

Supplementary Table 1. Plasmids used in the study

Plasmid	Description	Source
pKDsgRNA-ack	Encodes λ Red system under the control of <i>araBAD</i> promoter and gRNA under thr control of <i>tet</i> promoter, <i>Str^R</i>	[1]
pKDsgRNA_(1-6)	pKDsgRNA with default gRNA substituted with gRNA_(1-6), <i>Str^R</i>	This work
pCas9	Provides constitutive expressoin of <i>Streptococcus pyogenes</i> Cas9, <i>Cf^R</i>	[2]
pBAD/His B	Empty vector, was used for the cloning of recombination templates, <i>Amp^R</i>	[3]
pBAD_T7_BREX_(insert)	A series of pBAD vectors carrying a 600 bp T7 gDNA inserts with mutations or inversions of native BREX sites at positions 4527 or 7165 or novel BREX site in forward or reverse orientation at position 537, <i>Amp^R</i>	This work
pBAD_T7_ <i>gp10B::EYFP</i>	pBAD with an insert of the 3' 155 bp of the <i>gp10B</i> fused with <i>eyfp</i> an followed by 155 bp downstream of the <i>gp10B</i> , <i>Amp^R</i>	This work
pBAD_T5_EcoRV	pBAD with 600 bp T5 gDNA insert carrying a novel EcoRV site at position 6153, <i>Amp^R</i>	This work
pBAD_T3_ $\Delta 0.3$	pBAD with 180 bp 5' and 200 bp 3' regions of T3 gDNA flanking <i>0.3</i> gene, <i>Amp^R</i>	This work

1. Reisch, C. R., and Prather, K. L. J. (2015) The no-SCAR (Scarless Cas9 Assisted Recombineering) system for genome editing in *Escherichia coli*, *Sci. Rep.*, **5**, 1–12.
2. Jiang, W., Bikard, D., Cox, D., Zhang, F., and Marraffini, L. A. (2013) RNA-guided editing of bacterial genomes using CRISPR-Cas systems, *Nat. Biotechnol.*, **31**, 233–239.
3. Guzman, L.-M., Belin, D., Carson, M. J., and Beckwith, J. O. N. (1995) Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter, *J. Bacteriol.*, **177**, 4121–4130.

Supplementary Table 2. Primers used in the study

Primer	Sequence	Purpose
S_G453A_F	CTGGCGAAAGGTAAACCAAT GTTTTAGAGCTAGAAATAGC	Cloning of gRNA_1
S_G453A_R	ATTGGTTTACCTTTCGCCAGG TGCTCAGTATCTCTATCAC	Cloning of gRNA_1
S_check_F	GAAAGGTAAACCAAT	Screening of gRNA_1 insert
S_BREX_for_F	CAATCGACACGTTTTAGAGC TAGAAATAGC	Cloning of gRNA_2
S_BREX_for_R	GGATGGCTATGTGCTCAGTA TCTCTATC	Cloning of gRNA_2
BREX_for_check_F	ATAGCCATCCCAATCG	Screening of gRNA_2 insert
S_G231A_F	TCTATAAGCGTTTTAGAGC TAGAAATAGC	Cloning of gRNA_3
S_G231A_R	TACACATCGGGTGCTCAGTA TCTCTATC	Cloning of gRNA_3
S_G231A_check_F	CCGATGTGTATCTATAAGCG	Screening of gRNA_3 insert
pkd_for	CCAATTGTCCATATTGCATCA	Cloning of gRNA_4
pkd_gp10B_rev	CCGCTGAGCAATAACTAGCA GTGCTCAGTATCTCTATCACT GA	Cloning of gRNA_4
pkd_gp10B_for	TGCTAGTTATTGCTCAGCGG GTTTTAGAGCTAGAAATAGC AAG	Cloning of gRNA_4
pkd_rev	TTTATAACCTCCTTAGAGCTC GA	Cloning of gRNA_4
EcoRV_FST_F	ATTAACCTTGACACAATAGGA GTTTTAGAGCTAGAAATAGC	Cloning of gRNA_5
EcoRV_FST_R	TCCTATTGTGTCAAGTTAATG TGCTCAGTATCTCTATCAC	Cloning of gRNA_5
EcoRV_FST_check_F	ATTAACCTTGACACAATAGGA	Screening of gRNA_5 insert
03_spc_F	ATACAAATCACAGACTCACA GTTTTAGAGCTAGAAATAGC	Cloning of gRNA_6
03_spc_R	TGTGAGTCTGTGATTTGTAT GTGCTCAGTATCTCTATCAC	Cloning of gRNA_6
T3d3_spc_check_F	ATACAAATCACAGACTCAC	Screening of gRNA_6 insert
S_seq_R	TCTGAGATGAGTTTTTGTTT	Sequencing of gRNA_(1-6) insert
S_seq_F	ATTTTGGTCATGAGATTATC	Sequencing of gRNA_(1-6) insert

pBAD_T7wt_F	TTTTGGGCTAACAGGAGGAA ATGAAACCGGAAGACATC	Cloning of wt T7 insert
pBAD_T7wt_R	CTTGCGGCCGCGAGCTCCAT TCACTAGGAAGCAAGTTAAC	Cloning of wt T7 insert
M_G453A_F	GTAACCAATCGCTAAGGAA	Mutation of the first BREX site
M_G453A_R	TTCCTTAGCGATTGGTTTAC	Mutation of the first BREX site
G453A_check_F	GAAAGGTAAACCAATCGC	Screening of insert
pBAD_rec_BREX_for_F	TTTTGGGCTAACAGGAGGAA CTAAAGACCCATCAAGTCAA	Introduction of the novel BREX site
pBAD_rec_BREX_for_F1R	CTTATCCGGTTGACCCCGCTT ACCGTGTCGATTGGGATGGC	Introduction of the novel BREX site
pBAD_rec_BREX_for_R	CTTGCGGCCGCGAGCTCCAT TAAAGAGCGTCTTAGGTC	Introduction of the novel BREX site
pBAD_rec_BREX_for_F2F	GGTAAGCGGGTCAACCGG ATAAG	Introduction of the novel BREX site
pBAD_rec_BREX_rev_F1R	CTTATCCGGTTGACCCGGG TAAGGTGTCGATTGGGATGG C	Introduction of the novel BREX site
pBAD_rec_BREX_rev_F2F	CTTACCCGGGGTCAACCGGA TAAG	Introduction of the novel BREX site
pBAD_rec_BREX_flip_F	TAAACCAATCCTTACCGAAG GTTACTACTGGCTGAAAATC CACGGTGC	Mutation of the first BREX site
pBAD_rec_BREX_flip_R	CCTTTCGCCAGCGTAAGC	Mutation of the first BREX site
pBAD_rec_G231A_F	TTTTGGGCTAACAGGAGGAA CAATCCGTAAGAAAGATAAA G	Mutation of the second BREX site
pBAD_rec_G231A_F1R	TTCCACCAGCCAGATTTCTTC GCGCGCTTATAGATACACA	Mutation of the second BREX site
pBAD_rec_G231A_R	CTTGCGGCCGCGAGCTCCAT CATGTAGCTAATTTGACAC	Mutation of the second BREX site
pBAD_rec_G231A_F2F	TGTGTATCTATAAGCGCGCG AAGAAATCTGGCTGGTGGAA	Mutation of the second BREX site
Recomb_gp10B_for	GGCTAACAGGAGGAATTAAC CATGGGCTGGGGGTGGCCTC AACG	Creation of the Gp10B-EYFP fusion
Recomb_gp10B_rev	TTGCTCAGCGGTGGCAGCA	Creation of the Gp10B-EYFP fusion
Recomb_EYFP_for	GGCTGCTGCCACCGCTGAGC AAGTGGTGAGCAAGGGCGA GGAGC	Creation of the Gp10B-EYFP fusion
Recomb_EYFP_rev	CCCGTTTAGAGGCCCAAGG GGGTGTTACTTGTACAGCTC GTCCATGCCG	Creation of the Gp10B-EYFP fusion

Recomb_T7_for	CCCCTTGGGGCCTCTAAACG	Creation of the Gp10B-EYFP fusion
Recomb_T7_rev	CCATATGGTACCAGCTGCAG ATCTCGAGTTCAGCGTTGA TACCGGAGGTTCC	Creation of the Gp10B-EYFP fusion
pBAD_rec_T5-1_F	CCGTTTTTTGGGCTAACAGG AGGAATCATTAGCAAGCGAA TAATC	Introduction of the novel EcoRV site
pBAD_rec_T5-1_R	CTATAAAGCCTTCCTATTGGA TATCTTAATTTACAAAATGTA TAAGGC	Introduction of the novel EcoRV site
pBAD_rec_T5-2_F	GATATCCAATAGGAAGGCTT TATAG	Introduction of the novel EcoRV site
pBAD_rec_T5-2_R	CCAAGCTTGCGGCCGCGAGC TCCATGTTAATCGGTGAGTG AATG	Introduction of the novel EcoRV site
pBAD_T3d03_5'_F	CTCCATACCCGTTTTTTGGGC TAACGAAACGACAGTGAGG CG	Deletion of the <i>0.3</i> gene
pBAD_T3d03_5'_R	CATGACCTTGAGTTTAACT TTGGTGTTACCTCATGTTAG	Deletion of the <i>0.3</i> gene
pBAD_T3d03_3'_F	CTAACATGAGGTAACACCAA AGTGTTAAACTCAAGGTCAT G	Deletion of the <i>0.3</i> gene
pBAD_T3d03_3'_R	CCAAGCTTGCGGCCGCGAGC TCCATAAACCGTGGGATGAA TCC	Deletion of the <i>0.3</i> gene
T7_1f_BREX_F	ATCCCAATCGACACGGTAAG	Screening of mutations in T7 genome
T7_1_BREX_R	CTTGCGATACCCTTGAGTTAT C	Screening of mutations in T7 genome
T7_1r_BREX_F	ATCCCAATCGACACCTTACC	Screening of mutations in T7 genome
T7_flip_BREX_F	AAGGTAAACCAATCCTTACC	Screening of mutations in T7 genome
pBAD_T7wt_R	TCACTAGGAAGCAAGTTAAC	Screening of mutations in T7 genome
T7_2_BREX_F	TGTGTATCTATAAGCGCGCG	Screening of mutations in T7 genome
T7_2_BREX_R	CATCAGGTGTTTCCTCCATG	Screening of mutations in T7 genome
T7_head_for	TGCTGGTGCAGTGGTT	Screening of mutations in T7 genome
T7_head_rev	ATTGAATCTGTCGGTTAATCT TGTT	Screening of mutations in T7 genome
gT5_EcoRV_check_F	CCTTATACATTTTGTAATTA AGATATC	Screening of mutations in T5 genome
T5_check3	CATGTTTTTCCGTGTTGTC	Screening of mutations in T5 genome

del0.3_check_F	TGACTTAAGCGCACCCACGGC AC	Screening of mutations in T3 genome
del0.3_check_R	CCAGACCGCGCTTGAAGTCT CG	Screening of mutations in T3 genome