

Bacteriophage genome engineering with CRISPR-Cas13a

Table S1. Bacterial strains and phages used in this work.

Name	Description	Source/Reference
Bacterial strains		
PAO1	<i>P. aeruginosa</i> , wild-type isolate	RefSeq: NC_002516.2 [1]
SDM084	PAO1 <i>tn7::Lse cas13a</i>	[2]
BF#	<i>P. aeruginosa</i> CF clinical strains	Paul Turner Lab
Phages		
ΦKZ		Alan Davidson Lab
JBD30		Alan Davidson Lab
OMKO1	Clinical <i>P. aeruginosa</i> phage	Paul Turner Lab
PaMx41		Gabriel Guarneros Peña at Centro de Investigación y de Estudios Avanzados (GenBank Accession No.: KU884563)

Table S2. Plasmids.

Plasmid	Description	Source
pHERD30T	Arabinose inducible, gentamicin resistant (<i>Gent^R</i>) shuttle vector	[3]
pAM383	Amplification template of <i>acrVIA1</i>	[4]
pSDM057	pHERD30T with Version 1 Cas13a crRNA cassette	[2]
pSDM180	pHERD30T with Version 2 Cas13a crRNA cassette	This study
pSDM177	pHERD30T with Version 3 Cas13a crRNA cassette	This study
pJG003	pHERD30T with Φ KZ <i>orf93-mNeonGreen</i>	This study
pJG021	pHERD30T with HDR cassette to insert <i>acrVIA1</i> downstream of Φ KZ <i>orf120</i>	This study
pJG025	pHERD30T with HDR cassette to fuse Φ KZ <i>orf54</i> with <i>mCherry</i> at the N terminus	This study
pJG030	pHERD30T with Φ KZ <i>orf54</i> at the NheI site and <i>orf120</i> -crRNA	This study
pJG031	pHERD30T with HDR cassette to substitute Φ KZ <i>orf54</i> with <i>acrVIA1</i>	This study
pJG033	pHERD30T with Φ KZ <i>orf54</i>	This study
pJG039	pHERD30T with operon#24 at the NheI site and <i>orf120</i> -crRNA	This study
pJG040	pHERD30T with HDR cassette to insert <i>acrVIA1</i> upstream of Φ KZ <i>orf54</i> or the OMKO1 shell gene	This study
pJG041	pHERD30T with HDR cassette to insert a barcode and <i>acrVIA1</i> downstream of the OMKO1 capsid gene	This study
pJG050	pHERD30T with HDR cassette to substitute Φ KZ <i>orf146</i> with <i>acrVIA1</i>	This study
pJG053	pHERD30T with HDR cassette to substitute Φ KZ <i>orf241</i> with <i>acrVIA1</i>	This study
pJG055	pHERD30T with HDR cassette to fuse Φ KZ <i>orf54</i> with <i>gfp11</i> at the N terminus using a short linker	This study
pJG069	pHERD30T with HDR cassette to substitute Φ KZ <i>orf39</i> with <i>acrVIA1</i>	This study
pJG071	pHERD30T with HDR cassette to substitute Φ KZ <i>orf241</i> and <i>orf242</i> with <i>acrVIA1</i>	This study
pJG073	pHERD30T with HDR cassette to fuse Φ KZ <i>orf54</i> with <i>gfp11</i> at the N terminus using a long linker	This study
pJG074	pHERD30T with HDR cassette to substitute Φ KZ <i>orf93</i> with <i>acrVIA1</i>	This study
pJG075	pHERD30T with HDR cassette to substitute PaMx41 <i>orf24</i> (94 a.a. protein) with <i>acrVIA1</i>	This study
pJG076	pHERD30T with Φ KZ <i>orf39</i>	This study
pJG077	pHERD30T with HDR cassette to fuse Φ KZ <i>orf54</i> with <i>mCherry</i> at the C terminus	This study
pJG078	pHERD30T with HDR cassette to substitute Φ KZ <i>orf89</i> , <i>orf90</i> , <i>orf91</i> , <i>orf92</i> , <i>orf93</i> with <i>acrVIA1</i>	This study
pJG079	pHERD30T with HDR cassette to fuse Φ KZ <i>orf93</i> with <i>mNeonGreen</i> at the C terminus	This study

pJG084	pHERD30T with HDR cassette to fuse Φ KZ <i>orf39</i> with <i>mCherry</i> at the N terminus	This study
pJG085	pHERD30T with HDR cassette to fuse Φ KZ <i>orf39</i> with <i>mNeonGreen</i> at the N terminus	This study

Table S3. crRNA sequences.

Phage	Target	Type	No.	Sequence (5' - 3')
JBD30	<i>orf38</i>	VI-A		acgccaatctctgctacgacggtc
ΦKZ	<i>orf54</i>	VI-A	#1	tcaaactcaagctactgctcctgc
			#2	atgctcggtcagcagatgccctac
			#3	ggatggctggctactcattgtt
	<i>orf120</i>		#1	cttaagccgtgagctggaaagggt
			#2	gctgatcttaagccgtgagctgga
			#3	agcttgctgatcttaagccgtgag
<i>orf146</i>	#1	tcatactgggttgaactaatga		
	#2	ggcttctgcattgaagattatgt		
PaMx41	<i>orf11</i>	VI-A	#1	ccaccctgaatcctcaagaactca
			#2	agactggctgatctcggctgcta
			#3	aaagagcgcgtatatctgaccgcc
			#4	gtcaagcgtcagactggctgatc
			#5	tcaatctaccgtcaagcgtcaga
			#6	ctcggctgctatcatcgctgcgaa
	<i>orf04</i>	I-C	#1	tgatacgaaggaaattgctgcaattggcgataga
			#2	atttctgacttaatagggaggccgaattccata
	<i>orf14</i>	I-F	#1	tctcagcgtaatagtctgttcttctggcag
			#2	ttcttctatttctccagaatcttccgcaat
	<i>orf06</i>	II-A	#1	tttattactgttgaccctgc
			#2	ttccagcatagttctatac
	<i>orf20</i>	V	#1	tggagaatgaagaaggtaaggta
#2			agaatatgccttgcataggatag	
<i>orf23</i>	V	#1	tggagaatgaagaaggtaaggta	
		#2	agaatatgccttgcataggatag	
<i>orf46</i>	V	#1	tggagaatgaagaaggtaaggta	
		#2	agaatatgccttgcataggatag	

Table S4. Primers.

No.	Description	Name	Sequence (5' - 3')
JG003	mNeonGreen	mGreen-F	CCAATATTCTCGAGGGTGGCGGTGGCTCGATGGTGAGCAAGGGCG
JG004		mGreen-pHERD-R	GACTCTAGAGGATCCCCGGGTACCTTACTTGTACAGCTCGTCC
JG010	gp93	IB3-Sacl-F	AGCATC GAATTCGAGCTCC ATGTCTCTACTTAAAATGCT
JG011		IB3-XhoI-R	AGCATC CTCGAG CTTCTCATCATTCTCAGGTG
JG075	H1 for insertion at gp120 3'UTR (contains point mutations to avoid targeting)	pHERD-gp120H1-F	CCATGGGATCTGATAAGAATTC CTAGG GTAATGCGTCCGACCTATCG
JG064		gp120-muH1stop-R	GATCATTTTTTATTTCCTACGTAGG CTCGAG CTAAGGTTTTAAGTCGGCAAGCTTACC
JG029	H2 for insertion at gp120 3'UTR	gp120-H2-KpnI-F	AGCATC GGTACC CATATG GCTGGAAAGGGTTACCGTTA
JG030		gp120-H2-Sall-R	AGCATC GTCGAC CAACAAAACGTGAGACAGGG
JG046	Check insertion at gp120 3' UTR	CheckFP-inphage-R	AGTGCTTCTTCTTCTGGACTAAGTC
JG047		CheckFP-inphage-F	GTGGTACTATCAGCCTATCCAAAGG
JG065	AcrVIA1	AcrVIA1-XhoI-F	AGGCCCTCTCGAG CCTACGTAGGAATAAAAA ATGATCTACTATATAAAAG
JG066		AcrVIA1-NdeI-R	CGGCCCTCATATG TTAATTTAGCTCCTCTTTTA
JG088	Check the existence of AcrVIA1	AcrVIA1-mid-F	TCCGTTTCGCTCCCGATACGA
JG095	H1 for mCherry-gp54 fusion	pHERD-Op24NheI-F	GCCTTTTGCTGGCCTTTTGCTCACATAAG TTGAAGACCCGCCACTATATTTAGG
JG096		Op24H1-AcrVIA-R	CACTTTTAAATCTTTTATATAGTAGATCAT TTTAAATACCTTTACGATTATGG
JG097	AcrVIA1 for mCherry-gp54 fusion	Op24H1-AcrVIA-F	CAAGAAACCATAATCGTAAAGGTATTTAAA ATGATCTACTATATAAAAGA
JG098		AcrVIA-mCherry-R	CACCATTTTTTATTTCCTACGTAGGCCTAGG TTAATTTAGCTCCTCTTTTA
JG099	H2 for mCherry-gp54 fusion	AcrVIA-mCherry-F	AATTAACCTAGGCCTACGTAGGAATAAAAA ATGGTGAGCAAGGGCGAGGA
JG100		pHERD-gp54NheI-R	TGCTCTGCGAGGCTGGCCGATAAGCTAG ATGTGGGTCGCAATAGAGAACGGAG
JG104	H1 for gp54-gfp11 fusion	pHERD-gp54end-F	GCCTTTTGCTGGCCTTTTGCTCACATAAG GAGCAAGTCATCCTAGATGCA
JG105		gp54end-gfp11-R	CATGTGGTCACGCGAGCCGCCCGACCCGCC GTACCAGGTACCCGGTGCAT
JG106	H2 for gp39 deletion	KO39H2-F	ATTAACTTATACTGGAGCCCTTCGG
JG107		pHERD-KO39H2-R	TGCTCTGCGAGGCTGGCCGATAAGCTAG CTTTTTAGCAGTGTCTTTGCC

JG050		gp54-mid-R	CAGTGGTCGGAGTCCAATGTAGATC
JG108	Check gp54 editing	Check-recomb54-F	AGTTTGAGGACCAAGTGTAACACC
JG118		Check-KO54-R	GTGGGTTAATTAGGCTACGTACGTG
JG110		pHERD-gp54NheI-F	GCCTTTTGCTGGCCTTTTGCTCACATAAG CTGCATTTTATAAAAATACTG
JG111		pHERD-gp54NheI-R	TGCTCTGCGAGGCTGGCCGATAAGCTAG TTAGTACCAGGTACCCGGT
JG127	gp54	gp54-PstI-F	AGCATC CTGCAG ATGGCTGTTAACGAAAACGA
JG128		gp54-SacI-R	AGCGTC GAGCTC TTAGTACCAGGTACCCGGTGCATTATAG
JG112	AcrVIA1 for gp54 deletion (pair with JG097)	AcrVIA-Op24H2-R	TAACATCCCTATCTACCGAGGTATTTCTTA TTAATTTAGCTCCTCTTTTA
JG113		AcrVIA-Op24H2-F	GCAAACAAATTTTAAAAGAGGAGCTAAATTA TAGGAAATACCTCGGTAGATAGG
JG114	H2 for gp54 deletion	pHERD-Op24H2NheI-R	TGCTCTGCGAGGCTGGCCGATAAGCTAG CTTTCTCATCTGAGTTAC
JG119	H2 for gp93 deletion	KO93H2-F	CAAACATAATGAGGAACCCCTTCGG
JG120		pHERD-KO93H2-R	TGCTCTGCGAGGCTGGCCGATAAGCTAG CATAAAAGCAGCTGATTGTTTA
JG121	Check gp93 deletion	Check-KOgp93-R	AGTTAGTACACGCTGTGCCGCT
JG122	gp39	pHERD-gp39-NheI-F	GCCTTTTGCTGGCCTTTTGCTCACATAAG ATTATATTAATCACAATGAGG
JG123		pHERD-gp39-NheI-R	TGCTCTGCGAGGCTGGCCGATAAGCTAG TTAATCGAATACAAGACCACTACT
JG124	H1 for gp241-gp242 deletion	pHERD-KO2412-H1Acr-F	GCCTTTTGCTGGCCTTTTGCTCACATAAG TTAATAAAGATAACTTCTTTTC
JG125		KO2412-H1Acr-R	CCTTTTAGTTAATTTAATTTAGCTCCTCTTTTAAAATTTG
JG126	Check gp241-gp242 deletion	Check-KO2412-R	GGCTAAGTCTTTTTCTCGATACTGG
JG133	Operon#24	pHERD-Op24NheI-F	GCCTTTTGCTGGCCTTTTGCTCACATAAG TACGTGACGGAGCATTCTTAAATA
JG134		pHERD-Op24NheI-R	TGCTCTGCGAGGCTGGCCGATAAGCTAG CCCGAAGGAGCCGCTATATCAG
JG135		H1 for insertion at the 3'UTR of the capsid gene in OMKO1	OmK-capH1-GibsonF
JG136		OmK-capmuH1-GibsonR	GATCATTTTTTATTCTACGTAGG GCTAGC CTAAGGTTTTAAGTCGGCAAGCTTACC
JG137	H1 for insertion at gp54 5'UTR	pHERD-Op24InH1-F	GCCTTTTGCTGGCCTTTTGCTCACATAAG GGAGCATTCTTAAATAAATTA
JG138		Op24InH1-RBS-R	GATCATTGTATATTCTATTGTAAT CTCGAG CGTAACGAACACTATGTT

JG139	AcrVIA1 for insertion at gp54 5'UTR	gp53RBS-AcrVIA1-F	CTCGAG ATTACAAATAGGAATATACA ATGATCTACTATATAAAAAGA
JG140		AcrVIA1-Op24InH2-R	CGCTATAATCTGCTATCAGCAGAT CCTAGG TTAATTTAGCTCCTCTTTTA
JG141	H2 for insertion at gp54 5'UTR	AcrVIA1-Op24InH2-F	AGGAGCTAAATTAA CCTAGG ATCTGCTGATAGCAGATTATAGCG
JG142		pHERD-Op24InH2-R	TGCTCTGCGAGGCTGGCCGATAAGCTAG GTTGTAAACGGTTCGGTAG
JG146	H1 for gp146 deletion	pHERD-KOgp146H1-F	GCCTTTTGCTGGCCTTTTGCTCACATAAG AGCTATTGCAAATGCGGTACC
JG147		KOgp146H1-AcrR	TAGATCATTTTTATTTCTTGCTGTATAAGAA CATTATACTACTTCTCAAGTTCCG
JG148	Check gp146 deletion	Check-gp146edit-R	AATCTATCCCAGTTC AAGCTAAGCC
JG152	AcrVIA1 for gp146 deletion	gp145RBS-AcrVIA1-F	TTCTTATACAGCAAGGAAATAAAA ATGATCTACTATATAAAAAGAT
JG153		AcrVIA1-Op63InH2-R	GGAGGGCAATATGTCGTT TTAATTTAGCTCCTCTTTTAAAAT
JG154	H2 for gp146 deletion	AcrVIA1-Op63InH2-F	AAAGAGGAGCTAAATTAA AACGACATATTGCCCTCCCTTCGG
JG155		pHERD-Op63InH2-R	TGCTCTGCGAGGCTGGCCGATAAGCTAG ATTCCATTTATTATTCAATTAATAGCT
JG156	gp146	pMMB-gp146-GbF	GGAAACAGAATTAATTAAGCTTGCATGC CTGCAG ATGGCTTCTGCATTTGAAGA
JG157		pMMB-gp146-GbR	CCGCCAAAACAGCCAAGCTGAATTC GAGCTC TTAATAACAGCACCTTTGG
JG160	H2 for gp241 deletion	AcrVIA1-KO241H2-F	CAAACAAATTTTAAAAGAGGAGCTAAATTAATAAGAGCATATGGCCCTCCCTT CGG
JG161		pHERD-KO241H2-R	TGCTCTGCGAGGCTGGCCGATAAGCTAG TAATAGTGGTATTAACCTTGTAG
JG162	Check gp241 deletion	Check-KOgp241-R	CGGGTGTAATCGTACGTAGATCAATCGC
JG166	H2 for gp241-gp242 deletion	KO2412-H2-F	GAGGAGCTAAATTAATTAACATAAAAGGTAAAAAATGAATAATC
JG167		pHERD-KO2412-H2-R	TGCTCTGCGAGGCTGGCCGATAAGCTAG CATCCTTATTATGGAAGCTATA
JG168	Check orf24 deletion in PaMx41	Check-PaM24-R	CTCGTGGTGATGTTAACCTAGGG
JG169		Check-PaM24-F	CAACTCTGAATTCCGAAGTGAAGCC
JG170	Check gp93 deletion	Check-KOgp93-F	ACTCTTGATATCCAATCTGTAGCGG
JG173	gp39	gp39-Sacl-F	ACCCATGGGATCTGATAAGAATTCGAGCTC ATGATGTCTAAAGTAAAACTCG
JG174		gp39-KpnI-R	CAGGTCGACTCTAGAGGATCCCCGGGTACC TTAATCGAATACAAGACCACTACT
JG175		pHERD-KOOp42H1-F	GCCTTTTGCTGGCCTTTTGCTCACATAAG TAACCTACCTTCTTTTACTAATGCAGAATAG

JG176	H1 for Operon#42 deletion	KOop42H1-R	CTTTTAAATCTTTTATATAGTAGATCAT TTTTCTTCTTCTATATGGGTTTGCAGAG
JG178	gfp1-10	pHERD-gfp10-GbF	ACCCATGGGATCTGATAAGAATTC GAGCTC ATGGTGAGCAAGGGCGAGGA
JG179		pHERD-gfp10-GbR	GGCCAGTGCCAAGCTTGCATGC CTGCAG CTTCTCGTTGGGGTCTTTGCTC
JG180	Check gfp11-gp54 and gp54-gfp11 fusion	gfp11-mid-F	TGGTCCTTCATGAGTATGTAAATGC
JG181	Check gp39 deletion	Check-KOgp39-R	CTTCAGCCCATTTGTCTACCCG
JG182	H1 for gp93-mNeonGreen fusion	gp93-mNGH1-Gb-F	GCCTTTTGCTGGCCTTTTGCTCACATAAG AATCCGAAGACTATTGCAGACTTCA
JG183		gp93-mNGH1-Gb-R	CTATATGGGTTTGC GGTTCCCTCATTATGTTTGTTA TTACTTGTACAGCTCGTCCAT
JG186	Check Operon#42 deletion	Check-KOop42-F	CACCAATAAAGTCACCAGTACGTCC
JG187	Check gp93-mNeonGreen fusion	gp93-mid-F	GCCCTAGCTGATTGGAATCCTGACG
JG195	H1 for FP-gp39 fusion	FP-gp39H1-F	GCCTTTTGCTGGCCTTTTGCTCACATAAGTTTAATTTGAAAATAGAAAAATTG G
JG196		FP-gp39H1-R	CCATTACACCTCATTGTGATTAATATAATTTAATTTAGCTCCTCTTTTAAAATTT G
JG197	H2 for FP-gp39 fusion	FP-gp39H2-F	TAAATTATATTAATCACAATGAGGTGTAATGGTGAGCAAGGGCGAGGAGG
JG198		FP-gp39H2-R	TGCTCTGCGAGGCTGGCCGATAAGCTAGAAGACTTTGGTGTCTTGATTA
oSDM455	pHERD30T-crRNA Version 3	Lse_crRNA_V3_Top	ACTCTCTACTGTTTCTCCATGGTAAGAGACTACCTCTATATGAAAGAGGACTA AAACCGA
oSDM456		Lse_crRNA_V3_Bot	CGGGTACCGAGCTCGAATTCGAGACCGTACGTACGTGGTCTCGGTTTTAGT CCTCTTTCA
oSDM457	pHERD30T-crRNA Version 2	p30T_TSS-R	ATGGAGAAACAGTAGAGAGTTG
oSDM458		p30T_TSS-F	GAATTCGAGCTCGGTACC
oSDM465		LseCas13 crRNA V2 F	ACTCTCTACTGTTTCTCCATGGTAAGAGACTACCTCTATATGAAAGAGGACTA AAACCGAGACCACGTACGTACGGTC
oSDM466		LseCas13 crRNA V2 R	CGGGTACCGAGCTCGAATTCGTTTTTGTGGAGATTCATATTCTCCAAGTCTC TTACCGGAGACCGTACGTACGTGGTC

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