(1) annealing of the guide RNA: the guide RNA was diluted to an appropriate concentration (5 μM), and annealed in the PCR instrument. The annealing procedure: denaturing at 75°C for 5 min, and then cooling from 75°C to 20°C at a decreasing rate of 1°C per minute.

(2) incubation of the guide RNA with Cas12b: the annealed guide RNA was mixed with Cas12b at an equal molar concentration, and allowed to stand at 30°C for 20-30 min.

(3) Cas12b reaction: a 20 μL reaction system was added with the mixture of the guide RNA and Cas12b incubated in step (2) (the final concentrations of the guide RNA and Cas12b were both 250 μM), 1 μL of a target DNA or 1 μL of a LAMP product, a fluorescence probe (HEX-N12-BHQ1) (with a final concentration of 500 nM), 2 μL of a 10X NEB Buffer 3.1, and 0.5 μL of an RNase inhibitor (40 U/μL). After mixing homogenously, the reaction was conducted at 48°C for 30min. After that, it was inactivated fire heating at 98°C for 5 min in a PCR instrument.

4. Detection of trans-cleavage activity of Cas12b by a fluorescence microplate reader method:

20 μL of the inactivated reaction solution was added into a 96-well plate, and detected by a microplate reader (with an excitation light at 535 nm and an emission light at 556 nm). Combined with the LAMP amplification, Cas12b could produce a significant collateral single-stranded DNA trans-cleavage activity for a target DNA concentration as low as 10 aM. The results were as shown in FIG. 24.

Cis-cleavage characteristics of Cas12a in cleaving a target single-stranded DNA:

First, in order to test the single-stranded DNA cleavage characteristics of Cas12a, several crRNAs targeting a short single-stranded DNA (DNMT1-3) (Table 1) are designed, which are labeled with 5(6)-carboxyfluorescein (FAM) at the 3' terminal. After the cleavage by FnCas12a, the reaction product is analyzed by denatured urea-polyacrylamide gel electrophoresis (urea-PAGE). It is found that the single strand DNA cleavage by Cas12a is programmed. That is, the cleavage site is near the 22nd base (21st to 23rd bases) of the target sequence counted from the 3' end base to the 5' end of the first target sequence paired with the crRNA guide sequence, as shown in FIGs. 1A and 1C. The cleavage of a double-stranded DNA by Cas12a requires a PAM sequence, while the cleavage of a single-stranded DNA by Cas12a does not require the PAM sequence (FIGs.

1A, 1B and 2), which is similar to the single-stranded DNA cleavage mediated by Cas9. However, the single-stranded DNA cleavage activity mediated by Cas12a depends on a stem-loop structure in crRNA, as shown in FIG. 1A, while Cas9 still shows weak cleavage activity for a single-stranded DNA with only a 20-nt complementary RNA sequence. The stem-loop structure of the crRNA is important for stabilizing the structure of Cas12a, which is the reason why the ring structure of the crRNA is necessary for the cleavage of the single-stranded DNA by Cas12a. It is further tested whether a shorter lead sequence crRNA can pass through the single-stranded DNA cleavage site of Cas12a in such a manner that the cleavage is outside the recognition site. When the length of the guide sequence is 16 nt, 18 nt and 20 nt, all of these crRNAs lead to cleavage by Cpf1 near the 22nd base, as shown in FIGs. 1B and 1D, meaning that the cleavage site is 4 nt, 2 nt or 0 nt outside the recognition site. Next, the cleavage efficiency of Cas12a on different substrates is tested by using substrates of a double-stranded DNA and a single-stranded DNA respectively, as shown in FIG. 1F. Similar to the situation of Cas9 cleavage, the cleavage of the single-stranded DNA is slower than that of the double-stranded DNA, as shown in FIGs. 1E to 1G. These results indicate that the mechanism of recognizing and cleaving a single-stranded DNA by Cas12a may be different from that of a double-stranded DNA, which is a low-efficiency recognition and cleavage manner independent of PAM; and the PAM sequence accelerates the recognition and/or cleavage of the target double-stranded DNA by Cas12a.

Trans-cleavage characteristics of Cas12a in cleaving a single-stranded DNA:

When the target single-stranded DNA is labeled at the 3' terminal, Cas12a cleaves at the vicinity of the 22nd base, as shown in FIG. 1. However, when it is labeled at the 5' terminal, no cleavage product band of predicted size is observed, but a short (< 6 nt) FAM-labeled product is produced, as shown in FIG. 3B. Through detailed experiments, once the ternary complex Cas12a/crRNA/target single-stranded DNA is formed, the target single-stranded DNA (DNMT1-3) (Table 1) labeled at 5'-terminal is cleaved, and a short FAM-labeled product is produced, as shown in FIG. 3C. In addition, the ternary complex also cleaves a single-stranded DNA that has no sequence that is complementary with the crRNA (i.e., a collateral single-stranded DNA) in any

other reaction system, as shown in FIGs. 3C and 3D. This cleavage phenomenon is called trans-cleavage, which is different from programmable cis-cleavage. When the target single-stranded DNA is labeled at the 3' terminal, trans-cleavage is also observed, but many cis-cleavage products are left, as shown in FIG. 3B, This may be due to the complex formed by Cas12a/crRNA/target single-stranded DNA, and the target single-stranded DNA is protected, such that its labeled 3' terminal is protected from exposure to a nuclease active site of the ternary complex. These cleavage processes can be as shown in FIG. 3A.

In addition to the FnCas12a tested above, 9 Cas12as from other species sources are also tested (Table 2 and FIG. 4A). Except Lb4Cas12a, all Cas12as have good endonuclease activity to the plasmid DNA (as shown in FIG. 4B), and all Cas12a ternary complexes show cis and trans cleavage activities on the single strand (as shown in FIGs. 4C and 4D). This shows that cis and trans activity of Cas12a on the single-stranded DNA is a common phenomenon.

The cis and trans key sites and mechanism for cleaving a single-stranded DNA by Cas12a.

In order to determine the key amino acid residues related to cis and trans activities on the single-stranded DNA in Cas12a, several candidate residues of Cas12a are mutated to conduct the activity test. First, three single-amino-acid mutants of FnCas12a (H843A, K852A and K869A) are purified and tested, and their residues are related to an RNase activity. The results of the study of the trans-activity on the single-stranded DNA show that no obvious difference in cis- and trans-cleavage activities on the single-stranded DNA between the wild-type FnCas12a and the three mutants is found, as shown in FIGs. 5A and 5C.

Next, when endonuclease active sites in the FnCas12a, i.e., the RuvC domain (D917A, E1006A or D1255A) and the Nuc domain (R1218A) sites are mutated, the cis and trans cleavage activities of these mutated Cas12a on the single-stranded DNA are both affected, as shown in FIGs. 5B and 5D. These results indicate that the key site of Cas12a for cleavage of a target double-stranded DNA is closely related to cis and trans cleavage activities on the single-stranded DNA.