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In vitro digestion of DNA with Cas9 Nuclease, S. pyogenes (M0386) V.1

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New England Biolabs (NEB)
Tech. support phone: **+1(800)632-7799** email: **info@neb.com**

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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).

Guidelines

OVERVIEW:
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).

REQUIRED MATERIALS:
- Cas9 Nuclease, S. pyogenes (NEB **#M0386**)
- 10X Cas9 Nuclease Reaction Buffer
- Nuclease-free water
- sgRNA containing the targeting sequence in the region of interest
- sgRNAs can be generated by in vitro transcription using the HiScribe T7 Quick High-Yield RNA synthesis Kit (NEB **#E2050**) using linearized plasmid, PCR products, or oligonucleotides as templates
- sgRNAs must contain sequence complementary to the target DNA (1,2)For information on design of sgRNA transcription templates please visit **Addgene**
- DNA substrate containing the target sequence
- The substrate DNA can be circular or linearized plasmid, PCR products, or synthesized oligonucleotides

OPTIONAL MATERIALS:
Apparatus and reagents for DNA fragment analysis
- E. g. Agarose gel electrophoresis apparatus
- DNA Loading Dye (e.g. Gel Loading Dye, Purple (6X) NEB **#B7024S**)
- E.g. Agilent Bioanlyzer or similar

BEFORE YOU START:
We strongly recommend wearing gloves and using nuclease-free tubes and reagents to avoid RNase contamination. Further recommendations for avoiding ribonuclease contamination can be found **here**.
Reactions are typically 30 µl but can be scaled up as needed. Reactions should be assembled in nuclease-free microfuge tubes or PCR strip tubes.
It is essential to keep the molar ratio of Cas9 and sgRNA per target site at 10:10:1 or higher to obtain the best cleavage efficiency. A calculator can be found **here**.
Prepare 300nM sgRNA by diluting the stock with nuclease-free water on ice.
Prepare 30nM substrate DNA with a single target sequence by diluting the stock with nuclease-free water on ice.

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

	Component	Volume (for 30 µl reaction)
	Nuclease-free water	20 µl
	10X Cas9 Nuclease Reaction Buffer	3 µl
	300nM sgRNA	3 µl (30nM final)
	1 µM Cas9 Nuclease, S.pyogenes (M0386S)	1 µl (~30nM final)

Reaction volume	27 µl
30nM substrate DNA	3 µl (3nM final)
Total reaction volume	30 µl

*The substrate DNA and sgRNA, and nuclease-free water are not included.

2. Mix thoroughly and pulse-spin in a microfuge.
3. Incubate at 37°C for 1 hour.
4. Proceed with fragment analysis.

REFERENCES:
1. Jinek et al. (2012) Science 337 (6096) 816-821.
2. Larson et al. (2013) Nature Protocol 8 (2180-2196).
3. Mali et al. (2013) Science 339 (6121): 823-826.

Materials

MATERIALS

Cas9 Nuclease, S. pyogenes - 70 pmol **New England Biolabs Catalog #**M0386S

Before start

We strongly recommend wearing gloves and using nuclease-free tubes and reagents to avoid RNase contamination. Further recommendations for avoiding ribonuclease contamination can be found **here**.

1 Prepare 300nM sgRNA by diluting the stock with nuclease-free water on ice.

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

3 Assemble the reaction at room temperature in the following order (total volume **30 µl**)

Protocol

NAME

Cas9 M0386 Mixture

CREATED BY

New England Biolabs

PREVIEW

3.1 Nuclease-free water **20 µl**

3.2 10X Cas9 Nuclease Reaction Buffer **3 µl**

3.3 300nM sgRNA **3 µl** (30nM final)

3.4 1 µM Cas9 Nuclease, S.pyogenes (~30nM final)

1 µL

Cas9 Nuclease, S. pyogenes - 70 pmol **New England Biolabs Catalog #**M0386S

4 Pre-incubate for 10 minutes at 37ºC

00:10:00

5 Add 30nM substrate DNA

6 Mix thoroughly.

7 Incubate at 37°C for 1 hour.
01:00:00

8 Proceed with fragment analysis.