

Additional file 13, Table S3. Oligonucleotides.

Experiment	Substrate name	Primer	Sequence (5'→3')	Description
Cleavage, P1 footprints, Cloning	SP-CC	TS169	GACCACCCTTTTTGATATCCATACCTATATCAATGGCCTCCCACGCATAAGCGCAGATACGTTCTGAGGGAA	Target protospacer, CC PAM, ALQ13.2 phage surrounding sequences
		TS170	TTCCCTCAGAACGTATCTGCGCTTATGCGTGGGAGGCCATTGATATAGGTATAGGATATCAAAAAGGGTGGTC	
	NT	TS140	GACCACCCTTTTTGATATAACGCAACCCCTCCTTAGACATGGGAACAGTACTAGCAGATACGTTCTGAGGGAA	Non-target protospacer, AA PAM, ALQ13.2 phage surrounding sequences
		TS141	TTCCCTCAGAACGTATCTGCTAGTACTGTTCCCATGTCTAAGGAGGGGTTGCGTTATATCAAAAAGGGTGGTC	
	SP-AA	TS132	GACCACCCTTTTTGATATAATATACCTATATCAATGGCCTCCCACGCATAAGCGCAGATACGTTCTGAGGGAA	Target protospacer AA PAM, ALQ13.2 phage surrounding sequences
TS133		TTCCCTCAGAACGTATCTGCGCTTATGCGTGGGAGGCCATTGATATAGGTATATATATCAAAAAGGGTGGTC		
EMSA	SP-CC-1	TS276	GTGATGACGGTGAAAACCTCTG	800 bp long oligoduplex with CC PAM and complementary protospacer; PCR amplified using pSP-CC as template.
		TS911	AAAAGCCATTCAGGCGCTGATACCGCTCGCCGCAG	
EMSA	NT-1	TS276	GTGATGACGGTGAAAACCTCTG	800 bp long oligoduplex without target sequence; PCR amplified using pNT as template
		TS911	AAAAGCCATTCAGGCGCTGATACCGCTCGCCGCAG	
EMSA	SP-AA-1	TS276	GTGATGACGGTGAAAACCTCTG	800 bp long oligoduplex with AA PAM and complementary protospacer, PCR amplified using pSP-AA as template
		TS911	AAAAGCCATTCAGGCGCTGATACCGCTCGCCGCAG	
EMSA	SP-CC-2	pUC57-Forw	GCCAGGGTTTTCCAGTCACGA	420 bp long oligoduplex with CC PAM and complementary protospacer; PCR amplified using pSP-CC as template
		pUC-LguR	GCGAGGAAGCGGAAGAGCGCCC	
EMSA	SP3-AA-2	pUC57-Forw	GCCAGGGTTTTCCAGTCACGA	420 bp long oligoduplex without target sequence; PCR amplified using pNT as template
		pUC-LguR	GCGAGGAAGCGGAAGAGCGCCC	
Cleavage, Footprinting	SP-CC-3 (also as PS-WT)	TS930 [#]	GCGGGCAGTGAGCGCAACG	substrate for R-loop KMnO ₄ footprinting and Cas2/3 cleavage assays; PCR amplified using pSP-CC as template
		TS931 [*]	GCTCGGTACCCGACCACCCTTTTTGATATCC	
	PS-M6	TS930	GCGGGCAGTGAGCGCAACG	substrate for R-loop KMnO ₄ footprinting and Cas2/3 cleavage assays; PCR amplified using pPS-M6 as template
		TS931 [*]	GCTCGGTACCCGACCACCCTTTTTGATATCC	
	PS-T6	TS930	GCGGGCAGTGAGCGCAACG	substrate for R-loop KMnO ₄ footprinting and Cas2/3 cleavage

		TS931*	GCTCGGTACCCGACCACCCTTTTTGATATCC	assays; PCR amplified using pPS-T6 as template
	PS-T30	TS930	GCGGGCAGTGAGCGCAACG	substrate for R-loop KMnO ₄ footprinting and Cas2/3 cleavage assays; PCR amplified using pPS-T30 as template
		TS931*	GCTCGGTACCCGACCACCCTTTTTGATATCC	
Markers	M-32	TS932	CTTATGCGTGGGAGGCCAT	Marker for 32-bp R-loop boundary in KMnO ₄ footprinting; PCR amplified using pSP-CC as template
		TS931*	GCTCGGTACCCGACCACCCTTTTTGATATCC	
	M-62	TS933	AGGATCCCCTTCCCTCAGA	Marker for 62-bp R-loop boundary in KMnO ₄ footprinting; PCR amplified using pSP-CC as template
		TS931*	GCTCGGTACCCGACCACCCTTTTTGATATCC	
	M-116	TS934	AAACAGCTATGACCATGAT	Marker for 116-bp R-loop boundary in KMnO ₄ footprinting; PCR amplified using pSP-CC as template
		TS931*	GCTCGGTACCCGACCACCCTTTTTGATATCC	
	M-176	TS935	CTTTATGCTTCCGGCTCGT	Marker for 176-bp R-loop boundary in KMnO ₄ footprinting; PCR amplified using pSP-CC as template
		TS931*	GCTCGGTACCCGACCACCCTTTTTGATATCC	
		TS166	TTCCCTCAGAACGTATCT	³² P-5'-end-labelled oligonucleotides used for target strand dideoxy sequencing of SP-CC (complementary to TS169)
		TS153	GACCACCCTTTTTGATAT	³² P-5'-end-labelled oligonucleotides used for target strand dideoxy sequencing of SP-CC (complement to TS170)
		TS239	TTCCCTCAGAACGTAT	³² P-5'-end-labelled oligonucleotides used as markers (37, 39, 42, 44, and 46) for the target strand of the SP-CC.
		TS240	TTCCCTCAGAACGT	
		TS241	TTCCCTCAGAAC	
		TS242	TTCCCTCAGA	
		TS168	TTCCCTCA	
		TS232	GACCACCCTTTTTGAT	
		TS233	GACCACCCTTTTTG	³² P-5'-end-labelled oligonucleotides used as markers (-5, -7, -9, -11, and -13) for the non-target strand of the SP-CC.
		TS234	GACCACCCTTTT	
	TS235	GACCACCCTT		
	TS155	GACCACCC		
Cloning		TS336	<u>tcatatgaatacttacttqtagccaatg</u>	cas2/3 + NdeI (fw)
		TS337	<u>tctcgagatcttctgccaaaaaccagc</u>	cas2/3 + XhoI (rv)
		TS382	<u>tattcgtctcccataaactaaaacaacagtagtaaaagcc</u>	cas8f + Esp3I (fw)
		TS394	<u>gtgttaaftaactattcctaccgcttgcgcc</u>	cas7 + PaeI (rv)
		TS397	<u>tattgatcctatgacagtgcaaaccattacattg</u>	cas6f + BamHI (fw)

	TS417	<u>agttaatcaatcttctgcccacccagc</u>	cas3 + PacI (rv)
	TS543	<u>tatccatggtcactgccgaataggcagcttagaaatgaagagccgagctcgccgctgcaggtcgacaagc</u>	pACYC-Duet1 + repeat + NcoI (fw)
	TS544	<u>ctcttaatatttctaagctgcctattcggcagtgaaatgaagagcgcttgcgacctgcagccgcccagctcg</u>	pACYC-Duet1 + repeat + PacI (rv)
	TS545	<u>aaatatacctatatcaatggcctcccacgcataag</u>	32 bp (WT) spacer (fw)
	TS546	<u>aagcttagcgtgggagccattgatataggata</u>	32 bp (WT) spacer (rv)
	TS615	<u>tttgcgacttagaaatgtggaacgggtgtagttg</u>	cas6f + Sall (rv)
	TS616	<u>tttcatatgcctaagacccttacattc</u>	cas1 + NdeI (fw)
	TS714	<u>aaatatacctatatcaatggcctcccacg</u>	26 bp spacer (fw)
	TS715	<u>aagcgtgggagccattgatataggata</u>	26 bp spacer (rv)
	TS716	<u>aaatatacctatatcaatggcct</u>	20 bp spacer (fw)
	TS717	<u>aagagccattgatataggata</u>	20 bp spacer (rv)
	TS718	<u>aaatatacctatatcaa</u>	14 bp spacer (fw)
	TS719	<u>aagttgatataggata</u>	14 bp spacer (rv)
	TS720	<u>aaatataccta</u>	8 bp spacer (fw)
	TS721	<u>aagtaggtata</u>	8 bp spacer (rv)
	TS723	<u>atagctctcaaaaatacctatatcaatggcctccc</u>	elongated spacers + SapI (fw)
	TS724	<u>atagctctcaaaagctgccttatgcgtggga</u>	38 bp spacer + SapI (rv)
	TS725	<u>atagctctcaaaagacgtatctgcgcttatgcg</u>	44 bp spacer + SapI (rv)
	TS726	<u>atagctctcaaaagcctcagaacgtatctgcgc</u>	50 bp spacer + SapI (rv)
	TS727	<u>atagctctcaaaagccctccctcagaacgtat</u>	56 bp spacer + SapI (rv)
	TS728	<u>atagctctcaaaagggatcccctccctcaga</u>	62 bp spacer + SapI (rv)
	TS757	<u>tttcatatgataactaaacaacagtagtaaaagcc</u>	cas8f + NdeI (fw)
	TS758	<u>tttgcgacttagaaatgtggaacgggtgtagttg</u>	cas6f + Sall (rv)
	TS772	<u>aaatcttcaactgccgaataggcagcttagaaat</u>	2bp spacer + repeat (fw)
	TS773	<u>taatttctaagctgcctattcggcagtgaaqfa</u>	2bp spacer + repeat (rv)
	TS776	<u>atagctctcaaaagcttatgctccggctcgtatg</u>	176 bp spacer + SapI (rv)
	TS829	<u>atagctctcaaaagtcacgctgcaggtcgac</u>	86 bp spacer + SapI (rv)

	TS830	<u>atagctcttcaagaacagctatgacatgattacg</u>	116 bp spacer + SapI (rv)
	TS843	GTATTC <u>cg</u> cagatacgttctgaggg	6 bp mismatch (within 27-32 bp positions) at the PAM distal end of the 32 bp protospacer (fw)
	TS844	cgtgggaggccattgatatag	6 bp mismatch (within 27-32 bp positions) at the PAM distal end of the 32 bp protospacer (rv)
	TS846	ctagagtcgacctgcaggc	6 bp mismatch and 30 bp deletion of the PAM distal end of the 62 bp protospacer (fw)
	TS847	TCCTAG <u>cccctccctcagaacgtatc</u>	6 bp mismatch (within 57-62 bp positions) at the PAM distal end of the 62 bp protospacer (rv)
	TS849	<u>Acttatgcgtgggaggccattg</u>	30 bp deletion (within 33-62 bp positions) at the PAM distal end of the 62 bp protospacer (rv)
	TS911	aaaagccattcagggcgtgataccgctcgcgcag	pUC19 + BglI (rv); for cloning into M13mp18
	pUC-Ehe	<u>ccgcatcaggcgccattcgcc</u>	for cloning into M13mp18 (fw)

³²P-5'-end-labelled oligonucleotide was used for the DNA production with the labelled target strand;

* ³²P-5'-end-labelled oligonucleotide was used for the DNA production with the labelled non-target strand.