

## SUPPLEMENTARY TABLE

Table S1. Oligonucleotides used in this study

Name	Sequence	Use
OCN482	CATGCCCATGGGCCATCATCATCATCACGGAATGACCTGGCTTCCCCTT	Amplification of <i>cas1</i> (N-terminal His <sub>6</sub> tag)
OCN483	AAAAACTGCAGTCAGCTACTCCGATGGCCT	Amplification of <i>cas1</i> (C-terminus)
OCN504	CATGCCCATGACCTGGCTTCCCCTT	Amplification of <i>cas1</i> (N-terminus)
OCN506	GATCCGCATATGAGTATGTTGGTCGTG	Amplification of <i>cas2</i> (N-terminus)
OCN507	GCATGGTACCTCATCCGTGATGATGATGATGATGGCCAACAGGTAAAAAGAC	Amplification of <i>cas2</i> (C-terminal His <sub>6</sub> tag)
-62Leader-rep-F	CTTAAGTTGGTGGGTTGTTTTATGGGAAAAAATGCTTTAAGAACAAATGTAT ACTTTTAGAGAGTTCCCCGCGCCAGCGGGGATAAACCGAGCT	Construction of pBS-Z0 and pCOLA-Z0
-62Leader-rep-R	CGGTTTATCCCCGCTGGCGCGGGGA ACTCTCTAAAAGTATACATTTGTTCTTAA AGCATTTTTTCCCATAAAAACAACCCACCAACCTTAAGGTAC	
Leader-repeat F	CATGGGAAAAAATGCTTTAAGAACAAATGTATACTTTTAGAGAGTTCCCCGCGC CAGCGGGGATAAACCGAGCT	Construction of pBS-Z2
Leader-repeat R	CGGTTTATCCCCGCTGGCGCGGGGA ACTCTCTAAAAGTATACATTTGTTCTTAA AGCATTTTTTCCCATGGTAC	
-62Leader-F	CTTAAGTTGGTGGGTTGTTTTATGGGAAAAAATGCTTTAAGAACAAATGTAT ACTTTTAGAGGAGCT	Construction of pBS-Z3
-62Leader-R	CCTCTAAAAGTATACATTTGTTCTTAAAGCATTTTTTCCCATAAAAACAACCCAC CAACCTTAAGGTAC	
Repeat-F	CGAGAGTTCCCCGCGCCAGCGGGGATAAACCGAGCT	Construction of pBS-Z4
Repeat-R	CGGTTTATCCCCGCTGGCGCGGGGA ACTCTCGGTAC	
Repeat-F2	CGAGAGTTGCCGCGTGGCCGCGGCATAAACCGAGCT	Construction of pBS-Z5
Repeat-R2	CGGTTTATGCCGCGGCCACGCGGCAACTCTCGGTAC	
Repeat-F3	CCTCTCAAGGGGCGCGTGCGCCCTATTTGGGAGCT	Construction of pBS-Z6
Repeat-R3	CCCAAATAGGGGCGCACGCGCCCTTGAGAGGGTAC	
Repeat-F4	CGAGAGTTCCCCGCCGTGGCGGGGATAAACCGAGCT	Construction of pBS-Z7
Repeat-R4	CGGTTTATCCCCGCCACGCGGGGA ACTCTCGGTAC	
Repeat-F5	CCTCTCAACCCCGCGCCAGCGGGTATTTGGGAGCT	Construction of pBS-Z8
Repeat-R5	CCCAAATACCCCGCTGGCGCGGGGTTGAGAGGGTAC	

<b>Name</b>	<b>Sequence</b>	<b>Use</b>
Repeat-F6	CGAGAGTAGGGCGCGCCAGCGCCCTTAAACCGAGCT	Construction of pBS-Z9
Repeat-R6	CGGTTTAAGGGCGCTGGCGCGCCCTACTCTCGGTAC	
Repeat-F7	CGAGAGTTCCCCGCTCCAGCGGGGATAAACCGAGCT	Construction of pBS-Z10
Repeat-R7	CGGTTTATCCCCGCTGGAGCGGGGA ACTCTCGGTAC	
Repeat-F8	CGAGAGTTCCCCGCGTCAGCGGGGATAAACCGAGCT	Construction of pBS-Z11
Repeat-R8	CGGTTTATCCCCGCTGACGCGGGGA ACTCTCGGTAC	
Repeat-F9	CGAGAGTTCCCCGCGCTAGCGGGGATAAACCGAGCT	Construction of pBS-Z12
Repeat-R9	CGGTTTATCCCCGCTAGCGCGGGGA ACTCTCGGTAC	
Repeat-F10	CGAGAGTTCCCCGCGCCTGCGGGGATAAACCGAGCT	Construction of pBS-Z13
Repeat-R10	CGGTTTATCCCCGCAGGCGCGGGGA ACTCTCGGTAC	
Repeat-F11	CGAGAGTTGCCCGCGCCAGCGGGCATAAACCGAGCT	Construction of pBS-Z14
Repeat-R11	CGGTTTATGCCCGCTGGCGCGGGCA ACTCTCGGTAC	
Repeat-F12	CGAGAGTTCGCCGCGCCAGCGGCGATAAACCGAGCT	Construction of pBS-Z15
Repeat-R12	CGGTTTATCGCCGCTGGCGCGGCGA ACTCTCGGTAC	
Repeat-F13	CGAGAGTTCCGCGCGCCAGCGCGGATAAACCGAGCT	Construction of pBS-Z16
Repeat-R13	CGGTTTATCCGCGCTGGCGCGCGGA ACTCTCGGTAC	
Repeat-F14	CGAGAGTTCCCGGCGCCAGCCGGGATAAACCGAGCT	Construction of pBS-Z17
Repeat-R14	CGGTTTATCCCGGCTGGCGCCGGGA ACTCTCGGTAC	
Repeat-F15	CGAGAGTTCCCCCGCCAGGGGGGATAAACCGAGCT	Construction of pBS-Z18
Repeat-R15	CGGTTTATCCCCCCTGGCGGGGGGA ACTCTCGGTAC	
Repeat-F16	CGAGAGTTCCCCGGGCCACCGGGGATAAACCGAGCT	Construction of pBS-Z19
Repeat-R16	CGGTTTATCCCCGGTGGCCCGGGGA ACTCTCGGTAC	
Repeat-F17	CCTCTCATCCCCGCGCCAGCGGGGATAAACCGAGCT	Construction of pBS-Z20
Repeat-R17	CGGTTTATCCCCGCTGGCGCGGGGATGAGAGGGTAC	
Repeat-F18	CGAGAGTTCCCCGCGCCAGCGGGGAATTTGGGAGCT	Construction of pBS-Z21
Repeat-R18	CCCAAATTCCCCGCTGGCGCGGGGA ACTCTCGGTAC	
Repeat-F19	CGAGAGTACCCCCGCGCCAGCGGGGTTAAACCGAGCT	Construction of pBS-Z22
Repeat-R19	CGGTTTAACCCCGCTGGCGCGGGGTACTCTCGGTAC	

Name	Sequence	Use
Repeat-F20	CGAGAGTTCCGCGCGCCAGCGGGGATAAACCGAGCT	Construction of pBS-Z23
Repeat-R20	CGGTTTATCCCCGCTGGCGCGCGGAACTCTCGGTAC	
Repeat-F21	CGAGAGTTCCCCGCGCCAGCGCGGATAAACCGAGCT	Construction of pBS-Z24
Repeat-R21	CGGTTTATCCGCGCTGGCGCGGGGAACTCTCGGTAC	
Repeat-F22	CGAGAGTTCCCCGCGCTCAGCGGGGATAAACCGAGCT	Construction of pBS-Z25
Repeat-R22	CGGTTTATCCCCGCTGAGCGCGGGGAACTCTCGGTAC	
Repeat-F23	CTCTAGTTCCCCGCGCCAGCGGGGATAAAAGAAGCT	Construction of pBS-Z26
Repeat-R23	TCTTTTATCCCCGCTGGCGCGGGGAACTAGAGGTAC	
Repeat-F24	CTCTAGTTCCCCGCGCCAGCGGGGATAAACCGAGCT	Construction of pBS-Z27
Repeat-R24	CGGTTTATCCCCGCTGGCGCGGGGAACTAGAGGTAC	
Repeat-F25	CGAGTTTCAGACGAACCCTCGTGGGGTTGAAGCGAGCT	Construction of pBS-Z28
Repeat-R25	CGCTTCAACCCACGAGGGTTCGTCTGAAACTCGGTAC	
Repeat-F26	CGATTCCTTTTTCAATATTATTGAAGCATTGAGCT	Construction of pBS-Z29
Repeat-R26	CAAATGCTTCAATAATATTGAAAAAGGAATCGGTAC	
OMF13-782	DY782-ATTTACGCTACTCCGATGGCCCATATGCGGATC	Oligonucleotides P1 (top strand) and S2, DY782-labeled
RevOMF13v1	CTAGGGCATATGGGCCATCGGAGTAGCTATGTA	Oligonucleotide P1 (bottom strand)
OMF13	ATTTACGCTACTCCGATGGCCCATATGCGGATC	Oligonucleotide P1 (top strand), unlabeled
OMF13-682	DY682-ATTTACGCTACTCCGATGGCCCATATGCGGATC	Oligonucleotide P1 (top strand), DY682-labeled
OMF4	GATCCGCATATGGGCCATCGGAGTAGCTGAAAT	Oligonucleotides Q1 (top strand) and P5 (bottom strand)
RevOMF4v1	TAAAGAGCTACTCCGATGGCCCATATGCATTAC	Oligonucleotide Q1 (bottom strand)
Reverse-3	ATTTACGCTACTAACAGCTATGACCATGATTAC	Oligonucleotide R1 (top strand)
Reverse-R7	TCTCGCATGGTCATAGCTGTTAGTAGCTCTTTA	Oligonucleotide R1 (bottom strand)
OMF13 C3'T	DY782-ATTTACGCTACTCCGATGGCCCATATGCGGATT	Oligonucleotides P0, P2 (top strand) and S1, DY782-labeled
OMF13 +5T	DY782-ATTTACGCTACTCCGATGGCCCATATGCGGATCTTTTT	Oligonucleotides P8 to P11 (top strand), DY782-labeled
RevOMF13v2	CTAGGGCATATGGGCCATCGGAGTAGCTATGTC	Oligonucleotides P2, P3 and P8 (bottom strand)
RevOMF13v9	CTAGGGCATATGGGCCATCGGAGTAGCTGAAAT	Oligonucleotide P4 (bottom strand)

RevOMF13v6	CTAGGGCATATGGGCCATCGGAGTAGCTATGTCTT	Oligonucleotides P6 and P9 (bottom strand)
RevOMF13v7	CTAGGGCATATGGGCCATCGGAGTAGCTATGTCTTTT	Oligonucleotides P7 and P10 (bottom strand)
RevOMF13v16	CTAGGGCATATGGGCCATCGGAGTAGCTATGTACAGTG	Oligonucleotide P11 (bottom strand)
RevOMF13v3	GCATATGGGCCATCGGAGTAGCTATGTA	Oligonucleotide P12 (bottom strand)
RevOMF13v13	CTAGGGCATATGGGCCATCGGAG	Oligonucleotide P13 (bottom strand)
RevOMF13v11	CTAGGGCATATGGGCCATCGGAGTAGC	Oligonucleotide P14 (bottom strand)
RevOMF13v5	GCATATGGGCCATCGGAGTAGCT	Oligonucleotide P15 (bottom strand)

## SUPPLEMENTARY FIGURES

Figure S1. Nucleotide sequence of the DNA fragment introduced into pBluescript SK+ to give pBS-Z1. Cloning sites (KpnI and SacI) are underlined.

Figure S2. Formation of a stable ternary complex between Cas1-Cas2, protospacer P1 and CRISPR-containing plasmid pCOLA-Z0. Samples contained combinations of the indicated compounds: 400 nM of the DY782-labeled protospacer P1 (Fig. 1), 140 nM of Cas1 or of Cas1-Cas2 complex and 7.5 nM of either supercoiled pCOLA-Z0 or pCOLA-Duet1. All samples were analyzed by EMSA. The sample with Cas1-Cas2, protospacer P1 and plasmid pCOLA-Z0, was also treated with SDS at a final concentration of 0.5% (w/w) prior to electrophoresis (lane 1). The gel was scanned for fluorescence of DY782 (in green) and stained with ethidium bromide. The right side of the Figure is a zoom of the central part of the gel.

Figure S3. Binding of Cas1-Cas2 to acceptor DNA, in the presence or absence of protospacer P1. Samples contained the indicated concentrations of Cas1-Cas2 (abscissa of the top graph) and 7.5 nM of either supercoiled pBS-Z0 or pBluescript SK+. If present, DY782-labeled protospacer P1 was included at a concentration in excess by a factor 1.2 over that of Cas1-Cas2. After 4 h incubation at 22°C, samples were analyzed by EMSA. Gels were stained with ethidium bromide and, when applicable, scanned for fluorescence of DY782. Bound and free plasmid concentrations were calculated from the gel experiments. Experiments were made in triplicate. Error bars in the top graph represent standard deviations (s. d.). The gels under the top graph display representative examples of the experiments. Shown are the parts of the gels where free and bound plasmids migrate. With pBS-Z0 plus protospacer, DY782-labeled bound plasmid could be followed, not with pBluescript SK+. The weighted least-square fit of the data obtained with pBS-Z0 plus protospacer (thick blue line) yielded an apparent  $K_d$  value of  $8.7 \pm 1.3$  nM and a plateau value of  $6.4 \pm 0.1$  nM.

Figure S1. Sequence of the insert of plasmid pBS-Z1

GGTACCTTAAGGTTGGTGGGTTGTTTTTATGGGAAAAAATGCTTTAAGAACAAATGTATA  
CTTTTAGAGAGTTCCCCGCGCCAGCGGGGATAAACCGCTTGGAAGTGAAGACAGCGGA  
AGCTGTGGGGAGTTCCCCGCGCCAGCGGGGATAAACCGAGCTC

Figure S2. Formation of a ternary complex between Cas1-Cas2, protospacer P1 and plasmid pCOLA-Z0

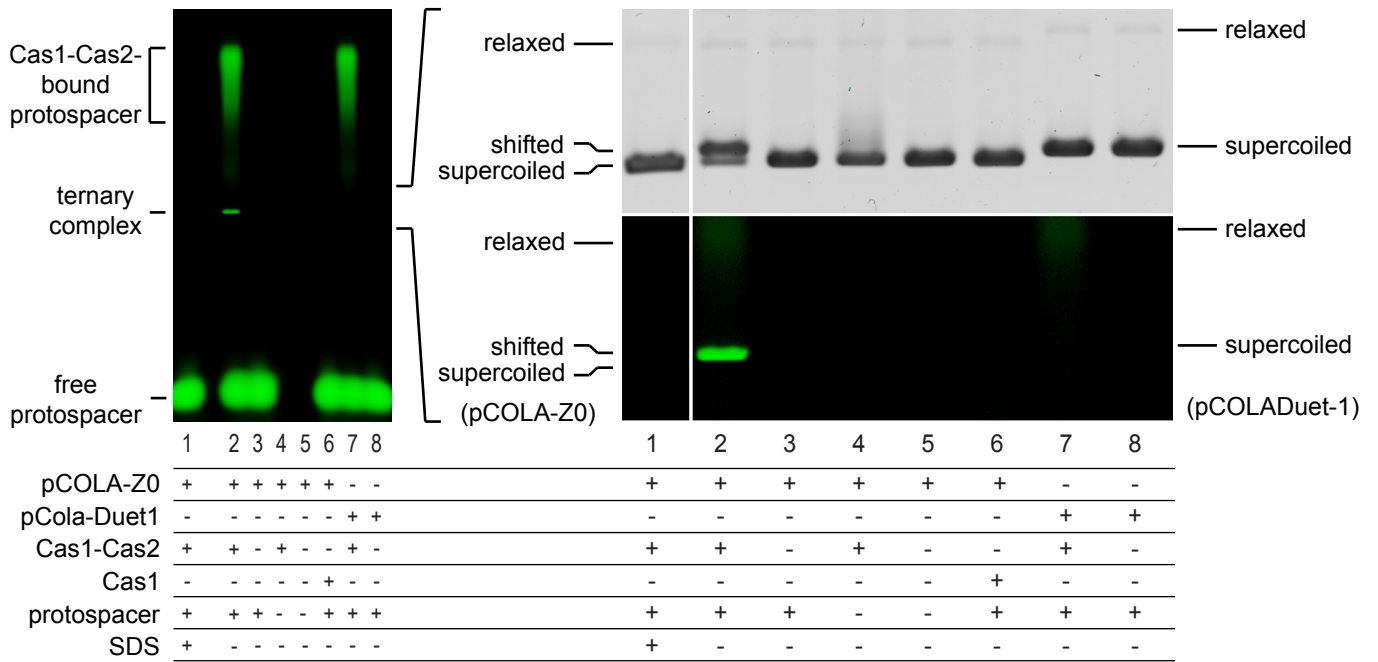


Figure S3. Binding of Cas1-Cas2 to acceptor DNA in the presence or absence of protospacer P1

