Protocol

TD-P Revision 1.0

Creation Date: 7/9/2018
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Gene Editing

Utilizing Cas9 Nuclease

Introduction

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems provide bacteria and archae with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids via endonuclease Cas9. Scientists have adapted this system to target any undesired coding sequence in mammalian cells. The CRISPR system consists of a short non-coding guide RNA (sgRNA) made up of a target complementary CRISPR RNA (crRNA) and an auxiliary transactivating crRNA (tracrRNA). The sgRNA guides the Cas9 endonuclease to a specific genomic locus via base pairing between the crRNA sequence and the target sequence, cleaving the DNA to create a double-strand break (Figure 1). The location of the break is within the target sequence 3 bases from the NGG PAM (Protospacer Adjacent Motif). The PAM sequence, NGG, must follow the targeted region on the opposite strand of the DNA with respect to the region complementary sgRNA sequence.

GoldBio Cas9 Nuclease is the purified recombinant Streptococcus pyrogenes Cas9 enzyme containing a nuclear localization signal (NLS) at the C-terminal for targeting to the nucleus. This enzyme is designed to perform CRISPR/Cas9-mediated genome editing.

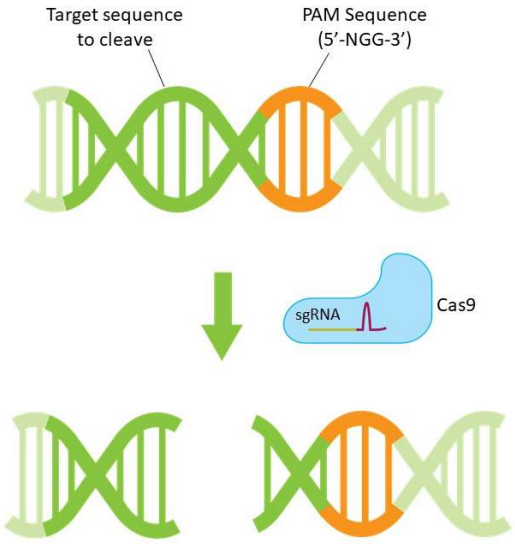

Figure 1. GoldBio Cas9, guided by sgRNA, cuts both strands of DNA creating a double-strand break.

Materials

- Cas9 Nuclease (GoldBio Catalog # C-519 or C-529)

Gold Biotechnology
St. Louis, MO
Ph: (314)890-8778
Web: www.goldbio.com
Email: contactgoldbio86@goldbio.com

- 10x Cas9 Nuclease Reaction Buffer

Not supplied

- Target DNA
- sgRNA
- H_2O PCR Grade

Note: The storage buffer contains 50mM Tris-HCl, 50mM KCl, 1mM DTT, 0.1mM EDTA and 50% glycerol, pH 7.5 at 25°C.

Note: The 1x Cas9 Reaction Buffer contains 20mM HEPES, 100mM NaCl, 5mM $MgCl_2$, 0.1mM EDTA, pH 6.5 at 25°C.

Note: The physical purity of this enzyme is ≥ 98% as assessed by SDS-PAGE with Coomassie® blue staining.

Storage and Handling

- Store Cas9 Nuclease and the 10x Cas9 Nuclease Reaction Buffer at -20°C.
- This product may be shipped on blue ice and should be stored at -20°C immediately upon arrival. When stored under the recommended conditions and handled correctly, this product should be stable for at least 1 year from the date of receipt.

Method

Cas9 Nuclease functional testing was done by *in vitro* DNA cleavage assay with the following protocol, which gives more than 95% digestion of the substrate DNA as determined by agarose gel electrophoresis.

1. Set up a 30 µl reaction in a microcentrifuge tube on ice with the following combinations (see Table 1).

Table 1. Set up of Cas9 reaction.

Component	20 µl Reaction
Target DNA	x µl (~100 ng)
sgRNA	x µl (~4000 ng)
10X Cas9 Reaction Buffer	3.0 µl
Cas9 Nuclease	1.0 µl
H_2O	Up to 30.0 µl

2. Gently mix the reaction mixture and centrifuge briefly.
3. Incubate at 37°C for 60 minutes.
4. Add 1 µl RNase (4 mg/ml)

5. Incubate at 37°C for 20 minutes.
6. Run 0.7 to 1% agarose TBE gel.

Associated Products

- dNTP mix (GoldBio Catalog # D-900)
- Hot Start *Taq* DNA Polymerase (GoldBio Catalog # T-510)
- Hot Start *Taq* DNA Polymerase plus dNTP (GoldBio Catalog # T-511)
- Hot Start *Taq* 2x Master Mix – 50 µl reaction (GoldBio Catalog # T-512)
- Hot Start *Taq* 2x Master Mix – 20 µl reaction (GoldBio Catalog # T-513)
- *Taq* DNA Polymerase (GoldBio Catalog # T-514)
- *Taq* DNA Polymerase plus dNTP (GoldBio Catalog # T-515)
- *Taq* DNA Polymerase with Dye (GoldBio Catalog # T-516)
- *Taq* DNA Polymerase with Dye plus dNTP (GoldBio Catalog # T-517)
- *Taq* DNA Polymerase 2x Premix with Dye (GoldBio Catalog # T-518)
- Proteinase K (GoldBio Catalog # P-480)

References

Jinek M., Chylinski K., Fonfara I., Hauer M., Doudna JA., Charpentier E. 2012. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*. Aug 17;337(6096):816-21.

Mali P., Yang L., Esvelt KM., Aach J., Guell M., DiCarlo JE., Norville JE., Church GM. 2013. RNA-guided human genome engineering via Cas9. *Science*. Feb 15;339(6121):823-6.