

**Table S6.** Instructions for preparing target DNA and Cas9 recombinant protein.

gRNA in vitro transcription (NEB T7 quick high yield RNA synthesis kit)

Reagents	Volume
NTP Buffer Mix	7.5 $\mu$ l
Template DNA	8 $\mu$ l
T7 RNA Polymerase	1.5 $\mu$ l
Nuclease free water	3 $\mu$ l
Total reaction volume	20 $\mu$ l
Incubate reactions in a PCR machine at 37 °C for 4 h.	

The linearized system of recombinant vector

Reagents	Volume
DNA sample	20 $\mu$ L
Restriction enzyme	10 $\mu$ L
10 $\times$ Buffer	20 $\mu$ L
dH2O	150 $\mu$ L
Incubate at 37 °C for 3 h	

In vitro cleavage assay

Reagent	Volume
Cas9 recombinant protein (1mg/mL)	2 $\mu$ L
gRNA	2 $\mu$ L
Linearized target DNA	12 $\mu$ L
Phosphate buffer (pH 7.5, 1 M)	0.8 $\mu$ L (50 mM final)
MgCl <sub>2</sub> (100 mM)	1 $\mu$ L (5 mM final)
DTT (1 M)	0.02 $\mu$ L (1 mM final)
PBS	2.18 $\mu$ L
Total volume	20 $\mu$ L
Incubate assays at 37 °C for 1 h	