SUPPLEMENTARY INFORMATION

Supplementary Table S1. oligos used in this work

| oligos	sequence
target-DNMT1-3-F	aatgtttcctgatggtccatgtctgttactcgcctgtcaagtggcgtgac
target-DNMT1-3-R	gtcacgccacttgacaggcgagtaacagacatggaccatcaggaaacatt
target-DNMT1-3-F-FAM	FAM-aatgtttcctgatggtccatgtctgttactcgcctgtcaagtggcgtgac
target-DNMT1-3-F-FAM-3'	aatgtttcctgatggtccatgtctgttactcgcctgtcaagtggcgtgac-FAM
target-DNMT1-3-R-FAM	gtcacgccacttgacaggcgagtaacagacatggaccatcaggaaacatt-FAM
target-DNMT1-3-R-FAM-5'	FAM-gtcacgccacttgacaggcgagtaacagacatggaccatcaggaaacatt
DNMT1-795-F	aatgcccaggttgtcctccatctgag
DNMT1-795-R	catgttggggattcctggtgccagaa
pSB1A2-DNMT1-795-F	caccaggaatccccaacatgggatccactagtctctagctcgag
pSB1A2-DNMT1-795-R	tggaggacaacctgggcattggatcctttctcctctttctag
T7-crRNA-F	GAAATTAATACGACTCACTATAGGG
T7-T1-14-R	gaaagttgcgataaATCTACAACAGTAGAAATTCCCTATAGTGAGTCGTATTAATTTC
T7-T1-15-R	agaaagttgcgataaATCTACAACAGTAGAAATTCCCTATAGTGAGTCGTATTAATTTC

T7-T1-16-R	tagaaagttgcgataaATCTACAACAGTAGAAATTCCCTATAGTGAGTCGTAT TAATTTC
T7-T1-17-R	gtagaaagttgcgataaATCTACAACAGTAGAAATTCCCTATAGTGAGTCGTA TTAATTTC
T7-T1-18-R	agtagaaagttgcgataaATCTACAACAGTAGAAATTCCCTATAGTGAGTCGT ATTAATTTC
T7-T1-19-R	cagtagaaagttgcgataaATCTACAACAGTAGAAATTCCCTATAGTGAGTCG TATTAATTTC
T7-T1-20-R	tcagtagaaagttgcgataaATCTACAACAGTAGAAATTCCCTATAGTGAGTCG TATTAATTTC
T7-T1-21-R	ttcagtagaaagttgcgataaATCTACAACAGTAGAAATTCCCTATAGTGAGTC GTATTAATTTC
T7-T1-22-R	attcagtagaaagttgcgataaATCTACAACAGTAGAAATTCCCTATAGTGAGTC GTATTAATTTC
T7-T1-23-R	aattcagtagaaagttgcgataaATCTACAACAGTAGAAATTCCCTATAGTGAGT CGTATTAATTTC
T7-T1-24-R	gaattcagtagaaagttgcgataaATCTACAACAGTAGAAATTCCCTATAGTGAG TCGTATTAATTTC
T7-T1-30-R	aagcttgaattcagtagaaagttgcgataaATCTACAACAGTAGAAATTCCCTATAG

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	TGAGTCGTATTAATTTC
T7-DNMT1-3-R	GAGTAACAGACATGGACCATCAGATCTACAACAGTAGAAATTCCCTA
	TAGTGAGTCGTATTAATTTC
T7-DNMT1-3-crRNA-R-18	acagacatggaccatcagATCTACAACAGTAGAAATTCCCTATAGTGAGTCG
	TATTAATTTC
act-crRNA1	cagctcgctgcactgatatctacaacagtagaaattccctatagtgagtcgtattaatttc
act-crRNA2	acacgtcccaataagttatctacaacagtagaaattccctatagtgagtcgtattaatttc
Ermp-F1	<u>cagtgca</u>gctcgctgcactgattaaagcccgacccgagcacgcgc
Ermp-R1	<u>catggac</u>acgtcccaataagttgaatctcaccgctggatcctaccaaccggc
actII-orf4ck-F	ttccgaggacccagccgtatc
actII-orf4ck-R	accaattcccggtcgtcgctc
orf4-RTF	aaggcgacccagctcctggtg
orf4-RTR	tggtccgcccacaactcctcg
actI-RTF	cagttcgccgtggcctgtgc
actI-RTR	atactcgcgctccaggctgg
actIII-RTF	tgcaccacaccctgctttccg
actIII-RTR	acgaactctggctcgatgtcg

The overhang sequences in target DNA primers were underlined.

## Supplementary Table S2. Strains and plasmids used in this work

| Strains/Plasmids	Description	Reference/source
Strains		
E. coli DH10B	F⁻ *endA1 deoR⁺ recA1 galE15 galK16 nupG rpsL Δ(lac)X74 φ80lacZ ΔM15 araD139 Δ(ara,leu)7697 mcrA Δ(mrr-hsdRMS-mcrBC)* Str^R λ⁻	Invitrogen
E. coli BL21(DE3)	F– *ompT gal dcm lon hsdSB(rB- mB-) λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5]*)	Invitrogen
E. coli ET12567	*dam*, *dcm*, *hsdM*, *hsdS*, *hsdR*, *cat*, *tet*, for intergeneric conjugation	Our lab
Streptomyces sp. 4F	A thermo-streptomyces found in soil	(1)
Plasmids		
pSB1A2	pMB1 replication origin (copy number of 100-300 per cell) and ampicillin resistance	iGEM[a]
pET28a-TEV	pET28a carrying the TEV protease cleavage site	(2)
pSB1A2-DNMT1-3	pSB1A2 carrying the target of DNMT1-3	This study
pET28a-TEV-FnCpf1	pET28a-TEV carrying the FnCpf1 encoding gene	(3)
pET28a-TEV-AsCpf1	pET28a-TEV carrying the AsCpf1 encoding gene	(3)
pET28a-TEV-LbCpf1	pET28a-TEV carrying the LbCpf1 encoding gene	This study
pHIW	A vector carrying the *act* cluster for actinorhodin expression in *Streptomyces* sp. 4F	(1)
pHIW-*erm*P	pHIW with the *actII-orf4* promoter in *act* replaced with *erm*P promoter	This study
pEASY-blunt-zero	A commercial vector for cloning of genes in a TOPO-cloning way	Transgen
pEASY-*erm*P	pEASY-blunt-zero carrying the *erm*P promoter	This study
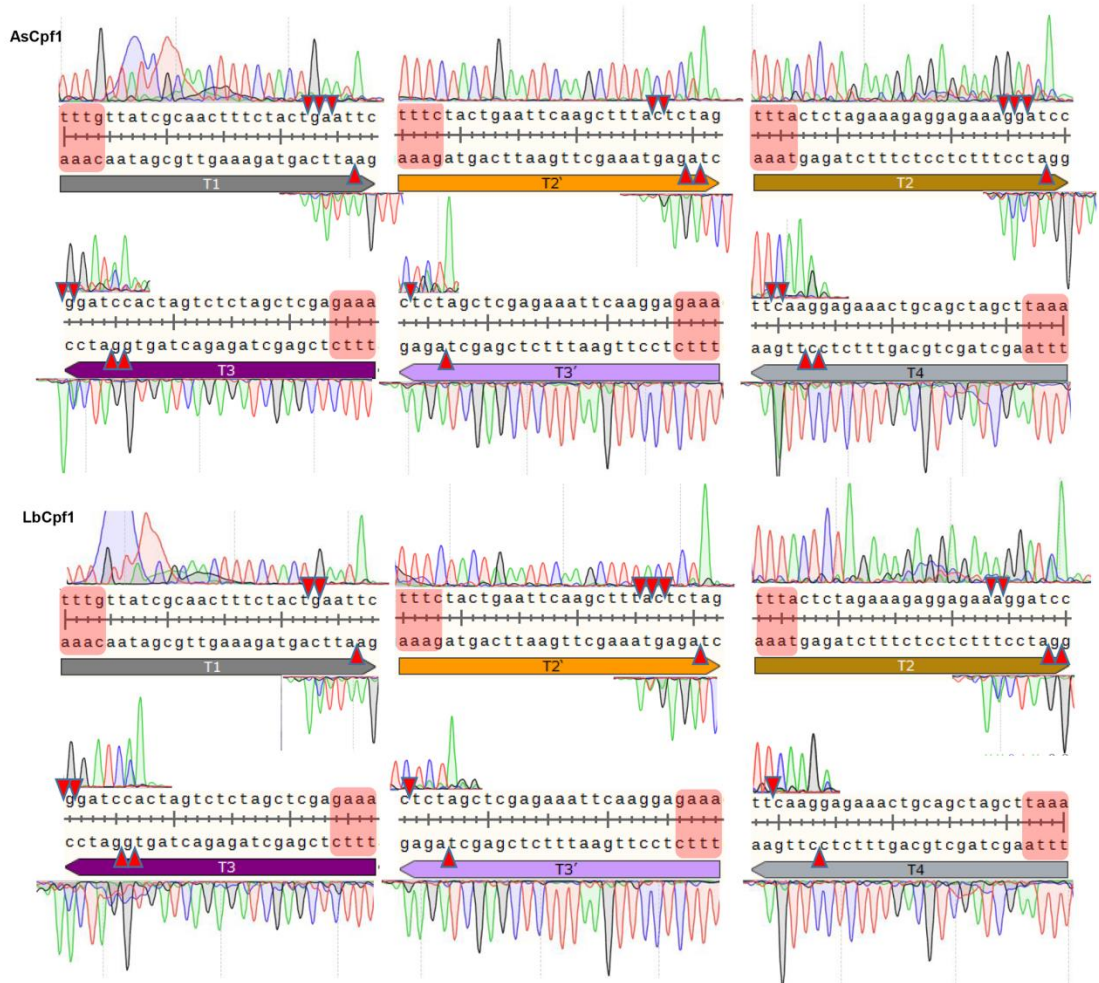
Supplementary Figure S1. Identification of the cleavage sites of AsCpf1 and LbCpf1 by Sanger DNA sequencing. The cleavage sites were around the 18th base on the non-target strand and the 23rd base on the target strand, which were indicated by red triangles based on the Sanger sequencing results. The accuracy of the cleavage varied among the targets and the origins of Cpf1.
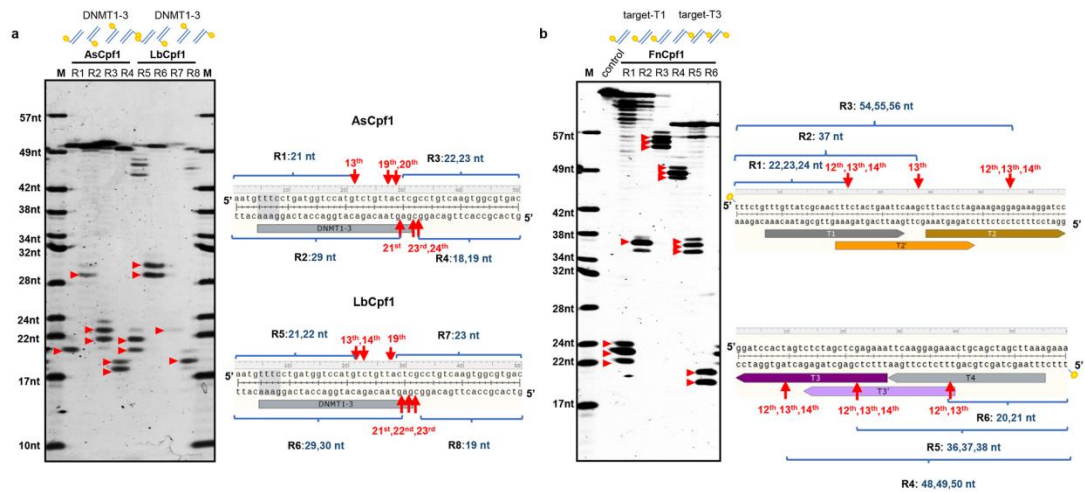
Supplementary Figure S2. Identification of the Cpf1 cleavage sites by urea PAGE. (a) Target of DNMT1-3 was labelled with FAM on either the 5'-end or 3'-end on the target or non-target strand. Both AsCpf1 and LbCpf1 showed cleavage activities around 14th base on the non-target strand. (b) Digestion of another six targets by FnCpf1. Target DNA was labelled on the 5'-end of the non-target strand. For all reactions (*i.e.* with different crRNAs), FnCpf1 showed obviously cleavage activities around the 14th base on the non-target strand.
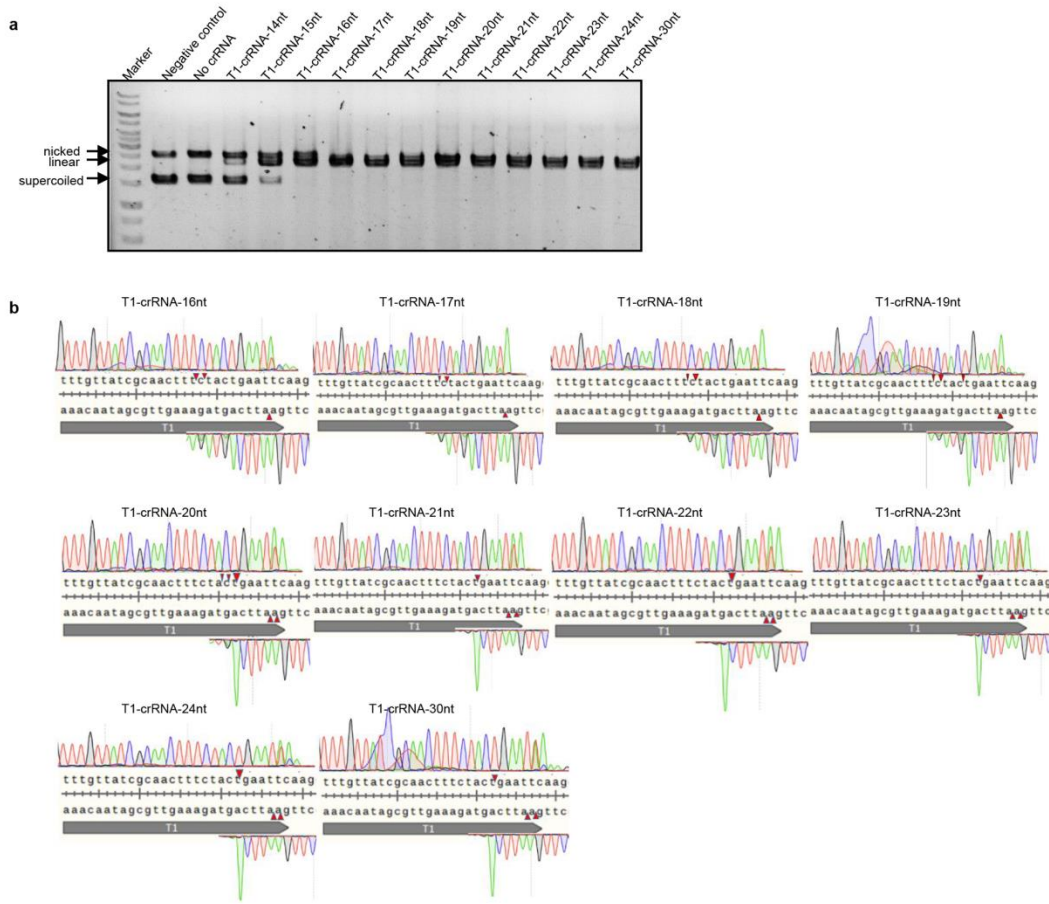
Supplementary Figure S3. Identification of the FnCpf1 cleavage sites with different length of spacers by Sanger sequencing. (a) Agarose gel electrophoresis analysis of the products of plasmid pCB1A2 digested by FnCpf1 with different length of spacers in crRNAs. Longer than 17-nt spacer crRNA enabled FnCpf1 with full double-stranded break activity. (b) FnCpf1 with shorter spacer crRNAs (less than 20 nt) had different cleavage sites from those with full-length crRNA (*e.g.* T1-crRNA-24nt). And FnCp1 cleavage with spacers longer than 20 nt showed similar cleavage profiles in the cleavage sites.
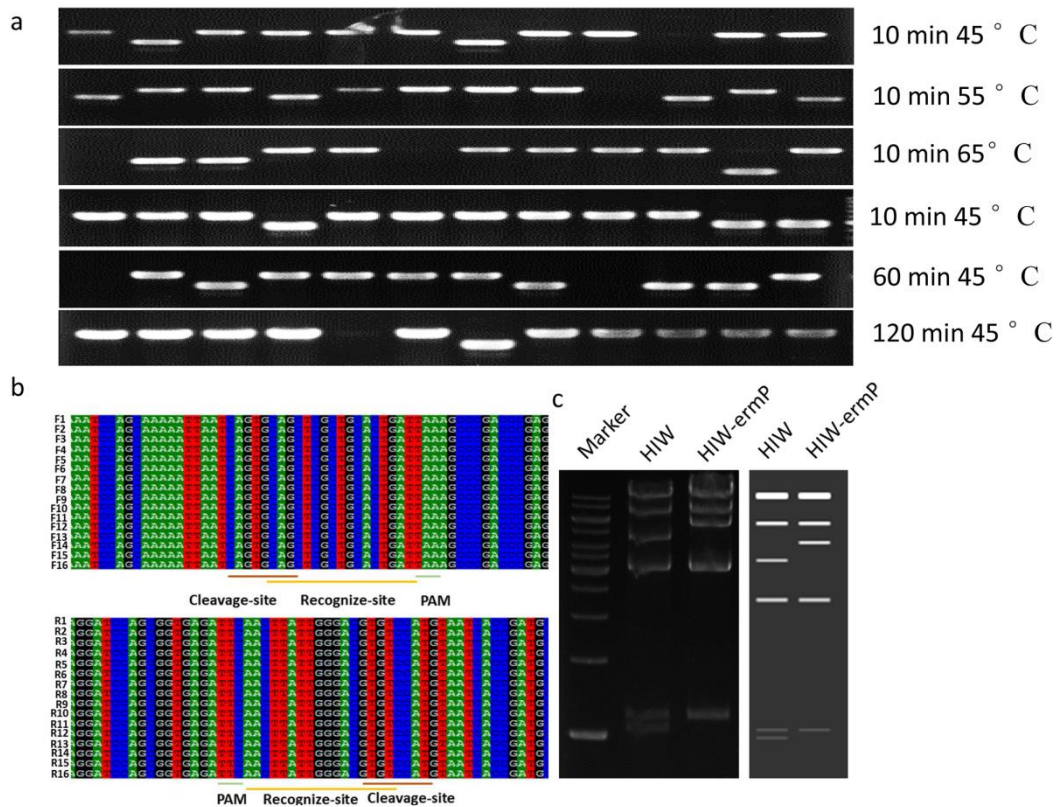
Supplementary Figure S4. Optimal reaction conditions for the CCTL method using Taq DNA ligase. (a) Positive rates of CCTL with different reaction temperatures and time courses, which was analyzed by PCR using primers of actII-orf4CKF and actII-orf4CKR. In each reaction condition, PCR bands with larger sizes showed the correct size and indicated positive clones. (b) Verification of the ligation accuracy of the positive clones by Sanger sequencing. The red line represented the cleavage sites, which produced sticky ends for DNA ligation. Spacer sequences were indicated by yellow lines (the Recognize-site), while the PAM sites were shown by green lines. The upper panel showed the sequencing results for the ligation 5'-end of the promoter, while the lower panel showed the 3'-end ligation results. (c) The StuI digestion results of plasmids pHIW and pHIW-*erm*P (left panel), which had the same theoretical restriction profiles as predicted (right panel).

REFERENCES

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