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# Supplemental Material to:

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# Efficient engineering of a bacteriophage genome using the type I-E CRISPR-Cas system

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# SUPPLEMENTARY INFORMATION

## Plasmid Construction

Plasmids pAnti-1.7 (Table S1) and pAnti-4.3 (Table S1) encode spacers against genes 1.7 and 4.3 of bacteriophage T7, respectively, in addition to other non-relevant spacers (spacers EcoRI, K12 sp3, BamHI, K12 sp5, NsiI, and K12 sp7 on plasmid pWUR477[1]). The plasmids were constructed by PCR amplification of pWUR477 (Table S1) using primers DS24F + DS24R for pAnti-1.7, and primers RK77F + RK77R for pAnti-4.3. The amplified DNAs were phosphorylated and self-ligated to yield pAnti-1.7 and pAnti-4.3. Plasmids pBAD-$1.7_{60}$ and pUC-$4.3_{60}$ (Table S1) encode 60 bp of the sequences flanking genes 1.7 and 4.3, respectively. These two plasmids were constructed by PCR amplification of pBAD283 using primers DS20F + RK76R for pBAD-$1.7_{60}$ and pUC19 using primers RK82F + RK82R for pUC-$4.3_{60}$. The amplified DNAs were phosphorylated and self-ligated to yield pBAD-$1.7_{60}$ and pUC-$4.3_{60}$.

## Preparation of Recombinant Lysate

*Escherichia coli* NEB5α harboring pBAD-$1.7_{60}$, pBAD18 (control), pUC-$4.3_{60}$, or pUC19 (control) plasmids were aerated overnight in LB supplemented with 100 μg/ml ampicillin at 37 °C. Each overnight culture was diluted in fresh LB supplemented with 100 μg/ml ampicillin at 37 °C and aerated until the $OD_{600}$ reached 1. The culture was then infected with the WT-T7 phage at a multiplicity of infection of 0.1. The infected bacteria were aerated at 37 °C until the culture became clear, which was indicative of a successful phage infection.

## Selection of Recombinant Phage

Phages were plated on BL21AI *E. coli*, with all bacteria harboring plasmids encoding *cas3* and *cascade* as well as different spacers. *E. coli* strains used were DS7045, a strain targeting a protospacer encoded on gene 1.7 (Table S1); RK6617, a strain targeting a protospacer encoded on gene 4.3 (Table S1); and RK6161, a strain encoding control spacers (Table S1). Bacteria were aerated overnight in LB supplemented with 50 μg/ml

kanamycin, 35 μg/ml chloramphenicol, and 50 μg/ml streptomycin. Each overnight culture was diluted in fresh LB, supplemented with 25 μg/ml kanamycin, 17.5 μg/ml chloramphenicol, and 25 μg/ml streptomycin at 37 °C and aerated until the $OD_{600}$ reached 1. Thereafter 0.2% L-arabinose and 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) were added, and the cells were aerated at 37 °C for an additional hour. A 100 μl suspension of induced bacteria, 10 μl of undiluted phage lysate, and $10^{-1}$ and $10^{-2}$ dilutions of the phage lysate in LB medium supplemented with 0.7% agar, 0.2% L-arabinose, and 0.1 mM IPTG were plated onto LB plates, supplemented with 50 μg/ml kanamycin, 35 μg/ml chloramphenicol, and 50 μg/ml streptomycin, and incubated overnight at room temperature. Plaques were then counted, and their size measured. Efficiency of plating was calculated by dividing the number of plaques obtained on *E. coli* encoding targeting spacers by the plaques obtained on *E. coli* encoding control spacers. Plaques larger than 0.4 cm growing on the targeting bacteria lawns were picked. The plaques served as a DNA template for a PCR using primers 101F + 67R (Table S1) for determining gene 1.7 deletion and primers 114F + RK58R (Table S1) for determining gene 4.3 deletion. Plaques were streaked three times on the induced targeting bacteria for isolation, and deletions were confirmed by DNA sequencing.

## Table S1. Bacterial strains, phages, plasmids and oligonucleotides used in this study

| Bacteria/plasmids/ phages/ oligonucleotides | Description/sequence | Source or reference |
|---|---|---|
| **Bacterial strains** | | |
| BL21-AI | $F^-$ *ompT hsdSB*($rB^-$, $mB^-$) *gal dcm araB*::*T7RNAP-tetA*, $tet^r$ | Invitrogen |
| NEB5α | $F^-$, ϕ80*lacZ*ΔM15Δ*(lacZYA-argF)*, U169, *deoR*, *recA1*, *endA1*, *hsdR17* ($r_k^-$, $m_k^+$), *gal*$^-$, *phoA*, *supE44*, λ$^-$, *thi-1*, *gyrA96*, *relA1* | New England Biolabs |
| RK6161 | BL21AI/pWUR397/pWUR400/pWUR477 | [1] |
| DS7045 | BL21AI/pWUR397/pWUR400/pAnti-1.7 | This study |
| RK6617 | BL21AI/pWUR397/pWUR400/pAnti-4.3 | This study |
| **Plasmids** | | |
| pWUR397 | cas3 under T7 promoter, $Kan^R$ | [1] |
| pWUR400 | cascade genes under T7 promoter, $Str^R$ | [1] |
| pWUR477 | pACYCDuet-1 (Novagen) cloned with control spacers under T7 promoter, $cam^r$ | [1] |
| pAnti-1.7 | pWUR477 cloned with anti-1.7 spacer | This study |
| pAnti-4.3 | pWUR477 cloned with anti-4.3 spacer | This study |
| pBAD18 | Arabinose-inducible expression vector, $amp^r$ | [2] |
| pBAD283 | pBAD18 cloned with T7 gp1.7 ORF | Laboratory collection |
| pUC19 | Cloning vector, $ampicillin^r$ | [3] |
| pBAD-$1.7_{60}$ | pBAD18 cloned with a segment composed of 60 bp flanking gene 1.7 from each side | This study |
| pUC-$4.3_{60}$ | puC19 cloned with a segment composed of 60 bp flanking gene 4.3 from each side | This study |
| **Phages** | | |
| WT-T7 | Wild-type bacteriophage T7 | Laboratory collection |
| T7Δ1.7 | Bacteriophage T7 deleted in 1.7 gene | This study |
| T7Δ4.3 | Bacteriophage T7 deleted in 4.3 gene | This study |
| **Oligonucleotides** | **5′→3′** | |
| DS20F | CGAAAGCAGACTTCTATAAA | |
| DS24F | TCCGAGCGATGGACCCGAGTTCCCCGCGCCAGCGGGGATA AACCGCAGCCGAAGCCAAAGAATTC | |
| DS24R | TTTCACCTAAGGTTATCGGTTTATCCCCGCTGGCGCGGGGA ACTCTCTAAAAGTATACATTTGTT | |

| | |
|---|---|
| RK58R | TAAGCAGCTGTGCTTGGATAACTTTAGGGTCACGCATGGT<br>AATATCTCCTATAGTGAGTCGAATAGTATTCATGAAGGCC |
| RK76R | TGGCGGGTTTTCTTTTTCCC |
| RK77F | TGGTTCGTATGTACAAGAGTTCCCCGCGCCAGCGGGGATA<br>AACCGCAGCCGAAGCCAAAGAATTC |
| RK77R | GCAGTTGGCCTAACTTCGGTTTATCCCCGCTGGCGCGGGG<br>AACTCTCTAAAAGTATACATTTGTT |
| RK82F | TCAAACAGGAGAAACCATTATGTCTAACGTAGCTGAAACT<br>ATCCGTCTATCCGATACAGCCGCGGGGAGAGGCGGTTTGC |
| RK82R | GGTGTGTCTCCTTTAGTGAGTCGTATTAGAATGGGACTCTC<br>CAGCGAAACTTCTCGTAGTCGTTGGCCGATTCATTAATG |
| 67R | GCACCACTCTGAGCAAGATG |
| 101F | TTCCTTTGGCACCGAGCGTC |
| 114F | CGGATGGCTTGAACCATCAA |

**Table S2. Mutations in phages selected on hosts encoding anti gene 1.7 spacers**

| Phage isolate[a] | Mutation[b] |
|---|---|
| 1 | Δ8403-8620 |
| 2 | **Δ8211-8697** |
| 3 | **Δ8211-8697** |
| 4 | Δ8403-8620 |
| 5 | Δ8403-8620 |
| 6 | **Δ8211-8697** |
| 7 | **Δ8211-8697** |
| 8 | **Δ8211-8697** |

[a] Random large (>4 mm) T7 phage plaques isolated from a single representative experiment

[b] Gene 1.7 coordinates: 8166-8756. Protospacer coordinates: 8423-8455 (NCBI reference sequence NC_001604.1); Bolded coordinates represent the specifically engineered recombinants

## References

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2. Guzman LM, Belin D, Carson MJ, Beckwith J. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. J Bacteriol 1995; 177:4121-30.
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