In vitro digestion of DNA with Cas9 nuclease and sgRNA

Overview:

This protocol describes how to digest double-stranded DNA *in vitro* using OriGene Cas9 protein (TP790148) and a synthetic guide RNA (sgRNA).

Required Materials:

- Cas9 Nuclease, S. pyogenes (Cat. No. TP790148)
- 10X Cas9 Nuclease Reaction Buffer (20mM HEPES, 100mM NaCl, 5mM MCl2, 0.1mM EDTA, pH 6.5 at 25°C, pH is critical for Cas9 activity, make sure buffer has the right pH value).
- Nuclease-free water
- sgRNA containing the targeting sequence
  - ✓ Synthetic pure sgRNA can be conveniently ordered from OriGene sgRNA service. http://www.origene.com/CRISPR-CAS9/
  - ✓ Alternatively, sgRNAs can be generated by *in vitro* transcription Kit using linearized plasmid, PCR products, or oligonucleotides as templates
  - ✓ sgRNAs must contain sequence complementary to the target DNA (guide sequence)
- DNA substrate
  - ✓ Substrate can be circular or linearized plasmid, PCR products, or synthesized oligonucleotides. Linearized DNA is preferred.
- Equipment and Reagents for DNA fragment analysis (agarose, DNA loading dye, Gel electrophoresis apparatus, etc.).

Before Starting

- We strongly recommend wearing gloves and using nuclease-free tubes and reagents to avoid RNase contamination.
- Reconstitution of synthetic sgRNA
  - ➢ Spin down briefly before opening the tube.
  - ➢ Add 100µl nuclease-free water to make 10µM stock solution.
  - ➢ Dilute stock solution to make 1µM working solution.
- Prepare 200-400 ng linearized DNA as substrate.

Procedure

1. Assemble the reaction in a nuclease free PCR tube in the following order:

Component	Volume (30ul final)
Nuclease-free water	21 µl
10X Cas9 Nuclease Reaction Buffer	3 µl
1 µM sgRNA	1 µl (~30 nM final)
1 µM Cas9 Nuclease, S. pyogenes (TP790148)*	1 µl (~30 nM final)
20 nM substrate DNA	4 µl (~3 nM final)
Total reaction volume	30 µl

* 1 µM equals 165 ng/µl.

2. Mix thoroughly and spin down briefly.
3. Incubate at 37°C for 1 hour, then heat at 65°C for 10 min to deactivate Cas9 nuclease. Hold the reactions at 4°C if you want to take a break.
4. Proceed with fragment analysis by gel electrophoresis.