**Supplementary Table S1.**

|  |  |
| --- | --- |
| DNA oligo | 5’-3’ sequence |
| Bth\_wt\_nt | GGGATCCGATCGATTGCATGCAAACGGATTGTTATATAAATCTTCTTGAACATATCCATA |
| Bth\_wt\_t | TATGGATATGTTCAAGAAGATTTATATAACAATCCGTTTGCATGCAATCGATCGGATCCC |
| Bth\_noPAM\_nt | GGGATCCGATCGGCACCATGCAAACGGATTGTTATATAAATCTTCTTGAACATATCCATA |
| Bth\_noPAM\_t | TATGGATATGTTCAAGAAGATTTATATAACAATCCGTTTGCATGGTGCCGATCGGATCCC |
| Bth\_T3G\_nt | GGGATCCGATCGATTGCAGGCAAACGGATTGTTATATAAATCTTCTTGAACATATCCATA |
| Bth\_T3G\_t | TATGGATATGTTCAAGAAGATTTATATAACAATCCGTTTGCCTGCAATCGATCGGATCCC |
| Bth\_C9A\_nt | GGGATCCGATCGATTGCATGCAAAAGGATTGTTATATAAATCTTCTTGAACATATCCATA |
| Bth\_C9A\_t | TATGGATATGTTCAAGAAGATTTATATAACAATCCTTTTGCATGCAATCGATCGGATCCC |
| Bth\_G15A\_nt | GGGATCCGATCGATTGCATGCAAACGGATTATTATATAAATCTTCTTGAACATATCCATA |
| Bth\_G15A\_t | TATGGATATGTTCAAGAAGATTTATATAATAATCCGTTTGCATGCAATCGATCGGATCCC |
| Bth\_G15C\_nt | GGGATCCGATCGATTGCATGCAAACGGATTCTTATATAAATCTTCTTGAACATATCCATA |
| Bth\_G15C\_t | TATGGATATGTTCAAGAAGATTTATATAAGAATCCGTTTGCATGCAATCGATCGGATCCC |
| Bth\_G15T\_nt | GGGATCCGATCGATTGCATGCAAACGGATTTTTATATAAATCTTCTTGAACATATCCATA |
| Bth\_G15T\_t | TATGGATATGTTCAAGAAGATTTATATAAAAATCCGTTTGCATGCAATCGATCGGATCCC |
| Bth\_sgRNA | TAATACGACTCACTATAGGCAGGACAACCGTCTAGCTATAAGTGCTGCAGGGGTGTGAG  AAACTCCTATTGCTGGACGATGTCTCTAAGCGAGGCATTAGCACCATGCAAACGGATTGTTAT |
| T7prom\_mod | TAATACGACTCACTATAGG |
| Bth\_sgRNA\_rev | ATAACAATCCGTTTGCATGGTGC |

**Supplementary Figure S1 (Related to Figure 2A).** **Determining the effects of substitutions introducing single-nucleotide mismatches between crRNA spacer and plasmid protospacer on interference by *B. thermoamilovorans* CRISPR-Cas system.** Results of analysis of two additional replicates of library experiment are shown.

