ARIA

# In Vitro gRNA-Cas12a Digestion Protocol

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# In Vitro gRNA-Cas12a Digestion

## Introduction

**Protocol obtained from NEB, specific for the purified protein that was ordered.**
EnGen Lba Cas12a (Cpf1) from Lachnospiraceae bacterium ND2006 is a site-specific DNA endonuclease guided by a single 41-44 nucleotide guide RNA (gRNA) (1). Targeting requires a gRNA complementary to the target site as well as a 5´ TTTN protospacer adjacent motif (PAM) on the DNA strand opposite the target sequence. Cleavage by EnGen® Lba Cas12a occurs ~18 bases 3´ of the PAM and leaves 5 nucleotide 5´ overhanging ends. EnGen Lba Cas12a has Simian virus 40 (SV40) T antigen nuclear localization sequences (NLS) at both the N and C-termini of the protein.

## Materials

- EnGen Lba Cas12a (Cpf1) (NEB #M0653)
- 10X NEBuffer r2.1 Reaction Buffer
- Nuclease-free water
- Proteinase K, Molecular Biology Grade (NEB #P8107)
  - Subtilisin-related serine protease hydrolyzes a variety of **peptide bonds** and is frequently used to cleanup enzymatic reactions or cell lysates. Probably won't be needed if we amplify the DNA target beforehand.
- Guide RNA containing the targeting sequence in the region of interest --> our construct
  - AmpR
  - ChloR
  - EryR
  - KanR
  - SpecR
- DNA substrate containing the target sequence --> lab plasmids with AR sequences
  - The substrate DNA can be circular or linearized plasmid, PCR products, or synthesized oligonucleotides
- Optional Materials
  - Apparatus and reagents for DNA fragment analysis --> Agarose gel electrophoresis apparatus

## Procedure

### Before starting

1. Wear gloves and use **nuclease-free tubes** and reagents to avoid RNase contamination.
2. 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.
3. It is essential to keep the **molar ratio** of Cas12a and gRNA per target site at **10:10:1** or **higher** to obtain the best cleavage efficiency. A calculator can be found here: https://nebiocalculator.neb.com/#!/ligation
4. Prepare **300 nM gRNA** by diluting the stock with nuclease-free water on ice.

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

6. If planning to use higher concentration EnGen Lba Cas12a (NEB #M0653T) for *in vitro* digestion of DNA, the enzyme can be diluted to **1 µM** in EnGen Lba Cas12a Diluent prior to the reaction assembly.

## In vitro Assay

7. Assemble the reaction at **room temperature** in the following order:

Table1

| | A | B |
|---|---|---|
| 1 | **COMPONENT** | **AMOUNT** |
| 2 | Nuclease-free water | 20 µl |
| 3 | NEBuffer r2.1 Reaction Buffer (10X) | 3 µl |
| 4 | 300 nM gRNA | 3 µl (30 nM final) |
| 5 | 1 µM EnGen Lba Cas12a (Cpf1) | 1 µl (~30 nM final) |
| 6 | **Total Reaction Volume** | **27 µl** |

8. **Pre-incubate** for 10 minutes at 25°C.

9. **Add** 3 $\mu$l of 30 nM substrate DNA (30 $\mu$l final volume).

10. **Mix** thoroughly and pulse-spin in a microfuge.

11. **Incubate** at 37°C for 10 minutes.

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12. NOT: Add 1 $\mu$l of Proteinase K (NEB #P8107) to each sample, Mix thoroughly, and pulse-spin in a microfuge. (Stop reaction, Cas12a digestion)

13. **Incubate** at room temperature for 10 minutes.

14. **Analyse** with the detection protocol [2].

## Bibliography

1. New England Biolabs. (n.d.-b). In vitro digestion of DNA with EnGen® Lba Cas12a (Cpf1) (M0653). Retrieved October 17, 2021, from Neb.com website: https://international.neb.com/protocols/2017/12/19/in-vitro-digestion-of-dna-with-engen-lba-cas12a-cpf1-neb-m0653
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