!!! RNase-free working environment is required for the whole procedure!!!

Protein preparation

. Prepare 1µM (1pmol/µL, 161.1ng/µL) CAS9/CPF1 with transduction buffer

a. Thaw the CAS9/CPF1 at r/t.
b. Centrifuge the sample in a desktop microcentrifuge at maximum speed for 5min at r/t.
c. Dilute CAS9/CPF1 10x with its corresponding 5xtransduction buffer.
d. Check the concentration with Nanodrop-Protein280.
e. Dilute to 161.1ng/µL (1µM) with its corresponding 1x transduction buffer.

Experimental procedure of the *in vitro* endonuclease activity assay

1) Assemble the reaction at room temperature, in the following order:

Nuclease free water	12µL
NEB buffer 3	2µL
1µM sgRNA	4µL
1µM CAS9 / CPF1	1µL
Reaction volume	19µL
Pre-incubate at room temperature for 20min	
100fmol/µL substrate DNA fragment	1ul
Total reaction volume	20µL

2) Mix thoroughly and briefly spin down
3) Incubate in a thermocycler for 1hour at 37^{o}C
4) Add 4µL of 6xDNA loading buffer
5) Checking on a 1.5% gel