USER MANUAL CAS-CLOVER™

Hera BioLabs

Cas-CLOVER™

2022 User Manual
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Cas-CLOVER™ : A Dimeric RNA Guided Targeted Nuclease for Precision Gene-Editing.

CRISPR-Cas9 System

Synthetic CRISPR (Clustered, Regularly Interspaced, Short Palindromic Repeats) single guide RNA (sgRNA) together with CRISPR-associated proteins (Cas9) can be engineered to bind and cut target sequences in the genome. Site-specific cleavage of the target DNA occurs at a location determined by the complementary base pairing of the gRNA and target DNA and a small motif, the protospacer adjacent motif (PAM) shown in Figure 1. In the type II CRISPR-Cas9 system, the PAM sequence (NGG) is essential for DNA cleavage.

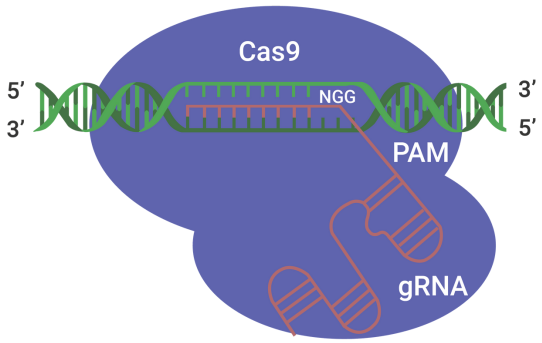

Figure 1: Mechanism of CRISPR-Cas9 directed Mutagenesis. The synthetic gRNA interacts with the complementary sequence within the target region. Recruitment of the Cas9 endonuclease cleaves the DNA 5' of the PAM. Creation of a double-stranded break result in DNA repair mechanism activation and repair of the break via non-homologous end joining (NHEJ) or homologous recombination (HR).

The Cas-CLOVER Gene Editing System

Cas-CLOVER is a modified CRISPR-Cas9 system that has demonstrated a lack of off-target activity associated with CRISPR-Cas9. Cas-CLOVER makes use of a catalytically inactive Cas9 protein, or "dead Cas9" (abbreviated as dCas9), fused to the dimeric Clo051 endonuclease domain.

Two gRNAs can be designed to target genes of interest to create double-stranded breaks, like other dimeric gene editing technologies (i.e. ZFN and TALEN). The Cas-CLOVER system requires two PAM sequences and a flexible spacer range of 16–30 nucleotides for the dual-complex to function. This enables high fidelity gene editing with no detectable off-target activity when screened via next-generation sequencing (NGS) reads, while also maintaining flexibility to target any gene of interest.

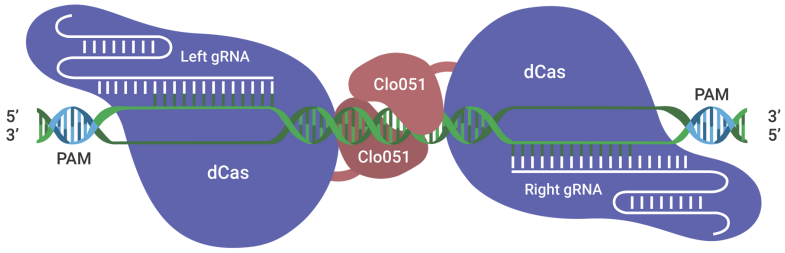

Figure 2: The Cas-CLOVER mechanism of directed mutagenesis.

Cas-CLOVER demonstrates:

- Ease of use; double-gRNA guided cuts for higher accuracy
- High efficiency; dCas-guided DNA recognition
- High fidelity, cuts only when Clo051 nuclease dimerizes

Dual synthetic gRNA/Cas-CLOVER complexes interact with the left and right complementary sequences within the targeted locus, which leads to dimerization of the Clo051 nuclease domains and cleavage of the targeted locus. Creation of a double-stranded break results in DNA repair mechanism activation and repair of the break via non-homologous end joining (NHEJ) or homologous recombination (HR).

Designing Guide-RNA Pairs for Cas-CLOVER

A variety of open source gRNA design tools are available online for you: platforms that accommodate gRNA designs for paired Cas9 Nickase such as ZiFit, CHOPCHOP or Benchling work well for CasCLOVER designs. The two guide-RNAs should be designed in the PAMs-out orientation, separated by a spacer region of 16–20 nucleotides (Figure 3). You simply need to ensure that the gRNA pairs are in the PAMs-out orientation and the targeted sequences are separated by 16–30 nucleotides. The recruited Clo051 nuclease domains dimerize and introduces a single double-stranded break in this spacer region between the two gRNA target sites.

B **Indicator: AAVS1**

mCherry-GAATTcctCCACCCCACAGTGGGGCCACtagggacaggattggtgACAGAAAAGCCCCATCCTTaggGATCC-GFP 17bp

Indicators with different gap length

12bp GAATTcctCCACCCCACAGTGGGGCCACtgtgacagcgagACAGAAAAGCCCCATCCTTaggGATCC
13bp GAATTcctCCACCCCACAGTGGGGCCACtgtgacagatgagACAGAAAAGCCCCATCCTTaggGATCC
14bp GAATTcctCCACCCCACAGTGGGGCCACtgtgacagatggagACAGAAAAGCCCCATCCTTaggGATCC
15bp GAATTcctCCACCCCACAGTGGGGCCACtgtgacagattggagACAGAAAAGCCCCATCCTTaggGATCC
16bp GAATTcctCCACCCCACAGTGGGGCCACtgtgacaggattggagACAGAAAAGCCCCATCCTTaggGATCC
17bp GAATTcctCCACCCCACAGTGGGGCCACtagggacaggattggtgACAGAAAAGCCCCATCCTTaggGATCC
18bp GAATTcctCCACCCCACAGTGGGGCCACtgtgacagtcgattggtgACAGAAAAGCCCCATCCTTaggGATCC
19bp GAATTcctCCACCCCACAGTGGGGCCACtgtgacagtcgacttggtgACAGAAAAGCCCCATCCTTaggGATCC
20bp GAATTcctCCACCCCACAGTGGGGCCACtgtgacagtcgactgtggtgACAGAAAAGCCCCATCCTTaggGATCC
21bp GAATTcctCCACCCCACAGTGGGGCCACtgtgacagtcgactgctggtgACAGAAAAGCCCCATCCTTaggGATCC
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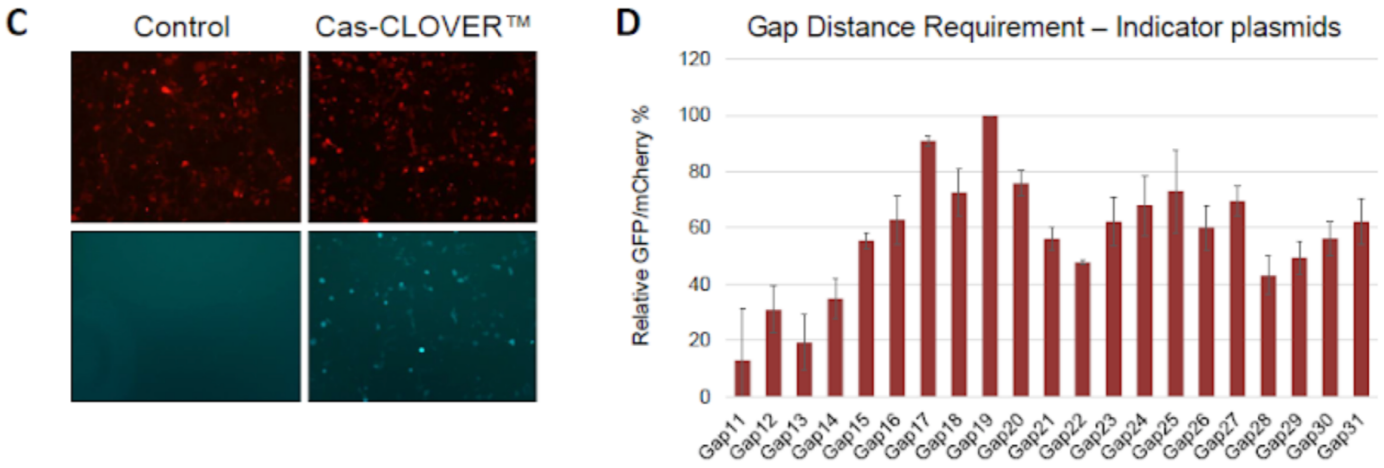

Figure 3: Cas-CLOVER design parameters includes PAMs facing in an outward direction separated by a gap distance between gRNAs of 15–40 base pairs. The ideal spacer distance spacer region of 16–20 nucleotides.

Hera provides the Cas-CLOVER mRNA as a reagent and service for designing gRNAs for your target. Hera can provide gRNAs directly or you can use any commercially available gRNAs from your prefered provided. Once both components are ordered use the following delivery protocol.

Cas-CLOVER mRNA and sgRNA Delivery

Instructions for use with Cas-CLOVER mRNA. Cat# CCL-100
Note: TransIT®-mRNA Transfection kit for CRISPR/Cas9 mRNA and gRNA Delivery by Mirus Bio is recommended for use with Cas-CLOVER mRNA

Cas-CLOVER REAGENT SPECIFICATIONS

Storage	Store Cas-CLOVER mRNA reagent immediately at -80°C.
Handling	Ensure work area and equipment used are RNase free. Minimize the amount of time reagent is outside of -80°C. Aliquot mRNA into working units upon first thawing. Use each aliquot in its entirety upon subsequent thawing. Avoid repeated freeze/thaw cycles.
Product Guarantee	Year from date of purchase, when properly stored and handled.

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Cas-CLOVER™ mRNA AND sgRNAs TRANSFECTION PROTOCOL

Fill in volumes below based on culture vessel used for transfection (Table 1).

A. Plate cells

1. Approximately 18-24 hours before transfection, plate cells in ___ml complete growth medium (per well). Most cell types should be ~80% confluent on day of transfection.
2. For adherent cells: Plate cells at a density of 0.8—3.0 x 105 cells/ml.
3. For suspension cells: Plate cells at a density of 2.5—5.0 x 105 cells/ml.
4. Culture cells overnight.

B. Transfect CasCLOVERTM mRNA

1. Warm TransIT®-mRNA and Boost Reagent to room temperature and vortex gently.
2. Place ___µl of OptiMEM® I Reduced-Serum Medium in a sterile tube.
3. Add ___µl of mRNA encoding Cas-CLOVER mRNA (1µg/µl, 0.6µM stock solution; 0.6nM final concentration per well). Mix gently by pipetting. Note: the amount of Cas-CLOVER mRNA per transfection can be increased and should be optimized for your cell line.
4. Add ___µl of mRNA Boost Reagent. Mix gently by pipetting.
5. Add ___µl TransIT®-mRNA Transfection Reagent. Mix gently by pipetting.
6. Incubate at room temperature for 2-5 minutes to allow complexes to form.
7. Add the complexes drop-wise to different areas of the wells.
8. Gently rock the culture vessel to evenly distribute the complexes.
9. Incubate 6-8hrs to allow for mRNA translation

C. Transfect sgRNAs

1. Add ___µl of sgRNA mix (1.6µg/ul, 50 µM stock solution; 50 nM final concentration per well). Mix gently by pipetting. NOTE: First combine at a 1:1 molar ratio of the two sgRNAs then add to tube containing OptiMEM®. Mix gently by pipetting.
2. Add ___µl of mRNA Boost Reagent. Mix gently by pipetting.
3. Repeat steps 4 – 8 outlined in section B above.
4. Incubate 24-72 hours. NOTE: A post-transfection media exchange is not necessary.
5. Harvest cells and assay as desired.

Culture vessel	24-well plate	12-well plate	6-well plate
Complete growth medium	0.5 ml	1 ml	2.5 ml
Serum-free medium	50 µl	100 µl	250 µl
Cas-CLOVER mRNA (1ug/ul stock) OR sgRNA mix (50uM stock)	1 µl	2 µl	5 µl
mRNA Boost Reagent	0.5 µl	1 µl	2.5 µl
TransIT®-mRNA Transfection Reagent	0.5 µl	1 µl	2.5 µl