Supporting Information

Plasmids, Parts, and Strains

The *E. coli* strain S17-1 was purchased from ATCC, and the recipient *E. coli* strain, an MJ1655 strain with chromosomal insertion of sfGFP and mRFP, was obtained from Lei S. Qi[1]. *E. coli* strain Top10 (Life Technologies) was used for cloning purposes.

Plasmid pARO190 was purchased from ATCC. Two additional plasmids, pdCas9_bacteria containing *S. pyogenes* dCas9 protein-coding gene under control of an anhydrotetracycline (aTc)-inducible promoter (pLTetO-1), and pgRNA_RFP containing sgRNA placed under a constitutive promoter (iGEM Parts Registry BBa_J23119), were obtained from Lei S. Qi[1].

E. coli strains transformed with plasmids pARO190, pdCas9_bacteria, and pgRNA_RFP, were maintained in 100 ug/mL carbenicillin, 34 ug/mL chloramphenicol, and 100 ug/mL carbenicillin, respectively. Recipient *E. coli* strain was routinely maintained in 50 μg/mL kanamycin, and strain S17-1 was routinely maintained in 100 μg/mL spectinomycin.
All *E. coli* strains were cultured in LB (Luria-Bertani) broth in culture tubes at 37 °C with 300 rpm shaking speed unless otherwise specified.

Supplemental Table 1: Primer Sequences

No.	Name	Sequence
	Amplicon(+overhang)-direction	overhangsequenceAMPLICONSEQUENCE
1	dCas9(+pARO190)-forward	tgcatgcctgcaggtcgactTTAAGACCCACTTTCACATTTAAGT
2	dCas9(+pARO190)-reverse	ggtacccggggatcctctagTATAAACGCAGAAAGGCCCA
3	dCas9(+sgRNA)-reverse	aggactgagctagctgtcaaTATAAACGCAGAAAGGCCCA
4	sgRNA(+dCas9)-forward	tgggcctttctgcgtttataTTGACAGCTAGCTCAGTCC
5	sgRNA(+pARO190)-reverse	ggtacccggggatcctctaGAAAAAAAGCACCGACTCGG
6	pARO190(+sgRNA)-forward	accgagtcggtgctttttttCTAGAGGATCCCCGGGTA
7	pARO190(+dCas9)-forward	tgggcctttctgcgtttataCTAGAGGATCCCCGGGT
8	pARO190(+dCas9)-reverse	aatgtgaaagtgggtcttaaAGTCGACCTGCAGGCA

Supplementary Methods

Plasmid Construction

To construct plasmid dCas9-pARO190, DNA fragments of dCas9 and pARO190 backbone were amplified by PCR from plasmids pdCas9_bacteria and pARO190, respectively, with a 20 bp overhang for each fragment. Primers 1 and 2 were used to amplify dCas9, and primers 7 and 8 were used to amplify pARO190 backbone. GeneArt® Seamless Cloning and Assembly Enzyme Mix (Life Technologies) was then used for assembly of the two fragments.

To construct plasmid dCas9-sgRNA-pARO190, DNA fragments of dCas9, sgRNA and pARO190 backbone were amplified by PCR from plasmids pdCas9_bacteria, pgRNA_RFP, and pARO190, respectively, with a 20 bp overhang for each fragment. Primers 1 and 3 were used to amplify dCas9, 4 and 5 for sgRNA, and 6 and 8 for pARO190 backbone. GeneArt® Seamless Cloning and Assembly Enzyme Mix (Life Technologies) was then used for assembly of the three fragments.

Primers were chemically synthesized at Integrated DNA Technologies Inc, and PCR amplification was performed with Phusion High-Fidelity DNA Polymerase (New England Biolabs). Chemical transformation was used for cloning purposes, and electroporation was used to transform plasmids into *E. coli* strain S17-1.

Conjugation

Overnight cultures of donor and recipient strains were washed three times by centrifuging at 3000 rpm for 2 minutes and then resuspending in fresh LB broth without antibiotics. The resuspended cultures were diluted and co-cultured into 10 mL fresh LB broth without antibiotics, to OD_{600} of 0.05 for both strains in the co-culture. The co-culture was then incubated at 37°C with 100 rpm shaking speed to allow for conjugation. After a specific time, the co-culture was vigorously mixed on a vortex mixer to stop conjugation and separate connecting donor and recipient cells.

Calculation of Conjugation Efficiency

S17-1 strains transformed with dCas9-sgRNA-pARO190 served as donor strains and the reporter strains served as recipients. After conjugation, co-cultures were plated on LB agar plates containing antibiotics of spectinomycin, kanamycin, and both carbenicillin and kanamycin, for the selection of donor, recipient, and trans-conjugant strains respectively. Serial dilutions (10^3, 10^4, 10^5, 10^6 fold) of the co-cultures were made prior to plating, and 200 μl of each dilutions were plated. After overnight incubation, colonies on each plate were counted, and the results were presented as colony forming units per milliliter culture (cfu/ml). Conjugation efficiencies were calculated by dividing counting results of trans-conjugant cells to that of recipient cells. Time points of 2, 5, and 8 hours of conjugation were tested for estimation of optimal conditions.

Supplemental Table 2: Conjugation Efficiency

		2h			5h			8h		
		pARO190	dCas9-pARO190	sgRNA-dCas9-pARO190	pARO190	dCas9-pARO190	sgRNA-dCas9-pARO190	pARO190	dCas9-pARO190	sgRNA-dCas9-pARO190
cfu/ml	Donor	1.66E+06	2.50E+06	2.22E+06	1.80E+07	1.85E+07	7.50E+06	5.60E+07	7.00E+07	4.20E+07
	Recipient	2.69E+07	2.42E+07	2.77E+07	3.39E+08	7.40E+07	2.19E+08	6.48E+08	8.49E+08	5.18E+08
	Transconjugant	1.28E+05	1.83E+05	1.25E+05	1.00E+06	9.80E+05	5.25E+05	2.34E+06	1.14E+06	2.30E+06
Efficiency	%	**0.47**	**0.76**	**0.45**	**0.29**	**1.32**	**0.24**	**0.36**	**0.13**	**0.44**

Induction and Flow Cytometry

The reporter strains were separately co-cultured with S17-1 strains transformed with plasmids dCas9-pARO190 and sgRNA-dCas9-pARO190 (conjugative plasmid) for 8 hours for conjugation. Co-cultures were then diluted 10^4 fold and plated on LB agar plates containing both carbenicillin and chloramphenicol to select for trans-conjugants. After overnight incubation, liquid cultures of trans-conjugant colonies were made and grown overnight for saturation. The overnight cultures were then diluted to OD 0.05 into fresh carbenicillin and chloramphenicol containing LB broth in 2 ml 96-well deep well plates in duplicate, with 10 μg/mL aTc supplemented to one replicate of each conjugation to induce production of the dCas9 protein. Cultures were incubated at 37°C with shaking (1200 rpm) for 8 hours, washed and resuspended in PBS, and run on a LSRII flow cytometer (BD Biosciences) equipped with a high-throughput sampler to determine the levels of fluorescent proteins. Recipient strains without conjugation

were also in parallel tested and served as a negative control experiment. Data were analyzed by FlowJo 7.6.1, and plotted using GraphPad Prism 6. For each experiment, triplicate cultures were measured, and their standard deviation was indicated as error bar.

Microscopy

Trans-conjugants were cultured and induced as above for flow cytometry analysis. After 8 hours induction, 5μl of cells were spotted on glass slides under a coverslip and edges sealed with a PAP Pen (Fisher Scientific). Images were acquired in mRFP and sfGFP channels with 5ms exposure on an Olympus BX51 fluorescence microscope with 60x PlanApoN 1.42 NA oil objective equipped with a Hamamatsu Orca-ER CCD camera. Images were processed with ImageJ and presented with identical contrast settings.