

Table S1. Bacterial strains and plasmids used in this study.

Strains and Plasmids		Relevant characteristics		Reference or source	Accession no
Strains					
<i>E. coli</i>					
DH5α	<i>F- gyrA96 recA1 relA1 endA1 thi-1 hsdR17 glnV44 deoR D (lacZYA-argF) U169[f80dD(lacZ)M15]</i>			[1]	
sExpress	NEB Express (R702): <i>fhuA2 [lon] ompT gal sulA11 R(mcr-73::miniTn10--TetS)2 [dcm] R(zgb-210::Tn10--TetS) endA1 Δ(mcrC-mrr)114::IS10</i>			[2]	
<i>C. difficile</i>					
		Ribotype	Isolation date		
500/12	Clinical strain	176	2012	MUW ^a	JBCJLD000000000
CKH08	Derived from 500/12, Δ <i>phi027</i>			This work	
137/12	Clinical strain	027	2012	MUW	JBCJLK000000000
1974/12	Clinical strain	176	2012	MUW	JBCJLI000000000
2163/12	Clinical strain	027	2012	MUW	JBCJLH000000000
2282/12	Clinical strain	027	2012	MUW	JBCJLG000000000
3136/12	Clinical strain	176	2012	MUW	JBCJLF000000000
3290/12	Clinical strain	176	2012	MUW	JBCJLM000000000
700/13	Clinical strain	027	2013	MUW	JBCJLJ000000000
OR/03/B	Clinical strain	027	2022	MUW	JBCJLL000000000
KB/04/B	Clinical strain	027	2022	MUW	JBCJLE000000000
WL/14/B	Clinical strain	027	2022	MUW	JBCJLR000000000

SL/15/Ba	Clinical strain	027	2023	MUW	JBCJLN000000000
BM/25/D	Clinical strain	027	2022	MUG ^b	JBCJLO000000000
AA/01/By	Clinical strain	027	2022	MUS ^c	JBCJLQ000000000
SM/02/By	Clinical strain	027	2022	MUS	JBCJLP000000000

Plasmids

pUC19	<i>ori(pMB1) lacZ Amp^R</i>			[3]	
pEcCdH01	Derived from pUC19, <i>sRNAP::crRNA</i> (23-nt repeat), PCR template for generating <i>sRNAP::crRNA</i> for retargeting			This work	
pWH34	Derived from pMTL82151, <i>E. coli</i> - <i>C. difficile</i> shuttle vector, <i>iLacP::AsCpf1</i> , BtgZI-BtgZI double sites, “Chassis” plasmid for gene-targeting plasmid construction, Cm ^R /Tm ^R			[4]	
pEcCdH07	Derived from pWH34, targeting <i>phi027</i> with one spacer, Cm ^R /Tm ^R			This work	
pEcCdH08	Derived from pWH34, targeting <i>phi027</i> with two sets of spacers, Cm ^R /Tm ^R			This work	

^aUniversity Clinical Center, Medical University of Warsaw, Poland

^bUniversity Clinical Center, Medical University of Gdańsk, Poland

^cPulmonology and Thoracic Surgery Center in Bystra, Medical University of Silesia, Poland

References

- [1] Woodcock DM, Crowther PJ, Doherty J, Jefferson S, DeCruz E, Noyer-Weidner M, Smith SS, Michael MZ, Graham MW. Quantitative evaluation of *Escherichia coli* host strains for tolerance to cytosine methylation in plasmid and phage recombinants. *Nucleic Acids Res.* 1989, 17:3469-78.
- [2] Woods C, Humphreys CM, Rodrigues RM, Ingle P, Rowe P, Henstra AM, Köpke M, Simpson SD, Winzer K, Minton NP. A novel conjugal donor strain for improved DNA transfer into *Clostridium* spp. *Anaerobe* 2019, 59:184-191.
- [3] Yanisch-Perron C, Vieira J, Messing J. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* 1985, 33:103-19.

- [4] Hong W, Zhang J, Cui G, Wang L, Wang Y. Multiplexed CRISPR-Cpf1-Mediated Genome Editing in *Clostridium difficile* toward the Understanding of Pathogenesis of C. difficile Infection. ACS Synth Biol 2018, 7:1588-1600.