

S4 Table. Plasmids used in this study. S5 Table indicates genes expressed from pZE21_tetR.

| Plasmid | crRNA promoter, sequence (5'-3') | Notes | Refs | Purpose |
|----------------|--|--|-------------------------|--|
| pZE21_tetR | n/a | Contains tetR behind pLac promoter for inducible expression of candidate Acrs. Targeted by crRNA_A; PAM = AGG. | (Forsberg et al., 2019) | Expressing genes to test <i>in vivo</i> anti-CRISPR activity |
| pSpyCas9_crA | pJ23100, GTTCATTCAGGGCAC CCGAC | Arabinose-inducible SpyCas9 with pZE21 targeting pZE21_tetR | (Forsberg et al., 2019) | Target pZE21_tetR for elimination with SpyCas9 |
| pSpyCas9_crMu | pJ23100, GTAATACTTGTCCCGC AAAG | Mu-targeting spacer for phage Mu immunity testing. Otherwise identical to pSpyCas9_crA | (Forsberg et al., 2019) | Phage Mu immunity testing |
| pSpyCa9_crNT | pJ23100, GAACGAAAAGCTGCG CCGGG | non-targeting spacer used as control. Otherwise identical to pSpyCas9_crA | (Forsberg et al., 2019) | Phage Mu immunity testing, Western blots |
| pCloDF13_GFP | pJ23100, GAACGAAAAGCTGCG CCGGG | <i>eGFP</i> gene replaces <i>spyCas9</i> in pSpyCas9_crA | | Measure generic protein expression from pSpyCas9 expression vector |
| pIDTsmart | n/a | Plasmid used for <i>in-vitro</i> SpyCas9 digestion. Sequence available at: https://www.idtdna.com/pages/products/genes-and-gene-fragments/custom-gene-synthesis | | Plasmid template for <i>in-vitro</i> nuclease reactions |
| pET15b/HE | n/a | Novagen Cat. No. 69661-3; pET15 variants 'b' and 'HE' differ only by a few bases upstream of the N-terminal thrombin cut site | | Protein purification |
| pSpyCas9_Fig3C | n/a | J23100 promoter expressing a theophylline inducible SpyCas9, used in Fig 3C | (Uribe et al., 2019) | Testing AcrIIA22 activity against a panel of Cas9 and Cas12 effector nucleases |
| pNmCas9_Fig3C | n/a | J23100 promoter expressing a theophylline inducible NmCas9, used in Fig 3C | (Uribe et al., 2019) | Testing AcrIIA22 activity against a panel of Cas9 and Cas12 effector nucleases |
| pLbCas12_Fig3C | n/a | J23100 promoter expressing a theophylline inducible LbCas12, used in Fig 3C | (Uribe et al., 2019) | Testing AcrIIA22 activity against a panel of Cas9 and Cas12 effector nucleases |
| pFnCas12_Fig3C | n/a | J23100 promoter expressing a theophylline inducible FnCas12, used in Fig 3C | (Uribe et al., 2019) | Testing AcrIIA22 activity against a panel of Cas9 and Cas12 effector nucleases |
| pDual4_Spy | P _{BAD} , GTTCATTCAGGGCAC CCGAC | Arabinose inducible gRNA for SpyCas9 targeting pZE21 | (Uribe et al., 2019) | Testing AcrIIA22 activity against a panel of Cas9 and Cas12 effector nucleases |
| pDual4_Nm | P _{BAD} , GAACACGGCGGCATC AGAGC | Arabinose inducible gRNA for NmCas9 targeting pZE21 | (Uribe et al., 2019) | Testing AcrIIA22 activity against a panel of Cas9 and Cas12 effector nucleases |
| pDual4_Lb | P _{BAD} , TCAAGACCGACCTGT CCGGTGCCCTGAATG | Arabinose inducible gRNA for LbCas12 targeting pZE21 | (Uribe et al., 2019) | Testing AcrIIA22 activity against a panel of Cas9 and Cas12 effector nucleases |
| pDual4_Fn | P _{BAD} , TCAAGACCGACCTGT CCGGTGCCCTGAATG | Arabinose inducible gRNA for FnCas12 targeting pZE21 | (Uribe et al., 2019) | Testing AcrIIA22 activity against a panel of Cas9 and Cas12 effector nucleases |

References for S4 Table.

Forsberg, K.J., Bhatt, I.V., Schmidtke, D.T., Javanmardi, K., Dillard, K.E., Stoddard, B.L., Finkelstein, I.J., Kaiser, B.K., and Malik, H.S. (2019). Functional metagenomics-guided discovery of potent Cas9 inhibitors in the human microbiome. *Elife* 8.

Uribe, R.V., van der Helm, E., Misiakou, M.A., Lee, S.W., Kol, S., and Sommer, M.O.A. (2019). Discovery and Characterization of Cas9 Inhibitors Disseminated across Seven Bacterial Phyla. *Cell Host Microbe* 25, 233-241 e235.