In vitro digestion of DNA with Cas9 Nuclease, S. pyogenes (M0386)

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Abstract

Cas9 Nuclease, *S. pyogenes*, (Cas9) is a double-stranded DNA endonuclease that is guided to its target by sequence complementarity of a small RNA loaded into the protein. This protocol describes how to digest double-stranded DNA *in vitro* using Cas9 and a single guide RNA (sgRNA).

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Guidelines

It is essential to keep the molar ratio of Cas9 and sgRNA per target site at 10:10:1

Before start

Prepare 300 nM sgRNA by diluting the stock with nuclease-free water on ice.
Prepare 30 nM substrate DNA with a single target sequence by diluting the stock with nuclease-free water on ice.

Materials

Cas9 Nuclease, S. pyogenes - 250 pmols M0386L by Contributed by users

Proteinase K, Molecular Biology Grade - 2 ml P8107S by New England Biolabs

Protocol

1. Assemble the reaction at room temperature in the following order:

Step 1.

Components	30 µl
Nuclease-free water	20 µl
10X Cas9 Nuclease Reaction Buffer	3 µl
300nM sgRNA	3 µl (30 nM final)
1 µM Cas9 Nuclease, *S. pyogenes* (M0386S)	1 µl (30 nM final)

Reaction volume	27 µl
Pre-incubate for 10 minutes at 25^{0}C	
30nM substrate DNA	3 µl (3 nM final)
Total reaction volume	30 µl

Mix thoroughly and pulse-spin in a microfuge.

Step 2.

Incubate at 37°C for 15 minutes.

Step 3.

Add 1 ul of Proteinase K to each sample, Mix thoroughly and pulse-spin in a microfuge.

Step 4.

Incubate at room temperature for 10 minutes.

Step 5.

Proceed with fragment analysis.

Step 6.

Warnings

We strongly recommend wearing gloves and using nuclease-free tubes and reagents to avoid RNase contamination.