Supporting Information

Engineering of Bacteriophage T4 Genome Using CRISPR-Cas9

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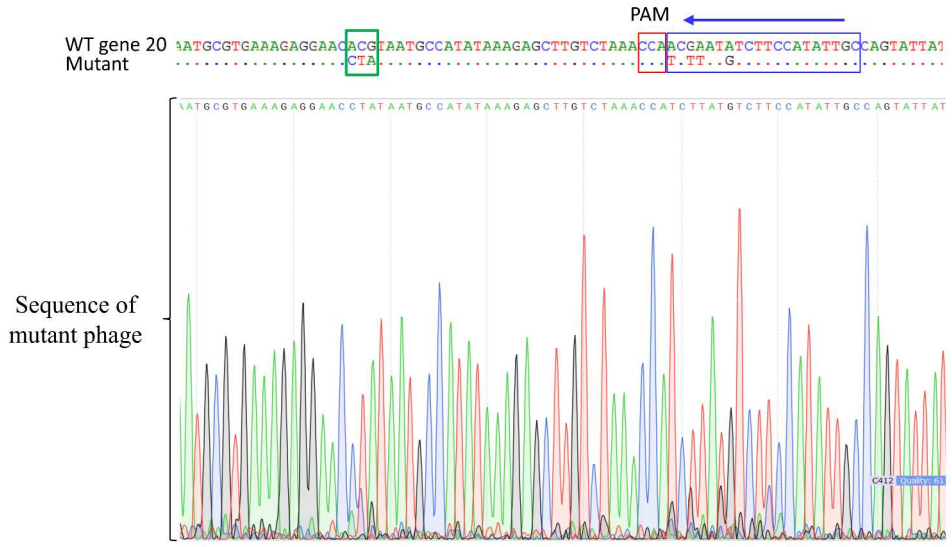

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Figure S1. Generation of ag20 amber mutant by CRISPR-Cas editing. *E. coli* B40 containing the spacer 20-1070 (Figure 2 and Table 1 in text) was infected with 500 PFU WT T4 according to the basic scheme shown in Figure 1 of the text. Plaques generated were purified, amplified by PCR, and sequenced. Sequencing shows the presence of amber mutation in the edited plaque. The blue arrow shows the 5' to 3' direction of the spacer sequence marked with a blue box. The PAM sequence and amber mutation are marked with red and green boxes, respectively.

Supplementary Table 1. Primers used for donor DNA constructions

Primers	Sequence (5' to 3')
23FW	TCAT AGATCT cacaaatgactatcaaaactaaagc
23BW	CTA GGATCC cgttagagattagatacctttaac
23am FW	gctccatatgtagctctgactccgctgcgtggttccgatccg aagaacttcTaGccAgtGatgggattc
segD BW	ACT CTCGAG aatcatatacaaacggagtgatg
50Bgl FW:	ATCG AGATCT ctccatatgtagctctgactcc
100Bgl FW:	ATCG AGATCT cactgtaggttataaaggtccg
200Bgl FW:	ATCG AGATCT ctaccggctttagcactgatac
500Bgl FW:	ATCG AGATCT aaatcaaccgtgaagttgttg
50Xho BW:	GTG CTCGAG cggattctgcaaatgggttg
100Xho BW:	GTG CTCGAG cagaatagaaggcataccgc
200Xho BFW:	GTG CTCGAG aagggaacccgaaggttccc
500Xho BW:	GTG CTCGAG caaaggcaaaaacaggcgt
rnlB FW	GTG CTCGAG cctttcggtccctttttatttaac
rnlB BW	gtttaaaaagtatagcagtcttg
rnlB amDSFW	agc gaattc gGA aaa Tac CtA ataagatacaaccgctgag
24.3 Xba BW	GTT TCTAGA gtatataatggcaccaactcg
24.2EcoR FW	TCC gaattc TTA TTA gtgttttaccttgttcagctgc
HocXba BW	GTT TCTAGA ggctaatgttacgggtgctc