Supporting Information

Towards a translationally-independent RNA-based synthetic oscillator using deactivated CRISPR-Cas

James Kuo[a,b], Ruoshi Yuan[a], Carlos Sánchez[a], Johan Paulsson[a] and Pamela A. Silver[a,b,1]

[a]Department of Systems Biology, Blavatnik Institute at Harvard Medical School, Boston, MA 02115, USA
[b]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, USA

Methods and Materials

Plasmid and Strain Construction. CRISPR DNA fragments were PCR amplified from existing plasmids: dCas9 system from pdCas9-bacteria, pAN-AND and pAN-OR (Addgene # 62308, 62307, 44249) [1,2]. For dCas12a, Cas12a pieces were PCR amplified with added D917A mutation from pY001 (pFnCpf1_full; Addgene #69973) [3]. Other DNA pieces not obtained by PCR were synthesized as IDT gBlock gene fragments (Integrated DNA Technologies). Plasmids were made by Gibson assembly [4] or restriction enzyme digestion and ligation with T4 ligase in *E. coli* DH5a. Either a high (ColE1), medium (p15A) or low (SC101) copy number origin of replication was used. Genomic integration into the attB site of *E. coli* was performed with the pOSIP plasmid system [5], with clonetegration plasmid cloning done in TransforMax EC100D pir+ electrocompetent *E. coli* (Lucigen).

All mother machine strains were in *E. coli* MG1655 with ΔmotA, a motility knockout for mother machine growth and an integrated constitutive red fluorescent protein reporter for use as a segmentation marker (strain NDL162) [6].

Mother Machine and Microscopy. Mother machine microfluidic chips made of polydimethylsiloxane (PDMS) were cast from a patterned silicon wafer and bonded to a No. 1.5 glass coverslip (Fisher Scientific), as previously described [6,7]. Most runs used chips with trench dimensions of 1.5 µm wide and high, with different lengths (10+ µm) used throughout. EZ Rich Defined Medium (EZ-RDM, TekNova) was used with 0.85 g/L Pluronic F108 (Sigma-Aldrich) to prevent cell adhesion to PDMS. Runs did not have supplemented antibiotic. For induction, typical concentrations were 1.88 ng/mL anhydrotetracycline (aTc) and 50 µM 2,4-diacetylphloroglucinol (DAPG). For most runs, media was flown at 20 µL/min through Tygon microfluidic tubing (US Plastic Corp 56515) with NE-300 syringe pumps (New Era Pump Systems).

Cells were imaged on modified Nikon Ti2-E inverted microscopes with Plan Apo 40x air objective (NA 0.95, Nikon), Perfect Focus System (PFS, Nikon), Nikon Ei-S-ER motorized stage with encoders, and high-resolution CMOS camera (Andor Zyla 4.2 PLUS), all controlled with NIS-Elements software (Nikon). All runs were kept at 37°C with a temperature-controlled cage incubator (Okolab). Exposure times varied per experimental run, but RFP was imaged at

100-200 ms and YFP for 10-50 ms using an LED excitation system (SPECTRA X light engine, Lumencor). Images were acquired at frame rates of 6-10 min, as noted throughout.

Image processing and analysis. Microscopy images were processed using ImageJ and MATLAB to segment mother cells using code originally developed by Somenath Bakshi. Further analysis was performed in MATLAB with code from S. Bakshi, J. Kuo and R. Yuan. Autocorrelation function (ACF) and power spectrum of the windowed ACF were calculated in MATLAB and as described previously [7]. Interpeak distances and peak heights were determined using MATLAB *findpeaks* function to find local maxima on a Savitzky-Golay filter smoothed signal. After minimal smoothing by fitting a polynomial order 5 with an 11-frame window using MATLAB function *sgolayfilt*, the peak distances were found using MATLAB function *findpeaks*. The minimum peak prominence value was adjusted if the fluctuation heights were not captured based on visual inspection. Inducible cascade measurements plotted as average plus shaded region standard deviation used MATLAB function *confplot* from Michele Giugliano (Brain Mind Institute, EPFL, Lausanne, Switzerland).

Bulk strain analysis: plate reader and agarose pads. Strains were evaluated in 200 µL test cultures in a 96-well flat-bottom black with clear bottom fluorescence plates (Corning 3631) with low evaporation lids (Corning 3931). Plates were grown in a BioTek Synergy H1 plate reader with continuous shaking at 37ºC, including a higher gradient temperature above to reduce condensation. Seed cultures were grown overnight in a separate 96-well plate. Agarose pads to qualitatively look at cultures were prepared with 1% agarose with microscope slides [8].
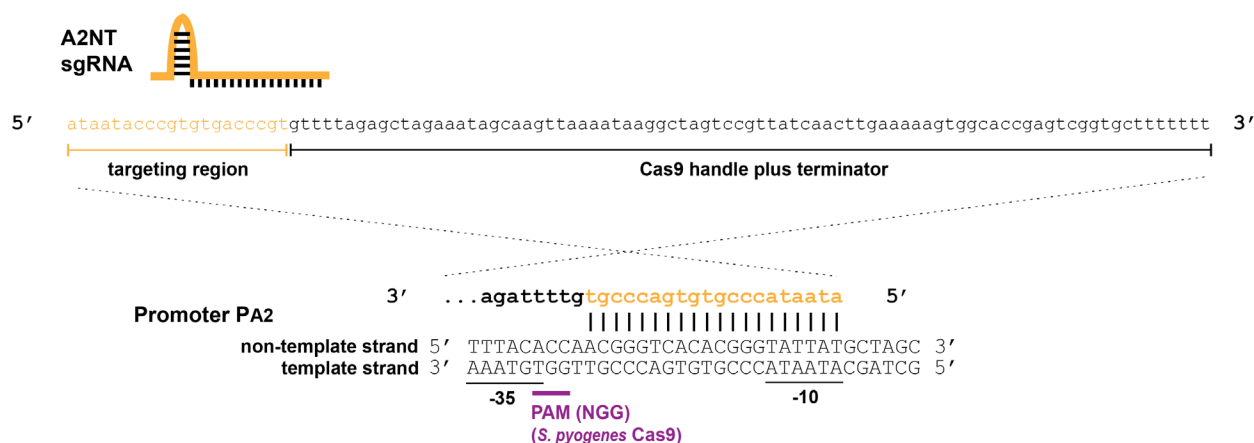
Supplementary Figures

Figure S1. Single guide RNA (sgRNA) targeting of promoter at the sequence level. The dCas9-sgRNA complex targets a promoter region between the -35 and -10 boxes. In this case, A2NT sgRNA targets the non-template strand of Promoter PA2 in the inter-region of the -10 and -35 boxes. The *S. pyogenes* dCas9 is targeted to "NGG" protospacer adjacent motif (PAM) site. Other sgRNA and promoter pair designs target the same regions with different sequences for the -35 and -10 inter-region. Parts are from [2].
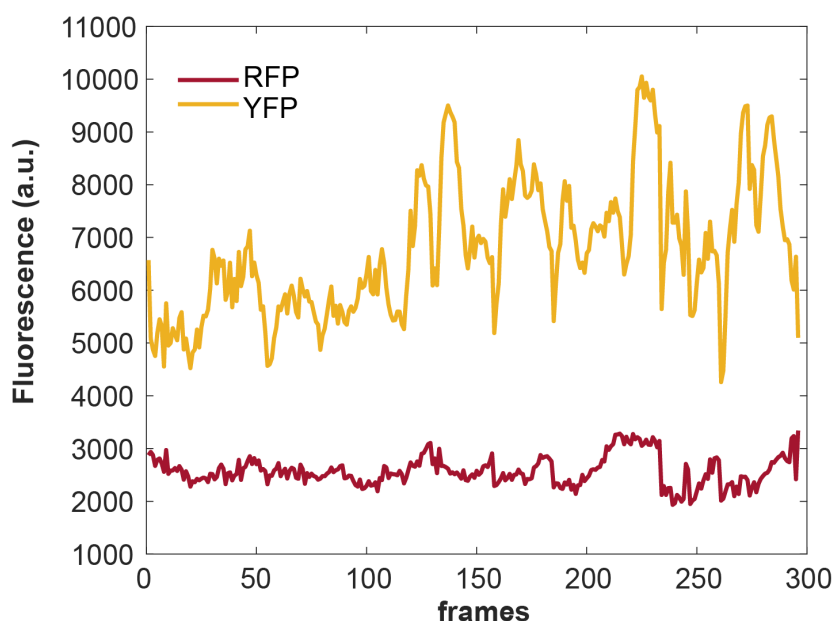

Figure S2. Mother machine reporter and segmentation traces for dCas9 RNA oscillator, one-plasmid form. RFP segmentation marker (modified mKate2) and YFP reporter (mVenus) traces from mother machine run for one-plasmid dCas9 oscillator (JK412) cell in mother machine. EZ-RDM, 8 min/frame. Significant fluctuations are from YFP.
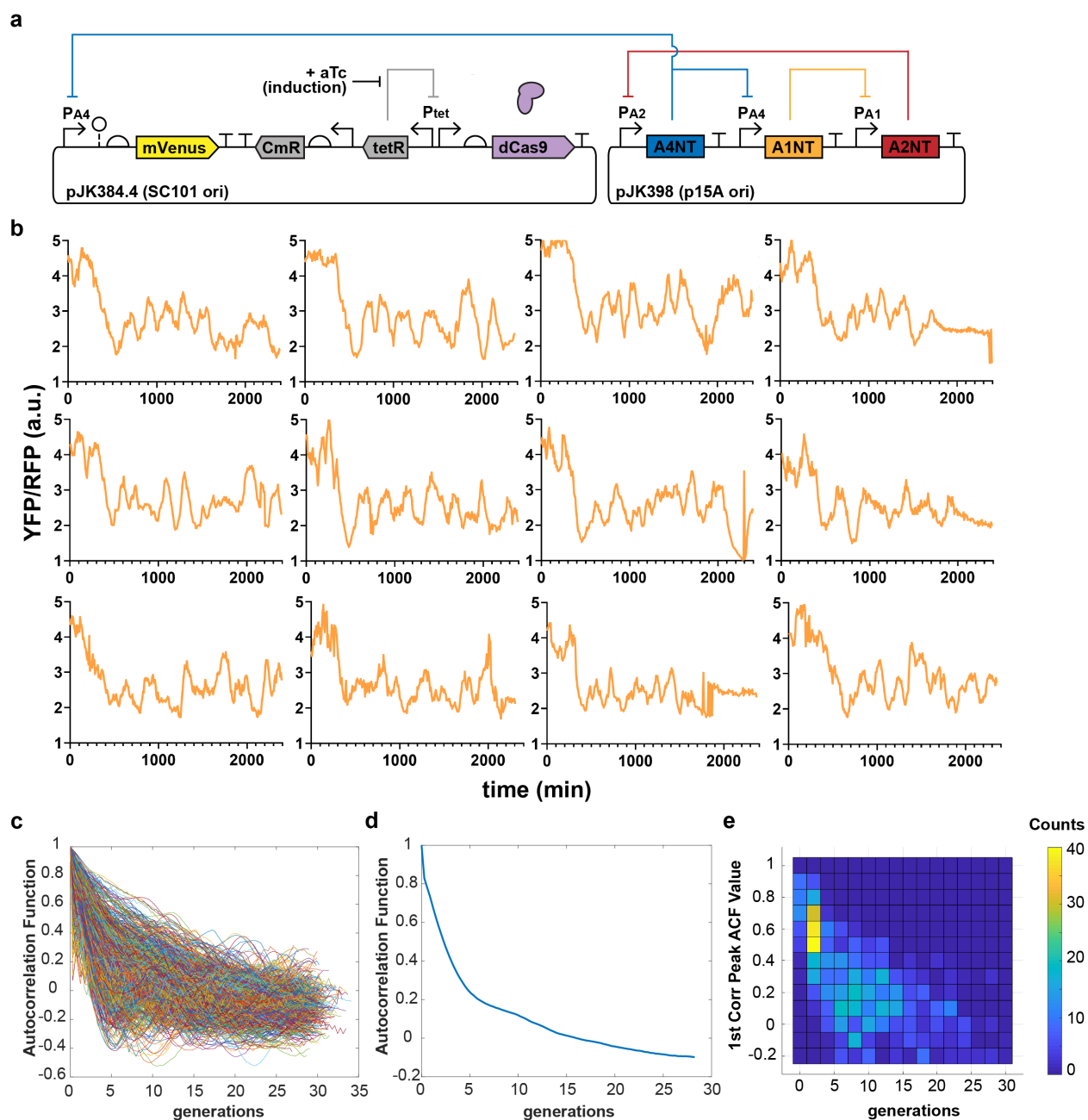
Figure S3. Two-plasmid dCas9 RNA oscillator with PA4 promoter. (a) Diagram of circuit on two plasmids, with PA4-mVenus reporter. (b) Example mother cell traces from mother machine run in EZ-RDM with 0.625 ng/mL aTc added at ~280 min and changed to 1.3 ng/mL aTc at ~2000 min. Mean generation time ~28 min. Acquired every 8 minutes. (c) Autocorrelation function (ACF) of 887 individual cells. (d) Population-averaged ACF. (e) Individual first correlation peaks have a wide distribution.
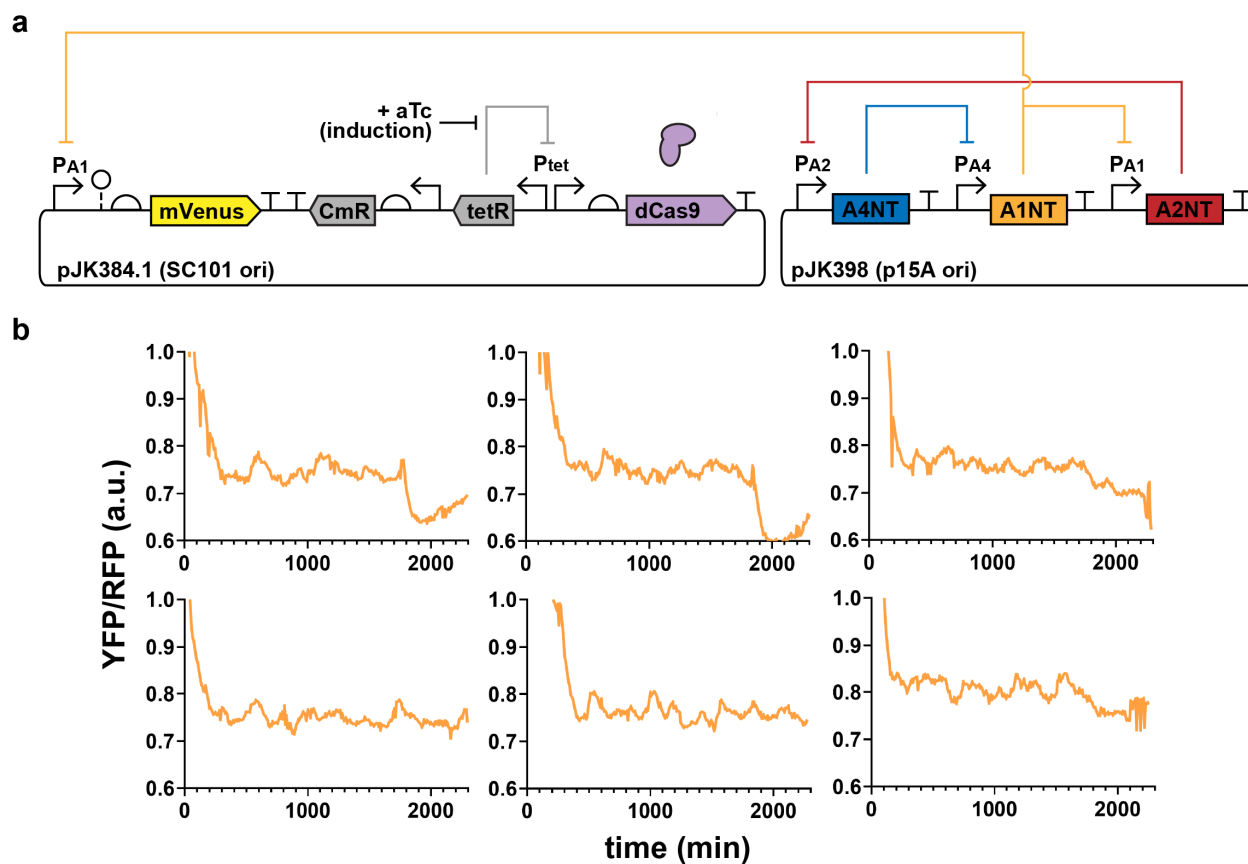
Figure S4. Two-plasmid dCas9 RNA oscillator with PA1 reporter. (a) Diagram of circuit on two plasmids, with PA1-mVenus reporter. (b) Example mother cell traces from mother machine run in EZ-RDM with 0.625 ng/mL aTc added ~142 min. Median generation time ~26.5 min. Acquired every 8 minutes.

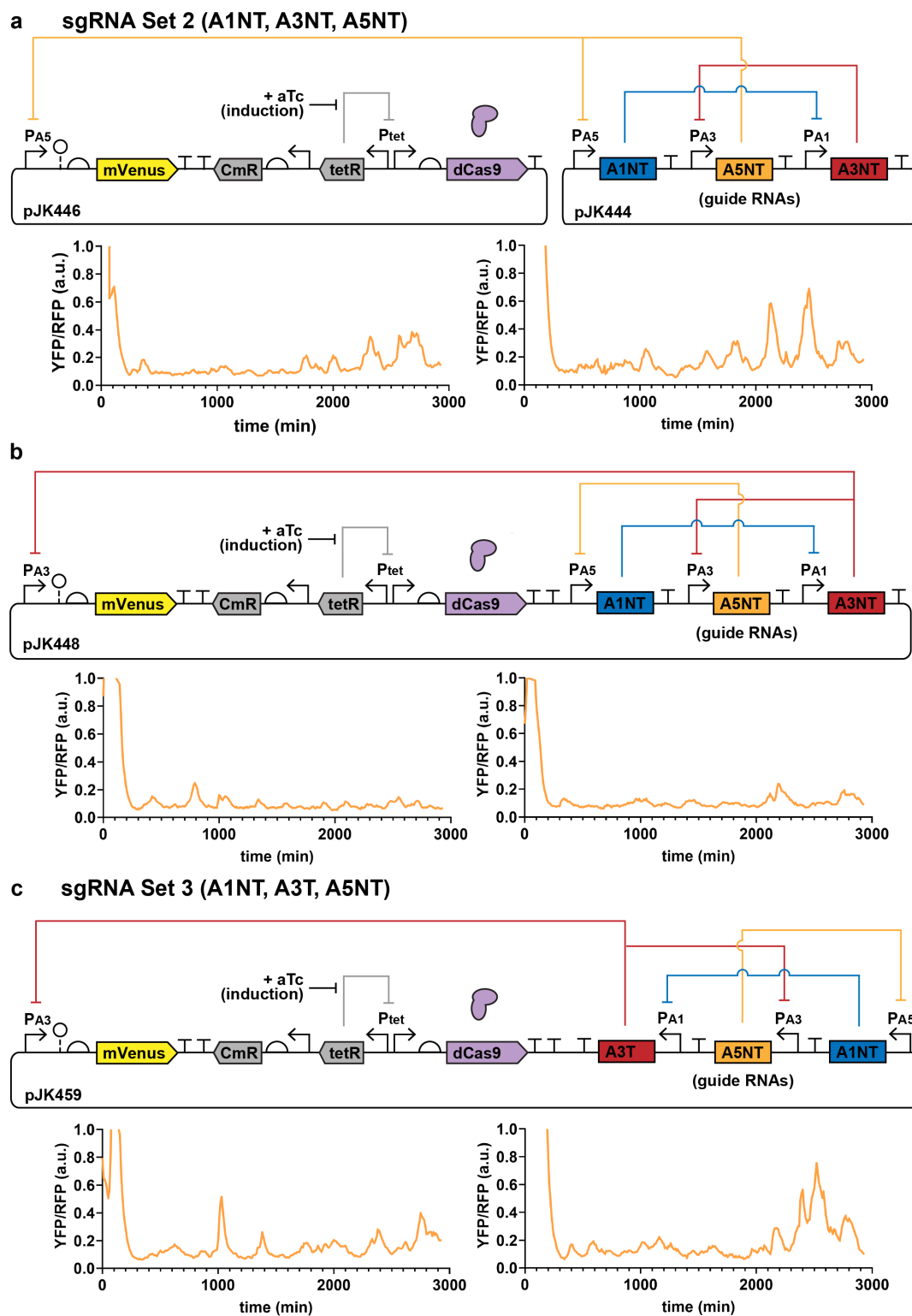**Figure S5. Other guide RNA sets for dCas9 oscillator.** Designs with different sets of orthogonal sgRNAs [2]. Signals were repressed to low levels after aTc induction at 90 min. Some fluctuations were preserved, as shown in the example cell traces, but signal-to-noise was too low for many peaks to characterize. Cells grown in mother machine chip with EZ-RDM. Average generation times ~27 min. Acquired every 10 min.

Attempted Synchronization

We tried to synchronize dCas9 RNA oscillator fluctuations across a population by adding an accessory plasmid with an inducible extra guide RNA (Fig. S6). Previous systems likely had high levels of each sgRNA to start. The intended effect was for all cells to have an initial high amount of the induced sgRNA relative to other sgRNAs, to strongly bias cells into that starting state. This could also reduce cell fluctuations between guide RNA states initially because of similar levels of each sgRNA. Neither induction overnight or for a few hours before dCas9 induction changed performance when compared to the genome integrated dCas9 oscillator circuit (JK422) without an extra sgRNA plasmid, suggesting little effect of the extra initial sgRNA.
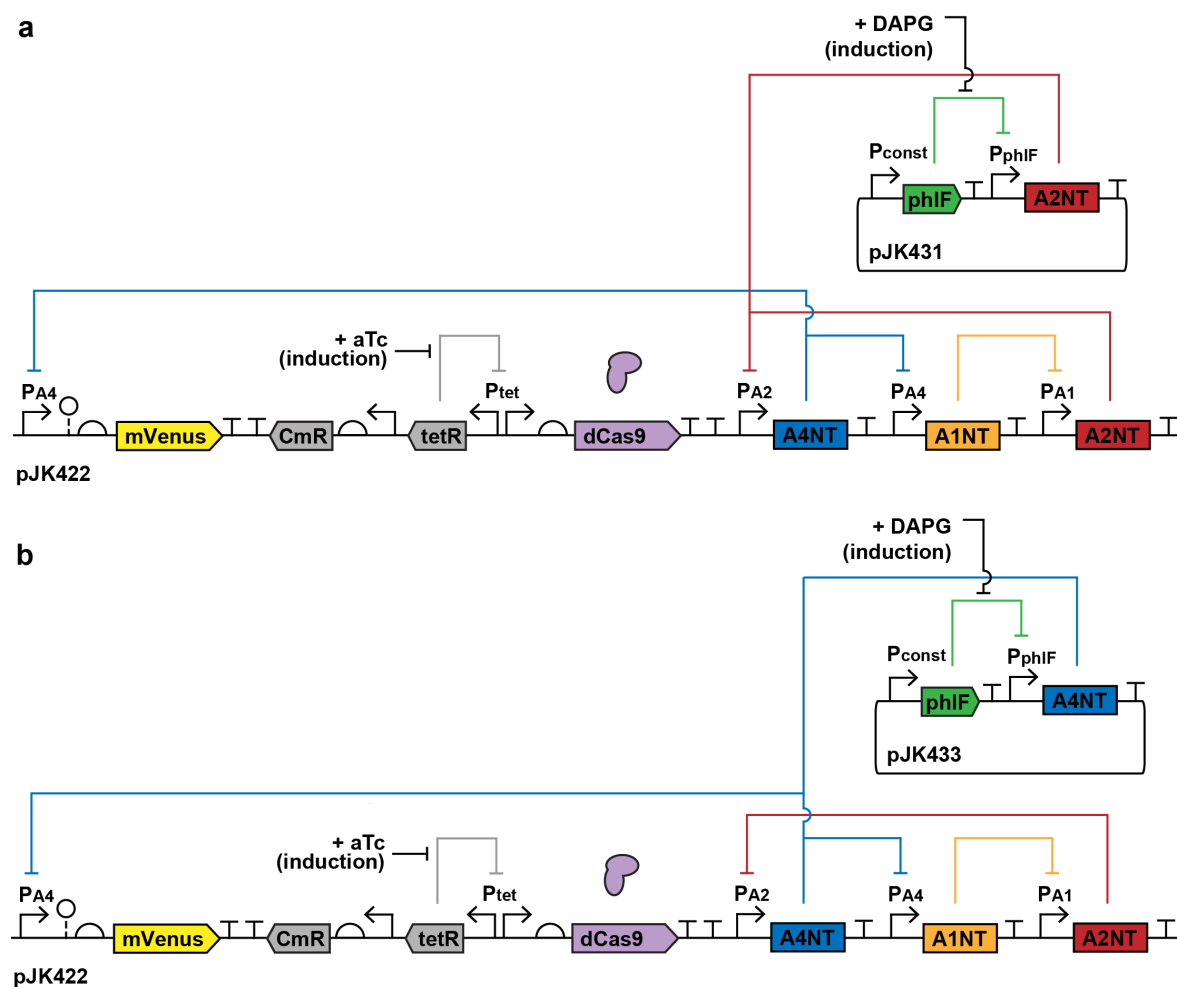
Figure S6. Genomic dCas9 RNA oscillator (JK422) plus accessory sgRNA plasmid. JK422 strains had an accessory plasmid with small molecule DAPG-inducible production of one of the sgRNAs. The intention was to help maintain cells in one RNA state prior to dCas9 induction, for population synchronization. pJK433 plasmid was also transformed into JK423.

Figure S7. Signals for YFP and RFP. Response times for one-plasmid dCas9 cascade (pJK430) shown in Fig. 3b. In the pre-DAPG sgRNA induction phase (frames 20-70), the YFP reporter signals span roughly $0.5 - 1 \times 10^4$, which leads to the 10-20 YFP/RFP signals. Traces shown for 388 cells. 6 min/frame.

a 1x cascade

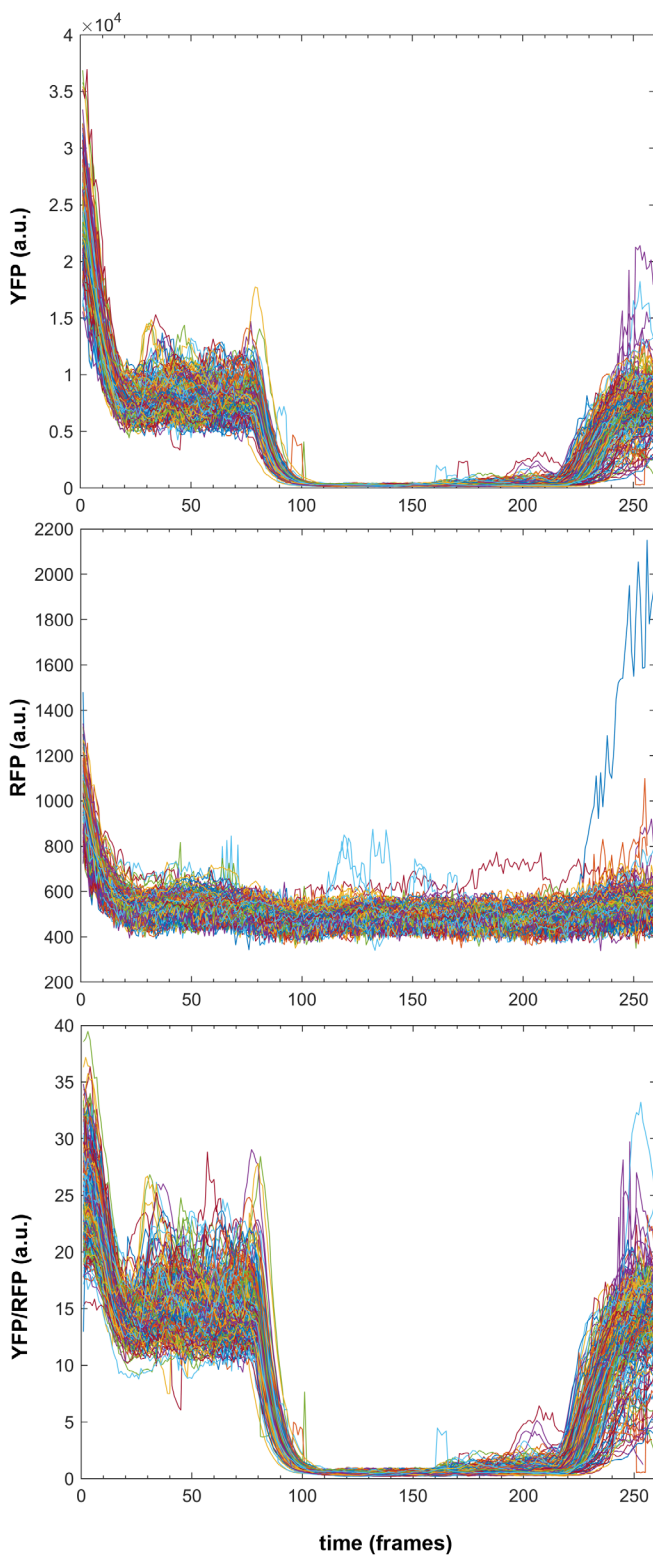|	A1NT	A2NT	A4NT	YFP
Initial	1	1	0	1
+aTc	1	0	0	1
+DAPG	0	1	1	0

1 = species produced
0 = not produced / low amount

JK434 (genomic, attB site)

b 2x cascade

	A1NT	A2NT	A4NT	YFP
Initial	1	0	1	0 / 1
+aTc	1	0	0	0
+DAPG	1	1	0	1

JK435 (genomic, attB site)

c

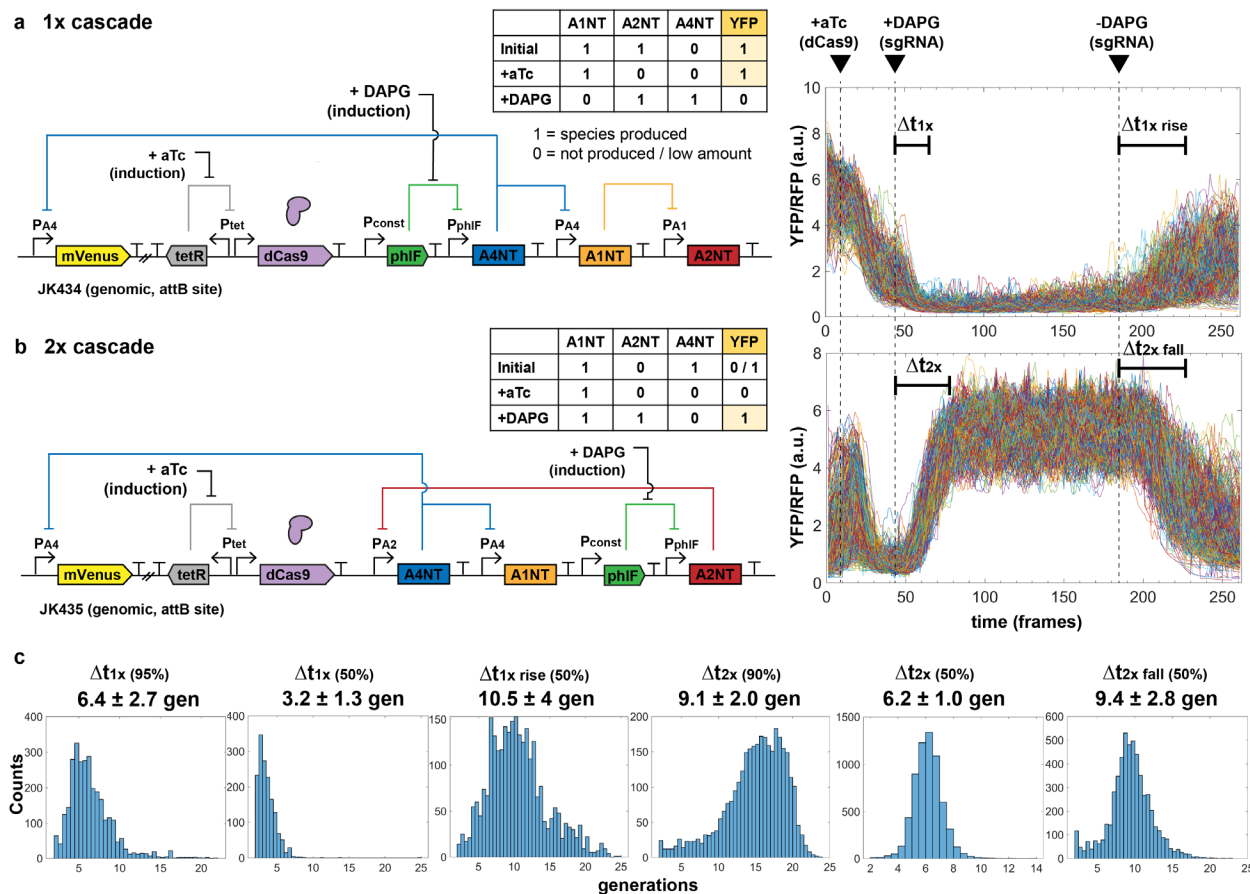| Δt_{1x} (95%)	Δt_{1x} (50%)	Δt_{1x} rise (50%)	Δt_{2x} (90%)	Δt_{2x} (50%)	Δt_{2x} fall (50%)
6.4 ± 2.7 gen	3.2 ± 1.3 gen	10.5 ± 4 gen	9.1 ± 2.0 gen	6.2 ± 1.0 gen	9.4 ± 2.8 gen

Figure S8. dCas9 cascade response plots for genomic versions. Analogous plot as Fig. 3 for genomic versions of dCas9 cascade step circuits. Times are similar, with lower signals as expected. 6 min/frame. Distributions were measured from (a) 2988 cells for pJK434 and (b) 6379 cells for pJK435.
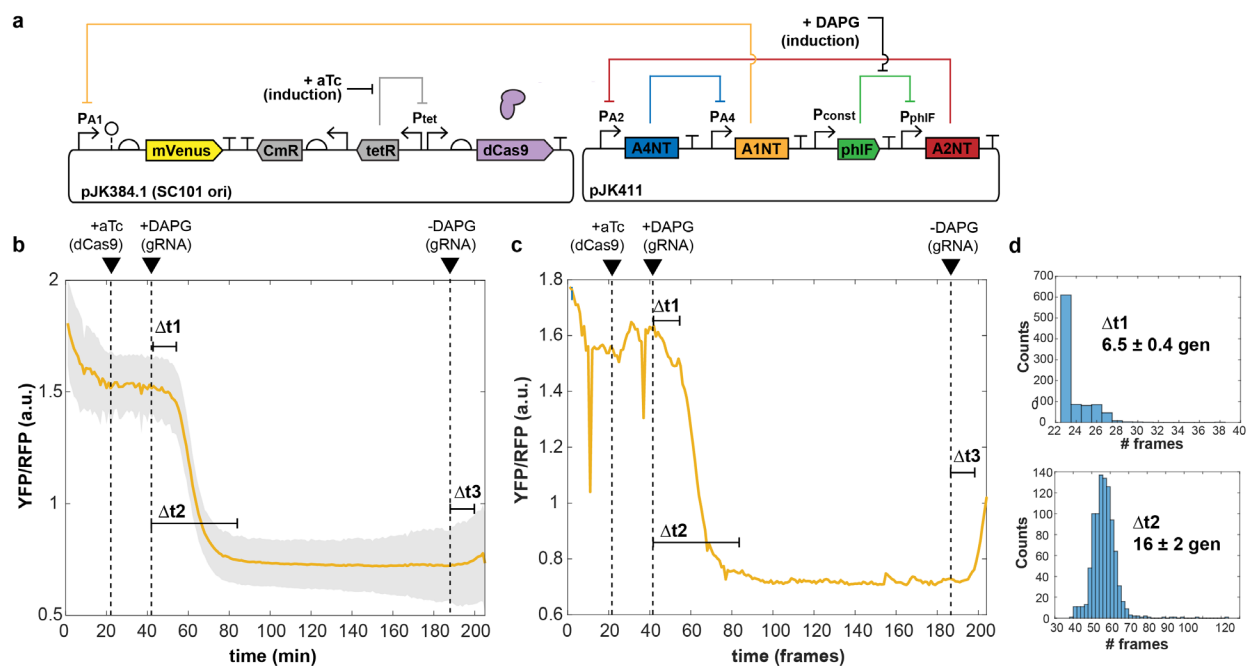
Figure S9. Response times for two-plasmid dCas9 sgRNAs. (a) Two-plasmid 3x cascade diagram. Upon DAPG-induced production of A2NT sgRNA, two more steps are needed for A1NT sgRNA repression of mVenus YFP signal. (b) Average reporter fluorescence trace of 1048 cells, with shaded region showing standard deviation. (c) Single trace of representative mother cell, highlighting fluorescence recovery at end upon DAPG removal. (c) Histograms of characteristic time lengths for each step.

dCas9 Degradation

Since dCas9 binding was previously shown to be very tight, with off rates longer than *E. coli* division times [9], we thought dCas9 protein degradation might be important. However, tests of adding four ssrA-based (small stable RNA A) tags [10] C-terminal to dCas9 did not noticeably improve behavior. These ssrA tags have the sequence "AADENYALAA", where the underlined portion varies to determine strength. The strong tags ("LVA" and "LAA") reduced dCas9 repression in a plate reader bulk assay, as did a medium strength tag ("AAV"), while the weakest tag ("ASV") performed similarly to without degradation (Fig. S10). Mother machine runs also did not show improved performance, with the strongest LAA tag abolishing fluctuations. Because of this, we used an untagged dCas9 in our other designs.
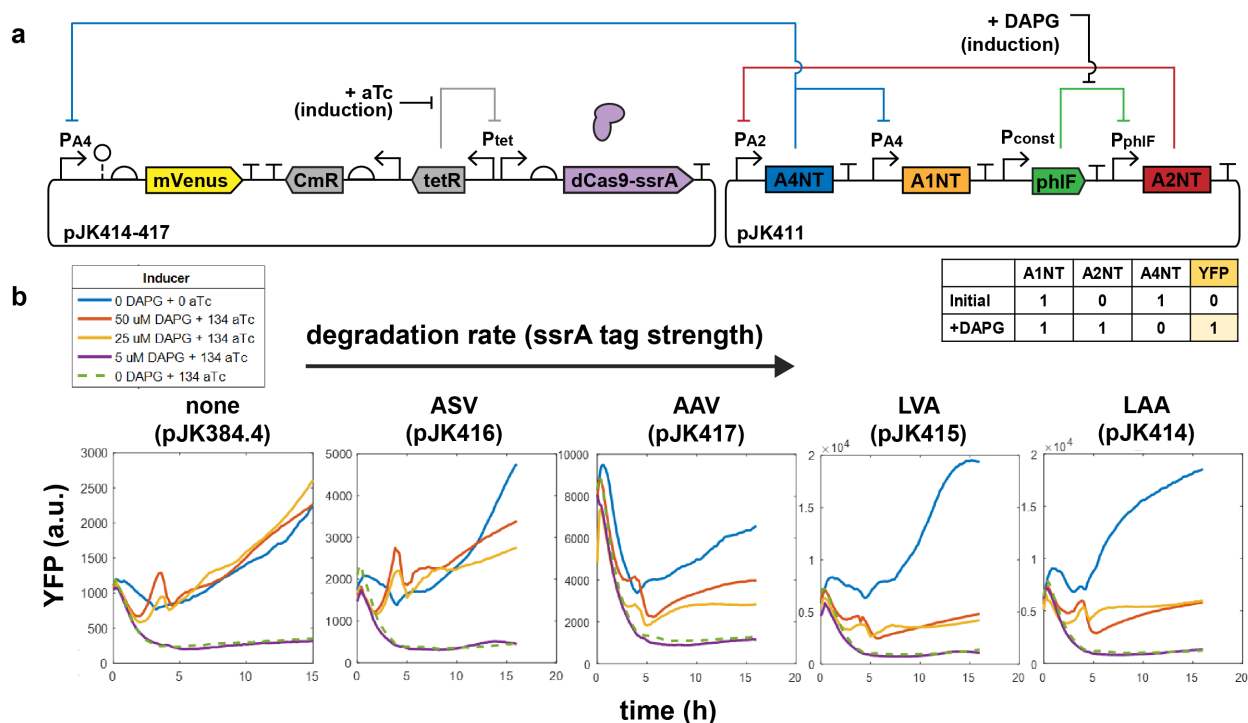

Figure S10. Degradation tag effects on dCas9 in bulk plate reader assay. (a) An inducible 3x sgRNA cascade was used with a PA4-mVenus reporter to test dCas9 constructs with C-terminal ssrA degradation tags (ex., dCas9 + AADENYALAA). (b) Responses of YFP signal in system with added degradation tags to dCas9. Tags are shown in order of increasing putative strength [10], from none to ASV, AAV, LVA and LAA. Inducer added was 0-50 µM DAPG and 134 nM (62.5 ng/mL) aTc. No induction (blue line) allowed free mVenus reporter production, while dCas9 induction without sufficient sgRNA production (purple and dashed green lines) repressed mVenus. DAPG-induced sgRNA production led to repression of promoter PA2 and A4NT sgRNA, allowing mVenus expression (red and orange lines) to varying degrees depending on dCas9 degradation tag.

aTc Titration

In addition to adjusting aTc levels during mother machine runs, plate reader assays helped determine aTc effects. Looking at different YFP reporter promoters with different induced sgRNAs showed even low amounts of aTc could be effective for repression (Fig. S11).
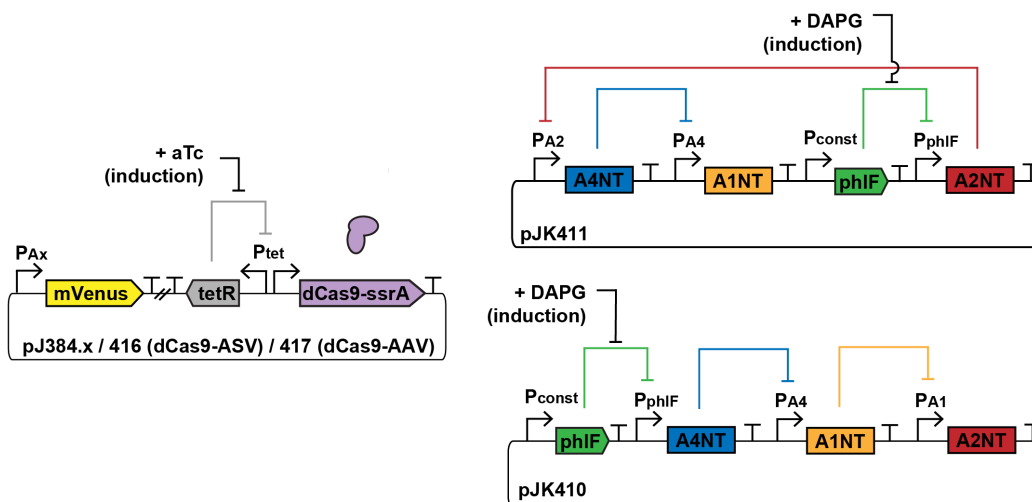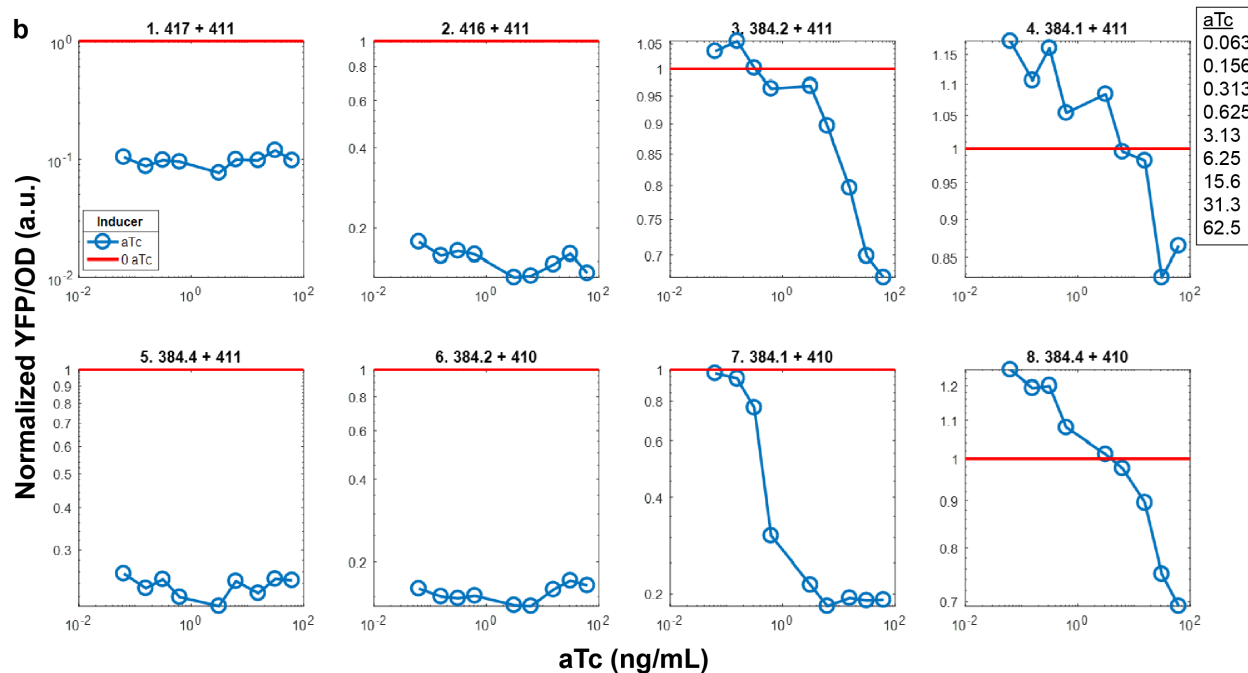
Figure S11. Inducer aTc titration for dCas9 expression in bulk plate reader assay. (a) Inducible sgRNA cascades were used with a varying PAx-Venus reporters (384.1,2,4 for PA1, PA2, PA4) to produce constant levels of sgRNAs. pJK416 and pJK417 both have PA4 promoter for mVenus, with added ssrA tags ASV and AAV on dCas9. (b) Plate assays were run. Red line shows level without aTc added (aTc = 0). All values are normalized to aTc = 0 for that strain. Concentrations of aTc, increasing order (ng/uL): 0.063, 0.156, 0.313, 0.625, 3.13, 6.25, 15.6, 31.3, 62.5. DAPG to induce sgRNA was not added. Without DAPG added, pJK410+411 act as constitutive producers of sgRNAs.

In Fig. S11b panels #1,2,5,6, even the lowest level of aTc (0.063 ng/mL) was enough dCas9 production to provide repression comparable to 1000x more aTc (62.5 ng/mL). In panel #7, a clearer response curve was seen for PA1 repression, with similar repression from 62.5 down to 3.13 ng/mL but a drop in effectiveness at lower concentrations.

Along with these results, we noticed higher toxicity to cells in mother machine runs at higher aTc amounts. Because of this, we tried to use a low amount but enough such that degradation over the 24+ of growth and imaging would not be a significant factor. A balance of this was 0.625 ng/mL.

Testing dCas9 induction levels in the mother machine. After previous bulk plate reader assays showed a lower amount of dCas9 may be helpful, we used these lower aTc levels to test in single-cell mother machine experiments. When using 62.5 ng/mL aTc for induction, cells showed signs of stress – much higher filamentation rates and halting of growth. Lowering aTc 100-fold to 0.625 ng/mL, fluctuations persisted, but somewhat better performance was observed at three-fold higher to 1.88 ng/mL (Fig. S12). When further raised to 6.25 ng/mL aTc, cells again showed the greater toxicity effects seen at 62.5 ng/mL, with an increased rate of halted growth while maintaining high levels of YFP reporter.
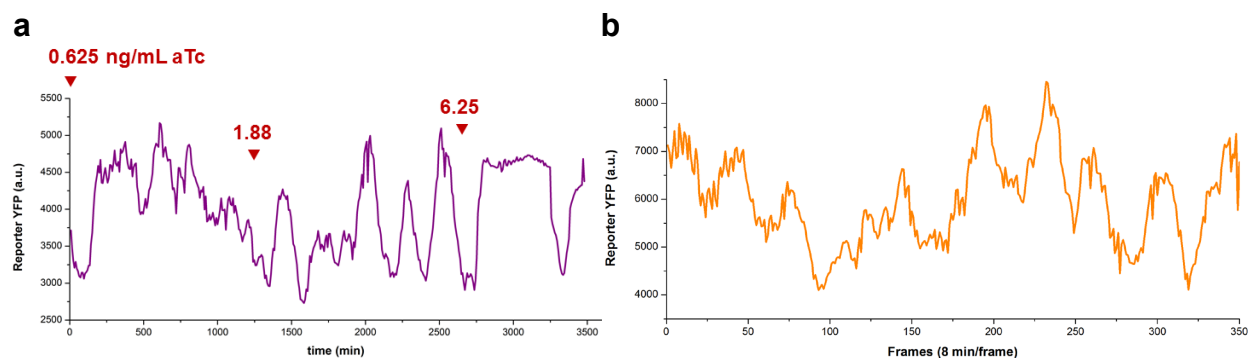
Figure S12. aTc titration in mother machine. Following a cross-section of a mother cell, the reporter YFP signal fluctuates over time. One-plasmid dCas9 oscillator (JK412) cells grown in mother machine chips with EZ-RDM. (a) Marked timepoints are where aTc concentration was changed to the indicated amount (ng/mL). Prior to addition of aTc for dCas9 induction, cells had a consistently high level of fluorescence. (b) In a separate run, dCas9 induction was held constant at 1.88 ng/mL. A 350-frame (~46 h) window of a 96+ h run is shown of a representative trace of a clearly fluctuating cell.
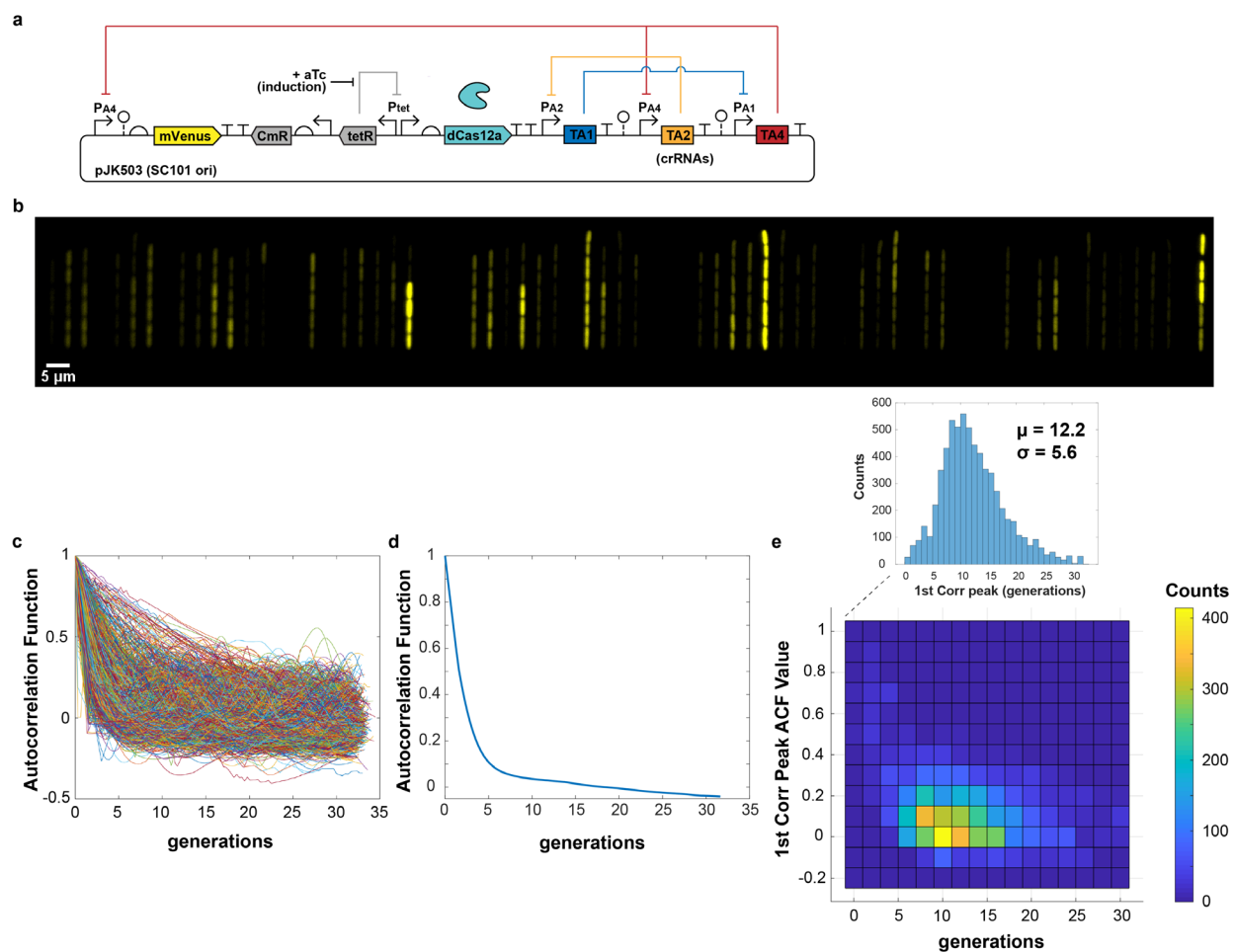
Figure S13. One-plasmid dCas12a RNA oscillator, with crRNAs in same transcriptional direction as dCas12a (JK503). (a) Diagram of 1-plasmid dCas12a RNA oscillator design with dCas12a and crRNAs in same transcriptional direction (pJK503). (b) Snapshot of *E. coli* MG1655 cells with plasmid in mother machine. Cells show variation, with strong repression. (c) Individual autocorrelation function (ACF) traces. (d) The population-averaged ACF shows a slower decay. (e) Individual first correlation peaks have a wide distribution. Measured from 6653 cells.
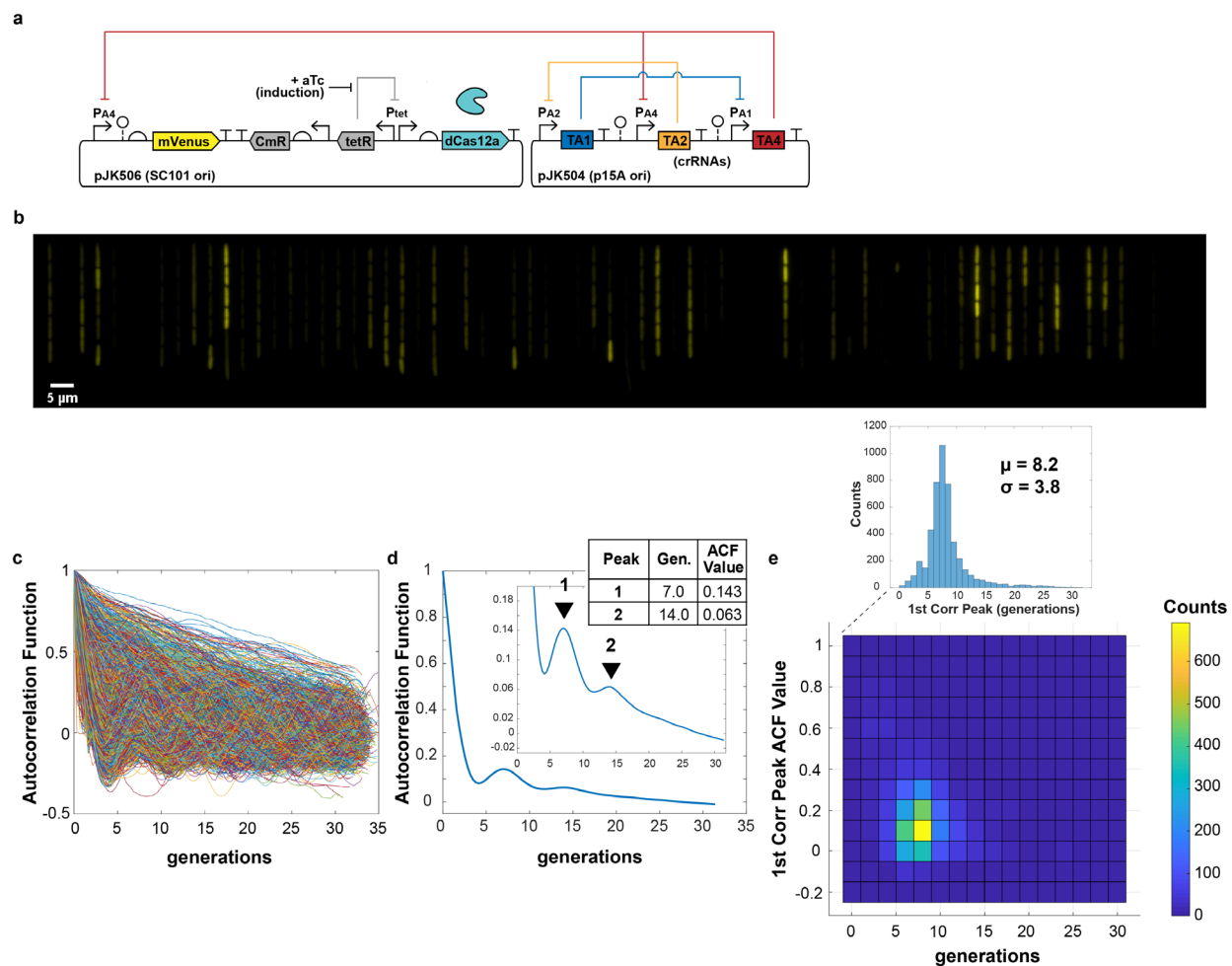
Figure S14. Two-plasmid dCas12a RNA oscillator (JK504+506). (a) Diagram of 2-plasmid dCas12a RNA oscillator design (pJK504 + pJK506). (b) Snapshot of *E. coli* MG1655 cells with plasmids in mother machine. Cells show variation, with strong repression. (c) Individual autocorrelation function (ACF) traces. (d) The population-averaged ACF showed distinct correlation peaks. (e) Individual first correlation peaks had a narrower distribution. Measured from 4962 cells.
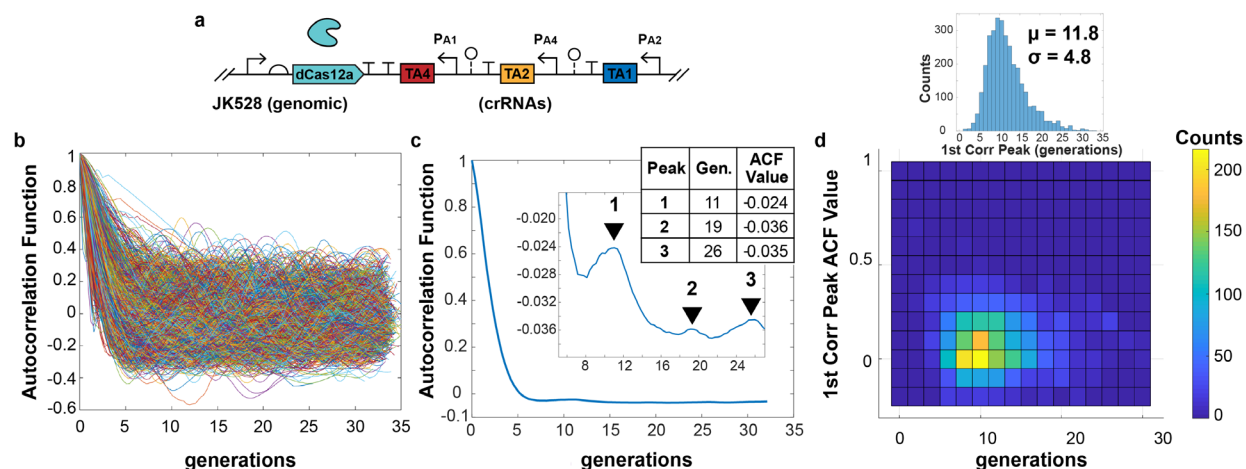
Figure S15. Genome-integrated dCas12a RNA oscillator (JK528). (a) Diagram of genome-integrated dCas12a RNA oscillator design (JK528), with transcriptionally convergent crRNAs. (b) Individual autocorrelation function (ACF) traces. (c) The population-averaged ACF showed distinct correlation peaks, though small. (d) Individual first correlation peaks had a wide distribution. Measured from 6636 cells.
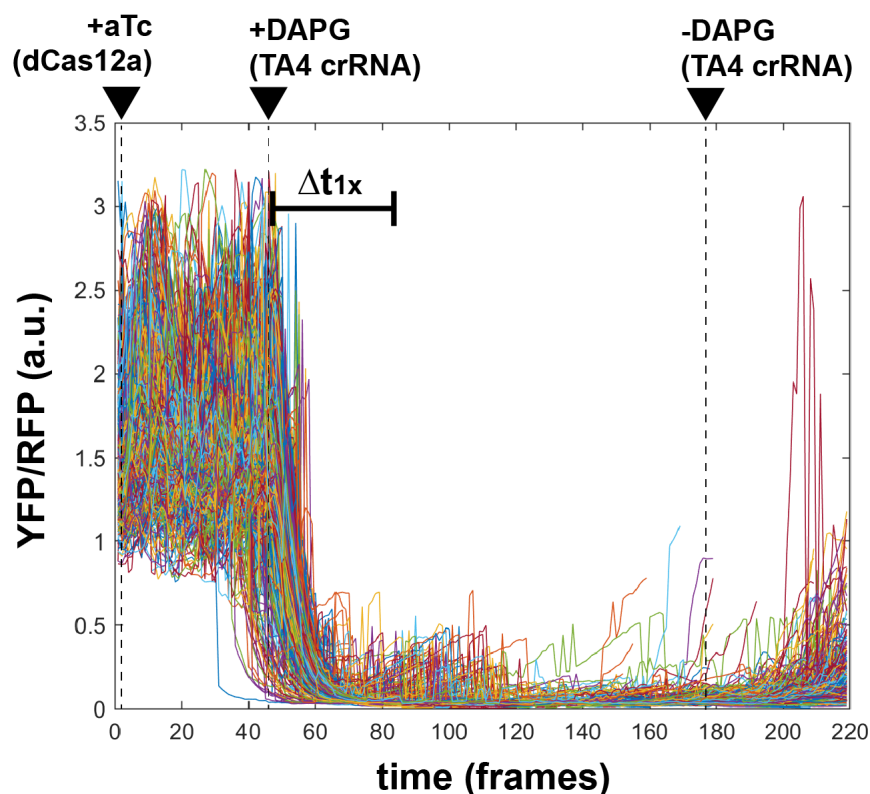
Figure S16. dCas12a crRNA response times single cells. Individual cell traces for dCas12a 1x (pJK526). 1049 cells. Average shown in main text Fig. 6b.

a

	TA1	TA2	TA4	YFP
Initial	0	1	1	1
+aTc	0	0	1	0
+DAPG	1	1	0	1

1 = species produced
0 = not produced / low amount

b

c
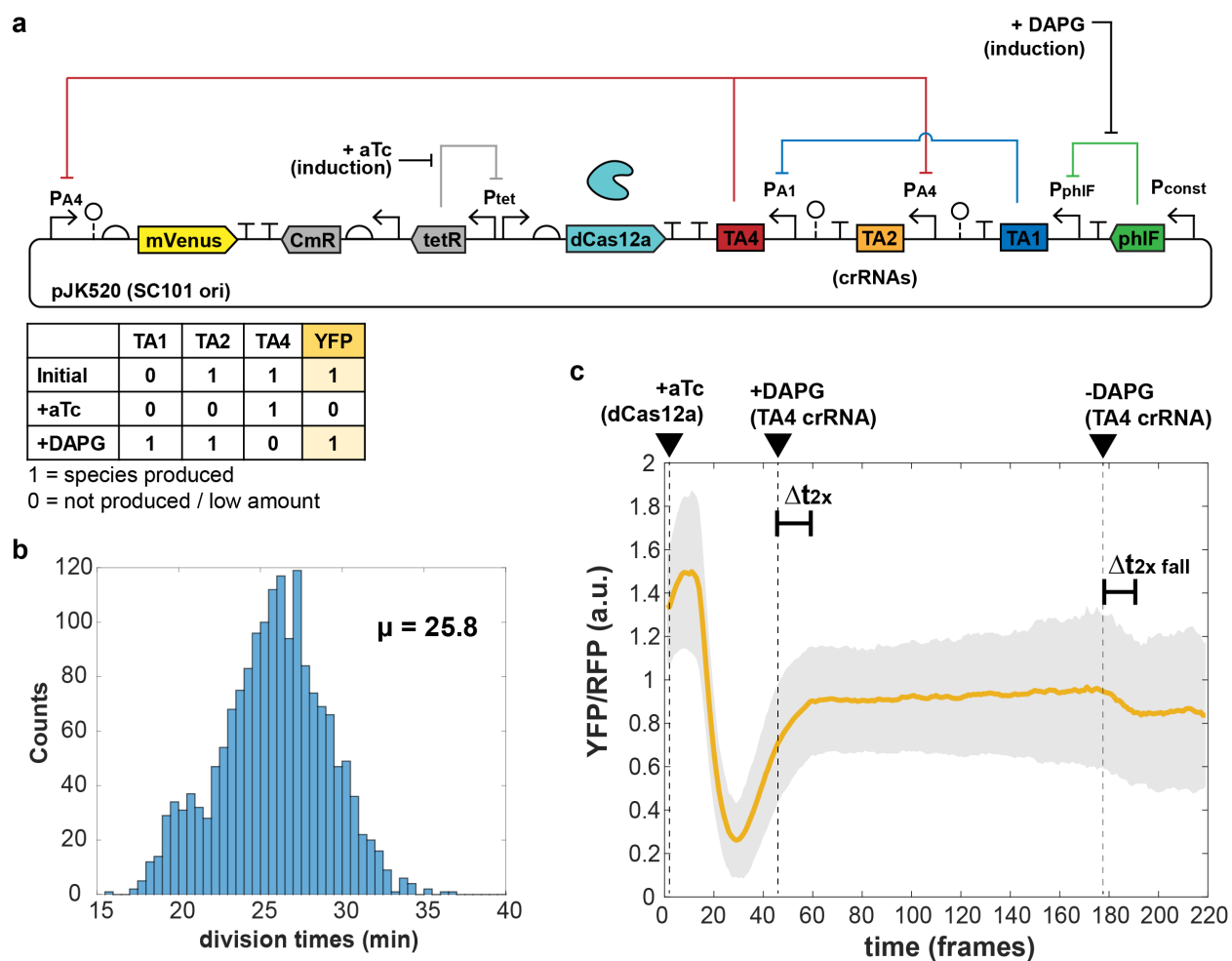
Figure S17. dCas12a crRNA dCas12a crRNA two-step response times. (a) A single constitutive promoter of the free-running dCas12a oscillator was replaced with a PhlF repressor-based promoter system, allowing inducible control with small molecule DAPG. *E. coli* MG1655 cells with plasmid were grown in EZ-RDM in the mother machine. (b) Histogram of average division times. (c) Averaged trace from 1698 cells of plasmid version (pJK520), with shaded region showing one standard deviation of the averaged values. 1 frame = 6 minutes.

Supplementary Videos

Video S1. dCas9 RNA oscillator (JK412) in mother machine.

E. coli MG1655 cells with dCas9 RNA oscillator plasmid (pJK412) are in a mother machine chip with EZ-RDM for exponential growth. YFP reporter fluorescence shown, 8 min per frame. Inducer aTc begins at 0.625 ng/mL and is changed to 1.88 ng/mL at time 20:00.

Video S2. dCas12a RNA oscillator (JK511) in mother machine.

E. coli MG1655 cells with dCas12a RNA oscillator plasmid (pJK511) are in a mother machine chip with EZ-RDM for exponential growth. YFP reporter fluorescence shown, 8 min per frame. Inducer aTc (1.88 ng/mL) added at video start.

Video S3. dCas12a RNA oscillator (JK503) in mother machine.

E. coli MG1655 cells with dCas12a RNA oscillator plasmid (pJK503, which has crRNAs in the same transcriptional direction as dCas12a) are in a mother machine chip with EZ-RDM for exponential growth. YFP reporter fluorescence shown, 8 min per frame. Inducer aTc (1.88 ng/mL) added at time 2:56.

Video S4. dCas12a RNA oscillator as two plasmids (pJK504+506) in mother machine.

E. coli MG1655 cells with dCas12a RNA oscillator plasmids (pJK504+506) are in a mother machine device with EZ-RDM for exponential growth. YFP reporter fluorescence shown, 8 min per frame. Inducer aTc (1.88 ng/mL) added at time 2:56.

Supplementary Tables

Table S1. Plasmids for dCas9 designs. ORI: origin of replication. Res: antibiotic resistance: Cm, chloramphenicol; Amp, ampicillin; K, kanamycin.

Name	Description	ORI	Res
pJK384.4	dCas9 + PA4-mVenus	SC101	Cm
pJK384.1	dCas9 + PA1-mVenus	SC101	Cm
pJK384.2	dCas9 + PA2-mVenus	SC101	Cm
pJK388	full dCas9 sgRNA oscillator v1 (dCRv1) (ColE1)	ColE1	Amp
pJK398	full dCas9 sgRNA oscillator v1 (dCRv1) (p15A)	p15A	Amp
pJK410	PphlF_A4NT in dCRv1	p15A	Amp
pJK411	PphlF_A2NT in dCRv1	p15A	Amp
pJK412	dCas9 oscillator v1 (dCRv1) + [dCas9 + PA4-mVenus]	SC101	Cm
pJK413	dCas9 oscillator v1 (dCRv1) + [dCas9 + PA4-mVenus] (sgRNAs flip of 412)	SC101	Cm
pJK414	dCas9-LAA + PA4-mVenus	SC101	Cm
pJK415	dCas9-LVA + PA4-mVenus	SC101	Cm
pJK416	dCas9-ASV + PA4-mVenus	SC101	Cm
pJK417	dCas9-AAV + PA4-mVenus	SC101	Cm
pJK418	dCas9-LAA + PA1-mVenus	SC101	Cm
pJK419	dCas9-LVA + PA1-mVenus	SC101	Cm
pJK421	dCas9-AAV + PA1-mVenus	SC101	Cm
pJK422	dCas9 oscillator v1 (dCRv1) + [dCas9 + PA4-mVenus]. (Integration of 412; pOSIP)	R6K gamma	Cm/K
pJK423	dCas9 oscillator v1 (dCRv1) + [dCas9 + PA4-mVenus]. (Integration of 413; pOSIP)	R6K gamma	Cm/K
pJK424	[PA4-mVenus]. (Integration; pOSIP)	R6K gamma	Cm/K
pJK425	dCRv1 sgRNAs only (412 direction). (Integration; pOSIP)	R6K gamma	K
pJK426	dCRv1 sgRNAs only (413 direction). (Integration; pOSIP)	R6K gamma	K
pJK427	[PA4-mVenus + dCas9]. (Integration; pOSIP)	R6K gamma	Cm/K
pJK428	TetR-dCas9. (Integration; pOSIP)	R6K gamma	K
pJK429	PphlF_A4NT in dCRv1, 1x cascade + [dCas9 + PA4-mVenus]	SC101	Cm
pJK430	PphlF_A2NT in dCRv1, 2x cascade + [dCas9 + PA4-mVenus]	SC101	Cm
pJK431	PphlF-A2NT (p15A ori)	p15A	Amp
pJK433	PphlF-A4NT (p15A ori)	p15A	Amp
pJK434	PphlF_A4NT, 1x cascade + [dCas9 + PA4-mVenus]. (Integration of 429; pOSIP)	R6K gamma	Cm/K
pJK435	PphlF_A2NT, 2x cascade + [dCas9 + PA4-mVenus]. (Integration of 430; pOSIP)	R6K gamma	Cm/K
pJK436	PphlF_A1NT in dCRv1	p15A	Amp
pJK437	PphlF_A1NT in dCRv1 + [dCas9 + PA4-mVenus]	SC101	Cm
pJK439	PphlF_A1NT in dCRv1 + [dCas9 + PA4-mVenus]. (Integration of 437; pOSIP)	R6K gamma	Cm/K
pJK444	dCRv2 (PA5-A1NT + PA3-A5NT + PA1-A3NT) in p15A	p15A	Amp
pJK446	dCas9 + PA3-mVenus	SC101	Cm
pJK447	dCRv3 (PA5-A1NT + PA3-A5NT + PA1-A3T) in p15A	p15A	Amp
pJK448	dCRv2 + [dCas9 + PA3-mVenus]	SC101	Cm
pJK458	dCRv2 flip + [dCas9 + PA3-mVenus]	SC101	Cm
pJK459	dCRv3 flip + [dCas9 + PA3-mVenus]	SC101	Cm

Table S2. Plasmids for dCas12a designs. ORI: origin of replication. Res: antibiotic resistance: Cm, chloramphenicol; Amp, ampicillin; K, kanamycin.

Name	Description	ORI	Res
pJK503	dCas12a oscillator, same direction crRNAs. dCas12a (D917A) + PA4-mVenus	SC101	Cm
pJK504	dCas12a crRNAs	p15A	Amp
pJK506	dCas12a (D917A) + PA4-mVenus. (removed crRNAs)	SC101	Cm
pJK507	dCas12a crRNAs. (flipped middle crRNA)	p15A	Amp
pJK511	dCas12a oscillator, reverse direction crRNAs. dCas12a (D917A) + PA4-mVenus	SC101	Cm
pJK517	PphlF-TA4 in crRNAs	p15A	Amp
pJK520	PphlF-TA1 crRNAs, 2x cascade. dCas12a (D917A) + PA4-mVenus.	SC101	Cm
pJK526	PphlF-TA4 crRNAs, 1x cascade. dCas12a (D917A) + PA4-mVenus.	SC101	Cm
pJK528	dCas12a oscillator, reverse crRNAs + PA4-mVenus. (Integration of 511; pOSIP)	R6K gamma	Cm/K

Table S3. Strains.

Name	Description
NDL162	*E. coli* MG1655 ΔmotA PRNA1-mKate2 (ref. [6])
	(mKate2 N-term 4 amino acids replaced by mCherry N-term 11 amino acids)
JK422	NDL162 attB::dCas9 oscillator v1 [dCRv1 + dCas9 + PA4-mVenus]
JK423	NDL162 attB::dCas9 oscillator v1 [dCRv1 flip sgRNAs + dCas9 + PA4-mVenus]
JK434	PphlF_A4NT in dCRv1, 1x cascade + [dCas9 + PA4-mVenus]
JK435	PphlF_A2NT in dCRv1, 2x cascade + [dCas9 + PA4-mVenus]
JK528	NDL162 attB::dCas12a oscillator, reverse crRNAs + PA4-mVenus.

Table S4. Sequences. (For full plasmid maps/sequences, see Addgene.)

Name	Description	DNA Sequence (5' to 3')
mVenus	RiboJ ribozyme + RBS + mVenus + rrnB T1 terminator	Agctgtcaccggatgtgctttccggtctgatgagtccgtgaggacgaaacagcctctacaaataatttt tgtttaactttacaaagaggagaaaggtaccgcatgagtaaaggagaagaacttttcactggagttgt cccaattcttgttgaattagatggtgatgttaatgggcacaaattttctgtcagtggagagggtgaag gtgatgcaacatacggaaaacttacccttaaatttatttgcactactggaaaactacctgttccatgg ccaacacttgtcactactttgggttatggtgttcaatgctttgcgagatacccagatcatatgaaaca gcatgacttttttcaagagtgccatgcccgaaggttatgtacaggaaagaactatattttttcaaagatg acgggaactacaagacacgtgctgaagtcaagtttgaaggtgataccccttgttaatagaatcgagtta aaaggtattgattttaaagaagatggaaacattcttggacacaaattggaatacaactataactcaca caatgtatacatcacggcagacaaacaaagaatggaatcaaagcgaacttcaaaattagacacaaca ttgaagatggaggtgttcaactagcagaccattatcaacaaaatactccaattggcgatggccctgtc cttttaccagacaaccattacctgtcctaccaatctaagctttcgaaagatcccaacgaaaagagaga ccacatggtccttcttgagtttgtaacagctgctgggattacacatggcatggatgaactatacaaat aagcttgatatcgaattcctgcagcccggggatcccattgtacgcgtgctagaggcatcaaataaaa cgaaaggctcagtcgaaagactgggcctttcgttttatctgttgtttgtcggtgaacgctctcctgag taggacaaat
TetR cassette	TetR protein and Ptet promoter	ttaagacccactttcacatttaagttgtttttctaatccgcatatgatcaattcaaggccgaataagaa aggctggctctgcaccttggtgatcaaataattcgatagcttgtcgtaataatggcggcatactatca gtagtaggtgtttccctttcttctttagcgacttgatgctcttgatcttccaatacgcaacctaaagt aaaatgccccacagcgctgagtgcatataatgcattctctagtgaaaaaccttgttggcataaaaagg ctaattgattttcgagagtttcatactgtttttctgtaggccgtgtacctaaatgtactttttgctcca tcgcgatgacttagtaaagcacatctaaaactttagcgttattacgtaaaaaatcttgccagctttc cccttctaaagggcaaaagtgagtatggtgcctatctaacatctcaatggctaaggtcgcgagcaaag cccgcttatttttacatgccaatacaatgtaggctgctctcacacctagcttctgggcgagtttacgg gttgttaaaccttcgattccgacctcattaagcagctctaatgcgctgttaatcactttactttatc taatctagacatcattaattcctaatttttgttgacactctatcgttgatagagttattttttaccactc cctatcagtgatagagaaaagaattcaaaagatctaaagaggagaaaggatct
PhlF cassette	PhlF protein + rrnB T1 and T7Te terminators and PphlF promoter	ctatggactatgtttgaaagggagaaatactagatggcacgtaccccgagccgtagcagcattggtag cctgcgtagtccgcatacccataaagcaattctgaccagcaccattgaaatcctgaaagaatgtggtt atagcggtctgagcattgaaagcgttgcacgtcgtgccggtgcaagcaaaccgaccatttatcgttgg tggaccaataaagcagcactgattgccgaagtgtatgaaatgaaagcgaacaggtgcgtaaatttcc ggatctgggtagctttaaagccgatctggattttcgtgctgcgtaatctgtggaaagtttggcgtgaaa ccatttgtggtgaagcatttcgttgtgttattgcagaagcacagctggaccctgcaaccctgacccag ctgaaagatcagtttatggaacgtcgtcgtgagatgccgaaaaaactggttgaaaatgccattagcaa tggtgaactgccgaaagataccaatcgtgaactgctgctggatatgattttggttttttgttggtatc gcctgctgaccgaacagctgaccgttgaacaggatattgaagaatttaccttcctgctgattaatggt gtttgtccgggtacacagcgttaactagggcccataccccaggcatcaaataaaacgaaaggctcag tcgaaagactgggcctttcgttttatctgttgtttgtcggtgaacgctctctactagagtcacactgg ctcaccttcgggtgggcctttctgcgtttatatacggcacgtacggtggaatctgattcgttaccaat tgacatgatacgaaacgtaccgtatcgttaaggtctagggcccataccccaggcat
dCas9 Parts		
PA1	promoter [2]	tttacaccTAGCTCAGTCCTAggtattatgctagc
PA2	promoter [2]	tttacaccAACGGGTCACACGggtattatgctagc
PA4	promoter [2]	tttacaccTCCACAACTAGCTggtattatgctagc
A1NT	sgRNA [2]	ataatacctaggactgagctgttttagagctagaaatagcaagttaaaataaggctagtccgattgca acttgaaaaagtggcaccgagtcggtgcttttttt
A2NT	sgRNA [2]	ataatacccgtgtgacccgtgttttagagctagaaatagcaagttaaaataaggctagtccgttatca acttgaaaaagtggcaccgagtcggtgcttttttt
A4NT	sgRNA [2]	ataataccagctagttgtgggttttagagctagaaatagcaagttaaaataaggctagtccgttatca acttgaaaaagtggcaccgagtcggtgcttttttt
L3S2P11	terminator [11] for A1NT sgRNA, TA2 crRNA	ctcggtaccaaattccagaaaagagacgctttcgagcgtcttttttcgttttggtcc
L3S2P55	terminator [11] for A2NT sgRNA, TA4 crRNA	ctcggtaccaaagacgaacaataagacgctgaaaagcgtcttttttcgttttggtcc
L3S2P21	terminator [11] for A4NT sgRNA, TA1 crRNA	ctcggtaccaaattccagaaaagaggcctcccgaaaggggggcctttttttcgttttggtcc

S. pyogenes dCas9	RBS + dCas9 (D10A, H840A) [1] + rrnB T1 terminator	aaagaggagaaaggatct atggataagaaatactcaataggcttagctatcggcacaaatagcgtcgg atgggcggtgatcactgatgaatataaggttccgtctaaaaagttcaaggttctgggaaatacagacc gccacagtatcaaaaaaaatcttatagggggctcttttatttgacagtggagagacagcggaagcgact cgtctcaaacggacagctcgtagaaggtatacacgtcggaagaatcgtatttgttatctacaggagat tttttcaaatgagatggcgaaagtagatgatagtttctttcatcgacttgaagagtctttttggtgg aagaagacaagaagcatgaacgtcatcctattttttggaaatatagtagatgaagttgcttatcatgag aaatatccaactatctatcatctgcgaaaaaaattggtagattctactgataaagcggatttgcgctt aatctatttggccttagcgcatatgattaagtttcgtggtcattttttgattgagggagatttaaatc ctgataatagtgatgtggacaaactatttatccagttggtacaaacctacaatcaattatttgaagaa aaccctattaacgcaagtggagtagatgctaaagcgattctttctgcacgattgagtaaatcaagacg attagaaaatctcattgctcagctccccggtgagaagaaaaatggcttatttgggaatctcattgctt tgtcattgggtttgacccctaattttaaatcaaattttgatttggcagaagatgctaaattacagctt tcaaaagatacttacgatgatgatttagataatttattggcgcaaattggagatcaatatgctgattt gttttttggcagctaagaatttatcagatgctattttactttcagatatcctaagagtaaatactgaaa taactaaggctcccctatcagcttcaatgattaaacgctacgatgaacatcatcaagacttgactctt ttaaaagctttagttcgacaacaacttccagaaaagtataaagaaatctttttttgatcaatcaaaaaa cggatatgcaggttatattgatgggggagctagccaagaagaattttataaatttatcaaaccaattt tagaaaaaatggatggtactgaggaattattggtgaaactaaatcgtgaagatttgctgcgcaagcaa cggacctttgacaacggctctattccccatcaaattcacttgggtgagctgcatgctattttgagaag acaagaagactttatccatttttaaaagacaatcgtgagaagattgaaaaaatcttgacttttcgaa ttccttattatgttggtccattggcgcgtggcaatagtcgttttgcatggatgactcggaagtctgaa gaaacaattaccccatggaattttgaagaagttgtcgataaaggtgcttcagctcaatcatttattga acgcatgacaaactttgataaaaatcttcccaaatgaaaaagtactaccaaaacatagtttgctttatg agtattttacggtttataacgaattgacaaagtcaaatatgttactgaaggaatgcgaaaaccagca tttctttcaggtgaacagaagaaagccattgttgatttactcttcaaaacaaatcgaaaagtaaccgt taagcaattaaaagaagattatttcaaaaaaatagaatgttttgatagtgttgaaatttcaggagttg aagatagatttaatgcttcattaggtacctaccatgatttgctaaaaattattaaagataaagatttt ttggataatgaagaaaatgaagatatcttagaggatattgttttaacattgaccttatttgaagatag ggagatgattgaggaaagacttaaaacatatgctcacctctttgatgataaggtgatgaaacagctta aacgtcgccgttatactggttggggggacgtttgtctcgaaaattgattaatggtattagggataagcaa tctggcaaaacaatattagattttttgaaatcagatggttttgccaatttatgcaatttttatgcagctgat ccatgatgatagtttgacatttaaagaagacattcaaaaagcacaagtgtctggacaaggcgatagtt tacatgaacatattgcaaatttagctggtagccctgctattaaaaaaggtattttacagactgtaaaa gttgttgatgaattggtcaaagtaatggggcggcataagccagaaaaatatcgttattgaaatggcacg tgaaaatcagacaactcaaaagggccagaaaaattcgcgagagcgtatgaaacgaatcgaagaaggta tcaaagaattaggaagtcagattcttaaagagcatcctgttgaaaatactcaattgcaaatgaaaag ctctatctctattatctccaaaatggaagagacatgtatgtggaccaagaattagatattaatcgttt aagtgattatgatgtcgatgccattgttccttcacaaagttccttaaagacgcttcaatagacaataagg tcttaacgcgttctgataaaaatcgtggtaaatcggataacgttccaagtgaagaagtagtcaaaaag atgaaaaactattggagacaacttctaaacgccaagttaatcactcaacgtaagtttgataatttaac gaaagctgaacgtggaggtttgagtgaacttgataaagctggtttttatcaaacgccaattggttgaaa ctcgccaaatcactaagcatgtggcacaaattttggatagtcgcatgaatactaaatacgatgaaaat gataaacttattcgagaggttaaagtgattaccttaaaatctaaattagtttctgacttccgaaaaga tttccaattctataaagtacgtgagattaacaattaccatcatgcccatgatgcgtatctaaatgccg tcgttggaactgctttgattaagaaatatccaaaacttgaatcggagtttgtctatggtgattataaa gtttatgatgttcgtaaatgattgctaagtctgagcaagaaataggcaagaataggcaaagcaaccgcaaaatattt cttttactctaatatcatgaacttcttcaaaacagaaattacacttgcaaatggagagattcgcaaac gccctctaatcgaaactaatggggaaactggagaaattgtctgggataaagggcgagattttgccaca gtgcgcaaagtattgtccatgccccaagtcaatattgtcaagaaaacagaagtacagacaggcggatt ctccaaggagtcaattttaccaaaaagaaattcggacaagcttattgctcgtaaaaaagactgggatc caaaaaaatatggtggttttgatagtccaacggtagcttattcagtcctagtggttgctaaggtggaa aaagggaaatcgaagaagtttaaaatccgttaaagagttactagggatcacaattatggaaagaagttc ctttgaaaaaatccgattgacttttttagaagctaaaggatataaggaagttaaaaagacttaatca ttaaactacctaaatatagtcttttttgagttagaaaacggtcgtaaacggatgctggctagtgccgga gaattacaaaaaggaaatgagctggctctgccaagcaaatatgtgaattttttatatttagctagtca ttatgaaaagttgaagggtagtccagaagataacgaacaaaaacaattgtttgtgtgagcagcataagc attatttagatgagattattgagcaaatcagtgaatttctaagcgtgttatttttagcagatgccaat ttagataaagttcttagtgcatataacaaacatagagacaaaccaatacgtgaacaagcagaaaatat tattcattttttttacgttgacgaatcttggagctcccgctgcttttaaatattttgatacaacaattg atcgtaaacgatatacgtctacaaaagaagtttttagatgccactcttatccatcaatccatcactggt ctttatgaaacacgcattgattttgagtcagctaggaggtgactaactcgagtaaggatctccaggcat caaataaaacgaaaggctcagtcgaaagactgggcctttcgttttatctgttgtttgtcggtgaacgc tctc
dCas12a Parts		
TA1 crRNA	crRNA	TAATTTCTACTGTTGTAGATcaccTAGCTCAGTCCTAggt
TA2 crRNA	crRNA	TAATTTCTACTGTTGTAGATcaccAACGGGTCACACGggt
TA4 crRNA	crRNA	TAATTTCTACTGTTGTAGATcaccTCCACAACTAGCTggt
SarJ ribozyme	ribozyme spacer [12]	AGACTGTCGCCGGATGTGTATCCGACCTGACGATGGCCCAAAAGGGCCGAAACAGTCCTCTACAAATA ATTTTGTTTAA

PlmJ ribozyme	ribozyme spacer [12]	AGTCATAAGTCTGGGCTAAGCCCACTGATGAGTCGCTGAAATGCGACGAAACTTATGACCTCTACAAA TAATTTTGTTTAA
F. novicida dCas12a	RBS + dCas12a (D917A) [3] + ECK120029600 terminator [11]	aaagaggagaaaggatctatgtcaatttatcaagaatttgttaataaatatagtttaagtaaaactct aagatttgagttaatcccacagggtaaaacacttgaaaacataaaagcaagaggtttgattttagatg atgagaaaagagctaaagactacaaaaaggctaaacaaataattgataaatatcatcagttttttata gaggagatattaagttcggtttgtattagcgaagatttattacaaaactattctgatgtttattttaa acttaaaaagagtgatgatgataatctacaaaaagattttaaaagtgcaaaagatacgataaagaaac aaatatctgaatatataaaggactcagagaaatttaagaatttgtttaatcaaaaccttatcgatgct aaaaaagggcaagagtcagatttaattctatggctaaagcaatctaaggataatggtatagaactatt taaagccaatagtgatatcacagatatagatgaggcgttagaaataatcaaatcttttaaaggttgga caacttattttaagggttttcatgaaaatagaaaaaatgtttatagtagcaatgatattcctacatct attatttataggatagtagatgataatttgcctaaatttctagaaaataaagctaagtatgagagttt aaaagacaaagctccagaagctataaactatgaacaaattaaaaaagatttggcagaagagctaacct ttgatattgactacaaaacatctgaagttaatcaaagagttttttcacttgatgaagttttgagata gcaaactttaataattatctaaatcaaagtggtattactaaatttaatactattattggtggtaaatt tgtaaatggtgaaaatacaaagagaaaaggtataaatgaatatataaatctatactcacagcaaataa atgataaaacactcaaaaaatataaaatgagtgtttttatttaagcaaattttaagtgatacagaatct aaatcttttgtaattgataagttagaagatgatagtgatgtagttacaacgatgcaaagttttttatga gcaaatagcagcttttaaaacagtagaagaaaatctattaaagaaacactatctttattatttgatg atttaaaagctcaaaaacttgatttgagtaaaatttattttaaaaatgataaatctcttactgatcta tcacaacaagttttttgatgattatatgtgttattggtacagcggtactagaatatataactcaacaaat agcacctaaaaatcttgataaccctagtaagaaagagcaagaattaatagccaaaaaaactgaaaaag caaatacttatctctagaaactataaagcttgccttagaagaatttaataagcatagagatatagat aaacagtgtaggtttgaagaaatacttgcaaactttgcggctattccgatgatatttgatgaaatagc tcaaaacaaagacaatttggcacagatatctatcaaatatcaaatcaaggtaaaaaagacctacttc aagctagtgcggaagatgatgttaaagctatcaaggatcttttagatcaaactaataatctcttacat aaactaaaaatatttcatattagtcagtcagaagataaggcaaatatttagacaaggatgagcattt ttatctagtatttgaggagtgctactttgagctagcgaatatagtgcctctttataacaaaattagaa actatataactcaaaagccatatagtgatgagaaatttaagctcaattttgagaactcgactttggct aatggttgggataaaaataaagagcctgacaatacggcaatttttatttatcaaagatgataaatatta tctgggtgtgatgaataagaaaaataacaaaatatttgatgataaagctatcaaagaaaataaaggcg aggttataaaaaaattgtttataaacttttacctggcgcaaataaaatgttacctaaggttttctttt tctgctaaatctataaaattttataatcctagtgaagatatacttagaataagaaatcattccacaca tacaaaaaatggtagtcctcaaaaaggatatgaaaaatttgagtttaatattgaagattgccgaaaat ttatagattttttataaacagtctataagtaagcatccggaatgtgaaaagtttggatttagattttct gatactcaaagatataattctatagatgaattttataggaagttgaaaatcaaggctacaaactaac ttttgaaaatatatcagagagctatattgatagcgtagttaatcagggtaaattgtacctattccaaa tctataataaagatttttcagcttatagcaaagggcgaccaaatctacatactttatattggaaagcg ctgtttgatgagagaaatcttcaagatgtggtttataagctaaatggtgaggcagagctttttttatcg taaacaatcaatacctaaaaaaatcactcacccagctaaagaggcaatagctaataaaaacaaagata atcctaaaaaagagagtgttttttgaatatgatttaatcaaagataaacgctttactgaagataagttt ttctttcactgtcctattacaatcaattttaaatctggagctaataagtttaatgatgaaatcaa tttattgctaaaagaaaaagcaaatgatgttcatatattaagtatagCtagaggtgaaagacatttag cttactatactttggtagatggtaaaggcaatatcatcaaacaagatactttcaacatcattggtaat gatagaatgaaaacaaactaccatgataagcttgctgcaatagagaaagatagggattcagctaggaa agactggaaaaagataaataacatcaaagagatgaaagagggctatctatctcaggtagttcatgaaa tagctaagctagttatagagtataatgctattgtggttttttgaggatttaaattttggatttaaaaga gggcgtttcaaggtagagaagcaggtctatcaaaagttagaaaaaatgctaattgagaaactaaacta tctagttttcaaagataatgagtttgataaaactgggggagtgtgcttagacgttatcagctaacagcac cttttgagacttttaaaaagatgggtaaacaacaggtattatctactatgtaccagctggtttttact tcaaaaatttgtcctgtaactggttttgtaaatcagttatatcctaagtatgaaagtgtcagcaaatc tcaagagttctttagtaagtttgacaagatttgttataaccttgataagggctattttgagtttagtt ttgattataaaaactttggtgacaaggctgccaaaggcaagtggactatagctagctttgggagtaga ttgattaactttagaaattcagataaaaatcataattgggatactcgagaagtttatccaactaaaga gttggagaaattgctaaaagattattctatcgaatatgggcatggcgaatgtatcaaagcagctattt gcggtgagagcgacaaaaagtttttttgctaagctaactagtgtcctaaatactatcttacaaatgcgt aactcaaaaacaggtactgagttagattatctaatttcaccagtagcagatgtaaatggcaatttctt tgattcgcgacaggcgccaaaaaaatatgcctcaagatgctgatgccaatggtgcttatcatattgggc taaaaggtctgatgctactaggtaggatcaaaaataatcaagagggcaaaaaactcaatttggttatc aaaaatgaagagtattttgagttcgtgcagaataggaataactaattgagaagagaaaagaaaaccgc cgatcctgtccaccgcattactgcaaggtagtggacaagaccggcggtcttaagttttttggctgaa

(red: ribozyme, orange: gene, purple: terminator, green: promoter)

Table S5. Primers. Select primers used for cloning.

Use	Name	DNA Sequence (5' to 3')
Reporters	dCR-PA4-F	atat actagtatgcgtctcctttacacc tccacaactagct ggtattatgctagcagctg
	dCR-PA1-F	atat actagtatgcgtctcctttacacc TAGCTCAGTCCTA ggtattatgctagcagctg
	dCR-PA2-F	atat actagtatgcgtctcctttacacc AACGGGTCACACG ggtattatgctagcagctg
	Vs-RiboJ-R	TGCGGTACCTTTCTCCTCTTTGTAAAGTTAAACAAAATTATTTGTAGAGGCTGTTTCGTC
	dCR-Venus-F	CTTTACAAAGAGGAGAAAGGTACCGC
	XbaI-rrnB-R	TTAATCTAGAATTTGTCCTACTCAGGAGAGCG
sgRNAs	385-BglII-F	ttaa agatctcagaaatcattttacaccaacgg
	385-HindIII-R	atat AAGCTTAAGCGGACCAAAACGAAAAAAGG
	386-BgH-F	ttaa AGATCTAAGCTTTTTACACCTCCACAACTAGCTGG
	386-Bam-R	atat GGATCCTATCGGACCAAAACGAAAAAAG
	387-BB-PA1-F	ttaa AGATCTGGATCCTTTACACCTAGCTCAGTCCTAGGTATTATGCTAGCATAATACCCGTGTGACCCGT
	387-Xba-R	atat TCTAGATAAAGGACCAAAACGAAAAAAGACGC
pJK398	398-388-R	gagtcagtgagcgaggaagcctgcagtctagataa
	398-p15A-F	gactgcaggcttcctcgctcactgactcgctac
	398-p15A-R	cgtgagttttcgttccactgccaagcactagtaacaactt
	398-388-F	gttactagtgcttggcagtggaacgaaaactcacg
PphlF sgRNA (pJK410, 411)	410-Bg-PhlF-F	TTAA AGATCT ACTTTTACAGCTAGCTCAGTCC
	410-Hind-R	ATAT AAGCTT CTTAAGTTTTTTGGCTGAAGGACC
	411-Bam-PhlF-F	TTAA ggatcc ACTTTTACAGCTAGCTCAGTCC
	411-Pst-R	ATAT CTGCAG GAATTCTAAAGGACCAAAACGA
ssRA-tagged dCas9	dCas9-DENYA-R	AGCGTAGTTTTCGTCGTTTGCTGCGTCACCTCCTAGCTGACTCAAATCAATGCG
	LAA-F	GCAGCAAACGACGAAAACTACGCT TTAGCAGCT TAACTCGAGTAAGGATCTCCAGGCATC
	LVA-F	GCAGCAAACGACGAAAACTACGCT TTAGTAGCT TAACTCGAGTAAGGATCTCCAGGCATC
	ASV-F	GCAGCAAACGACGAAAACTACGCT GCATCAGTT TAACTCGAGTAAGGATCTCCAGGCATC
	AAV-F	GCAGCAAACGACGAAAACTACGCT GCAGCAGTT TAACTCGAGTAAGGATCTCCAGGCATC
	SC101-R	ACACCAGAACAGCCCGTTTG
	414-chk-F	TAGGAGGTGACGCAGCAAAC
	414-chk-Rv	AAGCTGTTCACCATGAACAG
dCRv1 clonetegration (pJK422 + 423)	SpeI-398-F	ATATATactagtaataggcgtatcacgaggcaga
	Bss-412-F	TATAGCGCGCCTAGTATGCGTCTCCTTTACACC
	SpeI-412-R	AATTACTAGTAGCAAAACCCGTACCCTAGAT
	SpeI-413-Rv	AATTACTAGTAATAGGCGTATCACGAGGCA
	SpeI-lt0Vs-R	AATTACTAGTGACTCCTGTTGATAGATCCAGTA
	Bss-425-412-F	TATAGCGCGCTACTAGAGTCACACTGGCTCAC
	SpeI-427-R	ATATATACTAGTATAAACGCAGAAAGGCCCACC
	Bss-428-F	TATAGCGCGCACGTCTCATTTTCGCCAGATATCG
	433end-R	ATCTTCCAGGAAATCTCCGC
	SC101R2	GCGATGCTGCTTATCGA
	435-SpeI-R	AATTACTAGTAGCAAAACCCGTACCCTAGA
sgRNA Set 2+3 (1,3,5)	440-PA5-F	ATAT AGATCTCAGAAATCATTTTACACCAAAACACTCGGAGGGTATTATGCTAGCA
	445-PA5-F	TACACCAAAACACTCGGAGGGTATTATGCTAGCAGCTG
	445-PA5-R	TAATACCCTCCGAGTGTTTTGGTGTAAAGGAGACGCATAC
	446-PA3-F	TACACCCGAAATGGAGCATGGTATTATGCTAGCAGCTG
	446-PA3-R	TAATACCATGCTCCATTTCGGGTGTAAAGGAGACGCATAC
dCas12a	500-vR	ataaattgacatagatcctttctcctcttt
	500-Cp-F	ggatctatgtcaatttatcaagaatttg
	500-Cp-R	gctaaatgtctttcacctctaGctatacttaatatatgaacatcatttgc
	500-Cp-2F	atagCtagaggtgaaagacatttagc

	500-Cp-2R	tagttattcctattctgcacgaactc
	500-vF	TTAATTAAgtacgggttttgctgcccg
	504-v-F	CCGTTAATTAACTGCAGGCTTCCTCGCTCACTGACTCGCTAC
	504-v-R	CCGTTAATTAAATGGTTTCTTAGACGTCAGG
	516-105-F	tttacggctagctcagtcctaggtactatgctagcagtacgtctg
	516-Phlf-R	AggtgATCTACAACAGTAGAAATTAaccttaacgatacggtacg
	516-v-F	TCTACTGTTGTAGATCACCTAGCTCAGTCCTAGGTCTCGG
	516-v-R	TACCTAGGACTGAGCTAGCCGTAAAATGATTTCTGAGATC
	517-v-F	TCTACTGTTGTAGATcaccTCCACAACTAGCTggtctcgg
	517-v-R	tacctaggactgagctagccgtaaaatcctatcTTAAACA
	528-511-F	cagtatgcgtatttgcgcgcttggcctagtatgcgtctcc
	528-v-R	ggagacgcatactaggccaagcgcgcaaatacgcatactg
	528-511-R	ccatgcatctcgaggcatgcacaccagaacagcccgtttg
	528-v-F	caaacgggctgttctggtgtgcatgcctcgagatgcatgg
PhlF-TA1	520-phlF-F	ATCTACAACAGTAGAAATTAACCTTAACGATACGGTACGTT
	520-phlF-R	TGATTTCTGGAATTCTAAAGATCTTTTACGGCTAGCTCAGTC
	520-v-F	AGATCTTTAGAATTCCAGAAATCATCCTTAGC
	520-v-R	GTTAATTTCTACTGTTGTAGATCACCTAGCTCAGTCCTAGGT

References

(1)   Qi, L. S.; Larson, M. H.; Gilbert, L. A.; Doudna, J. A.; Weissman, J. S.; Arkin, A. P.; Lim, W. A. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell* **2013**, *152*, 1173.

(2)   Nielsen, A. A.; Voigt, C. A. Multi-input CRISPR/Cas genetic circuits that interface host regulatory networks. *Mol Syst Biol* **2014**, *10*, 763.

(3)   Zetsche, B.; Gootenberg, J. S.; Abudayyeh, O. O.; Slaymaker, I. M.; Makarova, K. S.; Essletzbichler, P.; Volz, S. E.; Joung, J.; van der Oost, J.; Regev, A.; Koonin, E. V.; Zhang, F. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell* **2015**, *163*, 759.

(4)   Gibson, D. G.; Young, L.; Chuang, R. Y.; Venter, J. C.; Hutchison, C. A.; Smith, H. O. Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nat Methods* **2009**, *6*, 343.

(5)   St-Pierre, F.; Cui, L.; Priest, D. G.; Endy, D.; Dodd, I. B.; Shearwin, K. E. One-step cloning and chromosomal integration of DNA. *ACS Synth Biol* **2013**, *2*, 537.

(6)   Luro, S.; Potvin-Trottier, L.; Okumus, B.; Paulsson, J. Isolating live cells after high-throughput, long-term, time-lapse microscopy. *Nat Methods* **2020**, *17*, 93.

(7)   Potvin-Trottier, L.; Lord, N. D.; Vinnicombe, G.; Paulsson, J. Synchronous long-term oscillations in a synthetic gene circuit. *Nature* **2016**, *538*, 514.

(8)   Skinner, S. O.; Sepulveda, L. A.; Xu, H.; Golding, I. Measuring mRNA copy number in individual Escherichia coli cells using single-molecule fluorescent in situ hybridization. *Nat Protoc* **2013**, *8*, 1100.

(9)   Jones, D. L.; Leroy, P.; Unoson, C.; Fange, D.; Curic, V.; Lawson, M. J.; Elf, J. Kinetics of dCas9 target search in Escherichia coli. *Science* **2017**, *357*, 1420.

(10)   Andersen, J. B.; Sternberg, C.; Poulsen, L. K.; Bjorn, S. P.; Givskov, M.; Molin, S. New unstable variants of green fluorescent protein for studies of transient gene expression in bacteria. *Appl Environ Microbiol* **1998**, *64*, 2240.

(11)   Chen, Y. J.; Liu, P.; Nielsen, A. A.; Brophy, J. A.; Clancy, K.; Peterson, T.; Voigt, C. A. Characterization of 582 natural and synthetic terminators and quantification of their design constraints. *Nat Methods* **2013**, *10*, 659.

(12)   Lou, C.; Stanton, B.; Chen, Y. J.; Munsky, B.; Voigt, C. A. Ribozyme-based insulator parts buffer synthetic circuits from genetic context. *Nat Biotechnol* **2012**, *30*, 1137.