

Additional file 12, Table S2. Expression vectors and plasmid substrates.

Vector	Description	Cloning sites	Primers	Source
Expression				
pCDF-Duet1	T7 promoter based expression vector; Sm ^R			Novagen
pCDF-SH	Derivative of pCDF-Duet1 where His ₆ and S-tag sequences are replaced with Strep-II and His ₆ sequences, respectively; Sm ^R			[61]
pACYC-Duet1	T7 promoter based expression vector, Cm ^R			Novagen
pCOLA-Duet1	<i>araC</i> promoter based expression vector, Kn ^R			Novagen
pET-SH	Derivative of T7 promoter based expression vector pET-Duet1 where His ₆ and S-tag sequences are replaced with Strep-II and His ₆ sequences, respectively; Ap ^R			[61]
pCd	<i>cas8f1-cas5f1-cas7f1-cas6f</i> cassette in pCDF-SH, no tags	NcoI/Sall	TS382/TS615	This study
pCd-H	<i>cas8f1-cas5f1-cas7f1</i> cassette and gene of Cas6f fused at N-terminus with His ₆ sequence in pCDF-Duet1	NdeI/PacI	TS757/TS394	This study
		BamHI/Sall	TS397/TS758	
pCas1-2/3	<i>cas1-cas2/3</i> cassette in pCDF-SH, no tags	NdeI/PacI	TS616/TS417	This study
pCOLA-Cas1-2/3	<i>cas1-cas2/3</i> cassette in pCOLA-Duet1, no tags	NdeI/PacI	TS616/TS417	This study
pCas2/3-H	<i>cas2/3</i> gene in pET-SH; C-terminus of Cas2/3 is fused with His ₆ tag	NdeI/XhoI	TS336/TS337	This study
pCd-Cas1-2/3	<i>cas1-cas2/3</i> cassette cloned into pCDF-Cd; no tag	NdeI/PacI	TS616/TS417	This study
pCd-Cas2/3	<i>cas2/3</i> gene cloned into pCDF-Cd; no tag	NdeI/PacI	TS336/TS417	This study
pCR-cloning	Repeats cloned into pACYC-Duet1	NcoI/PacI	TS543/TS544	This study
pCR(WT)	32 bp spacer cloned into pCR-cloning; spacer assembled from oligos	SapI	TS545/TS546	This study
pCR(-30)	2 bp spacer cloned into pCR-cloning; spacer assembled from oligos	SapI/PacI	TS772/TS773	This study
pCR(-24)	8 bp spacer cloned into pCR-cloning; spacer assembled from oligos	SapI	TS720/TS721	This study
pCR(-18)	14 bp spacer cloned into pCR-cloning; spacer assembled from oligos	SapI	TS718/TS719	This study
pCR(-12)	20 bp spacer cloned into pCR-cloning; spacer assembled from oligos	SapI	TS716/TS717	This study
pCR(-6)	26 bp spacer cloned into pCR-cloning; spacer assembled from oligos	SapI	TS714/TS715	This study
pCR(+6)	38 bp spacer cloned into pCR-cloning; spacer was PCR amplified using pSP-CC as template	SapI	TS723/TS724	This study
pCR(+12)	44 bp spacer cloned into pCR-cloning; spacer was PCR amplified using pSP-CC as template	SapI	TS723/TS725	This study

pCR(+18)	50 bp spacer cloned into pCR-cloning; spacer was PCR amplified using pSP-CC as template	<i>SapI</i>	TS723/TS726	This study
pCR(+24)	56 bp spacer cloned into pCR-cloning; spacer was PCR amplified using pSP-CC as template	<i>SapI</i>	TS723/TS727	This study
pCR(+30)	62 bp spacer cloned into pCR-cloning; spacer was PCR amplified using pSP-CC as template	<i>SapI</i>	TS723/TS728	This study
pCR(+54)	86 bp spacer cloned into pCR-cloning; spacer was PCR amplified using pSP-CC as template	<i>SapI</i>	TS723/TS829	This study
pCR(+84)	116 bp spacer cloned into pCR-cloning; spacer was PCR amplified using pSP-CC as template	<i>SapI</i>	TS723/TS830	This study
pCR(+144)	176 bp spacer cloned into pCR-cloning; spacer was PCR amplified using pSP-CC as template	<i>SapI</i>	TS723/TS776	This study
Substrates				
pSP-CC (also pPS-WT) [Target]	CC PAM and target protospacer sequences in pUC19 (pSP1-CC)			[34]
pNT [Non-target]	AA PAM and non-target protospacer sequences in pUC19 (pSP3-AA)			[34]
pSP-AA	AA PAM and target protospacer sequences in pUC19 (pSP1-AA)			[34]
pSP-AG	AG PAM and target protospacer sequences in pUC19 (pSP1-AG)			[34]
pSP-AC	AC PAM and target protospacer sequences in pUC19 (pSP1-AC)			[34]
pSP-AT	AT PAM and target protospacer sequences in pUC19 (pSP1-AT)			[34]
pSP-GA	GA PAM and target protospacer sequences in pUC19 (pSP1-GA)			[34]
pSP-GG	GG PAM and target protospacer sequences in pUC19 (pSP1-GG)			[34]
pSP-GC	GC PAM and target protospacer sequences in pUC19 (pSP1-GC)			[34]
pSP-GT	GT PAM and target protospacer sequences in pUC19 (pSP1-GT)			[34]
pSP-CA	CA PAM and target protospacer sequences in pUC19 (pSP1-CA)			[34]
pSP-CG	CG PAM and target protospacer sequences in pUC19 (pSP1-CG)			[34]
pSP-CT	CT PAM and target protospacer sequences in pUC19 (pSP1-CT)			[34]
pSP-TA	TA PAM and target protospacer sequences in pUC19 (pSP1-TA)			[34]
pSP-TG	TG PAM and target protospacer sequences in pUC19 (pSP1-TG)			[34]
pSP-TC	TC PAM and target protospacer sequences in pUC19 (pSP1-TC)			[34]
pSP-TT	TT PAM and target protospacer sequences in pUC19 (pSP1-TT)			[34]
pPS-T6	6 bp mutations was introduced within 57-62 positions from the CC		TS846/TS847	This study

	PAM in pSP-CC			
pPS-T30	Sequence within 33-62 positions from the CC PAM was deleted in pSP-CC		TS846/TS849	This study
pPS-M6	6 bp mutations was introduced within 27-32 positions from the CC PAM in pSP-CC		TS843/TS844	This study
M13mp18	ssDNA phage; ssDNA of this phage was used for helicase, nuclease and ATPase assays; replicative dsDNA was used for cloning procedures			NEB
M13-SP-CC [Target]	Target sequence was PCR amplefied from pSP-CC and cloned into M13mp18 phage	<i>EheI/BglI</i>	pUC-Ehe/TS911	This study
M13-NT [Non-target]	Non-target sequence was PCR amplefied from pNT and cloned into M13mp18 phage	<i>EheI/BglI</i>	pUC-Ehe/TS911	This study

REFERENCES

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61. Drabavicius G, Sinkunas T, Silanskas A, Gasiunas G, Venclovas C, Siksnys V: **DnaQ exonuclease-like domain of Cas2 promotes spacer integration in a type I-E CRISPR-Cas system**. *EMBO reports* 2018, **19**(7).