

High-Efficiency gRNA-Cas9-GFP Plasmid Assembly Kit

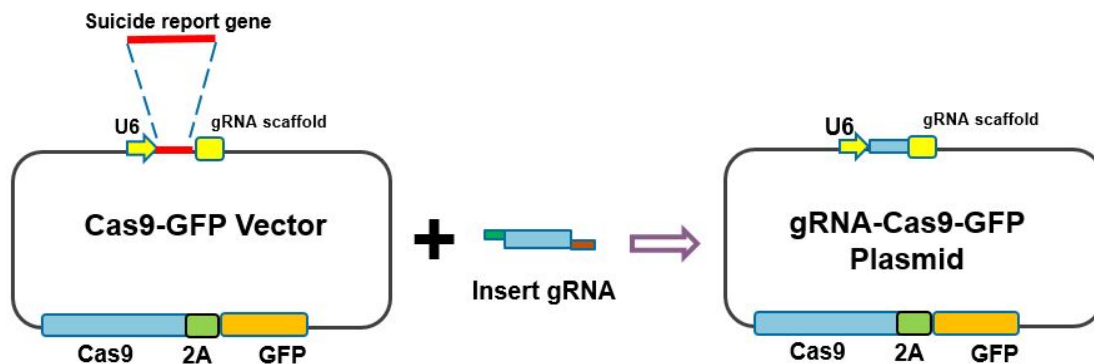
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I	Description.....	1
II	Kit Contents.....	1
III	Key Features.....	2
IV	Quality Control Analysis.....	2
V	Product Usage.....	2
VI	Storage.....	2
VII	Protocol.....	2
VIII	FAQ.....	4

I Description

The High-Efficiency gRNA-Cas9-GFP plasmid Assembly Kit is used for easier and highly efficient cloning of gRNA fragments into Cas9 vectors. This vector has been constructed by integrating a suicide reporter gene into the gRNA insert site. Under the selection of this suicide report gene, almost 100% of clones are positive clones without screening. The High-Efficiency gRNA-Cas9-GFP plasmid is provided in both the circular and linear formats. The GFP fluorescence reporter is linked by 2A, a self-cleavage peptide, and is expressed separately from Cas9, keeping the maximum activity of Cas9.



II Kit Contents

Components	Quantity		Concentration
	10-reaction	25-reaction	
Cas9-GFP Vector	20 µL	50 µL	50 ng/µL
Control gRNA insert	10 µL	25 µL	0.05 µM
Control gRNA primer F	10 µL	25 µL	10 µM
Control Screening primer R	50 µL	125 µL	10 µM

III Key Features

- **Simplicity** –Only a pair of primers is needed. The digestion and ligation can be done in a single reaction.
- **Efficiency** –Up to 100% positive clones.
- **Fidelity** –No mutations.
- **Time-saving** – The construction reaction can be completed within 30 minutes.

IV Quality Control Analysis

- Positive clone rate: up to 100%.
- None colonies could be grown in DH5α competent cells without the insert.

V Product Usage

Rapid construction of gRNA-Cas9 plasmid for the subsequent gene editing research.

VI Storage

Please store at -20°C.

VII Protocol

1. Design specific gRNA primers

Forward primer gRNA-F: **CACC**+N₁₉₋₂₁ (gRNA target sequences)

Reverse primer gRNA-R : **AAAC**+ N₁₉₋₂₁ (gRNA Reverse complementary sequence)

e.g : If the target sequence of gRNA is CATCATTGGAAGACGTTCTT, the forward primer is **CACCCATCATTGGAAGACGTTCTT**, and the reverse primer is **AAACAAGAACGTCTTCCAATGATG**.

2. Anneal the primers

- Dissolve specific gRNA primers F and R to 10 μM.
- Dilute to 0.1 μM.
- Mix the primers F and R 1:1 by volume.
- Denature at 95°C for 5 minutes.
- Place the mixture at room temperature for another 10 minutes, so that the primers cool down to room temperature gradually.

3. Ligation reaction

	Experimental reaction	Positive control reaction
10×T4 DNA ligase buffer	1 µL	1 µL
Cas9-GFP Vector	1 µL	1 µL
annealed specific gRNA insert	0.5 µL	-
Control gRNA insert	-	0.5 µL
BbsI(10 U/µL)	0.25µL	0.25µL
T4 DNA ligase(400 U/µL)	0.25 µL	0.25 µL
H ₂ O	7 µL	7 µL
Total reaction volume	10 µL	10 µL

Reaction conditions :

Temperature	Time	Cycle
37°C	5 minutes	3×
16°C	5 minutes	
4°C	Hold	-

4. Transformation

- Add 5 µL of the above reaction mixture to 50 µL DH5α competent cells.
- Place the cells on ice for 30 min.
- Heat shock at 42°C for 1 min followed by ice bath for 2 min.
- Add 500 µL LB Broth and shake for 30 min at 37°C under 180 rpm.
- Add 100 µL to LB Agar plate with 100 µg/ml *Ampicillin* and incubate overnight at 37°C.

5. Colony PCR screening

	Experimental reaction screening	Positive control reaction screening
10×Taq buffer	2.5 µL	0.25 µL
10mM dNTP	0.5 µL	0.5 µL
Specific gRNA-F (10µM)	0.5 µL	-
Control gRNA primer F (10µM)	-	0.5 µL
Control Screening primer R (10µM)	0.5 µL	0.5 µL
Taq DNA polymerase (5U/µL)	0.25 µL	0.25 µL
Colony	a little	a little
H ₂ O	20.75 µL	20.75 µL
Total reaction volume	25 µL	25 µL

Note: Less than 10 clones is enough for screening in each experimental plate.

Reaction conditions :

Temperature	Time	Cycle
94°C	5 minutes	1
94°C	10 seconds	25×
55°C	10 seconds	
72°C	10 seconds	
72°C	5 minutes	1
4°C	Hold	-

PCR products are detected using agarose gel electrophoresis. The positive control band runs at 270bp. The negative control should remain blank.

Note : This is a basic protocol. The reagent concentrations, conditions, and parameters may need to be optimized.

VIII FAQ

1. Which kinds of competent cells can be used for transformation?

Answer: Suitable competent cells include, but are not limited to DH5 α , Stbl2, Stbl3, EPI300, EPI400, JM108, ER2925 and DH10B-N. Inappropriate competent cells include, but are not limited to Top10, XL1-Blue, and SURE2.

2. What is the size of the plasmid before and after transformation?

Answer: The size of Cas9-GFP vector provided in the kit is 9629bp, and the size of the target plasmid after transformation is about 9290-9292 bp depending on the inserted gRNA.

3. Are there any other versions of gRNA-Cas9 constructs?

Answer: GenScript carries a series of different versions of gRNA-Cas9 constructs, including “High-Efficiency gRNA-Cas9-GFP Plasmid (linear) Assembly Kit (Cat. No. L00690)”, “High-Efficiency gRNA-Cas9-Puro Plasmid (linear) Assembly Kit (Cat. No. L00691)”, and “High-Efficiency gRNA-Cas9-Puro Plasmid Assembly Kit (Cat. No. L00693)”.

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