

Table S5 Oligonucleotides and target sequences used in this study.

ID #	Assay	Description	Sequences (5'-3')
oligo-001	Cy5-labeled dsDNA cleavage of Cas π	Forward PCR primer for cy5-labeled dsDNA target amplification	Cy5-CGGTACCCGGGGATCC
oligo-002	Cy5-labeled dsDNA cleavage of Cas π	Reverse PCR primers for cy5-labeled dsDNA target amplification of Cas π	HO-TATGACCATGATTAGGCCAAGCT TGTTACCAGGGTGTGCGCCCTGG GCAGGATCCCCGGGTACCG
oligo-003	Cy5-labeled dsDNA cleavage, cleavage sites determination and <i>trans</i> -cleavage assay of Cas π	DNA non-target strand containing a TGCCC PAM (blue) and a protospacer region (red) for Cas π cleavage assay and cleavage sites determination	NH ₂ -CGGTACCCGGGGATCCTGCCCAG GGCGACACCCTGGTGAACAAGCT TGGCCTAATCATGGTCATA
oligo-004	Cy5-labeled dsDNA cleavage, cleavage sites determination and <i>trans</i> -cleavage assay of Cas π	Non-labelled complementary strand of oligo-003 and oligo-006	OH-TATGACCATGATTAGGCCAAGCT TGTTACCAGGGTGTGCGCCCTGG GCAGGATCCCCGGGTACCG
oligo-005	Cy5-labeled dsDNA cleavage, ssDNA cleavage, cleavage sites determination and <i>trans</i> -cleavage assay of Cas π	DNA target strand for Cas π cleavage assay and cleavage sites determination of Cas π	NH ₂ -TATGACCATGATTAGGCCAAGCT TGTTACCAGGGTGTGCGCCCTGG GCAGGATCCCCGGGTACCG
oligo-006	Cy5-labeled dsDNA cleavage, cleavage sites determination and <i>trans</i> -cleavage assay of Cas π	Non-labelled complementary strand containing a TGCCC PAM (blue) and a protospacer region (red) of oligo-004 and oligo-005.	OH-CGGTACCCGGGGATCCTGCCCAG GGCGACACCCTGGTGAACAAGCT TGGCCTAATCATGGTCATA
Oligo-007	Cy5-labeled dsDNA cleavage and <i>trans</i> -cleavage assay of LbCas12a	Reverse PCR primer for cy5-labeled dsDNA target amplification and non-labelled DNA target strand for Cas12a <i>trans</i> -cleavage assay of LbCas12a.	HO-TATGACCATGATTAGGCCAAGCT TGTTACCAGGGTGTGCGCCCTGA AAAGGATCCCCGGGTACCG
Oligo-008	<i>Trans</i> -cleavage assay of LbCas12a	Non-labelled DNA non-target strand containing a TTTTC PAM (blue) and a protospacer	HO-CGGTACCCGGGGATCCTTTTCAG

		region (red) for Cas12a <i>trans</i> -cleavage assay.	GGCGACACCCTGGTGAACAAGCT TGGCCTAATCATGGTCATA
Oligo-010	<i>Trans</i> -cleavage assay	ssDNA substrate.	NH ₂ - CTGAAAACCTCGCAGCTACCTTAA CGGCCGCCATTTCCTATGCGTAC ATCTGGATCAAGCC
Oligo-011	Cas π R-loop ternary complex reconstitution	DNA non-target strand containing a TTTTC PAM (blue) and a protospacer region (red) for Cas π R-loop complex reconstitution.	HO- CGGGATGCCCAGGGCGACAC AAGTTGTCCAAGATCC
Oligo-012	Cas π R-loop ternary complex reconstitution	DNA target strand for Cas π R-loop complex reconstitution.	HO- GGATCGTTCACCAGGGTGTCCGCC CTGGGCATCCCCG
Oligo-013	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primes for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 1 (brown).	HO- TATGACCATGATTAGGCCAAG CTTGTTACCAGGGTGTCCGCC CGGGGCAGGATCCCCGGGTACCG
Oligo-014	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primes for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 2 (brown)	HO- TATGACCATGATTAGGCCAAG CTTGTTACCAGGGTGTCCGCC ATGGGCAGGATCCCCGGGTACCG
Oligo-015	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primes for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 3 (brown)	HO- TATGACCATGATTAGGCCAAG CTTGTTACCAGGGTGTCCGCA CTGGGCAGGATCCCCGGGTACCG
Oligo-016	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primes for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 4 (brown)	HO- TATGACCATGATTAGGCCAAG CTTGTTACCAGGGTGTCCGAC CTGGGCAGGATCCCCGGGTACCG
Oligo-017	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primes for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 5 (brown)	HO- TATGACCATGATTAGGCCAAG CTTGTTACCAGGGTGTCTCC CTGGGCAGGATCCCCGGGTACCG

Oligo-018	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primes for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 6 (brown)	HO-TATGACCATGATTAGGCCAAG CTTGTTACCAGGGTGTAGCC CTGGGCAGGATCCCCGGGTAC CG
Oligo-019	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primes for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 7 (brown)	HO-TATGACCATGATTAGGCCAAG CTTGTTACCAGGGTGGCGCC CTGGGCAGGATCCCCGGGTAC CG
Oligo-020	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primes for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 8 (brown)	HO-TATGACCATGATTAGGCCAAG CTTGTTACCAGGGTTTCGCC CTGGGCAGGATCCCCGGGTAC CG
Oligo-021	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primes for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 9 (brown)	HO-TATGACCATGATTAGGCCAAG CTTGTTACCAGGGGGTCGCC CTGGGCAGGATCCCCGGGTAC CG
Oligo-022	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primes for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 10 (brown)	HO-TATGACCATGATTAGGCCAAG CTTGTTACCAGGTTGTTCGCC CTGGGCAGGATCCCCGGGTAC CG
Oligo-023	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primes for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 11 (brown)	HO-TATGACCATGATTAGGCCAAG CTTGTTACCAGTGTGTTCGCC CTGGGCAGGATCCCCGGGTAC CG
Oligo-024	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primes for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 12 (brown)	HO-TATGACCATGATTAGGCCAAG CTTGTTACCA TGGTGTTCGCC CTGGGCAGGATCCCCGGGTAC CG

Oligo-025	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primes for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 13 (brown)	HO-TATGACCATGATTAGGCCAAG CTTGTTACCCGGGTGTCGCC CTGGGCAGGATCCCCGGGTAC CG
Oligo-026	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primes for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 14 (brown)	HO-TATGACCATGATTAGGCCAAG CTTGTTACAGGGGTGTCGCC CTGGGCAGGATCCCCGGGTAC CG
Oligo-027	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primes for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 15 (brown)	HO-TATGACCATGATTAGGCCAAG CTTGTTCAACAGGGGTGTCGCC CTGGGCAGGATCCCCGGGTAC CG
Oligo-028	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primes for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 16 (brown)	HO-TATGACCATGATTAGGCCAAG CTTGTTCCCAGGGGTGTCGCC CTGGGCAGGATCCCCGGGTAC CG
Oligo-029	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primes for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 17 (brown)	HO-TATGACCATGATTAGGCCAAG CTTGTTAACAGGGGTGTCGCC CTGGGCAGGATCCCCGGGTAC CG
Oligo-030	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primes for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 18 (brown)	HO-TATGACCATGATTAGGCCAAG CTTGTCACCAGGGGTGTCGCC CTGGGCAGGATCCCCGGGTAC CG
Oligo-031	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primes for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 19 (brown)	HO-TATGACCATGATTAGGCCAAG CTTGTCACCAGGGGTGTCGCC CTGGGCAGGATCCCCGGGTAC CG

Oligo-032	Cy5-labeled dsDNA mismatch cleavage assay	Reverse PCR primers for cy5-labeled dsDNA target amplification and mismatch cleavage assay with mismatch at position 20 (brown)	HO-TATGACCATGATTAGGCCAAGCTTCTTCACCAGGGTGTCCGCTGGGCAGGATCCCCGGGTACCG
RNA-001	<i>In vitro</i> plasmids cleavage	crRNA containing target spacer (red) of Cas π -1	ACACUCUAAAGGAAUGAAAGAGGGCGACACCCUGGUGAAC
RNA -002	<i>In vitro</i> plasmids cleavage	crRNA containing target spacer (red) of Cas π -2	AACGCUCUUAGGGAAUGAAAGAAGGGCGACACCCUGGUGAAC
RNA -003	<i>In vitro</i> plasmids cleavage	tracrRNA sequence of Cas π -1	GUCUGCCGAAGACGCCGCACGGAGCCUGGGCCGAAUCGUAGAUCGAACGCGGCAUCGAAGCCCUGCAGCCCUUCGGGGCCAAGGCGGCGCAGCAAGCCUCUUUCAGGGCGCAGAGUCCUUUAGAGUGU
RNA -004	<i>In vitro</i> plasmids cleavage	tracrRNA sequence of Cas π -2	GUCUCGACUAUGCCGUACCACUAGACCGAGCCUACACGGCACGGGUCAUAGCGUUAACCAAGGCGUGGUGACAAGCCUCUUUCAGGGCAGCGGACACUUAAGAGCGUU
RNA -005	<i>In vitro</i> plasmids cleavage, cy5-labeled dsDNA cleavage, cleavage sites determination, <i>trans</i> -cleavage and complex reconstitution	sgRNA joint by tracrRNA and crRNA, which contained target spacer (red), with GAGAG loop (green) of Cas π -1	GUCUGCCGAAGACGCCGCACGGAGCCUGGGCCGAAUCGUAGAUCGAACGCGGCAUCGAAGCCCUGCAGCCCUUCGGGGCCAAGGCGGCGCAGCAAGCCUCUUUCAGGGCGCAGAGUCCUUUAGAGUGUGAGAGACACUCUAAAGGAAUGAAAGAGGGCGACACCCUGGUGAAC
RNA -006	<i>In vitro</i> plasmids cleavage, cy5-labeled dsDNA cleavage, cleavage sites determination, <i>trans</i> -cleavage and complex reconstitution	sgRNA joint by tracrRNA and crRNA, which contained target spacer (red), with GAGAG loop (green) of Cas π -2	GUCUCGACUAUGCCGUACCACUAGACCGAGCCUACACGGCACGGGUCAUAGCGUUAACCAAGGCGUGGUGACAAGCCUCUUUCAGGGCAGCGGACACUUAAGAGCGUUGAGAGAACGCUCUUAGGGAAUGAAGAGGGCGACACCCUGGUGAAC
RNA -007	<i>In vitro</i> plasmids cleavage, cy5-labeled dsDNA cleavage, 12nt	sgRNA joint by tracrRNA and crRNA, which contained target spacer (red), with GAGAG loop	GUCUGCCGAAGACGCCGCACGGAGCCUGGGCCGAAUCGUAGAUCGAACGCGGCAUCGAAGCCCUGCAGCCCUUCGGGGCCAAGGCGG

	truncated A-II region	(green) and 12nt truncated A-II region of Cas π -1	CGCAGCAAGCCUCUUUCAGGGC GCAGAGUCCUUUAGAGAGUAAA GGAAUGAAAGAGGGCGACACCC UGGUGAAC
RNA - 008	<i>In vitro</i> plasmids cleavage, cy5-labeled dsDNA cleavage, 24nt truncated A-II region	sgRNA joint by tracrRNA and crRNA, which contained target spacer (red), with GAGAG loop (green) and 24nt truncated A-II region of Cas π -1	GUCUGCCGAAGACGCCGCACGG AGCCUGGGCCGGAAUCGUAGAU CGAACGCGGCAUCGAAGCCUCG CAGCCCUUCGGGGCCAAGGCGG CGCAGCAAGCCUCUUUCAGGGC GCAGAGUGAGAGAAUGAAAG AGGGCGACACCCUGGUGAAC
RNA - 009	<i>In vitro</i> cy5-labeled dsDNA cleavage, and <i>trans</i> -cleavage	sgRNA of LbCas12a, which contained target spacer (red)	GAGAUUUCUACUCUUGUAGAU GGGGCGACACCCUGGUGAAC
π -sg1	Cell genome editing	<i>EGFP</i> gene target 1 of Cas π , PAM of the targets: CCT	CTGCTCAGGTGGAGATGAAC
π -sg2	Cell genome editing	<i>EGFP</i> gene target 2 of Cas π , PAM of the targets: CCC	GCTTCTTGTTTCATCTCCACC
π -sg3	Cell genome editing	<i>EGFP</i> gene target 3 of Cas π , PAM of the targets: CCC	GAGCTCAGCCACACTGTCCG
π -sg4	Cell genome editing	<i>EGFP</i> gene target 4 of Cas π , PAM of the targets: CCC	CGAGCTCAGCCACACTGTCC
π -sg5	Cell genome editing	<i>EGFP</i> gene target 5 of Cas π , PAM of the targets: CCT	TCTCCAGCTTCTGCTTCACC
π -dest	Cell genome editing	<i>EGFP</i> gene non-target of Cas π ,	GGAAGAGCGAGCTCTTCC
12a-sg1	Cell genome editing	<i>EGFP</i> gene target 1 of Cas12a, PAM of the targets: TTTC	CCGCTTCTTGTTTCATCTCCA
12a-sg2	Cell genome editing	<i>EGFP</i> gene target 2 of Cas12a, PAM of the targets: TTTC	TGAAACTCAGTTTCCCGCTT
12a-sg3	Cell genome editing	<i>EGFP</i> gene target 3 of Cas12a, PAM of the targets: CTTC	TTCCGAAGAGCGGCTGCTGT
12a-sg4	Cell genome editing	<i>EGFP</i> gene target 4 of Cas12a, PAM of the targets: CTTC	GGAAGAAGCACGCGGACAGT
12a-sg5	Cell genome editing	<i>EGFP</i> gene target 5 of Cas12a, PAM of the targets: CTTC	TCCTTCTCCAGCTTCTGCTT
12a-dest	Cell genome editing	<i>EGFP</i> gene non-target of Cas12a	GGAAGAGCGAGCTCTTCC
Cas9-sg1	Cell genome editing	<i>EGFP</i> gene target 1 of SpyCas9, PAM of the targets: AGG	GTTTCATCTCCACCTGAGCAG
Cas9-sg2	Cell genome editing	<i>EGFP</i> gene target 2 of SpyCas9, PAM of the targets: GGG	GGTGGAGATGAACAAGAAGC

Cas9-sg3	Cell genome editing	<i>EGFP</i> gene target 3 of SpyCas9, PAM of the targets: GGG	CGGACAGTGTGGCTGAGCTC
Cas9-sg4	Cell genome editing	<i>EGFP</i> gene target 4 of SpyCas9, PAM of the targets: GGG	GGACAGTGTGGCTGAGCTCG
Cas9-sg5	Cell genome editing	<i>EGFP</i> gene target 5 of SpyCas9, PAM of the targets: AGG	GGTGAAGCAGAAGCTGGAGA
Cas9-dest	Cell genome editing	<i>EGFP</i> gene non-target of SpyCas9	GGAAGAGCGAGCTCTTCC
ccdB-T	Bacterial plasmids interference	<i>ccdB</i> gene target of Cas π	CGATAACGGAGACCGGCACA
CcdB-dest	Bacterial plasmids interference	<i>ccdB</i> gene non-target of Cas π	GGAAGAGCGAGCTCTTCC
<i>B2M</i> -1	Cell genome editing	<i>B2M</i> gene target 1 of Cas π , PAM of the targets: CCC	GATATTCCTCAGGTACTIONCA
<i>B2M</i> -2	Cell genome editing	<i>B2M</i> gene target 2 of Cas π , PAM of the targets: CCT	GAATCTTTGGAGTACCTGAG
<i>TP53</i> -1	Cell genome editing	<i>TP53</i> gene target 1 of Cas π , PAM of the targets: CCC	ACCATGAGCGCTGCTCAGAT