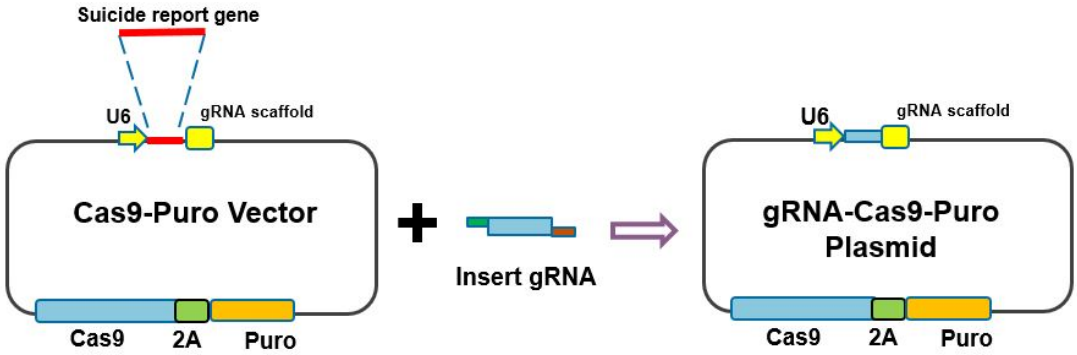
High-Efficiency gRNA-Cas9-Puro Plasmid Assembly Kit

Cat. No. L00693 Version 02202017

I Description

The High-Efficiency gRNA-Cas9-Puro 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-Puro plasmid is provided in both the circular and linear formats. The Puromycin resistant gene 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-Puro 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

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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+N_{19-21} (gRNA target sequences)

Reverse primer gRNA-R：AAAC+ N_{19-21} (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℃ for 5 minutes.
   - Place the mixture at room temperature for another 10 minutes, so that the primers cool down to room temperature gradually.

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3. Ligation reaction

	Experimental reaction	Positive control reaction
10×T4 DNA ligase buffer	1 μL	1 μL
Cas9-Puro 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_2O	7 μL	7 μL
Total reaction volume	10 μL	10 μL

Reaction conditions :

Temperature	Time	Cycle
37℃	5 minutes	3×
16℃	5 minutes	
4℃	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℃ for 1 min followed by ice bath for 2 min.
   - Add 500 μL LB Broth and shake for 30 min at 37℃ under 180 rpm.
   - Add 100 μL to LB Agar plate with 100 μg/ml *Ampicillin* and incubate overnight at 37℃.

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_2O	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.

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Reaction conditions：

Temperature	Time	Cycle
94℃	5 minutes	1
94℃	10 seconds	25×
55℃	10 seconds	
72℃	10 seconds	
72℃	5 minutes	1
4℃	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 DH5α, 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-Puro vector provided in the kit is 9515bp, and the size of the target plasmid after transformation is about 9178-9180 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-GFP Plasmid Assembly Kit (Cat. No. L00692)”.

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