

Figure S1. Mechanism of the Type II CRISPR System from *S. pyogenes*, Related to Figure 1

The system consists of a set of CRISPR-associated (Cas) proteins and a CRISPR locus that contains an array of repeat-spacer sequences. All repeats are the same and all spacers are different and complementary to the target DNA sequences. When the cell is infected by foreign DNA elements, the CRISPR locus will transcribe into a long precursor transcript, which will be cleaved into smaller fragments. The cleavage is mediated by a *trans*-acting antisense RNA (*tracrRNA*) and the host RNase III. After cleavage, one single protein, Cas9, recognizes and binds to the cleaved form of the crRNA. Cas9 guides crRNA to DNA and scans the DNA molecule. The complex is stabilized by basepairing between the crRNA and the DNA target. In this case, Cas9 causes double-stranded DNA breaks due to its nuclease activity. This usually removes cognate DNA molecules, and cells confer immunity to certain DNA populations.

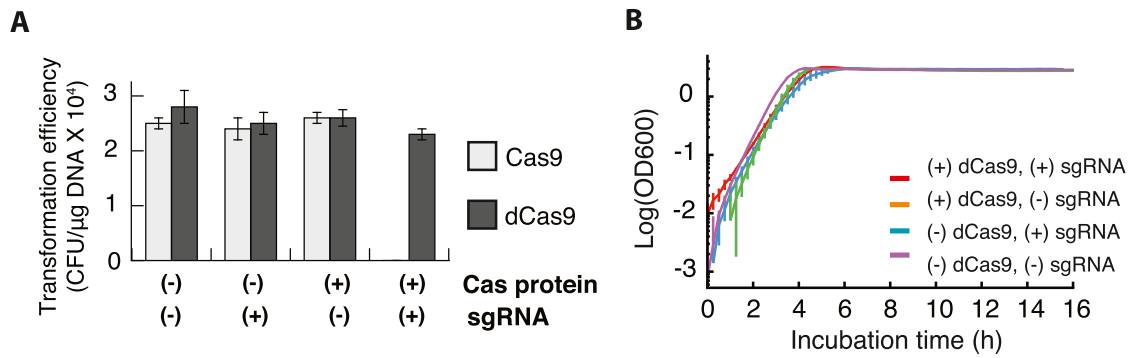


Figure S2. Growth of *E. coli* Cell Cultures Cotransformed with dCas9 and sgRNA, Related to Figure 2

(A) Transformation efficiency for transforming *E. coli* cells with two plasmids. One plasmid contains an sgRNA that targets to a genomic copy of mRFP and the other plasmid contains the wild-type Cas9 (light) or dCas9 (darker). Cotransformation of wild-type Cas9 and sgRNA is highly toxic, which can be alleviated using dCas9.

(B) The sgRNA (NT1) is designed to target the coding sequence of mRFP. Coexpression of dCas9 and sgRNA exhibits almost no effects on cellular growth rates, suggesting the dCas9-sgRNA interaction with DNA is strong enough to block RNA polymerase but not DNA polymerase or cell replication. The results represent average and SEM of at least three independent experiments.

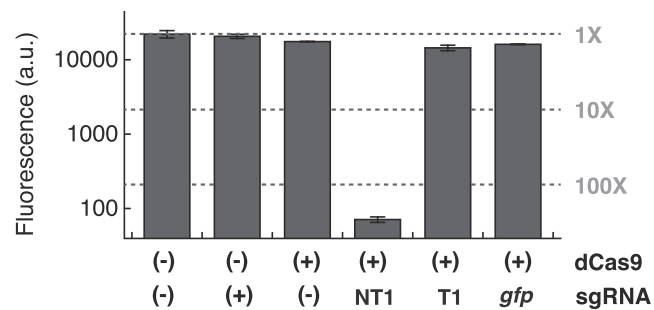


Figure S3. CRISPRi Could Silence Expression of a Reporter Gene on a Multiple-Copy Plasmid, Related to Figure 2C

The mRFP gene was cloned onto a p15A plasmid. Presence of the dCas9 and an mRFP-specific sgRNA (NT1) strongly represses mRFP (~300-fold). The repression effect is similar to that observed using the mRFP in the genome (Figure 2C). Silencing is only effective when the sgRNA acts on the non-template DNA strand but not the template DNA strand (T1). Also, silencing is highly specific, as a GFP-specific sgRNA (*gfp*) shows no effect on mRFP expression. Fluorescence results represent average and SEM of at least three biological replicates.

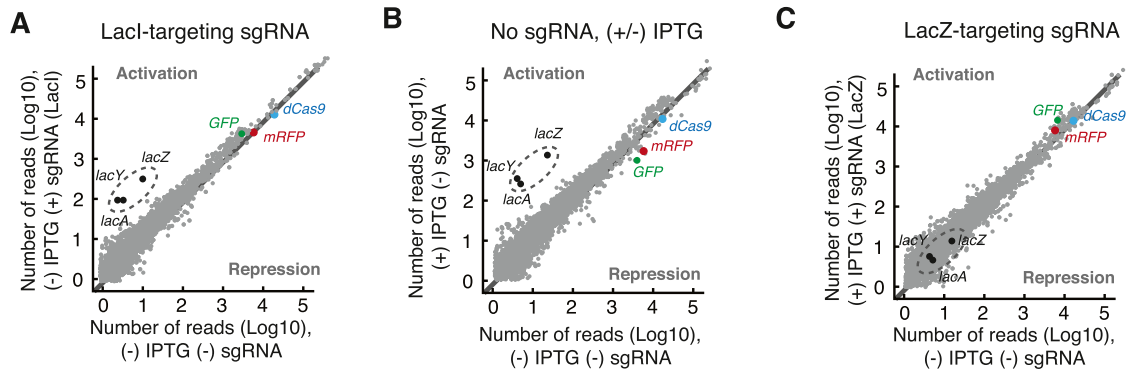


Figure S4. The RNA-Seq Data of Cells with sgRNAs that Target Different Genes, Related to Figure 4A

(A) (+/-) sgRNA that targets the promoter of the endogenous *lacI* gene in *E. coli*. The same *lacI*-targeting sgRNA is used as in Figure 6A.

(B) (+/-) 1 mM IPTG for cells with auto-inhibited sgRNA (sgRNA represses its own promoter).

(C) (+/-) sgRNA that targets the endogenous *lacZ* gene in *E. coli*. The same *lacZ*-targeting sgRNA is used as in Figure 6A. 1 mM IPTG is also supplemented to cells with the *lacZ*-targeting sgRNA.

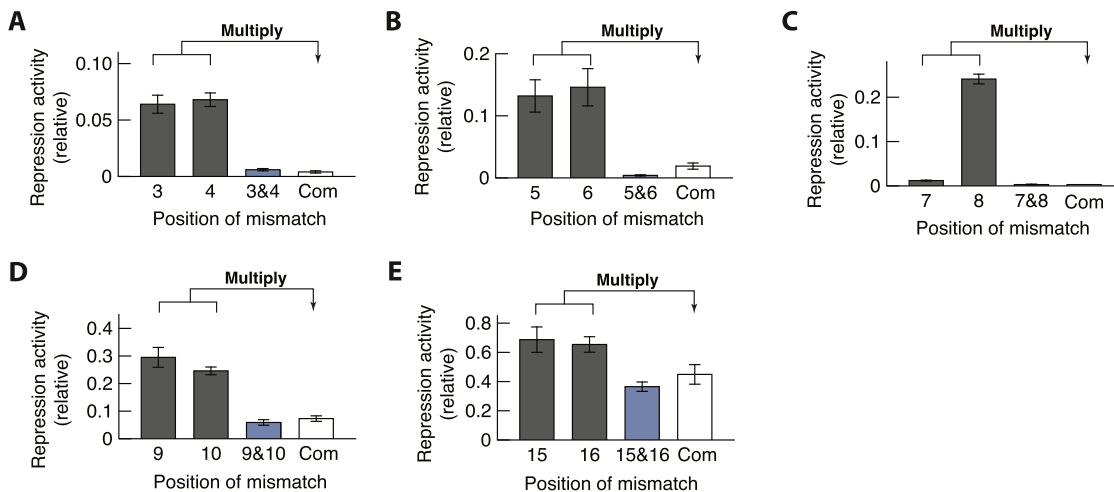


Figure S5. Silencing Effects of sgRNAs with Adjacent Double Mismatches, Related to Figure 5E

The relative repression activity of single-mismatched sgRNAs is shown in gray with the mismatch position labeled on the bottom. Experimentally measured activity of double-mismatched sgRNAs is shown in blue. Activity calculated by multiplying the effects of two single-mismatched sgRNAs is shown in white and labeled with "Com." Fluorescence results represent average and SEM of three biological replicates.

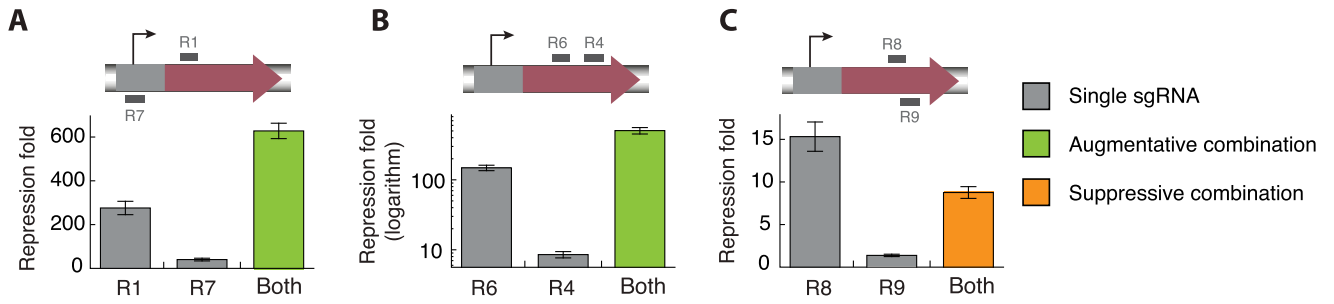


Figure S6. Combinatorial Silencing Effects of Using Two sgRNAs to Regulate a Single Gene, Related to Figure 5F

In all cases, nonoverlapping sgRNAs show augmentative silencing effects, and overlapping sgRNAs show suppressive effects. The combinatorial effect is independent of whether the sgRNA is targeting the template or nontemplate DNA strands. Fluorescence results represent average and SEM of three biological replicates.

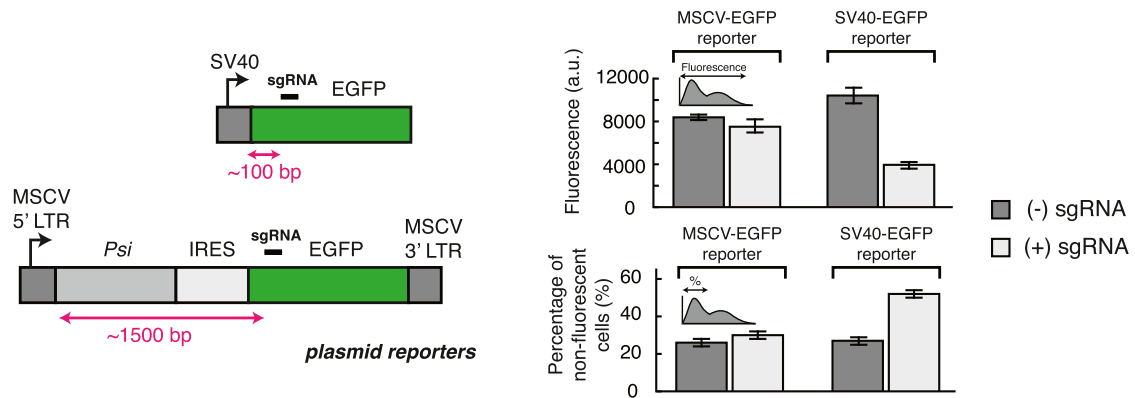


Figure S7. sgRNA Repression Is Dependent on the Target Loci and Relative Distance from the Transcription Start, Related to Figure 7

The same sgRNA is used to repress the same EGFP gene with different promoters. Cas9/sgRNA complexes repress transcription from transiently transfected plasmid DNA. The level of transcriptional repression is slightly better (63%) than that observed for genomic genes, and the percentage of GFP-negative cells increases in the presence of sgRNA. The target locus has different distance from the transcription start. While SV40-EGFP shows repression, LTR-EGFP has no effect. Fluorescence results represent average and error of two biological replicates.