In vitro DNA digestion with Cas9 Nuclease

Materials:

• Cas9 Nuclease (Vazyme, Catalog # EN301)
• Sterile and nuclease-free 1.5 or 2.0-mL Eppendorf tubes, PCR tubes or multi-well plates
• Nuclease-free, molecular biology-grade water

Procedure:

To obtain maximum cleavage efficiency, it is highly recommend to use the molar ratio of Cas9 Nuclease, sgRNA, and target DNA at 10:10:1 or higher. Total volume of reaction is 30 µl, which can be scaled up as needed. Dilute sgRNA with nuclease free water to 300 nM and DNA to 30 nM in advance.

1. Prepare the following reaction solution in order as follows:

table

Nuclease-free water	20 µl
10× Cas9 Nuclease Reaction Buffer	3 µl
sgRNA (300 nM)	3 µl
Cas9 Nuclease (1 µM)	1 µl
Total Volume	27 µl

2. Incubate at 37℃ for 10 min.
3. Add 3 µl of 30 nM DNA.
4. Vortex to mix well and spin down briefly to collect the liquid.
5. Incubate at 37℃ for 1 hour.
6. The digested product can be analyzed by agarose electrophoresis.

Note: Please wear gloves and hygiene mask, use nuclease-free consumables to avoid contamination of RNA nuclease during the operation.