCAAATAATTGATTGTAGGTTTGTAC CAACTGGATAAATAGTTTGTCCACATCACTATTATCAGGATTTAAATCTCCCT CAATCAAAAAATGACCACGAAACTTAATCATATGCGCTAAGGCCAAATAGATT AAGCGCAAATCCGCTTTATCAGTAGAATCTACCAATTTTTTTCGCAGATGATA GATAGTTGGATATTTCTCATGATAAGCAACTTCATCTACTATATTTCCAAAAA TAGGATGACGTTCATGCTTCTTGTCTTCTTCCACCAAAAAAGACTCTTCAAGT CGATGAAAGAAACTATCATCTACTTTCGCCATCTCATTTGAAAAAATCTCCTG TAGATAACAAATACGATTCTTCCGACGTGTATACCTTCTACGAGCTGTCCGTT TGAG**G**CGAGTCGCTTCCGCTGTCTCTCCACTGTCAAATAAAAGAGCCCCTATA AGATTTTTTTTGATACTGTGGCGGTCTGTATTTCCCAGAACCTTGAACTTTTT AGACGGAACCTTATATTCATCAGTGATCACCGCCCATCCGACGCTATTTGTGC CGATAGCTAAGCCTATTGAGTATTTCTTATCCAT (Scar/Spacer, **mutation**)
		Reverse RBS B0034	AGATCCTTTCTCCTCTTT (Scar/Spacer)
		tetR/tetA promoters	AGATCTTTT**C**AATTCTTTTCTCTATCACTGATAGGGAGTGGTAAAATAACTCT ATCAACGATAGAGTGTCAACAAAAATTAGGAATTAATG (**mutation**)
		tetR CDS	ATGTC**A**AGATTAGATAAAAGTAAAGTGATTAACAGCGCATTAGAGCTGCTTAA TGAGGTCGGAATCGAAGGTTTAACAACCCGTAAACTCGCCCAGAAGCTAGGTG TAGAGCAGCCTACATTGTATTGGCATGTAAAAAATAAGCGGGCTTTGCTCGAC GCCTTAGCCATTGAGATGTTAGATAGGCACCATACTCACTTTTGCCCTTTAGA AGGGGAAAGCTGGCAAGATTTTTTACGTAATAACGCTAAAAGTTTTAGATGTG CTTTACTAAGTCATCGCGATGGAGCAAAAGTACATTTAGGTACACGGCCTACA GAAAAACAGTATGAAACTCTCGAAAATCAATTAGCCTTTTTATGCCAACAAGG TTTTTCACTAGAGAATGCATTATATGCACTCAGCGCTGTGGGGCATTTTACTT TAGGTTGCGTATTGGAAGATCAAGAGCATCAAGTCGCTAAAGAAGAAAGGGAA ACACCTACTACTGATAGTATGCCGCCATTATTACGACAAGCTATCGAATTATT TGATCACCAAGGTGCAGAGCCAGCCTTCTTATTCGGCCTTGAATTGATCATAT GCGGATTAGAAAAACAACTTAAATGTGAAAGTGGGTCTTAA (**mutation**)
		Terminator L3S3P22	TACTAGAGCCAATTATTGAAGGCCGCTAACGCGGCCTTTTTTTGTTTCTGGTC TCCC (Scar/Spacer)
		BioBrick Suffix	TACTAGTAGCGGCCGCTGCAG
dxCas9 generator	Composite Module (This study)	BioBrick Prefix	GAATTCGCGGCCGCTTCTAGAG
		Reverse Terminator L3S3P00	GGGAGACCAGAAACAAAAAAAGGGGAGCGGTTTCCCGCTCCCCTTCAATAATT GG
		Reverse Standardized *dxCas9* CDS	CTCTAGTATTAGTCACCTCCTAGCTGACTCAAATCAATGCGTGTTTCATAAAG ACCAGTGATGGATTGATGGATAAGAGTGGCATCTAAAACTTCTTTTGTAGACG TATATCGTTTACGATCAATTGTTGTATCAAAATATTTAAAAGCAGCGGGAGCT CCAAGATTCGTCAACGTAAATAAATGAATAATATTTTCTGCTTGTTCACGTAT TGGTTTGTCTCTATGTTTGTTATATGCACTAAGAACTTTATCTAAATTGGCAT CTGCTAAAATAACACGCTTAGAAAATTCACTGATTTGCTCAATAATCTCATCT AAATAATGCTTATGCTGCTCCACAAACAATTGTTTTTGTTCGTTATCTTCTGG

Name	Type and source	Parts	DNA sequence (5’-3’)
			ACTACCCTTCAACTTTTCATAATGACTAGCTAAATATAAAAAATTCACATATT
			TGCTTGGCAGAGCCAGCTCATTTCCTTTTTGTAA**AA**CTCCGGCACTAGCCAGC
			ATCCGTTTACGACCGTTTTCTAACTCAAAAAGACTATATTTAGGTAGTTTAAT
			GATTAAGTCTTTTTTAACTTCCTTATATCCTTTAGCTTCTAAAAAGTCAATCG
			GATTTTTTTCAAAGGAACTTCTTTCCATAATTGTGATCCCTAGTAACTCTTTA
			ACGGATTTTAACTTCTTCGATTTCCCTTTTTCCACCTTAGCAACCACTAGGAC
			TGAATAAGCTACCGTTGGACTATCAAAACCACCATATTTTTTTGGATCCCAGT
			CTTTTTTACGAGCAATAAGCTTGTCCGAATTTCTTTTTGGTAAAATTGACTCC
			TTGGAGAATCCGCCTGTCTGTACTTCTGTTTTCTTGACAATATTGACTTGGGG
			CATGGACAATACTTTGCGCACTGTGGCAAAATCTCGCCCTTTATCCCAGACAA
			TTTCTCCAGTTTCCCCATTAGTTTCGATTAGAGGGCGTTTGCGAATCTCTCCA
			TTTGCAAGTGTAATTTCTGTTTTGAAGAAGTTCATGATATTAGAGTAAAAGAA
			ATATTTTGCGGTTGCTTTGCCTATTTCTTGCTCAGACTTAGCAATCATTTTAC
			GAACATCATAAACTTTATAATCACCATAGACAAACTCCGATTCAAGTTTTGGA
			TATTTCTTAATCAAAGCAGTTCCAACGACGGCATTTAGATACGCATCATGGGC
			ATGATGGTAATTGTTAATCTCACGTACTTTATAGAATTGGAAATCTTTTCGGA
			AGTCAGAAACTAATTTAGATTTTAAGGTAATCACTTTAACCTCTCGAATAAGT
			TTATCATTTTCATCGTATTTAGTATTCATGCGACTATCCAAAATTTGTGCCAC
			ATGCTTAGTGATTTGGCGAGTTTCAACCAATTGGCGTTTGATAAAACCAGCTT
			TATCAAGTTCACTCAAACCTCCACGTTCAGCTTTCGTTAAATTATCAAACTTA
			CGTTGAGTGATTAACTTGGCGTTTAGAAGTTGTCTCCAATAGTTTTTCATCTT
			TTTGACTACTTCTTCACTTGGAACGTTATCCGATTTACCACGATTTTTATCAG
			AACGCGTTAAGACCTTATTGTCTATTGAATCGTCTTTAAGGAAACTTTGTGGA
			ACAATGGCATCGACATCATAATCACTTAAACGATTAATATCTAATTCTTGGTC
			CACATACATGTCTCTTCCATTTTGGAGATAATAGAGATAGAGCTTTTCATTTT
			GCAATTGAGTATTTTCAACAGGATGCTCTTTAAGAATCTGACTTCCTAATTCT
			TTGATACCTTCTTCGATTCGTTTCATACGCTCTCGCGAATTTTTCTGGCCCTT
			TTGAGTTGTCTGATTTTCACGTGCCATTTCAATAACGATATTTTCTGGCTTAT
			GCCGCCCCATTACTTTGACCAATTCATCAACAACTTTTACAGTCTGTAAAATA
			CCTTTTTTAATAGCAGGGCTACCAGCTAAATTTGCAATATGTTCATGTAAACT
			ATCGCCTTGTCCAGACACTTGTGCTTTTTGAATGTCTTCTTTAAATGTCAAAC
			TATCATCATGGATCAGCTG**A**ATAAAATTGCGATTGGCAAAACCATCTGATTTC
			AAAAAATCTAATATTGTTTTGCCAGATTGCTTATCCCTAATACCATTAATCAA
			TTTTCGAGACAAACGTCCCCAACCAGTATAACGGCGACGTTTAAGCTGTTTCA
			TCACCTTATCATCAAAGAGGTGAGCATATGTTTTAAGTCTTTCCTCAATCATC
			TCCCTATCTTCAAATAAGGTCAATGTTAAAACAATATCCTCTAAGATATCTTC
			ATTTTCTTCATTATCCAAAAAATCTTTATCTTTAATAATTTTTAGCAAATCAT
			GGTAGGTACCTAATGAAGCATTAAATCTATCTTCAACTCCTGAAATTTCAACA
			CTATCAAAACATTCTATTTTTTTGAAATAATCTTCTTTTAATTGCTTAACGGT
			TACTTTTCGATTTGTTTTGAAGAGTAAATCAACAATGGCTTTCTTCTG**A**TCAC
			CTGAAAGAAATGCTGGTTTTCGCATTCCTTCAGTAACATATTTGACCTTTGTC
			AATTCGTTATAAACCGTAAAATACTCATAAAGCAAACTATGTTTTGGTAGTAC
			TTTTTCATTTGGAAGATTTTTATCAAAGTTTGTCATGCGTTCAATAAATGATT
			GAGCTGAAGCACCTTTATCGACAACTT**T**TTCAAAATTCCATGGGGTAATTGTT
			TCTTCAGACTTCCGAGTCATCCATGCAAAACGACTATTGCCACGCGCCAATGG
			ACCAACATAATAAGGAATACGAAAAGTCAAGATTTTTTCAATCTTCTCACGAT
			TGTCTTTTAAAAATGGATAAAAGTCTTCTTGTCTTCTCAAAATAGCATGCAGC
			TCACCCAAGTGAATTTGATGGGGAATA**AT**GCCGTTGTCAAAGGTCCGTTGCTT
			GCGCAGCAAATCTTCACGATTTAGTTTCACCAATAATTCCTCAGTACCATCCA
			TTTTTTCTAAAATTGGTTTGATAAATTTATAAAATTCTTCTTGGCTAGCTCCC
			CCATCAATATAACCTGCATATCCGTTTTTTGATTGATCAAAAAAGATTTCTTT
			ATACTTTTCTGGAAGTTGTTGTCGAACTAAAGCTTTTAAAAGAGTCAAGTCTT
			GATGATGTTCATCGTA**TAA**TTTAATCATTGAAGCTGATAGGGGAGCCTTAGTT
			ATTTCAGTATTTACTCTTAGGATATCTGAAAGTAAAATAGCATCTGATAAATT
			CTTAGCTGCCAAAAACAAATCAGCATATTGATCTCCAATTTGCGCCAATAAAT
			TATCTAAATCATCATCGTAAGTATCTTTTGAAAGCTGTAATTT**G**G**T**ATCTTCT
			GCCAAATCAAAATTTGATTTAAAATTAGGGGTCAAACCCAATGACAAAGCAAT
			GAGATTCCCAAATAAGCCATTTTTCTTCTCACCGGGGAGCTGAGCAATGAGAT
			TTTCTAATCGTCTTGATTTACTCAATCGTGCAGAAAGAATCGCTTTAGCATCT
			ACTCCACTTGCGTTAATAGGGTTTTCTTCAAATAATTGATTGTAGGTTTGTAC
			CAACTGGATAAATAGTTTGTCCACATCACTATTATCAGGATTTAAATCTCCCT
			CAATCAAAAAATGACCACGAAACTTAATCATATGCGCTAAGGCCAAATAGATT
			AAGCGCAAATCCGCTTTATCAGTAGAATCTACCAATTTTTTTCGCAGATGATA
			GATAGTTGGATATTTCTCATGATAAGCAACTTCATCTACTATATTTCCAAAAA
			TAGGATGACGTTCATGCTTCTTGTCTTCTTCCACCAAAAAAGACTCTTCAAGT
			CGATGAAAGAAACTATCATCTACTTTCGCCATCTCATTTGAAAAAATCTCCTG
			TAGATAACAAATACGATTCTTCCGACGTGTATACCTTCTACGAGCTGTCCGTT
			TGAGGCGAGTCGCTTCCGCTGTCTCTCCACTGTCAAATAAAAGAGCCCCTATA

Name	Type and source	Parts	DNA sequence (5'-3')
			AGATTTTTTTTGATACTGTGGCGGTCTGTATTTCCCAGAACCTTGAACTTTTT AGACGGAACCTTATATTCATCAGTGATCACCGCCCATCCGACGCTATTTGTGC CGATAGCTAAGCCTATTGAGTATTTCTTATCCAT (Scar/Spacer, **mutation**)
		Reverse RBS B0034	AGATCCTTTCTCCTCTTT (Scar/Spacer)
		tetR/tetA promoters	AGATCTTTT**C**AATTCTTTTCTCTATCACTGATAGGGAGTGGTAAAATAACTCT ATCAACGATAGAGTGTCAACAAAAATTAGGAATTAATG (**mutation**)
		tetR CDS	ATGTC**A**AGATTAGATAAAAGTAAAGTGATTAACAGCGCATTAGAGCTGCTTAA TGAGGTCGGAATCGAAGGTTTAACAACCCGTAAACTCGCCCAGAAGCTAGGTG TAGAGCAGCCTACATTGTATTGGCATGTAAAAAATAAGCGGGCTTTGCTCGAC GCCTTAGCCATTGAGATGTTAGATAGGCACCATACTCACTTTTGCCCTTTAGA AGGGGAAAGCTGGCAAGATTTTTTACGTAATAACGCTAAAAGTTTTAGATGTG CTTTACTAAGTCATCGCGATGGAGCAAAAGTACATTTAGGTACACGGCCTACA GAAAAACAGTATGAAACTCTCGAAAATCAATTAGCCTTTTTATGCCAACAAGG TTTTTCACTAGAGAATGCATTATATGCACTCAGCGCTGTGGGGCATTTTACTT TAGGTTGCGTATTGGAAGATCAAGAGCATCAAGTCGCTAAAGAAGAAAGGGAA ACACCTACTACTGATAGTATGCCGCCATTATTACGACAAGCTATCGAATTATT TGATCACCAAGGTGCAGAGCCAGCCTTCTTATTCGGCCTTGAATTGATCATAT GCGGATTAGAAAAACAACTTAAATGTGAAAGTGGGTCTTAA (**mutation**)
		Terminator L3S3P22	TACTAGAGCCAATTATTGAAGGCCGCTAACGCGGCCTTTTTTTGTTTCTGGTC TCCC (Scar/Spacer)
		BioBrick Suffix	TACTAGTAGCGGCCGCTGCAG

Supplementary Table 7: Sequences for activator generator and reporter

Name	Type and source	Parts	DNA sequence (5'-3')
PspFΔHTH::λN22plus	Composite Module (This study)	RBS B0032	TCACACAGGAAAGTACTAG (Scar/Spacer)
		pspFΔHTH::λN22plus CDS	ATGGCAGAATACAAAGATAATTTACTTGGTGAGGCGAACAGCTTTCTCGAA GTGCTGGAACAGGTTTCGCATCTCGCACCGCTGGACAAACCGGTGCTCATC ATCGGCGAACGCGGCACCGGTAAAGAGCTGATTGCCAGCCGCCTGCATTAT CTCTCCTCCCGTTGGCAAGGGCCGTTTATTTCCCTTAACTGCGCGGCGTTA AATGAAAATCTGCTGGATTCCGAACTGTTTGGTCACGAAGCGGGGGCGTTT ACCGGTGCGCAAAAACGTCATCCAGGGAGATTTGAACGTGCCGACGGCGGT ACGCTATTTCTTGATGAACTCGCTACGGCACCGATGATGGTGCAGGAGAAA TTATTGCGCGTGATTGAGTACGGTGAACTGGAGCGCGTTGGCGGCAGCCAA CCATTGCAGGTGAATGTGCGGTTGGTATGCGCGACGAATGCCGATCTCCCG GCGATGGTCAATGAAGGCACTTTTCGCGCTGACCTGCTCGACCGACTGGCT TTTGATGTTGTACAACTGCCACCACTGCGCGAGCGCGAAAGCGACATAATG TTGATGGCAGAATACTTTGCCATCCAGATGTGTCGGGAAATCAAGCTGCCT CTGTTCCCGGGGGTTTACGGAGCGCGCCAGAGAAACATTGCTGAATTATCGT TGGCCGGGAAATATTCGTGAATTGAAAAACGTGGTGGAACGTTCAGTGTAT CGCCACGGCACCAGCGATTATCCGCTTGATGACATCATTATTGATCCCTTT AAACGGCGTCCGCCTGAAGACGCTATCGCCGTTTCAGAAACCACCTCGCTT CCAACACTGCCGCTGGATTTACGTGAGTTTCAGATGCAGCAGGAAAAAGAG TTGCTGCAACTCAGTTTGCAAATGAATGCACGCACACGCCGCCGCGAACGT CGCGCAGAGAAACAGGCTCAATGGAAAGCAGCAAATTAA (λN22plus)
		Terminator L3S3P22	TACTAGAGCCAATTATTGAAGGCCGCTAACGCGGCCTTTTTTTGTTTCTGG TCTCCC (Scar/Spacer)
Reporter with sfGFP::ASV tag	Composite Module (This study)	RiboJ	AGCTGTCACCGGATGTGCTTTCCGGTCTGATGAGTCCGTGAGGACGAAACA GCCTCTACAAATAATTTTGTTTAACTAGAG
		RBS B0030	ATTAAAGAGGAGAAATACCAT (Scar/Spacer)
		sfgfp CDS	ATGCGTAAAGGCGAAGAGCTGTTCACTGGTGTCGTCCCTATTCTGGTGGAA CTGGATGGTGATGTCAACGGTCATAAGTTTTCCGTGCGTGGCGAGGGTGAA GGTGACGCAACTAATGGTAAACTGACGCTGAAGTTCATCTGTACTACTGGT AAACTGCCGGTACCTTGGCCGACTCTGGTAACGACGCTGACTTATGGTGTT CAGTGCTTTGCTCGTTATCCGGACCATATGAAGCAGCATGACTTCTTCAAG TCCGCCATGCCGGAAGGCTATGTGCAGGAACGCACGATTTCCTTTAAGGAT GACGGCACGTACAAAACGCGTGCGGAAGTGAAATTTGAAGGCGATACCCTG GTAAACCGCATTGAGCTGAAAGGCATTGACTTTAAAGAAGACGGCAATATC CTGGGCCATAAGCTGGAATACAATTTTAACAGCCACAATGTTTACATCACC GCCGATAAACAAAAAAATGGCATTAAAGCGAATTTTAAAATTCGCCACAAC GTGGAGGATGGCAGCGTGCAGCTGGCTGATCACTACCAGCAAAACACTCCA ATCGGTGATGGTCCTGTTCTGCTGCCAGACAATCACTATCTGAGCACGCAA AGCGTTCTGTCTAAAGATCCGAACGAGAAACGCGATCATATGGTTCTGCTG GAGTTCGTAACCGCAGCGGGCATCACGCATGGTATGGATGAACTGTACAAA **AGGCCTGCTGCAAACGACGAAAACTACGCTGCATCAGTTTAA** (**ASV tag**)
		Terminator L3S3P21	TAATACTAGAGCCAATTATTGAAGGCCTCCCTAACGGGGGGCCTTTTTTTG TTTCTGGTCTCCC (Scar/Spacer)
RiboJ	Insulator		AGCTGTCACCGGATGTGCTTTCCGGTCTGATGAGTCCGTGAGGACGAAACAGCCTCTACAAATA ATTTTGTTTAA

Supplementary Table 8: Sequences of gRNA, mRNA and related genetic elements

Part name	Type and source	DNA sequence (5'– 3')
sgRNA-LEA2	sgRNA (Previous study)	CTAGAAGTTATTATATAGTTCGGTCGTTTGAGAGCTAGGGCCCTGAAGAAGGGCCCTAGCA AGTTCAAATAAGGCTAGTCCGTTATCAACTTGGGCCCTGAAGAAGGGCCCAAGTGGCACCG AGTCGGTGCTTTTTTTGAAGCTTCTCGGTACCAAATTCCAGAAAAGAGGCCTCCCGAAAGG GGGGCCTTTTTTCGTTTTGGTCC (Scar, Spacer, sgRNA scaffold (Double BoxB), Terminator L3S2P21)
sgRNA-LEA2-ex2	sgRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCGTTTGAGAGCTAGAAATAGCAAGTTCAAATAAGGCT AGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCgggccctgaagaagGGCCCTA GCAAGTTCAAATAAGGCTAGTCCGTTATCAACTTGGGCCCTGAAGAAGGGCCCAAGTGGCA CCGAGTCGGTGCTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGAGGCCTCCCGA AAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Spacer, sgRNA scaffold (Double BoxB), Terminator L3S2P21)
sgRNA-LEA2-ex3	sgRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCGTTTGAGAGCTAGAAATAGCAAGTTCAAATAAGGCT AGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCgggccctgaagaagGGCCCAA GGCTAGGGCCCTGAAGAAGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAG CTCGGTACCAAATTCCAGAAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Spacer, sgRNA scaffold (Double BoxB), Terminator L3S2P21)
sgRNA-LEB3-ex2	sgRNA (This study)	CTAGAGCATAGTTCGTTTCCCATGTTTGAGAGCTAGAAATAGCAAGTTCAAATAAGGCTAG TCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCgggccctgaagaagGGCCCTAGC AAGTTCAAATAAGGCTAGTCCGTTATCAACTTGGGCCCTGAAGAAGGGCCCAAGTGGCACC GAGTCGGTGCTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGAGGCCTCCCGAAA GGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Spacer, sgRNA scaffold (Double BoxB), Terminator L3S2P21)
sgRNA-LEB3-ex3	sgRNA (This study)	CTAGAGCATAGTTCGTTTCCCATGTTTGAGAGCTAGAAATAGCAAGTTCAAATAAGGCTAG TCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCgggccctgaagaagGGCCCAAGG CTAGGGCCCTGAAGAAGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCT CGGTACCAAATTCCAGAAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Spacer, sgRNA scaffold (Double BoxB), Terminator L3S2P21)
crRNA-LEA2-WT	crRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCGTTTTAGAGCTATGCTGTTTTGTACTAGAGCCAATT ATTGAAGGCCGCTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Spacer, repeat, Terminator L3S3P22)
crRNA-LEA2-d1	crRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCGTTTTAGAGCTTTGCTGTTTTGTACTAGAGCCAATT ATTGAAGGCCGCTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Spacer, repeat, Terminator L3S3P22)
crRNA-LEA2-d2	crRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCGTTTTAGAGCATTGCTGTTTTGTACTAGAGCCAATT ATTGAAGGCCGCTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Spacer, repeat, Terminator L3S3P22)
crRNA-LEA2-d3	crRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCGTTTTAGAGGATTGCTGTTTTGTACTAGAGCCAATT ATTGAAGGCCGCTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Spacer, repeat, Terminator L3S3P22)
crRNA-LEA2-d4	crRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCGTTTTAGACGATTGCTGTTTTGTACTAGAGCCAATT ATTGAAGGCCGCTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Spacer, repeat, Terminator L3S3P22)
crRNA-LEA2-U1	crRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCGTTTTTGACGATTGCTGTTTTGTACTAGAGCCAATT ATTGAAGGCCGCTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Spacer, repeat, Terminator L3S3P22)
crRNA-LEA2-U2	crRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCGTTTATGACGATTGCTGTTTTGTACTAGAGCCAATT ATTGAAGGCCGCTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Spacer, repeat, Terminator L3S3P22)
crRNA-LEA2-U3	crRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCGTTAATGACGATTGCTGTTTTGTACTAGAGCCAATT ATTGAAGGCCGCTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Spacer, repeat, Terminator L3S3P22)

Part name	Type and source	DNA sequence (5'– 3')
crRNA-LEA2-U4	crRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCGTAAATGACGATTGCTGTTTTGTACTAGAGCCAATT ATTGAAGGCCGCTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Spacer, repeat, Terminator L3S3P22)
crRNA-LEA2-U5	crRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCGAAAATGACGATTGCTGTTTTGTACTAGAGCCAATT ATTGAAGGCCGCTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Spacer, repeat, Terminator L3S3P22)
crRNA-LEA2-U5A	crRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCAAAAATGACGATTGCTGTTTTGTACTAGAGCCAATT ATTGAAGGCCGCTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Spacer, repeat, Terminator L3S3P22)
crRNA-LEA2-U5C	crRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCCAAAATGACGATTGCTGTTTTGTACTAGAGCCAATT ATTGAAGGCCGCTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Spacer, repeat, Terminator L3S3P22)
crRNA-LEA2-U5T	crRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCTAAAATGACGATTGCTGTTTTGTACTAGAGCCAATT ATTGAAGGCCGCTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Spacer, repeat, Terminator L3S3P22)
crRNA-LEB3-WT	crRNA (This study)	CTAGAGCATAGTTCGTTTCCCATGTTTTAGAGCTATGCTGTTTTGTACTAGAGCCAATTAT TGAAGGCCGCTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Spacer, repeat, Terminator L3S3P22)
tracrRNA-WT	tracrRNA (This study)	CTAGAGGAACCATTCAAAACAGCATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGA AGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCCAATTATTGAAGGCCG CTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S3P22)
tracrRNA-d1	tracrRNA (This study)	CTAGAGGAACCATTCAAAACAGCAAAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCgggccctgaagaagGGCCCAAGGCTAGGGCCCTGAAGA AGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCCAATTATTGAAGGCCG CTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S3P22)
tracrRNA-d2	tracrRNA (This study)	CTAGAGGAACCATTCAAAACAGCAATGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCgggccctgaagaagGGCCCAAGGCTAGGGCCCTGAAGA AGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCCAATTATTGAAGGCCG CTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S3P22)
tracrRNA-d3	tracrRNA (This study)	CTAGAGGAACCATTCAAAACAGCAATCCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGA AGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCCAATTATTGAAGGCCG CTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S3P22)
tracrRNA-d4	tracrRNA (This study)	CTAGAGGAACCATTCAAAACAGCAATCGAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGA AGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCCAATTATTGAAGGCCG CTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S3P22)
tracrRNA-U1	tracrRNA (This study)	CTAGAGGAACCATTCAAAACAGCAATCGAAGTAAAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGA AGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCCAATTATTGAAGGCCG CTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S3P22)
tracrRNA-U2	tracrRNA (This study)	CTAGAGGAACCATTCAAAACAGCAATCGAAGTATAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGA AGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCCAATTATTGAAGGCCG CTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S3P22)
tracrRNA-U3	tracrRNA (This study)	CTAGAGGAACCATTCAAAACAGCAATCGAAGTATTAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGA

Part name	Type and source	DNA sequence (5'– 3')
		AGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCCAATTATTGAAGGCCG CTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S3P22)
tracrRNA-U4	tracrRNA (This study)	CTAGAGGAACCATTCAAAACAGCAATCGAAGTATTTATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGA AGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCCAATTATTGAAGGCCG CTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S3P22)
tracrRNA-U5	tracrRNA (This study)	CTAGAGGAACCATTCAAAACAGCAATCGAAGTATTTTTAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGA AGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCCAATTATTGAAGGCCG CTAACGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S3P22)
tracrRNA-ESI	tracrRNA (This study)	ACTAGT**GAGACC**CGGAACCATTCAAAGCTTTAAGGCTAGTC**GGTCTC**ACGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGA AGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCTCGGTACCAAATTCCA GAAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Scaffold, **BsaI site**, Terminator L3S2P21)
tracrRNA-R1	tracrRNA (This study)	CTAGAGGAACCATTCCTTGTAGATGAACAAGTGCCGTTAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGA AGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCTCGGTACCAAATTCCA GAAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S2P21)
tracrRNA-R2	tracrRNA (This study)	CTAGAGGAACCATTGCTCGGAGGAGGCCAAGTCCAGCTAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGA AGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCTCGGTACCAAATTCCA GAAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S2P21)
tracrRNA-R3	tracrRNA (This study)	CTAGAGGAACCATTAGGTGGTCTTGACCAAGTAGCGTTAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGA AGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCTCGGTACCAAATTCCA GAAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S2P21)
tracrRNA-R2L1	tracrRNA (This study)	CTAGAGGAACCATTCTCGGAGGAGGCCAAGTCCAGCTAAGGCTAGTCCGTTATCAACTTGA AAAAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAA GGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCTCGGTACCAAATTCCAG AAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S2P21)
tracrRNA-R2L3	tracrRNA (This study)	CTAGAGGAACCATTCGGAGGAGGCCAAGTCCAGCTAAGGCTAGTCCGTTATCAACTTGAAA AAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGG GCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAA AAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S2P21)
tracrRNA-R2L5	tracrRNA (This study)	CTAGAGGAACCATTGAGGAGGCCAAGTCCAGCTAAGGCTAGTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGC CCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAA GAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S2P21)
tracrRNA-R2L7	tracrRNA (This study)	CTAGAGGAACCATTGGAGGCCAAGTCCAGCTAAGGCTAGTCCGTTATCAACTTGAAAAAGT GGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGCCC GCAGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGA GGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S2P21)
tracrRNA-R2L9	tracrRNA (This study)	CTAGAGGAACCATTAGGCCAAGTCCAGCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGG CACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGCCCGC AGGGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGAGG CCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC

Part name	Type and source	DNA sequence (5'– 3')
		(Scar, Scaffold, anti-repeat, bulge, Terminator L3S2P21)
tracrRNA-R2L11	tracrRNA (This study)	CTAGAGGAACCATTGCCAAGTCCAGCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCA CCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGCCCGCAG GGCCCTGAAGAAGGGCCCTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGAGGCC TCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S2P21)
tracrRNA-R3L1	tracrRNA (This study)	CTAGAGGAACCATTGGTGGTCTTGACCAAGTAGCGTTAAGGCTAGTCCGTTATCAACTTGA AAAAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAA GGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTTACTAGAGCTCGGTACCAAATTCCAG AAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S2P21)
tracrRNA-R3L3	tracrRNA (This study)	CTAGAGGAACCATTTGGTCTTGACCAAGTAGCGTTAAGGCTAGTCCGTTATCAACTTGAAA AAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGG GCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAA AAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S2P21)
tracrRNA-R3L5	tracrRNA (This study)	CTAGAGGAACCATTGTCTTGACCAAGTAGCGTTAAGGCTAGTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGC CCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAA GAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S2P21)
tracrRNA-R3L7	tracrRNA (This study)	CTAGAGGAACCATTCTTGACCAAGTAGCGTTAAGGCTAGTCCGTTATCAACTTGAAAAAGT GGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGCCC GCAGGGCCCTGAAGAAGGGCCCTTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGA GGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S2P21)
tracrRNA-R3L9	tracrRNA (This study)	CTAGAGGAACCATTTGACCAAGTAGCGTTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGG CACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGCCCGC AGGGCCCTGAAGAAGGGCCCTTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGAGG CCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S2P21)
tracrRNA-R3L11	tracrRNA (This study)	CTAGAGGAACCATTACCAAGTAGCGTTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCA CCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGCCCGCAG GGCCCTGAAGAAGGGCCCTTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGAGGCC TCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S2P21)
tracrRNA-Ar1	tracrRNA (This study)	CTAGAGGAACCATTAGCTGCAATGGCGCAAGTACCGGTAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGA AGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTTACTAGAGCTCGGTACCAAATTCCA GAAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S2P21)
tracrRNA-Ar2	tracrRNA (This study)	CTAGAGGAACCATTAATGGTAATGTTGCAAGTATATCTAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGA AGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTTACTAGAGCTCGGTACCAAATTCCA GAAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar, Scaffold, anti-repeat, bulge, Terminator L3S2P21)
mRFP	mRNA (This study)	CTAGAG**ATTAAAGAGGAGAAA**TACTAGATGGTGAGCAAGGGCGAGGAGGATAACATGGCCA TCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTT CGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAG GTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACG GCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCC CGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACC CAGGACTCCTCACTTCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACT TCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCG GATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGAC GGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGC CCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCAT

Part name	Type and source	DNA sequence (5'– 3')
		CGTGGAACAGTACGAACGCGCTGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTAC AAGTAACCAATTATTGAAGGGGAGCGGGAAACCGCTCCCCTTTTTTTGTTTCTGGTCTCCC (Scar, coding, Terminator L3S3P00)
mRFP-ΔRBS	mRNA (This study)	CTAGAGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCA AGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGG CCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCC TTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACC CCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGT GATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCACTTCAGGACGGC GAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGC AGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCT GAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTC AAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCA AGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCTGA GGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAACCAATTATTGAAGGGGAG CGGGAAACCGCTCCCCTTTTTTTGTTTCTGGTCTCCC (Scar, partial coding, Terminator L3S3P00)
crRNA-R1	crRNA (This study)	CTAGACCCAGGACTCCTCACTTCAGGACGGCGAGTTCATCTACAAGGTACTAGAGCCAATT ATTGAAGGGGAGCGGGAAACCGCTCCCCTTTTTTTGTTTCTGGTCTCCC (Scar, Terminator L3S3P00)
crRNA-R2	crRNA (This study)	CTAGATAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCTACTAGAGCCAATT ATTGAAGGGGAGCGGGAAACCGCTCCCCTTTTTTTGTTTCTGGTCTCCC (Scar, Terminator L3S3P00)
crRNA-R3	crRNA (This study)	CTAGATGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTTACTAGAGCCAATT ATTGAAGGGGAGCGGGAAACCGCTCCCCTTTTTTTGTTTCTGGTCTCCC (Scar, Terminator L3S3P00)
mRNA-Ar	mRNA (This study)	CTAGACAATCAGGAGCGCAATATGTCATTTCTGTTACCCATCCAATTGTTCAAAATTCTTG CTGATGAAACCCGTCTGGGCATCGTTTTACTGCTCAGCGAACTGGGAGAGTTATGCGTCTG CGATCTCTGCACTGCTCTCGACCAGTCGCAGCCCAAGATCTCCCGCCACCTGGCATTGCTG CGTGAAAGCGGGCTATTGCTGGACCGCAAGCAAGGTAAGTGGGTTCATTACCGCTTATCAC CGCATATTCCAGCATGGGCGGCGAAAATTATTGATGAGGCCTGGCGATGTGAACAGGAAAA GGTTCAGGCGATTGTCCGCAACCTGGCTCGACAAAACTGTTCCGGGGACAGTAAGAACATT TGCAGTTAAAAATTTAGCTAAACACATATGAATTTTCAGATGTGTTTTATCCGGGAGGCAT TATGTTACTGGCAGGCGCTATCTTTGTCCTGACCATCGTATTGGTTATCTGGCAGCCGAAA GGTTTAGGCATCGGCTGGAGTGCAACGCTCGGCGCAGTACTGGCGTTAGTTACGGGCGTGG TCCATCCGGGTGATATTCCGGTGGTGTGGAATATCGTCTGGAACGCGACGGCTGCGTTTAT CGCCGTCATTATCATCAGCCTGCTGCTGGATGAGTCCGGCTTTTTTGAATGGGCGGCGCTG CACGTCTCACGCTGGGGTAATGGTCGTGGTCGCTTGCTGTTTACCTGGATTGTCCTGCTCG GTGCTGCCGTTGCCGCCCTGTTTGCCAATGATGGCGCGGCGCTTATTTTGACACCGATTGT CATCGCCATGCTGCTGGCTTTAGGGTTCAGTAAAGGCACTACGCTGGCGTTCGTGATGGCG GCCGGATTCATTGCCGATACCGCCAGCCTGCCGCTTATTGTCTCCAACCTGGTGAATATCG TTTCCGCTGATTTCTTTGGCCTCGGCTTTCGCGAATACGCCTCGGTGATGGTGCCGGTGGA TATCGCCGCGATTGTTGCCACGCTGGTGATGTTACATCTCTATTTTCGCAAAGATATTCCG CAGAACTACGATATGGCGCTGCTGAAATCTCCCGCAGAAGCGATCAAAGATCCTGCTACGT TCAAAACTGGCTGGGTTGTTTTACTGCTTCTGCTGGTGGGATTTTTCGTCCTGGAACCGCT CGGCATTCCGGTGAGCGCCATTGCAGCTGTGGGCGCGCTGATATTATTTGTCGTCGCTAAA CGCGGTCATGCGATTAATACGGGTAAAGTCCTGCGCGGTGCCCCCTGGCAGATTGTCATCT TCTCGCTCGGCATGTATCTGGTGGTTTATGGCCTGCGCAATGCCGGATTAACGGAATATCT TTCTGGCGTACTCAACGTGCTGGCGGATAACGGCCTGTGGGCCGCGACGCTCGGCACCGGA TTCCTCACCGCCTTCCTCTCTTCTATTATGAACAATATGCCGACGGTACTGGTTGGCGCGT TGTCCATTGATGGCAGCACGGCATCTGGCGTTATCAAAGAAGCGATGGTTTATGCCAATGT GATTGGCTGCGATTTGGGACCGAAAATTACCCCAATTGGTAGCCTGGCTACGCTACTCTGG CTGCACGTACTTTCGCAGAAGAATATGACTATCAGCTGGGGATATTACTTCCGTACAGGGA TTATCATGACCCTGCCTGTGCTGTTTGTGACGCTGGCTGCGCTGGCGCTACGTCTCTCTTT CACTTTGTAATGAGATACTGATATGAGCAACATTACCATTTATCACAACCCGGCCTGCGGC ACGTCGCGTAATACGCTGGAGATGATCCGCAACAGCGGCACAGAACCGACTATTATCCATT ATCTGGAAACTCCGCCAACGCGCGATGAACTGGTCAAACTCATTGCCGATATGGGGATTTC CGTACGCGCGCTGCTGCGTAAAAACGTCGAACCGTATGAGGAGCTGGGCCTTGCGGAAGAT AAATTTACTGACGATCGGTTAATCGACTTTATGCTTCAGCACCCGATTCTGATTAATCGCC CGATTGTGGTGACGCCGCTGGGAACTCGCCTGTGCCGCCCTTCAGAAGTGGTGCTGGAAAT TCTGCCAGATGCGCAAAAAGGCGCATTCTCCAAGGAAGATGGCGAGAAAGTGGTTGATGAA GCGGGTAAGCGCCTGAAATAATACTAGAGCCAATTATTGAAGGGGAGCGGGAAACCGCTCC CCTTTTTTTGTTTCTGGTCTCCC

Part name	Type and source	DNA sequence (5'– 3')
		(Scar, Terminator L3S3P00)
crRNA-Ar1	crRNA (This study)	CTAGAGTCCTGGAACCGCTCGGCATTCCGGTGAGCGCCATTGCAGCTTACTAGAGCCAATT ATTGAAGGGGAGCGGGAAACCGCTCCCCTTTTTTTGTTTCTGGTCTCCC (Scar, Terminator L3S3P00)
crRNA-Ar2	crRNA (This study)	CTAGACTAGATTCACTTTGTAATGAGATACTGATATGAGCAACATTACCATTTACTAGAGC CAATTATTGAAGGGGAGCGGGAAACCGCTCCCCTTTTTTTGTTTCTGGTCTCCC (Scar, Terminator L3S3P00)
crRNA-Ar3	crRNA (This study)	CTAGACTAGATGCTGCGTAAAAACGTCGAACCGTATGAGGAGCTGGGCCTTGTACTAGAGC CAATTATTGAAGGGGAGCGGGAAACCGCTCCCCTTTTTTTGTTTCTGGTCTCCC (Scar, Terminator L3S3P00)
crRNA-Ar4	crRNA (This study)	CTAGACTAGAAAGGCGCATTCTCCAAGGAAGATGGCGAGAAAGTGGTTGATGTACTAGAGC CAATTATTGAAGGGGAGCGGGAAACCGCTCCCCTTTTTTTGTTTCTGGTCTCCC (Scar, Terminator L3S3P00)
s-crRNA-LEB3	crRNA (This study)	CTAGACATAGTTCGTTTCCCATGTTTTAGAGCTATGCCCAATTATTGAAGGCCGCTAACGC GGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Terminator L3S3P22)
s-crRNA-LEA2-WT	crRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCGTTTTAGAGCTATGCCCAATTATTGAAGGCCGCTAA CGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Terminator L3S3P22)
s-crRNA-LEA2-C6	crRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCTACGTACCGGGTGAACCAATTATTGAAGGCCGCTAA CGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Terminator L3S3P22)
s-crRNA-LEA2-C9	crRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCAGAATCCTCGCTCAGCCAATTATTGAAGGCCGCTAA CGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Terminator L3S3P22)
s-crRNA-LEA2-C48	crRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCATTATTGCCGACACACCAATTATTGAAGGCCGCTAA CGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Terminator L3S3P22)
s-crRNA-LEA2-C74	crRNA (This study)	CTAGAAGTTATTATATAGTTCGGTCAATCGGGAGCTGCCTCCAATTATTGAAGGCCGCTAA CGCGGCCTTTTTTTGTTTCTGGTCTCCC (Scar, Terminator L3S3P22)
s-tracrRNA-WT	tracrRNA (This study)	CTAGGCATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGT CGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGCCCGCAGGGCCCT GAAGAAGGGCCCTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGAGGCCTCCCGA AAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar,Scaffold,Terminator L3S2P21)
s-tracrRNA-C6	tracrRNA (This study)	CTAGTTCACCCAAGTTACGTTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGT CGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGCCCGCAGGGCCCT GAAGAAGGGCCCTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGAGGCCTCCCGA AAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar,Scaffold,Terminator L3S2P21)
s-tracrRNA-C9	tracrRNA (This study)	CTAGCTGAGCGAAGTGATTCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGT CGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGCCCGCAGGGCCCT GAAGAAGGGCCCTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGAGGCCTCCCGA AAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar,Scaffold,Terminator L3S2P21)
s-tracrRNA-C48	tracrRNA (This study)	CTAGTGTGTCGAAGTAATAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGT CGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGCCCGCAGGGCCCT GAAGAAGGGCCCTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGAGGCCTCCCGA AAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar,Scaffold,Terminator L3S2P21)
s-tracrRNA-C74	tracrRNA (This study)	CTAGAGGCAGCAAGTCCGATTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGT CGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGCCCGCAGGGCCCT

Part name	Type and source	DNA sequence (5'– 3')
		GAAGAAGGGCCCTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGAGGCCTCCCGA AAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar,Scaffold,Terminator L3S2P21)
s-tracrRNA-R1	tracrRNA (This study)	CCTTGTAGATGAACAAGTGCCGTTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCG AGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGCCCGCAGGGC CCTGAAGAAGGGCCCTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGAGGCCTCC CGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar,Scaffold,Terminator L3S2P21)
s-tracrRNA-R2	tracrRNA (This study)	GCTCGGAGGAGGCCAAGTCCAGCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCG AGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGCCCGCAGGGC CCTGAAGAAGGGCCCTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGAGGCCTCC CGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar,Scaffold,Terminator L3S2P21)
s-tracrRNA-R3	tracrRNA (This study)	AGGTGGTCTTGACCAAGTAGCGTTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCG AGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGCCCGCAGGGC CCTGAAGAAGGGCCCTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGAGGCCTCC CGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar,Scaffold,Terminator L3S2P21)
CONAN	Sensor RNA	TTGCCATGTGTATGTGGGAGACGGTCGGGTCCAGATATTCGTATCTGTCGAGTAGAGTGTG GGCTCCCACATACTCTGATGATCCTTCGGGATCATTCATGGCAACTGCTGCTCTTCAACCT CGGTGACGAGGTTGAAGAGCAGCAGTTGCCATGTGTATGTGGGAGCCCACACTCTACTCGA CAGATACGTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG (Target, broccoli RNA ,PAM ,Terminator T7)
s-crRNA-13	crRNA (This study)	AGTTATTATATAGTTCGGTCGTTTTAGAGCTATGCCAATTATTGAAGGCCGCTAACGCGGC CTTTTTTTGTTTCTGGTCTCCC (Spacer ,Terminator L3S3P22)
s-crRNA-12	crRNA (This study)	AGTTATTATATAGTTCGGTCGTTTTAGAGCTATCCAATTATTGAAGGCCGCTAACGCGGCC TTTTTTTGTTTCTGGTCTCCC (Spacer ,Terminator L3S3P22)
s-crRNA-11	crRNA (This study)	AGTTATTATATAGTTCGGTCGTTTTAGAGCTACCAATTATTGAAGGCCGCTAACGCGGCCT TTTTTTGTTTCTGGTCTCCC (Spacer ,Terminator L3S3P22)
s-crRNA-10	crRNA (This study)	AGTTATTATATAGTTCGGTCGTTTTAGAGCTCCAATTATTGAAGGCCGCTAACGCGGCCTT TTTTTGTTTCTGGTCTCCC (Spacer ,Terminator L3S3P22)
s-tracrRNA-13	tracrRNA (This study)	CATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTG CGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGCCCGCAGGGCCCTGAAGA AGGGCCCTTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGAGGCCTCCCGAAAGGG GGGCCTTTTTTCGTTTTGGTCC (Scar,Scaffold,Terminator L3S2P21)
s-tracrRNA-12	tracrRNA (This study)	ATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGC GGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGCCCGCAGGGCCCTGAAGAA GGGCCCTTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGAGGCCTCCCGAAAGGGG GGCCTTTTTTCGTTTTGGTCC (Scar,Scaffold,Terminator L3S2P21)
s-tracrRNA-11	tracrRNA (This study)	TAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCG GGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGCCCGCAGGGCCCTGAAGAAG GGCCCTTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGAGGCCTCCCGAAAGGGGG GCCTTTTTTCGTTTTGGTCC (Scar,Scaffold,Terminator L3S2P21)
s-tracrRNA-10	tracrRNA (This study)	AGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCGG GCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGCCCGCAGGGCCCTGAAGAAGG GCCCTTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGAGGCCTCCCGAAAGGGGGG CCTTTTTTCGTTTTGGTCC (Scar,Scaffold,Terminator L3S2P21)

Part name	Type and source	DNA sequence (5'– 3')
s-tracrRNA-Ar2	tracrRNA (This study)	AATGGTAATGTTGCAAGTATATCTAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCG AGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGAAGGGCCCGCAGGGC CCTGAAGAAGGGCCCTTTTTTTTTACTAGAGCTCGGTACCAAATTCCAGAAAAGAGGCCTCC CGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar,Scaffold,Terminator L3S2P21)
tracrRNA- Ar3	tracrRNA (This study)	CTAGAGGAACCATTCAAGGCCCAGCTCCAAGTATACGTAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGA AGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTTACTAGAGCTCGGTACCAAATTCCA GAAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar,Scaffold,Terminator L3S2P21)
tracrRNA- Ar4	tracrRNA (This study)	CTAGAGGAACCATTCATCAACCACTTTCAAGTGCCATTAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGA AGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTTACTAGAGCTCGGTACCAAATTCCA GAAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar,Scaffold,Terminator L3S2P21)
tracrRNA- WT-21T	tracrRNA (This study)	CTAGAGGAACCATTCAAAACAGCATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCGGGCCCTGAAGAAGGGCCCAAGGCTAGGGCCCTGAAGA AGGGCCCGCAGGGCCCTGAAGAAGGGCCCTTTTTTTTTACTAGAGCTCGGTACCAAATTCCA GAAAAGAGGCCTCCCGAAAGGGGGGCCTTTTTTCGTTTTGGTCC (Scar,Scaffold,Terminator L3S2P21)

Supplementary Table 9: Sequences of promoters

Part name	Type and source	DNA sequence (5'– 3')
P_{pspA}-LEA2B2	σ^{54}-dependent promoter (This study)	AGTTATTATATAGTTCGGTCCGGTTTGAGACGTTGTTTTGCGGTTAGTGTAATTCGCTAACTCATCCTGGCATGTTGCTGTTGATTCTTCAATCAGATCTTTATAAATCAAAAAGATAAAAAATTGGCACGCAAATTGTATTAACAGTTCAGCAGGACAATCCTGAACGCAAA (-24 Box, -12 Box, TTS, UAS)
P_{pspA}-R1	σ^{54}-dependent promoter (This study)	CCCAGGACTCCTCACTTCAGCGGTTAGTGTAATTCGCTAACTCATCCTGGCATGTTGCTGTTGATTCTTCAATCAGATCTTTATAAATCAAAAAGATAAAAAATTGGCACGCAAATTGTATTAACAGTTCAGCAGGACAATCCTGAACGCAAA (-24 Box, -12 Box, TTS, UAS)
P_{pspA}-R2	σ^{54}-dependent promoter (This study)	TAATGCAGAAGAAGACCATGCGGTTAGTGTAATTCGCTAACTCATCCTGGCATGTTGCTGTTGATTCTTCAATCAGATCTTTATAAATCAAAAAGATAAAAAATTGGCACGCAAATTGTATTAACAGTTCAGCAGGACAATCCTGAACGCAAA (-24 Box, -12 Box, TTS, UAS)
P_{pspA}-R3	σ^{54}-dependent promoter (This study)	TGAAGGACGGCGGCCACTACCGGTTAGTGTAATTCGCTAACTCATCCTGGCATGTTGCTGTTGATTCTTCAATCAGATCTTTATAAATCAAAAAGATAAAAAATTGGCACGCAAATTGTATTAACAGTTCAGCAGGACAATCCTGAACGCAAA (-24 Box, -12 Box, TTS, UAS)
P_{pspA}-Ar1	σ^{54}-dependent promoter (This study)	GTCCTGGAACCGCTCGGCATCGGTTAGTGTAATTCGCTAACTCATCCTGGCATGTTGCTGTTGATTCTTCAATCAGATCTTTATAAATCAAAAAGATAAAAAATTGGCACGCAAATTGTATTAACAGTTCAGCAGGACAATCCTGAACGCAAA (-24 Box, -12 Box, TTS, UAS)
P_{pspA}-Ar2	σ^{54}-dependent promoter (This study)	TTCACTTTGTAATGAGATACCGGTTAGTGTAATTCGCTAACTCATCCTGGCATGTTGCTGTTGATTCTTCAATCAGATCTTTATAAATCAAAAAGATAAAAAATTGGCACGCAAATTGTATTAACAGTTCAGCAGGACAATCCTGAACGCAAA (-24 Box, -12 Box, TTS, UAS)
P_{pspA}-Ar3	σ^{54}-dependent promoter (This study)	AGTGCTGCGTAAAAACGTCGAACGGTTAGTGTAATTCGCTAACTCATCCTGGCATGTTGCTGTTGATTCTTCAATCAGATCTTTATAAATCAAAAAGATAAAAAATTGGCACGCAAATTGTATTAACAGTTCAGCAGGACAATCCTGAACGCAAA (-24 Box, -12 Box, TTS, UAS)
P_{pspA}-Ar4	σ^{54}-dependent promoter (This study)	AGAAGGCGCATTCTCCAAGGAACGGTTAGTGTAATTCGCTAACTCATCCTGGCATGTTGCTGTTGATTCTTCAATCAGATCTTTATAAATCAAAAAGATAAAAAATTGGCACGCAAATTGTATTAACAGTTCAGCAGGACAATCCTGAACGCAAA (-24 Box, -12 Box, TTS, UAS)
J23106	Anderson promoter	TTTACGGCTAGCTCAGTCCTAGGTATAGTGCTAGC
P_{T7}	T7 promoter	TAATACGACTCACTATAGG

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